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Carcinogenicity Bioassays of Vinyl Chloride Monomer: A Model of Risk Assessment on an Experimental Basis

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Data are presented regarding the final results of the Bentivoglio (Bologna) project on long-term carcinogenicity bioassays of vinyl chloride (VC).

The experimental project studied the effects of the monomer, administered by different routes, concentrations and schedules of treatment, to animals (near 7000) of different species, strains, sex and age. To our knowledge this is the largest experimental carcinogenicity study performed on a single compound by a single institution.

The results indicate that VC is a multipotential carcinogen, affecting a variety of organs and tissues. In the experimental conditions studied, the neoplastic effects of the monomer were also detected at low doses. The experimental and biological factors greatly affect the neoplastic response to VC. Long-term carcinogenicity bioassays are, at present, a unique tool for the identification and quantification of environmental and occupational risks. Precise and highly standardized experimental procedures are needed to obtain data for risk assessment.

Introduction

The present report deals with the presentation of the final results of our project on the long-term carcinogenicity bioassays of vinyl chloride (VC) (BT project).

To our knowledge this project is the most extensive experimental carcinogenesis study ever performed on one industrial compound by a single institution.

Planning, Materials, Methods and Performance of the Experiment

Planning

The experiments of the project were planned (a) to test the carcinogenicity of the compound; (b) to obtain information on the site and type of tumors; (c) to evaluate the possible effects of the routes of administration, with particular regard to the ones reproducing potential human exposure; (d) to assess, in quantitative terms, the level of risk. The planning of the experiments was aimed at achieving these goals.

The compound was tested on animals of different species, strain, sex and age (Table 1), since it is known that these factors may modify the neoplastic response qualitatively and quantitatively. The choice of the animals was made with the intention of having an integrated system of complementary biological models which could express a range, as wide as possible, of neoplastic responses.

VC was administered by different routes: intraperitoneal (IP) injection, subcutaneous (SC) injection, inhalation and ingestion (by stomach tube), the latter two being the major routes of potential human exposure.

The monomer was administered at different concentrations: 14 by inhalation levels and 6 ingestion levels for various periods of time, by continuous or intermittent treatment (Table 2).

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Table 1. BT project on VC: animals used.

Species	Strain	Sex	Age
Rat	Sprague-Dawley	M, F	Adult (10–21 wk) Newborn (1 day) Embryo (12 days pregnancy)
Rat	Wistar	M	Adult
Mouse	Swiss	M, F	Adult
Hamster	Golden	M	Adult

The plan of the project is presented in Tables 3-9.

Material

VC was supplied from the same source in all cases, and it contained very low amounts of impurities (Table 10). The oil employed as a vehicle in the ingestion and injection experiments was pure virgin olive oil from Tuscany.

The animals (except for the golden hamsters) were breeds which have been routinely employed in our laboratory for many years. It should be pointed out that, whatever their use, all the animals of our colony undergo periodic examination and complete autopsy, giving us extensive information concerning their pathology.

The chambers for inhalation exposure were built basically of stainless steel and glass.

For the ingestion treatment glass syringes and stainless steel needles with round tips were used.

To control the level of exposure in the inhalation experiments, an automatic gas chromatography system was used.

Table 2. BT project on VC: routes, concentrations and schedules.

Route	Concentration	Schedule
Inhalation	30,000, 10,000, 6000, 2500, 500, 250, 200, 150, 100, 50, 25, 10, 5, 1 ppm	4 hr/day, 5 days/wk, 52 wk
	10,000, 6000, 2500, 500, 250, 50 ppm	4 hr/day, 5 days/wk, 17 wk
	10,000, 6000 ppm	4 hr/day, 5 days/wk, 5 wk
	10,000, 6000 ppm	4 hr/day, 1 day/wk, 25 wk
	10,000, 6000 ppm	1 hr/day, 4 days/wk, 25 wk
	10,000, 6000 ppm	4 hr/day, 7 days
Ingestion	50, 16.65, 3.33, 1.0, 0.3, 0.03, mg/kg body weight	5 times/wk, 52 wk
IP injection	4.25 mg	4, 3, or 2 times at 2 month intervals
	4.25 mg	Once only
SC injection	4.25 mg	Once only

Table 3. Plan of long-term experiments on the effects of exposure by inhalation for 1 year to different doses of VC on adult Sprague-Dawley rats (basic experiments).

	Treatment			Animals						
Expt. no.	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT1	Inhalation	10,000, 6000, 2500, 500 250, 50 ppm Untreated controls Treated controls VA, 2500 ppm	4 hr/day, 5 days/wk, 52 wk	Rat	Sprague-Dawley	13	240	240	480	60
BT2	Inhalation	200, 150, 100 ppm Untreated controls	4 hr/day, 5 days/wk, 52 wk	Rat	Sprague-Dawley	13	280	265	545	120–185
BT6	Inhalation	30,000 ppm	4 hr/day, 5 days/wk, 52 wk	Rat	Sprague-Dawley	17	30	30	60	60
BT9	Inhalation	50 ppm Untreated controls	4 hr/day, 5 days/wk, 52 wk	Rat	Sprague-Dawley	11	200	200	400	100 (c) 300 (t)
BT15	Inhalation	25, 10, 5, 1 ppm Untreated controls	4 hr/day, 5 days/wk, 52 wk	Rat	Sprague-Dawley	13	300	300	600	120

Table 4. Plan of long-term experiments on the effects of length of VC exposure on VC carcinogenicity.

Expt. no.	Treatment			Animals						
	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT3	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hr/day, 5 days/wk, 17 wk	Rat	Sprague-Dawley	12	262	288	550	60–190
BT10	Inhalation	10,000, 6000, ppm Untreated controls	4 hr/day, 5 days/wk, 5 wk; 4 hr/day, 1 day/wk, 25 wk; 1 hr/day, 4 days/wk, 25 wk	Rat	Sprague-Dawley	11	420	420	840	120

Table 5. Plan of long-term experiments on the effects of age on vinyl chloride carcinogenicity.

Expt. no.	Treatment			Animals						
	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT5	Transplacental	10,000, 6000 ppm	4 hr/day, 7 days (from 12th to 18th day of pregnancy)	Rat	Sprague-Dawley	19 (breeders) 12 days (embryos)	110	36	146	30–54
BT14	Inhalation	10,000, 6000 ppm	4 hr/day, 5 days/wk, 5 wk	Rat	Sprague-Dawley	1 day	45	44	89	43–46

Table 6. Plan of long-term experiments on the effects of strain on vinyl chloride carcinogenicity.

Expt. no.	Treatment			Animals						
	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT7	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hr/day, 5 days/wk, 52 wk	Rat	Wistar	11	0	220	220	30–40
BT17	Inhalation	1 ppm Untreated controls	4 hr/day, 5 days/wk, 52 wk	Rat	Wistar	13	0	250	250	120–130

Table 7. Plan of long-term experiments on the effects of species on vinyl chloride carcinogenicity.

Expt. no.	Treatment			Animals						
	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT4	Inhalation	10,000, 6000, 2500, 250, 50 ppm Untreated controls	4 hr/day, 5 days/wk, 30 wk	Mouse	Swiss	11	500, 250	260	510	60–150
BT8	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hr/day, 5 days/wk, 30 wk	Hamster	Golden	11	10	268	268	30–62

Table 8. Plan of long-term ingestion experiments on vinyl chloride carcinogenicity.

Expt. no.	Treatment			Animals						
	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT11	Ingestion	50, 16.65, 3.33 mg/kg body weight in olive oil Controls, olive oil	5 times/wk, 52 wk	Rat	Sprague-Dawley	13	160	160	320	80
BT27	Ingestion	1, 0.3, 0.03 mg/kg body weight in olive oil Controls, olive oil	5 times/wk, 52 wk or 59 wk[a]	Rat	Sprague-Dawley	10	300	300	600	150

[a]For 10 animals of each of the three exposed and control groups the treatment was planned to last 104 weeks, but it had to be stopped because of animal intolerance.

Table 9. Plan of long-term injection experiments on vinyl chloride carcinogenicity.

Expt. no.	Treatment			Animals						
	Route	VC dose	Duration	Species	Strain	Age, weeks	No. ♀	No. ♂	Total	No. per group
BT12	IP injection	4.25 mg in 1.0 cc olive oil Controls, 1.0 cc olive oil	4, 3, 2, or 1 times; two month intervals	Rat	Sprague-Dawley	13	150	150	300	60
BT13	SC 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

Table 10. Maximum level of impurities in the VC used.

Impurity	Concn, ppm
H_2O	10
Acetic aldehyde	5
Acetylene	2
Allene	5
Butane	8
1,3-Butadiene	10
Chlorophene	10
Diacetylene	4
Vinyl acetylene	10
Propine	3
Methyl chloride	100

Methods and Procedures

For the experiment on VC, as well as for any other long-term experimental bioassays performed in our laboratory, the procedure has been always the same highly standardized and controlled one. In particular, the following points in our laboratory standard procedures, should be emphasized.

Compounds. All shipments of VC used were examined in order to determine whether they meet the required standards.

Concentrations. The concentrations, particularly when VC was given by inhalation, were controlled by continuous gas chromatographic monitoring.

Modalities of Treatment. Treatment was always performed by the same people. This is particularly important for gavage, since the animals become accustomed to the same operator.

Control of the Animals. The conditions of the animals was checked three times daily. Every two weeks the animals were examined to detect any gross changes.

Weight of the Animals. The animals were weighed every two weeks during treatment and every eight weeks after the end of treatment.

Duration of the Experiments. In the VC project, as in any other long-term bioassays performed in our laboratory, the animals were kept alive until spontaneous death.

Autopsy. Full autopsy was performed on each animal. All parts of the body were explored, including the central nervous system. Specimens for histology included the brain, Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, adrenals, spleen, pancreas, stomach, intestine, bladder, uterus, gonads and any other organ with pathological lesions.

Histology. Specimens were trimmed in the standard way. Sections were routinely stained with Haematoxylin-Eosin and, when necessary, with special techniques.

Histopathological Examination. All sides were screened by a junior pathologist and then reviewed

by a senior pathologist. The same classification of the lesions were used by all pathologists.

Classification of Data. All the anatomical sites and the gross and microscopic observations were classified and coded following our laboratory codes (Tables 11-13).

Table 11. Codes of organs considered (sequence).

