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PERSPECTIVES

Volume 41, October 1981

POLYVINYL CHLORIDE

ENVIRONMENTAL HEALTH PERSPECTIVES

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Print Schedule: Volume 42 will present the proceedings from the Symposium on Environmental Epidemiology

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Conference to Reevaluate the Toxicity of Vinyl Chloride Monomer, Poly(vinyl Chloride) and Structural Analogs

National Institutes of Health, Bethesda, MD
March 20-21, 1980

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and
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Environmental Health Perspectives
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Introduction

It has been over 30 years since the first suggestive evidence was published by Tribukh to indicate that vinyl chloride monomer (VCM) is acutely toxic in man. Chronic toxicity in animals was subsequently demonstrated by Torkelson in 1961, but it was an outbreak to VCM-induced acroosteolysis in the 1960's that focused attention for the first time on the occupational toxicity of this important industrial chemical. The carcinogenicity of VCM was recognized in 1974 following the nearly simultaneous reports of an association between exposure to VCM and angiosarcoma of the liver (ASL) in man (reported by Creech and Johnson) and in animals (reported by Maltoni). These reports precipitated a thorough international review of the toxicity of VCM at the New York Academy of Sciences. Since then, enormous research efforts have been undertaken to expand our knowledge of the toxicity of vinyl chloride. Many of the research efforts stimulated by the events of the 1970's have now come to fruition, and are appropriately brought together in these proceedings.

The proceedings begin with a presentation of the most extensive animal research on the carcinogenicity of VCM ever published. In these data, dose-response curves can be examined by site and type of tumor, by route and schedule of exposure, and by species and strain of animal. This study and other research on the carcinogenicity of VCM reported in this volume indicate the importance of cofactors such as age at exposure and simultaneous exposure to ethyl alcohol in VCM toxicity. Evidence that even a single exposure to VCM induces experimental neoplasia is significant from the standpoint of both occupational and nonoccupational exposures.

The updated epidemiologic studies presented in this volume of mortality among workers exposed to vinyl chloride reinforce the laboratory findings of multisite carcinogenicity. Data demonstrating the mutagenic and transplacental effects of VCM are also updated and summarized. The lack of predictivity of one prospective liver screening program suggests the need for further work in identifying effective medical surveillance techniques for early recognition of liver abnormalities.

While thousands of workers are exposed to VCM vapor, many more are exposed to its polymer, poly(vinyl chloride) (PVC). The carcinogenicity of PVC dust is evaluated both experimentally and epidemiologically in these proceedings. The results are not definitive. Nonmalignant respiratory system effects from PVC dust are suggested by a number of papers at this conference.

Finally, the extension of the findings of carcinogenicity of VCM to its structural analogs, vinylidene chloride, trichloroethylene and tetrachloroethylene, and its brominated equivalent, vinyl bromide, serves as another lead in our effort to understand some fundamental concepts of chemical carcinogenesis.

Richard J. Waxweiler
Philip J. Landrigan
Peter Infante
Raymond Shapiro

Environmental Health Perspectives
Vol. 41, pp. 3-29, 1981

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

by Cesare Maltoni,* Giuseppe Lefemine,* Adriano Ciliberti,* Giuliano Cotti* and Donata Carretti*

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
		84	Osteoma
26	Cystic ectasia of blood vessels with fibrosis and dysplasia of perithelial cells	85	Angioma
		86	Fibroangioma
27	Cellular depletion and atrophy (with or without fibrosis)	87	Ossifying angioma
		88	Other benign tumors of connective tissue
28	Simple cyst	89	Fibroadenoma
29	Hemorrhagic cyst	90	Adenomyoma
30	Multiple simple cyst	91	Benign tumors of nervous ganglia (ganglioneuroma) and benign sympathetic tumors of adrenal medulla
31	Multiple hemorrhagic cyst		
32	Dilatation of organs with cavity (including hydronephrosis)	92	Benign tumors of peripheral nerves (neurilemoma)
		93	Carcinoma
33	Hyperplasia and squamous metaplasia	94	Carcinoma with metastases
34	Glandular simple and cystic hyperplasia	95	Basocellular carcinoma
35	Diffused parenchymal hyperplasia	96	Basocellular carcinoma with metastases
36	Nodular parenchymal hyperplasia	97	Squamocellular carcinoma
37	Cortical hyperplasia	98	Squamocellular carcinoma with metastases
38	Medullary hyperplasia	99	Transitional cell carcinoma
39	Hyperplasia of stroma	100	Transitional cell carcinoma with metastases
40	Reactive hyperplasia	101	Adenocarcinoma
41	Simple proliferation of lymphoreticular cells with myelopoiesis	102	Adenocarcinoma with metastases
		103	Biliary duct adenocarcinoma
42	Proliferation of angioblastic cells	104	Biliary duct adenocarcinoma with metastases
43	Fibroangioblastic proliferation	105	Hepatocellular carcinoma or hepatocarcinoma
44	Proliferation of lipocytes	106	Hepatocarcinoma with metastases
45	Proliferation of biliary ducts	107	Exocrine pancreas adenocarcinoma
46	Proliferation of renal tubules and/or of nephroblastema	108	Exocrine pancreas adenocarcinoma with metastases
		109	Cortical adenocarcinoma
47	Adenomatous hyperplasia	110	Cortical adenocarcinoma with metastases
48	Cholangiofibrosis	111	Pheochromoblastoma
49	Dysplasia (comprehensive of neoplastic parenchymal nodule of liver)	112	Pheochromoblastoma with metastases
		113	Nephroblastoma
50	Simple and cystic glandular dysplasia	114	Nephroblastoma with metastases
51	Cortical dysplasia	115	Seminoma
52	Medullary dysplasia	116	Seminoma with metastases
53	Dysplasia of angioblastic cells	117	Melanoma
54	Papillomatosis	118	Melanoma with metastases
55	Acanthomatosis	119	Other malignant epithelial tumors
56	Angiomatosis		

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	153	Malignant tumors of nervous ganglia and malignant sympathetic tumors of adrenal medulla with metastases
129	Leiomyosarcoma	154	Malignant tumors of peripheral nerves (malignant schwannoma)
130	Leiomyosarcoma with metastases	155	Malignant tumors of peripheral nerves (malignant schwannoma) with metastases
131	Rhabdomyosarcoma	156	Characteristic tumors of eyes
132	Rhabdomyosarcoma with metastases	157	Lymphoreticular neoplastic localizations
133	Chondrosarcoma	158	Secondary localizations of tumors from other anatomical districts
134	Chondrosarcoma with metastases	159	Neoplastic effusions
135	Osteosarcoma	160	Odontoma
136	Osteosarcoma with metastases	161	Chondromatosis
137	Angiosarcoma	162	Histiocytosis and benign histiocytoma
138	Angiosarcoma with metastases	163	Mesothelial hyperplasia
139	Ossifying angiosarcoma		
140	Ossifying angiosarcoma with metastases		
141	Angiopericytosarcoma		
142	Angiopericytosarcoma with metastases		
143	Other malignant tumors of connective tissue		
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 *Treatment protocol:* 4 hr/day, 5 days/ week, 52 weeks		II, 1 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 (liver angiosarcoma)			11, 158 (17, 138)
Pleura and pleural cavity	Secondary neoplastic localization (liver angiosarcoma)			12, 158 (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, 13 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	96.3	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	96.3	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	18.3	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	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary 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	Hepatomas	Nephro-BL	Neuro-BL	Zymbal Gl.Ca	Skin EpT	Forestomach Pa&Ac	Mammary 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	13.2	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 17	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 17	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	–	3.8	–
	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 gland M	Zymbal gland F	Zymbal gland 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.

Experiment	Concentration, ppm	Animals with LAS, %			
		Sprague-Dawley rats	Wistar rats	Swiss mice	Golden hamsters
BT1, BT2, BT4, BT5	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.

Experiment	Concentration, ppm	Animals with Zymbal gland CA, %	
		Sprague-Dawley rats	Wistar rats
BT1, BT2	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
BT1–BT14	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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Neoplastic and Nonneoplastic Effects of Vinyl Chloride in Mouse Lung

by Yasunosuke Suzuki*

Neoplastic effects of vinyl chloride were studied in lungs of 27 mice exposed to vinyl chloride monomer at 2500 and 6000 ppm for 5 and 6 months (large doses and long-term exposure). Pulmonary tumors were observed in 26 of 27 experimental animals. Light microscopy showed the tumors to be multiple and arranged in either tubulo-papillary or adenomatous formations. Although occasional mitotic divisions and invaginations into the bronchiolar lumen were observed, no metastases were found. By electron microscopy, short microvilli, tight junctions between two adjacent cells, appearance of osmiophilic lamellar bodies, large mitochondria of irregular shape, well developed Golgi complexes, continuous or discontinuous basement membranes, occasional appearance of "sequestration" and of crystalloids and lack of both cilia and mucous secretory granules were observed as characteristic features of the neoplastic cells. Some of the cells were poorly differentiated and were equipped with poorly developed organoids, without formation of osmiophilic lamellar bodies. The pulmonary tumors corresponded to "alveologenic" tumors. It is suggested that the neoplastic cells were transformed from type II alveolar epithelium via its hyperplastic form.

Nonneoplastic effects of the chemical were also studied in the 27 mice. Major light microscopic alterations observed were proliferation and hypertrophy of the terminal bronchiolar cells, consisting of ciliated and Clara cells, hypersecretion of the epithelial mucin in the goblet cells of both the bronchial and the proximal bronchiolar epithelium, hyperplasia of alveolar epithelium, mobilization of alveolar macrophages and occasional presence of peribronchial or bronchiolar chronic inflammation. Electron microscopically, Clara cells of the terminal bronchiolar epithelium showed proliferation of the rough and smooth surfaced endoplasmic reticulum and appearance of large and abnormally shaped mitochondria. Similar alterations were found in the ciliated cells. Submicroscopic changes of pulmonary alveoli were represented by focal thickening of the basement membrane, multiple foci of hyperplastic type II cell (the precondition of the alveologenic tumor), active discharge of osmiophilic lamellar bodies from the type II cell and phagocytosis of the bodies by macrophages, appearance of cholesterol crystalloids in the macrophages, degeneration of alveolar septal cells and occasional appearance of a large nucleus with swelling of the capillary endothelium.

The neoplastic effect of vinyl chloride of smaller doses (100, 10, 1 and 0 [control] ppm) and shorter exposure (four weeks) was studied in lungs of 120 mice. Our preliminary observation indicated that sacrificed animals at 40 weeks after the exposure showed productions of the alveologenic tumor in 5 of 9 (100 ppm), 2 of 9 (10 ppm), 1 of 9 (1 ppm) and 0 of 10 (control = 0 ppm). A dose-response relation was considered in the incidence of the alveologenic tumor production of vinyl chloride. It is concluded that mouse lung is an extremely sensitive indicator of the oncogenicity of vinyl chloride.

Introduction

Hepatic hemangiosarcoma has been accepted as a serious health hazard associated with vinyl chloride exposure among workers in vinyl chloride polymerization plants (*1-7*). A risk of lung cancer has also been reported among the workers on the basis of epidemiological studies (*8,9*).

Experimental studies in rats, mice, and hamsters have shown that, in addition to liver, various organs such as lung, brain, breast and skin, including sebaceous glands, were involved in induction of primary neoplasia by vinyl chloride. Although a

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number of studies have demonstrated that pulmonary tumors can be induced by vinyl chloride in mice, the significance of such occurrence and the nature of the tumors have not yet been appropriately explored (*10-17*).

The occurrence of the nonneoplastic pulmonary abnormalities among vinyl chloride polymerization workers has been reported on the basis of chest x-ray (*18*), pulmonary function (*19*) and smear cytology (*1*) of the worker's sputum. No histopathological evaluation of the abnormalities has been reported.

It is believed that a relationship between lung cancer and vinyl chloride exposure exists from epidemiological studies (*6,8,9,20*). A high incidence of pulmonary tumors in mice exposed to vinyl chloride has been reported by several investigators (*10-15*). However, the nonneoplastic pulmonary effects of the chemical have not been completely explored. We have, therefore, undertaken detailed light and electron microscopy of the mouse lung, to characterize the neoplastic and nonneoplastic pulmonary effects of vinyl chloride.

FIGURE 1. Two pulmonary tumors induced by vinyl chloride are seen in the peripheral part of a mouse lung (2500 ppm in group III). Hematoxylin and eosin; 64×.

Materials and Methods

Twenty-seven CDI Charles River white strain male mice, 4 to 5 weeks old at first exposure, were used. All of 27 mice used for this study were alive until they were sacrificed at three separate stages. The mice that died in the course of the experiment were excluded from the study. Group I consisted of six animals exposed to vinyl chloride at 2500 (three mice) and 6000 (three mice) ppm/hr, 5 hr/day, 5 days/week, for 5 months. They were then kept for 6 days without exposure before sacrifice. Group II included 13 mice exposed at 2500 (seven mice) and 6000 (six mice) ppm for 6 months and were kept for an additional 2 days for recovering before sacrifice. Group III included eight animals (seven at 2500 ppm and one at 6000 ppm) which were exposed to vinyl chloride monomer for 6 months followed by a 37-day recovery period. The inhalation exposures were accomplished at the Industrial Bio-Test Laboratory, Northbrook, Illinois. In addition to the experimental animals, 16 mice (four for group I, four for group II, three for group III, and five which were 12 months old) were used as controls.

To study the pulmonary effects of the chemical at smaller doses with shorter exposure, 120 mice of the same strain, sex and age were prepared. These animals were divided into four groups (30 mice in

Table 1. Pulmonary tumor production in CD1 male mice with lower doses of VC and shorter exposure (4 weeks).[a]

VC dose, ppm	1st sacrifice (immediately after dosing)	Between 1st and 2nd sacrifice	2nd sacrifice (12 weeks)	Between 2nd and 3rd sacrifice	3rd sacrifice (40 weeks)
100 (N = 30)	0/10[b]	0/4[c]	0/6[b]	0/1[c]	5/9[b]
10 (N = 30)	0/10[b]	0/1[c]	0/9[b]	0/1[c]	2/9[b]
1 (N = 30)	0/10[b]		0/10[b]	0/1[c]	1/9[b]
0 (N = 30)	0/10[b]	0/1[c]	0/9[b]		0/10[b]

[a]Reported as pulmonary tumor-bearing mice/total number of mice.
[b]Sacrificed.
[c]Found dead.

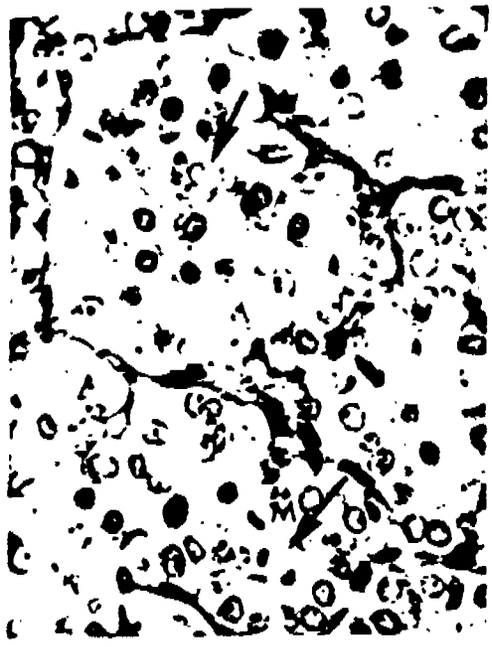

FIGURE 2. Neoplastic cells stained with PAS. An arrow indicates fine PAS-positive material digested by diastase. The arrow labeled M shows an abnormal mitosis (2500 ppm in group III). 560×.

each group) and were exposed to the chemical for 4 weeks (5 hr/day, and 5 days/week) at 100 ppm, 10 ppm, 1 ppm and 0 ppm (control). The low dose exposures were performed at the Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical U.S.A. As shown on Table 1, these animals were sacrificed at three different periods: immediately after, 12 weeks after and 40 weeks after exposure to the chemical. Six (four 100 ppm, one 10 ppm, and one control) were found dead between the first and second periods, and three (one 100 ppm, one 10 ppm and one 1 ppm) were found dead between the second and third periods.

Lungs were examined under a dissecting microscope after the organs were removed from sacrificed animals, to determine whether macroscopic abnormalities including tumor production were present.

For light microscopy, the organs were fixed in 10% neutral buffered formalin and embedded in paraffin after dehydration in alcohol. Sections (5-6 μm) were made and stained with hematoxylin–eosin, Masson's trichrome, Weigert's silver, periodic acid-Schiff's (PAS) with and without digestion by diastase, elastin and Van Gieson's picrofuchsin technique. For electron microscopy small pieces, smaller than 1 mm^3, were taken from both pulmonary tumors and nonneoplastic pulmonary tissues and were fixed in 1% phosphate-buffered osmic acid at pH 7.2-7.4 for 2 hr or in 2% paraformaldehyde fixative followed by the osmic acid. After alcohol dehydration, the blocks were embedded in epoxy resin. Ultrathin sections were obtained with an LKB microtome. The sections were stained with uranyl acetate and lead. A Siemens 101 electron microscope was used for ultrastructural observations.

Observations

Neoplastic Effects of Vinyl Chloride at Heavy Doses and Long-Term Exposures

Gross Anatomical Findings. Pulmonary tumors were observed in all experimental mice except one from the 6000 ppm series of group II (26 of 27). None were found in 16 controls. These tumors were round, whitish in color, multiple in number and variable in size from 1 to 5 mm in diameter. No metastases to regional lymph nodes or other organs were observed. Neither parenchymal fibrosis nor fibrotic adhesions of the pleura were detected.

Light Microscopy. As shown in Figure 1, the tumors were usually seen in the peripheral part of

FIGURE 3. Hyperplastic pulmonary cells are seen beneath the visceral pleura of a mouse lung (2500 ppm in group III). Hematoxylin and eosin; 430×.

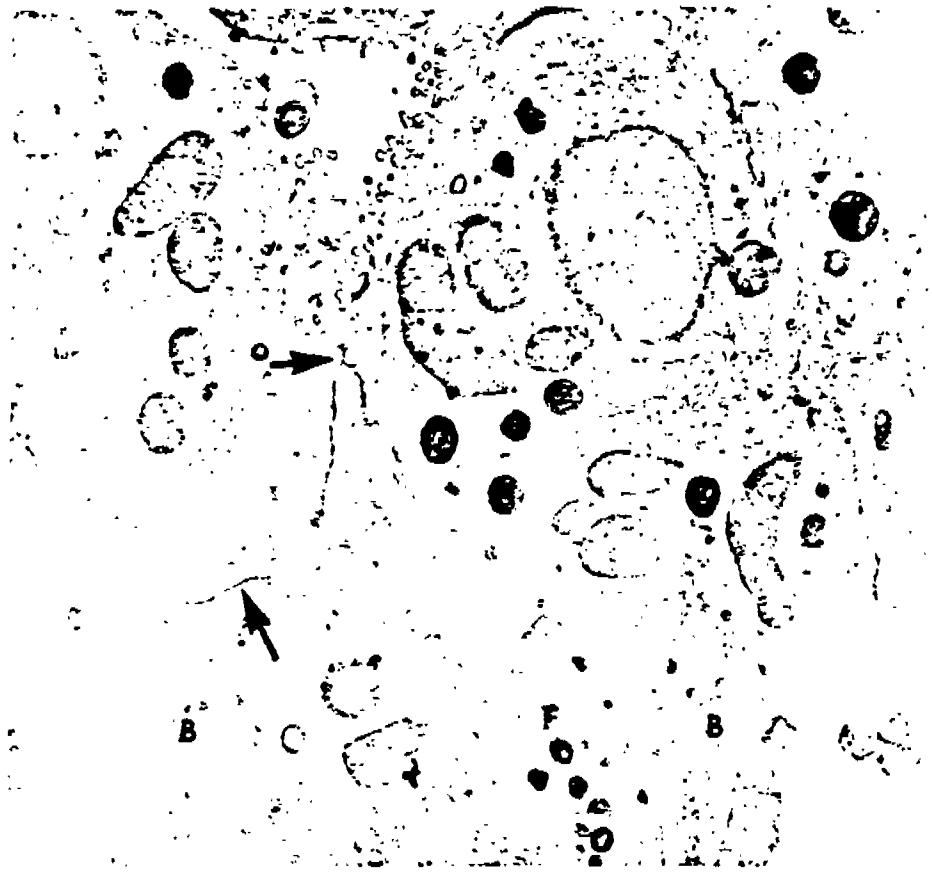

FIGURE 4. Low-power electron micrograph of a well-differentiated pulmonary tumor. Arrows indicate junctional structures between neoplastic cells. B, Basement membrane (2500 ppm in group III), OsO_4; 7900×.

lung parenchyma, although occasionally tumors were found in more proximal parts of the lung. No direct connections of the tumors with bronchi or bronchioles were observed. The neoplastic cells were arranged in various ways, such as tubulopapillary and adenomatous forms. Pleomorphism and atypical structures were not striking. However, sometimes abnormal mitoses were observed, as shown in Figure 2 (arrow labeled M). The nuclei were round in shape and small, and chromatin was generally finely distributed. Nucleoli were generally poor in development. Two different types, eosinophilic and basophilic, were distinguished in the neoplastic cells. Some of the cells stained with PAS, and the substance so stained was digested by diastase, suggesting that it was glycogen. The neoplastic tissue was not encapsulated by connective tissue. Often, air spaces separated neoplastic tissue from normal tissue. Although malignant invasion, such as destruction of preexisting tissue, was not observed in the animal lungs, invagination of the neoplastic tissue into bronchiolar air spaces was detected in instances of extremely large tumors. Collagen and reticular fibers showed little development in the neoplastic tissues. In addition to neoplastic changes, as shown in Figure 3, focal and multiple hyperplastic changes of the alveolar lining cells were noted in lungs exposed to vinyl chloride. In Figure 3, the hyperplastic cells are seen just beneath the visceral pleura. Since the lining cells beneath the thin connective tissue of the visceral pleura are known to be alveolar epithelium, the hyperplastic cells are assumed to be alveolar epithelial cells. Hyperplastic cells are also found in the deeper part of lung parenchyma. Occasionally, neoplasia and hyperplasia coexisted in the same lobe of the lung, and the distinction between neoplastic and hyperplastic cells with confidence was not always clear, as some cellular similarities were found between the two. Identification of the cell types of both

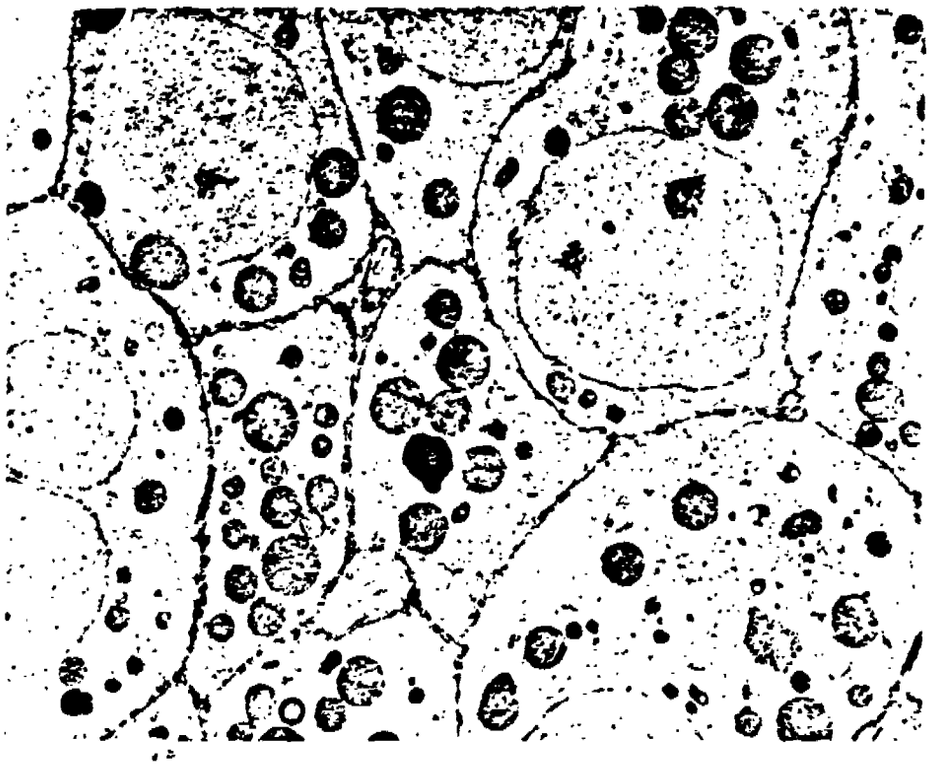

FIGURE 5. Differentiated neoplasm seen in the animal lung shown in Fig. 3. Formation of the tubular lumen and microvilli is poor. OsO_4; 6500 ×.

neoplastic and hyperplastic cells with confidence was not feasible at the level of light microscopy.

Electron Microscopy. Figure 4 is derived from neoplastic tissue of the tubulo-papillary form. Ultrastructural characteristics of the neoplastic cell included microvilli, large, round, or rod-shaped mitochondria, well-developed Golgi complexes, and osmiophilic lamellar bodies. Junctional structures (arrows in Fig. 4) between adjacent neoplastic cells and a basement membrane (B) were usually observed. Figure 5 was derived from an adenomatous area of the tumor. Neoplastic cells had poorly formed tubular lumens and microvilli and were smaller than those shown in Figure 4. However, other ultrastructural characteristics, such as mitochondria, Golgi complexes, and osmiophilic lamellar bodies, were almost identical in the two. Irregular arrangements of mitochondrial cristae were fairly common, and mitochondria were often wrapped by well-developed, smooth-surfaced endoplasmic reticulum (Fig. 6). Some of the neoplastic cells contained cytoplasmic compartments formed by membrane structures (Fig. 7). The occurrence of such compartments has been reported by Svoboda (*21*), who made electron microscopic observations on the neoplastic cells in mouse pulmonary tumors induced spontaneously or by urethane. Occasionally, crystalloid structures were observed in the cytoplasm of the neoplastic cells (Fig. 8). The above described neoplastic cells were quite similar in ultrastructure to type II alveolar epithelium. Capillaries in the neoplastic tissue consisted of single layers of nonfenestrated endothelium as seen in the normal alveolar capillary. In addition to well-differentiated neoplastic cells, poorly differentiated ones were also recognized (Fig. 9). As seen in Figure 9, the cells were cuboidal or cylindrical in shape and lacked formation of osmiophilic lamellar bodies. Mitochondria were small in size, although the cells were relatively rich in rough-surfaced endoplasmic reticulum. These cells seemed to correspond to the basophilic ones observed by light microscopy. Except for the lack of a large amount of glycogen, these cells resembled immature alveolar epithelium, as observed in fetal lung in late gestation. Neither cilia nor mucinous secretory granules were

FIGURE 6. Part of the cell cytoplasm of a neoplastic cell. The arrow indicates irregular mitochrondrial cristae (2500 ppm in Group III). OsO_4; 29, 400×.

FIGURE 7. Intracytoplasmic compartments formed by membrane structures (2500 ppm in group III). OsO_4; 20, 000×.

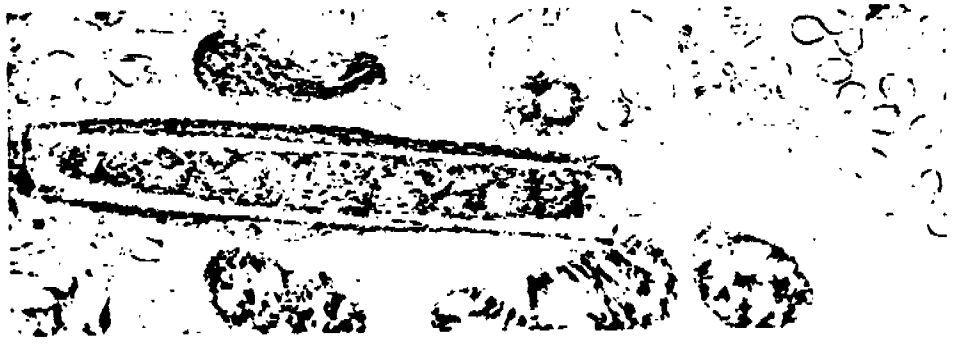

FIGURE 8. Crystalloid structure seen in the cytoplasm of a neoplastic cell (2500 ppm in group III). OsO_4; 30,000×.

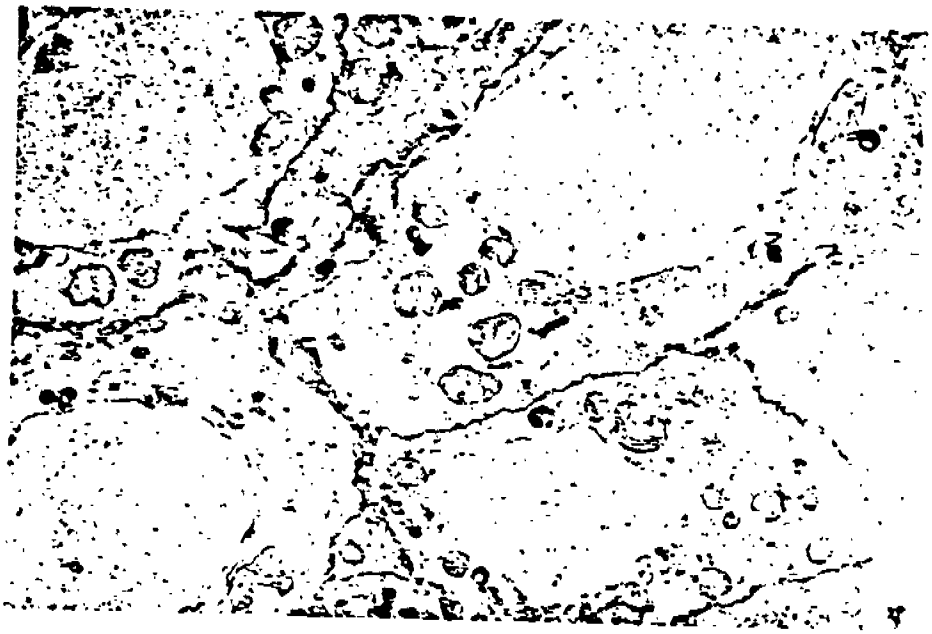

FIGURE 9. Poorly differentiated neoplastic cells. No osmiophilic lamellar bodies are observed (6000 ppm in group II). OsO_4; 6200 ×.

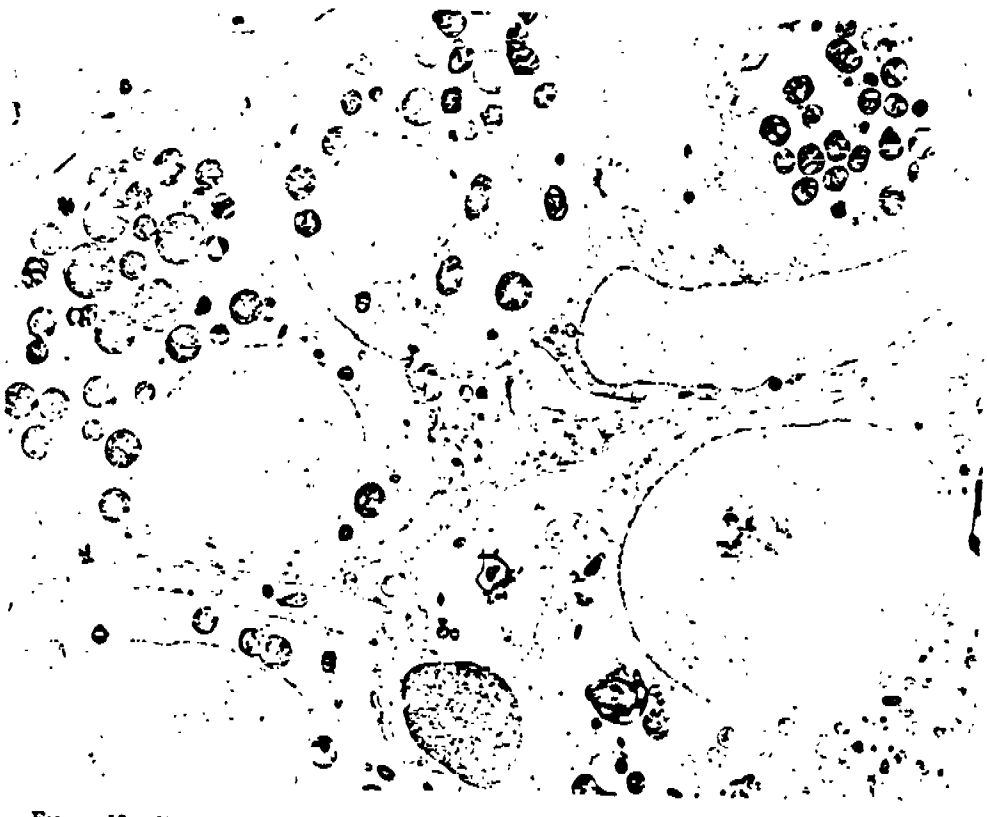

FIGURE 10. Hyperplastic type II cells are illustrated. Group 1; 6000 ppm; OsO_4; 5300 ×.

FIGURE 11. Light microscopy of a bronchiolo-alveolar area. Arrows indicate the site of transition between the respiratory bronchiole and the alveolus. Control mouse; Masson's trichrome staining; 420×.

FIGURE 12. Proliferated and hypertrophic bronchioles. Desquamated bronchiolar epithelial areas are illustrated. Masson's trichrome staining; Group I; 2500 ppm, 420×.

observed in the neoplastic cells. Based on these findings, it was strongly suggested that the neoplastic cells were derived from the alveolar epithelium, particularly from type II cells. A hyperplastic pulmonary alveolus is illustrated in Figure 10. Electron microscopically, aspects of the hyperplastic cells were evidently those of type II alveolar cells, although they showed some differences in ultrastructure from the normal type II cell. Mitochondria were large in size and irregular in shape, and, occasionally, retention of huge osmiophilic lamellar bodies was noted in the cell cytoplasm. Cristae mitochrondriales were arranged irregularly, and well-developed endoplasmic reticulum was frequently seen in the cytoplasm. Early stages of the membrane formation responsible for "cytoplasmic compartments" were observed in the hyperplastic cell. In many respects, the hyperplastic type II cell is assumed to be the precursor of the neoplastic cell. An intermediate form between type I and II cells was frequently observed in the hyperplastic pulmonary alveoli. The arrow in Figure 10 indicates a part of the cell cytoplasm which may represent a transitional form between type I and II cells. Though the secretory granules were observed in the neoplastic cells, based on these findings, it was strongly suggested that the neoplastic cells were derived from the alveolar.

FIGURE 13. Hypersecretion of epithelial mucin in bronchial epithelium. Arrows indicate mucinous substance stained with PAS. Group III; 6000 ppm, 420×.

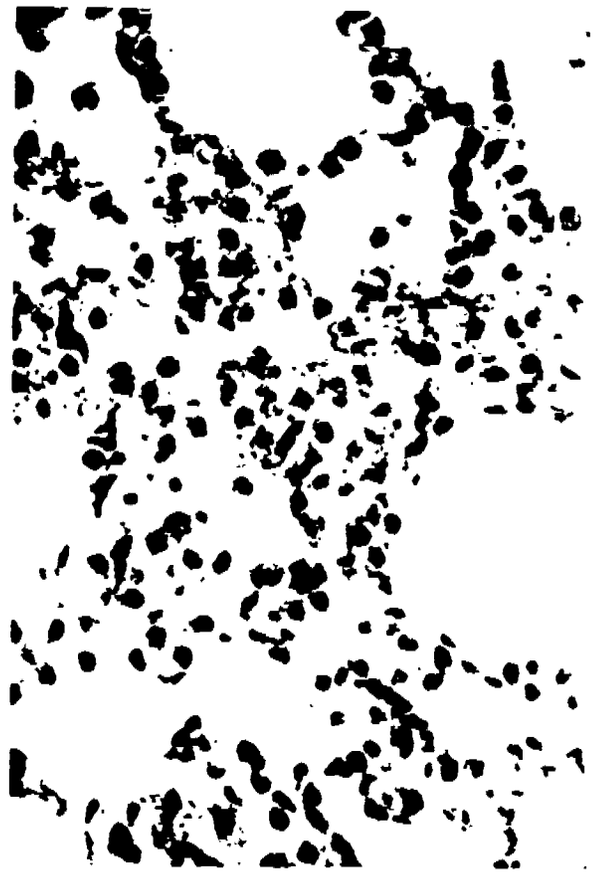

FIGURE 14. Hyperplasia of alveolar epithelium is shown. Hematoxylin-eosin: Group III: 2500 ppm; 670×.

Nonneoplastic Effects of Vinyl Chloride at Heavy Doses with Long-Term Exposure

Light Microscopy. In the bronchi and bronchioles, as a common finding in the treated animals, proliferation and hypertrophy of the bronchiolar epithelium were noted. As shown in Figure 11, the terminal and respiratory bronchioles of the control mice were relatively simple in structure and the transitional point (arrows) of the respiratory bronchiole into the alveolus was easily distinguished. To differentiate the two areas, we found that Masson's trichrome was a useful stain, since the cytoplasm of the bronchiolar cells was stained an intense brown red. The bronchioles of all the animals treated with vinyl chloride monomer showed proliferation, and cellular hypertrophy, through the degree varied among the animals (Fig. 12). The proliferated cells were irregular in arrangement (Fig. 12). Frequently, hypersecretion of epithelial mucin in goblet cells of the bronchi as well as the proximal bronchioles was observed (Fig. 13, arrows). It was noteworthy that those alterations were still found in the animals of group III, which had a recovery time of 37 days after vinyl chloride exposure. Chronic inflammatory changes represented by marked lymphocyte infiltration into the perivascular and peribronchiolar connective tissue were seen, particularly in group III.

The most significant finding in pulmonary alveoli was a high incidence of alveologenic tumors in the treated mice, as stated before. In addition to the neoplasm, multiple foci of hyperplastic alveolar epi-

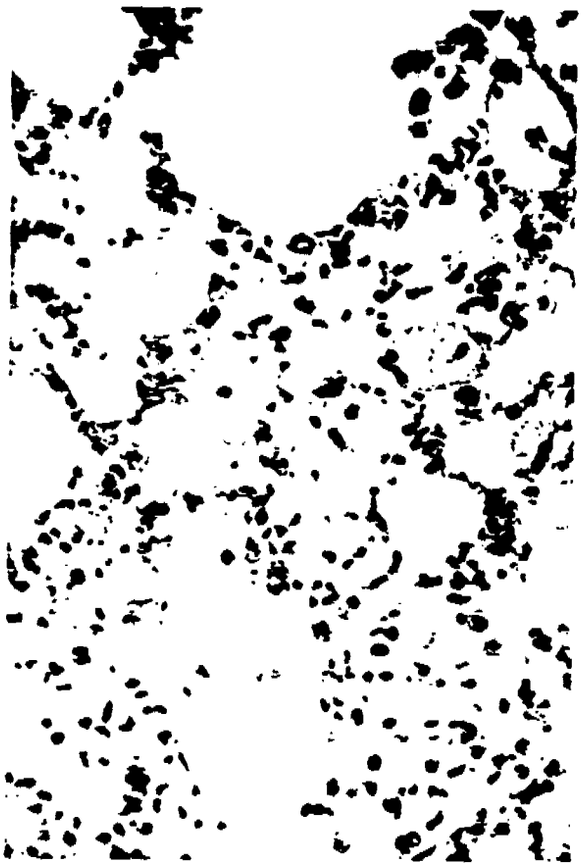

FIGURE 15. Large clear macrophages (foam cells) as well as small basophilic macrophages are seen in the alveolar air spaces. Hematoxylin-eosin: Group III: 2500 ppm; 420×.

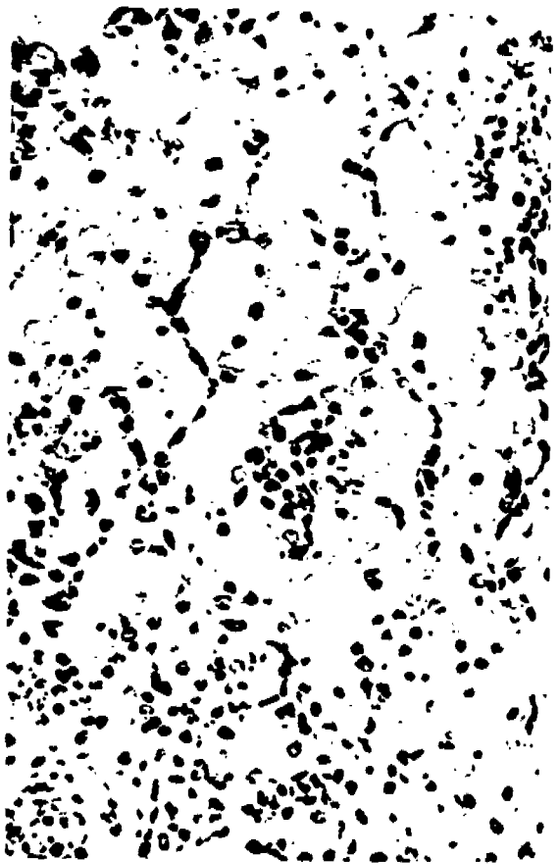

FIGURE 16. Striking accumulation of a large number of foam cells. Hematoxylin-eosin: Group III: 6000 ppm: 420×.

thelium (Fig. 14), which were strongly suggestive of being preneoplastic for alveologenic tumors (*22*), were frequently observed. Mobilization of alveolar macrophages in the alveolar space was fairly commonly seen in the three groups of animals (Fig. 15). In several cases, foam cells were markedly accumulated in alveolar space (Fig. 16). Two animals in group III showed bronchopneumonia-like changes. As shown in Figure 17, coexistence of the proliferated bronchioles with alveologenic tumors (arrow) was frequently observed.

Electron Microscopy. The epithelium of the terminal bronchiole consists of the Clara cell (nonciliated) and ciliated cells; it lacks mucous-producing cells in the mice. Both cell types are illustrated in Figure 18, obtained from a control mouse. Clara cells lack typical microvilli and their apical portion present a dome-like shape (Fig. 18). The smooth-surfaced endoplasmic reticulum and Golgi complex were well developed. Mitochondria were generally round in shape and their cristae were few in number (Fig. 18). Two distinct granules, a beadlike structure (electron-dense; the long axis was 1.6-0.2 μm) and a round phagolysosomal granule (electron-dense or opaque; 0.6 × 0.6 μm in size) were observed in the cells. It is noteworthy that the ultrastructure of the cell is not identical among animal species and, further different fixation methods result in different structural appearances of the endoplasmic reticulum in the cell.

Part of lung tissue showing coexistence of the alveolar tumor (arrow) with the proliferated bronchiole. Masson trichrome staining: Group I: 6000 ppm; 170×.

The cytoplasm of the ciliated cell was relatively clear and it contained rod-shaped mitochondria with cristae (Fig. 18). Cilia and microvilli were seen in the cell surface (Fig. 18). The two distinct granules were also occasionally seen in the cell. Ultrastructural changes seen in those cell types of the treated mice are described below.

A low-power view of the proliferated bronchiole is illustrated in Figure 19. Hypertrophic Clara cells frequently included dark cells rich in rough-surfaced endoplasmic reticulum and free ribosomes. The cell was usually large and its shape was occasionally irregular. Deep interdigitation of the lateral cell membranes of two adjacent cells was occasionally observed. Golgi complexes were well developed, and the two distinct granules described above were increased in number in the cytoplasm. The dark round granules are shown in Figure 19. Although smooth-surfaced endoplasmic reticulum of the normal Clara cell was generally vesicular (with a single osmic acid fixation as used in this study, though this cell organoid is cisternal with double fixation by glutaraldehyde and osmic acid), the hypertrophic cells contained various forms of the organoid and the transformation of the rough surfaced endoplasmic reticulum into smooth-surfaced endoplasmic reticulum was easily observed (Fig. 20). Mitochondria of abnormal shapes and large size occasionally appeared. Cristae were rather clearly shown in such abnormal mitichondria (Fig. 21).

Ciliated cells were also involved in the proliferated alteration. The shape of the cells was occasionally irregular. Golgi complexes were sometimes well developed and phagolysosomal granules as well as round dense granules frequently appeared in the cytoplasm (Fig. 22). Large round mitochondria in which cristae were fewer in number were seen. In some ciliated cells, the rough and smooth surfaced endoplasmic reticulum were markedly developed (Fig. 22).

Although light microscopic observations failed to detect details of damages in the pulmonary alveolus, various ultrastructural alterations of the alveolar cells were revealed by electron microscopy.

Mobilized alveolar macrophages were rich in phagolysosomal granules, as shown in Figure 23. Other cell organelles were also well developed. Basophilic macrophages contained a large number of free ribosomes as well as the rough-surfaced endoplasmic reticulum, while clear macrophages were represented by intracytoplasmic osmiophilic lamellar bodies (Fig. 23) which seemed to be phagocytosed from the alveolar space. Occasionally, cholesterol crystalloids were found in the macrophages (Fig. 24, arrows).

As shown in Figure 10, hyperplastic alveolar epithelium consisted of type II cells, precursors of alveolar tumor (*15*). The evolutional process of the neoplastic transformation has been reported (*15*). Deformation of the cell shape, appearance of giant mitochondria with abnormal cristae, retention of large osmiophilic lamellar bodies, early introcytoplasmic "sequestration," and occasional huge lipid granules were observed. Microvilli of the cell surface were frequently decreased in number. These alterations were observed in almost all mice exposed to vinyl chloride, regardless of difference in dose and duration of recovery time.

Swelling of the cytoplasm and appearance of lysosomal granules were occasionally observed in type I cells. Transformation of the type I cells into the

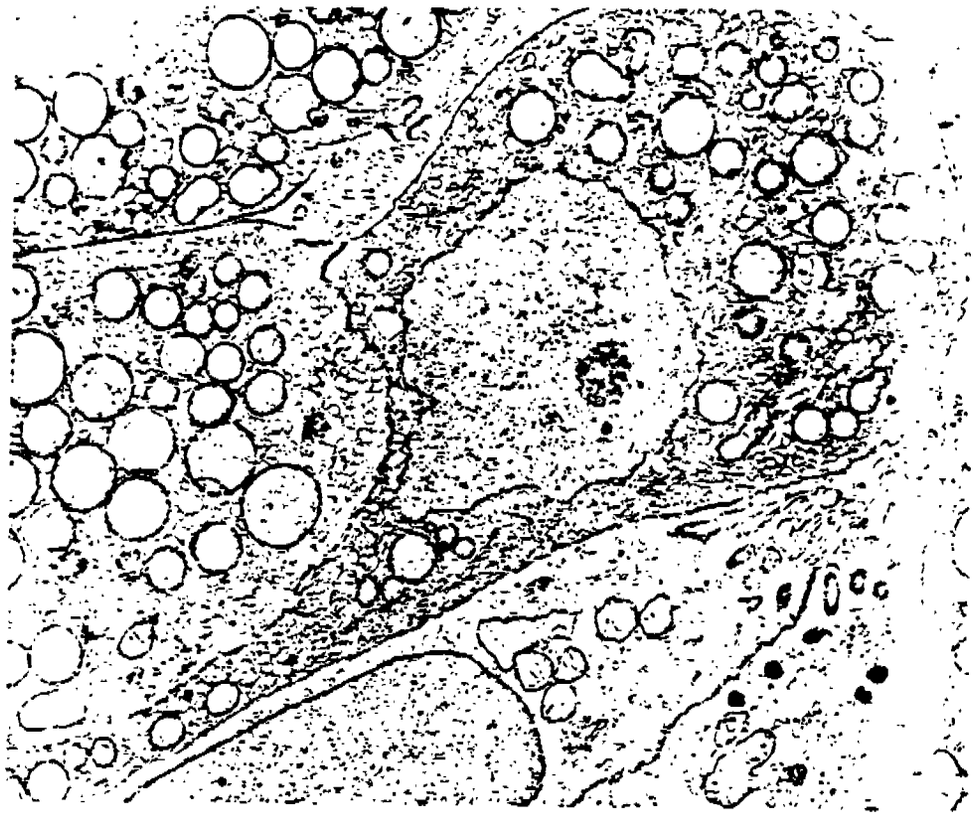

FIGURE 18. Part of the terminal bronchiole. Clara cells and ciliated cells are illustrated. Control mouse; OsO_4; 6700×.

type II cells was suggested, since intermediate cell types between the two were found on the alveolar lining.

Focal thickening of the basement membrane was commonly seen (Fig. 25). Sometimes, the thickened basement membrane showed a fibrillar appearance ("f" in Fig. 26) and contained cell debris (arrows in Fig. 26) which seemed to be derived from alveolar septal cells.

In addition to swelling of the cytoplasm, lysosomal granules and a large nucleus (Fig. 27), segmented or nonsegmented, were sometimes observed in the alveolar endothelium.

Alveolar septal cells frequently showed hypertrophy (h in Fig. 28) and degeneration (arrows in Fig. 28). In some cases, focal reticulosis was observed in the alveolar septum.

From these light and electron microscopic studies, mouse lungs exposed to vinyl chloride at 2500 and 6000 ppm for 5 and 6 months clearly showed nonneoplastic pulmonary changes in both bronchular and alveolar cells. Since these findings were not detected in control mice, they are considered related to vinyl chloride.

Neoplastic Effects of Vinyl Chloride at Smaller Doses with a Shorter Exposure

Detailed studies on pulmonary effects of vinyl chloride at smaller doses (1, 10 and 100 ppm) with a shorter exposure (4 weeks, 5 hr/day, 5 days/ week are still in a process of analyses in 90 mice. However, as shown in Table 1, our preliminary study that involved gross anatomical and histological observations revealed that alveologenic tumors were induced in 5 of 9 (100 ppm), 2 of 9 (10 ppm) and 1 of 9 (1 ppm), while the tumor was not seen in 10 control

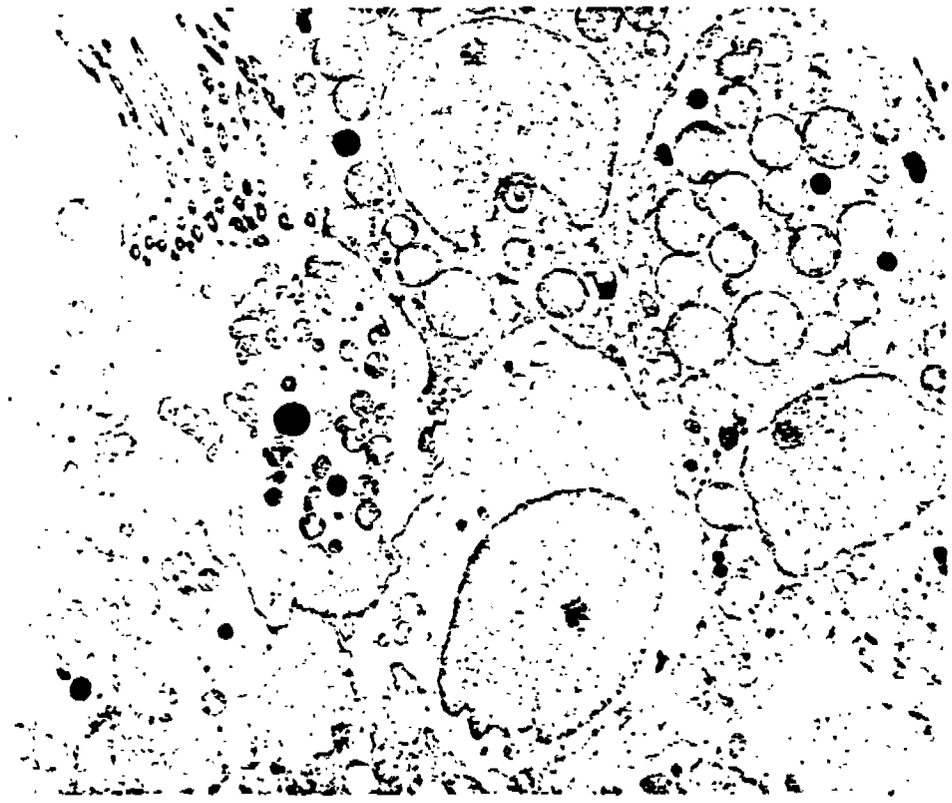

FIGURE 19. Proliferated bronchiolar epithelium. Group I: 6000 ppm; 3300×.

(0 ppm) at 40 weeks after vinyl chloride exposure. Size and number of the induced tumors were generally smaller than those of the tumors produced by the chemical at larger doses (2500 ppm and 6,000 ppm) and longer exposure (5 and 6 months).

A dose response relation was suggested in production of alveologenic tumor.

Discussion

Experimental studies (*10-17*) on the oncogenicity of vinyl chloride have revealed that the monomer can induce various neoplasms including hepatic hemangiosarcoma (rat, mice, hamsters), Zymbal gland carcinoma in the external auditory meatus (rats), breast cancer (mice), nephroblastoma (rats), "lung adenoma" (mice), skin trichoepithelioma (hamsters), lymphoma (hamsters) and forestomach papilloma (hamsters). Evidence of the pulmonary oncogenicity of vinyl chloride in animals has been obtained. Viola et al. (*16, 17*) have reported that rats exposed to vinyl chloride exhibited lung cancer (32%). Histological features of the cancers were stated to be those of adenocarcinoma, with the exception of a single epidermoid tumor. Maltoni and Lefemine (*13,14*), however, reviewed the histological slides of Viola et al. and stated that the lung cancers reported by the latter were not primary tumors of the lungs, but metastatic cancers from Zymbal glands. Maltoni and Lefemine (*13,14*) also have reported on the pulmonary oncogenicity of vinyl chloride on the basis of their own data. Though they could not find bronchogenic carcinoma in rats, rare pulmonary hemangiosarcomas and fibrosarcomas were induced in these animals. Unlike the case in rats, pulmonary tumors ("adenomas") were found in mice (89 of 471). Maltoni and Lefemine (*13,14*) noted that some of the adenomas underwent malignant transformation. Keplinger and associates (*11*) have found "alveologenic adenomas" in the lungs of mice exposed to vinyl chloride (44 of 49). Lee and associates (*12*) have stated that "bronchiolar ade-

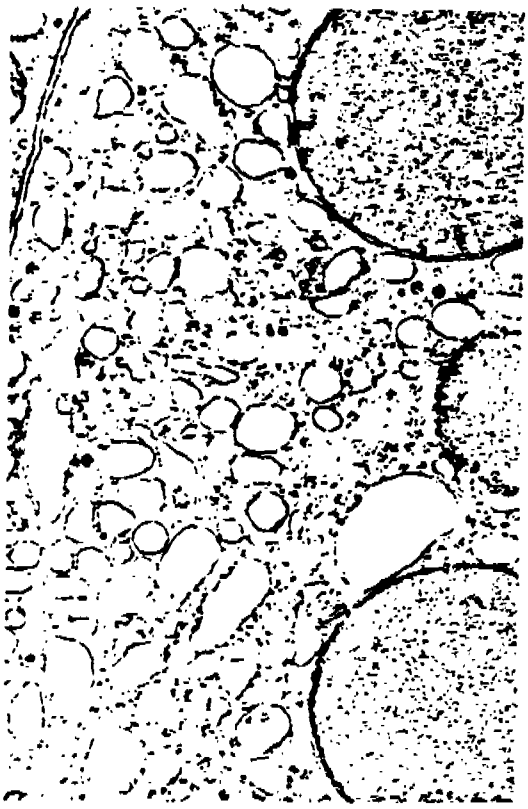

FIGURE 20. Part of the cytoplasm of a Clara cell. Various profiles of the endoplasmic reticulum are seen. Group I: 2500 ppm; 21,000 ×.

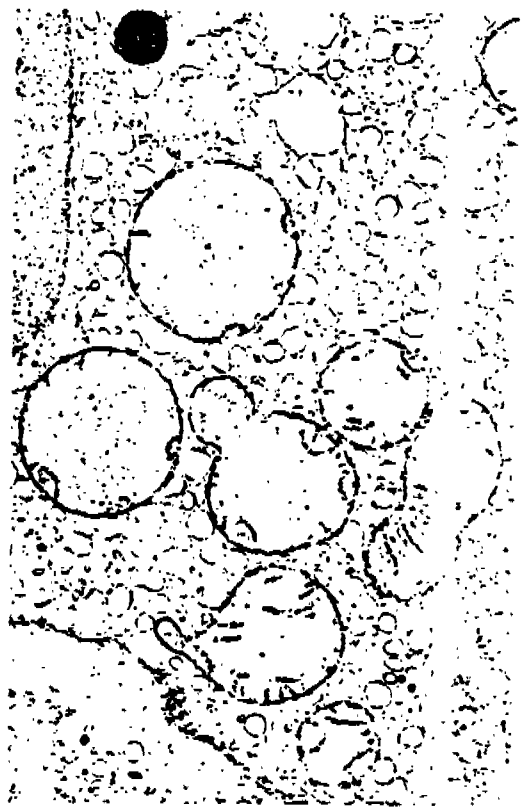

FIGURE 21. Irregular shaped mitochondria with cristae in the cytoplasm of a Clara cell. Group I: 6000 ppm; OsO_4; 12,600 ×.

noma" developed in mice 2 months after exposure to vinyl chloride at 50-1000 ppm. Holmberg and associates (*10*) found "alveologenic adenoma" in 13 of 24 mice exposed to vinyl chloride at 50 ppm for 24-52 weeks. Our present study has also confirmed that pulmonary tumors are induced by vinyl chloride of both the large doses (2,500 and 6,000 ppm) with long exposure (5 and 6 months) and the smaller doses (1, 10 and 100 ppm) with a short exposure (4 weeks).

Based on all the data available, it can be concluded that mouse lung is an extremely sensitive organ for demonstrating the oncogenicity of vinyl chloride. Gross anatomical and histological aspects of the tumors in our investigation corresponded to the "alveologenic tumor or cancer" of Steward et al. (*22-24*). The alveologenic tumor has been induced by various carcinogens (*22-28*), such as polycyclic hydrocarbons, urethane, nitrogen mustard, methylcholanthrene, and nitrofur derivatives and is known to occur spontaneously with aging (*6,29,30*). The cancer induced has been distinguished from that occurring spontaneously by multiple primary foci, occasional formation of huge tumors, and occurrence without any relation to aging. It is also known that in certain strains, such as A and DD, spontaneous tumors are quite common after 10 to 12 months of age. Spontaneous pulmonary tumors could be excluded in our experimental animals; in addition to the above-mentioned points, neoplastic changes in the lungs were absent in the controls.

Electron microscopically, alveolar epithelium, particularly the type II cell, was assumed to be the precursor of vinyl chloride-induced tumor in the mouse lung. This assumption was derived from ultrastructural similarities between the normal type II cell and the neoplastic cell. Similar suggestions have been made by other investigators (*21, 28, 31*) after studying pulmonary tumors induced by agents other than vinyl chloride.

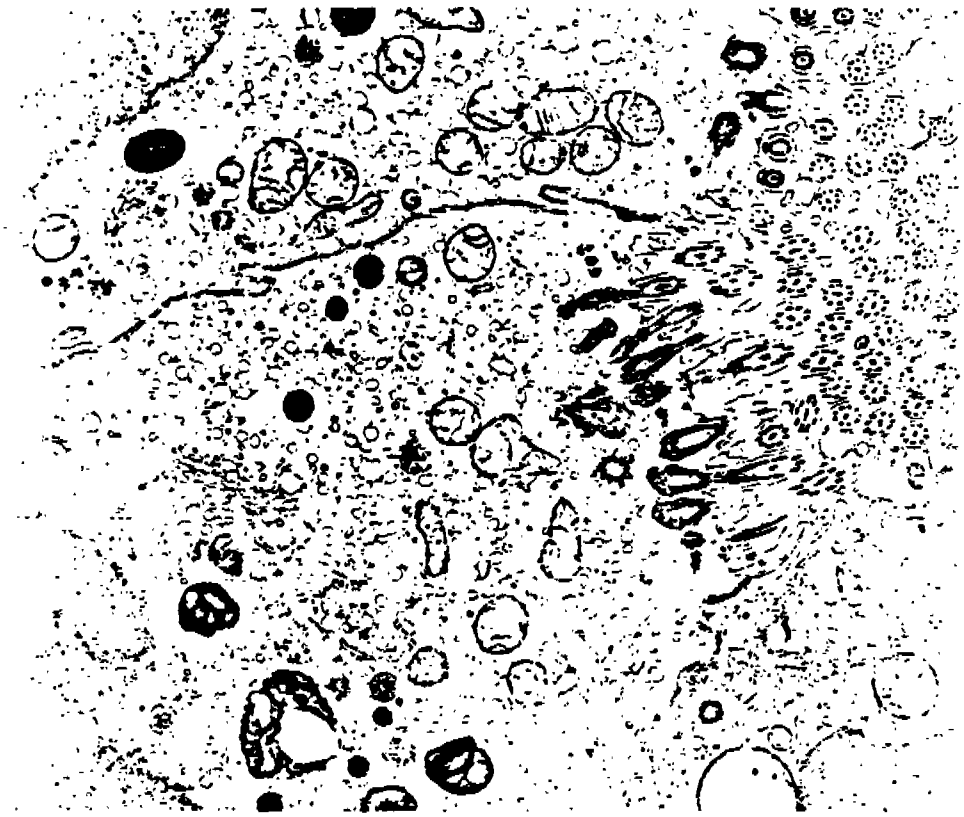

FIGURE 22. Two ciliated cells of a terminal bronchiole. Irregular shaped mitochondria, a well developed Golgi area, round dense granules and phagolysomes are shown. Group I: 6000 ppm; OsO_4; 9800 ×.

It was noteworthy that the processes of transformation of the normal alveolar epithelium into the neoplastic cell could be followed morphologically on the level of ultrastructure; the appearance of an intermediate form between type I and II cells in the alveolar lining, the disappearance of type II cell in the lining due to replacement by the hyperplastic type II cells, which were transformed from the intermediate form, and the neoplastic change of the type II cells were assumed to be a sequence of the process. The intermediate form appears in certain pathological conditions, prior to cuboidal metaplasia of the attenuated alveolar epithelium (*15*, *32*). Embryologically, both type I and II cells are of the same origin, the entodermal epithelum. Some of the neoplastic cells, distinguished as poorly differentiated, were similar in ultrastructure to the immature alveolar epithelium of fetal lung (*15*). From these perspectives, the process of neoplastic alteration observed in the epithelium may be interpreted as a retrograde process of the normal differentiations of the alveolar epithelium. Kaufman et al. (*33*) have reported that Clara cells of the mouse bronchioles developed into neoplasms which could occur in a malignant form, after transplacental exposure to ethylnitrosurea. However, such a Clara cell tumor was not produced in our material.

Waxweiler and associates reported an increased number of deaths due to lung cancer among vinyl chloride workers (*9*). They observed 12 cases compared to the 7.7 cases expected. Eight of the twelve were examined histologically and were classified as undifferentiated large-cell carcinoma (five cases) and adenocarcinoma (three cases). Since these human lung cancers are of bronchogenic origin, it may be that target pulmonary cells in vinyl chloride carcinogenesis are different in human and mouse lung.

Neoplastic invasion and metastases were not found in our material. However, it is known that sometimes both induced and spontaneous alveologenic

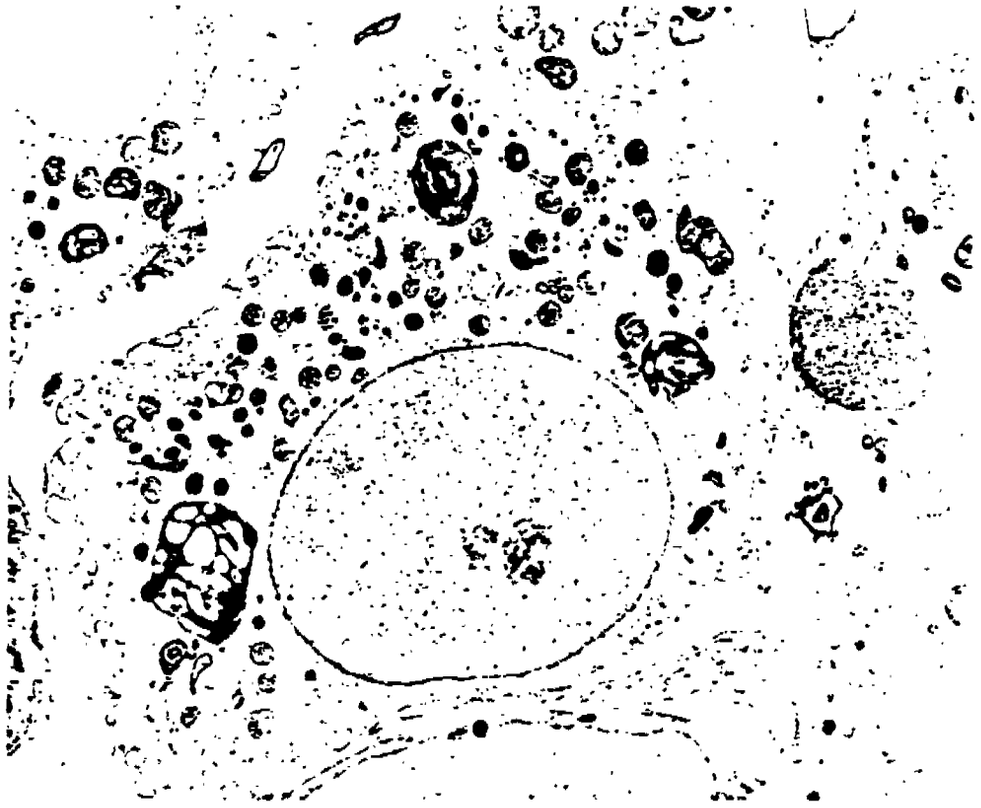

FIGURE 23. Alveolar macrophage including phagolysosomes and osmiophilic lamellar bodies. Group I: 2500 ppm; OsO_4; 5800 ×.

tumors (*22*, *34-37*) of mice show such changes and that the malignant transformation occurs with some delay after initiation of the tumor. In addition, transplantation of this tumor has been accomplished (*23*). Steward and associates (*22-24*) therefore dubbed it an "alveologenic tumor" or "alveologenic cancer." Maltoni and Lafemine (*13,14*) have found that some vinyl chloride-induced pulmonary tumors undergo transformation, although we did not observe this in our materials.

Alveologenic tumors may be considered unique in some ways, since it is possible to observe the process of malignant transformation sequentially from the precursor to the malignant cell via hyperplastic and benign neoplastic states.

Hepatic hemangiosarcoma is recognized as a characteristic malignant tumor related to vinyl chloride exposure. The tumor can be induced in a variety of experimental animals (mice, rats, and hamsters) (*13,14*), and histological features of the tumor are almost identical in humans and animals. In contrast, the intrapulmonary target cells of vinyl chloride oncogenesis may be different in humans and mice. Beyond these differences, moreover, the induction of alveologenic tumors in mice by vinyl chloride may be predictive or a risk of human bronchogenic cancer from the chemical. Consistent with this is the fact that various carcinogens, such as polycyclic aromatic hydrocarbons, nitrogen mustard and chromate compounds, are known to induce alveologenic tumors in mice, on one hand, and, on the other, to be associated with excess bronchogenic carcinoma among workers exposed to the carcinogens (*38*). It is noteworthy that a similar relation has been suggested for vinyl chloride.

Nonneoplastic pulmonary effects of vinyl chloride

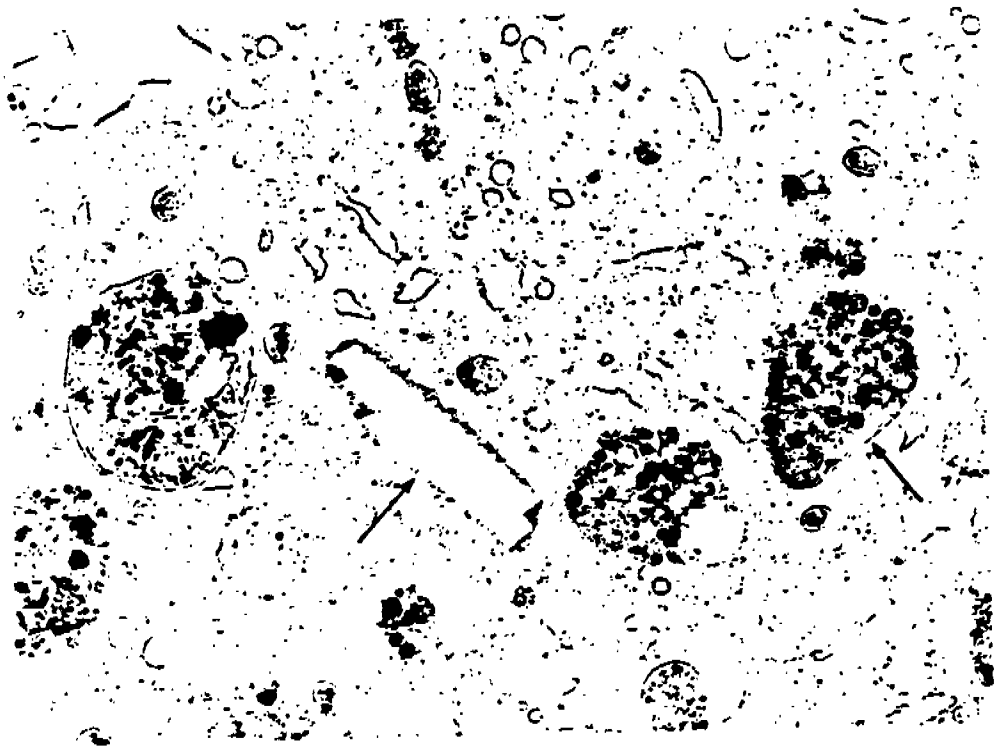

FIGURE 24. Part of a macrophage. Two cholesterol crystalloids are shown. Group III: 2500 ppm; OsO_4; 27,000×.

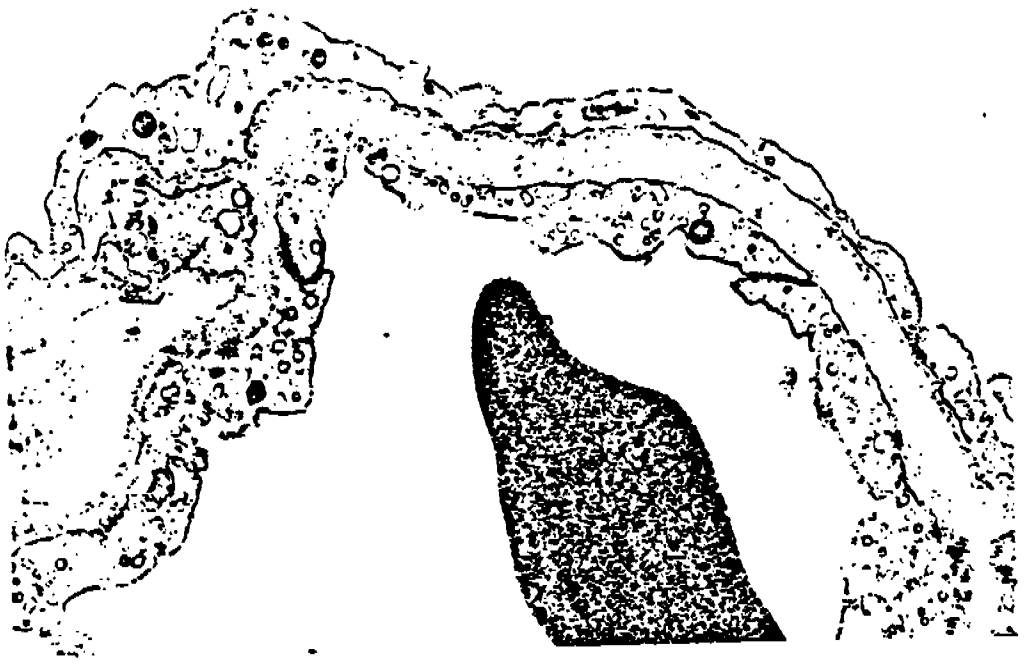

FIGURE 25. Thickened basement membrane of an alveolar capillary. Group I: 6000 ppm; OsO_4; 15,000×.

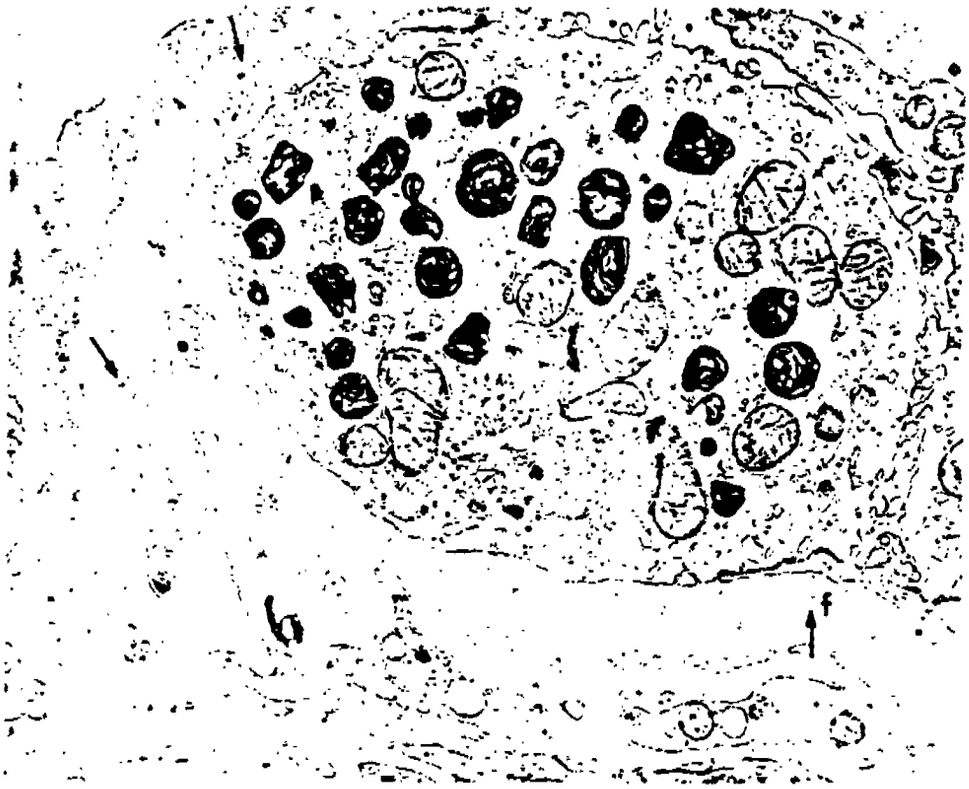

FIGURE 26. Cell debris of degenerated alveolar septal cells (arrows) and fibrillar appearance of a basement membrane (with an arrow) are illustrated. Group 1: 6000 ppm; OsO_4; 10,300×.

have been suggested by observations among workers in vinyl chloride polymerization plants. This suggestion was based on data obtained by chest x-ray examinations, pulmonary function tests, and sputum cytology studies among workers. Lilis and her associates (*18*) have found radiologic pulmonary changes, such as linear, reticular, and nodular opacities, in the lower and mid lung fields in a proportion of cases. They found that the prevalence of pulmonary changes increased with longer duration of exposure and that there was a significant association with peripheral circulatory abnormalities. However, pathological evaluation of these changes was not available. Miller et al. (*19*) have examined pulmonary function of 348 workers in a vinyl chloride polymerization plant. The major finding was diminution of air flow in 200 workers (57.7%). Again, no physicopathological relations were established. Maltoni and Lefemine (*14*) reported cytological studies of sputum in vinyl chloride and poly(vinyl chloride) workers. They found a significant increase in cellular changes of the bronchial epithelium; squamous metaplasia and squamous dysplasia were common among workers heavily exposed to vinyl chloride monomer.

Recently, McNamara and McLaughlin (*39*) have confirmed that a single 1-hr exposure to vinyl chloride in doses of 500 ppm or more induced pneumonitis in ICR mice and that aggravation of latent pulmonary changes, particularly bronchopneumonia, occurred in Fischer 344 rats.

Our present (*40*) study has shown that CD1 Charles River male mice exposed to vinyl chloride at a heavy dose (2500 and 6000 ppm), over relatively long term (5 and 6 months), obviously showed bronchiolo-alveolar changes. These alterations were recognized in almost all of the treated animals regardless of difference in doses (2500 and 6000 ppm), duration of exposure (5 and 6 months) and recovery time (2, 6 and 37 days).

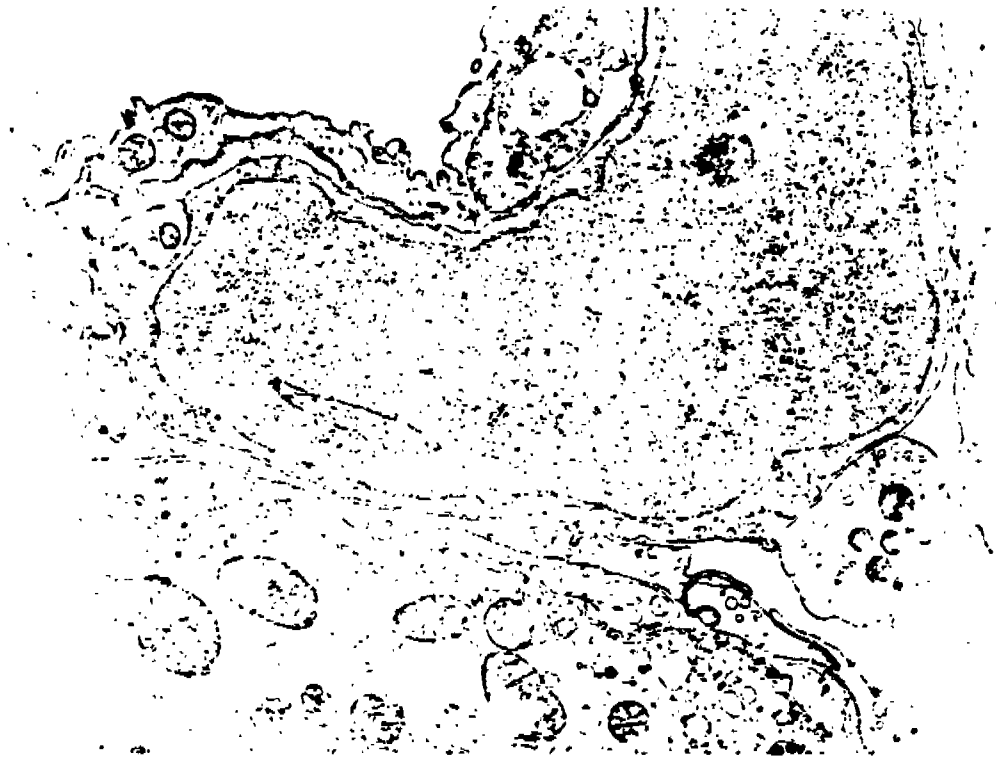

FIGURE 27. Large nucleus in the alveolar capillary endothelium. Group II: 2500 ppm; OsO_4; 7900×.

The types of pulmonary cells which were involved in the structural alterations varied. It was interesting that the ultrastructural changes seen in Clara cells and to some extent in the ciliated cells were similar to those of hepatic cells of animals exposed to vinyl chloride; cellular hypertrophy and proliferation of the endoplasmic reticulum were common responses, seen in both the bronchiolar epithelium and the hepatic cells. The endoplasmic reticulum of the hepatic cells has been suggested as the site where vinyl chloride is metabolized, to be transformed into a chemically reactive metabolite which is the ultimate carcinogen (*36*, *41-45*). Although it has not been shown that lung has the capacity to metabolize the chemical to produce the carcinogen; if it were so, the bronchiolar cells, particularly Clara cells, may provide this mechanism: the cells are normally equipped with well-developed smooth-surfaced endoplasmic reticula and the organelles have shown a proliferative response after mice are exposed to vinyl chloride.

The fact (*46*) that vinyl chloride has a tendency to be soluble in lipid substances suggests that lipid-rich pulmonary cells, such as type II cells (rich in osmiophilic lamellar bodies) and alveolar septal cells (containing lipid granules) may bind to vinyl chloride. If this assumption is correct, at least some of ultrastructural alterations seen in those cells might result from this mechanism. Both type II cells and Clara cells have been known as surfactant factor-producing cells (*47*). Hyperproduction of the surfactant factor was suggested, since hyperplasia of those cells was commonly seen in our material. Osmiophilic lamellar bodies and cholesterol crystalloids, which were seen in alveolar macrophages, may represent lipid substances bound to vinyl chloride. They may be removed from lung as part of the clearance mechanism for vinyl chloride. It is well accepted that mesenchymal elements such as bone, connective tissue, and blood vessels are involved in responses to vinyl chloride in various organs (*36*, *48-50*). Above all, malignant transformation of the blood capillary endothelium, induction of hemangioendothelioma in liver, and the subcutaneous con-

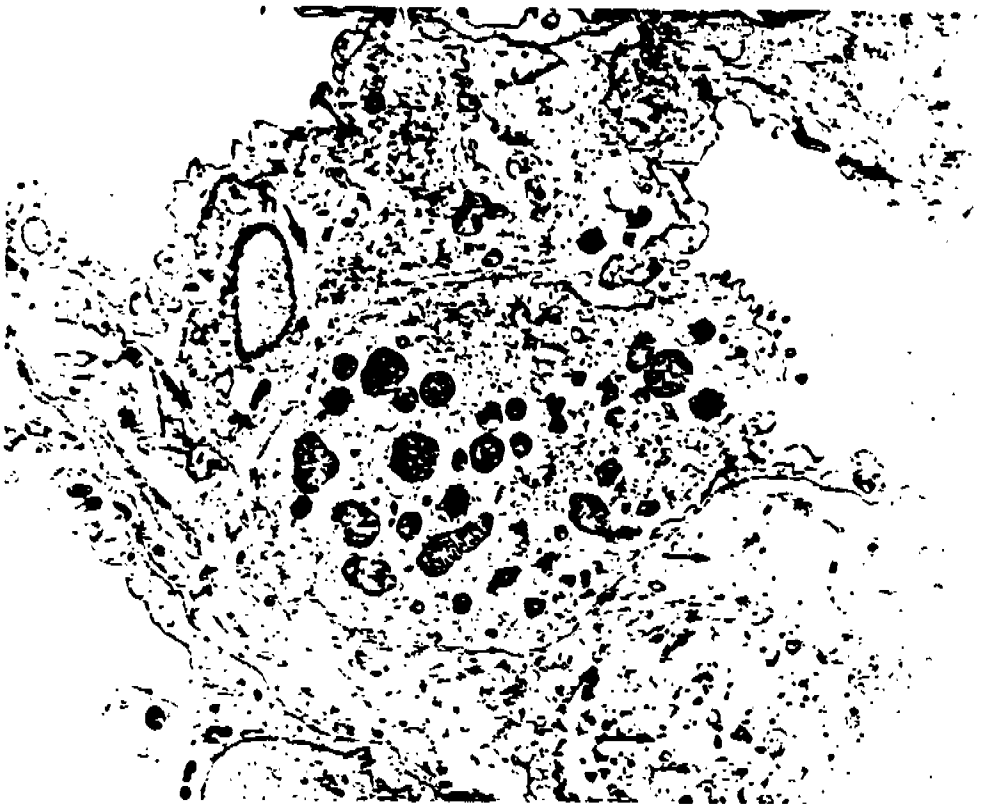

FIGURE 28. Degenerative alterations of the alveolar septal cells (arrows) and a hypertrophic septal cell (h with an arrow). Group I; 6000 ppm; OsO_4; 6300×.

nective tissue, lung, and adipose tissue have been well documented (*10*, *13*, *14*, *36*).

It is reasonable to assume that the alveolar capillary endothelium and the septal cell suffered toxic effects by vinyl chloride, since these cells are part of the mesenchymal elements in lung, sites of uptake and excretion of the chemical and its metabolites (*51*, *52*).

A dose-response relationship has been suggested in the production of alveolongenic tumors by vinyl chloride. The same relationship has been seen in occurrence of alveolitis (mouse) and bronchopneumonia (rats) with the chemical (*39*). A delayed appearance of these inflammatory changes after periods of recovery, following exposure to the chemical, has been postulated. A threshold dose for the induction of the nonneoplastic pulmonary lesions which are reported here has not been established.

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Effects of Aging on the Induction of Angiosarcoma

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Adult, Sprague-Dawley albino rats of four different ages (6, 18, 32 and 52 weeks) were exposed to 940 ppm vinyl chloride by inhalation for 24 weeks, 5 days/week, 7 hr/day. In each age group, there were 110 to 128 males and the same number of females. The similarly housed control group, which was not exposed to vinyl chloride, consisted of the same number of males and females in each age group. All animals that died spontaneously, or were sacrificed moribund, or were killed at scheduled times (3, 6 and 9 months after initial exposure) were autopsied. All organs were examined grossly, and several tissues from each animal were examined microscopically.

The older the rats were when they were first exposed, the greater the incidence of angiosarcomas. The incidences of angiosarcomas in the four age groups (from youngest to oldest) in the exposed males in the nonscheduled sacrifice groups were: 0/37 (0%); 0/44 (0%); 3/45 (6.7%); and 13/55 (24%). Similarly, for the females, these incidences were: 2/38 (5.3%); 7/47 (15%); 23/49 (47%); and 11/54 (20%). Most of the angiosarcomas were highly anaplastic, primary tumors in the livers that metastasized to the lungs. Only one angiosarcoma was seen in all the control rats; that occurred in subcutaneous tissue.

This study demonstrated that older adult animals and females are more susceptible to the angiosarcoma-inducing effects of vinyl chloride than young adult animals and males, respectively.

Introduction

Several epidemiological studies performed on occupational groups exposed to carcinogens have shown greater increased risks of cancer with increasing exposure durations. In addition, latent periods (time between initial exposure and increased risk of cancer) in these groups are commonly 10-30 years. These findings have been commonly attributed to greater total doses in those exposed for longer periods of time. However, there is one factor other than the total dose which could be responsible for contributing to the increased risk. That factor is aging. It is equally reasonable to postulate that older adults are more susceptible to the carcinogenic effect of some chemicals than young adults. That is, for the same dose of carcinogen, the incidence of cancer might be higher and the latent period shorter in older adults than in younger adults.

It has been suggested that only older people should work in carcinogenic environments, since the commonly observed latent periods for cancer induction would exceed their life expectancies. If, however, the latent periods were shorter in older individuals, that approach would be counterproductive.

The purpose of this study was to determine whether or not older adult rats were more or less susceptible to the angiosarcoma-inducing effects of vinyl chloride than young adult rats. This experiment was not designed to test the susceptibility of sexually immature animals or to determine the lifetime effect of relatively short-term exposures.

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Materials and Methods

A total of 473 male and 478 female Sprague-Dawley rats were exposed to 948 ppm of vinyl chloride monomer in a single 14 × 13 × 10 ft stainless steel walk-in chamber with a diffusion-screen false ceiling. Exposures were for 7 hr/day, 5 days/week for a mean duration of 24.5 weeks. An equal number of control male and female rats, kept in a similar exposure chamber, were exposed to conditioned outside air only. Air flow into the chambers was maintained at 300 ft^3/min. Air temperatures were kept at 75 ± 3°F. The concentration of vinyl chloride in the chambers was initially monitored by gas chromatography and later with an ultraviolet photoionization analyzer, 7 times/day on each day of exposure.

The animals were individually housed in suspended stainless steel wire-mesh cages equipped with automatic watering taps. Water was provided *ad libitum*, but food (Wayne Lab Blox) was removed during exposures.

Four differently aged sets of rats of both sexes were used in both the exposed and control groups. Their ages at initiation of exposure were 6, 17-18, 32-33 and 51-53 weeks. The numbers in each group and their disposition appear in Tables 1 and 2.

Because of the large number of animals, it was not practical to start exposures on all rats simultaneously. Consequently, the controls and exposed rats were divided into five squads, with proportionate representation of each age group and sex in each squad. Rats in each squad were assigned to the chambers over a 2-week period. Assignments of all rats to the chambers were completed throughout a 10-week period.

The original plan was to expose the rats for 12 months, but because of an epidemic of pneumonia among the exposed animals during the 28th week the exposures were terminated 29 weeks after initiating exposures. As the results will show, this exposure period was adequate to demonstrate the differences in effects between groups.

The experiment consisted of two subsets. Animals scheduled for sacrifice at 3, 6 and 9 months constituted one subset, and animals that died e

Table 1. Number and disposition of rats exposed to vinyl chloride.

Group	Age at first exposure, weeks	Mean body weights at first exposure, g	Sex	Initial number of rats	Number of deaths and moribund sacrificed	Number at final sacrificed	Number sacrificed at 3, 6 and 9 months	Total number of rats autopsied
2	6	228	M	110	36	6	66	108
2	6	167	F	110	16	23	70	109
4	17–18	541	M	119	39	13	67	119
4	17–18	304	F	120	33	16	70	119
6	32–33	693	M	116	43	4	69	116
6	32–33	350	F	120	45	6	69	120
8	51–53	739	M	128	60	0	67	127
8	51–53	375	F	128	52	6	70	128
Totals			M	473	178	23	269	470
			F	478	146	51	279	476

Table 2. Number and disposition of control rats.

Group	Age at first exposure, weeks	Mean body weights at first exposure, g	Sex	Initial number of rats	Number of deaths and moribund sacrificed	Number at final sacrificed	Number sacrificed at 3, 6 and 9 months	Total number of rats autopsied
1	6	230	M	110	9	6	69	84
1	6	173	F	110	6	23	71	100
3	17–18	550	M	119	11	13	70	94
3	17–18	304	F	120	9	16	70	95
5	32–33	701	M	115	12	4	70	86
5	32–33	353	F	120	10	6	69	85
7	51–53	772	M	128	23	0	70	93
7	51–53	385	F	127	27	6	69	102
Totals			M	472	55	23	279	357
			F	477	52	51	279	382

to group 6. The incidence in group 8, however, is lower than in group 6 and closer to that in group 4. The first angiosarcoma, however, was seen in group 8, the oldest group.

In comparing the males and females, it can be seen in Table 3 that the first angiosarcoma appeared earlier in the females in each group and that the incidence was higher in females for each age group, with the exception of group 8, the oldest age category. Since the mean survival times and exposure durations for males and females in groups 4 and 6 were similar, the differences in incidence can be attributed to sex.

The incidences of angiosarcomas in the interim sacrifice, nonscheduled sacrifice and combined groups appear in Table 4. The females sacrificed at 6 and 9 months had a much lower incidence of angiosarcomas than those in the nonscheduled sacrifice groups, whereas, the difference in incidence between similar groups of males was not as great.

The incidences of angiosarcomas in the different age groups were compared using Fisher's exact test. Because of multiple comparisons, the α-level for each individual comparison needed to be ≤ 0.01 for the incidences to be significantly different with an overall $\alpha = 0.05$. In the nonscheduled sacrifice groups, the incidence of angiosarcomas in group 8 males was significantly different from those incidences in group 4 and group 2 males; the incidence of angiosarcomas in group 6 females was significantly different from those in group 4, group 2 and group 8 females. The same comparisons between the same groups in the combined nonscheduled and interim sacrifice groups were also found to be significant.

The effect of age on angiosarcoma incidence in males was examined using the method of weighted least squares regression. A quadratic equation was shown to be a good model for predicting angiosarcoma incidence as a function of age in weeks. The percent of variability in incidence rates (R^2) explained by this model was: $R^2 = 99.96\%$ for nonscheduled sacrifice groups and $R^2 = 99.85\%$ for combined nonscheduled and interim sacrifice groups (Fig. 1). Both of these models were statistically significant ($p = 0.02$ and $p = 0.001$, respectively).

The fact that most of the angiosarcomas in the females occurred in the animals that died spontaneously suggested that their deaths might be directly related to the angiosarcomas. A comparison of mean survival times, however, revealed that animals with angiosarcomas survived as long and frequently longer than other animals in their respective groups (Table 5). At 4-week intervals, the accumulative incidence of angiosarcomas was tabulated (Table 6) for exposed females. Although the incidence of angiosarcomas at all intervals in groups 2 and 6 is about the same, in group 8 the incidence decreases with time, and in group 4 it increases with time.

Although a few brain tumors, mammary adenocarcinomas and zymbal gland tumors occurred in exposed animals, their incidences did not appear to be significantly different from those of the controls.

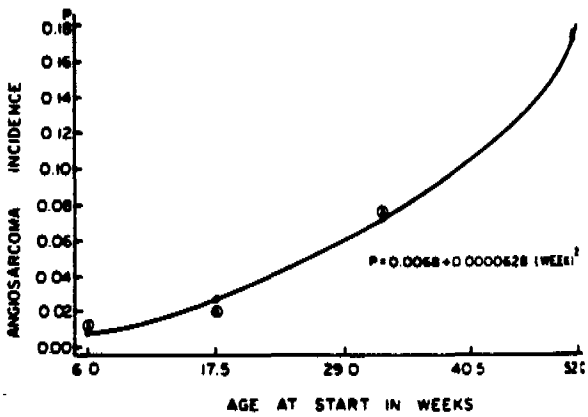

FIGURE 1. Angiosarcoma incidence: males (combined deaths, interim sacrifices and final sacrifice). Curve fitted by regression methods showing a significant quadratic fit. $R^2 = 99.85\%$.

Table 4. Incidence of angiosarcomas in exposed rats, nonscheduled (NS), interim sacrifice (IS) and combined groups.

Group	Sex	NS	IS	Combined NS + IS
2	M	0/37	1/46 (2.2%)	1/83 (1.2%)
2	F	2/38 (5.3%)	0/50	2/88 (2.3%)
4	M	0/44	2/47 (4.3%)	2/91 (2.2%)
4	F	7/47 (15%)	0/50	7/97 (7.2%)
6	M	3/45 (6.7%)	4/49 (8.2%)	7/94 (7.4%)
6	F	23/49 (47%)	4/49 (8.2%)	27/98 (28%)
8	M	13/55 (24%)	5/47 (11%)	18/102 (18%)
8	F	11/54 (20%)	3/50 (6%)	14/104 (13%)

Table 5. Mean survival of exposed rats in nonscheduled sacrifice groups.

	Mean survival, weeks			
	Males		Females	
Group	All rats	Rats with angiosarcomas	All rats	Rats with angiosarcomas
2	30.6	—	37.5	36.5
4	31.6	—	33.8	38.9
6	30.5	35.3	30.0	32.0
8	28.9	31.2	30.2	27.3

Table 6. Accumulative angiosarcoma incidence in exposed females, nonscheduled sacrifice group.