Code	Organ	Code	Organ
1	Skin (epidermis and dermis)	32	Adrenals
2	Epidermal appendages	33	Cerebrum
3	Zymbal glands	34	Cerebellum
4	Subcutaneous tissues	35	Spinal marrow
5	Mammary glands	36	Peripheral nervous system: ganglia
6	Parotid glands	37	Peripheral nervous system: nerves
7	Submaxillary glands	38	Eyes
8	Nasal and paranasal cavities	39	Harderian glands
9	Oral cavity	40	Skeletal muscles (diaphragm not included)
10	Tongue	41	Diaphragm
11	Lung	42	Bones
12	Pleura and pleural cavity	43	Articulations
13	Esophagus	44	Heart
14	Forestomach	45	Pericardium and pericardial cavity
15	Glandular stomach	46	Large vessels
16	Intestine	47	Thymus
17	Liver	48	Spleen
18	Pancreas	49	Axillary and inguinal lymph nodes
19	Peritoneum and peritoneal cavity	50	Head-neck lymph nodes
20	Kidneys	51	Interthoracic and parathymic lymph nodes
21	Pelves	52	Intrabdominal lymph nodes
22	Ureters	53	Lymph nodes of other sites
23	Bladder	54	Bone marrow
24	Ovaries	55	Soft tissues of support
25	Uterus	56	Interscapular fat pad
26	Seminal vesicles	57	Trachea
27	Prostate	58	Ear
28	Testicles	59	Female external sex organs
29	Epididymis	60	Male external sex organs
30	Hypophysis	61	Odontogenic apparatus
31	Thyroid	62	Gall bladder

Table 12. Codes of macroscopic changes.

Code	Change	Code	Change
1	No change	19	Dilatation of organ with cavity
2	Alopecia	20	Protrusion of eyeball
3	Keratosis	21	Simple cyst
4	Degenerative pathosis	22	Hemorrhagic cyst
5	Ulceration	23	Multiple simple cyst
6	Hyperemia, edema and hemorrhage	24	Multiple hemorrhagic cyst
7	Phlogosis (including of abscess)	25	Polypoid formation
8	Pulmonary hepatization	26	Papillomatous formation and horn
9	Pulmonary emphysema	27	Solid nodule
10	Irregular surface	28	Hemorrhagic nodule
11	Granulations and plaques	29	Cystic mass
12	Simple thickening	30	Solid mass
13	Thickening of capsule	31	Solid necrotic mass
14	Fibrosis	32	Hemorrhagic mass
15	*In toto* reduction	33	Ossifying mass
16	Atrophy	34	Serous effusion
17	*In toto* enlargement	35	Fibrinous-purulent effusion
18	Augmentation in consistency	36	Hemorrhagic effusion

Table 13. Codes of microscopic changes.

Code	Change	Code	Change
1	No changes	57	Fibroangiomatosis
2	Mild regressive changes	58	Simple polyp
3	Serious regressive changes	59	Polyp with cellular distypias
4	Necrosis	60	Papilloma
5	Ulcer	61	Fibropapilloma
6	Amyloidosis	62	Acanthoma
7	Hyalinosis	63	Trichoepithelioma
8	Colloid-cystic degeneration	64	Simple adenoma
9	Calcifications	65	Muciparous adenoma
10	Emphysema	66	Colloid-cystic adenoma
11	Vascular changes (hyperemia, dilatation of sinusoids and other vessels, edema and hemorrhage)	67	Exocrine pancreas adenoma
		68	Endocrine pancreas adenoma (Islet cell adenoma)
12	Hematic cyst	69	Chromophobe adenoma
13	Organized fibrinous coagulum	70	Chromophilic adenoma
14	Hemorrhagic effusion	71	Cortical adenoma
15	Acute phlogistic changes (including abscess)	72	Medullary adenoma
16	Chronic phlogistic changes (also reactive)	73	Cholangioma
17	Particular granulomatous changes	74	Hepatocellular adenoma or hepatoma
18	Phlogistic effusion	75	Tumor of granulosa and of theca
19	Thickening of capsule	76	Leydig cell tumor
20	Thickening of submesothelial tissues	77	Other epithelial benign tumors
21	Fibrosis	78	Fibroma
22	Post necrotic fibrosis (comprehensive of cirrhosis)	79	Mixoma
23	Fibrous thickening of vessels	80	Lipoma
24	Cystic ectasia of blood vessels with fibrosis	81	Leiomyoma
25	Cystic ectasia of blood vessels with fibrosis and hyperplasia of perithelial cells	82	Rhabdomyoma
		83	Chondroma
26	Cystic ectasia of blood vessels with fibrosis and dysplasia of perithelial cells	84	Osteoma
		85	Angioma
27	Cellular depletion and atrophy (with or without fibrosis)	86	Fibroangioma
		87	Ossifying angioma
28	Simple cyst	88	Other benign tumors of connective tissue
29	Hemorrhagic cyst	89	Fibroadenoma
30	Multiple simple cyst	90	Adenomyoma
31	Multiple hemorrhagic cyst	91	Benign tumors of nervous ganglia (ganglioneuroma) and benign sympathetic tumors of adrenal medulla
32	Dilatation of organs with cavity (including hydronephrosis)	92	Benign tumors of peripheral nerves (neurilemoma)
33	Hyperplasia and squamous metaplasia	93	Carcinoma
34	Glandular simple and cystic hyperplasia	94	Carcinoma with metastases
35	Diffused parenchymal hyperplasia	95	Basocellular carcinoma
36	Nodular parenchymal hyperplasia	96	Basocellular carcinoma with metastases
37	Cortical hyperplasia	97	Squamocellular carcinoma
38	Medullary hyperplasia	98	Squamocellular carcinoma with metastases
39	Hyperplasia of stroma	99	Transitional cell carcinoma
40	Reactive hyperplasia	100	Transitional cell carcinoma with metastases
41	Simple proliferation of lymphoreticular cells with myelopoiesis	101	Adenocarcinoma
		102	Adenocarcinoma with metastases
42	Proliferation of angioblastic cells	103	Biliary duct adenocarcinoma
43	Fibroangioblastic proliferation	104	Biliary duct adenocarcinoma with metastases
44	Proliferation of lipocytes	105	Hepatocellular carcinoma or hepatocarcinoma
45	Proliferation of biliary ducts	106	Hepatocarcinoma with metastases
46	Proliferation of renal tubules and/or of nephroblastema	107	Exocrine pancreas adenocarcinoma
		108	Exocrine pancreas adenocarcinoma with metastases
47	Adenomatous hyperplasia	109	Cortical adenocarcinoma
48	Cholangiofibrosis	110	Cortical adenocarcinoma with metastases
49	Dysplasia (comprehensive of neoplastic parenchymal nodule of liver)	111	Pheochromoblastoma
		112	Pheochromoblastoma with metastases
50	Simple and cystic glandular dysplasia	113	Nephroblastoma
51	Cortical dysplasia	114	Nephroblastoma with metastases
52	Medullary dysplasia	115	Seminoma
53	Dysplasia of angioblastic cells	116	Seminoma with metastases
54	Papillomatosis	117	Melanoma
55	Acanthomatosis	118	Melanoma with metastases
56	Angiomatosis	119	Other malignant epithelial tumors

Table 13. (cont.)

Code	Change	Code	Change
120	Other malignant epithelial tumors with metastases	145	Carcinosarcoma
121	Mesothelioma	146	Carcinosarcoma with metastases
122	Mesothelioma with metastases	147	Neuroblastoma
123	Fibrosarcoma	148	Neuroblastoma with metastases
124	Fibrosarcoma with metastases	149	Glioma (astrocytoma, oligodendroglioma, microglioma)
125	Mixosarcoma	150	Ependymoma
126	Mixosarcoma with metastases	151	Meningioma
127	Liposarcoma	152	Malignant tumors of nervous ganglia and malignant sympathetic tumors of adrenal medulla
128	Liposarcoma with metastases		
129	Leiomyosarcoma	153	Malignant tumors of nervous ganglia and malignant sympathetic tumors of adrenal medulla with metastases
130	Leiomyosarcoma with metastases		
131	Rhabdomyosarcoma	154	Malignant tumors of peripheral nerves (malignant schwannoma)
132	Rhabdomyosarcoma with metastases		
133	Chondrosarcoma	155	Malignant tumors of peripheral nerves (malignant schwannoma) with metastases
134	Chondrosarcoma with metastases		
135	Osteosarcoma	156	Characteristic tumors of eyes
136	Osteosarcoma with metastases	157	Lymphoreticular neoplastic localizations
137	Angiosarcoma	158	Secondary localizations of tumors from other anatomical districts
138	Angiosarcoma with metastases		
139	Ossifying angiosarcoma	159	Neoplastic effusions
140	Ossifying angiosarcoma with metastases	160	Odontoma
141	Angiopericytosarcoma	161	Chondromatosis
142	Angiopericytosarcoma with metastases	162	Histiocytosis and benign histiocytoma
143	Other malignant tumors of connective tissue	163	Mesothelial hyperplasia
144	Other malignant tumors of connective tissue with metastases		

For each animal an individual final card was prepared, which included data on experimental factors, survival, weight at 6, 12, 18 and 24 months, and any gross and microscopic lesions. Samples are shown in Figures 1 and 2.

Presentation of Pathological Data. The results of all VC experiments, as well as those of any other experiment performed in our laboratory, will be presented in the final report (now in press) with the same types of tables, in the same sequence.

This type of presentation has been made possible by the knowledge of the basic pathology of the animal used, which enabled us to make an approximated census of the expected lesions.

Such a procedure permits a quick comparison among the results of different experiments of the same project and possibly of the results of projects studying different compounds.

Interpretation of the Data. The data were subjected to statistical analysis. Although statistical analysis provides an extremely important tool for interpreting the meaning of the results of long-term bioassays, it should be stressed that there may be smaller differences between exposed and control groups which do not reach statistical significance, while these differences could still have meaning from an oncological point of view (particularly in the case of tumors which are infrequent in the animal colony).

Therefore, the most important data should be commented on both in the light of the statistical analysis performed and from a biological point of view.

The methodological protocol adopted meets the requirements of the recent Good Laboratory Practice Act.

Results

Part of these results, namely those dealing with seven basic experiments on the effects of long-term exposure to a range of 14 doses by inhalation (from 30,000 to 1 ppm) and of six doses by ingestion (from 50 mg to 0.03 mg/kg bw), on Sprague-Dawley rates, were presented previously (*1*, *2*).