Interval, weeks	Angiosarcoma incidence			
	Group 2	Group 4	Group 6	Group 8
16–19	0/0	0/1	0/2	2/5 (40%)
20–23	0/1	0/5	3/7 (43%)	3/9 (33%)
24–27[a]	0/6	0/14	8/23 (35%)	6/22 (27%)
28–31	1/13 (7.7%)	1/22 (4.5%)	12/31 (39%)	8/34 (24%)
32–35	1/14 (7.1%)	2/28 (7.1%)	14/37 (38%)	10/40 (25%)
36–39	1/15 (6.7%)	3/29 (10%)	18/41 (44%)	11/46 (24%)
40–43	2/38 (5.3%)	7/47 (15%)	23/49 (47%)	11/54 (20%)

[a]Exposures terminated.

Discussion

The results clearly indicate that the incidence of angiosarcomas is higher and these tumors occur earlier in older rats of both sexes. In addition, the incidence of angiosarcomas is generally higher and these tumors occur earlier in female rats than in male rats. Therefore, it can be concluded that older rats are more susceptible to the angiosarcoma-inducing effect of vinyl chloride than are young adult rats and that female rats are more susceptible than males.

The authors have been unable to find comparable studies in the scientific literature. However, Maltoni (*1*) reported in a summary article of his research with vinyl chloride that exposure duration was an important factor in determining angiosarcoma incidence. In that article, he reported that rats exposed to 10,000 ppm vinyl chloride, 4 hr/day, 5 days/week for 17 weeks did not develop liver angiosarcomas within a 155-week period, whereas, 13/60 (22%) of rats exposed to 6,000 ppm vinyl chloride 4 hr/day, 5 days/week for 52 weeks (and held for a 155-week period) developed liver angiosarcomas. In the authors' opinion, the difference in total dose between the two groups was not sufficient to account for the large difference in the angiosarcoma incidences, and that the major factor, therefore, was probably the difference in ages while they were being exposed.

There is suggestive evidence in the literature that older adult humans might be more susceptible than young adults to the carcinogenic effects of Thorotrast. Curry et al. (*2*) stated in their article that "although the latent period between thorium injection and liver malignancy has varied from 3 years to 35 years, all of these 123 cases occurred in patients between 49 and 55 years of age." There is suggestive evidence that older beryllium workers are more susceptible to the carcinogenic effects of beryllium. The data of Mancuso's study (*3*) of beryllium extraction workers show an extremely high risk for lung cancer in workers between the ages of 38 and 65 who were exposed for relatively short periods of time. Studies specifically designed to test this theory in humans, as well as in animals, with other compounds are needed.

If these findings can be reproduced in animals with a wide variety of classes of compounds, then it would be justifiable to modify chronic bioassay experiments by utilizing older animals at the beginning of the studies and shortening the durations of the experiments by 6-12 months. In many cases, this should result in decreasing costs by 20-30% and, thereby, permit a greater number of chemicals to be tested.

The observation in this study that the accumulative incidence of angiosarcomas in the 6-week-old group of rats did not increase with time after discontinuation of the exposures suggests that the carcinogen is metabolized and inhibited or excreted before most of the animals became susceptible. Whether or not these animals would have exhibited a higher incidence of angiosarcoma at some later time is not known. However, as mentioned above, Maltoni (*1*) was unable to induce liver angiosarcomas in young adult rats exposed to 10,000 ppm vinyl chloride 4 hr/day, 5 days/week for 17 weeks and held for a lifetime. The total dose in his experiment was 10,000 ppm × 340 hr (3,400,000 ppm-hr). The total dose in our experiment was 948 ppm × 858 hr (813,384 ppm-hr). Therefore, it is unlikely that the youngest groups in our experiment would have developed a higher incidence of angiosarcomas if they were held for a lifetime.

The results of this study also suggest that older people should not be preferentially placed in carcinogenic working environments.

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Discussion

In this animal model, ingested ethanol is a cocarcinogen in relation to VC induction of tumors in the liver. The incidence of angiosarcoma in VC-ethanol dosed rats (50%) was more than double that resulting from VC exposure alone (23%). VC-ethanol also induced a greater number of hepatocellular carcinomas (60%) than occurred in VC-treated animals (44%). The first deaths from angiosarcoma and carcinoma of the liver occurred earlier in VC-ethanol dosed animals indicating a shortened latent period.

The Sprague-Dawley rats used by Maltoni and Lefemine in 1974 (*4*) were more resistant to the effects of VC on the liver than the animals used in this VC-ethanol study. Following a year-long exposure to 500 ppm VC 4 hr/day, 5 days/week and observation from first VC exposure for 135 weeks, only 7 of 59 rats (12%) were reported to have angiosarcoma in the liver (*4*). Angiosarcomas developed in other sites such as in subcutaneous tissue, the abdominal cavity, neck, lung and uterus. Hepatocellular carcinomas were not reported. Zymbal gland carcinomas were seen in 8% of Maltoni's VC exposed animals; none were seen in this study.

Ethanol has been reported to potentiate the toxicity of other halogenated hydrocarbons. In whole animals, a variety of alcohols increased the hepatotoxicity of carbon tetrachloride (CCl_4) when ingested 16 to 18 hr prior to inhalation of CCl_4 (*7*). Following acute exposures, CCl_4 toxicity was enhanced by ethanol as judged by the elevation of serum glutamic-oxaloacetic transaminase levels. In similar studies, trichloroethylene (TCE) or 1,1,1-trichloroethane hepatotoxicity was potentiated by ethanol, but only at high concentrations of inhaled TCE or 1,1,1-trichloroethane (*8*).

Ethanol potentiation of VC carcinogenesis in the liver is probably due to an effect on VC metabolism. Inhibition of VC uptake (*5*) and metabolism (*6*) by ethanol suggests there is a shared step in the oxidation of the two toxic agents. The most like candidates for competition are acetaldehyde an chloroacetaldehyde. The normal substrate, acet dehyde, would be oxidized preferentially result in an increased half-life of chloroacetaldehyde.

Toxic manifestations of exposures were fou which were not related to the neoplastic respons Many animals died because of neoplasms or secon ary changes caused by tumors; however, dea directly attributable to toxicity was not seen. T toxicity of ethanol will be discussed in anoth publication.

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Cancer Induction Following Single and Multiple Exposures to a Constant Amount of Vinyl Chloride Monomer

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Vinyl chloride monomer (VCM), already identified as a human animal carcinogen, was selected as a model agent to explore an area of concern for single and intermittent low level exposure. In traditional cancer bioassay, animals are repeatedly exposed over their lifespan to a dose of suspected chemical.

In the current studies rats and mice were exposed in an inhalation chamber to single one-hour doses of VCM ranging from 50 to 50,000 ppm.

A second group was given 10 one-hour exposures to 500 ppm or 100 one-hour exposures to 50 ppm of the same chemical. All animals were then observed for the remainder of their lives, generally 18-24 months. Moribund animals were euthanized, and survivors were sacrificed on schedule and their tissues examined for pathological changes. Specifically, the oncogenic study demonstrated dose related effects for single one-hour exposure of VCM at high levels, i.e., 5,000 and 50,000 ppm. These concentrations increased the incidence of pulmonary adenomas and carcinomas in mice.

Repeated exposure of A/J mice to the same chemical at 500 ppm × 10 one-hour exposures also increased the incidence of pulmonary adenomas and carcinomas which are considered highly significant ($p = 0.001$) when compared to match controls. At the lower dose of 50 ppm × 100 one-hour exposure, no significant increase in tumors was observed. Rats exposed to identical concentrations of VCM failed to elicit a tumorigenic response.

Introduction

In the last decade there have been concerted efforts by industry and the Federal government to identify carcinogenic substances in the workplace. However, carcinogens are not limited to occupational exposures. Many of these same chemicals are also used in the formulation of household products. Therefore, consumers in all walks of life may be exposed to suspect chemicals in their homes without their knowledge. This type of consumer exposure is usually brief and at very low levels. The suspect chemical may be modified in the process of manufacturing the consumer product, i.e., as in the case of polymerization of vinyl chloride (VC) to poly(vinyl chloride) (PVC), or trace quantities of the unreacted monomer may be present in the product which may be leached out under normal conditions of use. There are other situations wherein a chemical like VC is used because it is chemically inert, for example as a propellant, and as an unreacted gaseous chemical it could become a potential health problem.

While regulatory bodies try to establish safe exposure levels and industry attempts to control exposures in the production facilities, no such control measures would be of practical value in the

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home environment. Another complication associated with making value judgements about potential risks of consumer exposure is that traditional cancer bioassays involve daily exposures to the test chemical over the animal's lifetime. There were no comparable studies on short-term exposure to a carcinogen like VC followed by a lifetime monitoring for toxicological symptoms with complete histopathological examination at death. Therefore, our approach was to explore what happens to laboratory animals following brief or intermittent exposure to a known carcinogen like VC. Vinyl chloride appeared to be the ideal chemical to use in these experiments because of its widespread use in industrial and consumer products. Furthermore, the relationship for inducing unique pathological lesions, i.e., angiosarcoma of the liver for high exposure levels in animal and man was already established by Viola et al. (*1*) Maltoni (*2*) and Creech et al. (*3*).

Quite apart from the need to establish a bench mark for acute short-term intermittent exposures to a carcinogen, such as vinyl chloride, was the question of "threshold effects" or "safety factor" for low level exposures. In setting up our experiments we were mindful of the many factors that could have an impact on the study design. Some of the more important factors are that (1) the substance may not reach a susceptible cell; (2) the substance may make noncarcinogenic biochemical combination with the cell; (3) the "initiated" lesion may not receive adequate "promotion"; (4) host factors may be unfavorable to carcinogenesis; (5) biochemical repair of the DNA lesion may occur; (6) morphological regression of tumorigenic proliferation may occur; (7) carcinogenic cells may be destroyed by the body's immune system. These items need to be addressed in any conventional cancer bioassay but are even more critical in searching for noncarcinogenic exposure levels.

Experimental Conditions

Two strains of rats, Fischer 344 (Charles River Laboratory, Wilmington, Mass.) and Sprague-Dawley/Wistar (Chemical Systems Lab, Edgewood, Md.), and two strains of mice, A/J (Jackson Lab, Bar Harbor, Maine) and ICR (Charles River Laboratory) were treated in single or multiple intermittent inhalation exposures to VC. The chambers were Rochester type, stainless steel, 1000 liter, constructed to provide laminar air flow and insure uniform exposures to VC to test animals.

Chamber concentrations were established by proportioning the amounts of VC being dispersed with the air flow through the chamber. Airflow through the chamber was created by a blower motor located in the flow pipe on the exhaust side of the chamber. A negative pressure was maintained in the chamber at all times when operational and the exhaust gas completely filtered (M6A1 gas-particulate filter) before discharge to the environment. The concentration of gas in the inhalation chamber was monitored by using a Hewlett-Packard 5830A gas chromatograph with a dual flame ionization detector.

Exposure Procedures for VC Lifetime Cancer Studies

Male and female rats and Fischer 344 and ICR mice were totally exposed for 1 hr to 50, 500, 5000 and 50,000 ppm VC. Fischer rats and A/J mice equally divided by sex received ten 1 hr exposures to 500 ppm VC (1 hr/day, 5 days/wk for 2 weeks) or 100 1-hr exposures to 50 ppm (1 hr/day, 5 days/wk for 20 weeks). Male and female parents (Sprague-Dawley/Wistar) rats (obtained from Veterinary Medical Division, Edgewood Arsenal, Md.) from the reproduction study were also maintained and observed for 24 months post exposure to 50 to 500 ppm VC 1 hr per day, 5 days per week for 10 weeks (49 exposures).

Following exposure, all animals were air washed until the chamber concentration was less than 1 ppm VC. Animals of the same sex were segregated in stainless steel cages with a maximum number of 5 rats in each compartment and placed in the animal holding area. Mice were similarly treated, but smaller cages were used and the number of mice per compartment was limited to two. The animals were observed twice daily for general health, sores, masses, alertness, activity or mortality. All animals were weighed weekly for the first 8 weeks post exposure and monthly thereafter. The exposure schedules for the studies described above are shown in Tables 1-3. No blood chemistries or hematology studies were performed.

Pathology

Gross and Light Microscopy. A complete gross and microscropic examination was performed on most control and exposed animals which died or were sacrificed. Autolysis precluded such examination in a few cases.

All rodents were to be serially sacrificed at 8, 16 and 24 months post exposure. However, the life span of the mice forced some changes in the later times of sacrifice and termination of mouse experiments. For single exposure the planned 16 and 24 month sacrifice were replaced by 18 month sacrifices.

Table 1. Single exposure schedule of animals to VC.

Species	Sex	Dose, ppm	Exposure date AM	Exposure date PM	Exposure group size	Age at time of exposure, weeks
Fischer 344 rat	M	50	3/4/75		90	15
		500	3/11/75		90	16
		5000	3/18/75		85	17
		50000	3/25/75		90	18
	F	50		3/4/75	90	15
		500		3/11/75	100	16
		5000		3/18/75	95	17
		50000		3/25/75	88	18
ICR mouse	M	50	3/4/75		90	15
		500	3/11/75		90	16
		5000	3/18/75		90	17
		50000	3/25/75		90	18
	F	50		3/4/75	90	15
		500		3/11/75	90	16
		5000		3/18/75	90	17
		50000		3/25/75	90	18
Rat	M	Neg/Cont.			92	15–18
	F	Neg/Cont.			79	15–18
Mouse	M	Neg/Cont.			82	15–18
	F	Neg/Cont.			88	15–18

Table 2. Exposure schedule for animals exposed repeatedly to VC.

Species	Sex	Dose, ppm	Exposure periods, days	Exposure dates From	Exposure dates To	Exposure group size Start	Exposure group size End	Age, weeks Start	Age, weeks End
Fischer rat	M	50	100	8/27/75	1/26/76	90	86	21	41
		500	10	7/7/75	7/28/75	90	90	14	16
	F	50	100	8/27/75	1/26/76	90	87	21	41
		500	10	7/7/75	7/18/75	90	90	14	16
A J mouse	M	50	100	7/7/75	7/18/75	90	87	15	35
		500	10	8/27/75	1/26/76	90	90	8	10
	F	50	100	7/7/75	7/18/75	90	88	15	35
		500	10	8/27/75	1/26/76	90	90	8	10
Fischer rat	M	Neg.	100(c)[a]	—		50	50	21	41
		Control	10(c)	—		50	50	14	16
	F	Neg.	100(c)	—		50	47	21	41
		Control	10(c)	—		50	50	14	16
A J mouse	M	Neg.	100(c)	—		40	39	15	35
		Control	10(c)	—		50	50	8	10
	F	Neg.	100(c)			50	50	15	35
		Control	10(c)	—		50	50	8	10

[a](c) denotes control for corresponding dose above.

Table 3. VC multigeneration study, F_0 parents.[a]

Group	Compound	Dose[b]	Number of males	Number of females
I	Air	Control	25	25
II	VC	Low dose (50 ppm)	25	25
III	VC	High dose (500 ppm)	25	25

[a]Sprague-Dawley/Wistar rats.
[b]Exposure to 50 or 500 PPM of VC 1 hr/day, 5 days/week for 10 weeks (49 exposures) before mating.

This observation period was the minimum suggested by the National Cancer Institute (4) for cancer bioassays in small animals. For multiple dose studies in mice the final sacrifice was at 20 months rather than 24 months. The change was made in consideration of the risk of animal loss through death and possible cannibalism. Tissues examined were lung, trachea, heart, liver, stomach, small intestines, spleen, kidney, bladder, bone marrow (sternum), adrenals, pancreas, duodenum, brain, eye, zymbal gland, ear, nose, muscle and bone (femur).

Electron Microscopic Studies. Groups of five male and five female Fisher (344) rats from each of the single 1-hr exposure studies (50, 500, 5000 and 50,000 ppm) with equal numbers of their corresponding control groups were sacrificed at 8, 16, 24 months.

Groups of five male and five female Fischer (344) rats from the multiple 1-hr exposure studies at 50 and 500 ppm VC along with equal numbers of control animals were sacrificed at 16 and 24 months.

Results

Toxicity During Exposure

Rats and mice exposed for 1 hr to concentrations of VC ranging from 50 to 50,000 ppm or for repeated exposures, i.e., 50 ppm or 500 ppm, produced no remarkable signs of toxicity with the exception of mice at the 50,000 ppm level. At 50,000 ppm VC 50% of the male mice exhibited hyperventilation at 45 minutes together with twitching and possible ataxia. Female mice became hyperactive after 40 min exposure, and respiratory difficulty and ataxia was observed in approximately 25% of the female mice after 55 min. Upon removal from the test atmosphere, all animals recovered to normal appearance within 24 hr. After exposure there were no consistent or dose-related differences between control and exposed (single or multiple) mice or rats in death rate, toxic signs or change in body weight.

Gross Pathology

There was a suggestion of higher frequency of masses in the lungs and livers of mice exposed once or repeatedly to VC at the higher dose levels, i.e. 500, 5000 or 50,000 ppm.

Histological Examination of ICR Mice: Single Exposure

Changes ascribable to vinyl chloride were apparent primarily in the lungs with the induction of pulmonary adenomas and pneumonitis. Pneumonitis was evident in all animal groups which were exposed to VC at 500 ppm and above.

The development of bronchio-alveolar adenomas increased with exposure to higher dose levels of VC. Tables 4 and 5 summarize the significant histological changes observed in ICR mice at 8 and 18 months following single exposure to graded doses of VC.

Mice were more susceptible than rats to pneumonitis following exposure to VC. The effect however was not incremental with dose level. The incidence of pneumonitis, adenoma and carcinoma in ICR mice following single exposure is presented in Table 6. No correlation was observed between the incidence of pneumonitis and adenoma or pneumonitis and carcinoma. However, males seemed more prone to the induction of pneumonitis, particularly at 50,000 ppm, i.e., 34% M vs. 13% F. At 5000 ppm and below, male and females were about equally sensitive.

There was an increase in bronchio-alveolar adenomas with exposure to higher doses of VC and the condition manifests itself more frequently in males, i.e., at 50,000 ppm: 51% M vs. 18% F. This trend continued at the 5000 ppm: 22% M vs. 13% F. Males and females were equally susceptible at dose level 500 ppm and below. The upper respiratory tract (nasal turbinates) and trachea revealed no unusual changes specifically attributable to VC.

Table 4. Histological examination of ICR mice at 8 and 18 months following a single (1-hr) exposure to vinyl chloride monomer.

Vinyl chloride concentration, ppm	Histological changes attributable to vinyl chloride	
	Induction of pulmonary adenomas	Progression to carcinoma
50,000[a]	45/137 (33.3%)	3/137 (2.2%)
5,000[a]	24/143 (16.8%)	1/143 (0.7%)
500[a]	18/139 (12.9%)	1/139 (0.7%)
50	14/139 (10.1%)	0/139 (0%)
0 (control)	12/120 (10.0%)	0/120 (0%)

[a]Pneumonitis was evident in all animal groups which were exposed to VCM at doses of 500 ppm or more.

Histological Examination of A/J Mice at 8, 16 and 20 Months Following Multiple Exposure

500 ppm, 10 days, 1 hr. Changes ascribable to VC were apparent in the lung only, primarily the induction of pulmonary adenomas. Bronchio-alveolar adenomas were induced in the test group with approximately equal frequency in males and females, i.e., 73.7% M vs. 75.6% F, respectively. The increase in incidence as shown in Table 7 for the test group over that of the controls was substantial, by a factor of 2.2, i.e., controls, 34.4%; 500 ppm; 74.7.

Progression to malignancy (carcinoma) in the test group vs. controls was also highly significant: controls, 3/90 or 3.3%; 500 ppm, 22/166 or 13.3%. In animals scheduled for evaluation (survivors) pulmonary adenomas were observed as early as 8 months post exposure in 60% of males and females; at 20 months, 75% of animals were affected.

The incidence of pneumonitis, adenoma and carcinoma in A/J mice following multiple exposure to VC is presented in Table 8. Pneumonitis was observed more frequently in the control animal than

Table 5. Overall summary: incidence of nonneoplastic changes and histologically proven neoplasms within the liver and lungs of ICR Swiss mice exposed to vinyl chloride in single inhalation exposures.

	Incidence of response for various vinyl chloride concns									
	0		50,000 ppm		5,000 ppm		500 ppm		50 ppm	
Tissue response	M (62)[a]	F (77)[a]	M (74)[a]	F (82)[a]	M (76)[a]	F (82)[a]	M (72)[a]	F (75)[a]	M (81)[a]	F (80)[a]
Liver (number evaluated)	50	75	63	78	68	76	67	72	64	68
Hepatic cell necrosis	2	10	3	5	5	7	4	6	2	3
Hepatic cell vacuolation (lipidosis)	2	11		4	2	12	3	5	1	4
Hepatic cell hypertrophy	1		2	8		8	4	13	1	
Hepatic cell hyperplasia					4	4				
Angiectasis				1		4				
Sinusoidal reticulosis				5						
Hepatic cell adenoma	2		1			1			2	
Hepatic cell carcinoma	2		4	1	6	1	9		2	
Hemangioma					1					
Hemangiosarcoma	1									
Lung (number evaluated)	50	70	61	76	65	78	66	73	71	68
Pneumonitis	1	6	21	10	13	17	19	15	4	7
Bronchio-alveolar adenoma	4	8	31	14	14	10	8	10	8	6
Bronchio-alveolar carcinoma			1	2	1			1		

[a]Numbers in parentheses denote animals per group. Total includes animals from scheduled sacrificed (8 and month periods) and spontaneous deaths.

Table 6. Single inhalation exposure of ICR mice to vinyl chloride monomer.

VC exposure concn, ppm	Group	Lung tissue: incidence of response/number evaluated[a]		
		Pneumonitis	Adenoma	Carcinoma
0 (control)	Male	1/50 (2%)	4/50 (8%)	0/50 (0%)
	Female	6/70 (9%)	8/70 (11%)	0/70 (0%)
	Combined (M + F)	7/120 (6%)	12/120 (10%)	0/120 (0%)
50,000	Male	21/61 (34%)	31/61 (51%)	1/61 (2%)
	Female	10/76 (13%)	14/76 (18%)	2/76 (3%)
	Combined (M + F)	31/137 (23%)	45/137 (33%)	3/137 (2%)
5,000	Male	13/65 (25%)	14/65 (22%)	1/65 (2%)
	Female	17/78 (22%)	10/78 (13%)	0/78 (0%)
	Combined (M + F)	30/143 (21%)	24/143 (17%)	1/143 (1%)
500	Male	19/66 (29%)	8/66 (12%)	0/66 (0%)
	Female	15/73 (21%)	10/73 (14%)	1/73 (1%)
	Combined (M + F)	34/139 (24%)	18/139 (13%)	1/139 (1%)
50	Male	4/71 (6%)	8/71 (11%)	0/71 (0%)
	Female	7/68 (10%)	6/68 (9%)	0/68 (0%)
	Combined (M + F)	11/139 (8%)	14/139 (10%)	0/139 (0%)

[a]Total includes animals from scheduled sacrifice (8, 16, 20 months period) and spontaneous deaths.

in either test group. No correlation was observed between the incidence of pneumonitis and adenoma or for pneumonitis and carcinoma. These results are similar to those observed in the single exposure study.

50 ppm, 100 days, 1 hr. Again changes attributable to VC were apparent in the lung only, primarily pulmonary adenomas. However, the incidence in the induction of adenomas and progression to carcinoma are considered only marginal and not statistically significant.

Comparatively, the potential for development of pulmonary adenomas as shown in Table 7 was greater in A/J mice following multiple exposures at 500 ppm than at 50 ppm, i.e., incidence at 500 ppm, 74.7%; at 50 ppm, 44.1%, despite an equivalent total dose of 5000 ppm VC. Also, pulmonary adenomas were induced earliest (8 months) following multiple exposures at 500 ppm. It is also of interest that the control groups of A/J mice for both multiple inhala studies showed a baseline incidence for pulmo adenomas which was nearly identical, i.e., at ppm, 34.4%; at 50 ppm, 34.5%.

Table 9 provides an overall summary of the in dence of histologically proven neoplasms obser in both strains of mice, i.e., ICR and A/J, follow single and multiple exposures to VC. The neopl tic and nonneoplastic changes observed in the li and/or lungs of A/J mice following multiple ex sures to VC at 50 and 500 ppm are shown in Tab 10 and 11. Although other neoplasms and nonn plastic changes occurred variously in all remain organs and tissues, the response appeared eith sporadically or was shared by all test groups incl ing controls. Relationship by incidence and sever to test exposure was not evident. Furthermo morphologic deviations were not unlike those no mally observed in aging A/J mice maintained und standard laboratory conditions.

Table 7. Histological examination of A/J mice at 8, 16 and 20 months following multiple (1-hr) exposures to vinyl chloride monomer.

Number of exposures and concentration of VCM	Histological changes attributable to vinyl chloride monomer	
	Induction of pulmonary adenomas	Progression to carcinoma
0 (controls)	31/90 (34.4%)	3/90 (3.3%)
10 × 500 ppm[a]	124/166 (74.7%)	22/166 (13.3%)
0 (controls)	29/84 (34.5%)	2/84 (2.4%)
100 × 50 ppm[b]	65/158 (44.1%)	7/158 (4.4%)

[a]Highly significant difference in the number of pulmonary adenomas ($p \simeq 0.001$) observed at the 500 ppm × 10 hr exposure level versus control by Z test.

[b]No significant difference for pulmonary adenomas ($p < 0.14$) and carcinomas ($p \simeq 0.19$) observed at the lower multiple exposure level.

Electron Microscopic Results

In general, these studies indicate that expos to vinyl chloride increased organelle turnover as w as loss of volume control (bleb formation) and i creased lysosomal activity in the liver of rats. The alterations progressively decreased as recovery af exposure increased.

Hepatocellular carcinoma was seen in one ma Fischer rat which had received 10 exposures of 5 ppm. Lymphosarcoma was noted in one fema Fischer rat which had received a single exposure a 500 ppm. Since these were individual cases at since no cancers were seen at 50,000 ppm, th lymphosarcoma and the hepatocellular carcino are not likely related to vinyl chloride exposure.

Table 8. Multiple inhalation exposure of A/J mice to vinyl chloride monomer.

VC Exposure			Lung tissue: incidence of response/number evaluated[a]		
Frequency	concn, ppm	Group	Pneumonitis	Adenoma	Carcinoma
10 × 1	0	Male	0/43 (0%)	15/43 (35%)	0/43 (0%)
		Female	2/47 (4%)	16/47 (34%)	3/47 (6%)
		Combined (M + F)	2/90 (2%)	31/90 (34%)	3/90 (3%)
10 × 1	500	Male	0/76 (0%)	56/76 (74%)	12/76 (16%)
		Female	0/90 (0%)	68/90 (76%)	10/90 (11%)
		Combined (M + F)	0/166 (0%)	124/166 (75%)	22/166 (13%)
100 × 1	0	Male	5/39 (13%)	11/39 (28%)	2/39 (5%)
		Female	3/45 (7%)	18/45 (40%)	0/45 (0%)
		Combined (M + F)	8/84 (10%)	29/84 (35%)	2/84 (2%)
100 × 1	50	Male	4/77 (5%)	27/77 (35%)	3/77 (4%)
		Female	5/81 (6%)	38/81 (47%)	4/81 (5%)
		Combined (M + F)	9/158 (6%)	65/158 (41%)	7/158 (4%)

[a]Total includes animals from scheduled sacrifice (8, 16, 20 months period) and spontaneous deaths.

Reproduction Carcinogenesis Study

One hundred and fifty colony rats (Sprague-Dawley/Wistar) were divided into three groups with equal numbers of males and females. These specific-pathogen-free, random bred animals were obtained from the Animal Resources Branch at Edgewood Arsenal. The rats were 12 weeks old when initially exposed to VC. One hundred rats were exposed as described and the remaining 50 were carried as unexposed controls. The parental generations of S-D/W rats were maintained 24 months post exposure for carcinogenic evaluation.

A complete gross and microscopic pathological examination of all tissues was performed on each control and exposed animal. Particular emphasis was placed on examination of brain, lung, and liver tissues and zymbal gland in the rodent ear. The livers of randomly selected rats were prepared and examined by electron microscopic techniques.

Neoplastic and nonneoplastic lesions were observed in approximately equal frequency in control and F_0 parent generation of Sprague-Dawley/Wistar rats exposed to 50 ppm or 500 ppm of VC, 1 hr per day, five days per week for 10 weeks (49 exposures).

The only lesions that occurred in higher frequency in the exposed animals than in control rats were eosinophilic cellular alterations presented as foci/ or areas. The appearance of these foci was related to the dosage of VC. The nature of these lesions are of interest but as yet are controversial, so that no inference can be drawn.

Table 9. Summary of incidence of histologically proven neoplasms observed within tissues of mice at various intervals following single or multiple exposures to vinyl chloride monomer.

Inhalation exposure	Exposure concentration, ppm	Number of neoplasms observed (scheduled sacrifice) 8 months	16 months	18 months	20 months	Totals	Total number of neoplasms observed
Single (1 hr)	50,000	5		24		29	110
	5,000	3		18		21	78
	500	1		29		30	82
	50	0		16		16	62
	0 (control)	0		14		14	72
Multiple (1 hr)	500 × 10	12	22		70	104	170
	0 (control)	0	2		38	40	45
	50 × 100	4	16		63	83	137
	0 (control)	1	5		27	33	41

[a]ICR strain used in single (1 hr) exposure studies; and A/J mice used in the multiple (1 hr) exposure studies.

Table 10. Overall summary: incidence of nonneoplastic changes and histologically proven neoplasms within the lungs of A/J mice exposed to vinyl chloride in multiple inhalation exposures.

Tissue response	Incidence of response: Control (0 ppm), 100 × 1 M (39)[a]	Control (0 ppm), 100 × 1 F (47)[a]	Vinyl chloride, 50 ppm, 100 × 1 M (81)[a]	Vinyl chloride, 50 ppm, 100 × 1 F (83)[a]
Lung (number evaluated)	39	45	77	81
Edema	2			
Congestion	4	1	1	
Focal hemorrhage	2		2	
Pneumonitis	5	3	4	5
Bronchio-alveolar hyperplasia		3		3
Osseous metaplasia				1
Bronchio-alveolar adenoma	11	18	27	38
Bronchio-alveolar carcinoma	2		3	4
Reticulum cell sarcoma				1
	13	18	30	43

[a]Numbers in parentheses denote animals per group. Total includes animals from scheduled sacrifice (8, 16, 20 month periods) and spontaneous deaths.

Discussion

The fact that the severity of carcinogenic effects of VC had been described by various investigators like Maltoni (*2,5*) and Lee et al. (*6*) to coincide with dose and length of exposure implies that the total dosage (concentration × exposure time) may be an important factor in the carcinogenicity of VC. Therefore, the inhaled dose was approximated by the Haber (*7*) concept. In its simplest form this concept states that the dosage, Ct is the product of C (concentration in milligrams/cubic meters) and t (time in minutes). The total concentration can also be expressed in parts per million (ppm) and the time can be expressed in hours, producing Ct ppm-hr. Factors for breathing rate and detoxication can be added. However, this simplified approximation of total inhaled dose is relatively useful, as is, for comparative purposes.

In his experiments Maltoni (*5, 10*) exposed rats and mice as shown in Table 12 to a series of doses of VC ranging from 50 to 10,000 ppm for 4 hr/day, 5 days per week for 52 weeks. The results indicated a questionable carcinogenic effect at 50 ppm or total dosage of 52,000 ppm-hr after 135 weeks. In another experiment (BT3), Sprague-Dawley rats, treated in a similar fashion to BT1 but for 17 weeks only, show after 86 weeks a negative carcinogenic re-

Table 11. Overall summary: incidence of nonneoplastic changes and histologically proven neoplasms within the liver and lung of A/J mice exposed to vinyl chloride in multiple inhalation exposures.

	Incidence of response			
	Control (0 ppm), 10 × 1		Vinyl chloride, 500 ppm, 10 × 1	
Tissue/response	M (46)[a]	F (48)[a]	M (78)[a]	F (92)[a]
Liver (number evaluated) [c, b]	45	48	78	89
Hepatic cell necrosis	4	6	6	13
Lymphoid cell infiltrate	1		1	
Hepatic cell lipidosis	2		2	
Neutrophil infiltrate	2			1
Bile duct hyperplasia	1			
Granulomatous foci			1	
Sinusoidal reticulosis				1
Hepatocyst				1
Amyloidosis			1	
Angiectasis				1
Hepatic cell adenoma	1			
Cholangiocarcinoma	—	—	1	—
	11	6	13	16
Lung (number evaluated)	43	47	76	90
Edema				1
Pneumonitis		2		
Bronchio-alveolar hyperplasia	2	1		2
Bronchio-alveolar adenoma	15	16	56	68
Bronchio-alveolar carcinoma	—	3	12	10
	17	22	68	81

[a]Numbers in parentheses denote animals per group. Total includes animals from scheduled sacrifice (8, 16, 20 month periods) and spontaneous deaths.

Table 12. Maltoni vinyl chloride studies.[a]

Test	Species	Results (carcinogenesis), ppm-hr
BT1	RATS	Questionable at 52,000
BT3	RATS	Negative at 17,000
		Questionable at 85,000
		Positive at 170,000; 850,000; 2,000,000; 3,000,000
BT6	RATS	Positive at 24,600,000
BT7	RATS	Negative at 52,000 and 260,000
		Questionable at 520,000
		Positive at 2,600,000; 6,200,000; 10,400,000
BT4	MICE	Positive at 30,000; 150,000; 300,000; 600,000; 1,500,000; 3,600,000

sponse at a total dose of 17,000 ppm-hr, a questionable response at 85,000 ppm and a positive carcinogenic response at 170,000 ppm-hr and above. In experiment BT7, Wistar rats treated with VC at doses ranging from 50 to 10,000 ppm, for 4 hr daily, 5 days/week for 52 weeks in the same manner as described above show positive carcinogenic affects at total dosage of 2,600,000 ppm-hr. The author suggests from comparison of the experimental data obtained with two different strains of rats (BT1/BT3), Sprague-Dawley and (BT7) Wistar, that the strain seems to be a factor in neoplastic response. Based upon neoplastic lesions observed, the Wistar strain was less responsive than Sprague-Dawley. Experiment BT4 with Swiss mice, involving exposure to VC at 50 to 10,000 ppm, 4 hr per day, 5 days per week for 30 weeks produced carcinogenic effects with a total dosage *Ct* of 30,000 to 3,600,000 ppm-hr.

In the studies of Viola, Bigotti and Caputo (*1*), tumors were seen in rats which had been exposed to 30,000 ppm VC, 4 hr/day, 5 days/week, for 12 months. The total dose (*Ct*) of VC was 28,800,000 ppm-hr.

In the studies of Caputo, Viola and Bigotti (*8*), rats and rabbits were exposed to VC for 4 hr/day, 5 days/week for 12 months at six dose levels. The exposure to 50 ppm VC or total dose of 48,000 ppm-hr produced no tumors. Carcinogenic effects were observed at total dosage of 320,000 ppm-hr and above for rats and at 7,800,000 ppm-hr for rabbits.

Keplinger et al. (*9*) exposed rats, hamsters and mice to VC. Only the data on mice were sufficiently complete for examination of total dose effect. Total dosage of 56,000 ppm-hr and above produced tumors in mice. The final report on the rats and hamsters is still unavailable for evaluation.

Lee et al. (*6*) exposed mice and rats to three levels of VC: 50, 250 and 1000 ppm, 5 hr/day, 5 days/week for up to 12 months. All tests with a total dose of 48,000 ppm-hr and below were essentially negative for mice. Positive carcinogenic effects were noted, however, in mice above 78,000 ppm-hr.

The results of our study indicate that a positive carcinogenic effect in rats may not appear until the total dose (*Ct*) of VC exceeds 50,000 ppm-hr. The single exposure study with ICR mice was negative at 50 and 500 ppm-hr, borderline at 5000 ppm-hr but did produce neoplastic lesions in the lung at 50,000 ppm-hr. The A/J mice exposed to multiple doses of VC, i.e., 50 ppm × 100 days × 1 hr or 500 ppm × 10 days × 1 hr for a total cumulative dose of VC of 5000 ppm-hr show a significant tumorigenic response, but only at the higher dose level.

In our studies Fischer rats exposed to a total dosage of 50, 500, 5000, and 50,000 ppm VC for 1 hr showed no chemically induced tumor response. Neither did the Sprague-Dawley/Wistar rats that were exposed to 500 ppm VC 1 hr/ day, 5 days/week for 10 weeks (total dosage 24,500 ppm-hr).

Table 13 summarizes various investigators' test results, including our own, on vinyl chloride. The tests with ICR mice, single exposure to VC, show an increased frequency of adenomas at total dosage of 5000 ppm-hr and above. For A/J mice, repeated exposures to 50 and 500 ppm VC for a total dosage of 5000 ppm-hr shows a similar dose response pattern for adenomas. In general, in terms of total dosage *Ct*, carcinogenic effects are seen in the two mice strains at *Ct* levels of 5000 to 50,000 ppm-hr. Thus total dosage for carcinogenicity in mice is in general agreement with Lee et al. (*6*) (78,000 ppm-hr) and Maltoni (*5*) (between 30,000 and 150,000 ppm-hr) and other investigators.

There is no doubt that risk is related to length of exposure and hence total dose. In our discussion much attention has been focused on the risk of cancer associated with total dosage of VC. However, the multiple exposure experiment at 50 and 500 ppm dose levels with A/J mice for an equivalent total exposure to 5000 ppm-hr appears inconsistent with the thesis of total dosage. Indeed, it appears

Table 13. Other vinyl chloride studies.

Authors	Species	Results (carcinogenesis), ppm-hr
Viola, Bigotti and Caputo (*1*)	Rats	Positive 28,800,000
Caputo, Viola and Bigotti (*8*)	Rats	Negative at 48,000
		Positive at 320,000; 1,280,000; 3,200,000; 6,400,000; 12,800,000
	Rabbits	Positive at 7,200,000
Keplinger et al. (*9*)	Mice (rats and hamsters)	Positive at 56,000
Lee (*6*)	Mice	All tests essentially negative below 78,000 and positive above 78,000
Consumer Product Safety Commission (1979)	ICR mice	50 and 500, negative 5000, borderline positive
	A/J mice	50,000 positive
	Fischer rats	50, 500, 5000 and 50,000, negative 24,500, eosinophilic loci, no cancers

from our own data that concentration may be the dominant factor for acute or low level intermittent exposures. This result may be explained on the basis of a number of factors, i.e., metabolism, detoxication, DNA repair or a time for tumor development for low level exposure beyond the animals' lifespan.

As is evident from Table 13, the two strains of rats, Fischer (344) and S-D/W, were more resistant to the adverse effects of single and multiple intermittent exposures of VC.

Our studies are in agreement as to the dose-time relationship for carcinogenesis related to the VC exposure. All of the continuous exposure studies considered collectively indicate that there is a lifetime total dose (Ct) for VC. However, from our own studies with single or intermittent low level exposures to VC we believe that concentration may be the most dominant factor in whether or not carcinogenic effects are observed. For single dose studies with VC in ICR mice, neoplastic lesions were produced at 5000 ppm. For multiple intermittent exposures studies with A/J mice the critical concentration for VC was 500 ppm, total dosage 5000 ppm-hr.

Approximate carcinogenic Ct levels of VC based on data for mice and rats are Ct = 5,000–50,000 ppm-hr, carcinogenic tendencies; 500,000 Ct > 50,000 ppm-hr, definite carcinogenicity; Ct > 500,000 ppm-hr, high incidence of carcinogenicity.

Considerations of Carcinogenicity of VC: Conclusions

Cancer seems dependent on total dose of vinyl chloride, especially in life-time exposure studies, but for short-term exposure the concentration may be the most critical factor.

One dose is sufficient if dose is high enough. The carcinogenic total dose was 5000 ppm-hr for mice and 50,000 ppm-hr for rats.

There were apparent noncarcinogenic doses in the study.

Mice were more sensitive indicators than rats for carcinogenic effects of vinyl chloride.

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Pneumoconiosis in Animals Exposed to Poly(vinyl Chloride) Dust

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Rats, guinea pigs and monkeys were exposed by inhalation (6 hr/day, 5 days/week) for up to 22 months to a 13 mg/m³ concentration of PVC dust. Autopsies on rats and guinea pigs were performed after 12 months of exposure and on monkeys after 22 months of exposure. Lung function tests were performed on monkeys after 9, 14 and 22 months of exposure. Aggregates of alveolar macrophages containing PVC particles were found in the lungs of all animals. These aggregates were more numerous in the monkey lungs. No fibrosis or significant cellular infiltrates were present in or near these cellular aggregates. No significant effects on pulmonary function could be demonstrated in the monkeys exposed to PVC. Under the conditions of this experiment, inhaled PVC produced a benign pneumoconiosis.

Introduction

Approximately seven billion pounds of poly(vinyl chloride) (PVC) were produced in the United States in 1979. There are two major types of PVC production processes, suspension polymerization and emulsion polymerization. The latter process produces particles which are respirable and much smaller than those in the former process. These polymers of vinyl chloride are usually compounded with a variety of ingredients (e.g., plasticizers, light and heat stabilizers, pigments and fillers) and processed in several different ways to produce thousands of end products in common use in our society. About 40% of PVC is used to make sewage pipes, water pipes and conduits. It is also used to make construction siding, window sashes, electrical wire and cable insulation, packaging films (for meat, etc.), vinyl floor tile, wall coverings, phonograph records, shower curtains, bottles, fabrics for clothes, furniture, automotive parts and many other products.

Chest x-ray abnormalities, respiratory dysfunction and pulmonary granulomas have been reported in workers exposed to PVC dust during PVC manufacturing and fabrication (*1–6*). Consequently, experimental studies were initiated in 1975 to study the effects of inhaled PVC dust in animals.

Materials and Methods

Test Material

Ten pounds of PVC (trade name Geon 121) were obtained from Goodrich Chemical Company and used in this experiment. The manufacturer's specifications stated a particle size range of 0.5–1.5 μm. Electron microscopic examinations in this laboratory confirmed that at least 90% of the particles were less than 1.5 μm in diameter. The PVC was stored in its covered, fiberboard shipping container throughout the duration of the study.

Dust Generation and Measurements

PVC powder was packed daily or every other day into a Wright dust feeder at a pressure of 3000

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lb/in.^{2}. The generated dust was fed into the mainstream of the inhalation exposure chamber intake air supply and through a static eliminator before entering the 160 ft^3 chamber. Airflow through the chamber was maintained at 40 ft^3/min with a negative chamber pressure of 0.2 in. water. Animals were exposed to PVC dust 6 hr/day, 5 days/week for up to 22½ months.

Four chamber dust samples were collected each day by drawing chamber environment air, at a rate of 10 liters/min for 20 min, through DM 5 μm pore size Metricel filters with a vacuum pump. In addition, simultaneous samples consisting of particles with an aerodynamic diameter ≥ 7 μm (respirable fraction) were collected with a 10-plate, horizontal elutriator. All samples were weighed immediately after each sampling period and the chamber dust concentrations calculated. Adjustments in the dust generating system after each sampling period were made when necessary to maintain a concentration approximating 10 mg of respirable dust/m^3 of air. Daily and grand means (derived from averaging the daily means) for the total and respirable dust fractions were calculated.

Midway through the experiment, two 6-hr chamber air samples were collected on charcoal tubes while PVC dust was being generated. Those two samples and the bulk PVC were analyzed for vinyl chloride by gas chromatography. Samples of dust that were allowed to settle on Formvar-coated copper grids placed in the chamber during the PVC dust generation were examined by transmission electron microscopy.

Animals

Animals used in this study were male, cesarean-derived, Sprague-Dawley rats (Laboratory Supply Company, Inc., Indianapolis, Indiana); male, Hartley guinea pigs (Sweetwater Farms, Hillsboro, Ohio); and imported, adult, male Cynomolgus monkeys (Primate Imports Corp., Long Island, New York). The treatment and control groups each contained 80 rats, 40 guinea pigs and 10 monkeys. Because of difficulties in obtaining imported monkeys from the supplier, the control group of monkeys was received several months before the exposed group of monkeys. They were not randomly assigned, because the control group was also used for the control group in another ongoing experiment. Consequently, at time of first exposure, the mean weight of the control monkeys was 4219 and that of the exposed group 3361 g. In the ensuing 22½ months, the controls gained 552 g in weight, whereas, the exposed group gained 2116 g.

The rats and guinea pigs were quarantined for two weeks and the monkeys were quarantined for one month prior to the initiation of the inhalation exposures. Stainless and galvanized steel open wire mesh cages were used as exposure caging to provide adequate distribution of the dust aerosols within the exposure chambers. All of the animals in the study were individually marked by toe-clipping or tattoo. All three species were individually housed during the 6-hr exposures, whereas the rats and guinea pigs were housed two to four animals per cage at all other times. Control animals were housed in similar cages in separate animal quarters and exposed to filtered air 24 hr/day. The exposed animals were also housed in the animal quarters except during the 6-hr inhalation exposure. Rats, guinea pigs, and monkeys were fed standard laboratory pellet diets (Rodent Laboratory Chow, Guinea Pig Chow, and Monkey Chow-Jumbo from Ralston Purina, St. Louis, Missouri). Monkeys were given fresh fruit (oranges, bananas or apples) twice a week. Tap water was available *ad libitum* except during the exposure period. Food was available to the rodents at all times except during exposure. The monkeys were fed once daily at the cessation of the exposure period.

Pathology

Within one day after the last exposure day, all the surviving rats and guinea pigs were autopsied. Their lungs were inflated with 10% buffered formalin and sections of liver, spleen, heart, kidney, pancreas, adrenals, thyroid, testis and urinary bladder from each animal were fixed in 10% buffered

Table 1. *Results of pulmonary function tests in monkeys after 14 months exposure to PVC dust.*[a]

Pulmonary function test	Groups	
	Control	Exposed
RL, cm $H_2O/l./sec$	12.1 ± 4.0	13.8 ± 3
CL, ml/cm H_2O	22.9 ± 10	17.5 ± 7
TCL, ml	357 ± 39	360 ± 2
VC, ml	334 ± 36	339 ± 7
IC, ml	185 ± 19	210 ± 2
RV, ml	40 ± 10	36 ± 1
RV/TCL, %	11.3 ± 2	9.9 ± 2
FeV 0.5/FVC, %	87.7 ± 5.3	86.5 ± 9
PF, ml/sec	943 ± 91	960 ± 12
FEF 50%, ml/sec	920 ± 91	905 ± 19
FEF, 25%, ml/sec	648 ± 173	655 ± 9
FEF 10%, ml/sec	262 ± 114	269 ± 7
CV, ml	21 ± 9	21 ± 1
ΔN_2/100 ml	1.05 ± 0.3	0.67 ± 0
Viso V, ml	28.7 ± 19	19.9 ± 1

[a]Data are presented as means ± one standard deviation for each of the parameters.

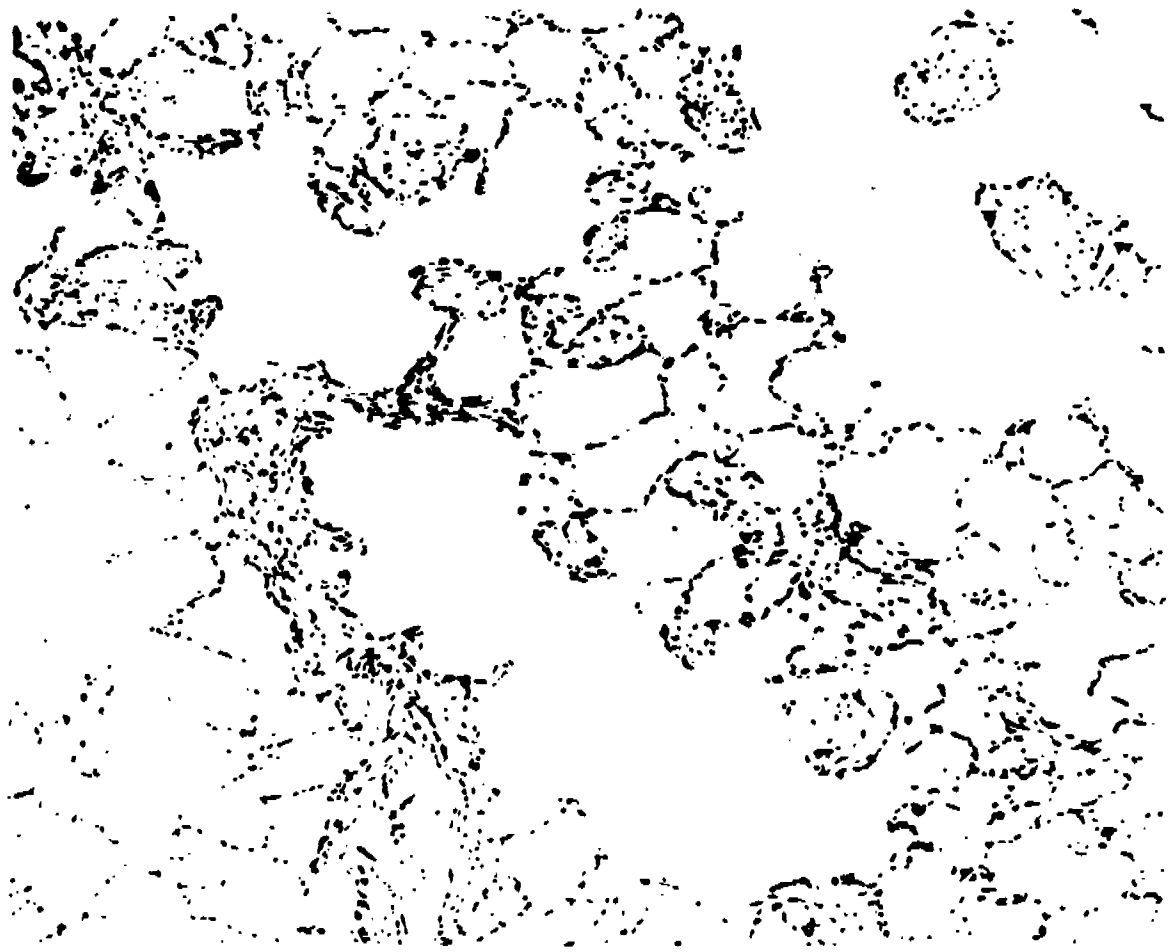

FIGURE 1. Macrophage aggregates in lung of monkey exposed to PVC dust for 22 months. Aggregates are primarily in respiratory bronchioles and alveolar ducts. (Original magnification 40 ×).

formalin. Hematoxylin and eosin stained sections of each lobe of each lung and of each of the above tissues were prepared for and examined by light microscopy. Within 48 hr of the last exposure day, all surviving monkeys were autopsied and sections of the same aforementioned tissues plus tracheobronchial and mesenteric lymph nodes, prostate, stomach, duodenum and skin were fixed and processed in the same manner as those for the rodents with the exceptions that two sections from each lobe of the lungs were examined, and some of the lung tissue was processed for examination by transmission electron microscopy.

Pulmonary Function

Before onset of exposures and after 9, 14 and 22½ months of exposure to PVC dust, the following pulmonary function tests were performed on fasted, anesthetized (pentobarbital anesthetic) exposed and control monkeys: total lung capacity (TLC), vital capacity (VC), inspiratory capacity (IC), residual volume (RV), forced expiratory volume in 0.5 sec ($FeV^{0.5}$), peak expiratory flow (PF), maximum expiratory flow volume (FEF) at 50%, 25% and 10% of vital capacity, resistance (RL), compliance (CL), closing volume (CV), N_2 washout (ΔN_2/100 ml) and volume of isoflow (VisoV). These tests were performed with a variable pressure, whole-body plethysmograph. Means and standard deviations for all parameters in each group were calculated at each interval and comparisons made between groups using parametric and nonparametric statistical analyses.

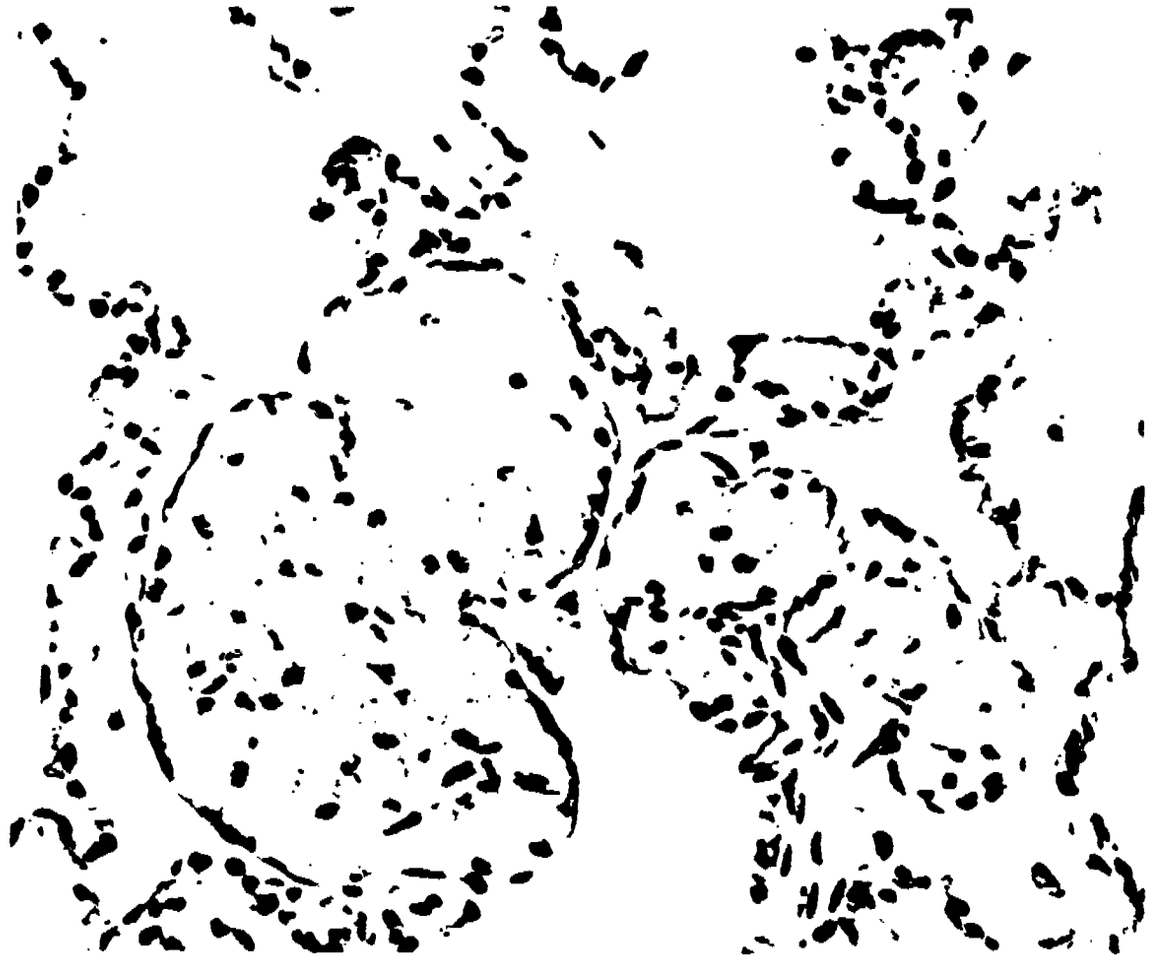

FIGURE 2. Macrophage aggregates in lung of monkey exposed to PVC dust for 22 months. (Magnification 385 ×).

Results

The mean total dust concentration in the chambers for the rodents was 13.0 mg/m^3 (SD = 2.34) and for the monkeys 12.9 mg/m^3 (SD = 4.69). The mean respirable dust concentration was 10.4 mg/m^3 for both the rodents and the monkeys. The monkeys were exposed on 464 days for a total of 2818 hr, while the rodents were exposed on 245 days for a total of 1426 hr.

The vinyl chloride concentrations in the chambers at the two sampling periods were 0.01 and 0.02 ppm. A small nonquantified amount of vinyl chloride was detected in the bulk PVC.

Electron micrographs of the settled chamber dust showed individual particles ranging from 0.13 to 1.68 μm in diameter and agglomerates measuring up to 12.8 μm in diameter. Although the agglomerates measuring greater than 7 μm in diameter accounted for only 2.3% (5/222) of all the particles counted, they were estimated to account for more than 20% of the mass.

Pulmonary Function

A summary of the extensive pulmonary function evaluations indicated some signs of loss of lung recoil pressure, probably a result of the animals' aging process. In most cases, differences were noted during the second and third testing periods (exposure months 9 and 14) and were indicative of some small airway obstruction. At this time, however, these differences were not statistically significant (see Table 1). At the last evaluation (month 22) compared with baseline (preexposure) data, there were no significant differences for any parameter tested.

Because of the differences in animal sizes be-

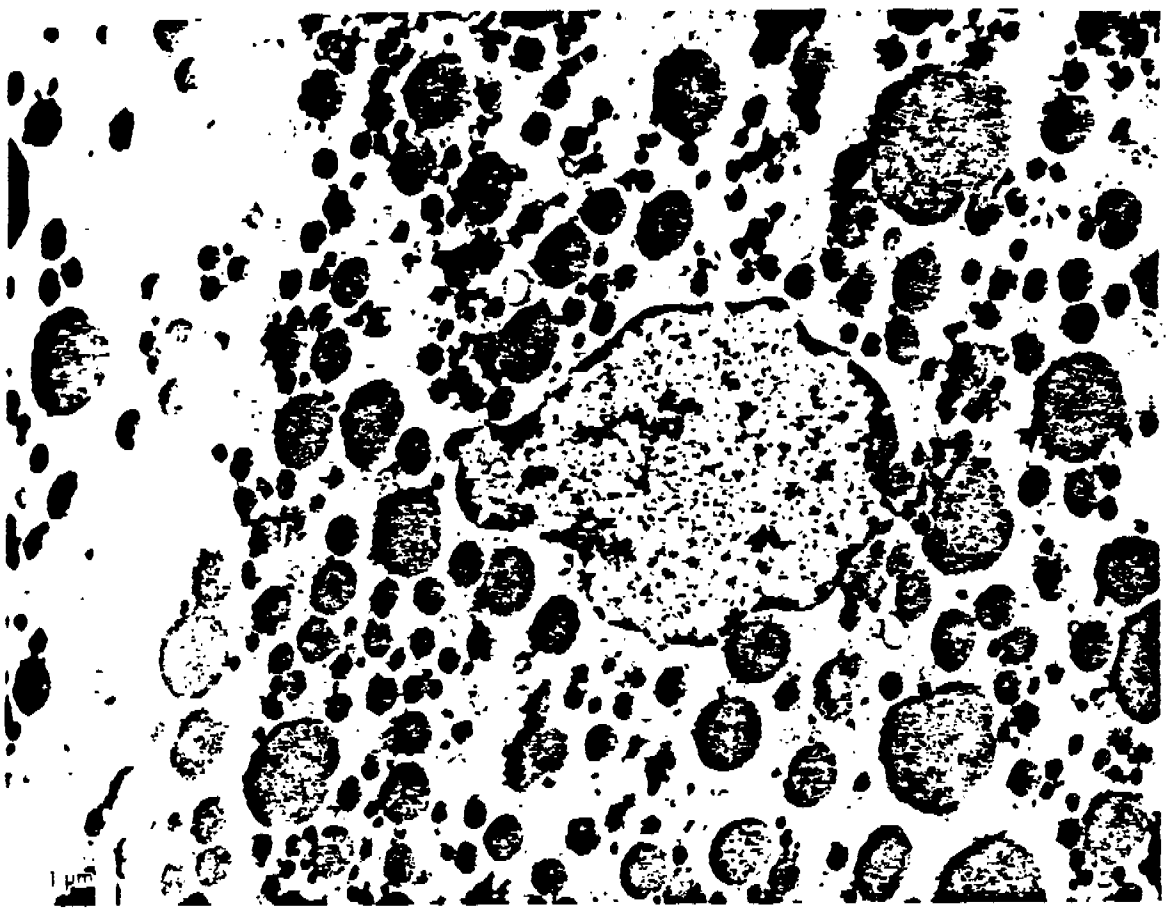

Fig. 3. Transmission electron micrograph of a macrophage from the lung of a monkey exposed to PVC dust for 22 months. Note the numerous round and oval, electron dense particles comprising most of the cytoplasmic space. These are the inhaled PVC particles.

tween the control and exposed groups for all except the 14-month interval, meaningful comparisons could be made only at that time period. At that time, the mean weight of the controls was 4470 g and that of the exposed animals was 4190 g. The results appear in Table 1. No statistically significant differences existed. Impairment of respiratory function does not appear to be indicated under the conditions of this study from exposure to respirable PVC dust.

Pathology

Ten exposed and ten control monkeys were autopsied 22[illegible] months after initiating exposures. No gross abnormalities were seen in the lungs or other organs that could be related to the exposures. Light microscopic examination of the lung sections of exposed animals revealed macrophage aggregates (Fig. 1 and 2) in the alveolar walls, alveoli, alveolar ducts, respiratory bronchioles and around veins. The majority of the aggregates were 100-200 μm in diameter and some were as large as 475 μm in diameter. Their concentration varied from three to seven per microscopic field at 100 × magnification. The tightly packed, large, spherical macrophages contained colorless cytoplasm which appeared light blue under phase contrast microscopy. Only a rare aggregate contained any other type of infiltrating cells, which usually consisted of a few polymorphonuclear leukocytes. No other lesions that could be related to experimental treatment were present, and no metaplasia, interstitial fibrosis, nodular fibrosis or pneumonitis were present. The tracheobronchial lymph nodes contained aggregates of the same type of macrophages described above in the lungs. Transmission electron microscopic examination of the macrophage aggregates revealed numerous round particles that do not normally occur in macrophages

FIGURE 4. Scanning electron micrograph of a 5-μm section of lung of monkey exposed to PVC dust for 22 months. This photo shows the numerous PVC particles which obscure the cellular outlines of the macrophages in which they are residing.

and were of the same size and shape as PVC particles (Fig. 3). Analysis of these particles with the microprobe revealed high concentrations of chlorine (Fig. 4 and 5), thus, further establishing their identity as PVC particles. A few aggregates of birefringent particles appeared in the lungs and high concentrations in the lymph node of both the exposed and control monkeys. These particles were identified as mica, kaolin and quartz by the use of microprobe, electron and x-ray diffraction methods. The bulk PVC did not contain these minerals. No macrophage aggregates were present in the control monkey lungs. No treatment related lesions were seen in sections of the other organs examined.

The 57 exposed and 64 control rats were autopsied 12 months after initiating exposures. Microscopic examination of the lungs of the exposed animals revealed 0–3 macrophage aggregates/microscopic field at 100× magnification (Fig. 6). These aggregates were smaller and much less frequent than those seen in the monkey lungs. Chronic pneumonitis, typical for rats, was present with equal severity and frequency in the control and exposed rats. No treatment related lesions were seen in sections of the other organs examined.

The 36 exposed and 39 control guinea pigs were autopsied 12 months after initiating exposures. Light microscopic examinations of the lungs of exposed guinea pigs revealed fewer collections of giant macrophages with clear foamy cytoplasm than those

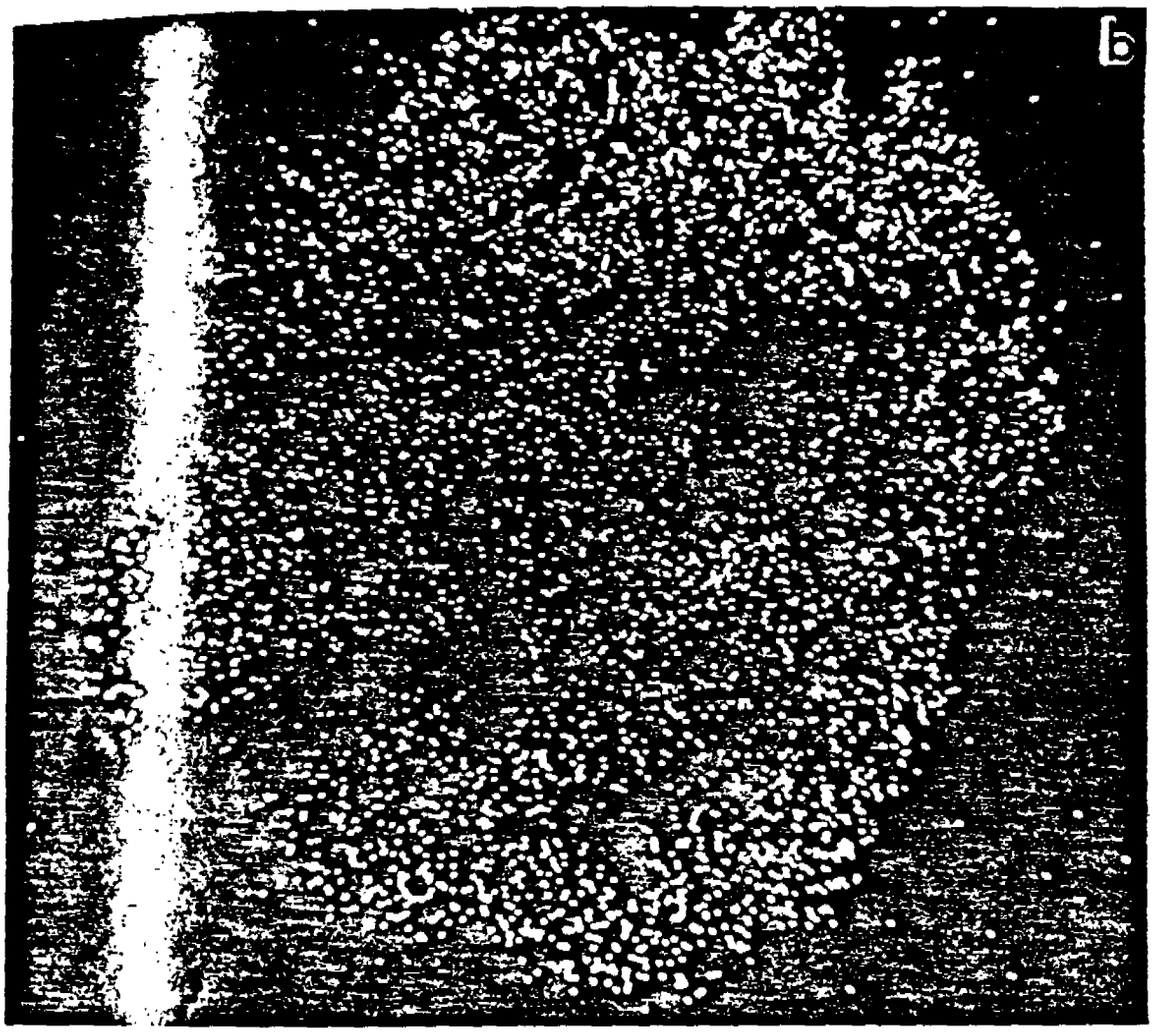

FIGURE 5. Chlorine x-ray map of the same area and magnification depicted in Fig. 4. The white dots indicate the presence of the element chlorine. Note that the dots are clustered in the same area as the PVC particles, thus confirming the identification of the PVC particles.

seen in the exposed monkeys or rats. Interstitial lymphocytic pneumonia was a constant finding in most of the exposed and control animals, and the severity and incidence were not related to treatment. Few macrophage aggregates similar to those seen in the lungs were present in the tracheobronchial lymph nodes of the exposed guinea pigs. No treatment-related lesions were seen in sections of the other organs examined.

Discussion

The monkeys in this study developed a benign, simple pneumoconiosis resulting from the inhalation of PVC dust at a mean concentration of 13 mg/m^3 for 464 days (2818 hr). The cellular response visible at 22 months of exposure consisted of PVC-laden macrophages aggregated into clusters in the lungs. On the other hand, the lungs of the rats and guinea pigs contained very few macrophage aggregates and in a much lower concentration than in the monkey lungs. This difference in degree of cellular response could possibly be explained on the basis of fewer PVC particles being deposited in the lungs of these rodents.

Rodents at rest in the chambers during the day breathe exclusively through their noses. Monkeys under the same conditions do not. The rodents in this experiment had extensive bronchitis and

FIGURE 6. Macrophage aggregates in the lung of a rat exposed to PVC dust for 12 months. Note vacuolated appearance of cel (Magnification 385 ×).

pneumonitis, whereas the monkeys did not. Both nose breathing and bronchitis are factors which reduce the percentage deposition of inhaled particles in the alveolar regions of the lungs.

Two studies have been published on the pathological effects of inhaled PVC in rats (*7*, *8*). In one study (*7*) rats and guinea pigs were placed in a PVC bagging facility. Two guinea pigs and an unstated number of rats were autopsied 2, 4 and 7 months after their continuous residence in the facility. Although the animals developed pulmonary diseases, the small number of animals studied and the lack of adequate controls makes it impossible to assess the significance of the exposure to PVC. In the other study (*8*) rats exposed to high concentrations (97 gm/m^3) of PVC dust in a static air chamber for 1 hr/day for up to 12 months developed bronchiectasis, emphysema, purulent pneumonia, lung abscesses and squamous metaplasia in bronchial epithelium. Although the control rats were free of these diseases, there were only 10 controls examined. In view of the propensity for rodents (controls) to contract acute and chronic pulmonary diseases, the experimental conditions under which control animals are kept is extremely important, and should be stated in the protocol. No mention of this was made in that experiment.

Two cases reports showing granulomas in lungs of two PVC workers have been reported (*3*, *5*). Except for the more closely packed macrophages in the lesions shown by Aranaud et al. (*5*), the morphology of the lesion is similar to that seen in our exposed monkey lungs. Part of the compactness of the cells in their lesion could be explained on the basis of artifactual compression caused during the taking of the lung biopsy. No excess collagen was

apparent in the light microscopic pictures of the granulomas they showed in their article, although they did refer to their case as a "case of discrete pulmonary fibrosis." Their electron microscopic pictures of the PVC particles in *in vitro* cell systems were very similar to what we saw in the macrophages in the monkey lungs. The PVC particles they showed in a macrophage from the lung biopsy appear to be clustered in lysosomes and the individual particles are not as dense as the ones we found in the monkey lungs. That difference might be related to the different exposure durations and differing intervals since last exposure. Their patient was exposed to PVC for 23 years (our monkeys were exposed for 22½ months) and had no PVC exposure for 6 years (our tissue was sampled within 48 hr of last exposure). It is possible that some of the ingredients in the PVC particles in the human lung were altered and leached out over a period of time.

Szende et al. (*3*) reported that the lung biopsy specimen from a worker who shoveled PVC for one year showed moderate diffuse fibrosis and contained a granuloma with concentrically arranged connective tissue fibers. We did not see this in the monkey lungs.

The apparent difference in response of the lung tissue from two workers exposed to PVC compared to the monkeys exposed to PVC might be related to a variety of factors, including differences in the PVC dusts. Since various types of emulsifiers and initiators are used in making PVC, and since these are not completely removed from the powdered PVC, they might explain the differences observed. It would be prudent in the future to obtain more information on PVC manufacturing processes and content of PVC dusts when studying their health effects.

Mastrangelo et al. (*6*) published health findings on employees who were currently working in a PVC production factory. Twenty cases of pneumoconiosis were found in a population of 1216 workers. Although the data were not given, the authors stated that slight restrictive respiratory function impairments were associated with chest x-ray changes in a small percentage of cases. In our study, no differences in pulmonary function tests could be detected between exposed and control monkeys after 14 months of exposure to 13 mg PVC/m^3. Whether any pulmonary function deficits would have occurred upon further exposure cannot be ascertained from this study. The lack of any significant pulmonary function abnormality in these monkeys, however, does not mean that animals or humans exposed to other types of PVC dust or mixtures of PVC dust with vinyl chloride (or a multitude of other ingredients that might be used in fabricating PVC products) might not exhibit pulmonary function changes.

For the first time, we have been able to show that a PVC dust generated under controlled experimental conditions is respirable and is deposited in cell aggregates in the lungs of monkeys, that PVC particles within macrophages are fairly unique in appearance, and that their identity can be confirmed with microprobe analyses. No pulmonary function deficits were found after 14 months of exposure to PVC concentrations of 13 mg/m^3, thus, confirming the pathological diagnosis of a benign pneumoconiosis.

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Preliminary Observations of the Effect of Inhalation of PVC in Man and Experimental Animals

by J. C. Wagner* and N. F. Johnson*

Attention has been focussed, both in man and experimental animals, on the effects of inhalation of the gas monomer, vinyl chloride. Recently, note is being taken of the possible effects of the inhalation of the polymer in man. The particles in question are those produced commercially as paste polymer or dispersion polymer or having an average diameter of 0.15 μm, and accounting for more than 10% of the production in Britain. There are now strict regulations for the control of the monomer gas, but the particles are regarded as nuisance dust and their emission is not covered by specific legislation.

Our studies on rats, where both inhalation and implantation methods of exposure have been used, and examination of tissue from human cases exposed to paste polymers, indicate that these small particles can only be regarded as evidence of exposure, and on present evidence there is no indication of causation of significant pulmonary disease. Techniques have been developed by which these particles can be demonstrated in ordinary histological preparations and by transmission electron microscopy.

The finer particles of PVC known in the United States as dispersion polymer, are usually less than 1 μm in diameter. During the last five years we have conducted a number of preliminary investigations using this material, and have also observed these small particles in the lungs of men exposed to this type of PVC, and have recovered these particles from the lungs and livers of human cases using maceration techniques. A second series of experiments has been started but no results are available.

The recognition of PVC in tissue has been greatly facilitated by a staining method developed by Wilson (*1*). Normally processed histological sections are stained with a Sudanophilic dye, which stains the particles a bright red. As the tissue has been processed and all lipid removed, only the PVC and some other related plastics will stain. The particles can also be recognized under the transmission electron microscope, and the morphological appearances supported by the confirmation of the presence of chloride when examined by x-ray microanalysis.

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Animal Experiments

Experiment 1

The experimental animals were caesarean-derived, barrier-maintained rats of the I.C.I. Alderley Park Wistar strain. Six-week old rats were randomized, with equal numbers of each sex, and inoculated intrapleurally by using a previously described technique (*2*). Forty-eight animals received 20 mg of PVC dispersion polymer into the right pleural cavity. The control animals were inoculated in a similar manner (Table 1). The rats were allowed to live out their lives and all are now dead.

Between 12 and 18 months after inoculation, nine of the animals receiving the PVC died. Of these, five had tumors of the liver and one had a tumor originating from the site of inoculation. None of the other animals injected with PVC, nor any of the control animals, had a tumor of this type. These tumors were shown to pathologists in Britain and the United States. It was generally agreed that they were poorly differentiated sarcomas, and in three of the animals, the possibility of Kupffer cell origin was considered. As no other animals devel-

Table 1. Intrapleural inoculation (rats 6 weeks old).

No. rats	Treatment
48 rats	20 mg PVC in saline
24 control rats	20 mg UICC crocidolite in saline
24 control rats	20 mg Min-U-Sil (quartz) in saline
24 control rats	saline

oped these tumors, two further experiments have been started. In the first, dispersion polymer from three British manufacturers was used, and in the second, freshly produced dispersion polymer from two sources has been inoculated, as well as similar material from which all evidence of detergent or vinyl chloride monomer has been removed. These investigations are still in progress, but in the first of these studies, the animals have passed the 18 month period without any occurrence of the tumors.

Inhalation Studies

A group of 48 rats, 24 of each sex, of the same stock as used in the previous experiments were exposed in an inhalation chamber to PVC dust, the apparatus and methods being the same as previously described (*3*). The animals were exposed to dispersion polymer, at a concentration of 12 mg/m^3 for 7 hr/day, 5 days/week, for 5 months. There were also 48 nonexposed controls. The cumulative dose of the exposed rats was 8552 mg/m^3 (Table 2). Six rats from each group were killed at the end of the exposure, and a further six a year after the start of the exposure. The remaining animals were allowed to survive until they died of natural causes.

Table 2. Inhalation study.

No. of rats	Exposure
48 rats 6 weeks old	22 weeks, 12 mg/m^3; cumulative dose 8552 mg/m^3 -hr
48 control rats	None

The PVC particles were present in the macrophages within the alveoli arising directly from the respiratory bronchioles immediately after the exposure. The distribution of these accumulations was widespread but less than a third of the primary units were involved. Occasional dust particles were observed in sections from the bronchopulmonary lymph glands, the Kupffer cells in the liver and in the spleen. At the end of a year, the dust was still present in the spleen and around some foci of macrophages there was evidence of a slight proliferation of reticulin fibers. This early dust reticulination did not show evidence of progression in any of the other animals, some of which survived for more than two years after the initial exposure.

Experience with Human Tissue

We have had the opportunity of examining a limited amount of human tissue. This included three cases of accepted angiosarcoma of the liver, a man with severe hepatic fibrosis who underwent a subsequent porto-caval shunt and material from people who had been employed in a PVC factory. These people had either had pulmonary biopsies or had died of diseases unconnected with their occupation. In all but one case we found small amounts of PVC in the lung tissue. In two of three angiosarcomas particles of PVC were found after the maceration of the liver tissue.

At this stage of the investigation, these particles can only be regarded as evidence of exposure to dispersion polymer.

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Results of Sputum Cytology among Workers Exposed to Vinyl Chloride Monomer and to Poly(vinyl Chloride)

by Cesare Maltoni* and Paola Lodi*

The results of systematic cytological sputum examinations of 3,380 Italian VC-PVC industry workers and of 2,287 workers in other industries at different potential risk and chosen as control groups are reported.

The results indicate an increase in cellular abnormalities and dysplasias in the epithelium of the respiratory tract among VC-PVC workers. These data are in line with experimental results showing that VC produces lung tumors in mice and with early epidemiological evidence among exposed workers.

Introduction

In 1975, it was decided to include the cytological examination of sputum in the protocol of systematic medical surveillance of the workers in all the Italian plastic factories exposed to vinyl chloride (VC) and poly(vinyl chloride) (PVC).

This program was co-sponsored by the Italian Federation of Unions of Chemical Workers (FULC) and by the Public Health Service of the Italian regions where the factories were located.

The decision to investigate cytologically all Italian VC-PVC industry workers was taken on the basis of the following facts: (1) the observation of a marked increase in the incidence of lung adenomas in mice exposed by inhalatory route, to a spectrum of doses of VC ranging from 10,000 down to 250 ppm (*1-3*); (2) the unusual finding of cellular abnormalities in a high portion of the lung adenomas of exposed mice (*1-3*); (3) the observation of frequent severe abnormalities (squamous dysplasia and atypical adenomatous hyperplasia) in the sputa of a small group of heavily exposed workers (*3*); (4) the report of some increase in lung carcinomas among workers from two VC-PVC factories (*4*); (5) the finding that the majority of lung carcinomas observed among exposed workers were not of a common histotype i.e., giant cell carcinomas (*5*).

Planning and Methods

The investigation was carried out on workers from 13 Italian VC-PVC factories located in the northern, central and southern parts of Italy, namely, in Villadossola (1), Portomarghera (3), Bollate (1), Ferrara (1), Ravenna (1), Rosignano (1), Terni (1), Brindisi (1), Ferrandina (1), Porto Torres (1) and Cagliari (1).

A total of 3380 workers was examined. These workers have been and/or are exposed to the monomer and part of them also to PVC dust. For comparison 2287 workers in other industries at different potential risk were examined: namely, workers manufacturing PVC products, workers in chemical industries not dealing with plastics, metal workers, miners and workers in the chromium industry.

For each sputum examination four cytological slides were prepared. The smears were stained by the Papanicolaou technique.

The slides were screened and evaluated independently by the pathologists of the hospitals in the area where the factories were located, then by a trained cytologist in our institute. All abnormal smears were reviewed by the senior pathologist of

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Table 1. Type, sequence and classification of local changes in the genesis of the various histotypes of pulmonary carcinoma

Stage					Cytologic class
		Normal respiratory epithelium			
		Nonciliated cuboid epithelium			
Initial nonspecific stage		Adenomatous hyperplasia	Squamous metaplasia	Basal cell hyperplasia	II, II-(III), II-III
Intermediate specific state		Atypical adenomatous proliferation	Squamous dysplasia	Basaloid cell dysplasia	(II)-III, III, III-IV
Tumor stage	Preclinical tumor	*In situ* adenocarcinoma	*In situ* squamous carcinoma	*In situ* anaplastic carcinoma	IV and V
	Clinical tumor	Invasive adenocarcinoma	Invasive squamous carcinoma	Invasive anaplastic carcinoma	

Table 2. Classification, characterization, distribution by classes and code of sputum cytology findings.

Class	Cell character: Type[a]	Degree	Quantity	Code
Not performed				99
I	Normal epithelium (LRE, URE, OCA)		1, 2, 3	01
	Histocytes		1, 2	
I-II	Normal epithelium (LRE, URE, OCA)		1, 2, 3	02
	Histocytes		3	
II	Squamous metaplasia LRE	+ / + +	1, 2, 3	03
	Hypersecretive mucous cells			
II-(III)	Squamous metaplasia LRE	+ + +	1, 2, 3	04
	Adenomatous hyperplasia LRE			
II-III	Mild atypical adenomatous hyperplasia LRE		1	05
	Mild squamous dysplasia LRE		1	
	Mild dysplasia URE, OCA		1	
(II)-III	Mild atypical adenomatous hyperplasia LRE		2, 3	06
	Mild squamous dysplasia LRE		2, 3	
	Mild dysplasia URE, OCA		2, 3	
III	Well defined atypical adenomatous hyperplasia LRE			07
	Well defined squamous dysplasia LRE			
	Well defined dysplasia URE, OCA			
III-IV	Grave atypical adenomatous hyperplasia LRE			08
	Grave squamous dysplasia LRE			
	Grave dysplasia URE, OCA			
IV	Suspected Adenocarcinoma			09
	Suspected Squamous carcinoma			
	Suspected Undifferentiated carcinoma			
V	Adenocarcinoma			10
	Squamous carcinoma			
	Undifferentiated carcinoma			
Not evaluable				11

[a]LRE = lower respiratory epithelium; URE = upper respiratory epithelium; OCA = oral cavity.

Table 3. Incidence of various types of cytological changes in sputa: comparison of results among VC-PVC workers and workers of the reference groups.

	No. of cases	Non-evaluable cases (insufficient material)	Evaluable cases	Cases without epithelial changes		Cytological changes: Hypersecretive mucous cells and squamous metaplasia LRE +/+ +		Squamous metaplasia LRE + + +		Squamous metaplasia with horn pearls LRE		Adenomatous hyperplasia LRE		Atypical adenomatous hyperplasia LRE		Squamous dysplasia LRE		Dysplasia URE, OCA	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Workers of VC-PVC industry	3380	822	2558	799	31.2	1153	45.1	374	14.6	70	2.7	794	31.0	38	1.5	219	8.6	44	1.7
PVC manufacturers	422	79	343	99	28.9	253	73.8	36	10.5	1	0.3	74	21.6	4	1.2	4	1.2	1	0.3
Workers of various chemical industries[a]	1019	130	889	165	18.6	604	66.9	83	9.3	3	0.3	227	25.5	8	0.9	10	1.1	–	–
Metal workers	264	54	210	73	34.8	109	51.9	30	14.3	1	0.5	5	2.4	–	–	6	2.8	–	–
Miners	402	48	354	47	13.3	254	71.7	41	11.6	1	0.3	59	16.7	5	1.4	4	1.1	1	0.3
Workers of chromium industry	180	28	152	9	5.9	89	58.5	69	45.4	1	0.6	7	4.6	6	3.9	28	18.4	–	–

[a] Other than VC-PVC and chromium industries and not exposed to a known risk.
[b] Not exposed to a known risk.

Table 4. Distribution by classes of cytological changes in sputa: comparison of results among VC-PVC workers and workers of the reference groups.

	No. of cases	Not evaluable cases (insufficient material)	Evaluable cases	Classes: I, I-II		II		II-(III)		II-III		(II)-III		III		III-IV		IV		V	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Workers of VC-PVC industry	3380	822	2558	799	31.2	705	27.5	780	30.4	211	8.6	21	0.8	35	1.3	7	0.2	–	–	–	–
PVC manufacturers	422	79	343	99	28.9	135	39.3	102	29.7	4	1.2	1	0.3	1	0.3	1	0.3	–	–	–	–
Workers of various chemical industries[a]	1019	130	889	165	18.6	399	44.8	313	35.2	6	0.7	6	0.7	–	–	–	–	–	–	–	–
Metal workers	264	54	210	73	34.8	102	48.6	28	13.3	6	2.8	–	–	1	0.5	–	–	–	–	–	–
Miners	402	48	354	47	13.3	220	62.1	82	23.1	2	0.6	1	0.3	1	0.3	1	0.3	–	–	–	–
Workers of chromium industry	180	28	152	9	5.9	63	41.5	51	33.6	18	11.8	2	1.3	5	3.3	4	2.6	–	–	–	–

[a] Other than VC-PVC and chromium industries and not exposed to a known risk.
[b] Not exposed to a known risk.

Table 5. Distribution of classes II-(III) and over among VC-PVC workers and the workers in reference groups.

	Classes II-(III), %	Classes II-III, (II)-III, %	Classes III, III-IV, %
Workers of VC-PVC industry	30.4	9.4	1.5
PVC manufacturers	29.7	1.5	0.6
Workers of various chemical industries	35.2	1.4	–
Metal workers	13.3	2.8	0.5
Miners	23.1	0.9	0.6
Workers in chromium industry	33.6	13.1	5.9

the same institute. A joint critical reevaluation was carried out for those cases where there was not full agreement.

The same criteria of classification of the lesions and their distribution by classes were adopted by all pathologists.

Such criteria are based on our knowledge of the local cytological changes and of their sequence in the genesis of the various histotypes of pulmonary carcinomas (Table 1).

The list of the most relevant changes correlated with environmental and occupational exposure, their distribution by classes following qualitative and quantitative parameters, and the code, are given in Table 2. This list takes into account not only cells from the pulmonary tree, but also cells from the upper respiratory tract and oral cavity.

Results

The number of valuable cases, the distribution of the cytological changes and the distribution of the results expressed by classes in the group of workers in the VC-PVC industries and in the control groups, are given in Tables 3 and 4. The incidence of dysplastic changes is particularly high in the plastics industry workers when compared to the other control groups, with the exception of chromium workers, which can be considered a positive control. The situation is well illustrated by the distribution of classes II-(III) and above (Table 5).

It may be stated that, in relation to the incidence of respiratory cell abnormalities, the workers in the VC-PVC industries can be placed halfway between chromium workers and other groups.

A finding which deserves attention is the occurrence of atypical cells in the oral cavity and upper respiratory tract in 44 workers (17%) among VC-PVC workers (Table 3).

The results were highly homogeneous in different factories, suggesting that variations in climate which may affect the incidence of common pneumopathies, is not a determining factor.

The difference in the distribution of the pathological findings was not dependent on tobacco smoking.

Conclusions

The results presented are consistent with experimental data and early epidemiological evidence on the potential oncogenic effects of VC exposure on the lungs.

Furthermore, our data suggest a possible oncogenic risk for the upper respiratory tract which should now be explored by proper epidemiological investigation.

Much remains to be done to clarify the part played by the monomer and by polymer dust in determining the observed cytological changes. Our data are now being re-examined, on the basis of the complete exposure history of single workers, to better establish the role played by PVC particles.

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Observations of the Site-Specific Carcinogenicity of Vinyl Chloride to Humans

by Peter F. Infante*

A review of epidemiologic studies of workers exposed to vinyl chloride (VC) was conducted. Some of these studies comprised small cohorts and thus were insensitive in the evaluation of carcinogenic response for sites that do not demonstrate a high relative risk. Other larger studies used methodology and design that precluded an interpretation of the results. Such limitations were acknowledged by some authors.

Use of restrictive disease rubrics also lead to the submerging of sites that would have demonstrated significant excesses. For example, some investigators analyzed data for liver cancer deaths with the board category of digestive system cancer deaths, while others combined data for CNS cancer deaths with the broad category of "other and unspecified cancer," and most studies analyzed information for lymphatic and hematopoietic system cancer deaths with all data combined. Only four of eight studies reviewed could demonstrate a significant excess of liver cancer among VC-exposed workers—a site confirmed in humans by 1974. In contrast, five of eight studies appear to demonstrate a significant excess of CNS cancer mortality. Workers exposed to VC also demonstrate a significant excess of mortality for lung cancer, while the data for lymphatic and hematopoietic system cancer are suggestive. Interpretation of cancer of the latter systems may have been clarified if investigators had not analyzed their data by broad disease classifications.

In 1930, shortly after vinyl chloride (VC) was introduced into commerce, VC toxicity was reported in experimental animals (*1*). Over the next two decades, study throughout the world indicated that employment in the VC industry was associated with a wide range of toxicity in humans. This toxicity has included nonmalignant pathologic effects or symptoms, involving (but not limited to) the bones, liver, central nervous system (CNS), lungs, and blood (*2*).

Between 1970 and 1974, experimental bioassay demonstrated VC-induced cancer in multiple organs including the liver, brain, lungs and lymphatic system (*3, 4*). This carcinogenic response has been observed in several species, given a wide range of doses, by various routes of administration.

Between 1973 and 1977, several epidemiologic studies were undertaken to assess the site-specific cancer risk among workers exposed to VC. For this presentation, findings of these studies will be limited to an assessment of cancer of four organs or organ systems in humans. These same sites are known to be associated with nonmalignant VC-related disease or symptoms in humans and cancer in experimental animals.

Liver Cancer in Humans

Table 1 shows a summation of data from the epidemiologic studies as related to liver cancer. Some authors did not present site-specific analyses for liver cancer deaths. Therefore, the most specific information available is presented.

Waxweiler et al. (*5*) conducted a cohort study of workers who had been exposed to VC in the U.S.

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The views expressed in this paper do not necessarily represent those of the Occupational Safety and Health Administration.

Table 1. Epidemiologic study results of VC-exposed workers as related to biliary and liver or digestive system cancer deaths

Investigation	Site	Deaths		
		Observed	Expected	SMR
Waxweiler et al. (5)	Biliary and liver			
Total cohort		7	0.6	1155[a]
Latency > 15 yr		7	0.4	1606[a]
Byren et al. (6)	Liver and pancreas			
Total cohort		4	0.97	413[b]
Latency > 10 yr		4	0.68	589[a]
Fox and Collier (7)	Liver			
Total cohort		4	1.64	244
Plant #2 > 15 yr		3	0.13	2308[a]
Seven other plants		1	1.51	66
Monson et al. (8)	Biliary and liver			
Total deaths		8	0.7	11.0[b,c]
Tabershaw and Gaffey (9)	Digestive organs			
Total cohort		19	21.7	94
Highest exposure, > 5 yr employment		11	7.5	151
Buffler et al. (10)[d]				
Ott et al. (11)[e]				
EEH (12)	Digestive organs			
Total cohort		29	40.8	71
Latency > 20 yr		9	13.6	70

[a] $p < 0.01$.
[b] $p < 0.05$.
[c] Proportional mortality study risk ratio.
[d] No liver cancer identified among 8 cancer deaths.
[e] No liver cancer identified among 20 cancer deaths.

for at least five years and who had achieved a period of ten or more years since initial exposure (latency). On the basis of seven liver and biliary cancer deaths that fit the cohort definition, the study demonstrated an 11-fold to 16-fold excessive risk of death from cancer of this site among VC-exposed workers. These findings represent an underestimate of the risk because seven additional individuals who died from liver/biliary cancer at the plants being studied were not included in the analyses. Of these latter seven cases, four individuals diagnosed with liver angiosarcoma were still alive at the study cut-off date, while two individuals who died from biliary cancer had incomplete information on length of exposure to VC. A seventh individual did not fit the study cohort definition as he was exposed for only three years. He died from liver angiosarcoma 17 years after his initial exposure to VC.

Byren et al. (6) conducted a cohort study of all Swedish workers ever employed in positions where exposure to VC could have occurred. The investigators combined deaths from cancer of the liver and pancreas because they believed that there was some overlap in reporting. There were four liver/pancreatic cancer deaths observed, compared to 0.77 expected ($p < 0.02$). An additional death from liver angiosarcoma was identified as having occurred after the study cut-off date and thus was not included in the study.

Fox and Collier (7) studied U.K. workers exposed to VC. In eight factories studied, they observed a total of four liver cancer deaths as compared to 1.64 expected. Three of these deaths occurred in factory 2, where only 0.13 would have been expected ($p < 0.01$). The authors stated that it was difficult to identify angiosarcoma of the liver from death certificates (the usual method of identification), since some of these deaths were classified as primary, some as secondary liver cancer, and others were not certified as cancer deaths at all.

The results from the Fox and Collier study probably represent an underestimate of the observed risk of death from cancer for the following reasons: (1) 75% of the study cohort was employed for less than ten years; (2) only 8% of the cohort was employed for more than 20 years; and (3) even

for those who completed 20 years of service, the authors stated that "because their service has only recently been completed, the follow-up period is too short to evaluate the carcinogenic effect of VCM." This statement obviously applies to the liver as well as to other sites.

Monson et al. (*8*) conducted a proportional mortality study of 161 deceased workers from two plants where VC was used. These plants were also studied by Waxweiler et al. (*5*). They observed eight deaths from liver and biliary tract cancer versus 0.7 expected; the risk ratio was 11.0.

Tabershaw and Gaffey reported the results of a cohort study of workers exposed to VC in 33 U.S. industrial facilities. The authors did not analyze the data separately for liver and biliary cancer. Thus, deaths for these causes are accounted for in the category of digestive organ cancer. As noted in Table 1, for the total cohort there were 19 deaths from digestive organ cancer and 21.7 expected. For the highest exposure cohort, with more than five years of exposure, there were 11 digestive organ cancer deaths observed versus 7.5 expected. None of the observations are statistically significant.

However, the risk of liver cancer in this cohort is submerged by including data for this cause of death in the analysis with digestive organ cancer deaths. Of the 19 digestive cancer deaths, the authors noted that seven were from liver cancer. Two of these liver cancer deaths were listed as angiosarcoma according to the diagnosis on the death certificate. An additional four deaths from liver angiosarcoma were identified in this cohort by the time the study was published in 1974. Two of these deaths had been categorized by death certificate diagnosis as primary liver cancer, and two had been certified under causes of death other than cancer, i.e., cirrhosis of the liver and hepatoma.

This study again demonstrates one of the inherent limitations of epidemiologic studies (incorrect diagnosis in relatively rare causes of death) that result in an underestimate of the relative cancer risk. An additional factor, indicated by the authors, that may have led to an underestimate of the risk in this study was that the group with no vital status determination, 15% of the population, began their exposure ten years before the group for whom followup was completed. As a result, some individuals with longer latency periods were omitted from the study. In addition, 57% of the cohort actually studied had less than 15 years of latency. Because of the healthy worker effect, the authors appropriately stated that SMRs higher than expected may be worthy of attention even if they are not statistically significant.

Buffler et al. (*10*) and Ott et al. (*11*) did not observe any liver cancer deaths among eight and twenty deaths, respectively, in their studies.