A report, for limited circulation, dealing with part of the results has also appeared (*3*).

The results of the whole project, with detailed tables, will appear in a monograph which will encompass data on survival rate, body weight, regressive and inflammatory changes, benign and malignant tumors, neoplastic precursors, and the most important proliferative changes.

With this report we are presenting only tables summarizing the most outstanding results and information, and what we do believe to be the integrative documentation and strictly necessary comments.

		Code
Agent: Vinyl chloride		XVIII, 1
Experiment No.: BT 6		
Group No.: I	No. animal: 10	
Type of exposure	Inhalation	I, 1
Concentration: 30,000 ppm		II, 1
Treatment protocol: 4 hr/day, 5 days/ week, 52 weeks		VI, 5
Species: Rat		X, 1
Strain: Sprague-Dawley		XI, 1
Sex: Female		XII, 1
Age at start of experiment (weeks): 17		XIII, 10
Total dose received:		
Age at death (weeks): 85		
Period from start of treatment (weeks): 68		
Weight (g)		
6 months: 247		
12 months: 278		
18 months: 290		
24 months:		

FIGURE 1. Sample treatment protocol card.

Tables 15-31 presented data on the incidence of the tumors which have been considered as dependent or possibly correlated to VC exposure, in 17 different experiments on the effects of VC in different animal systems, by different routes, at different doses and with various schedules of treatment. Explanations of abbreviations used in the tables are given in Table 14.

The possible leukemogenic effect of VC in golden hamsters is expressed both by the slight increase in incidence but more by the decrease in latency time (from 16 weeks in animals treated at 10,000 ppm to 36 weeks in control animals).

Examples of the most characteristic microscopic features of these tumors were given in a previous publication (*4*).

The data on dose-response relationship in long-term treatment experiments, by inhalation and by ingestion, in Sprague-Dawley rats, Wistar rats and Swiss mice, with reference to the incidence of total malignant and benign tumors, and the most important neoplasias observed, are shown in Tables 32-63.

The striking effect of the influence of scheduled treatment is pointed out by the results shown in Table 64.

Examples of the marked influence of the animals used in determining the neoplastic response are shown in Tables 65-67, which point out the effects of species, strain and age.

Conclusions

VC-dependent tumors are identified on the basis of one or more of the following parameters: (a) sharply enhanced incidence; (b) rare or exceptional occurrence in the colony of the animal used; (c) dose-response relationship; (d) association of precursor lesions.

From the presented data the following conclusions may be drawn.

(1) VC causes tumors in all the different animal systems tested.

(2) VC is a multipotential carcinogen, since it causes tumors of different types in different sites (Table 68).

(3) Some types of tumors are observed in all the animals studied, i.e., liver angiosarcoma, whereas others are observed in only one animal system.

(4) The degree of evidence of correlation between VC treatment and the tumors considered as VC-dependent varies from tumor to tumor.

(5) VC shows carinogenic effects both when given by inhalation and ingestion and possibly by injection.

(6) Both through inhalation and ingestion experiments there is a clear-cut dose-response relationship.

(7) The duration of treatment and schedule of treatment greatly affects the neoplastic response.

(8) The neoplastic response, in qualitative and quantitative terms, is greatly affected by the species, the strain and the sex of the animals studied.

(9) Newborn animals appear to be extremely responsive and easily develop liver tumors, both hepatocarcinomas and angiosarcomas.

(10) VC produces carcinogenic effects on embryos via the placenta.

(11) With the above criteria for identifying VC-dependent tumors, VC shows carcinogenic effects even at low doses, namely down to 50 ppm and less.

(12) The results of the seven basic experiments studying the effects of doses of VC as given by inhalation (BT1, 2, 6, 9, 15), and ingestion (BT11, 27), have been subject to statistical analysis following the Fisher exact probability test ($p \leq 0.05$). The total cancer-bearing animals and the tumors

Macroscopic changes:

Site	*Type*	*Side*	*No.*	*Code*
Subcutaneous tissues	Hemorrhagic nodule		1	4, 28, D1
Lung	Hemorrhagic nodule			11, 28, D12
Pleura and pleural cavity	Hemorrhagic effusion			12, 36
Forestomach	No changes			14, 1
Liver	Hemorrhage			17, 6
Peritoneum and peritoneal cavity	No changes			19, 1
Adrenals	Hemorrhagic mass	Sn	1	32, 32, C1, D1
Harderian glands	No changes	Sn		39, 1, C1
Intrathoracic and parathymic lymph node	In toto enlargement			51, 17

Microscopic changes:

Site	*Type*	*Side*	*No.*	*Code*
Subcutaneous tissues	Fibroangioma			4, 86
Lung	Secondary neoplastic localization			11, 158
	(liver angiosarcoma)			(17, 138)
Pleura and pleural cavity	Secondary neoplastic localization			12, 158
	(liver angiosarcoma)			(17, 138)
Forestomach	Papilloma			14, 60
Liver	Hepatocarcinoma			17, 105
	Angiosarcoma with metastases			17, 138
Peritoneum and peritoneal cavity	Secondary neoplastic localization (liver angiosarcoma)			19, 158 (17, 138)
Adrenals	Cortical adenoma	Sn		32, 71, C1
Harderian glands	Abscess	Sn		39, 15, C1
Intrathoracic and parathymic lymph node	No changes			51, 1

FIGURE 2. Sample record of macroscopic and microscopic changes.

Table 14. Abbreviations used in tables.[a]

Abbreviation	
T	Tumor
Ca	Carcinoma
Ep T	Epithelioma
Pa	Papilloma
Ac	Acanthoma
Ad	Adenoma
Ad ↑	Adenoma in malignant transformation
MT	Malignant tumors (total if not otherwise specified)
BT	Benign tumors (total if not otherwise specified)
LAS	Liver angiosarcoma
LA	Liver angioma
ELAS	Extra-liver angiosarcoma
ELA	Extra-liver angioma
Nephro-BL	Nephroblastoma
Neuro-BL	Neuroblastoma
A	Angioblastic hyperplasia in liver
A ↑	Angioblastic dysplasia in liver
Neop. nod.	Neoplastic nodules of liver
Nod. hyp.	Nodular hyperplasia of liver
Dif. hyp.	Diffused hyperplasia of liver
+ +	Marked
+ + +	Very marked

[a]The incidence of total malignant and benign tumours is given as the total number of tumors per 100 animals (one animal may bear more than one malignant or benign tumor) on the basis of the tumors observed among the animals alive, when the first tumor was observed in the experiment.

The incidence of specific tumour is given, as percent of the animals bearing the tumor considered, referred to the animals alive when the first tumor was observed (in parentheses).

significantly in excess in these experiments, in relation to dose, are given in Tables 69 and 70.

The Fisher exact probability test at 95% confidence is, in relation to the above, not "sensitive" enough, in our experimental conditions.

Biologically, in our opinion, the following results, although not statistically significant according to the test used, should be given proper attention.

Extrahepatic angiosarcomas of different sites are observed at a very low incidence dose in untreated Sprague-Dawley rats of our colony. Results of experiments BT1 and particularly BT9, however, strongly suggest a relationship between these tumors and VC exposure. This relationship is supported by the excessive incidence of extrahepatic vascular tumors in mice treated with VC (BT4).

Few cases of hepatomas have been observed in treated groups, particularly in BT1. This tumor is exceptionally rare in our colony of animals, and none have been observed in the control group of the 17 experiments. Moreover the relationship with treatment is supported by the fact that a high incidence of hepatomas has been observed in Sprague-Dawley rats, following neonatal exposure to a high dose for a short period (BT14).

In view of their rareness or nonobservation in the colony of animal used, for the following tumors it should be stressed that attention should be paid to

Table 15. Experiment BT1.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 10,000 ppm	81.7	23.3	11.7 (7/60)	–	5.0 (3/60)	5.0 (3/60)	1.7 (1/60)	8.3 (5/60)	11.7 (7/60)	26.7 (16/60)	5.0 (3/60)	–	5.0 (3/60)
II 6000 ppm	60.0	38.3	22.0 (13/59)	3.4 (2/59)	5.1 (3/59)	6.8 (4/59)	1.7 (1/59)	8.5 (5/59)	5.1 (3/59)	11.9 (7/59)	3.4 (2/59)	1.7 (1/59)	–
III 2500 ppm	63.3	20.0	21.7 (13/60)	–	5.0 (3/60)	3.3 (2/60)	3.3 (2/60)	10.0 (6/60)	6.7 (4/60)	3.3 (2/60)	1.7 (1/60)	–	3.3 (2/60)
IV 500 ppm	51.7	13.3	10.0 (6/60)	–	1.7 (1/60)	1.7 (1/60)	8.3 (5/60)	10.0 (6/60)	–	6.7 (4/60)	1.7 (1/60)	–	1.7 (1/60)
V 250 ppm	30.0	25.0	5.1 (3/59)	1.7 (1/59)	3.4 (2/59)	–	1.7 (1/59)	8.5 (5/59)	–	–	3.4 (2/59)	–	3.4 (2/59)
VI 50 ppm	15.0	36.7	1.7 (1/60)	–	1.7 (1/60)	3.3 (2/60)	–	1.7 (1/60)	–	–	1.7 (1/60)	1.7 (1/60)	3.3 (2/60)
VII No treatment (control)	13.3	43.3	–	–	–	3.4 (2/58)	–	–	–	–	1.7 (1/58)	–	–

[a]Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 135 weeks (end of experiment).

Table 16. Experiment BT2.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 200 ppm	35.0	21.7	10.0 (12/120)	3.3 (4/120)	0.8 (1/120)	0.8 (1/120)	2.5 (3/120)	5.8 (7/120)	–	3.3 (4/120)	4.2 (5/120)	–	5.0 (6/120)
II 150 ppm	35.0	25.0	5.0 (6/119)	–	–	0.8 (1/119)	–	9.2 (11/119)	–	3.4 (4/119)	3.4 (4/119)	1.7 (2/119)	5.0 (6/119)
III 100 ppm	21.7	27.5	0.8 (1/120)	0.8 (1/120)	–	–	–	8.3 (10/120)	–	0.8 (1/120)	0.8 (1/120)	3.3 (4/120)	3.3 (4/120)
IV No treatment (control)	15.7	21.6	–	–	1.1 (2/185)	–	–	–	–	1.1 (2/185)	1.1 (2/185)	1.6 (3/185)	1.0 (2/185)

[a]Exposure by inhalation to VC in air at 200, 150, 100 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 143 weeks (end of experiment).