An unpublished study conducted by Equitable Environmental Health (*12*) reported the mortality of a cohort of workers from 37 U.S. industrial facilities. This study included data for the facilities studied by Tabershaw and Gaffey (*9*). Data were not analyzed separately for liver cancer. However, the category of death from cancer of the digestive organs, which includes liver cancer deaths, indicates a deficit of mortality. As shown in Table 1, even for those individuals who had achieved 20 or more years of latency, the SMR was only 70. It is somewhat unusual to observe such a deficit of mortality among workers who had achieved such a long latency period.

Brain Cancer in Humans

Brain cancer also has been associated with exposure to VC. A summary of the results of mortality studies is shown in Table 2. Although the number of cases upon which observations were based are small, Waxweiler et al. (*5*) and Byren et al. (*6*) demonstrated significant excesses of brain cancer. The relative risks were five and six, respectively. Waxweiler et al. (*5*) also noted an unusual distribution in the cell type of brain cancer. Of 10 brain cancer deaths identified among the VC-exposed workers, nine (90%) had a histologic diagnosis of glioblastoma multiforme. The tenth case had no confirmation of cell type. The authors contrasted this high proportion with that of the Yale autopsy series in which 33% of primary intracranial neoplasms were glioblastoma multiforme.

Fox and Collier (*7*) observed two brain cancer deaths as compared to 3.7 expected for the entire cohort. For those cohort members categorized as having high exposure, one brain cancer death was observed versus 0.4 expected. Monson et al. (*8*) demonstrated a fourfold risk of brain cancer.

Tabershaw and Gaffey (*9*) categorized brain cancer with "other and unspecified causes of cancer death;" therefore, it is not possible to determine the actual brain cancer risk identified in this study. Since the Tabershaw and Gaffey study (*9*) was a subset of the EEH study (*12*), the latter study was used to estimate the expected number of brain cancer deaths in the former study under the assumption that the age distributions were similar. The proportion of expected brain cancer deaths from "other and unspecified cancer deaths" in the EEH study (*12*) was then applied to the expected from this same category in the Tabershaw and

Table 2. Epidemiologic study results of VC-exposed workers as related to central nervous system.

Investigation	Site	Deaths		
		Observed	Expected	SMR
Waxweiler et al. (5)	Brain and CNS			
Total cohort		3	0.9	329
Latency > 15 yr		3	0.6	498[a]
Byren et al. (6)	Brain			
Total cohort		2	0.3	612[a]
Fox and Collier (7)	Brain			
Total cohort		2	3.7	55
Highest exposure		1	0.4	278
Monson et al. (8)	Brain			
Total deaths		5	1.2	4.2[a,b]
Tabershaw and Gaffey (9)	Other and unspecified			
Total cohort		17	11.8	155
Highest exposure, > 5 yr employment		7	3.5	204
All exposure levels	Brain	6	2.4[c]	250[a]
Buffler et al. (10)[d]				
Ott et al. (11)	All sites other than			
Total cohort	digestive and respiratory	6	6.8	88
Total cohort	Brain	2	0.7[c]	286
EEH (12)	Brain and CNS			
Total cohort		12	5.9	203[a]

[a] $p < 0.05$.
[b] Proportional mortality study risk ratio.
[c] Estimated.
[d] No brain cancer identified among 8 cancer deaths.

Gaffey study (9). As a result, 2.4 brain cancer deaths were estimated to have been expected and compared to six observed in the study. This difference is significant.

Buffler et al. (10) did not identify any deaths from brain cancer in their small cohort. Ott et al. (11) did not analyze their data separately for brain cancer, but rather included brain cancer deaths with cancer deaths from "all sites other than digestive and respiratory." Therefore, using an analytical technique similar to that described above, the proportion of expected brain cancer deaths among "all sites other than digestive and respiratory" from the EEH study (12) was applied to the expected in the study by Ott et al. (11). As shown in Table 2, there was a resultant estimated 0.7 brain cancer deaths expected as compared to two observed. The excess risk is estimated to be about threefold. The EEH study (12) also demonstrates a significant excess brain cancer among workers occupationally exposed to VC. In summary, the data for brain cancer appear to be more consistent between studies than the data for liver cancer, although the magnitude of the excessive risk is not as great for brain cancer.

Lung Cancer in Humans

As shown in Table 3, Waxweiler et al. (5) observed 11 lung cancer deaths as compared to 5.7 expected ($p < 0.005$) for cohort members who had achieved 15 or more years of latency. The authors also noted what appeared to be an unusual distribution in the histologic types of lung cancer. Of eight histologically confirmed lung cancer cases, five were classified as large cell undifferentiated, and three were categorized as adenocarcinoma. These cell types are different from those usually associated with a cigarette smoking etiology, i.e., small cell undifferentiated and epidermoid carcinomas.

With the exception of the study by Fox and Collier (7), the remaining studies show excess risks of lung cancer ranging from 7 to 200%. However the reservation expressed by Fox and Collier (7), as mentioned earlier, about the short period of follow-up limits the interpretation of their study. This concern is supported by the observation that the SMR for total mortality was only 75, 75% of what would be expected on the basis of comparison to the standard population.

Table 3. Epidemiologic study results of VC-exposed workers as related to respiratory system cancer deaths.

Investigation	Site	Deaths		
		Observed	Expected	SMR
[illegible] et al. (5)	Respiratory			
Total cohort		12	7.7	156
Latency > 15 yr		11	5.7	194[a]
[illegible] et al. (6)	Lung			
Total cohort		3	1.8	168
[illegible] and Collier (7)	Lung			
Total cohort		46	51.2	90
Highest exposure		2	3.7	54
[illegible] et al. (8)	Lung			
Total deaths		13	7.9	1.6[b]
[illegible]shaw and Gaffey (9)	Respiratory			
Total cohort		25	23.9	112
Highest exposure, 5 yr employment		12	8.5	144
[illegible] et al. (10)	Lung			
Total cohort		5	1.7	289[a]
Long exposure duration		4	1.05	381[a]
Short exposure duration		0	0.45	—
[illegible] et al. (11)	Respiratory			
Total cohort		7	5.8	121
EEH (12)	Respiratory			
Total cohort		45	44.3	107
Highest exposure		7	5.1	141
Medium exposure		19	17.0	116
Low exposure		19	22.2	92

[illegible] 0.05
[illegible]portional mortality study risk ratio.

Table 4. Epidemiologic study results of VC-exposed workers as related to lymphatic and hematopoietic system cancer deaths.

Investigation	Site[a]	Deaths		
		Observed	Expected	SMR
[illegible]weiler et al. (5)	(200–205)	4	2.50	159
Latency > 15 yr	(200–205)	3	1.70	176
[illegible] and Collier (7)	(200–207)	9	9.01	100
[illegible] (8)	(200–205)	5	3.4	1.5[b]
[illegible]shaw & Gaffey (9)	(200–203, 205)	6	6.06	106
Highest exposure, > 5 years employment		4	1.84	222
EEH (12)	(200–203, 205)	11	10.36	112
Latency > 20 years		4	3.10	136

[a]International Classification of Diseases, 7th Revision.
[b]International Classification of Diseases, 8th Revision.
[c]Proportional mortality study risk ratio.

As shown in Table 3, data from the EEH (*12*) and Buffler et al. (*10*) studies suggested a qualitative dose-response relationship between VC exposure and lung cancer risk. However, retrospective categorization of high, medium and low exposure, as was used in the EEH study (*12*), must be viewed with caution because of the subjective nature of judgment. The study by Buffler et al. (*10*) is particularly noteworthy; within a small cohort of only 464 workers, a fourfold risk of lung cancer was observed. When the investigators examined the effect of smoking, under the extreme assumption that those with unknown smoking habits were smokers, the data still demonstrated a significant

excess of lung cancer (5 observed versus 1.98 expected.)

Cancer of the Lymphatic and Hematopoietic System in Humans

Table 4 summarizes data on cancer of the lymphatic and hematopoietic systems among workers exposed to VC. The relative risks for various studies ranged from 1.0 to 2.2, and none of the results demonstrated a significant excess. Although somewhat suggestive, the data need to be further analyzed by latency period and exposure levels combined. Analysis of data separately for lymphatic cancers and leukemia might also lead to meaningful observations.

Summary

In summary, epidemiologic evidence demonstrates that the carcinogenic effects of VC in humans extend beyond the liver. The brain and lung should also be considered target organs. Some studies indicate that the lymphatic and hematopoietic systems are also involved. These observations in humans are supported by studies demonstrating the induction of cancer of these same sites in experimental animals.

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German Investigations on Morbidity and Mortality of Workers Exposed to Vinyl Chloride

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Two studies on mortality and morbidity of workers exposed to vinyl chloride monomer (VCM) which have been carried out on behalf of the Ministry of Labour, Health and Social Affairs on Northrhine-Westphalia are reported.

Vinyl Chloride Mortality Study

The aims of this study were to determine standardized mortality ratios (SMR) for male workers exposed to VCM, using the mortality rates of the West German male population as reference, to study the SMRs of a cohort of workers of the chemical industry comparable concerning age distribution and observation period but not exposed to VCM and to determine the SMR of a cohort of workers in PVC-processing plants.

The study was designed as a historic cohort study, covering the period from the beginning of the VCM- and PVC-production in all of the German plants till the end of 1974.

Table 1 shows the main characteristics of the three cohorts investigated. Only Germans and Austrians were included in data analysis because of insufficient mortality data on various foreign nationals employed in German factories. To determine the mortality rates of Austrians, West German rates were used. To calculate expectations of total mortality, the mortality rates of the adequate years have been used. To calculate expectations of specific causes of death for all years before 1968, the rates of 1968 have been used; for the following years the rates of the corresponding years. Following the procedure applied by Tabershaw (*1*), weighting of observed cases of specific causes of death according to unknown causes of death has been done with weighting factors calculated separately for three observation periods (up to 1959, 1960-1969, 1970-1974) as well as for six age groups.

In all of the cohorts, follow-up rates have been near or above 90%. The percentage of causes of death that could not be investigated due to loss or deletion of death certificates varied from 7.3% to 13.1%. To calculate age-standardized mortality ratios of specific causes of death, weighting has been done according to the procedure used by Tabershaw and Gaffey (*1*) to compensate for unknown or unidentified causes of death.

Table 2 displays total mortality as well as some of the relevant specific causes of death. It can be observed that the otherwise observed "healthy worker effect" cannot be demonstrated in the German cohorts exposed to VCM or employed in PVC-processing plants.

In the VCM cohort there are significant elevations of SMR of malignancies of the lymphatic and hematopoietic tissues (ICM 200-209), and of malignancies of the GI tract (ICD 150-159). The latter is due to the paramount elevation of SMR of tumors of the liver (ICD 155).

It must be noted that there is a modest elevation of SMR of tumors of the liver also in the cohort not exposed to VCM nor employed in PVC-processing plants. No obvious explanation for this observation can be offered. In addition elevated SMRs for ischemic heart disease (ICD 410-414) can be found in all of the three cohorts. Due to methodological

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shortcomings of the study no assessment of cardiovascular risk factors has been made. Therefore these results are of minor interest.

When subdividing the VCM-exposed cohort according to time of exposure there is a clear-cut increase of the SMR of liver tumors with time (Table 3). This seems to be highly suggestive of a time-response pattern. As it has been impossible to determine concentrations of VCM retrospectively due to technological and methodological problems no dose-response pattern can be established. However time of exposure seems to be the best available guess for dose.

Subclassification according to observation period

Table 1. Characteristics of study cohorts.

	Group I, VCM/PVC production	Group II, reference group	Group III, PVC processing
Population (Germans + Austrians)	7.021	4.910	4.007
Man years	73.734	76.029	52.896
Follow-up completed till 12/31/74, % deceased	93.2	89.8	92.1
Observed	414	417	360
Expected	435	533	380
Unknown causes of death			
No.	30	47	47
%	7.3	11.3	13.1
Total mortality (SMR)	95	78	95
Foreigners (excluding Austrians)	882	711	1.454
Deceased	6	6	10

Table 2. Standardized mortality ratios.

		VCM/PVC production		Reference group		PVC processing	
ICD 8	Cause of death	Obs.	SMR	Obs.	SMR	Obs.	SMR
Total mortality		414	95	417	78	360	95
140–209	All malignant tumors	94	112	83	83	62	85
140–199	Malignant tumors of organs	79	103	77	83	60	89
200–209	Malignancies of lymphatic and hematopoetic tissues	15	214[b]	6	77	2	34
150–159	Malignant tumors of GI tract and peritoneum	45	149[a]	27	71	15	56
155	Malignant tumors of the liver	12	1523[b]	4	401[a]	3	434
191	Malignant tumors of the brain	2	162	2	184	5	535[b]
410–414	Ischemic heart disease	91	127[a]	115	131[a]	96	158[b]
410	Acute myocardial	66	114	83	120	69	143[b]
800–949	Accidents	61	137[a]	44	99	32	110

[a]Beyond 95% confidence interval (2).
[b]Beyond 99% confidence interval (2).

Table 3. Standardized mortality ratios by duration of exposure.

		Duration of exposure, months							
		< 12		13–16		61–120		> 121	
ICD 8	Cause of death	Obs.	SMR	Obs.	SMR	Obs.	SMR	Obs.	SMR
Total mortality		53	93	138	102	93	87	130	96
140–199	Malignant tumors of organs	6	74	20	88	22	116	31	115
200–209	Malignancies of lymphatic and hematopoetic tissues	1	92	4	186	5	287	5	249
150–159	Malignant tumors of GI tract and peritoneum	3	101	12	135	13	173	17	156
155	Malignant tumors of the liver	0	–	2	874[a]	3	1525[b]	7	2528[b]
191	Malignant tumors of the brain	0	–	0	–	1	350	1	278

Table 4. Standardized mortality ratios by period of observation.

ICD 8	Cause of death	Observation period: To 1959 Obs.	To 1959 SMR	1960–69 Obs.	1960–69 SMR	1970–74 Obs.	1970–74 SMR	Total Obs.	Total SMR
140–199	Malignant tumors	12	160	29	84	194	103	414	95
200–209	Malignancies of lymphatic and hematopoetic tissues	1	147	9	275[b]	5	168	15	214[b]
150–159	Malignant tumors of GI tract and peritoneum	8	270[a]	13	94	24	177[b]	45	149[a]
155	Malignant tumors of the liver	1	1282	3	834[a]	8	2264[b]	12	1523[b]
191	Malignant tumors of the brain	1	557	0	–	1	223	2	162

[a]Beyond 95% confidence interval.
[b]Beyond 99% confidence interval.

Table 5. Standardized mortality ratios by age.

ICD 8	Cause of death	Age group: ≤ 24 Obs.	≤ 24 SMR	25–34 Obs.	25–34 SMR	35–44 Obs.	35–44 SMR	45–54 Obs.	45–54 SMR	55–64 Obs.	55–64 SMR	≥ 65 Obs.	≥ 65 SMR	Total Obs.	Total SMR
Total mortality		10	68	45	111	65	104	90	101	105	80	90	103	414	95
140–199	Malignant tumors of organs[b]	0	–	4	141	13	188[a]	19	141	22	76	21	100	79	103
200–209	Malignancies of lymphatic and hematopoetic tissues	0	–	2	194	4	303	4	264	3	162	2	197	15	214[b]
150–159	Malignant tumors of GI tract and peritoneum	0	–	3	397	10	365[b]	10	145	10	89	12	140	45	149[b]
155	Malignant tumors of the liver	0	–	0	–	6	6865[b]	0	–	3	934[b]	3	1664[b]	12	1523[b]
191	Malignant tumors of the brain	0	–	0	–	0	–	1	254	1	362	0	–	2	162

[a]Beyond 95% confidence interval.
[b]Beyond 99% confidence interval.

Table 6. Groups for subdivision of laboratory examinations.

Group	Specification
A-I	VCM PVC production
A-II	PVC processing
B-I	VCM PVC production and PVC processing, work capacity loss < 20%
B-II	VCM PVC production and PVC processing, work capacity loss ≥ 20%
C-I	Germans and Austrians
C-II	Foreigners (Excl. Austrians)
D-I	Plants with high morbidity
D-II	Plants with low morbidity

Table 7. Bromsulfalein retention.[a]

	VCM/PVC Production	PVC Processing	Total
Retention normal	26	21	47
Retention abnormal	44	10	54
Total	70	31	101

[a]Chi square$_1$ = 8.09 ($p < 1\%$).

Table 8. Bromsulfalein retention.[a]

	Germans and Austrians	Foreigners	Total
Retention normal	17	30	47
Retention abnormal	33	21	55
Total	50	51	101

[a]Chi square$_1$ = 6.25 ($p < 2.5\%$).

Table 9. Bromsulfalein retention.[a]

	Work capacity loss < 20%	Work capacity loss > 20%	Total
Retention normal	41	6	47
Retention abnormal	32	22	54
Total	73	28	101

[a]Chi square$_1$ = 9.81 ($p < 1\%$).

(Table 4) reveals a rather inconsistent pattern: malignancies of the lymphatic tissues (ICD 200-209) are significantly elevated in the sixties only, whereas SMRs for tumors of the liver increase till the end of the study period. This might be referred to different latency periods for both kinds of malignancies, but other causes might likewise have contributed to these results. However, it has to be reported that the number of angiosarcomas confirmed histologically in the Federal Republic of Germany in patients previously exposed to VCM has actually come to 17 in contrast to mere 4 at the endpoint of the mortality study (12/31/1974).

The distribution of SMRs by age (Table 5) demonstrates an obvious susceptibility of males aged 35-44 for malignancies in general as well as for malignancies of the liver.

The data base for the German morbidity study consists of all of the reports of suspected cases of occupational disease due to VCM or PVC production or PVC processing. The reference population for these reports has to be defined as the total

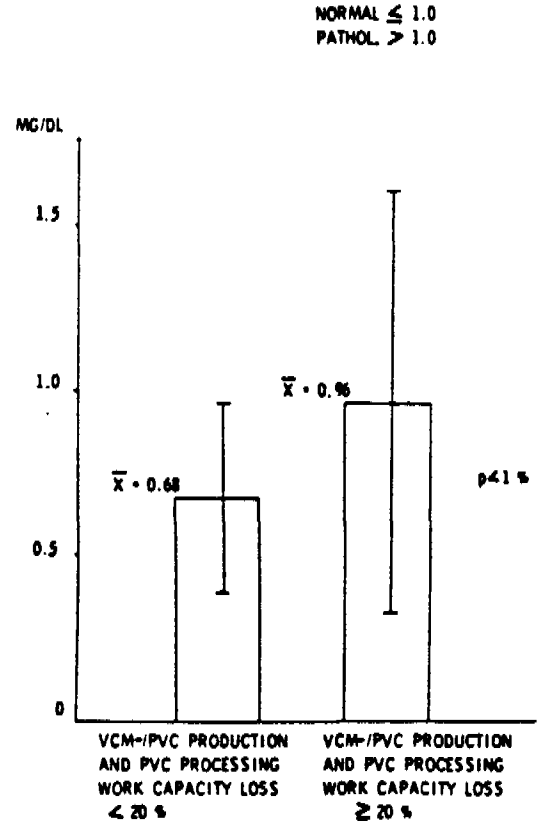

FIGURE 1. Vinyl chloride morbidity study: total bilirubin (normal 1.0).

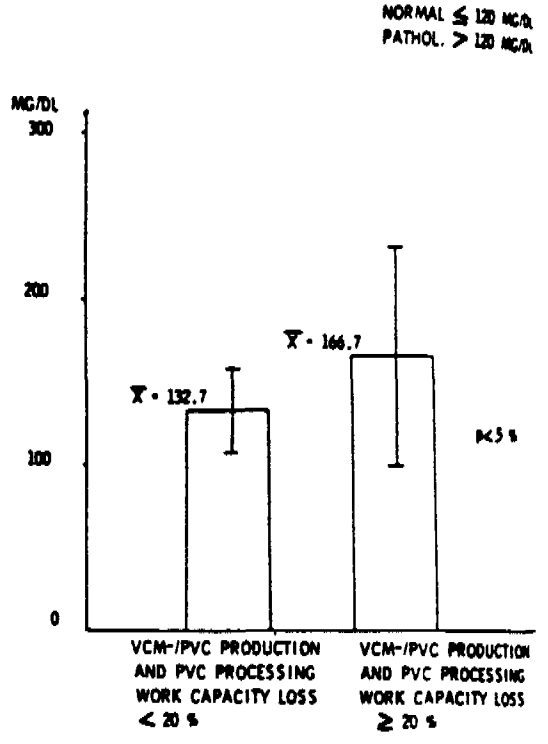

FIGURE 2. Vinyl chloride morbidity study: oral glucose tolerance test, 120 min after loading (normal 120 mg dl).

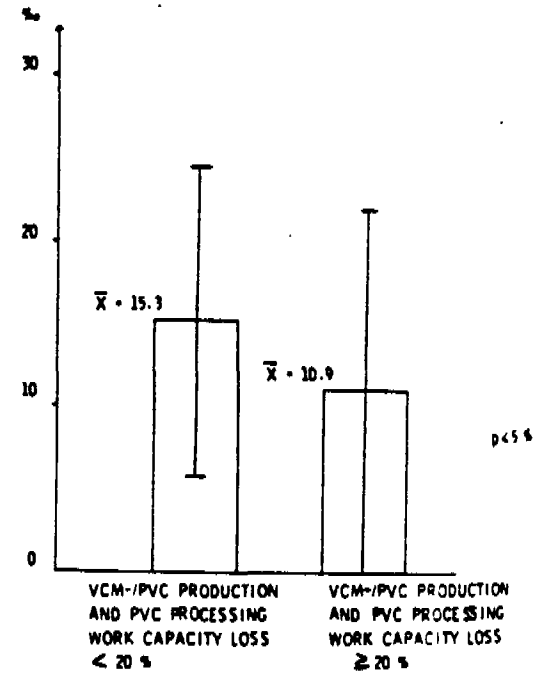

FIGURE 3. Vinyl chloride morbidity study: reticulocytes (normal 15‰).

working population in 1974 in the above mentioned branches, i.e., 6,500 workers in VCM or PVC production and 42,800 workers in PVC processing (as given by the German Association of Plastic Producing Industries). Till the end of 1974, 269 reports of suspected cases of occupational disease had been received. As there has been no consistent set of examinations performed on each of the cases, numbers of observations for various variables analyzed vary according to examination method performed. Insofar as the results of this study are of much lower validity than those of the mortality study, one should regard them as hints for further investigations.

Four attempts to subclassify observation on the 269 cases have been undertaken (Table 6). Only those results showing significant differences when applying *t*-tests or chi-square tests are so classified. Amazingly none of the more sensitive lab examinations of liver functions showed a marked difference in all of the subclassifications besides bromsulfalein retention. In this instance there is a significant difference when subdividing by VCM/PVC production versus processing (Table 7), as well as by nationality (Table 8) and most pronounced when subdividing by extent of work capacity loss (Table 9). This latter result, however, must be expected when an effect of exposure on liver function is anticipated. An impairment of the excretory liver function is suggested by elevated total bilirubin values in the subgroup with work capacity loss greater than 20% (Fig. 1). There seems to be an impaired glucose tolerance in this group, although observed in a small subsample only (Fig. 2), as well as a lower number of reticulocytes (Fig. 3). The thromocyte count in both groups was the same. These results, however, lead to no sensible interpretation, as all results attempts failed to standardize the methods applied for thrombocyte counts by various laboratories.

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Epidemiologic Study of Vinyl Chloride Workers: Mortality through December 31, 1972

by W. Clark Cooper*

A population of 10,173 men, employed in 37 plants, was identified as having worked for at least one year in jobs involving probable exposure to vinyl chloride monomer (VCM) prior to January 1, 1973. Of the 9677 men whose vital status was determined, 707 were known to have died. For 699, death certificates were obtained. The standardized mortality ratio (SMR) for all causes was 89, that for all malignancies was 104. The only type of malignancy found in significant excess was in the category of malignant neoplasms of the brain and other parts of the nervous system; 12 deaths occurred where 5.9 were expected, for an adjusted SMR of 203. There were slight but inconclusive upward trends in all malignancies, and for malignancies of the respiratory tract, digestive tract, and central nervous system associated with reported levels of maximum exposure to VCM. When groups in whom less than 20 years had elapsed from the first exposure were compared with those with 20 or more elapsed years, and 25 or more elapsed years, no significantly different SMR's were detected for major primary sites of malignancy. Plans for an updated study of mortality, to include deaths in the period 1973–1979 are briefly discussed.

The epidemiologic studies of vinyl chloride workers summarized in this report were carried out during the period June 15, 1973 through December, 1976 by Tabershaw-Cooper Associates, Inc., and Equitable Environmental Health, Inc., for the Manufacturing Chemists Association (MCA) (now the Chemical Manufacturers Association).

An initial report, dealing with 8,384 workers from 34 plants, was prepared May 3, 1974 (*1*). A summarized version (*2*) was published in 1974. The study population was subsequently increased and follow-up was improved. After an interim report in 1976 (*3*), a final report based on 10,173 workers was prepared in January, 1978 (*4*). In all of these studies the observation period ended December 31, 1972.

Participating Plants

In mid-1973, the MCA identified 43 plants in the United States, belonging to 19 companies, which either produced vinyl chloride monomer (VCM) or used it in the production of poly(vinyl chloride) (PVC). Of these, 34 were included in the initial study; four were excluded because they had been in operation less than 5 years, one had stopped production in 1966, and in others information on job histories or exposures was deficient. Three plants were subsequently added to the original 34, so the 1978 report included 37 plants. Of these, 11 produced only VCM, 18 produced only PVC, three produced both, and five plants produced homopolymers and copolymers, with or without VCM and PVC.

The geographical distribution of those in the study, as shown in Table 1, indicates a disproportionate number of workers from the South, particu-

Table 1. Geographical distribution of 10,173 vinyl chloride workers in 1978 report.

Region	U.S. males (1970), %	Workers in study, %
Northeast	23.8	25.9
North Central	27.9	18.4
South	30.9	64.8
West	17.4	0.9

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larly from the East South Central and West South Central States. The South, with 30.9% of the total U.S. male population in 1970, supplied 64.8% of the study population.

Of the plants participating, the one which had first begun operations with VCM had done so in 1935, the most recent was in 1968. Four plants had begun in 1942 or earlier, 13 in 1952 or earlier, and 27 in 1962 or earlier.

The Study Population

The objective was to include all male employees whose work had involved exposure to vinyl chloride for at least one year prior to December 31, 1972. The designation of jobs which involved exposure to VCM was made by staff members at individual plants or by a corporate industrial hygienist. In approximately two thirds of the study population, TCA staff copied personnel records on individuals who were identified as having been in exposed jobs. In the remaining portion of the population, detailed information on exposed individuals was provided by plant personnel. The methods used in doing this are described in the complete reports cited earlier.

Study Period

The period of time in which the work-force of a plant was included in the study depended upon the date it began making or using VCM and also upon the earliest date when personnel records were complete for all employees, if that was later than the foregoing. This was done to eliminate periods when there was differential record retention of workers terminated, deceased, or retired. The end of the study period was December 31, 1972.

Estimates of Exposure

In each plant, every job and location with VCM exposure was graded in terms of probable exposure. Originally, a job history form was designed in the expectation that the exposures could be quantified in parts per million. This proved impossible in practice. However, for each plant, jobs and locations involving the highest exposures could be classified as "high", and other jobs classified as "medium" or "low" relative to the "high". It is recognized that this subjective classification is of questionable validity in categorizing the past and present exposure of a given worker. From the number of months spent in jobs with classifications of 3 (high), 2 (medium) and 1 (low), a number of exposure categories were developed for use in later analyses. As shown in Table 2, individual exposures in various papers were classified in a number of ways, including the maximum level at which an individual had been exposed for at least 12 months, an integrated or cumulative exposure, and an exposure index based on the cumulative score divided by the number of months.

Table 2. Bases for development of VCM exposure categories

Criterion	Unit
Duration of exposed employment	Months
Interval from beginning of exposure to end of observation	Months
Estimated maximum level to which an individual was exposed for at least 12 months, classified as high, medium, or low.	High Medium Low
Integrated or cumulative exposure, crediting 1 for each month at low, 2 for each month at medium and 3 for each month at high exposure to VCM	1 2 3
Exposure index (EI) = Cumulative score/average number of months	

Follow-Up

As is customary in historical prospective studies, all who had left employment were traced when possible. Methods included form letters sent by mail and use of retail credit follow-up. For the first report, there was insufficient time to utilize Social Security Administration records, but for subsequent reports such follow-up was used.

In the first, or 1974 report, 85% of the study population was located; for the final or 1978 report, the percentage had been increased to 95%.

The mortality calculations were based only on those who were successfully traced, which is equivalent to assuming that mortality among those not found was the same as among those who were found. This usually, but not always, results in some overestimation of mortality.

Calculation of Standardized Mortality Ratios

Each worker in the study, i.e., everyone whose vital status was ultimately known, was considered to have been under observation from the date on which he attained a year of exposed employment or from the date when his plant's records were complete, whichever came later. Observation periods ended December 31, 1972, or on the date of death, whichever occurred first.

Observed deaths were classified by cause according to the 7th (1955) revision of the International

Classification of Diseases. The expected number of deaths by cause were calculated by using age and cause-specific mortality rates for United States males with the same birth years and age distribution for the years 1950, 1955, 1959, 1965, 1967 and 1970.

Deaths for which death certificates could not be obtained were assumed to have the same cause distribution as the death certificates that were obtained. Appropriate increases were made in calculated SMR's.

The statistical significance of the deviation of each SMR in the study population from an expected value of 100 was tested by a method derived from Chin Leong Chiang (5). The formula for determining the standard error of the SMR was

$$SE = \sqrt{\frac{100 \times SMR}{\text{No. expected deaths}}}$$

If an observed SMR differed from 100 by more than 1.96 standard errors, it was regarded as significant at the 5% level; if it differed by more than 2.57 standard errors, it was regarded as significant at the 1% level. SMR's based on fewer than five observed deaths were usually not tested for significance.

Results

Table 3 summarizes the numbers of individuals, success of follow-up, person-years and deaths in successive phases of the study. The number of deaths per 1000 man-years of observation, which in general reflects the age distribution of the work force, suggests that the proportion of older individuals increased in the study population as it was expanded and follow-up improved. Even so, the 5.56 deaths per 1000 man-years indicates that a relatively young population was being observed; the U.S. male population 20 and above has about 11 deaths per 1000 man-years. Some published occupational epidemiologic studies will show 20 or more.

Table 3. Numbers of individuals, success of follow-up, person-years, and deaths analyzed in successive phases of the study.

	Report 1, 1974	Report 2, 1976	Report 3, 1978
No. of men	8,384	9,109	10,173
No. found	7,128	8,714	9,677
% found	85%	96%	95%
No. deaths	352	525	707
No. certificates	328	511	669
Total man-yr	77,846	94,221	120,203
Deaths/1000 man-yr	4.52	5.57	5.88

Duration of Exposure to VCM

In the first report, only 15.2% of those studied had begun exposure prior to 1950 (22 years before end of the observation period). In the third report, 33.4% had had 20 or more years for observation since exposure began. This resulted from finding more early employees by improved follow-up, and the inclusion of an older group from one plant in the augmented population.

Standardized Mortality Ratios

Standardized mortality ratios (SMR's) for selected causes of death are shown in Table 4, based on the 352 deaths analyzed in Report 1 and the 707 deaths analyzed in Report 3. Results in the first report led Tabershaw and Gaffey (1) to conclude that vinyl chloride may be associated with cancer of a number of sites, notably digestive cancer, respiratory cancer, cancer of other and unspecified sites (primarily those of the central nervous system) and lymphomas. This was based not on statistically significant excesses in each category, but upon apparent trends when different levels and durations of exposure were compared.

With the enlarged study group, the SMR for malignancies in the entire population dropped slightly, as did the SMR's for malignancies of the buccal cavity and pharynx, digestive tract and respiratory tract. However, tumors of the brain and central nervous system, when examined separately, still appeared to be in excess.

A number of analyses were done in the third report in an attempt to sharpen the focus on work exposures.

There appeared to be a slight but definite trend in the SMR's for all malignancies, malignancies of the digestive tract, the respiratory tract, and for other and unspecified sites with increasing levels of estimated maximum exposure (Table 5). However, the numbers of expected deaths were relatively few in some categories and the groups differed widely in age distribution as manifested by deaths per 1000 person-years.

To reduce dilution of the study population by men whose exposures had begun only recently, a separate analysis was carried out on those whose exposures to VCM had begun 20 years or more prior to 1972, and on those whose exposures had begun 25 years or more prior to 1972 (Table 6).

Another analysis was made of a population of men who had worked in plants producing only PVC

(where VCM exposures were presumably high), whose exposures had begun 20 years or more before the end of the study period and who had been reported as having medium or high VCM exposures for a year or more (Table 7). This group experienced 210 deaths where 249.7 had been expected. The pattern of mortality from malignancies was not appreciably different from that of the total study group.

In summary, increasing the study population and

Table 4. Observed and expected deaths (O/E) and standardized mortality ratios for selected causes (SMR's adjusted for missing death certificates).

Cause (ICD Mo, 7th Rev)	1974 Report		1978 Report	
	O/E	SMR	O/E	SMR
All causes	352/467	75[a]	707/795	89[a]
All malignancies (140–205)	79/77	110	139/141	104
Buccal and pharynx (140–148)	5/2.84	189	5/5.19	102
Digestive (150–159(	19/21.7	94	29/40.8	76
Respiratory (160–164)	45/44.3	112	25/23.9	107
Other and unspecified (190–199)	17/11.75	155	28/20.2	147
Brain and CNS (193)	—	—	12/5.9	203[b]
Leukemia and aleukemia (204)	3/3.77	85	9/6.65	143
Lymphomas (200–203, 205)	6/6.06	106	11/10.36	112
Major cardiovasc. renal (330–334, 400–468, 592–594)	155/207	80[a]	347/385	95
Cirrhosis liver (581)	3/15.6	21	14/26.5	56[b]
No. of workers	7,128		9,677	
Person-yr	77,846		120,203	

[a]Significant at 1% level.
[b]Significant at 5% level.

Table 5. Observed and expected deaths (O/E) and standardized mortality ratios for selected causes as related to maximum level of reported exposure to vinyl chloride monomer (SMR's adjusted for missing death certificates).

Cause (ICD Mo, 7th Rev)	Reported maximum exposure VCM					
	Low		Medium		High	
	O/E	SMR	O/E	SMR	O/E	SMR
All malignancies (140–205)	65/71	98	56/53.5	109	18/16.5	112
Digestive (150–159)	14/20.9	72	10/15.6	67	5/4.4	117
Respiratory (160–164)	19/22.2	92	19/17.1	116	7/5.1	141
Other and unspecified (190–199)	11/9.9	119	13/7.5	180	4/2.7	150
No. of workers	4,925		3,021		1,731	
Person-yr	58,741		39,927		21,535	
Deaths/1000 person-yr	6.16		6.6		3.9	

Table 6. Analysis of deaths based on time from beginning of exposure to end of study period (SMR's adjusted for missing death certificates).

Cause of death (ICD No. 7th revision)	< 20 yr		> 20 yr		> 25 yr	
	No.	SMR	No.	SMR	No.	SMR
All causes	158	77[a]	549	93	393	96
All malignancies (140–205)	31	95	108	107	73	104
Digestive (150–159)	8	96	21	70	16	74
Respiratory (160–164)	8	80	37	116	22	100
Other and unspecified (190–199)	6	108	22	162	13	146
Leukemia (204)	3	155	6	137	4	137
Cardiovascular-renal	68	84	279	97	211	105

[a]Significant at 1% level.

improving follow-up did not strengthen the suggested associations between VCM exposure and malignancies other than those caused by hepatic angiosarcoma, as will be pointed out later, and a suggested association with tumors of the brain and central nervous system.

Table 7. Deaths in plants producing only PVC, based on workers whose first exposures began before 1952, and who had medium or high VCM exposures (SMR's corrected for missing death certificates).

Cause of death (ICD No. 7th revision)	O/E	SMR
All causes	210/249.7	84[a]
All malignancies (140–205)	46/46.04	106
Digestive (150–159)	9/13.63	70
Respiratory (160–164)	17/14.75	122
Other and unspecified (190–199)	10/ 6.33	167
Angiosarcoma of liver (581)	6/7.95	80

[a] Significant at 1% level.

Angiosarcomas

Nine angiosarcomas are known to have occurred in the U.S. during the study period, i.e., prior to 12/31/72. As shown in Table 8, eight of these were found in the study, but only three were coded as angiosarcoma on the death certificate. However, four others were coded as tumors of the digestive tract. Unfortunately, two were coded 230x so as to fall out of the category for malignant tumors of the GI tract, and one was coded as cirrhosis of the liver.

As shown in Table 8, the angiosarcoma which was not found was in a man who had died in 1961. We have not determined how he failed to be in the study population. The years of exposure for the eight cases ranged from 4 to 23 years, while elapsed time from beginning of exposure to death ranged from 16 to 24 years.

Tumors of the Central Nervous System

The 12 tumors of the brain had been diagnosed on death certificates as follows: glioblastoma multiforme, 4 (1 confirmed by autopsy); astrocytoma, 2 (2 au-

Table 8. Angiosarcoma deaths and data on VCM exposures.

ICI No.[a]	First exposed	Year of death	Time from first exposure to death, yr	Total yr exposure	Est. max. exposure	Age at death
USA-02	1955	1971	16	14	High	38
-04	1949	1968	19	18	High	43
-05	1944	1964	20	20	High	52
-07	1944	1968	24	14	Med	45
-10	1946	1970	24	23	Low	70
-11	1951	1968	17	17	Med	60
-12	1949	1969	20	20	High	50
-16	1950	1969	19	4	High	41
-08	—	1961		Not in study group		

[a] Number used in registry periodically prepared by J. Stafford, Imperial Chemical Industries Ltd., Plastics Division.

Table 9. Summary of brain tumor deaths (ICD No. 205) and data on VCM exposures.

Case no.	First exposed	Yr of death	Time from 1st exposure to death, yr	Total yr exposure	Max. exposure	Age at death
1	1958	1972	14	5	Low	67
2	1967	1972	5	5	Low	43
3	1957	1968	11	7	Med	54
4	1941	1958	17	6	Med	61
5	1950	1970	20	8	High	43
6	1956	1971	15	3	Med	54
7	1947	1971	24	23	Low	57
8	1945	1963	18	18	High	44
9	1949	1971	22	21	Low	58
10	1947	1971	24	23	High	49
11	1935	1956	21	18	Low	59
12	1935	1967	32	22	Low	57

topsies); ependymoma of the 4th ventricle (autopsy); "malignant brain tumor" or "carcinoma of the brain," 5 (with no autospies). This group is currently being made the subject of more rigorous review. The information obtainable from our records, summarized in Table 9, is insufficient to prove or disprove a cause-and-effect relationship between occupational exposure and these tumors.

Conclusions

A study of 707 deaths in a population of 9677 men who had worked for one year or more in jobs involving exposure to vinyl chloride and whose vital status had been determined as of December 31, 1972, did not show a significant excess of deaths due to malignancies. There did appear, however, to be a significant excess of tumors of the brain and central nervous system, based on 12 such deaths. There also continued to be slight but inconclusive trends toward higher SMR's for deaths from digestive tract and respiratory tract tumors associated with maximum levels of past exposure. No striking changes in malignancy patterns were apparent when analyses were directed toward individuals in whom 20 to 25 years had elapsed since first exposure. The results suggest that, except for a proven association with hepatic angiosarcoma and a strongly suggestive association with central nervous system tumors, vinyl chloride probably is not associated with significant excess cancers of other sites.

It should be emphasized that the epidemiologic study summarized in this report was planned, the populations defined, and analysis under way before cases of hepatic angiosarcoma had been diagnosed in workers exposed to vinyl chloride (*6*). An update is scheduled with inclusion of additional deaths in the cohort during the years 1973 through 1979. The study can be improved by a separate analysis of data from the plants which began operations before 1960, and by separating, insofar as possible, exposures to vinyl chloride monomer, polyvinyl chloride, and various copolymers. It is also hoped that criteria for defining exposure and for rating levels of exposure can be improved to permit better indices of integrated exposure.

This study was begun June 15, 1973 under a contract between the Manufacturing Chemists Association, 1825 Connecticut Avenue, N.W., Washington, D.C. (now the Chemical Manufacturers Association) and Tabershaw/Cooper Associates, Inc. It was continued under later contracts with TCA and with Equitable Environmental Health, Inc.

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Epidemiology of Hepatic Angiosarcoma in the United States: 1964-1974

by Henry Falk,*† John Herbert,* Steven Crowley,* Kamal G. Ishak,‡ Louis B. Thomas,** Hans Popper § and Glyn G. Caldwell*

A nationwide survey of hepatic angiosarcoma (HAS) in the United States during the years 1964 through 1974 identified 168 cases. Of these, 42 cases (25%) were associated with known etiologic factors, such as vinyl chloride monomer exposure during preparation of poly(vinyl chloride), use of Thorotrast in angiography, exposure to inorganic arsenic, and treatment with androgenic-anabolic steroids; 126 cases (75%) are of uncertain etiology. HAS most often affects males (ratio of approximately 3:1), peaks in the sixth and seventh decades of life (somewhat earlier than other sarcomas of the liver) and appears to occur more often in the industrialized Northeast and Midwest (although reporting artifact may be a factor). There is an extraordinary relative risk for poly(vinyl chloride) polymerization workers; there may also be other chemical-industrial associations that require further investigation. Prospective epidemiologic studies of HAS should be considered as a means of identifying other causative factors (e.g., chemicals or drugs) related to HAS.

Introduction

This report is an overview of the nationwide hepatic angiosarcoma (HAS) case-finding study for the years 1964-1974 conducted by the Centers for Disease Control (CDC).

At the start of this study, three causative factors had been identified for HAS: vinyl chloride monomer (VCM) (*1, 2*), Thorotrast (*3*), and inorganic arsenic (*4*). The recently discovered association between VCM and HAS has served as a stimulus for this investigation, and multiple epidemiologic studies of polyvinyl chloride (PVC) polymerization workers exposed to VCM have since demonstrated very high relative risks for the development of HAS (*5, 6*). Thorotrast is a colloidal suspension of thorium dioxide, a radioactive alpha-emitter with markedly prolonged radiologic and biologic half-lives, which was used for carotid angiography and liver-spleen scans in the period 1930-1955. The thorium dioxide is sequestered by the reticuloendothelial system, primarily in the Kupffer cells of the liver; radiation injury to adjacent cells is the presumed carcinogenic mechanism. Epidemiologic studies of Thorotrast recipients have shown very high relative risks for the development of HAS as well as hepatocellular tumors (*7, 8*). The association between arsenic and HAS is based on data from several small autopsy series in German vintners in the 1940s and 1950s (*4, 9*), which demonstrated an increased incidence of liver disease, including HAS. These workers were exposed to inorganic arsenical pesticides during application of the pesticide and also by drinking beverages prepared from the skins of the sprayed grapes. Subsequently, HAS cases were reported following long-term ingestion of Fowler's solution (potassium arsenite) (*10, 11*) and

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arsenic-contaminated well water (*12*). Individual cases suggesting associations between HAS and hemochromatosis (*13*) and copper exposure (*14*) have also been reported.

Earlier reports from this study reviewed cases associated with single causative factors (*15-18*), including androgenic-anabolic steroids which we feel are implicated as a fourth cause of HAS (*19*). In this paper, we present an overview of the case-finding effort, placing the known causative factors and cases of idiopathic origin in perspective.

Methods

Information relating to cases of HAS occurring in the United States during the years 1964 through 1974 was solicited by CDC in a variety of ways: (1) announcements were placed in seven medical journals; (2) a mailing was sent to all pathologists in the country; (3) separate mailings were sent to all state epidemiologists, major tumor referral centers, and statewide tumor registries; (4) a death certificate review for International Classification of Diseases (ICD), Eighth Revision, Code 197.8 (liver tumors, unspecified primary or secondary) for the period 1966-1973 was conducted with the assistance of the National Center for Health Statistics (NCHS) and the 50 state health departments (Code 155.0, primary liver tumors, was not reviewed because it would have been impractical to obtain the much larger number of certificates in this category and because it was felt that the majority of cases of HAS listed as such on the death certificate would have been coded as 197.8); (5) arrangements were made with the Armed Forces Institute of Pathology (AFIP) to include cases in their files (1943-1975) in the review; (6) a number of cases were identified from industrial surveys of PVC polymerization workers and others potentially exposed to VCM; and (7) permission was requested to include the previously published cases of HAS occurring in 1964-1974.

The evaluation procedure for each identified case was as follows: Initially, permission was requested to review the appropriate pathologic specimens (all submitted non-AFIP case material was reviewed at the National Cancer Institute's Laboratory of Pathology by H. P. and L. B. T.). Following confirmation of the diagnosis, the local physician was notified, and, with his permission, the nearest of kin was identified and contacted. Consent was obtained to review medical records, and a questionnaire was administered by telephone to obtain detailed occupational, residential, and chemical exposure histories. In selected instances, friends, employers, previous physicians or others were also interviewed.

In all, approximately 350 submitted cases were reviewed by the pathology review group, some outside our requested time period of 1964-1974. Pathologic specimens were obtained for review in approximately 95% of cases, many of which were considered by our pathology panel not to be HAS. Since survival after diagnosis is very brief and many cases were only diagnosed at autopsy, we have included all 168 confirmed cases with death during 1964-1974 in the study group.

The above effort is essentially a case review. Subsequently, the 22,432 death certificates obtained from the review of Code 197.8 were used to carry out a case-control study of occupation as recorded on the death certificate in the following manner. Death certificates were sought for all 168 confirmed HAS cases; 166 were available. Controls were sought from among the 22,432 death certificates in ICD Eighth Revision Code 197.8 (liver tumor, unspecified primary or secondary) that had been obtained in the HAS case-finding effort (see above). certificates of confirmed HAS cases were excluded from the control selection process. Although deaths in Code 197.8 might not represent the ideal control group, the rationale was that these deaths resulted from a broad variety of tumors of multiple sites metastatic to the liver or represented cases which were diagnostically uncertain; it was therefore unlikely that any single diagnostic category would predominate or that the occupational listings would be heavily biased by any single group of cases.

Up to four controls (as many as were available) were matched to each case ≥ 30 years of age on the basis of the following criteria: age (±6 years), sex, race, county of residence, and year of death (±3 years). One hundred thirty-five cases were successfully matched (79 of these had 4 matched controls; the overall ratio of controls to cases was approximately 3:1). Individuals who could not be matched were primarily young females and residents of sparsely populated counties.

A combined occupation and industry coding scheme was developed to accommodate all listings recorded on the certificates and to group listings on the basis of potential hazardous exposures. The data were analyzed by Rothman's method for matched groups with multiple controls per case (*20*).

Results

Our study identified 168 deaths from confirmed HAS during the years 1964 through 1974. For this rare tumor, the best sources of case identification were the pathologists, with the largest number of cases identified through the single mailing to all pathologists and the second largest number of cases

identified through pathology referral centers (particularly the AFIP).

Table 1 summarizes the results of the death certificate review portion of the case-finding effort and points up some of the inadequacies of using this method in epidemiologic studies of uncommon tumors. Only 42% of the cases initially identified on the death certificate as HAS were confirmed as such on pathologic review; 50% of the cases were confirmed not to be HAS. Furthermore, only 23% of all the total cases in our study during the years 1966-1973 would have been identified by the death certificate search alone. It can be seen that an epidemiologic study based only on a death certificate search of Code 197.8 would have been quite inadequate.

Figure 1 compares age, race and sex data for the confirmed HAS cases in our study with 131 cases of non-angio hepatic sarcoma identified in the death certificate survey (these latter cases were not confirmed by pathologic review). HAS has a striking male preponderance of approximately 3:1; this is not true for other hepatic sarcomas. HAS also appears to occur more often in younger age groups.

The higher proportion of males than females with HAS first appears in the 40- to 49-year-old group (Fig. 2) and is associated with a younger peak age for males than females, although the mean age for female HAS cases (50.1 years) is lower than that for males (57.9 years). These findings are not solely related to the VCM-induced cases among polymerization workers. The male preponderance is also present in the Thorotrast, arsenic, and androgenic-anabolic steroid associated cases, as well as in the idiopathic cases (Table 2). Cases with known etiology, however, have a younger age distribution than idiopathic cases (Table 3), with a mean age of 51.0 years compared to 57.6 years for the idiopathic cases.

Table 4 presents the 168 cases of HAS by year of death and etiologic status. The VCM-, Thorotrast- and androgenic-anabolic steroid-associated cases were more common during the latter part of the study

Table 1. HAS cases, CDC survey: death certificate review—code 197.8 (8th revision ICD), 1966–1973

Length of review: 7.5 years (1966–1971, 1973, 1/2 of 1972)	
Total number of death certificates reviewed	22,432
Sarcomas of the liver, death certificate diagnoses:	
Angiosarcoma (HAS)	74 (36.1%)
Leiomyosarcoma	24 (11.7%)
Fibrosarcoma	14 (6.8%)
Sarcoma (type unspecified)	64 (31.2%)
Sarcoma (other types)	29 (14.1%)
Total	205
Final pathologic diagnoses of 74 cases identified on death certificate as HAS	
Confirmed HAS	31 (42%)
Confirmed not HAS	37 (50%)
No pathology available	6 (8%)

Table 2. HAS cases, CDC survey, 1964–1974: sex ratios, etiologic categories.

	Male	Female	Ratio
Vinyl chloride	12	0	(12 : 0)
Thorotrast	15	5	(3 : 1)
Arsenic	4	2	(2 : 1)
Androgenic-anabolic steroids	3	1	(3 : 1)
Subtotal	34	8	(4.3: 1)
Idiopathic	93	33	(2.8: 1)

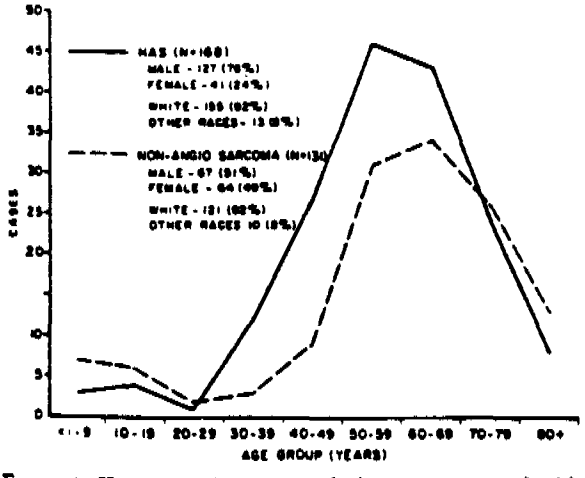

FIGURE 1. Hepatic angiosarcoma (HAS) cases compared with non-angio hepatic sarcoma cases, by age group: U.S., 1964-1974.

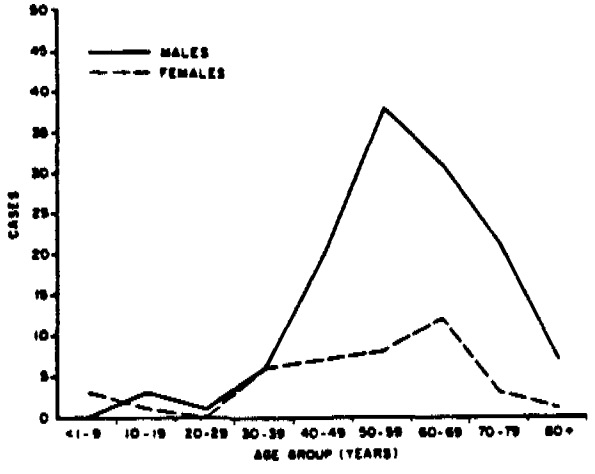

FIGURE 2. Hepatic angiosarcoma cases, by age group and sex: U.S., 1964-1974

Table 3. HAS cases, CDC survey, 1964–1974: age distribution for cases of known cause and for idiopathic cases.

Age, yr	Known etiology No. %	Idiopathic No. %
0–9	1 (2)	2 (2)
10–19	0 (–)	4 (3)
20–29	0 (–)	1 (1)
30–39	4 (10)	8 (6)
40–49	8 (19)	19 (15)
50–59	22 (52)	24 (19)
60–69	4 (10)	39 (31)
70–79	3 (7)	21 (17)
≥80	0 (–)	8 (6)
Total	42	126

period. For the VCM- and androgenic-anabolic steroid-associated cases, this is due to the relatively recent introduction of these causative agents. For the Thorotrast-induced cases, however, this pattern was an unexpected finding. Based on the originally calculated latent period of approximately 20 years (*21*), the general impression was that such cases would be diminishing by 1975. However, the number of cases appeared to still be increasing as of 1974, apparently due to an increase in cases having relatively low-dose angiographic procedures and prolonged latent periods (*16*). The arsenic-associated cases (including five adults with a history of prolonged use of Fowler's solution and one child with environmental exposure) occurred primarily during the earlier years of the study and may represent the tail end of a larger problem in previous years. The number of idiopathic cases was low during the first few years of the study (perhaps related to poor recall by pathologists, and to the fact that the death certificate search started with 1966). Case numbers, however, remained steady for most of the study period except for a spurt in 1974. The latter occurred right after the original report and the attendant publicity of the first cases of VCM-induced HAS (January 1974) and may be related to improved diagnostic evaluation. Review of the idiopathic cases occurring in 1974 demonstrated a large number of elderly cases with no evidence of clustering in a particular exposure setting.

The HAS mortality rate for the entire United States during the study period was 0.75 cases per 10^7 population per year. Crude mortality rates by region, based on county of residence at time of diagnosis (Table 5), suggest a somewhat higher incidence in the Northeast and in the industrial portions of the Midwest. There was also an increase in the mountain region, although this is based on a very small number of cases. The rates were low in farming states and in the South; however, since

Table 4. HAS cases, CDC survey, 1964–1974: distribution by causative factors and year of death.

	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	Total
Idiopathic	4	10	9	13	12	13	11	12	13	11	18	126
Vinyl chloride	1	–	–	–	3	2	1	1	–	3	1	12
Arsenic	1	–	1	2	1	–	–	–	–	1	–	6
Thorotrast	–	–	–	1	2	2	3	2	4	1	5	20
Androgenic-anabolic steroids	–	–	–	1	–	–	–	1	–	1	1	4
Total	6	10	10	17	18	17	15	16	17	17	25	168

Table 5. HAS cases, CDC survey, 1964–1974: distribution by region.

Region	States	Pop. 1970	No. of cases (HAS/10^7/yr.)	No. of idiopathic cases (idiopathic HAS/10^7/yr.)
1. New England	ME, NH, VT, MA, RI, CT	11,847,186	12 (0.92)	9 (0.69)
2. Mid-Alantic	NY, NJ, PA	37,152,813	36 (0.93)	28 (0.69)
3. E.N. Central	OH, IN, IL, MI, WI	40,252,678	35 (0.79)	30 (0.68)
4. W.N. Central	MN, LA, MO, ND, SD, NB, KS	16,324,389	10 (0.56)	9 (0.50)
5. S. Atlantic	DE, MD, DC, VA, WV, NC, SC, GA, FL	30,671,337	23 (0.68)	15 (0.44)
6. E.S. Central	KY, TN, AL, MS	12,804,552	7 (0.50)	1 (0.07)
7. W.S. Central	AR, LA, OK, TX	19,322,458	12 (0.56)	10 (0.47)
8. Mountain	MT, ID, WY, CO, NM, AZ, UT, NV	8,283;585	10 (1.10)	7 (0.77)
9. Pacific	WA, OR, CA, AK, HI	26,525,744	21 (0.72)	17 (0.58)
Total		203,184,742	168 (0.75)	126 (0.56)

many cases are diagnosed only after referral to major hospital and tumor centers, it is possible that the rural rates could be artifactual.

To summarize this portion of the study, it is apparent that HAS has a strong male preponderance, that it appears to occur more often in younger age groups than other sarcomas of the liver, and that there may be geographic differences which are unrelated simply to the distribution of VCM associated cases (the largest number of which were in Kentucky and West Virginia). As part of the follow-up effort we looked at occupation in the death certificate case-control study. Table 6 shows that the case-control ratios were elevated only for the last two occupational categories. The first of these (#7) shows a highly significant difference for chemical workers that is entirely related to exposure to VCM: all 10 cases were PVC polymerization workers. The last category (#8) combined a large number of laboring groups with potential exposure to a variety of chemicals; included are laborers, machinists, maintenance workers and others. The association of this heterogeneous grouping of occupations with HAS is not statistically significant, nor in reviewing the individual occupational data from the interviews with family members were we able to identify a specific chemical, plant, or process as a causative factor. Nevertheless, taken together with the earlier findings, this association suggests that some chemical exposure or industrial factors may be related to a portion of the idiopathic cases. Such an association would not be unreasonable; machinists, e.g., are potentially exposed to trichloroethylene (an experimental hepatocarcinogen that is structurally related to vinyl chloride), inorganic arsenic and nitrosamines (which have been identified in cutting fluids and are a known cause of HAS in animals) (*22, 23*).

Discussion

A particularly intriguing feature of HAS is that there are now four probable causes of this tumor, and yet about 75% of the cases are of uncertain etiology. Given that the HAS cases associated with the four quite distinct etiologic factors (VCM, Thorotrast, arsenic, and androgenic-anabolic steroids) share a common morphologic progression to HAS which is indistinguishable from that of idiopathic cases (*24*), it is likely that additional causal factors are (or will be) associated with HAS. In this study, the male preponderance, the younger age distribution, the possible geographic relationship with industrial portions of the country, and the suggestion from the death certificate case-control study that groups with chemical exposure may be at higher risk, raise the possibility that some of the idiopathic cases may be related to presently unidentified environmental or occupational exposures.

This study and that of Baxter et al. (*25*) confirm the limited diagnostic reliability of death certificate diagnosis of HAS and the need to assess a variety of

Table 6. Occupation as recorded on death certificate, case-control study, CDC HAS survey, 1964–1974.

Occupation	Cases (N - 135)	Control (N = 421)
Professional, technical, engineers	16	40
Managers, administrators	5	23
Sales, wholesale/retail trade	3	29
Clerical	9	13
Communications	1	5
Finance, insurance, real estate	2	4
Business, store owners, proprietors	5	11
Service workers	8	36
Housewife, homemaker	18	58
Military, government	2	3
Unlisted, retired, disabled	5	12
	74 (55%)	234 (56%)
Agricultural (farming, stock, feed, fisherman)	4 (3%)	23 (5%)
Mining	1 (1%)	8 (2%)
Transport	4 (3%)	21 (5%)
Carpenters, craftsmen	2 (1%)	9 (2%)
Manufacturing (unspecified, untitled, other)	2	13
Lumber, logging, wood products	0	3
Metal, steel	0	5
Food, beverages, tobacco, packing	2	6
Textiles	3	9
Paper, printing	1	5
Rubber/plastics	1	2
Automobile	0	3
	9 (7%)	46 (11%)
Chemical	10 (7%)	1 (0%)
		$p = 4.29 \times 10^{-7}$[a]
Machinist-metal cutter	7	14
Machine operators	0	8
Forgers, molders, casters	1	3
Painters	1	4
Sanitation, water works, power	3	2
Maintenance, custodians	5	9
Laborers	8	20
Construction	1	2
Heavy equipment operators	2	6
Repair services	1	5
Mechanics	2	6
	31 (23%)	79 (19%)
		$p = 0.11$[a]

[a]Statistical analysis for case-control study with multiple matches (*20*).

sources of data in order to identify cases for studies of rare tumors such as HAS.

The Thorotrast data are instructive because the initial estimate of the latency period has lengthened with time; recent cases have been in individuals who had relatively low-dose procedures but prolonged latent periods (*16*). This accentuates the need to continue evaluation of VCM exposed groups for the possible appearance of a similar pattern. The time frame covered by our study is perhaps too early to detect a sizable number of cases with relatively low VCM exposure either in occupational or environmental settings. In addition, Thorotrast-induced HAS was noted approximately 10 years before Thorotrast-induced hepatocellular tumors were recognized; given the identical morphologic appearance after VCM exposure, involving mixed hyperplasia of sinusoidal cells and hepatocytes, and the occurrence of hepatocellular tumors in experimental animals exposed to VCM (*26*), we need to continue to follow-up VCM-exposed cohorts for the possible appearance of hepatocellular tumors.

Cases of HAS associated with various factors have previously been reported (*14*, *27*, *28*); individual cases associated with hemochromatosis, prior radiotherapy, chloroprene exposure and chemotherapy with urethane were also noted in this study, but we have not discussed them in detail here.

It would appear valuable to continue epidemiologic studies of this rare tumor. This might enable early detection of additional etiologic factors for this disease, such as the various structural analogs of vinyl chloride (e.g., vinyl bromide and vinylidene chloride) which have had industrial use. However, because of the difficulty and time involved in establishing adequate surveillance for rare tumors such as HAS or mesothelioma, it would be best to establish a single framework for studying a number of rare tumors or marker illnesses at once. One would then be able, e.g., to survey pathologists or other groups at a single time to obtain information on a variety of such conditions.

HAS is a rare tumor with at least four probable causes and undoubtedly others that have not yet been identified. Ascertainment of the range of causative agents for HAS, as well as for more common tumors, presents a challenge to epidemiologists.

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The British Hepatic Angiosarcoma Register

by Peter J. Baxter*

A register of British cases of primary hepatic angiosarcoma (HAS) was established in 1974 to monitor the occurrence of cases from 1963 onwards. Details of cases dying in 1963-77 have been obtained. Thirty-five cases were agreed as HAS by a panel of liver pathologists, and occupational and medical information was obtained in the majority of these. Two cases were attributable to VCM exposure, and eight others had received intra-arterial Thorotrast. In 1978-79, two more cases were confirmed in VCM polymerization workers.

Introduction

In 1974, the Health and Safety Executive embarked upon a mortality study of all workers in Great Britain who had been exposed to vinyl chloride in the manufacture of vinyl chloride (VCM) or poly(vinyl chloride) (PVC), and a register of British cases of primary hepatic angiosarcoma (HAS). The mortality study embraced over 7000 people, and an analysis of deaths up to the end of 1974 has been published (*1*). The main finding was four certified cases of liver cancer, two of which were histologically confirmed HAS. An update of this analysis has been postponed while, with the collaboration of the factories concerned, exposure data are being refined to take into account job changes and exposure to PVC dust. A mortality study of PVC fabricators was considered, but remains in the planning stage. The register is also the basis for a case-control study of occupation for those cases occurring from 1974 onwards (*2*).

To determine the annual occurrence of HAS and to monitor its incidence, cases dying in 1963-73 were reviewed as well as those occurring after this period. Information on cases for the 15 years 1963-77 has now been obtained and will be outlined here. Further details may be found elsewhere (*3*, *4*).

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Methods

Cases were identified by a search of death certificates in seven appropriate categories of the International Classification of Diseases for a diagnosis of HAS (or one of its synonyms). Hospital pathologists and cancer registries were requested to report cases, as were the medical departments of factories manufacturing VCM or PVC. Some cases were identified from publications. When available, histological material was sought for all the reported cases and submitted to a panel of three liver pathologists (Drs. P. P. Anthony, R. N. M. MacSween and P. J. Scheuer), who reviewed the slides independently and without knowledge of the prior diagnoses. For control purposes, histological material from other liver cancers was included. For those cases agreed by the panel as HAS, the full medical records were sought and further information, for example, occupational histories and alcohol consumption, was obtained when possible by interview with the next of kin.

Results and Discussion

Eighty-eight cases were reported from all sources. The panel agreed the diagnosis in 32 cases and three more were agreed from among the controls. In half of the confirmed cases the diagnosis had been recorded on the death certificate, but the remainder would have been missed if reliance had been placed upon death certificates as the sole method of ascertainment. At least some clinical and

necropsy details were obtained in all but two patients. Occupational histories were obtained in 91%, the occupation recorded on the death certificate being accepted for the remainder.

The annual numbers of both reported cases and those agreed by the panel increased after 1975, so that in 1977, 12 cases were reported, 10 of which were agreed by the panel. Between 1968 and 1977 an average eight cases per annum were reported and three per annum were agreed by the panel, the population of Britain during this period being about 50 million. The observed increase in incidence was almost entirely due to 10 cases of known etiology; eight were patients who had received intra-arterial Thorotrast (a colloidal suspension of thorium dioxide), and two others had been VCM polymerization workers. Plotting on a map the last places of residence of all 35 agreed cases revealed a cluster of six Thorotrast cases in or around Edinburgh, where the use of Thorotrast in neurological diagnosis had been greater than at any other British center. One hundred and nine patients who had received intra-arterial Thorotrast in Edinburgh during 1933-48 have been followed up in detail (*5*): about three quarters of these patients have died, 13 (17%) from liver tumors. Histological material for review by the panel was available in nine of these. In those cases dying in earlier years, the tumors were predominantly cholangiocarcinomas, but later cases were all HAS, confirming that the recent emergence of Thorotrast-induced HAS in Britain is a real increase in incidence and not an artifact due to under-reporting of cases occurring in the past.

Twenty-eight of the 35 cases were males; one female infant died aged 8 months. For idiopathic cases, the male:female ratio was 4:1. Adult cases were assigned to one of the Registrar General's five social classes according to the occupation on the death certificates. The social class distribution was unremarkable, except that the only cases in social classes one and two were four men, all of whom were designated as electrical engineers. In fact, six out of the 28 males had worked in the electrical industry at some time, a figure in excess of any other industry. Three other men had been employed in workplaces where PVC was fabricated, but the occupational histories were inadequate to confirm that these men had worked with PVC. One other male case had lived for six years before his death within a half mile of a plant manufacturing PVC. Occupational exposure to arsenic did not appear to be a factor in any of these cases. Arsenical drugs were commonly prescribed in Britain in the past and, in a few areas, even until the early 1960's. However, one case only was suspected to have clinical evidence of chronic arsenical intoxication, but nail and hair analyses for arsenic were negative and the medical records were inadequate for verifying the use of arsenical drugs. None of these cases was recorded to have taken androgenic steroids (*6*), but one woman had taken an estrogen preparation for several years. Alcohol did not appear to be an important factor in the majority of cases, only four men having a history of heavy consumption.

In its age distribution, clinical presentation and prognosis, HAS resembled primary liver carcinoma (*4*). Upper abdominal pain and a hepatic mass were the commonest presentation, and extrahepatic metastases were found in 23% of cases only. Excluding the Thorotrast cases, and for men only, the median interval between the onset of symptoms and death was six months for those aged under sixty years, and only six weeks for those aged sixty and over, a difference which was statistically highly significant. Hemochromatosis was not evident in any of the cases.

In 1978-79, two more cases of HAS have been confirmed in polymerization workers, and another case has led to speculation that hydrazine derivatives, such as phenelzine, may induce angiosarcoma in man (*7*). It is too early to predict the full impact of past industrial exposure to VCM in Britain and at least until the picture becomes clearer the monitoring of HAS should continue.

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Effectiveness of Federally Required Medical Laboratory Screening in the Detection of Chemical Liver Injury

by Carlo H. Tamburro* and Richard Greenberg*

The increasing concern of industrialized societies over the potential health hazard of synthetic chemicals in the occupational environment has led to goverment requirements for medical laboratory screening of workers. The specific tests for such screening programs are most often selected on the basis of medical experience which utilized them in symptomatic or hospitalized populations. Required screening tests for hepatic injury including cancer in vinyl chloride workers has been systematically and prospectively studied in an industrial population working with synthetic rubber and plastics. Approximately 1300 employees were studied over a five-year period. A cohort of 969 male employees, for the purposes of analysis, were divided into a "standard" and "nonstandard" population based upon the absence or presence of significant medical disease (including liver disease). A subcohort of 120 individuals was further identified based on availabiliity of liver biopsy. Evaluation of federally required studies included alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGTP), alanine aminotranserase (ALT, SGPT), aspartic aminotransferase (AST, SGOT) and bilirubin (BR). Also studied were indocyanine green clearance (ICG) and radioisotopic liver spleen scans (L-S scans). The GGTP provided the highest positive predicted value as a screening test for identifying "nonstandard" individuals (individuals with all types of medical disease) followed by ICG, AST, ALT, L-S scan, AP, and BR.

In the identification of asymptomatic liver disease the GGTP had the least specificity due to a high false positive rate, while the AP provided the highest specificity. The ICG clearance however, provided the best combination of positive predictive value and sum of specificity and sensitivity. The AP provided additional increase in specificity as a follow-up study. There was no evidence that any of the other federally required tests added any additional benefit and did add significant increase in the false positive rate. These studies support the need for evaluating screening tests as to their sensitivity, specificity and positive predictive value, in asymptomatic individuals, before they are made established requirements.

Introduction

Industrialized societies throughout the world have become increasingly concerned over the potential health hazard of synthetic chemicals in the occupational environment. Governmental regulations have increased the number and types of medical laboratory screening required for a large variety of halogenated hydrocarbons as well as other potential environmental hazards. The primary objective of these screening programs is to reduce disability, morbidity and mortality in workers, especially as related to serious low-grade health hazards. In general, screening programs are instituted because of the presence in the work environment of a suspected or proven environmental toxin or carcinogen, which has the potential of producing low-grade injury over long periods of exposure.

Most screening studies are directed toward the detection of abnormalities in certain body systems. The specific tests are frequently selected on the basis of medical experience which utilized them in symptomatic or hospitalized populations. Prior experiences utilizing nonspecific multiphasic health surveillance screening and maintenance have not proven to be cost effective except under certain limited

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conditions (*1*). The cost effectiveness of such tests, however, in the determination of medical screening requirements, has played a limited role due to the potential seriousness of these occupational agents. Little attention has been paid as to whether the effectiveness of federally required screening provides the best or, more importantly, a necessary benefit when applied to asymptomatic and otherwise healthy worker populations.

The discovery in 1974 of hepatic toxicity and cancer formation in vinyl chloride workers provided the opportunity to systematically and prospectively study the effectiveness of federally required and federally recommended medical screening procedures for the detection of chemical liver injury, including cancer development (*2*). Table 1 lists the federally required medical screening procedures since 1974 for environments utilizing vinyl chloride or polyvinyl chloride. Table 2 lists the federally recommended studies for these same environments. This paper will present a preliminary assessment of the effectiveness of these federally required studies in the accurate detection and identification of chemically induced liver injury due to halogenated hydrocarbons, especially vinyl chloride.

Materials and Methods

The industrial population studied consisted of approximately 1200-1400 employees of a chemical plant whose two major products were synthetic rubber and plastics. The industrial plant had been in operation for over 35 years and had a predominance of male employees (96%), approximately 80-87% of the work force being white, 11-12% black, less than 1% of other racial origins. Turnover of the plant was approximately 10 to 15% per year with 65-70% of the work force having worked five years or more at the plant. Employee ages ranged from 18-65, with a mean of 52 years.

A cohort consisting of 969 male employees who worked continually from June 1, 1976 to May 31, 1977 was, for purposes of this analysis, divided into a "standard" and a "nonstandard" population. These designations were given on the basis of a review of

Table 1. Federally required studies for vinyl chloride workers.

History and physical
< 10 years as vinyl chloride worker—(annual)
> 10 years as vinyl chloride worker—(semiannual)
Biochemical studies
SGOT (AST)
SGPT (ALT)
GGTP
AP
TB

Table 2. Federally recommended (not required) studies.

Hepatic studies
LDH isoenzyme
Total protein
Protein electrophoresis
Hb_SA_C
Radioisotopic scan
Kidney dysfunction (urine examination)
Albumin
RBC
Exfoliative abnormal cells
Pulmonary system
FVC
FEV_1
Chest x-ray (PA and lateral)

all present standard medical data on each employee, including the federally required studies. Other screening studies of the medical surveillance programs were not utilized in the classification of overall medical status because, at that time, their clinical usefulness was unknown or controversial. All studies were performed on an annual basis. Those individuals with ten years or more of employment were examined and screened semiannually. Compliance with medical screening studies during the five-year study period showed a continuous participation in the history and physical examinations by over 75% of the work force, laboratory tests and chest x-rays by 86%, and liver-spleen scans by 85%. Seventeen percent failed to undergo at least one history and physical examination, 9% did not have any of the radiological studies, and only 4% failed to have laboratory studies during this period. Approximately 40-50% of these individuals who did not undergo an examination claimed to have been examined by their private physician.

A subcohort of 120 individuals was further identified based on the availability of a liver biopsy performed for medical reasons, both related and not related to their work.

The term "standard" is used for those individuals who, based upon the best medical opinion, demonstrated no evidence of any significant medical disease, occupational or nonoccupational in origin. The "nonstandard" population included all others not included in the standard population.

The subcohort population was divided into those individuals with and without histological evidence of liver injury and further subdivided into those with and without histological features characteristic of chemical injury.

All employees had individual work histories. These consisted of a standardized job classification for all jobs within the plant since its opening and a

rank ordering of exposure for 22 different suspected or potentially hazardous heptatoxic chemicals used within the work place (*3-5*). The agents were rank ordered on the basis of the intensity of exposure for each of the job classifications for each of the years that the plant was in operation. From this detailed work history, a cumulative exposure ran month ration (CERM) was determined for each employee for each of the 22 chemicals. All histological material was classified as to the presence or absence of liver disease, and to whether the abnormalities were consistent with chemical or nonchemical injury. This classification was conducted double blindly by three experienced physicians, two pathologists, and a hepatologist (*6*), without knowledge of any medical data, exposure or work history.

Results

Although 50 or more biochemical screening tests were performed during this study period, this paper will limit itself to the evaluation of the federally required studies, the indocyanine green clearance (ICG) study at the 0.5 mg/kg dose (*7, 8*) and radioisotopic liver and spleen scan (*9*). The

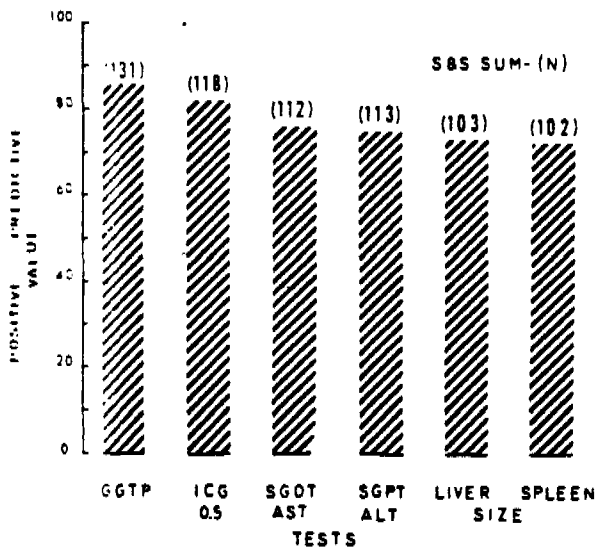

FIGURE 1. Positive predictive values of screening tests in identification of medical disease in an asymptomatic working population (N = 969). All those screening tests with positive predictive values of greater than 70 are shown except for indirect bilirubin (due to high number of congenital indirect hyperbilirubinemia) and triglyceride determination. Above each bar in the graph are shown the sum values for sensitivity and specificity of each test. They generally follow the same ranking.

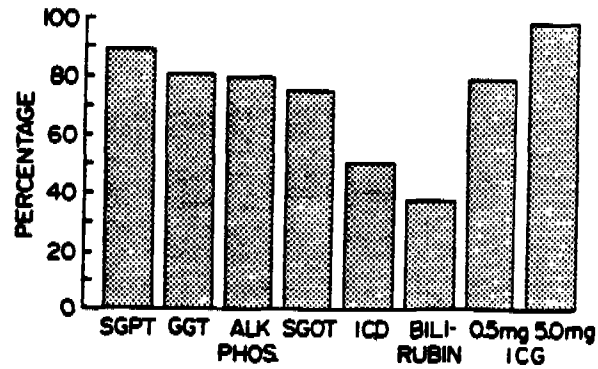

FIGURE 2. Frequency with which clinical biochemical tests correctly reflex the presence of hepatic damage in chemical workers suspected of having liver disease: (SGPT) alanine aminotransferase (ALT) (GGT) γ-glutamyl transpeptidase, (SGOT) aspartic aminotransferase (AST), (Alk. Phos.) alkaline phosphatase, (ICD) isocitric dehydrogenase (ICG) Indocyanine Green clearances at 0.5 and 5.0 mg/kg dose.

federally required biochemical studies include alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGPT), alanine aminotransferase (ALT/SGPT), bilirubin (ALT/SGPT), and the aspartic aminotransferase (AST/SGOT).

The positive predictive values of these screening tests in identifying medical disease (including liver disease) in this asymptomatic working population are shown in Figure 1. The GGTP provided the highest positive predictive value as a screening test for "nonstandard" individuals. It also provided the highest sensitivity and specificity sum shown in brackets. The predictive value of the other tests, in decreasing positivity were ICG, AST, ALT, liver and spleen scan, AP, and bilirubin.

Further evaluations were conducted on the subcohort population in whom we had both histological and biochemical data concerning hepatocellular damage. If one looks at only those individuals who received liver biopsies for suspected liver disease then one would find the percent of positive tests as illustrated in Figure 2. The ALT (SGPT), GGTP, AP and AST (SGOT) demonstrate a very high degree of sensitivity in identifying individuals with hepatic disease. As shown on the right, ICG clearances at the 0.5 mg/kg level provide a similar degree of sensitivity to SGOT and AP. The higher dose ICG clearance (5 mg/kg) appears to provide the most sensitivity for latent hepatic disease. These findings are consistent with the general medical experience with hospitalized patients.

Sensitivity alone however is not an adequate indicator of a test's screening value, especially when used in asymptomatic individuals. More appropriate evaluation of these tests' value as screening instruments are shown by their sensitivity, specificity

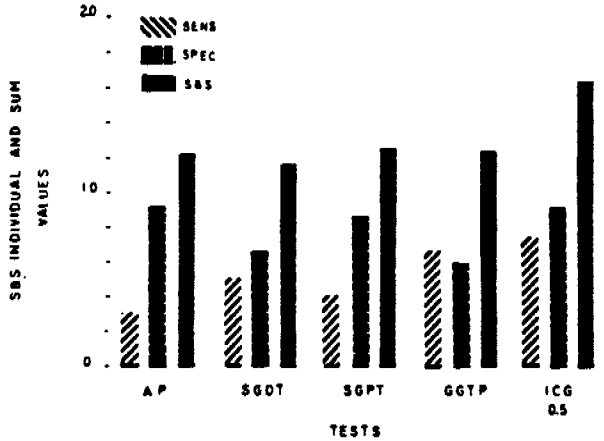

FIGURE 3. Sensitivity and specificity of various biochemical screening tests and their sensitivity and specificity sum values (S & S) based on 78 with biopsy documentation of their hepatic status and all of the biochemical screening studies listed. All screening tests with S & S sums less than 110 (e.g. bilirubin and isocitric dehydrogenase, are not illustrated.

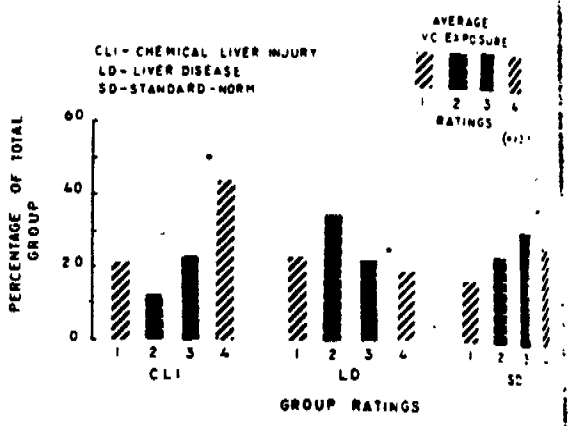

FIGURE 4. Correlation between the histologic findings on liver biopsy and each individual's average total vinyl chloride exposure based on their average CERM (Cumulative Exposure Rank Months) ratings. Rankings: 1 = lowest possible exposure; 2 = minimal exposure, low levels; 3 = moderate exposure; 4 = worked in areas subject to occasional high excursions, or frequently high and/or had intimate contact

and sum values shown in Figure 3 in the biopsied subpopulation. Here again, γ-glutamyl transpeptidase and ICG clearance (0.5 mg dose) show the greatest sensitivity for identifying individuals with liver disease. However, GGPT had the least specificity, reflecting its high incidence of false positives. Specificity increased with the use of AST, ALT, and ICG clearance. The alkaline phosphatase provided the highest specificity, suggesting that mild or low grade chronic hepatic injury due to environmental agents may be activating hepatic AP synthesis in the absence of biliary tract obstruction or cholestasis. The ICG clearances, even at the low dose (0.5 mg/kg), clearly remains the test with the best combined sensitive and specific screening study for detection of individuals with subclinical hepatic disease.

This subcohort biopsied group was further examined on the basis of the histological interpretation of their liver biopsies and their work exposure to vinyl chloride. All biopsied individuals were subdivided into three groups: 19 with histological evidence consistent with chemical liver injury; 30 with histological evidence of liver disease, nonchemical liver injury; and 29 with normal liver biopsies. Each of the histological subgroups were further subdivided based on their vinyl chloride exposure, on a scale of 1 to 4 (Fig. 4).

The chemical liver injury group contained the highest percentage of individuals with the highest average rating (CERM) for vinyl chloride exposure. In contrast, with those with liver disease, nonchemical, and those with normal livers have a more even distribution of individuals relative to their degrees of vinyl chloride exposure.

In our previous studies we noted that almost all individuals with histologically specific lesion of vinyl chloride injury or angiosarcoma had a total average CERM rating of 3.5 or greater. The asterisk in Figure 3 indicates the percentage of individuals in each of the three histological groups with exposure ratings of 3.5 or greater. Again, the chemical liver injury group have the highest percentage of individuals with the high exposure ratings. This further supports previous work (*J. 10*) identifying focal hepatocellular hyperplasia as the earliest histological characteristics of chemical injury in liver disease.

A study of the frequency with which these tests are positive among those individuals with liver disease, based on their histological findings (chemical versus nonchemical), provides additional data supporting the clinical observation that an increased AP has a greater specificity for chronic liver injury.

Figure 5 shows the ratio of the proportion of positive screening tests in those with histological chemical liver disease divided by the proportion of positive tests in those whose disease is not of chemical origin. All tests, independent of their sensitivity and specificity for liver injury, were more frequently abnormal in the presence of nonchemical, subclinical liver injury, except for AP. In contrast, AP was far more frequently abnormal in those individuals with chemical liver injury, which tended to be more chronic than acute and generally less severe.

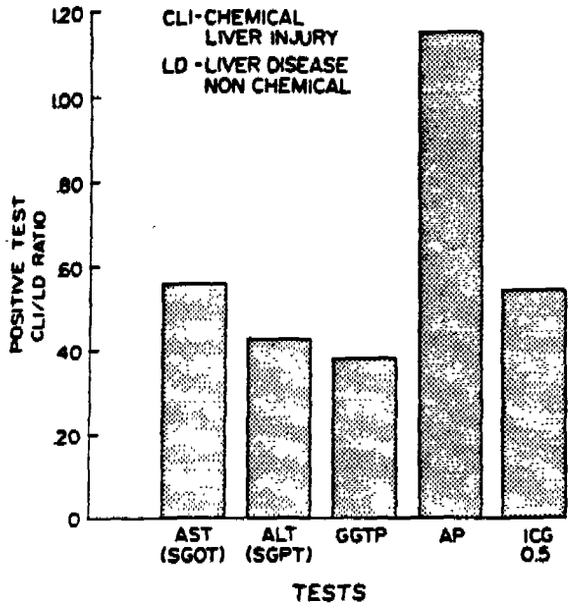

FIGURE 5. Frequency with which biochemical tests were abnormal in those with different type of hepatic injury expresses as a ratio: (CLI) chemical liver injury, (LD) liver disease, nonchemical.

Discussion

This preliminary systematic review of the positive predictive values and the sensitivity and specificity of federally and some non-federally required tests for chemical workers provides the first scientific and biological basis for the selection of medical screening tests for liver injury in occupational environments. Although these commonly used medical tests have been found by clinical experience to be effective as diagnostic tools in the symptomatically ill or hospitalized population, little clinical work has been done to determine their ability to accurately separate biological variations from early latent or underlying disease in asymptomatic individuals. Tests which provide very high false-positive rates (decreased specificity) such as GGTP, interfere with the screening process identification of the high risk worker by the extra time and cost required for repeat testing, the decreased productivity for the employer, the employees' increased anxiety, and by the loss of confidence in the effectiveness of the testing program by both employees and employer. Determination of the sensitivity and specificity of screening studies for asymptomatic individuals is essential if effective recommendations are to be made a federal requirement. This evaluation process also provided the best means of developing effective triage protocols for the screening program. For example, in this particular population of industrial workers, we have shown that the assessment of hepatic function is best accomplished by low dose ICG clearance (0.5 mg/kg). The ICG clearance is somewhat a more complicated technique (i.e., injection of substance and repeated blood sampling) but requires only 10 min to perform, and needs only one needle stick. In exchange it provides the best singular screening test for latent hepatic injury. If adequate medical facilities are not easily accessible, then ALT should be substituted. If either ICG clearance and/or ALT studies are found to be abnormal, an AP should be done and a diagnostic work-up instituted to determine the etiology (*11*).

The rationale for these recommendations is based on the actual study of chronic subacute chemical injury in an asymptomatic population, not preselected because of signs or symptoms. Therefore the test's ability to correctly differentiate disease from nondisease or one type of injury from another is more accurately determined. Chemical and environmental agents of low toxicity tend to produce repeated or persistent injury which accumulates over time. Tests which measure overall functional capacity quantitatively or semiquantitatively, rather than measuring acute low-grade injury over time are more likely to detect changes. For this reason, clearance or tolerance studies provide the best means for identifying latent hepatic disease, while enzyme studies like ALT, GGPT, and SGOT usually reflect acute cellular injury of higher grade or degree and cannot accurately reflex accumulative damage until very late in the disease process. Tests which provide information concerning the progression or nonprogression of injury will be far more helpful to the practicing occupational physician. They provide him/her with a better capability to discern between nonoccupational and occupational disease, and the best available reassurance for the worker of his or her safety while allowing the greatest possibility for continued productivity and employment.

Finally, these data provide a sound scientific basis upon which to modify federal requirements. The removal of specific testing requirements which, with field experience, prove not to have any significant positive predictive value, or effective sensitivity and specificity will aid in reducing overall cost and help maintain continued compliance by industry and workers. There may be theoretical reasons to maintain or continue some of the present federally required screening studies, but these reasons should be separately identified and not be confused with the purposes of the more effective test in the

detection of occupationally related liver injury.

The capabilities and limitations of any new tests or old tests for new screening purposes in detection of other potential occupational hazards, should be validated before making them a required screening procedure. This would lay the foundation for systematic determination of effectiveness of screening procedure against a proven standard. Unvalidated federal requirements provide the worker and employer with a false sense of security and safety by what is believed to be effective monitoring. More importantly, such a situation can lead to delay in effective correction of cause and disease prevention.

Conclusions

Biochemical screening for hepatic injury in asymptomatic chemical workers can be done most effectively by the use of liver specific clearance studies. ICG clearance provides the best combination of positive predictive value and sensitivity and specificity for functional hepatic testing at the present time.

None of the present federally required studies provide any significant degree of sensitivity without marked reduction in specificity in asymptomatic individuals.

The ALT (SGOT) is the most useful among those Federal tests presently required and the alkaline phosphatase may provide additional specificity as a follow-up study in those individuals with positive ICG or ALT screening studies.

There is no evidence that any of the other federal studies add any benefit and strong evidence that they significantly increase the false-positive results in well individuals.

All screening studies should undergo evaluation as to their positive predictive value and sensitivity and specificity in asymptomatic individuals before becoming permanent or established requirements.

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Poly(vinyl Chloride) Processes and Products

by R. N. Wheeler, Jr.*

Poly(vinyl chloride) resins are produced by four basic processes: suspension, emulsion, bulk and solution polymerization. PVC suspension resins are usually relatively dust-free and granular with varying degrees of particle porosity. PVC emulsion resins are small particle powders containing very little free monomer. Bulk PVC resins are similar to suspension PVC resins, though the particles tend to be more porous. Solution PVC resins are smaller in particle size than suspension PVC with high porosity particles containing essentially no free monomer. The variety of PVC resin products does not lend itself to broad generalizations concerning health hazards. In studying occupational hazards the particular PVC process and the product must be considered and identified in the study.

Poly(vinyl chloride) is a ubiquitous part of our environment today, in that it appears in clothing, upholstery, flooring, wire insulation, food containers, phonograph records and an almost infinite variety of other items. Despite this wide application and the size of the industry there is little public understanding of the term. A part of this confusion stems from the human inclination to abbreviate terms—thus, we say PVC when discussing poly(vinyl chloride) resins, poly(vinyl chloride) latexes, poly(vinyl chloride) compounds, poly(vinyl chloride) film and so forth. This confusion has been accentuated by governmental regulators, who define PVC as a polymer containing any amount of vinyl chloride. On taking these two factors into account it has become almost impossible to distinguish what is meant when the term PVC is used in the various media. In order to study possible PVC industry-related health problems one must know something of the various processes and products to properly evaluate study results. Many study results can have significance only if the source and composition of the PVC is stated.

Manufacture of synthetic resins from vinyl chloride and other monomers involves reacting these monomers in agitated pressure vessels in the presence of catalysts and converting these liquids and/or gases to solid resins. A considerable amount of heat is generated by the reaction. This is removed by cooling the vessel. As the monomer is converted to polymer during the reaction, the rate of reaction slows down; thus, after some optimum reaction time, the unconverted remaining monomer is removed from the reacting mass by heat and vacuum and the resin (PVC) is recovered as a dried white powder or as a liquid latex or solution. This polymerization reaction may take place in pure monomer, in a solution, in a water-monomer emulsion or in a water suspension of monomer. The nature of the polymerization process determines the nature of the subsequent recovery process, and the nature of the resin particles produced. Currently there are four basic vinyl chloride polymerization techniques which give use to the following four processes: (1) suspension polymerization, (2) emulsion polymerization, (3) bulk polymerization, and (4) solution polymerization.

Suspension polymerization is the major process used for the manufacture of PVC resins and is used for about 82–85% of U.S. production (Fig. 1). It involves the charging of one or two parts water and one part vinyl chloride monomer or comonomer mixture to an agitated reactor along with initiator and suspending agents such as poly(vinyl alcohol). The mass is reacted at 50–65°C until about 85–90% of the contained monomer is converted to resin. The resin-water mixture is heated, sometimes under vacuum, until the unconverted monomer is substantially removed. The resin is then removed

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from the water and dried in rotary, flash or fluid bed dryers by exposure to heated air. The dried resin is transferred to storage silos, whence it is shipped to fabricating plants in bulk containers or in paper bags. If only vinyl chloride monomer is reacted, the product is PVC homopolymer. If a monomer such as vinyl acetate is mixed with the vinyl chloride then a PVC copolymer is produced.

The advantages of the suspension resin process are its high productivity per unit reactor volume, its flexibility with regard to polymer composition and resin particle characteristics and the granular nature of its product. The relatively large resin particles (50 to 150 μm) cause problems in removing unconverted vinyl chloride monomer. The monomer tends to diffuse slowly from the center of the resin particle to the surface, where it is removed by the monomer recovery operation; thus removal often is incomplete. In the past, residual vinyl chloride monomer concentrations in suspension PVC resins ranged as high as 2000 ppm by weight until improved methods were developed in response to recognized need for lower residual levels.

The process produces a wide variety of products, each of which presents different monomer release characteristics as well as differing suitability for particular fabricating operations. As a general rule, small particle size resins have lower residual monomer than large particle size; porous particle resins have lower residual monomer than nonporous particle resins; and resins containing little or no comonomer have lower residual monomer than resins with significant amounts of comonomer. The relatively large suspension resin particle, while retaining monomer from the manufacturing process, results in a product that has excellent handling properties such as bulk flow. Recent improvements in monomer stripping technology have resulted in most PVC suspension homopolymer resins containing about 10 ppm residual vinyl chloride monomer at the time of shipment while the residual vinyl chloride monomer in most copolymers ranges from 25 to 200 ppm at the time of shipment.

The stress on residual monomer content at the time of shipment in the preceding paragraph is purposeful. Vinyl chloride monomer is not soluble in the PVC nor is it absorbed or adsorbed in the resin particle. It is entrapped, and, given an opportunity, it escapes to the ambient air. Heating tends to accelerate this escape. PVC resin in a bulk-container loses its residual monomer at a rate of 25 to 50% per month. PVC resin in a paper bag loses its residual vinyl chloride monomer at an approximate rate of 75% per week. Once the residual monomer is gone, there is no source of monomer from the PVC resin, i.e., the resin does not decompose to yield significant amounts of monomer. If heated, the resin gives off hydrogen chloride and turns black. Thus a year-old PVC resin contains almost no residual monomer; on heating until it blackens it will always give off hydrogen chloride. PVC resin fabricating operations drive off the free residual monomer in the first heating step; thus the probability of a toxic response from vinyl chloride monomer contained in the vinyl film or

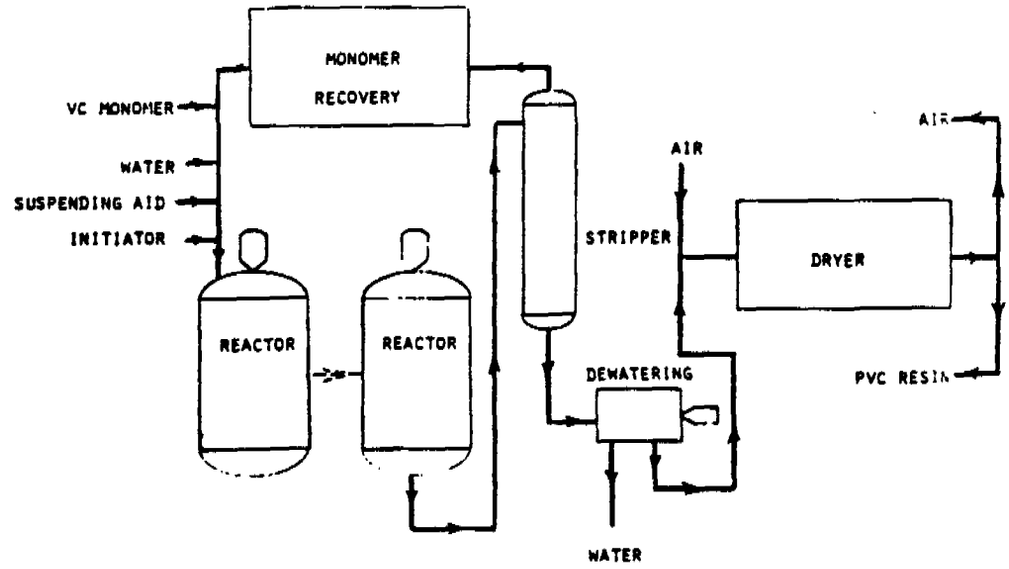

FIGURE 1. Suspension polymerization process.

moldings produced is remote.