Table 17. Experiment BT6.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 30,000 ppm	100.0	50.0	30.0 (18/60)	1.7 (1/60)	1.7 (1/60)	5.0 (3/60)	1.7 (1/60)	–	1.7 (1/60)	58.3 (35/60)	1.7 (1/60)	18.3 (11/60)	3.3 (2/60)

[a]Exposure by inhalation to VC in air at 30,000 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 17 weeks old. Results after 68 weeks (end of experiment).

Table 18. Experiment BT9.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 50 ppm	44.3	41.7	4.8 (14/294)	2.7 (8/294)	3.1 (9/294)	3.7 (11/294)	–	0.3 (1/294)	–	3.1 (9/294)	1.0 (3/294)	0.4 (1/294)	21.7 (62/294)
II No treatment (control)	23.0	24.0	–	–	–	–	–	–	–	–	–	1.0 (1/98)	10.2 (10/98)

[a]Exposure by inhalation to VC in air at 50 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 142 weeks (end of experiment).

Table 19. Experiment BT15.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 25 ppm	33.3	58.3	4.2 (5/120)	0.8 (1/120)	–	2.5 (3/120)	–	0.8 (1/120)	–	3.3 (4/120)	–	–	15.0 (17/120)
II 10 ppm	31.7	53.3	0.8 (1/119)	–	1.7 (2/119)	2.5 (3/119)	–	–	–	1.7 (2/119)	–	–	17.6 (21/119)
III 5 ppm	35.8	55.0	–	–	–	–	–	–	–	0.8 (1/119)	0.8 (1/119)	–	18.5 (22/119)
IV 1 ppm	22.5	44.2	–	–	–	–	–	–	–	0.8 (1/118)	0.8 (1/118)	–	12.7 (15/118)
V No treatment (control)	23.3	37.5	–	–	–	0.8 (1/120)	–	–	–	1.7 (2/120)	–	–	5.8 (7/120)

[a]Exposure by inhalation to VC in air at 25, 10, 5, 1 ppm; 4 hr/day, 5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 147 weeks (end of experiment).

Table 20. Experiment BT3.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 10,000 ppm	45.0	20.0	–	–	–	1.7 (1/58)	1.7 (1/58)	1.7 (1/58)	15.5 (9/58)	15.5 (9/58)	8.6 (5/58)	1.7 (1/58)	1.7 (1/58)
II 6000 ppm	53.3	25.0	1.7 (1/60)	–	–	3.3 (2/60)	1.7 (1/60)	1.7 (1/60)	20.0 (12/60)	15.0 (9/60)	8.3 (5/60)	3.3 (2/60)	1.7 (1/60)
III 2500 ppm	41.7	35.0	1.7 (1/60)	–	–	–	3.3 (2/60)	3.3 (2/60)	8.3 (5/60)	11.7 (7/60)	3.3 (2/60)	–	6.7 (4/60)
IV 500 ppm	15.0	35.0	1.7 (1/60)	–	–	–	–	–	–	1.7 (1/60)	–	–	5.0 (3/60)
V 250 ppm	21.7	25.0	–	1.7 (1/59)	–	1.7 (1/59)	–	10.2 (6/59)	–	1.7 (1/59)	–	5.1 (3/59)	1.7 (1/59)
VI 50 ppm	18.3	25.0	–	1.7 (1/58)	–	1.7 (1/58)	–	5.2 (3/58)	–	–	1.7 (1/58)	–	1.7 (1/58)
VII No treatment (control)	14.7	20.0	–	–	0.5 (1/190)	–	–	–	–	1.0 (2/190)	0.5 (1/190)	–	2.6 (5/190)

[a]Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 17 weeks. Sprague-Dawley rats, M and F, 12 weeks old. Results after 156 weeks (end of experiment).

Table 21. Experiment BT10.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 10,000 ppm	33.3	41.7	0.8 (1/118)	–	–	0.8 (1/118)	0.8 (1/118)	–	–	7.6 (9/118)	–	2.5 (3/118)	11.0 (13/118)
II 6000 ppm	30.0	45.0	–	0.8 (1/120)	–	1.7 (2/120)	–	0.8 (1/120)	0.8 (1/120)	7.5 (9/120)	–	1.7 (2/120)	10.8 (13/120)
III 10,000 ppm	35.8	45.0	0.8 (1/119)	1.7 (2/119)	–	0.8 (1/119)	–	–	–	7.6 (9/119)	2.5 (3/119)	2.5 (3/119)	13.4 (16/119)
IV 6000 ppm	30.8	39.2	2.5 (3/118)	–	1.7 (2/118)	–	–	–	–	4.2 (5/118)	3.4 (4/118)	1.7 (2/118)	9.3 (11/118)
V 10,000 ppm	41.7	45.0	0.8 (1/119)	1.7 (2/119)	–	0.8 (1/119)	–	0.8 (1/119)	0.8 (1/119)	6.7 (8/119)	0.8 (1/119)	0.8 (1/119)	16.8 (20/119)
VI 6000 ppm	33.3	50.8	0.8 (1/120)	1.7 (2/120)	0.8 (1/120)	–	1.7 (2/120)	0.8 (1/120)	–	7.5 (9/120)	–	0.8 (1/120)	10.0 (12/120)
VII No treatment (control)	16.6	41.0	–	–	–	0.4 (1/227)	–	–	–	–	0.9 (2/227)	2.2 (5/227)	7.5 (17/227)

[a]Exposure by inhalation to VC in air at 10,000, 6000, ppm; 4 hr/day, 5 days/week, for 5 weeks (groups I and II) or 1 hr/day, 4 days/week, for 25 weeks (groups III and IV) or 4 hr/day, once weekly, for 25 weeks (groups V and VI) (100 hr). Sprague-Dawley rats, M and F, 13 weeks old. Results after 154 weeks (end of experiment).

Table 22. Experiment BT5.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 10,000 ppm	6.7	36.7	–	–	–	–	–	–	–	3.3 (1/30)	–	–	–
II 6000 ppm	6.7	23.3	–	–	–	–	–	–	–	–	–	–	–
III 10,000 ppm	29.6	22.2	–	–	–	–	–	5.9 (3/51)	–	9.8 (5/51)	–	2.0 (1/51)	2.0 (1/51)
IV 6000 ppm	21.9	46.9	–	–	–	3.1 (1/32)	–	–	–	9.4 (3/32)	3.1 (1/32)	3.1 (1/32)	6.2 (2/32)

[a]Exposure by inhalation to VC in air at 10,000, and 6000 ppm of breeders; 4 hr/day for 1 week (from 12th to 18th day of pregnancy). Sprague-Dawley rats, M and F, 19 weeks old (breeders). Breeders (groups I and II) and offsprings (groups III and IV). Results after 143 weeks (end of experiment).

Table 23. Experiment BT14.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 10,000 ppm (breeders)	16.7	66.7	–	–	–	–	–	–	–	–	–	–	–
II 6000 ppm (breeders)	–	–	–	–	–	–	–	–	–	–	–	–	–
III 10,000 ppm (newborn)	100.0	55.5	34.1 (15/44)	–	–	6.8 (3/44)	45.4 (20/44)	–	–	2.3 (1/44)	2.3 (1/44)	–	–
IV 6000 ppm (newborn)	109.3	58.1	40.5 (17/42)	2.4 (1/42)	2.4 (1/42)	2.4 (1/42)	47.6 (20/42)	–	–	4.8 (2/42)	4.8 (2/42)	–	2.4 (1/42)

[a]Exposure by inhalation to VC in air at 10,000 and 6000 ppm, 4 hr/day, 5 days/week, for 5 weeks (from 1 day to 5 weeks of age). Sprague-Dawley rats, M and F, 21 weeks old (breeders) (groups I and II) and newborn (groups III and IV). Results after 124 weeks (end of experiment).

Table 24. Experiment BT7.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %									
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac
I 10,000 ppm	50.0	10.0	29.6 (8/27)	–	–	–	–	3.7 (1/27)	11.1 (3/27)	7.4 (2/27)	–	–
II 6000 ppm	53.3	20.0	11.5 (3/26)	7.7 (2/26)	3.8 (1/26)	3.8 (1/26)	7.7 (2/26)	7.7 (2/26)	3.8 (1/26)	7.7 (2/26)	–	–
III 2500 ppm	26.7	13.3	12.0 (3/25)	–	4.0 (1/25)	–	4.0 (1/25)	–	4.0 (1/25)	–	4.0 (1/25)	–
IV 500 ppm	30.0	10.0	10.7 (3/28)	3.6 (1/28)	–	–	–	7.1 (2/28)	–	–	–	–
V 250 ppm	13.3	16.7	3.7 (1/27)	–	3.7 (1/27)	3.7 (1/27)	–	–	–	–	3.7 (1/27)	–
VI 50 ppm	16.7	6.7	–	–	–	–	–	3.6 (1/28)	–	–	–	–
VII No treatment (control)	15.0	15.0	–	–	2.6 (1/38)	–	–	–	–	–	–	–

[a]Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 52 weeks. Wistar rats, M, 11 weeks old. Results after 165 weeks (end of experiment).

Table 25. Experiment BT17.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %									
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac
I 1 ppm	24.2	29.2	–	1.0 (1/99)	3.0 (3/99)	5.0 (5/99)	1.0 (1/99)	–	–	2.0 (2/99)	–	–
II No treatment (control)	20.0	18.5	–	–	–	–	–	–	–	3.2 (3/94)	–	1.1 (1/94)

[a]Exposure by inhalation to VC in air at 1 ppm; 4 hr/day, 5 days/week, for 52 weeks. Wistar rats, M, 13 weeks old. Results after 134 weeks (end of experiment).

Table 26. Experiment BT4.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %							
	MT	BT	LAS	LA	ELAS	ELA	Lung T	Mammary Ca	Skin EpT	Forestomach Pa&Ac
I 10,000 ppm	50.0	98.3	17.8 (10/56)	10.7 (6/56)	1.8 (1/56)	7.1 (4/56)	82.1 (46/56)	23.2 (13/56)	7.1 (4/56)	1.8 (1/56)
II 6000 ppm	56.7	100.0	21.7 (13/60)	11.7 (7/60)	1.7 (1/60)	5.0 (3/60)	78.3 (47/60)	13.3 (8/60)	11.7 (7/60)	1.7 (1/60)
III 2500 ppm	58.3	90.0	27.1 (16/59)	8.5 (5/59)	13.5 (8/59)	1.7 (1/59)	67.8 (40/59)	13.5 (8/59)	6.8 (4/59)	1.7 (1/59)
IV 500 ppm	58.3	103.3	23.3 (14/60)	8.3 (5/60)	11.7 (7/60)	5.0 (3/60)	83.3 (50/60)	13.3 (8/60)	3.3 (2/60)	–
V 250 ppm	63.3	98.3	30.0 (18/60)	18.3 (11/60)	5.0 (3/60)	5.0 (3/60)	68.3 (41/60)	20.0 (12/60)	1.7 (1/60)	1.7 (1/60)
VI 50 ppm	28.3	23.3	1.7 (1/60)	1.7 (1/60)	1.7 (1/60)	8.3 (5/60)	10.0 (6/60)	20.0 (12/60)	– –	1.7 (1/60)
VII No treatment (control)	14.7	14.7	–	–	0.7 (1/150)	0.7 (1/150)	10.0 (15/150)	0.7 (1/150)	1.3 (2/150)	–

[a]Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 30 weeks. Swiss mice, M and F, 11 weeks old. Results after 81 weeks (end of experiment).

Table 27. Experiment BT8.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELA	Hepatomas	Cholangio-Ca	Cholangiomas	Acoustic Duct EpT	Skin EpT	Melanomas	Forestomach Pa&Ac	Leukaemias[b]
I 10,000 ppm	50.0	73.3	–	3.3 (1/30)	6.7 (2/30)	–	6.7 (2/30)	13.3 (4/30)	3.3 (1/30)	23.3 (7/30)	3.3 (1/30)	33.3 (10/30)	16.7 (5/30)
II 6000 ppm	40.0	63.3	3.3 (1/30)	3.3 (1/30)	–	3.3 (1/30)	6.7 (2/30)	16.7 (5/30)	6.7 (2/30)	3.3 (1/30)	6.7 (2/30)	33.3 (10/30)	20.0 (6/30)
III 2500 ppm	43.3	103.3	–	6.7 (2/30)	–	–	–	26.7 (8/30)	3.3 (1/30)	10.0 (3/30)	3.3 (1/30)	56.7 (17/30)	30.0 (9/30)
IV 500 ppm	53.3	63.3	6.7 (2/30)	–	3.3 (1/30)	–	–	20.0 (6/30)	10.0 (3/30)	23.3 (7/30)	–	30.0 (9/30)	16.7 (5/30)
V 250 ppm	30.0	43.3	–	–	3.3 (1/30)	–	–	20.0 (6/30)	–	10.0 (3/30)	3.3 (1/30)	13.3 (4/30)	20.0 (6/30)
VI 50 ppm	50.0	40.0	–	–	–	–	–	23.3 (7/30)	–	30.0 (9/30)	3.3 (1/30)	10.0 (3/30)	20.0 (6/30)
VII No treatment (control)	20.0	46.7	–	–	–	–	–	36.7 (22/60)	–	5.0 (3/60)	–	5.0 (3/60)	13.3 (8/60)

[a]Exposure by inhalation to VC in air at 10,000, 6000, 2500, 500, 250, and 50 ppm; 4 hr/day, 5 days/week, for 30 weeks. Golden hamsters, M, 11 weeks old. Results after 109 weeks (end of experiment).
[b]Latency time in weeks: Group I, 16.7; Group II, 27.2; Group III, 30.8; Group IV, 19.0; Group V, 22.5; Group VI, 35.3; Group VII, 36.5.

Table 28. Experiment BT11.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 50.00 mg/kg	38.7	35.0	21.2 (17/80)	3.7 (3/80)	2.5 (2/80)	2.5 (2/80)	–	2.5 (2/80)	–	1.2 (1/80)	1.2 (1/80)	2.5 (2/80)	5.0 (4/80)
II 16.65 mg/kg	30.0	17.5	12.5 (10/80)	–	–	–	–	3.7 (3/80)	–	2.5 (2/80)	–	1.2 (1/80)	7.5 (6/80)
III 3.33 mg/kg	10.0	25.0	–	–	2.5 (2/80)	1.2 (1/80)	–	–	–	–	–	–	3.7 (3/80)
IV Olive oil (control)	13.7	22.5	–	–	–	–	–	–	–	1.2 (1/80)	1.2 (1/80)	–	5.0 (4/80)

[a]Exposure by ingestion (stomach tube) of VC in olive oil at 50.00, 16.65 and 3.33 mg/kg body weight, once daily, 4-5 days/week, for 52 weeks. Sprague-Dawley rats, M and F, 13 weeks old. Results after 136 weeks (end of experiment).

Table 29. Experiment BT27.[a]

Group and concentration	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary MT
I 1.0 mg/kg	24.7	35.3	2.0 (3/149)	–	0.7 (1/149)	–	0.7 (1/149)	–	–	3.3 (5/149)	–	2.0 (3/149)	8.0 (12/149)
II 0.3 mg/kg	13.3	28.0	0.7 (1/148)	0.7 (1/148)	–	–	0.7 (1/148)	–	–	–	0.7 (1/148)	1.3 (2/148)	2.7 (4/148)
III 0.03 mg/kg	18.0	31.3	–	–	–	–	–	–	–	–	0.7 (1/150)	0.7 (1/150)	9.3 (14/150)
IV Olive oil (control)	16.0	28.7	–	–	–	–	–	–	–	0.7 (1/150)	–	1.3 (2/150)	4.7 (7/150)

[a]Exposure by ingestion (stomach tube) of VC in olive oil at 1.0, 0.3, 0.03 mg/kg body weight, once daily, 4-5 days/week, for 59 weeks. Sprague-Dawley rats, M and F, 10 weeks old. Results after 136 weeks (end of experiment).

Table 30. Experiment BT12.[a]

Group and dose	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepa-tomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Fore-stomach Pa&Ac	Mam-mary MT
I 4.25 mg×4	13.8	25.0	–	–	–	–	–	–	–	–	–	1.8 (1/56)	1.8 (1/56)
II 4.25 mg×3	16.7	28.3	–	–	1.9 (1/53)	–	–	–	–	–	–	1.9 (1/53)	1.9 (1/53)
III 4.25 mg×2	11.7	18.3	–	–	1.8 (1/56)	–	–	–	–	–	1.8 (1/56)	–	5.3 (3/56)
IV 4.25 mg×1	20.0	35.0	–	–	–	1.8 (1/55)	–	–	–	1.8 (1/55)	–	3.6 (2/55)	3.6 (2/55)
V Olive oil (control)	8.3	31.7	–	–	–	–	–	–	–	–	–	3.6 (2/55)	–

[a]Exposure by intraperitoneal injection of VC, 4.25 mg in olive oil (1 ml), 4, 3, 2 times, at two month intervals or once only. Sprague-Dawley rats, M and F, 17 weeks old. Results after 144 weeks (end of experiment).

Table 31. Experiment BT13.[a]

Group and dose	Tumors/100 animals		Animals with tumors, %										
	MT	BT	LAS	LA	ELAS	ELA	Hepa-tomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Fore-stomach Pa&Ac	Mam-mary MT
I 4.25 mg	16.0	17.3	–	–	–	–	–	1.3 (1/75)	–	–	–	–	4.0 (3/75)
II Olive oil (control)	13.3	26.7	–	–	–	1.3 (1/75)	–	–	–	1.3 (1/75)	–	–	1.3 (1/75)

[a]Exposure by subcutaneous injection of VC, 4.25 mg, in olive oil (1 ml), single dose. Sprague-Dawley rats, M and F, 21 weeks old. Results after 145 weeks (end of experiment).

Table 32. Incidence of total MT and BT in Sprague-Dawley rats, in relation to concentration of VC administered by inhalation for 52 weeks.

Experiments	Concentration (ppm)	Tumors/100 animals					
		MT			BT		
		M	F	Total	M	F	Total
BT 6	30,000	76.7	123.3	100.0	40.0	60.0	50.0
BT 1	10,000	80.0	83.3	81.7	26.7	20.0	23.3
	6,000	46.7	73.3	60.0	13.3	63.3	38.3
	2,500	53.3	73.3	63.3	16.7	23.3	20.0
	500	23.3	80.0	51.7	13.3	13.3	13.3
	250	23.3	36.7	30.0	23.3	26.7	25.0
BT 2	200	40.0	30.0	35.0	10.0	33.3	21.7
	150	21.7	48.3	35.0	21.7	28.3	25.0
	100	23.3	20.0	21.7	20.0	35.0	27.5
BT 1	50	6.7	23.3	15.0	23.3	50.0	36.7
BT 9	50	20.7	68.0	44.3	23.3	60.0	41.7
BT 15	25	20.0	46.7	33.3	31.7	85.0	58.3
	10	18.3	45.0	31.7	30.0	76.7	53.3
	5	25.0	46.7	35.8	28.3	81.7	55.0
	1	15.0	30.0	22.5	18.3	70.0	44.2
Controls							
BT 1	0	–	26.7	13.3	23.3	63.3	43.3
BT 2	0	15.3	16.0	15.7	21.2	22.0	21.6
BT 9	0	16.0	30.0	23.0	4.0	44.0	24.0
BT 15	0	18.3	28.3	23.3	20.0	55.0	37.5

Table 33. Incidence of LAS, LA, A+ +/+ + + ↑ and A+ +/+ + + in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiments	Concentration, ppm	Animals with tumors and correlated changes, %											
		LAS			LA			A+ +/+ + + ↑			A+ +/+ + +		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total
BT 6	30,000	16.6	43.3	30.0	–	3.3	1.7	6.7	6.7	6.7	6.7	13.3	10.0
BT 1	10,000	10.0	13.3	11.7	–	–	–	–	3.3	1.7	3.3	6.7	5.0
	6,000	10.3	33.3	22.0	–	6.7	3.4	–	–	–	20.0	16.7	18.3
	2,500	20.0	23.3	21.7	–	–	–	–	–	–	13.3	13.3	13.3
	500	–	20.0	10.0	–	–	–	3.3	–	1.7	13.3	6.7	10.0
	250	3.4	6.7	5.1	–	3.3	1.7	–	–	–	20.0	10.0	15.0
BT 2	200	11.7	8.3	10.0	3.3	3.3	3.3	6.6	5.0	5.8	18.3	10.0	14.1
	150	1.7	8.3	5.0	–	–	–	8.3	10.0	9.2	18.3	6.6	12.5
	100	–	1.7	0.8	1.7	–	0.8	1.7	6.6	4.2	8.3	5.0	6.6
BT 1, BT 9	50	1.1	7.2	4.2	1.1	3.3	2.2	1.7	1.1	1.4	5.5	15.5	10.5
BT 15	25	1.7	6.7	4.2	–	1.7	0.8	1.7	–	0.8	3.3	5.0	4.2
	10	–	1.7	0.8	–	–	–	1.7	–	0.8	1.7	1.7	1.7
	5	–	–	–	–	–	–	–	–	–	–	–	–
	1	–	–	–	–	–	–	–	–	–	–	–	–
Control BT 1, BT 2, BT 9, BT 15	0	–	–	–	–	–	–	–	–	–	–	2.5	1.3