All PVC processes have a problem with reactor system fouling to some degree. The conversion of a liquid monomer to a high molecular weight solid polymer while contacting reactor walls, baffles and so forth results in the deposition of some of that solid polymer on surfaces. This deposition or fouling interferes with the reactor operation so that periodically it has to be removed. In earlier times, this was removed manually by a man working in a shutdown reactor. These reactor cleaners were exposed to very high concentrations of vinyl chloride monomer (several thousand ppm). It was within the group of men in this cleaning operation that the excess of angiosarcoma was observed by Creech (*1*). More recently, techniques to minimize fouling and techniques for cleaning such as solvent washing and hydroblasting have reduced or eliminated the need for reactor entry and cleaning.

Emulsion polymerization is the second most widely used process for the manufacture of PVC resins and comprises 10–12% of total U.S. production. One of the important things to understand about emulsion polymerization is that it is not a single process but a large family of processes, each producing specialized products that are defined or specified in terms of performance in a particular application. In the interest of brevity, the two major process families will be discussed: the water-soluble initiator system and the oil-soluble initiator system (Fig. 2).

In the water-soluble initiator system, one to two parts water, one part monomer, 0.01 to 0.03 parts surfactant and water-soluble initiator (a redox system or a persulfate salt) are charged or fed to an agitated reactor and reacted at 30–60°C to form a synthetic latex. The reactor agitation must be sufficiently vigorous to emulsify the monomer-water mixture but not so vigorous that the latex is coagulated. When 80–95% of the monomer is converted to polymer, the latex may be gently stripped of the unconverted monomer with heat and vacuum, or it may be subjected to a second initiator treatment and reacted to essentially 100% monomer conversion. The product of this polymerization may be simply filtered and shipped to consumers as a latex for coatings, mastics, and the like, or the polymer may be recovered as a dry resin. Recovery techniques vary. The most commonly used is simply spray-drying of the latex, though some resins are recovered by coagulating the latex and dewatering with subsequent drying of the coagulum (resin).

In the oil-soluble initiator system, one part monomer containing an organic peroxide is emulsified in one to two parts water containing 0.01 to 0.03 parts surfactant. The resulting emulsion is reacted at 30–60°C to form a synthetic latex. Approximately 80–90% of the monomer is converted to polymer. After reaction, the latex is gently heated and vacuum-treated to remove the unconverted monomer. After stripping, the latex may be shipped as a product though most of it is converted to a dry powder by spray-drying. The basic difference between the oil-soluble initiator process and the water-soluble initiator is that the size of the emulsified monomer particle determines the resin particle size in the oil-soluble initiator process, while polymerization technique determines the resin particle size

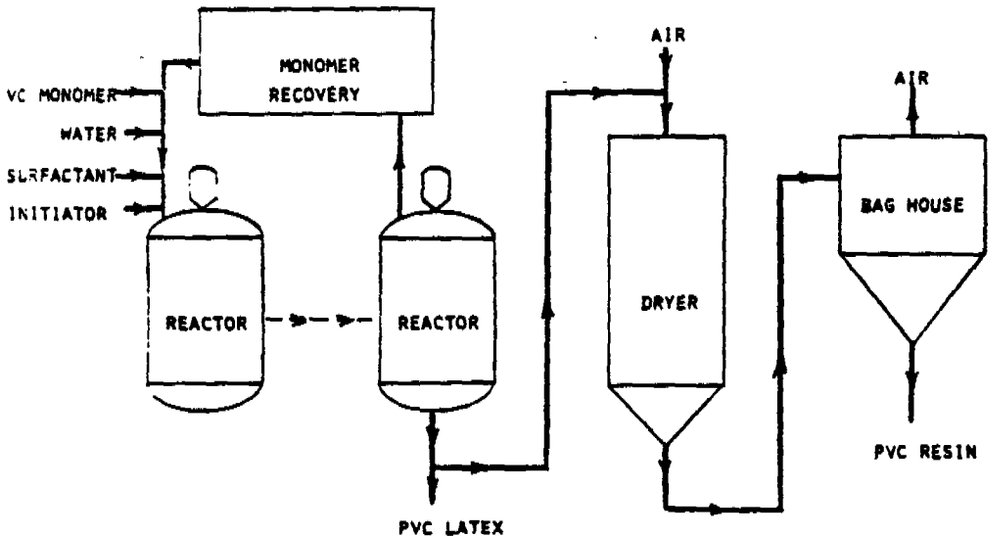

FIGURE 2. Emulsion polymerization process.

in the water initiator process. The resin particles formed during emulsion polymerization range in size from 0.05 to 2 μm. In the course of recovery and drying these may be agglomerated to particles as large as 30 μm. For some uses the agglomerates may be ground to a median particle size of about 2 μm. The small particle size results in rapid loss of residual vinyl chloride so that the resin contains about 1 ppm free monomer as produced. Because of handling problems, nearly all the resin is bagged, letting the small amount of monomer retained escape easily before it arrives at a fabricating plant.

Nearly all dried emulsion process PVC resin is utilized by paste or dispersion fabrication techniques. The resin, pigments and other additives are stirred into plasticizer, yielding a thick viscous liquid plastisol. This plastisol is spread on a cloth or a paper or poured into molds. The coated mold or cloth is heated, causing the resin and plasticizer to fuse into a solid forming the finished plastic article. In this fashion, the small manufacturer or the producer of elaborate shapes or highly styled items can make a product with a minimum capital investment.

The emulsion polymerization process, in addition to the reactor fouling problem noted for suspension polymerization, has less flexibility with regard to changes in process conditions. Each change in reaction, stripping or drying has an identifiable effect on product properties and quality. Some of these properties are well known, and quality tests keep them under control. Others are not well known and are recognized when customers complain. Emulsion process resins cost more to produce, and plant operators tend to be very conservative in the approach to process changes. Because of this difficulty, emulsion process plants tend to emit more vinyl chloride monomer to the ambient air per pound of resin produced. In Europe particularly there have been reports of worker dermatitis from surfactants and possible pneumoconiosis from resin dust inhalation in the emulsion process plants (2-4).

Bulk polymerization is the third major process in terms of volume for the manufacture of PVC resins but supplies only about 5% of U.S. production (Fig. 3). The process involves the charging of vinyl chloride monomer and initiator to a first-stage polymerizer, where about 10% of the monomer is converted to polymer. This batch is then transferred to a second-stage polymerizer where additional monomer, and sometimes initiator, are added. The polymerization is continued until about 80–85% of the container monomer is converted to polymer. The unreacted vinyl chloride is removed by heat and vacuum and the finished resin product transferred to storage bins for later shipment to fabricating plants. The absence of water in the polymerization stage eliminates the need for the drying step and provides some economy in capital and operating costs.

The advantages of the bulk process are its simplicity, the uniformity of the resin particle size, the high porosity of the resin particles and the purity of the polymer (no soaps or suspending aids). The disadvantages are: less flexibility in product mix (homopolymers only) than the suspension pro-

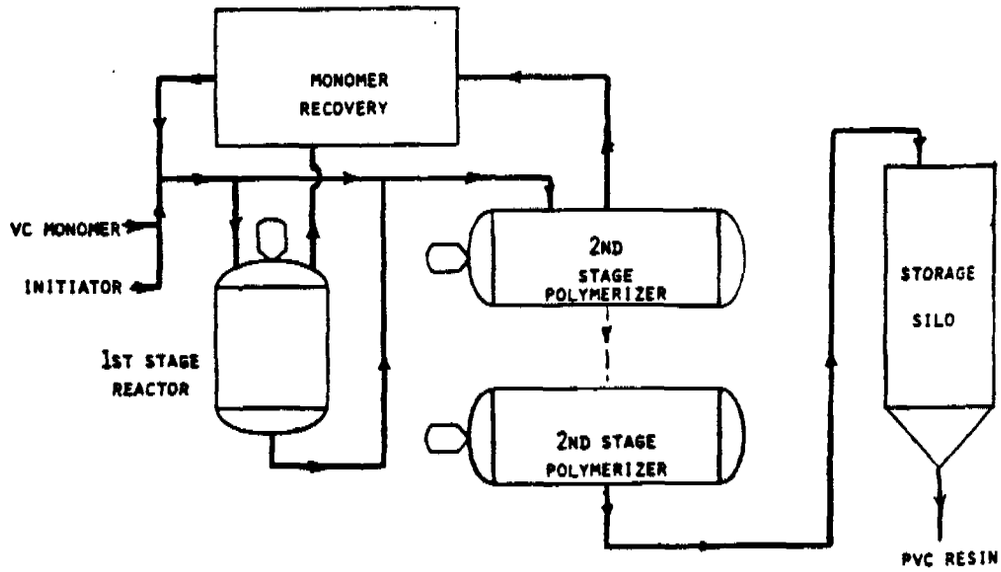

FIGURE 3. Bulk polymerization process.

ess and poorer removal of residual VCM. These bulk process resins are generally directly competitive with suspension process PVC homopolymers.

The relatively large porous resin particles (50 to 150 μm) tend not to retain monomer, thus removal of residual VCM is theoretically easy. The transfer of heat from the reactor wall to the resin particle for stripping is poor thus offsetting the advantages gained by the high particle porosity in removing residual VCM. In the early 1970s, residual VCM in this type of resin was of the order of 1000 ppm when produced, but more recently these resins have less than 50 ppm in residual VCM when produced.

Solution polymerization is a process unique to Union Carbide Corporation and accounts for about 2% of the total resin produced (Fig. 4). Vinyl chloride monomer, comonomer, solvent and initiator are fed to a continuous reactor system. The polymer formed is soluble in the reacting mass so that the reactor product is a viscous resin solution. This solution is distilled to remove the unconverted vinyl chloride monomer, and the resin product is recovered by treating the resin solution with water and drying the product. The resin particle is very porous, is always a copolymer, is free of soaps and suspending agents, has a median particle size of 75 μm and contains less than 0.2 ppm residual VCM. Manufacturing investment is high, and the product finds its greatest use as a coating material, i.e., paints and lacquers, that utilize its good dissolving qualities. It is used in relatively small quantities, and is nearly always shipped in bags rather than bulk.

In addition to the basic PVC resins described earlier, there is a wide variety of resin powders, pellets, liquids and latexes in commerce that fall under the general designation of PVC. These are chemically or mechanically converted PVC resins such as post-chlorinated resins and compounded resins containing plasticizers, stabilizers and the like. As a general rule, the additional processing has removed essentially all of the residual VCM and agglomerated the dusts. Converted PVC resins have no involvement with problems related to vinyl chloride monomer exposure.

Conclusions

Poly(vinyl chloride) resins are produced by four basic techniques: suspension, emulsion, bulk and solution polymerization.

The variety of PVC resin products does not lend itself to broad generalizations concerning hazards to worker health.

In evaluating occupational hazards, the PVC process, the PVC product and other materials present must be considered before valid conclusions can be reached.

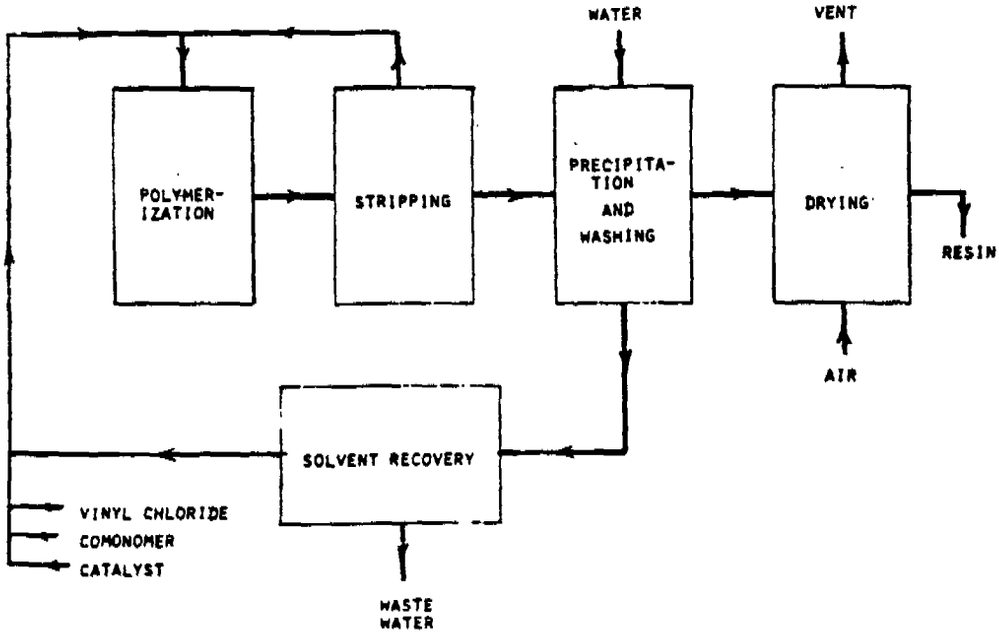

FIGURE 4. Solvent vinyl resin process.

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Worker Exposure to Vinyl Chloride and Poly(vinyl Chloride)

by James H. Jones*

The National Institute for Occupational Safety and Health (NIOSH) in early 1974 began industrial hygiene studies of vinyl chloride exposed workers. Three VC monomer plants, three VC polymerization plants, and seven PVC fabrication plants were surveyed. VC polymerization plant workers and workers in one job category in VC monomer plants were exposed to average levels above 1 ppm. The highest average exposure was 22 ppm. NIOSH health hazard evaluation studies since these initial surveys have primarily shown nondetectable levels of vinyl chloride. A NIOSH control technology study in 1977 showed that exposure levels in VC polymerization plants had been drastically reduced but exposure levels above 1 ppm were still found in several cases.

Introduction

The National Institute for Occupational Safety and Health (NIOSH) began studies of vinyl chloride (VC) early in 1974 following a report of increased incidence of angiosarcoma of the liver among VC exposed workers. A part of this work included retrospective cohort mortality and industrial hygiene studies of the VC polymerization industry. Later NIOSH contracted with Bendix Corporation, Launch Support Division, to conduct industrial hygiene studies in the VC monomer production industry and the polyvinyl chloride (PVC) fabrication industry. These industrial hygiene studies were done to document the levels of VC exposure occurring in industry at that time. Three VC monomer plants, three VC polymerization plants and seven PVC fabrication plants were studied. In addition to the VC industry-wide study, NIOSH, through their health hazard evaluation (HHE) program has sampled for PVC at 34 other plants, primarily VC fabrication operations. Also a study to document control methods utilized in the plastics industry was performed under contract by Enviro Control. This study included work at five VC polymerization plants (*1-36*).

Industry-Wide Study

VC Monomer Plants

One of the VC monomer plants sampled used the acetylene-hydrogen chloride process, the older process for making VC, and the other two used the ethylene dichloride pyrolysis process. It was not possible to assess the difference in worker exposure between the two processes because the plant using the acetylene-hydrogen chloride process was operating at only 10% capacity during the surveys in December 1974.

As can be seen in Figure 1, only the job of loader shows exposure substantially above 1 ppm. VC exposure for this job was being reduced, at the time of the surveys, by the redesign of tank car hookup systems and the use of air-supplied breathing apparatus while the tanks cars were connected or disconnected.

VC Polymerization Plants

Three VC polymerization plants were sampled. Located at these plants were three suspension resin operations, three dispersion resin operations, one mass resin operation and one solvent resin operation. As can be seen in Figure 2, suspension and dispersion resin operations had the highest exposure levels. Within plants the reactor area operators and helpers had the highest exposures.

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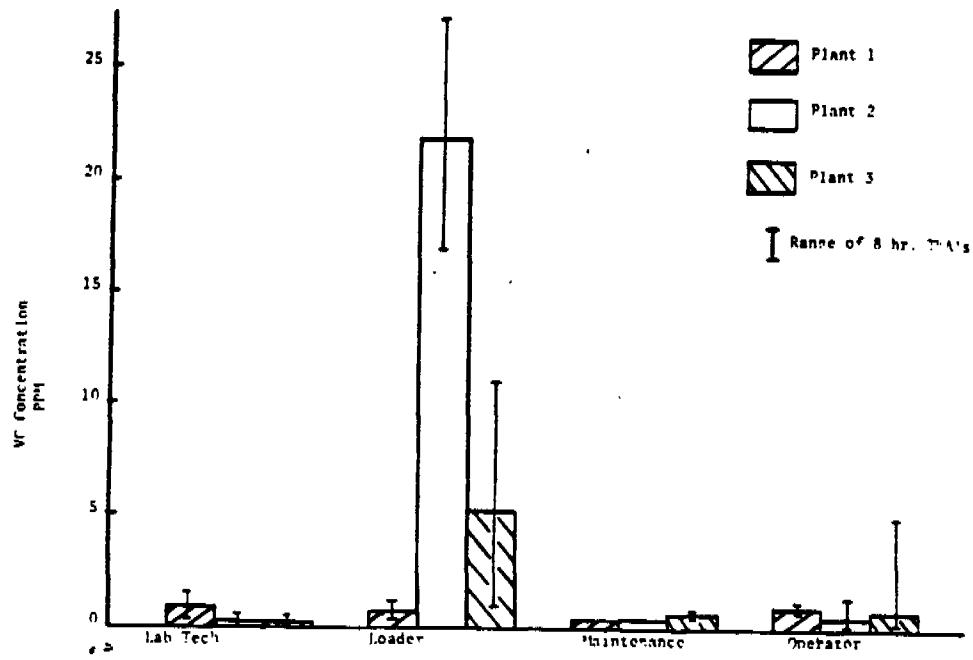

FIGURE 1. Means and ranges of VC exposures in VC monomer production plants.

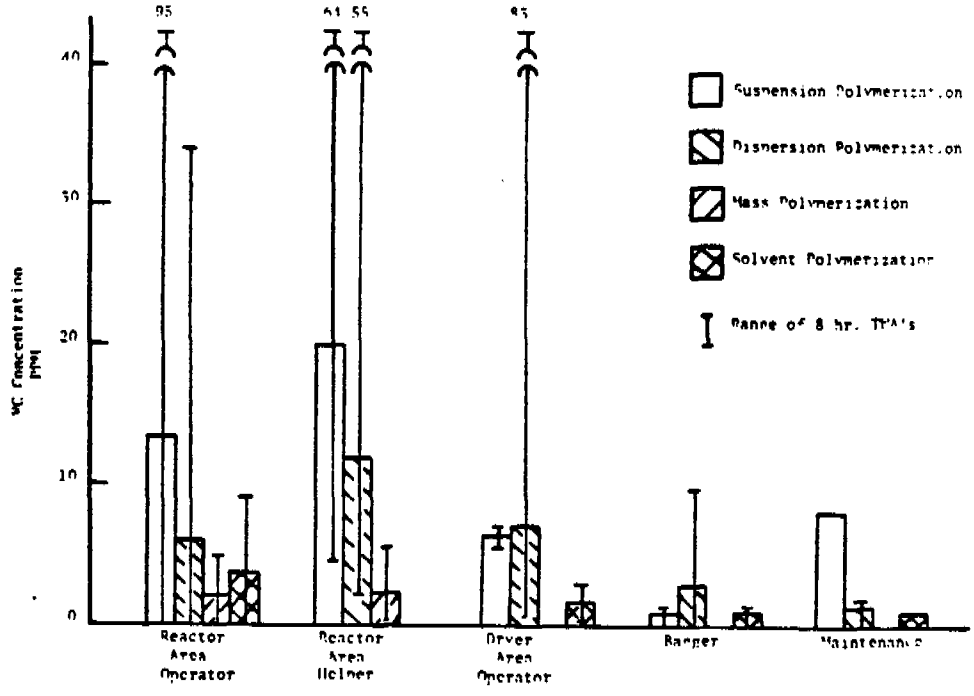

FIGURE 2. Means and ranges of VC exposures in VC polymerization plants.

PVC Fabrication Plants

In the seven PVC fabrication plants samples were collected in calendering, compounding, extrusion, molding, and plastisol operations. All exposures were quite low with calender operators having the only exposures above 1 ppm. Figure 3 shows the relationship of VC exposure between the segments of the PVC industry. As would be expected, VC polymerization had the highest exposures and PVC fabrication the lowest.

Changes in Exposure Since 1974

We have no information generated by NIOSH and know of none in the literature on how VC monomer plant exposures have changed since 1974, although it would be expected that the single "high" exposure category, loader, has had exposure reductions.

Information on how well VC polymerization plants were able to reduce exposures since 1974 was obtained during the control technology study. This information is in the form of company personnel sampling data for VC over a period of time. At the first plant, a mass polymerization plant, the percentage of personnel samples below 1 ppm has gone from 2% in 1974 to 73% in 1976 (Fig. 4). At the second plant, a suspension and dispersion polymerization plant, the percentage of personnel samples below 1 ppm has gone from 0% in 1974 to 65% in 1977 (Fig. 5). At the third plant, a mass polymerization plant, VC exposures are given by job for the period October 1974 to May 1975 and then for January 1977. In Figure 6, it can be seen that exposures dropped substantially, in most cases by a factor of almost 10. VC exposures at the fourth plant, a suspension polymerization plant, are given for both 1975 and 1976. As seen in Figure 7, in most cases a two-fold drop in exposure levels occurred. Although the percentage drop is lower than the third plant, the final exposures are about the same. At the fifth plant, a dispersion polymerization plant (Fig. 8), VC exposures are given by job for the period May 1975 to December 1975 and then for January 1976 to September 1976. Again reductions were sufficient to bring exposure averages to the same approximate level (less than 1 ppm) as the preceding two plants.

PVC fabrication plants had exposures predominantly below 1 ppm in early 1975 when the industry-wide study was done. In addition to this work, HHE's have been conducted at 26 PVC fabrication plants beginning May 1974 and ending in April 1978. Detectable levels of VC were found in only 10 plants with only 1 plant of the 15 sampled since April, 1975 having detectable levels. At that plant sampled in March, 1976, the highest level found was 0.38 ppm.

PVC dust exposures were also briefly examined

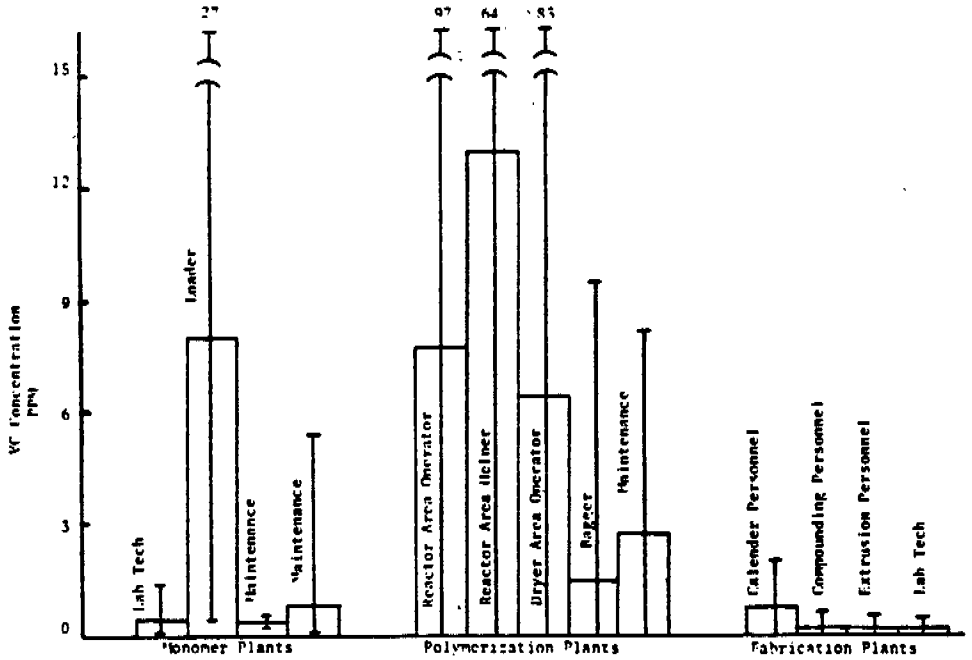

FIGURE 3. Means and ranges of VC exposures in VC related industries.

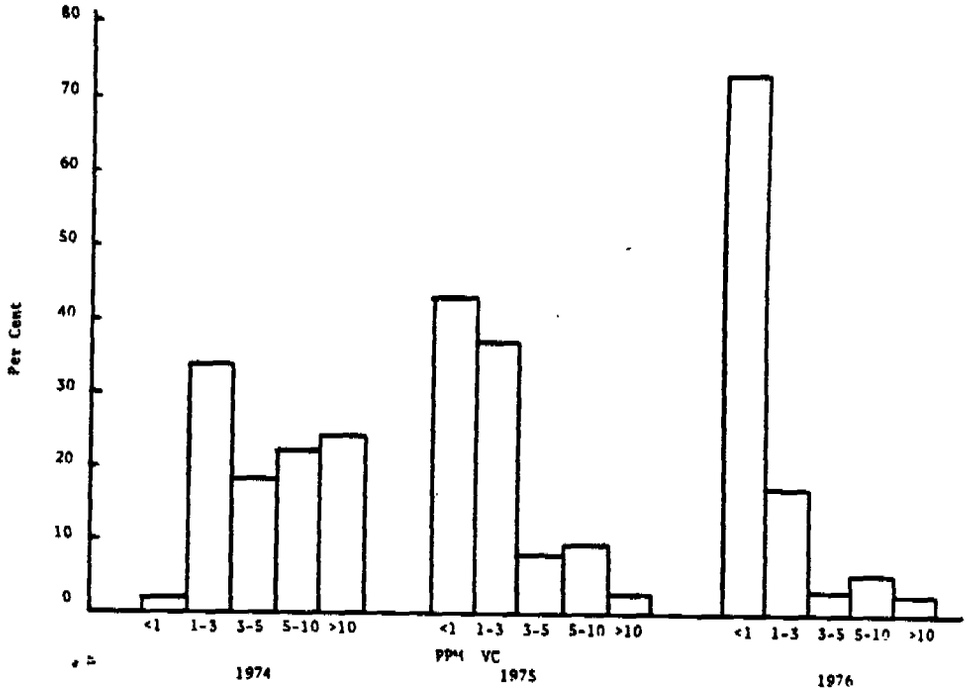

FIGURE 4. Distribution of VC exposures at a mass polymerization plant.

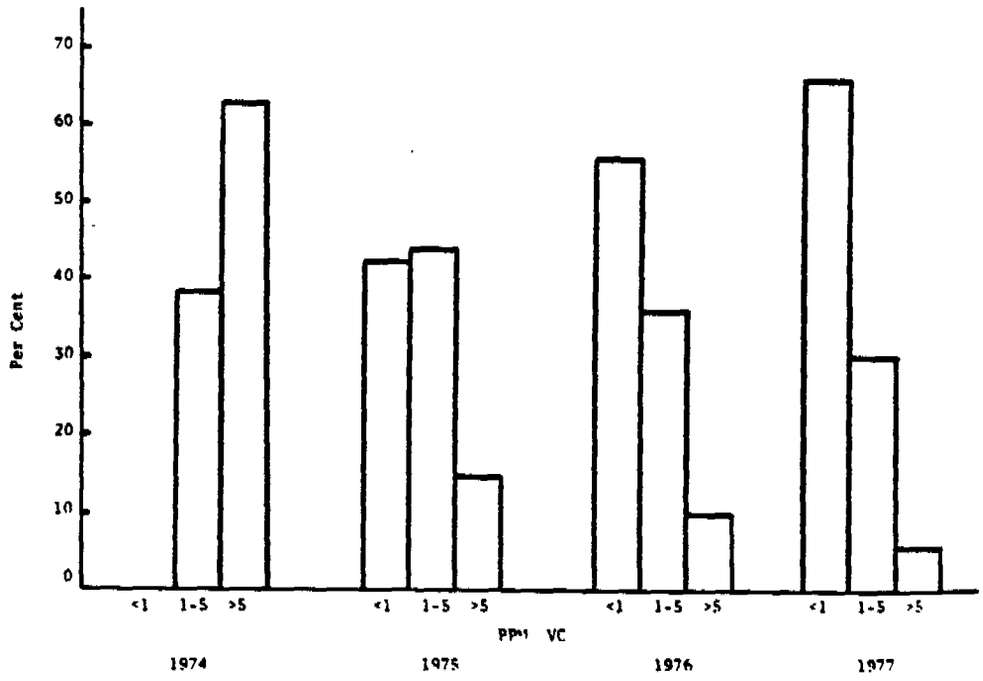

FIGURE 5. Distribution of VC exposures at a suspension and dispersion polymerization plant.

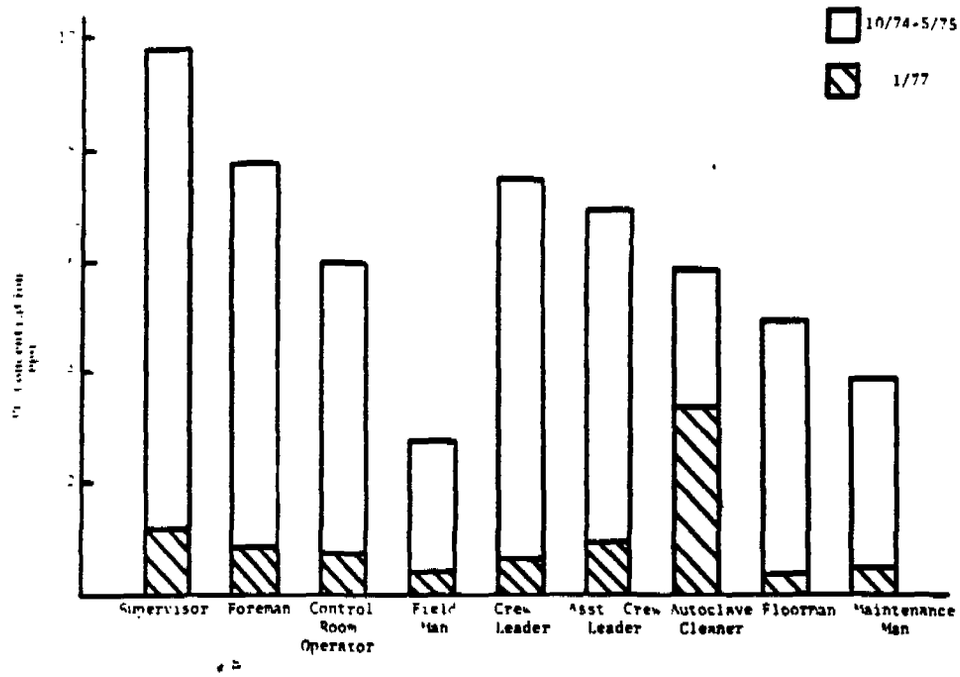

FIGURE 6. Change in VC exposures at a mass polymerization plant.

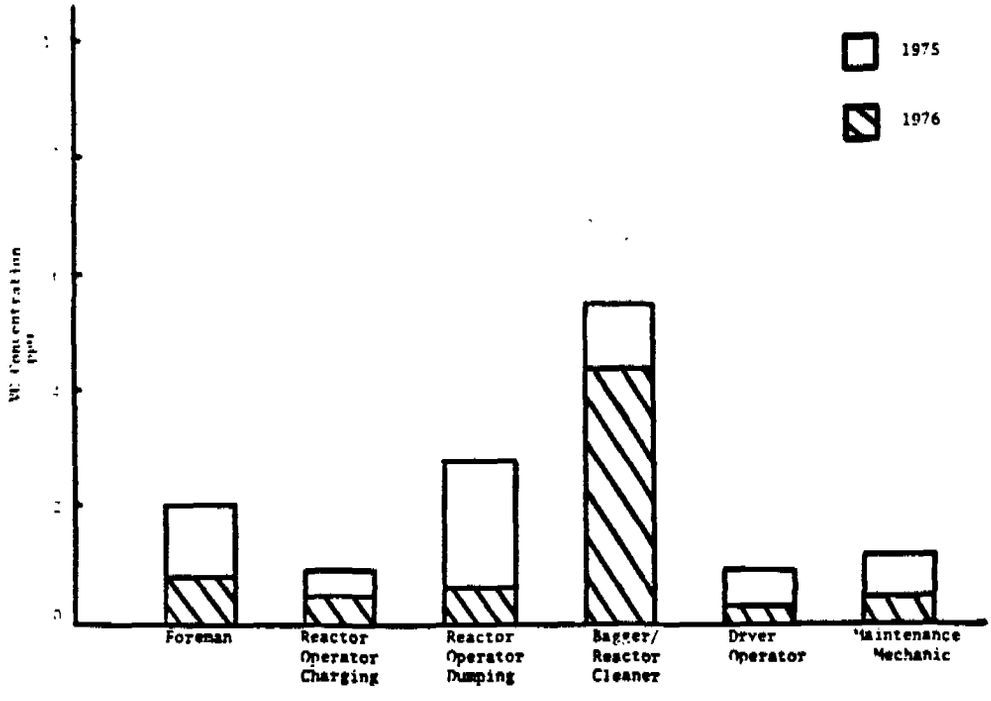

FIGURE 7. Change in VC exposures at a suspension polymerization plant.

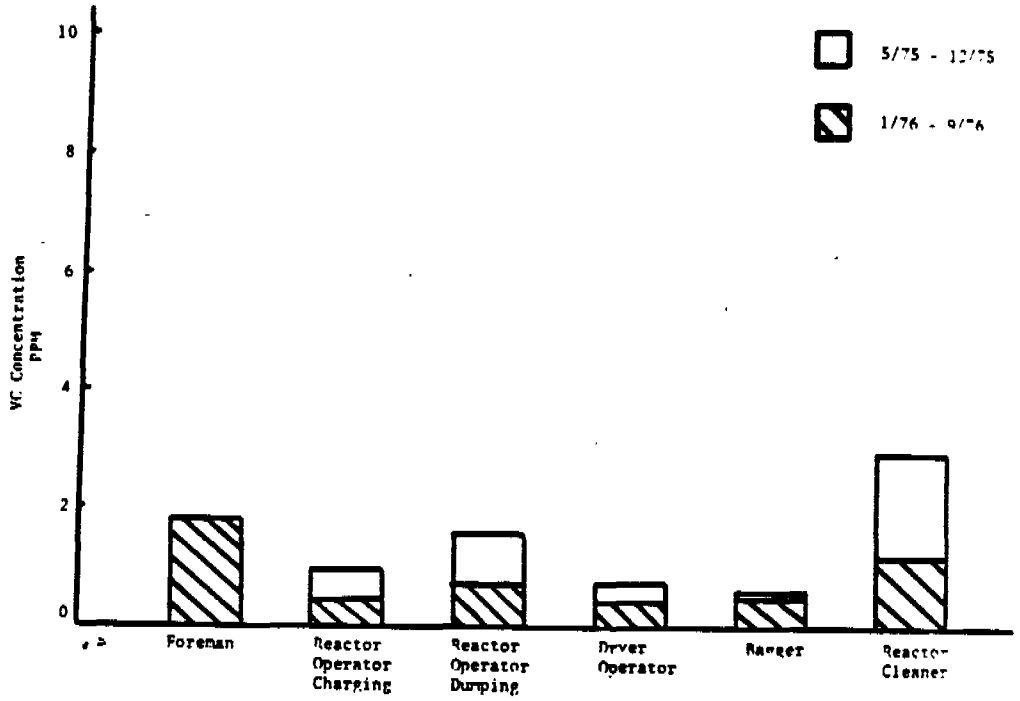

FIGURE 8. Change in VC exposures at a dispersion polymerization plant.

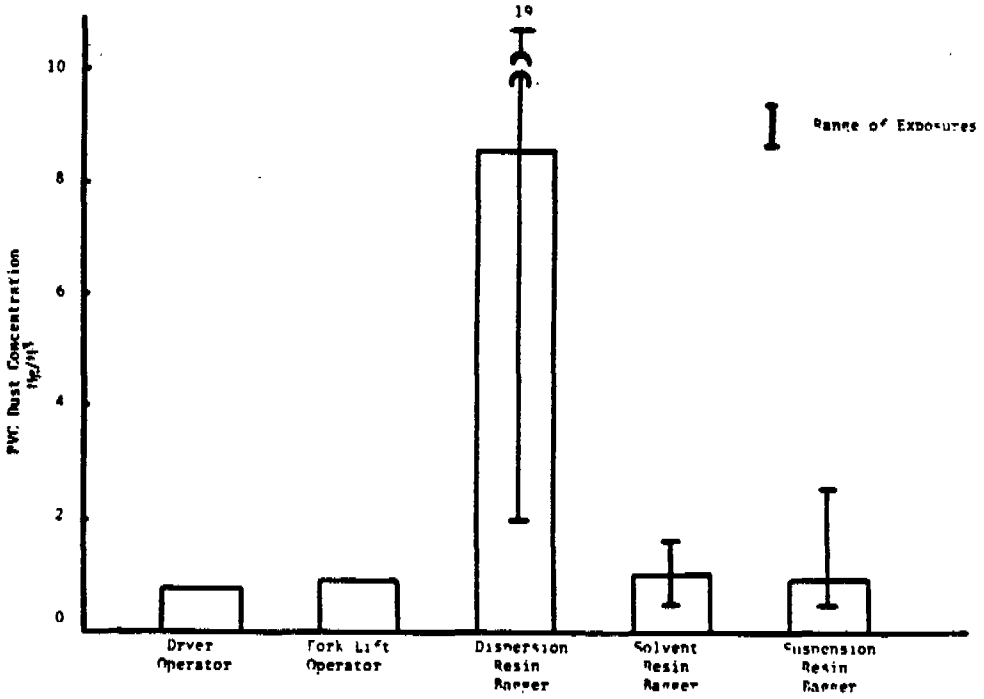

FIGURE 9. PVC dust exposures at VC polymerization plants.

during the industry-wide study. Total dust concentrations were determined. Because it was felt that PVC would be the primary constituent of the dust, no analysis for PVC was performed. Respirable samples were not collected because it was felt that the static charge on PVC particles would cause malfunctioning of the normally used sampling equipment, such as nylon cyclones. A few samples were examined by optical microscopy. In these samples all particles had diameters less than 7 μm, and 90% of the particles had diameters less than 2.5 μm. The samples examined were from dispersion resin operations.

This result was expected, since dispersion resins generally are in the range of 2-10 μm in diameter. Suspension resins, the major resins in terms of production, are generally much larger, about 100 μm in diameter. The sampling results displayed in Figure 9 show that dispersion resin bagging had by far the highest exposure, averaging 8.6 mg/m^3. We have no information on whether PVC dust exposures have been reduced since 1974.

It appears, based on the information that we currently have that the PVC fabrication industry has achieved good control of VC exposures. The VC polymerization industry was making big strides in controlling exposures, but we have no information to tell if they have continued to lower exposures since 1977. We have no information on exposure reductions in the VC monomer production industry, but it appeared that only one job category was significantly above the standard in 1974.

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Mortality Among PVC-Fabricating Employees

by Leonard Chiazze, Jr.,* and Lorraine D. Ference*

The results of a cross-sectional mortality study of 3847 deaths occurring among current and former (white) employees of 17 PVC fabricators during 1964-1973 are presented. Sex-race-cause-specific proportionate mortality ratios (PMR's) were computed by using two separate standards: one, the U.S. mortality in 1968; the second, U.S. mortality for the individual years 1964-1973. In addition, a case-control analysis, based upon 44 breast cancer deaths among white female employees, is presented. PMR's are significantly different from unity for all cancers, and for cancers of the digestive system among both white males and white females. Although observed deaths significantly exceeded expectations for cancer of the breast, a subsequent case-control analysis reveals no statistically significant relative risks for breast cancer.

Introduction

In March, 1974, Organization Resources Counselors (ORC) was requested by representatives of PVC producers who are members of ORC's Occupational Safety and Health Standards Group, to carry out a study of health risks to employees working in the PVC fabricating industry. After considering a number of alternative study designs, it was decided that a cross-sectional mortality study would best meet the need for providing information as rapidly as possible. Details regarding reasons for selecting this type of study, the actual study design and results of the inquiry have been reported previously (*1*).

In the original report, proportionate mortality ratios were calculated according to the method of Guralnick (*2*) and by using the sex-cause-age specific distribution of deaths among U.S. whites in 1968 as the basis for determining expectation (*3*). For the current report, we have analyzed the data using the Mantel-Haenszel procedure both for determination of PMR's and in order to test whether PMR's differ significantly from unity (*4*, *5*). In addition, PMR's are presented using two separate standards as the basis for expectation.

A follow-up to the cross-sectional mortality investigation, utilizing deaths among white women for whom the underlying cause of death was cancer of the breast, was undertaken (*6*), and results of the case-control analysis will be presented.

Materials and Methods

Although alternative study designs were considered, several factors led to the decision to carry out a cross-sectional mortality study. First, the primary study objective was to determine relatively quickly whether any angiosarcoma deaths could be identified among the study group and this was best accomplished by examining causes of death among relatively recent decedents. Second, it would not have been possible to identify clearly and completely (if at all) the cohort of workers necessary for a historical cohort mortality study within a reasonably short period of time. As a result, the study was based upon 4336 deaths which occurred during the years 1964-1973 among active or retired employees of 17 companies engaged in PVC fabrication (Table 1). This report will be restricted to the 3847 deaths among whites, since the number of deaths for most causes among nonwhites was quite small, making interpretation of cause specific mortality difficult at best.

Since the population at risk could not be deter-

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Table 1. Distribution of deaths among employees of 17 PVC fabricators by race and sex, 1964–1973.

Race	Total	Sex		
		Male	Female	Unknown
Total	4336	3676	658	2
White	3847	3252	595	0
Nonwhite	198	174	24	0
Unknown	291	251	39	2

mined, mortality rates as measures of risk could not be calculated. Rather, results are summarized in terms of proportionate mortality ratios (PMR) calculated according to the method of Mantel-Haenszel (5). Two separate standards have been employed. The first is the sex-cause-age specific distribution of deaths among U.S. whites in 1968 (1968 Standard). A second standard employs the sex-cause-age specific distribution of deaths among U.S. whites in each of the years 1964-1973 (1964-1973 standard). Cause-specific PMR's based upon the 1968 standard are adjusted for age, race and sex. Cause-specific PMR's based upon the 1964-1973 standard are adjusted for year of death as well as for age, race and sex.

The Mantel-Haenszel procedure for assessing the statistical significance of a difference between the observed and expected numbers of deaths for a specific cause requires the construction of a series of 2×2 contingency tables as shown in Table 2. Separate tables are constructed for each level of the factor(s) to be adjusted for. If, for example, we wish to compute a PMR for all decedents for cause X, adjusted for age (five age groups) and year of death (10 years: 1964-1973), i.e., 1964-1973 standard, we would have 50 2×2 tables: age $<$ 35, year of death 1964; age $<$ 35, year of death 1965; . . . ; age $<$ 65, year of death 1973. Observed deaths and expected deaths are summed over the 50 separate 2×2 tables to produce a PMR for cause X, adjusted for age and year of death. The resulting age-year of death-adjusted PMR compares the number of deaths from cause X observed in the study group to the number of deaths from cause X expected in the study group, if the proportion of total deaths

Table 2. Construction of 2×2 contingency tables.

	Number of deaths in group I, attributed to		Total deaths
	Specified cause	Other causes	
Study group	A_i	C_i	N_{1i}
Standard	B_i	D_i	N_{2i}
Total	M_{1i}	M_{2i}	T_i

ascribed to cause X was the same, age group by age group and year by year, as for the comparison group.

The statistical significance of the PMR (a test of the hypothesis that the PMR is 1.0 against the alternative that the PMR is different from 1.0) is evaluated by using the Mantel-Haenszel continuity corrected chi-square with one degree of freedom, i.e.,

$$\chi_{MH}^2 = \frac{(|\Sigma A_i - \Sigma E(A_i)| - \frac{1}{2})^2}{\Sigma \ \mathrm{Var}\ A_i} \qquad (1)$$

where

$$\mathrm{Var}\ A_i = \frac{N_{1i}N_{2i}M_{1i}M_{2i}}{T_i^2(T_i - 1)}$$

$$E(A_i) = \text{Expected value of } A_i = (M_{1i}N_{1i})/T_i$$

The case-control analysis is based on 44 deaths among white females in the study with cancer of the breast as the underlying cause of death. Controls were drawn from deaths due to diseases of the circulatory system and accidents, and matched by age plus or minus five years and company, if possible. Forty of the cases were matched on both criteria and four on the basis of age alone. The basis for the case-control analysis, then, is the 44 breast cancer deaths and 134 matched controls distributed among eight of the 17 companies.

Results

Proportionate Mortality Ratios

Cause specific PMR's adjusted for age are presented for white male and white female employees in Tables 3 and 4. For Table 3, expected numbers of deaths are based on the cause-sex-age specific distribution of deaths among U.S. whites applied to the study group. For Table 4, PMR's are adjusted for year of death as well as race, sex and age. Statistically significant departures from unity (i.e., PMR = 1) are evaluated by using the Mantel-Haenszel continuity corrected chi-square with one degree of freedom. Causes of death for which the calculated chi-square indicated a statistically significant excess or deficit at the $\alpha = 0.05$ level of significance are indicated in the table. It is important to remember that when carrying out a large number of significance tests, some number will turn out to be significant on the basis of chance alone. When dealing with a large number of tests for PMR's, it may be more appropriate to consider the Mantel-Haenszel test as a screening device rather

giving it a strict probabilistic interpretation. With these caveats, we see that based upon the standard, among white males there are statistically significant PMR's for all cancers, digestive cancer, respiratory cancer and other and unspecified cancers (Table 3). Among white females, PMR's are significantly different from one for all cancers, digestive cancers, breast cancer, urinary cancer and other and unspecified cancers. In contrast to white males, the PMR for urinary cancer among white

Table 3. Observed deaths and cause specific proportionate mortality ratios (PMR) for employees of 17 PVC fabricators by sex (white only), 1964–1973 (expectations based on U.S. mortality, 1968).

Cause of death	ICDA 8th Revision	Male		Female	
		Observed deaths	PMR	Observed deaths	PMR
All causes	000–999	3252	1.0000	595	1.0000
All cancer	140–209	670	1.1905[a]	181	1.3266[a]
Buccal cavity and pharynx	140–149	15	0.8382	3	—
Digestive organs and peritoneum	150–159	212	1.3058[a]	53	1.5134[a]
Stomach	151	41	1.3000	8	1.6226
Large intestine	153	72	1.3688[a]	24	1.5785[a]
Rectum	154	26	1.3230	8	2.0822
Liver	155	6	1.4306	0	—
Pancreas	157	37	1.1307	7	1.1612
Other and unspecified digestive	152, 156, 158, 159	30	1.3861	6	1.3680
Respiratory system	160–163	206	1.1647[a]	12	1.0119
Larynx	161	9	1.0695	0	—
Trachea, bronchus and lung	162	194	1.1704[a]	12	1.0788
Other	160, 163	3	—	0	—
Breast	174	0	—	44	1.3710[a]
Female genital organs	180–184	—	—	19	0.8232
Cervix	180	—	—	5	0.6827
Corpus	181, 182	—	—	6	1.2911
Ovary	183	—	—	7	0.6714
Other	184	—	—	1	—
Male genital organs	185–187	42	0.7936	—	—
Prostate	185	41	0.8223	—	—
Other	186, 187	1	—	—	—
Urinary organs	188–189	37	1.1153	11	2.9296[a]
Bladder	188	20	1.0053	4	—
Kidney	189	17	1.2500	7	3.4535[a]
Brain and nervous system	191–192	16	1.1499	4	—
Lymphomas	200–203, 208, 209	44	1.3560	9	1.1900
Leukemias	204–207	19	0.8117	1	—
Other and unspecified cancers	170–173, 190, 193–199	79	1.6132[a]	25	1.9584[a]
Other causes					
Diabetes	250	43	0.8655	10	0.6540
Diseases of circulatory system	390–458	1930	1.0516[a]	278	0.9087[a]
Heart disease	(390–398), 402, 404, (410–429)	1495	1.0625[a]	188	0.9009
Rheumatic heart disease	393–398	16	0.6373	7	0.7502
Hypertensive disease	400–404	36	1.0575	3	—
Ischemic heart disease	410–413	1381	1.0599[a]	158	0.8644[a]
Myocardial infarction	410	898	1.1040[a]	93	0.9888
Cerebrovascular disease	430–438	290	0.9913	65	0.9133
Other circulatory	450–458	40	1.5294[a]	8	1.2398
Diseases of respiratory system	460–519	144	0.6344[a]	17	0.5804[a]
Cirrhosis of liver	571	42	0.6675[a]	3	—
Cholelithiasis, cholecystitis and cholangitis	574–575	7	1.1018	3	—
Accidents, poisonings and violence	800–999	187	0.7007[a]	46	1.1364
Accidents	800–949	134	0.7043[a]	37	1.3369
Suicide	950–959	39	0.7427	2	—
All other causes	Residual	229	1.0410	57	1.1500

[a]PMR significantly different from one at $\alpha = 0.05$.

females is high and, although based upon only 11 deaths, is statistically significant. On the other hand, in contrast to the observation in white men, the PMR for respiratory cancer among white women is very close to unity. Similar to the observation in white men, mortality from cirrhosis of the liver is low, but the corresponding number of observed deaths is quite small.