Table 34. Incidence of ELAS, and ELA in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiments	Concentration, ppm	Animals with tumors, %					
		ELAS			ELA		
		M	F	Total	M	F	Total
BT 6	30,000	–	3.3	1.7	3.3	6.7	5.0
BT 1	10,000	6.7	3.3	5.0	6.7	3.3	5.0
	6,000	3.4	6.7	5.1	6.9	6.7	6.8
	2,500	6.7	3.3	5.0	3.3	3.3	3.3
	500	–	3.3	1.7	3.3	–	1.7
	250	3.4	3.3	3.4	–	–	–
BT 2	200	1.7	–	0.8	–	1.7	0.8
	150	–	–	–	–	1.7	0.8
	100	–	–	–	–	–	–
BT 1, BT 9	50	2.3	3.3	2.8	4.6	2.8	3.7
BT 15	25	–	–	–	5.0	–	2.5
	10	3.3	–	1.7	3.3	1.7	2.5
	5	–	–	–	–	–	–
	1	–	–	–	–	–	–
Controls BT 1, BT 2, BT 9, BT 15	0	0.9	–	0.4	0.4	0.8	0.6

Table 35. Incidence of hepatomas, neoplastic liver nodules, nodular hyperplasia of the liver and diffuse hyperplasia of the liver in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiments	Concentration, ppm	Animals with tumors and correlated changes, %											
		Hepatomas			Neopl. nod.			Nod. hyp.			Diff. hyp.		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total
BT 6	30,000	–	3.3	1.7	3.3	13.3	8.3	13.3	10.0	11.7	–	–	–
BT 1	10,000	3.3	–	1.7	–	–	–	3.3	3.3	3.3	3.3	6.7	5.0
	6,000	–	3.3	1.7	3.3	–	1.7	13.3	6.7	10.0	3.3	3.3	3.3
	2,500	–	6.7	3.3	–	3.3	1.7	6.7	13.3	10.0	–	3.3	1.7
	500	–	16.7	8.3	–	–	–	–	10.0	5.0	13.3	6.7	10.0
	250	3.3	–	1.7	–	–	–	23.3	–	11.7	3.3	–	1.7

Table 35 (cont.)

Experiments	Concentration, ppm	Animals with tumors and correlated changes, %											
		Hepatomas			Neopl. nod.			Nod. hyp.			Diff. hyp.		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total
BT 2	200	1.7	3.3	2.5	3.3	1.7	2.5	20.0	13.3	16.7	38.3	18.3	28.3
	150	–	–	–	1.7	–	0.8	8.3	13.3	10.8	16.7	25.3	20.8
	100	–	–	–	–	–	–	5.0	23.3	14.2	26.7	13.3	20.0
BT 1, BT 9	50	–	–	–	0.5	–	0.3	13.3	9.4	11.4	2.8	3.3	3.0
BT 15	25	–	–	–	–	–	–	15.0	8.3	11.7	5.0	10.0	7.5
	10	–	–	–	–	–	–	20.0	5.0	12.5	10.0	6.7	8.3
	5	–	–	–	–	–	–	1.7	–	0.8	–	–	–
	1	–	–	–	–	–	–	3.3	–	1.7	1.7	–	0.8
Controls BT 1, BT 2, BT 9, BT 15	0	–	–	–	–	0.4	0.2	0.4	0.8	0.6	0.9	2.9	1.9

Table 36. Incidence of nephroblastoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with NEPHRO-BL, %		
		M	F	Total
BT 6	30,000	–	–	–
BT 1	10,000	10.0	6.7	8.3
	6,000	13.8	3.3	8.5
	2,500	16.7	3.3	10.0
	500	6.7	13.3	10.0
	250	3.4	13.3	8.5
BT 2	200	8.3	3.3	5.8
	150	13.3	5.0	9.2
	100	13.3	3.3	8.3
BT 1, BT 9	50	–	1.1	0.6
BT 15	25	1.7	–	0.8
	10	–	–	–
	5	–	–	–
	1	–	–	–
Controls BT 1, BT 2, BT 9, BT 15	0	–	–	–

Table 37. Incidence of neuroblastoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiments	Concentration, ppm	Animals with NEURO-BL, %		
		M	F	Total
BT 6	30,000	3.3	–	1.7
BT 1	10,000	6.7	16.7	11.7
	6,000	6.9	3.3	5.1
	2,500	6.7	6.7	6.7
	500	–	–	–
	250	–	–	–
BT 2	200	–	–	–
	150	–	–	–
	100	–	–	–
BT 1, BT 9	50	–	–	–
BT 15	25	–	–	–
	10	–	–	–
	5	–	–	–
	1	–	–	–
Controls BT 1, BT 2, BT 9, BT 15	0	–	–	–

Table 38. Incidence of zymbal gland carcinoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with Zymbal gland CA, %		
		M	F	Total
BT 6	30,000	56.6	60.0	58.3
BT 1	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	–	–	–
BT 2	200	5.0	1.7	3.3
	150	–	6.7	3.4
	100	–	1.7	0.8
BT 1, BT 9	50	2.3	2.8	2.5
BT 15	25	5.0	1.7	3.3
	10	1.7	1.7	1.7
	5	–	1.7	0.8
	1	1.7	–	0.8
Controls BT 1, BT 2, BT 9, BT 15	0	0.9	0.8	0.9

Table 39. Incidence of forestomach papilloma and acanthoma in Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with forestomach Pa and Ac, %		
		M	F	Total
BT 6	30,000	16.7	20.0	18.3
BT 1	10,000	–	–	–
	6,000	–	3.3	1.7
	2,500	–	–	–
	500	–	–	–
	250	–	–	–
BT 2	200	–	–	–
	150	3.3	–	1.7
	100	3.3	3.3	3.3
BT 1, BT 9	50	1.1	–	0.6
BT 15	25	–	–	–
	10	–	–	–
	5	–	–	–
	1	–	–	–
Controls BT 1, BT 2, BT 9, BT 15	0	1.3	0.4	0.9

their onset, even at doses below the ones with statistically significant results.

An excess of Zymbal gland carcinomas is observed down to 50 and 25 ppm.

Liver angiosarcomas are extremely rare in the colony used (4 cases over several thousand untreated animals). Therefore, one must consider the onset of these tumors as important even at doses not shown by statistical analysis, and particularly 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 1 mg/kg (3 liver angiosarcomas out of 150 animals), and at 0.3 mg/kg (1 liver angiosarcoma out of 150 animals).

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

Neuroblastomas have never been observed by us, up to the present, in the Sprague-Dawley rats used in our laboratory as control or otherwise

Table 40. Incidence of mammary malignant tumor in female Sprague-Dawley rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with Mammary MT, %
BT 6	30,000	6.7
BT 1	10,000	10.0
	6,000	–
	2,500	6.7
	500	3.3
	250	6.7
BT 2	200	8.3
	150	10.0
	100	6.7
BT 1	50	6.7
BT 9	50	40.7
BT 15	25	28.3
	10	35.0
	5	38.3
	1	23.3
Controls		
BT 1	0	–
BT 2	0	2.0
BT 9	0	18.0
BT 15	0	10.0

Table 41. Incidence of total MT and BT in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Tumors/100 Animals	
		MT	BT
BT 7	10,000	50.0	10.0
	6,000	53.3	20.0
	2,500	26.7	13.3
	500	30.0	10.0
	250	13.3	16.7
	50	16.7	6.7
BT 17	1	24.2	29.2
Controls			
BT 7	0	15.0	15.0
BT 17	0	20.0	18.5

Table 43. Incidence of ELAS and ELA in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with tumors, %	
		ELAS	ELA
BT 7	10,000	–	–
	6,000	3.8	3.8
	2,500	4.0	–
	500	–	–
	250	3.7	3.7
	50	–	–
BT 17	1	3.0	5.0
Controls BT 7, BT 17	0	0.7	–

treated. Therefore we consider as dependent on treatment the onset of these tumors, even at doses below 10,000 ppm, i.e., 6000 and 2500 ppm.

The meaning in oncological terms of the results at the lowest doses may be better evaluated in considering, not singly, but together, the tumors found to be VC-dependent (Table 71).

None (or no increase) of the specifically VC related tumors shown in Table 71, observed in the seven basic experiments, was found at doses of 5 and 1 ppm (by inhalation) and 0.03 mg/kg (by ingestion).

General Comments

VC long-term experimental study led to the discovery of VC carcinogenicity, and as a direct consequence, to what probably has been the greatest effort ever made at controlling the exposure to an industrial carcinogen in the workplace (Table 72).

Moreover, long-term carcinogenicity bioassays on VC are a crucial step in the field of environmental and occupational carcinogenesis which, in turn,

Table 42. Incidence of LAS, LA, A+ +/+ + + ↑ and A+ +/+ + + in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with tumors and correlated changes, %			
		LAS	LA	A+ +/+ + + ↑	A+ +/+ + +
BT 7	10,000	29.6	–	3.3	–
	6,000	11.5	7.7	–	3.3
	2,500	12.0	–	3.3	–
	500	10.7	3.6	3.3	3.3
	250	3.7	–	3.3	–
	50	–	–	–	–
BT 17	1	–	1.0	1.7	0.8
Controls BT 7, BT 17	0	–	–	–	–

Table 44. Incidence of hepatomas, neoplastic liver nodules, nodular hyperplasia of the liver and diffuse hyperplasia of the liver in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with tumors and correlated changes, %			
		Hepatomas	Neop.nod.	Nod.hyp.	Dif.hyp.
BT 7	10,000	–	–	6.7	–
	6,000	7.7	6.7	3.3	6.7
	2,500	4.0	–	6.7	6.7
	500	–	6.7	–	3.3
	250	–	–	–	16.7
	50	–	–	–	10.0
BT 17	1	1.0	–	5.0	4.2
Controls BT 7, BT 17	0	–	–	1.2	2.3

Table 45. Incidence of NEPHRO-BL in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with NEPHRO-BL, %
BT 7	10,000	3.7
	6,000	7.7
	2,500	–
	500	7.1
	250	–
	50	3.6
BT 17	1	–
Controls BT 7, BT 17	0	–

Table 46. Incidence of NEURO-BL in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with NEURO-BL, %
BT 7	10,000	11.1
	6,000	3.8
	2,500	4.0
	500	–
	250	–
	50	–
BT 17	1	–
Controls BT 7, BT 17	0	–

Table 47. Incidence of Zymbal gland CA in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with Zymbal gland CA, %
BT 7	10,000	7.4
	6,000	7.7
	2,500	–
	500	–
	250	–
	50	–
BT 17	1	2.0
Controls BT 7, BT 17	0	2.3

Table 48. Incidence of forestomach Pa and Ac in male Wistar rats in relation to concentration of VC administered by inhalation for 52 weeks.

Experiment	Concentration, ppm	Animals with forestomach Pa and Ac, %
BT 7	10,000	–
	6,000	–
	2,500	–
	500	–
	250	–
	50	–
BT 17	1	–
Controls BT 7, BT 17	0	0.7

are among the most important areas of public health nowadays.

These studies have demonstrated that long-term carcinogenicity bioassays: may predict carcinogenic risk for humans; may give indication of the level of risk, in relation to dose; may provide information on possible target organs and, in general terms, on the quality of neoplastic response; may represent a tool for obtaining information on the relative risk represented by different compounds, provided that they are tested under the same standard conditions (Table 73); have revealed the need to identify

Table 49. Incidence of total MT and BT in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

Experiment	Concentration, ppm	Tumors/100 animals					
		MT			BT		
		M	F	Total	M	F	Total
BT 4	10,000	16.6	83.3	50.0	83.3	113.3	98.3
	6,000	26.7	86.6	56.7	100.0	100.0	100.0
	2,500	40.0	76.7	58.3	80.0	100.0	90.0
	500	36.7	80.0	58.3	93.3	113.3	103.3
	250	36.7	90.0	63.3	116.7	80.0	98.3
	50	6.7	50.0	28.3	20.0	26.7	23.3
	0	6.2	24.3	14.7	13.7	15.7	14.7

Table 50. Incidence of LAS and LA in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

Experiment	Concentration, ppm	Animals with tumors, %					
		LAS			LA		
		M	F	Total	M	F	Total
BT 4	10,000	3.8	30.0	17.8	3.8	16.7	10.7
	6,000	6.7	36.7	21.7	6.7	16.7	11.7
	2,500	20.7	33.3	27.1	6.9	10.0	8.5
	500	20.0	26.7	23.3	3.3	13.3	8.3
	250	30.0	30.0	30.0	20.0	16.7	18.3
	50	3.3	–	1.7	–	3.3	1.7
	0	–	–	–	–	–	–

Table 51. Incidence of ELAS and ELA in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

Experiment	Concentration, ppm	Animals with tumors, %					
		ELAS			ELA		
		M	F	Total	M	F	Total
BT 4	10,000	–	3.3	1.8	7.7	6.7	7.1
	6,000	–	3.3	1.7	6.7	3.3	5.0
	2,500	13.8	13.3	13.5	–	3.3	1.7
	500	6.7	16.7	11.7	3.3	6.7	5.0
	250	6.7	3.3	5.0	6.7	3.3	5.0
	50	3.3	–	1.7	3.3	13.3	8.3
	0	–	1.4	0.7	1.2	–	0.7

Table 52. Incidence of lung tumors (Ad and Ad ↑) in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

Experiment	Concentration, ppm	Animals with lung tumors (Ad and Ad ↑), %		
		M	F	Total
BT 4	10,000	76.9	86.7	82.1
	6,000	76.7	80.0	78.3
	2,500	62.1	73.3	67.8
	500	80.0	86.7	83.3
	250	80.0	56.7	68.3
	50	10.0	10.0	10.0
	0	10.0	10.0	10.0

Table 53. Incidence of mammary CA in female Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

Experiment	Concentration, ppm	Animals with mammary CA, %
BT 4	10,000	43.3
	6,000	26.7
	2,500	26.7
	500	23.3
	250	40.0
	50	40.0
	0	1.4

animal systems more equivalent to humans in neoplastic response, which in turn depends on partly-known factors, such as basic "spontaneous" tumorigram and enzymatic profiles.

Prospects

At present the most important goal of research on environmental and occupational carcinogenesis is, in our own view, the extrapolation of results

Table 54. Incidence of forestomach Pa and Ca in Swiss mice in relation to concentration of VC administered by inhalation for 30 weeks.

Experiment	Concentration, ppm	Animals with forestomach Pa and Ac, %		
		M	F	Total
BT 4	10,000	–	3.3	1.8
	6,000	3.3	–	1.7
	2,500	–	3.3	1.7
	500	–	–	–
	250	3.3	–	1.7
	50	3.3	–	1.7
	0	–	–	–

Table 55. Incidence of total MT and BT in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiment	Concentration, mg/kg	Tumors/100 animals					
		MT			BT		
		M	F	Total	M	F	Total
BT 11	50.00	35.0	42.5	38.7	20.0	50.0	35.0
	16.65	22.5	37.5	30.0	–	35.0	17.5
	3.33	5.0	15.0	10.0	2.5	47.5	25.0
BT 27	1.0	13.3	36.0	24.7	12.0	58.7	35.3
	0.3	12.0	14.7	13.3	20.0	36.0	28.0
	0.03	8.0	28.0	18.0	14.7	48.0	31.3
Controls							
BT 11	0	12.5	15.0	13.7	10.0	35.0	22.5
BT 27	0	8.0	24.0	16.0	9.3	48.0	28.7

Table 56. Incidence of LAS, LA, A+ +/+ + + ↑ and A+ +/+ + + in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiment	Concentration, mg/kg	Animals with tumors and correlated changes, %											
		LAS			LA			A+ +/+ + + ↑			A+ +/+ + +		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total
BT 11	50.00	20.0	22.5	21.2	2.5	5.0	3.7	7.5	5.0	12.5	7.5	10.0	8.7
	16.65	10.0	15.0	12.5	–	–	–	5.0	5.0	5.0	7.5	15.0	11.2
	3.33	–	–	–	–	–	–	2.5	7.5	5.0	15.0	22.5	18.7
BT 27	1.0	1.3	2.7	2.0	–	–	–	–	–	–	1.3	6.7	4.0
	0.3	–	1.4	0.7	–	1.4	0.7	–	1.3	0.7	1.3	1.3	1.3
	0.03	–	–	–	–	–	–	–	–	–	–	–	–
Controls BT 11, BT 27	0	–	–	–	–	–	–	–	0.9	0.4	–	0.9	0.4

Table 57. Incidence of ELAS and ELA in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiment	Concentration, mg/kg	Animals with tumors, %					
		ELAS			ELA		
		M	F	Total	M	F	Total
BT 11	50.00	–	5.0	2.5	2.5	2.5	2.5
	16.65	–	–	–	–	–	–
	3.33	–	5.0	2.5	–	2.5	1.2
BT 27	1.0	–	1.3	0.7	–	–	–
	0.3	–	–	–	–	–	–
	0.03	–	–	–	–	–	–
Controls BT 11, BT 27	0	–	–	–	–	–	–

Table 58. Incidence of hepatomas, neoplastic liver nodules, nodular hyperplasia of the liver, and diffuse hyperplasia of the liver in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiment	Concentration, mg/kg	Animals with tumors and correlated changes, %											
		Hepatomas			Neop.nod.			Nod.hyp.			Dif.hyp.		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total
BT 11	50.00	–	–	–	–	5.0	2.5	17.5	17.5	17.5	15.0	15.0	15.0
	16.65	–	–	–	–	–	–	17.5	30.0	23.7	25.0	32.5	28.7
	3.33	–	–	–	2.5	–	1.2	10.0	20.0	15.0	15.0	42.5	28.7
BT 27	1.0	1.3	–	0.7	1.3	2.7	2.0	6.7	13.3	10.0	6.7	14.7	10.7
	0.3	1.3	–	0.7	–	1.3	0.7	9.3	6.7	8.0	–	10.7	5.3
	0.03	–	–	–	–	–	–	4.0	6.7	5.3	–	10.7	5.3
Controls BT 11, BT 27	0	–	–	–	–	0.9	0.4	5.2	6.1	5.6	7.0	8.7	7.8

Table 59. Incidence of NEPHRO-BL in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiments	Concentration, mg/kg	Animals with NEPHRO-BL, %		
		M	F	Total
BT 11	50.00	2.5	2.5	2.5
	16.65	5.0	2.5	3.7
	3.33	–	–	–
BT 27	1.0	–	–	–
	0.3	–	–	–
	0.03	–	–	–
Controls BT 11, BT 27	0	–	–	–

Table 60. Incidence of NEURO-BL in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiments	Concentration, mg/kg	Animals with NEURO-BL, %		
		M	F	Total
BT 11	50.00	–	–	–
	16.65	–	–	–
	3.33	–	–	–
BT 27	1.0	–	–	–
	0.3	–	–	–
	0.03	–	–	–
Controls BT 11, BT 27	0	–	–	–

from animal to human, both in qualitative and in quantitative terms.

VC carcinogenicity may again provide an important tool towards solving this problem.

We now know a great deal about the effects of VC in experimental animal systems, both in qualitative and quantitative terms.

On the other hand, epidemiological investigations on occupationally exposed population groups have been made and are being carried out in different parts of the world, particularly in Western Europe and in the U.S.A., with reference to general pathology and neoplasias.

If these epidemiological investigations provide precise figures on the whole group considered, including figures on the level and length of expo-

Table 61. Incidence of Zymbal gland CA in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiments	Concentration, mg/kg	Animals with Zymbal gland CA, % M	F	Total
BT 11	50.00	2.5	–	1.2
	16.65	2.5	2.5	2.5
	3.33	–	–	–
BT 27	1.0	2.7	4.0	3.3
	0.3	–	–	–
	0.03	–	–	–
Controls BT 11, BT 27	0	–	1.7	0.9

Table 62. Incidence of forestomach Pa and Ac in Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiments	Concentration, mg/kg	Animals with forestomach Pa and Ac, % M	F	Total
BT 11	50.00	5.0	–	2.5
	16.65	–	2.5	1.2
	3.33	–	–	–
BT 27	1.0	1.3	2.7	2.0
	0.3	2.7	–	1.3
	0.03	–	1.3	0.7
Controls BT 11, BT 27	0	–	1.7	0.9

Table 63. Incidence of mammary MT in female Sprague-Dawley rats in relation to concentration of VC administered by ingestion for 52 (or 59) weeks.

Experiment	Concentration mg/kg	Animals with mammary MT, %
BT 11	50.00	10.0
	16.65	15.0
	3.33	5.0
BT 27	1.0	16.0
	0.3	5.5
	0.03	18.7
Controls		
BT 11	0	10.0
BT 27	0	9.3

sure (so as to define homogeneous exposed groups), and collect all possible available data on pathology, we shall have an opportunity, unique at present, to compare animal and human data, both in qualitative and quantitative terms, and to help find a possible key for extrapolating from animals to humans.

The Cost

With the presentation made in Paris last November (*2*) and with today's report, ten years of work on our VC experimental project seem to be nearly concluded. After having presented the results, we also wish to present the data of the cost of the project, which cannot be expressed only in financial terms.

The cost of the BT project of long-term carcinogenicity bioassays on vinyl chloride includes the cost of (1) the planning and setting-up of experimental apparatus, including inhalation facilities, of

Table 64. Incidence of LAS and Zymbal gland CA in Sprague-Dawley rats in relation to schedule of treatment with VC administered by inhalation.

Experiment	VC concentration, ppm	Schedule[a]	Animals with tumors, % LAS M	LAS F	LAS Total	Zymbal gl.ca M	Zymbal gl.ca F	Zymbal gl.ca Total
BT 1	10,000	I	10.0	13.3	11.7	33.3	20.0	26.7
BT 3	10,000	II	–	–	–	17.8	13.3	15.5
BT 10	10,000	III	1.7	–	0.8	13.5	1.7	7.6
		IV	1.7	–	0.8	8.5	6.7	7.6
		V	–	1.7	0.8	3.3	10.2	6.7
BT 1	6,000	I	10.3	33.3	22.0	10.3	13.3	11.9
BT 3	6,000	II	–	3.3	1.7	20.0	10.0	15.0
BT 10	6,000	III	–	–	–	10.0	5.0	7.5
		IV	3.4	1.7	2.5	8.5	–	4.2
		V	–	1.7	0.8	10.0	5.0	7.5

[a]Schedules: (I) 4 hr/day, 5 days/wk, 52 weeks; (II) 4 hr/day, 5 days/wk, 17 weeks; (III) 4 hr/day, 5 days/wk, 5 weeks; (IV) 1 hr/day, 4 days/wk, 25 weeks; (V) 4 hr/day, 1 day/wk; 25 weeks.

Table 65. Incidence of LAS in relation to species (male Sprague-Dawley Rats, Wistar rats, Swiss mice and golden hamsters), treated with VC administered by inhalation.

Experiments	Concentration, ppm	Animals with LAS, %			
		Sprague-Dawley rats	Wistar rats	Swiss mice	Golden hamsters
BT1, BT7, BT4, BT8	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	–	–	–	–

Table 66. Incidence of Zymbal gland CA in relation to strain (male Sprague-Dawley and Wistar rats) treated with VC administered by inhalation.

Experiments	Concentration, ppm	Animals with Zymbal gland CA, %	
		Sprague-Dawley rats	Wistar rats
BT1, BT7	10,000	33.3	7.4
	6,000	10.3	7.7
	2,500	3.3	–
	500	10.0	–
	250	–	–
	50	–	–
	0	–	–

a type uncommon in 1971, and the working out of a protocol for long-term bioassays; (2) the study of nearly 7000 animals up to the point of their natural death, equivalent to more than 3,000,000 rodent days; (3) ten years of work; (4) the routine examination of some 200,000 histological slides; (5) a financial commitment equivalent to more than $2,000,000 U.S. at present prices (the average cost of a rat throughout the world in this type of experiment is $300 U.S.); (6) the availability of the same team of scientists throughout the entire 10 years of the project, a prerequisite which may be difficult or even impossible to ensure in many countries at the present time; (7) the highly motivated commitment of those scientists to a type of work which is long-lasting, onerous and often tedious; (8) the effort involved in maintaining the

Table 67. Incidence of LAS in relation to age (newborn and adult) Sprague-Dawley rats treated with VC administered by inhalation 4 hr/day, 5 days/week, 52 weeks.

Experiment	Concentration, ppm	Animals with LAS, %					
		Newborn rats			11 week old rats		
		M	F	Total	M	F	Total
BT 10, BT 14	10,000	25.0	45.0	34.1	1.7	–	0.8
	6,000	27.8	50.0	40.5	–	–	–

Table 68. Tumors presently correlated to VC exposure, by experiments on rodents.

Species	Angiosarcomas of liver	Tumors of brain	Tumors of lung	Lymphomas and leukemias	Hepatomas	Angiosarcomas and angiomas of other sites	Nephroblastomas	Sebaceous cutaneous carcinomas	Other cutaneous epithelial tumors	Mammary carcinomas	Forestomach papillomas and acanthomas	Melanomas
Rat	+	+			+	+	+	+	(+)	+	+	
Mouse	+		+			+			(+)	+	(+)	
Hamster	+			(+)		(+)			(+)		+	(+)

Table 69. Total cancer-bearing animals significantly in excess by Fisher exact probability test ($p \leq 0.05$).

Sex	Dose level at which total cancer bearing animals in excess
Male	30,000
	10,000
	6,000
	2,500
	500
	250
	200
	50 ppm
	50 mg/kg
Female	30,000
	10,000
	6,000
	2,500
	500
	200
	150
	50 ppm
	50 mg/kg

Table 70. Tumors significantly in excess by Fisher exact probability test ($p \leq 0.05$).

Tumor type	Sex	Doses at which tumors in excess
Zymbal gland carcinoma	M	30,000; 10,000 ppm
	F	30,000; 10,000 ppm
Liver angiosarcoma	M	30,000; 2500; 200 ppm 50 mg/kg
	F	30,000; 6000; 2500; 500 200; 150; 50 ppm 50; 16.65 mg/kg
Nephroblastoma	M	2500; 200; 150; 100 ppm
	F	500; 250 ppm
Neuroblastoma	F	10,000 ppm
Mammary gland adenocarcinoma	F	150; 50; 25; 10; 5 ppm
Forestomach papilloma	M	30,000 ppm
	F	30,000 ppm

Table 71. Onset of tumors considered VC-correlated at the lowest doses.

Dose	Tumors
25 ppm	Over 120 animals, 5 liver angiosarcomas, 4 Zymbal gland carcinomas and 1 nephroblastoma
10 ppm	Over 120 animals, 1 liver angiosarcoma, 2 extrahepatic angiosarcomas, and 2 Zymbal gland carcinomas
1 mg/kg	Over 150 animals, 3 liver angiosarcomas, 1 extrahepatic angiosarcoma, 1 hepatoma, and 5 Zymbal gland carcinomas
0.3 mg/kg	Over 150 animals, 1 liver angiosarcoma and 1 hepatoma

Table 72. History of vinyl chloride carcinogenicity studies.

Date	
1961	VC was found to produce liver enlargement and microscopic hepatic degenerative changes (*5*)
1970	Zymbal gland carcinomas were reported in rats exposed to 30,000 ppm of VC, by inhalation (*6*)
1970	An increase in atypias in respiratory cells was observed among workers heavily exposed to VC (*7*)
July 1971	A vast project of long-term carcinogenicity bioassays on VC was started in Bentivoglio, near Bologna, Italy (BT project)
August 1972	Zymbal gland carcinomas, nephroblastomas and liver angiosarcomas were observed in rats exposed to VC by inhalation (Maltoni, BT project)
April 1973	The first data of the BT project were released to the scientific community: the oncogenic effect was observed up to 250 ppm (*4*)
1973	Splenomegalic liver disease was found among poly(vinyl chloride) production workers (*8*)
December 1973	For the first time a case of liver angiosarcoma in a poly(vinyl chloride) production worker was correlated to VC exposure (*9*)
February 1974	On the basis of the BT project data indicating a carcinogenic effect at 250 ppm, OSHA proposed a TLV of 50 ppm
February 1974	The BT project data showed that VC is a multipotential carcinogen, producing a variety of tumors, in different animal species
1974	The BT project data indicated a carcinogenic effect at 50 ppm (*10*); OSHA proposed new stricter rules
1974	Early epidemiological observations (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 (*11*)
1974–75	BT project data showed that VC had carcinogenic effects in rats also when given by ingestion (*12*)
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 (*13*)

consistency of the methodology, which has as its reverse side the limits placed on the exercise of imagination—the most positive element in scientific life; (9) the effort involved in establishing and preserving objectivity and balance in the evaluation and interpretation of data; (10) and finally, the strength required to withstand the sense of loneliness arising from the lack of co-operation of many of those bodies which should properly be concerned with the progress of science in this field, not excluding part of the scientific community whose

Table 73. Comparative effects of three related compounds—vinyl chloride (VC), vinylidene chloride (VDC) and ethylene dichloride (EDC) on the same animal systems.

Compound	Species	Angiosarcomas of liver	Tumors of the brain	Tumors of the lung	Hepatomas	Angiosarcomas and angiomas of other sites	Tumors of the kidney: Nephroblastomas	Tumors of the kidney: Adenocarcinomas	Sebaceous cutaneous carcinomas	Other cutaneous epithelial tumors	Mammary carcinomas	Forestomach papillomas and acanthomas
VC	Rat (Sprague-Dawley)	+	+		+	+	+		+	(+)	+	+
	Mouse (Swiss)	+		+		+				(+)	+	(+)
VDC	Rat (Sprague-Dawley)											
	Mouse (Swiss)			(+)				+				
EDC	Rat (Sprague-Dawley)											
	Mouse (Swiss)											

indifference sometimes degenerates into frank hostility.

The high costs probably represent the reason why, in the field of experimental and environmental carcinogenesis, words overlap facts, opinions overlap data, and meetings and commissions reports submerge good laboratory work.

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