Among both white male and female employees diseases of the circulatory system account for a large percentage of total deaths. In each case observed numbers of deaths are close to expected

Table 4. Observed deaths and cause specific proportionate mortality ratios (PMR) for employees of 17 PVC fabricators by sex (white only), 1964–1973 (expectations based on U.S. mortality, 1964–1973).

Cause of death	ICDA 8th Revision	Male		Female	
		Observed deaths	PMR	Observed deaths	PMR
All causes	000–999	3252	1.0000	595	1.0000
All cancer	140–209	670	1.1567[a]	181	1.2956[a]
Buccal cavity and pharynx	140–149	15	0.8340	3	—
Digestive organs and peritoneum	150–159	212	1.2667[a]	53	1.4813[a]
Stomach	151	41	1.3084	8	1.6541
Large intestine	153	72	1.3239[a]	24	1.5490[a]
Rectum	154	26	1.3164	8	2.1165
Liver	155	6	0.9801	0	—
Pancreas	157	37	1.1202	7	1.1461
Other and unspecified digestive	152, 156, 158, 159	30	1.3139	6	1.4548
Respiratory system	160–163	206	1.1155	12	0.9100
Larynx	161	9	1.0563	0	—
Trachea, bronchus and lung	162	194	1.1266	12	0.9787
Other	160, 163	3	—	0	—
Breast	174	0	—	44	1.3448[a]
Female genital organs	180–184	—	—	19	0.8161
Cervix	180	—	—	5	0.6869
Corpus	181, 182	—	—	6	1.2736
Ovary	183	—	—	7	0.6597
Other	184	—	—	1	—
Male genital organs	185–187	42	0.7687	—	—
Prostate	185	41	0.7964	—	—
Other	186, 187	1	—	—	—
Urinary organs	188–189	37	1.0942	11	2.8365[a]
Bladder	188	20	0.9884	4	—
Kidney	189	17	1.2517	7	3.4206[a]
Brain and nervous system	191–192	16	1.1267	4	—
Lymphomas	200–203, 208, 209	44	1.3525	9	1.1944
Leukemias	204–207	19	0.8019	1	—
Other and unspecified cancers	170–173, 190, 193–199	79	1.5975[a]	25	1.9564[a]
Other causes					
Diabetes	250	43	0.8993	10	0.6743
Diseases of circulatory system	390–458	1930	1.0538[a]	278	0.9132[a]
Heart disease	(390–398), 402, 404, (410–429)	1495	1.0600[a]	188	0.9035
Rheumatic heart disease	393–398	16	0.7030	7	0.8009
Hypertensive disease	400–404	36	1.1541	3	—
Ischemic heart disease	410–413	1381	1.0512[a]	158	0.8522[a]
Myocardial infarction	410	898	1.1148[a]	93	0.9962
Cerebrovascular disease	430–438	290	1.0011	65	0.9184
Other circulatory	450–458	40	1.5062[a]	8	1.2256
Diseases of respiratory system	460–519	144	0.6883[a]	17	0.6546
Cirrhosis of liver	571	42	0.6438[a]	3	—
Cholelithiasis, cholecystitis and cholangitis	574–575	7	1.1536	3	—
Accidents, poisonings and violence	800–999	187	0.6991[a]	46	1.1173
Accidents	800–949	134	0.7084[a]	37	1.3266
Suicide	950–959	39	0.7034[a]	2	—
All other causes	Residual	229	1.0246	57	1.1197

[a]PMR significantly different from one at $\alpha = 0.05$.

Table 5. *Distribution of 44 breast cancer deaths and 134 matched controls by exposure category.*

Exposure category	Breast cancer, cases		Matched control	
	Number	%	Number	%
[illegible] exposure	27	61.4	97	72.4
[illegible]bable exposure	6	13.6	17	12.6
[illegible]ble exposure	0	0	4	3.0
[illegible]te exposure	2	4.5	6	4.5
[illegible]known exposure	9	20.5	10	7.5
	44	100	134	100

although statistically significant.

Results based upon the 1964-1973 standard are, in general, consistent with those on the 1968 standard (Table 4). There are, however, some notable exceptions. In general, the PMR's based upon the 1964-1973 standard are lower than those based upon the 1968 standard. Further, the PMR for respiratory cancer among white males is not significantly different from one when based on the 1964-1973 standard. Among white women, the PMR for other (noncancer) respiratory deaths is significantly below unity based on the 1964-1973 standard.

Case-Control Analysis

Comparison of cases and controls on a variety of variables where information was available, including length of employment, ever married versus never married, and child-bearing history, reveals no statistically significant differences ($p > 0.05$) between cases and controls for any of these variables.

Review of employment histories for cases and controls revealed a wide variety of jobs ranging from office and clerical work to production jobs such as bench inspector, press operator, trimmer, assembler and sweeper. The wide range of job content and location made it impossible to determine precisely whether there was PVC exposure in every case, or the precise length of that exposure. Therefore, a subjective ranking system was developed to classify exposures. Work histories were reviewed with plant personnel and PVC exposure potential was categorized into five classes—no exposure, improbable exposure, possible exposure, definite exposure and unknown exposure. This classification scheme enabled some definitive exposure statement in 80% of the cases and 92% of the controls (Table 5).

After matching by age and company, 38 matched sets of cases and controls were developed from the 44 cases and 134 controls. There are fewer matched sets than cases because, in six instances, it was necessary to combine cases of similar age within the same set in order to have at least one control per matched set. The Mantel-Haenszel procedure was used to derive a summary estimate of relative risk and to test for significant departures from unity (4, 7). In this procedure, each of the 38 sets can be viewed as a 2×2 contingency table. Thus, the ith set can be represented as in Table 6.

Relative risk is defined as the ratio of the probability of dying from cancer of the breast among women exposed to PVC to the probability for women not exposed. Estimates of these individual probabilities are not available from a case-control study. However, a measure of estimated relative risk from case-control studies as suggested by Mantel and Haenszel has been calculated as in Eq. (2). To assess whether the departure from unity of an observed relative risk is too great to have occurred by chance alone, a summary chi-square test corrected for continuity was performed using the Mantel-Haenszel procedure. The calculated chi-square must be 3.84 or larger in order to conclude with 95% assurance that the observed relative risk did not differ from unity by chance alone.

$$R = \Sigma_i (A_i D_i T_i)/\Sigma_i (B_i C_i / T_i) \qquad (2)$$

Combining the five exposure categories into two may be accomplished in a variety of ways, resulting in several possible relative risk measures as shown in Table 7.

None of the calculated relative risks, including the second shown which treats anything other than no exposure as definitely exposed, are statistically

Table 6. *Representation of ith set.*

	PVC exposure		
	Yes	No	Total
Cases	A_i	B_i	N_{1i}
Controls	C_i	D_i	N_{2i}
Total	M_{1i}	M_{2i}	T_i

significant; i.e., they may have occurred by chance alone. Similar analyses were carried out on a company-by-company basis. None of the relative risks so calculated is significantly different from unity.

Discussion and Summary

The cross-sectional mortality study was designed with two objectives. The first was to determine if any angiosarcoma deaths had occurred among employees of the PVC fabricators under study. Since no angiosarcoma deaths were found among the employees studied, the first question has an unequivocal answer. A secondary objective was that of examining the distribution of deaths by cause among the employees under study. Implicit in that objective is the question of whether or not that distribution is, in some sense, unusual. There is no unequivocal answer to the latter question. Whether or not an observed distribution of causes of death is unusual clearly relates to the standard or comparison population as well as the analytic methodology (*8*). This is illustrated by the observation that there are PMR's which are significantly different from unity on the 1968 standard and not on the 1964-1973 standard. However, it seems much more important to focus on the large area of agreement between the two standards rather than on the few areas of disagreement.

On the basis of the PMR analyses, there are statistically significant excesses in total cancer mortality among both white males and white females when compared to the distribution of deaths for the total United States specific for color and sex and adjusted for age. Excesses in cancer mortality appear concentrated in cancers of the digestive system and, in particular, in cancers of the intestine for both men and women. In addition, there is a suggestion that mortality from cancer of the breast and urinary organs among white women employees is higher than that for the total U.S. There are, however, several reasons why definitive interpretation is difficult. Factors meant to suggest that proportionate mortality analyses must be interpreted cautiously have been reviewed previously (*9-11*). However, while these results must be interpreted with caution, they appear to be consistent with previously studied workers and suggest the need for some continuing investigation (*1*).

One such follow-up investigation has been presented here in the form of a case-control study involving the 44 breast cancer deaths. On the basis of a case-control analysis, estimates of relative risk were derived but none of these relative risk estimates is significantly different from one, although such results must be interpreted with caution.

Absence of a statistically significant relative risk does not demonstrate that there is not an excess risk of death from breast cancer among women employees with PVC exposure. In fact, when no statistically significant relative risks are found, it is pertinent to ask what the chances are of detecting an increase of a given magnitude from the available data. Using the method described by Walter (*12*) we have subjected each of the relative risk estimates (Table 7) to a least significant relative risk analysis under the conditions that we desire 95% assurance that a risk of such magnitude, if observed, did not occur by chance alone and 80% probability of detecting the least significant relative risk if it exists.

Even in the case where all but no exposure are counted as exposed, the smallest relative risk which could be detected from these data is nearly 3:1. Nearly 200 cases and 200 controls would have been necessary to detect a true doubling of the risk under the specified conditions. Given the sample size in this study and the percentage of controls exposed (a percentage which was unknown at the

Table 7. Relative risk (RR) and least significant relative risk (LSRR) estimated for contrasts of various exposure category combinations.

Contrast	Number of cases	RR (Relative risk) estimate[a]	LSRR (Least significant relative risk) estimate[b]
Definite exposure vs. no exposure	29	1.81	6.77
Definite + improbable + possible + unknown exposure vs. no exposure	44	1.94	2.92
Definite + possible exposure vs. no + improbable exposure	35	0.624	5.02
Definite + possible + unknown exposure vs. no + improbable exposure	44	2.73	3.41

[a]None of the relative risk estimates are statistically significantly different from unity ($p > 0.05$).
[b]The true relative risk would have to be at least this large to have an 80% assurance of detection (i.e., power = 0.80) with a type I error of 0.05 (i.e., $\alpha = 0.05$).

part of the study), it would be possible to detect only very large increases in risk. There seems reasonable assurance, therefore, that such very large increases in the risk for breast cancer do not exist among these PVC fabricators, but no such statement can be made for possibly smaller increases in risk.

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Mortality and Cancer Rates among Workers in the Swedish PVC Processing Industry

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Personnel lists from four PVC-processing industries were collected on production of employees with at least three months of employment at the beginning of 1945 and the last day of employment December 31, 1974. Of 2073 persons, 103 could not be followed up, because they had moved abroad. The remaining persons comprise the cohort of 1970 individuals who were analyzed and compared with the national population with respect to mortality from various diseases and cancer morbidity.

The death risk from myocardial infarction is elevated in the cohort. This elevation is most clearly apparent in the subcohort which had at least two years of exposure time and where the analysis was directed at circumstances chronologically close to the time of exposure. The myocardial infarction risk related to vinyl chloride exposure is discussed in relation to earlier studies on the vascular effects of vinyl chloride. An indication of an elevated risk of morbidity and mortality from tumors in the digestive organs is also present. However, this is not statistically confirmed. A few future follow-ups of the present study are necessary in order to clarify any possible elevated risk of tumors in the PVC-processing industry.

Vinyl chloride has been shown to cause scleroderma, Raynaud's phenomenon, acroosterolysis, liver damage and liver cancer (hemangiosarcoma) (*1*) in workers exposed to vinyl chloride monomer (VCM). This has been shown in studies (*2*, *3*) performed at companies which fabricate poly(vinyl chloride) (PVC). In animal experimental studies it has been reported that inhalation of VCM causes malignant tumors in different organs in rodents (*4-6*).

In Sweden, in 1974, two cases of liver hemangiosarcoma were diagnosed in employees at a company engaged in the processing of VCM and PVC (*7*). Later another two cases occurred at the same factory.

Studies on other forms of cancer (*8*, *9*) suggest that VCM-exposed workers in the PVC fabricating industries may possibly run an elevated risk of contracting forms of cancer other than hemangiosarcoma in the liver. Earlier, an excess mortality from cardiovascular diseases was also observed (*10*) in employees in the PVC manufacturing industry.

The present retrospective cohort study was performed for the purpose of determining the pattern of morbidity and mortality in the PVC processing industry. The PVC processing industry, generally speaking, has had a lower level of exposure to VCM than the fabrication industry. In the Swedish PVC processing industry, at present, about 5000 persons are employed in production.

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Material and Methods

Information for the study was collected from four PVC processing companies. The four companies all used PVC which, after additions of various chemicals, is heat-treated for fabrication into floor covering, lace, pipes, and food packaging.

Data Collection

The following data were collected from the personnel lists at the companies: personal number, name, beginning and end of exposure (year and month) and class of exposure.

In order for a person to be included in the original cohort, at least three months of employment was required in the period beginning in 1945 and the ending December 13, 1974. The exposure was classified as follows: class 3 (high), work in the mixing department; class 2 (medium), heat treatment machines; and class 1 (low), other production departments.

The collected data were transferred to punched cards and magnetic tape for statistical processing. The magnetic tape was coordinated with the national total population and the so-called death tapes for the 1961-1976 period and checked against the cancer registry by the Central Bureau of Statistics (SCB). The personnel numbers which could not be recovered at this time were checked by the national taxation office. The original cohort included a total of 2,073 persons. Of these, 103 persons (5%) dropped out, 70 or whom had moved abroad, 5 were found in the missing persons register of the tax office, and 28 could not be traced.

Study Cohorts

For the statistical processing, the results of the cohort of 1970 persons were divided into a number of subcohorts (study cohorts): (1) all persons with at least three months of exposure (follow-up time from the beginning of exposure and through 1976); (2) all persons with at least six months of exposure excluding those who stopped before 1961 (follow-up time from beginning of exposure but no earlier than 1961 and through 1976); (3) all persons with at least six months of exposure and where the exposure began no earlier than 1961 (follow-up time from beginning of exposure and through 1976); (4) all persons with at least two years exposure (follow-up time from two years after beginning of exposure but no earlier than 1961 and through 1976 but no more than ten years after exposure stopped); (5) all persons with at least two years exposure (follow-up time from ten years after exposure began but no earlier than 1961 and through 1976).

The two latter named study cohorts were chosen in order to study whether any differences existed in the death cause pattern with respect to when the deaths occurred after the beginning of exposure. The first of the study cohorts was intended to shed light on possible causes of death which occur relatively early, e.g., accidents caused by the job. The second was intended to shed light on such death causes as occurred after a longer time had passed. Tumors caused by occupational exposure, for example, often have a long latency period, 5-10 yr or longer.

Results

The original cohort was relatively young at the beginning of exposure. The age distribution in the different exposure classes is given in Table 1. One finds various dissimilarities between the exposure classes. In class 1 (low), 41.7% were younger than 35 years at the beginning of exposure; in class 2 (average), 47.7%; and in class 3 (high), 50.6%. There

Table 1. Age distribution in original cohort at beginning of exposure

Age	% in each exposure class			
	1	2	3	1–3
< 19	1.6	2.0	8.9	2.1
20–24	7.5	14.6	14.3	9.2
25–29	13.5	15.7	21.4	14.4
30–34	19.1	15.4	17.0	18.3
35–39	17.0	13.2	10.7	15.9
40–44	13.7	12.6	9.8	13.3
45–49	11.1	12.6	8.9	11.3
50–54	8.0	7.8	5.4	7.6
55–59	5.1	3.9	1.8	4.7
60–64	2.6	2.0	1.8	2.4
> 65	0.7	0.3	0.0	0.6
No. of persons	100% (1501)	100% (357)	100% (112)	100% (1970

Table 2. Distribution of exposure time in the original cohort

Months	% in each exposure class			
	1	2	3	1–3
< 5	13.1	0.3	0.0	10.1
6–23	38.4	8.4	8.9	31.3
24–59	25.0	17.4	10.7	22.6
60–119	15.3	45.7	15.2	20.8
> 120	8.2	28.3	65.2	15.1
	100% (M = 1510)	100% (M = 357)	100% (M = 112)	100% (M = 19

Table 3. Observed and anticipated number of deaths as of December 31, 1976.

[illegible]	Number	No. of deaths		Ratio O/E	Approx. 95% confidence interval
		Observed	Expected		
[illegible]: class 1	1303	53	55.5	0.95	± 0.26
[illegible]: class 2	356	14	21.9	0.64	± 0.34
[illegible]: class 3	112	6	10.3	0.70	± 0.47
[illegible]: class 1–3	1171	73	87.8	0.84	± 0.19

Table 4. Observed and anticipated number of deaths from certain causes during the 1969–1976 period in those with at least six months of exposure including those who stopped before 1961.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	17	14.0	1.21
Digestive organ tumors 150–159	8	4.9	1.63
Cardiovascular diseases VII	22	24.3	0.91
Myocardial infarction 410.90	15	20.0	1.49
Accidents, suicide, etc. XVII	13	9.2	1.42

[a]Risk calculated from the beginning of exposure but no earlier than 1961. Study cohort 2 (1771 persons).

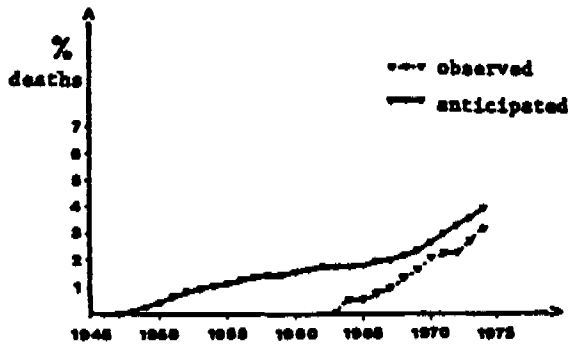

FIGURE 1. Cumulative deaths (in percent): (▽) observed; (▼) anticipated. Expected value calculated from beginning of exposure. Study cohort 1 (1970 persons). The percentage for a given year was calculated as 100 (number of persons dying through year in question divided by the number of persons beginning exposure up to and including the year in question).

were also dissimilarities in the length of exposure with respect to exposure class (Table 2). However, it should be noted that the table includes cases which were still under exposure at the final date for entrance into the cohort (December 31, 1974), for which reason, a certain bias toward short exposure times is found. Regardless of this, exposure class 3 has longer exposure times on the average.

The cohort as a whole reveals no noteworthy increase in the total risk compared with the national average, nor is there any indication of this in the subgroups making up the study cohort.

Study cohort 1, which includes everyone with at least six months of exposure and with calculation of the risk from the beginning of exposure, is somewhat remarkable in that the anticipated number of deaths is significantly higher than that observed up to 1964 (Fig. 1). This is commented on further in the discussion. Study cohort 2 (Tables 3 and 4; Fig. 2) includes persons with at least six months of exposure, excluding those who stopped before 1961. The risk calculation is made from the beginning of the exposure, but no earlier than 1961 and up to the end of the follow-up time (1976). The observed number of deaths is somewhat lower than expected, much lower in exposure class 2. Classes 2 and 3 are relatively small and are sensitive to random deviations in this type of analysis. In order for random deviations not to influence the results, the classes were combined. This is true of all study cohorts. This distribution with respect to the vari-

Table 5. Observed and anticipated number of deaths as of December 31, 1976 in those with at least six months of exposure beginning no earlier than 1961.[a]

Cohort	Number	No. of deaths		Ratio O/E	Approx. 95% confidence interval
		Observed	Expected		
Exp. class 1	1139	43	41.2	1.04	± 0.31
Exp. class 2	247	4	11.7	0.34	± 0.34
Exp. class 3	42	1	1.8		
Exp. class 1–3	1428	48	54.7	0.88	± 0.35

[a]Risk calculation from beginning of exposure. Study cohort 3 (1428 persons).

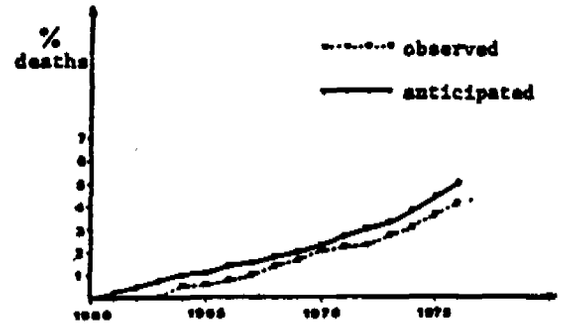

FIGURE 2. Cumulative deaths (in percent). Expected value calculated through 1961. Study cohort 2 (1771 persons). Percentage for a given year calculated as in Fig. 1.

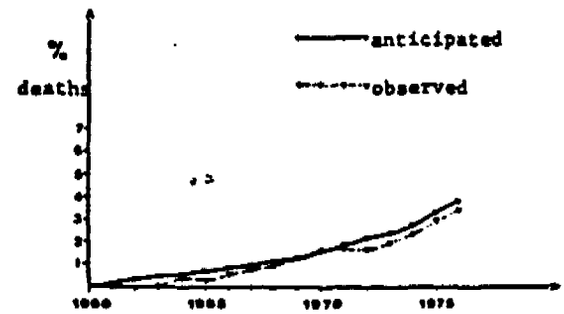

FIGURE 3. Cumulative deaths (in percent) of those who began exposure in 1960 or later. Study cohort 3 (1428 persons). Percentage for a given year calculated as in Fig. 1.

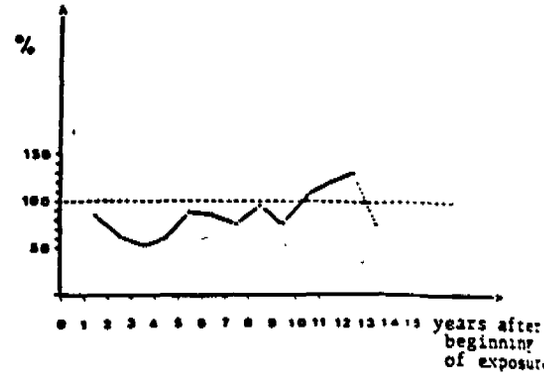

FIGURE 4. Observed death risk per year at different points of time after beginning of exposure expressed in percent of corresponding anticipated risk in those who began exposure in 1961 or later. Study cohort 3 (1428 persons).

Table 6. Observed and anticipated number of deaths from certain causes during the 1969–1976 among those with at least six months of exposure beginning in 1961 or later.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	9	9.7	0.93
Digestive organ tumors 150–159	4	3.3	1.2
Cardiovascular diseases VII	16	16.2	0.9
Myocardial infarction 410.90	14	11.2	1.25
Accidents, suicide, etc. XVII	11	7.3	1.51

[a]Risk calculated from the beginning of exposure. Study cohort 3 (1428 persons).

ous death causes is shown in Table 4. The observed and anticipated number of deaths during the 1961-1968 period is relatively small, only a few cases, and the death cause classification was modified as mentioned earlier in 1969, for which reason 1961-1968 period is not discussed separately. By and large, the picture is the same there as for the 1969-1976 period reported on. From Table 4, one sees that the observed number of deaths, especially those from tumors of the digestive tract, myocardial infarction and accidents, is somewhat higher than anticipated. However, the differences are not significant. Study cohort 3 (Tables 5 and 6; Figs. 3-5) which pertains to those who began working in 1961 or later but which otherwise satisfy the same criteria as study cohort 2, displays a similar picture.

An analysis of study cohort 3 according to formula B (Figs. 4 and 5) indicates that the annual risk during the first year of exposure is somewhat lower than the anticipated one, but that after about ten years, an increased risk occurs so that the observed risk becomes higher than the anticipated.

Table 7. Observed and anticipated number of deaths from certain causes during the 1969–1976 among those with at least two years of exposure.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	5 (9)	7.4 (8.9)	0.86[b] (1.01)
Digestive organ tumors 150–159	2 (4)	2.6 (3.2)	0.78[b] (1.27)
Cardiovascular diseases VII	15 (16)	12.7 (15.8)	1.18[b] (1.01)
Myocardial infarction 410.90	11 (12)	5.4 (6.6)	2.03[b] (1.82)[b]
Accidents, suicide, etc. XVII	4 (5)	4.6 (5.1)	0.87[b] (0.97)

[a]Risk calculated from two years after beginning of exposure and no more than five years (10 years) after end of exposure. Study cohort 4 (1155 persons).
[b]$p < 0.05$.

Table 8. Observed and anticipated number of deaths from certain causes during the 1969–1976 in those with at least two years of exposure.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	9	6.0	1.51
Digestive organ tumors 150–159	4	2.2	1.85
Cardiovascular diseases VII	12	11.1	1.08
Myocardial infarction 410.90	8	4.5	1.77
Accidents, suicide, etc. XVII	2	2.5	0.79

[a]Risk calculated from 10 years after beginning of exposure. Study cohort 5 (680 persons).

Table 9. Observed and anticipated number of deaths from cancer during 1961–1976 in those with at least six months of exposure excluding those who stopped before 1961.[a]

	Observed	Expected	Ratio O/E
Malignant tumors (total)	51	44.6	1.14
Digestive organ tumors (150–159)	11	8.5	1.29

[a]Risk calculated from beginning of exposure but no earlier than 1961. Study cohort 2 (1771 persons).

In study cohort 4 (Table 7) which concerns time during ongoing exposure or a relatively short time after the end of exposure, i.e., "short-term perspective," one sees an increased death risk from myocardial infarction. Other causes are somewhat lower here than expected.

In study cohort 5 (Table 8), finally, one finds an indication of an increase in the death risk as regards tumors, but also for myocardial infarction. The differences between the observed and anticipated numbers are not, however, statistically confirmed at the 5% level.

The result with respect to mortality can be summarized as follows. In the study cohorts, overall, one finds no noticeable increase in mortality. On the other hand, there are indications of a shift in the death cause pattern compared with the national average. This shift is expressed primarily in the fact that the number of myocardial infarctions is noticeably higher during ongoing exposure or within a relatively short period of time after the end of exposure. There are also indications that the death from tumors can be elevated among persons with a long latency period (Tables 7 and 8).

In the question of cancer morbidity, there is no certain increase in study cohort 2 (Table 9 and Fig. 6). In the question of tumors of the digestive organs, in the same study cohort, 11 cases were observed as opposed to an anticipated 8.5. The difference is not statistically verified. One of these eleven tumors was liver cancer (ICD 155.0).

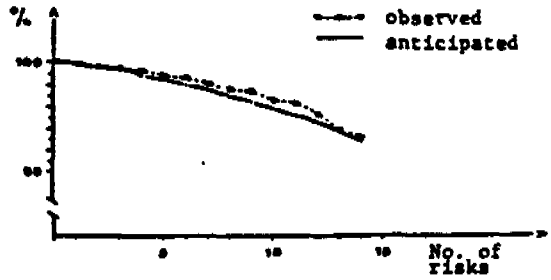

FIGURE 5. Cumulative survival probability (in percent) of those who began exposure in 1961 or later and have at least six months of exposure. Study cohort 3 (1428 persons).

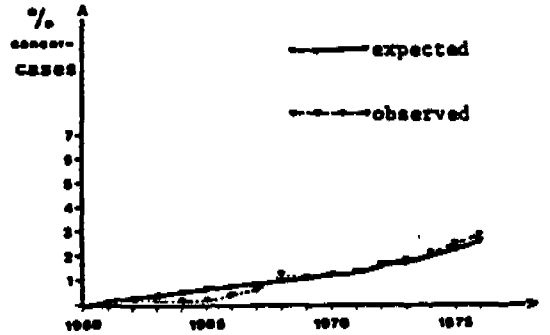

FIGURE 6. Cumulative rates of cases of all cancer (in percent). Study cohort 2 (1771 persons). The percentage for a given year was calculated as 100 (number of persons diagnosed through year in question divided by the number of persons beginning exposure through year in question).

Discussion

A noteworthy finding which arises in the analysis of the total cohort mortality (Fig. 1) is that the number of deaths at the beginning of the observation period (1947-1964) is significantly lower than one would expect in relation to the national average. This difference is so great that one cannot directly consider it to be randomly conditioned, nor can it be entirely ascribed to the so-called healthy worker effect. Theoretically, of course, the possibility exists that the selected cohort, in the question of mortality and the factors which influence said mortality, deviates from the general population. A more credible possibility is, however, that the personnel register that was available at the company involved at the time of this study was incomplete in the matter of hirings during this early period. A personnel register which, in the mid-1960's, was purged

of persons who began employment before 1960, could lead to the difference mentioned above. The companies involved reported that such a purging did not occur, so far as they knew.

If such a purging (thinning out) nevertheless occurred, this would have resulted in the elimination of persons with a long observation time at the time of follow-up. In the present study, the risk calculations were limited to beginning no earlier than 1961. This means a limitation of the analysis to pertain to the group of employees who were living at the beginning of 1961 and where the risk of an elimination is positively eliminated. This limitation, however, signifies a weakening of the analysis, since parts of the cohort with long follow-up times are excluded. Basically, this weakening signifies a poorer possibility of discovering an elevated incidence of cancer if one exists.

The myocardial infarction mortality (ICD 410.90) is elevated in the cohort. This elevation occurs most clearly in the category of the total cohort which has at least two years of employment time and where the analysis was directed at the period of time following two years after the beginning of employment and extending to no more than five years after the beginning of employment. Therefore, this involves that fraction of the mortality from myocardial infarction which chronologically is relatively closely connected to the time of employment. It is impossible on the basis of such observations to draw conclusions that the elevation was caused by exposure to vinyl chloride. The observed increase in myocardial infarction mortality is, however, so striking that it, in combination with the known facts about the toxic properties of vinyl chloride, must be given consideration. There are no reasons to assume that varying diagnostics, standards or practices in filling out the death certificates alone could provide an explanation. A natural conclusion is, therefore, that if one disregards the possibility of a random local phenomenon, the increased frequency is to be ascribed either to selection of individuals susceptible to the risk or an outbreak of risk factors in the close environment of employees. A combination of these two circumstances is, of course, also possible theoretically.

In this connection, it should be noted that many risk factors for myocardial infarction are environmentally conditioned in the fact that they constitute part of the lifestyle of the modern social environment in an industrialized country. Cigarette smoking, physical inactivity, overweight and high blood lipids constitute environmental factors which are related to social behavior. It is a well known fact that the risk of coronary vascular disease in the heart varies, inter alia, with the total load of risk factors. Among other risk factors, one can also name hereditary characteristics and high blood pressure. In this connection, there is reason to recollect that the causal network of coronary disease is multifactorial and that the disease has an environmental relationship in the broad sense. There is also reason to recall the aspect that the total risk increases when several risk factors, known or unknown, are allowed to collaborate (*11*, *12*).

It has not been possible to establish the distribution of such already known risk factors for coronary disease in the cohorts studied with respect to the national population in general. Therefore, no continued analysis of the matter of the causal relationship between close environment and heart disease morbidity can be made within the limits of the study.

Exposure classes 2 and 3 constitute subcohorts that are too small, in the present study, to allow a meaningful discussion of the myocardial infarction risks relative to the various exposure levels in the processing industry. In this connection, one should also consider the circumstance that the exposure classes in this study are based on interviews with the employees directed at the work environment at the time in question some 10 to 15 years ago. Therefore, this involves an environment which has subsequently undergone changes. Objective classification criteria in the matter of exposure, e.g., in the form of environmental measurements, do not exist. The distribution into exposure classes is, for this reason, fraught with uncertainty.

In animal experiments, it has been found that the toxicity picture in rodents chronically exposed to VCM involves the blood vessels. Besides hemangiosarcoma in the liver and the other organs (*4*, *5*) the inhalation of VCM is also believed to cause development of telangiectasis (*4*) in the liver of mice which can lead to death from hemocoele. Changes in the sinus cells have been observed in liver biopsies in VCM-exposed workers (*13*). Capillary changes in the skin of the fingers have also been observed (*14-16*), both in VCM-exposed workers with other vascular-involved diseases, such as acroosteolysis, Raynaud's phenomenon, and sclerodermia, and in VCM-exposed workers without such diseases. An over-representation of deaths from cardiovascular diseases has also been observed in a study on the PVC-fabricating industries (*10*). Animal experimental and previous medical studies of VCM-exposed populations therefore support the assumption that the increased risk of myocardial infarction observed in the present study could possibly be ascribed to VCM exposure.

As regards the mortality and morbidity from tumors, the results are uncertain. There are certain

-dications of an elevation, but the differences are ... statistically confirmed. One can think of two ...ssibilities here: (1) in reality, there is no increase ... the risk of tumors; (2) there is indeed an in-...reased risk of tumors. The results neither confirm ... refute this. Tumors do not occur until after a ...g latency period. The majority of the persons ...cluded in the study did not begin their exposure ...til the 60's and 70's and therefore could not be ...llowed for a sufficiently long time. An accurate ...llow-up of the present cohort during the coming ...ve-year period should bring greater clarity into ...is.

In the present connection, it is of interest that in a recently published mortality study (*17*) on almost ... deaths in the American PVC-processing in-...stry, an overrepresentation in cancer mortality ...ppears to exist (all cancer), especially gastrointes-...nal cancer in both sexes.

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Epidemiological Study of Pneumoconiosis in the Italian Poly(vinyl chloride) Industry

by G. Mastrangelo,* B. Saia,* G. Marcer* and G. Piazza†

Among 1216 workers employed in a poly(vinyl chloride) production factory, 20 cases of pneumoconiosis were found. None of these workers had had previous exposure to organic or inorganic dusts; 731 had been exposed to PVC dust (employed in drying, sacking and blending of polymer) and 485 had been exposed to monomer alone. Chest x-ray films were read by two independent physicians utilizing the ILO/UC Pneumoconiosis Classification, 1971. X-ray abnormalities were characterized by limited profusion, irregular type and low gravity; in a small percentage of cases these were associated with slight restrictive respiratory function impairments. All 20 workers with PVC-induced pneumoconiosis had been exposed to high PVC dust pollution for at least five years. Mild nonspecific alterations (profusion of 0/1 class) were found both in the group exposed to PVC dust and in the group exposed to VCM alone. Such changes (observed in 388 cases, 31.9% of the whole population), are related mainly to age and smoking habits, and the role of exposure is minor.

We examined the working population of plants producing poly(vinyl chloride) (PVC) in Porto Marghera, Italy; 1216 workers had no previous dust exposure. Of these 731 were exposed to PVC dust polymer alone while 485 were exposed to vinyl chloride monomer (VCM). In the drying, sacking and blending departments, PVC dust concentrations were over 10 mg/m^3 of total dust in about 60% of the samples, whereas in the polymerization departments no concentration over 10 mg/m^3 was found. In the samples taken, particles with diameters of 1 μm to 6 μm constituted 4.5 to 30.9% of total dust weight.

All the workers had chest x-rays according to ILO standards and a spirographic examination. Chest x-ray films were read by two independent physicians utilizing the ILO/UC pneumoconiosis classification. For statistical analysis, a consensus reading was used.

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Table 1 shows that there are no significant differences in age and smoking habits, but the exposure duration is higher in the workers not exposed to dust.

Table 1.

Group	No. of subjects	% of smokers	Age, yr (mean ± SD)	Exposure, yr (mean ± SD)
Exposed to PVC dust	731	73.9	37.7 ± 8.8	6.1 ± 4.0
Not exposed to PVC dust	485	68.7	35.7 ± 8.5	8.6 ± 4.5

Table 2.

PVC dust exposure, yr	Age distribution of cases[a]			
	≤ 30 yr	31–40 yr	41–50 yr	> 50 yr
< 5	–	–	–	–
5–10	–	2(2.4)	5(8.3)	1(5.9)
> 10	–	2(3.8)	8(9.0)	2(9.5)

[a]Values in parentheses are percentages of subjects with PVC pneumoconiosis in each class of age and exposure.

In 20 subjects we found chest x-ray changes of at least class 1 according to the ILO/UC classification. The mean age of this group was 44.9 ± 5.2 years and the mean length of exposure was 11.6 ± 5.4 years. Sixteen subjects (80%) were either smokers or ex-smokers.

Table 2 summarizes the distribution of cases in relation to age and length of exposure. In all age groups there is an increase of disease prevalence associated with the increasing length of exposure.

In the case of the x-ray changes, 16 subjects had class 1/0 profusion, 2 cases class 1/1, 1 case class 2/1, and 1 case class 2/2. Irregular opacities were prevalent: 10 were type s, three were type t, six were type p and one was type r. They were diffused, mainly over median lobe areas.

A chest x-ray of a worker exposed to PVC dust (Fig. 1) for 15 years shows a gross reticular pattern: profusion is class 2/2, type t. In the right hemithorax of the same subject (Fig. 2) there is a mottled reticular pattern. Figure 3 is an enhanced view of the right hemithorax showing the mottled reticular pattern more clearly.

Another case of PVC induced pneumoconiosis can be seen in Figure 4. The worker was exposed to PVC dust for 20 years. A fine, dense reticular micronodulation is evidenced. Profusion is class 2; type p-s. Figure 5 shows the right hemithorax of the same subject; pin-point opacities can be seen.

In spite of age and the considerable exposure, the majority of cases were in a low profusion category, indicating the slow evolution of the disease. All had worked in high air-borne dust level environments (mostly drying and sacking) for at least five years. None of the 20 subjects was in a group unexposed to dust and none had experienced previous occupational exposure to organic or inorganic dusts. We considered the alterations to be PVC pneumoconiosis.

In 388 subjects (31.9%) we found slight chest x-ray alterations consisting of linear or irregular vanishing opacities or both, classified as class 0/1; the remaining 808 subjects were class 0/0.

Table 3 reports the total population distribution excluding 20 subjects with PVC pneumoconiosis. Results are presented in a two-way table: each entry reports the number of observations. Samples are classified according to age and PVC dust exposure: PVC + represents presence, and PVC - absence of PVC; x-ray + indicates the group with

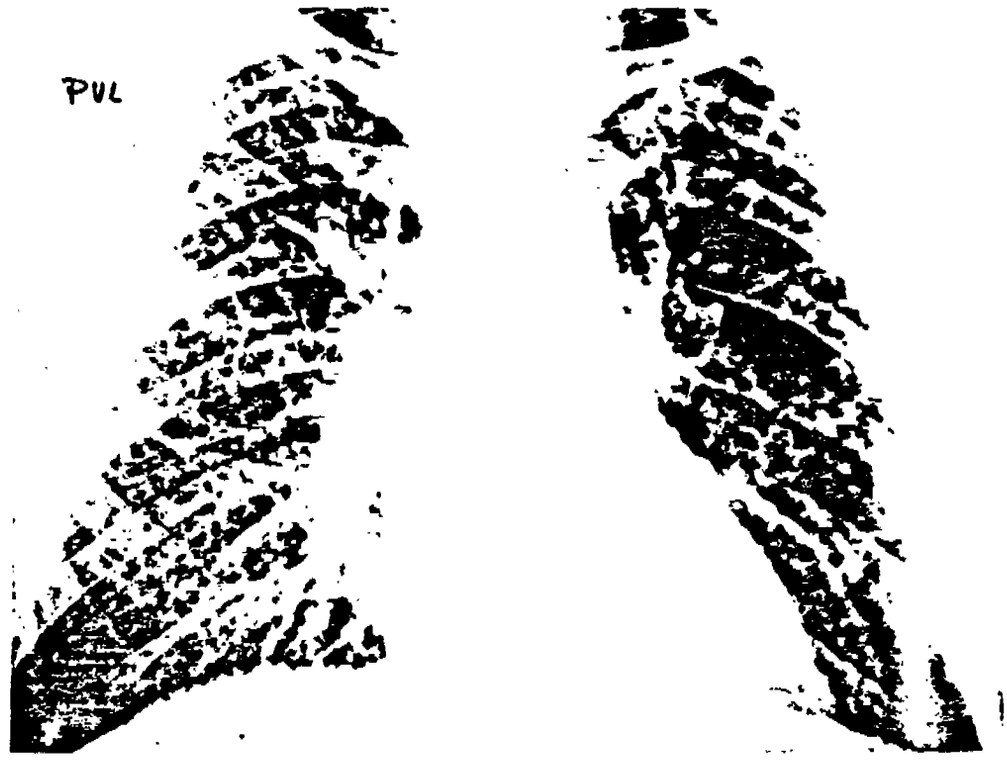

FIGURE 1. Chest x-ray of a worker exposed to PVC dust for 15 years. Profusion class 2/2: type t.

FIGURE 2. Right hemithorax of the same subject as in Fig. 1.

FIGURE 3. Enhanced view of the right hemithorax of Fig. 2, showing the mottled reticular pattern.

class 0/1 profusion. To assess the influence of both risk indicators we performed a two-way analysis of variance for proportions.

Table 4 shows that both age and exposure were significant factors influencing x-ray abnormalities. The square of a multiple partial association coefficient for qualitative data was calculated to measure the degree of association between the dependent variable (x-ray changes) and each of the two predictor variables (age and exposure). Age and exposure are alternately held constant. Age is a most important factor. When exposure is held constant, 33.2% of the x-ray changes are shown to depend upon age, when age is held constant, 6.5% are shown to depend upon exposure.

Table 5 summarizes the distribution of the cases according to smoking habits in workers exposed and not exposed to PVC dust.

Table 6 shows that chest abnormalities are significantly influenced by both risk indicators.

Table 3.

	Age distribution of subjects			
	≤ 30 yr	31–40 yr	41–50 yr	> 50 yr
PVC +				
X-ray +	20	78	123	35
X-ray −	133	197	108	17
PVC −				
X-ray +	17	45	55	15
X-ray −	119	158	67	9

Table 4.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Age	3	30.9713	10.3238	53.81[a]
PVC dust exposure	1	0.7843	0.7843	4.09[b]
Interaction	3	0.9882	0.3294	1.72
Error	1188	227.9403	0.1919	

[a] $p < 0.01$.
[b] $p < 0.05$.

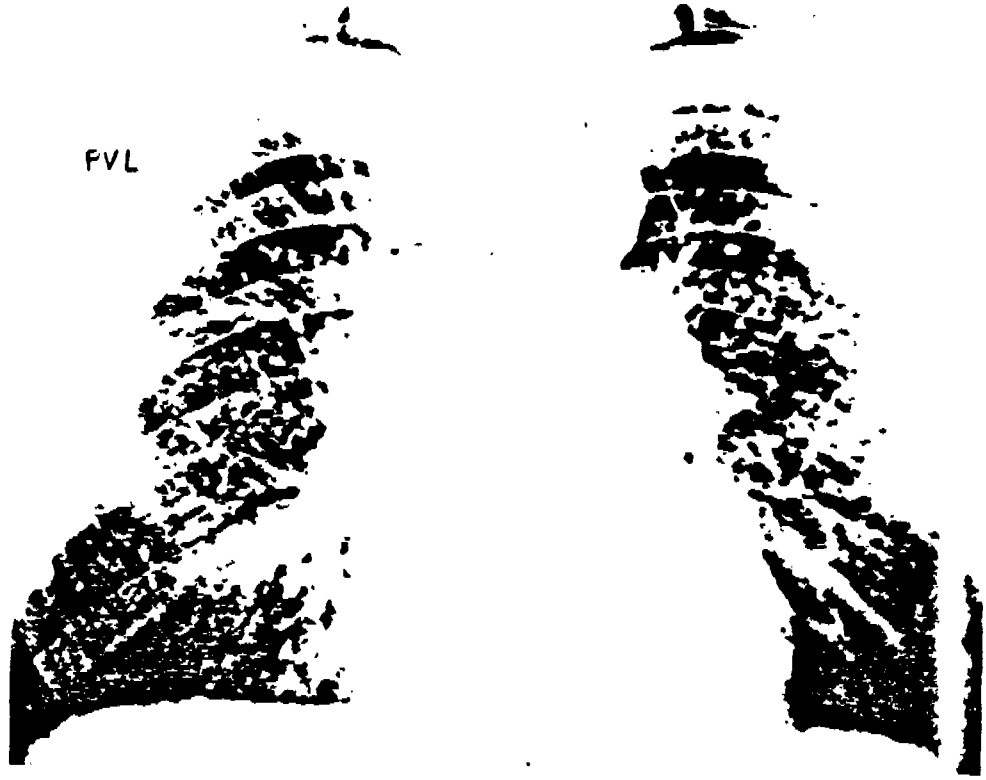

FIGURE 4. Chest x-ray of a worker exposed to PVC dust for 20 years. Profusion class 2/1; type p-s.

Table 5.

	Nonsmokers	Smokers
PVC +		
X-ray +	40	216
X-ray −	147	308
PVC −		
X-ray +	25	107
X-ray −	127	226

Table 6.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	*F*
Age	1	7.8850	7.8850	37.31[a]
PVC dust exposure	1	1.7847	1.7847	8.44[a]
Interaction	1	0.1023	0.1023	1.0
Error	1192	251.9125	0.2113	

[a] $p < 0.01$.

FIGURE 5. Right hemithorax of the same subject as in Fig. 4

When exposure is held constant, habitual smoking is responsible for 17.1% of the changes. When smoking habits are held constant, exposure to PVC dust is shown to be responsible for 8.9% of the x-ray abnormalities.

Our epidemiological study confirms experimental and pathological data already reported regarding the effects of PVC dust. Lung changes are directly related to PVC dust exposure, whereas VCM exposure alone fails to cause these changes. Therefore, we believe that pulmonary changes are not pathogenically similar to other vinyl chloride induced abnormalities, that is fibrosis of the liver, scleroderma-like skin changes, and peripheral vascular damage.

In our study there was only a 1.6% prevalence of pneumoconiosis in the total population, but in the workers exposed to effective risk of pneumoconiosis (731 subjects) the prevalence rose to 2.7%.

Apart from 20 cases of pneumoconiosis, mild nonspecific alterations (profusion of 0/1 class) were found in the group exposed to PVC dust and in the group exposed to VCM alone. Such changes are related mainly to age and smoking habits, and the role of exposure is minor.

as in Fig. 4.

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Excess Lung Cancer Risk in a Synthetic Chemicals Plant

by Richard J. Waxweiler,* Allan H. Smith,† Henry Falk‡ and Herman A. Tyroler**

A standardized mortality ratio of 1.49 for respiratory system cancer (42 observed deaths versus 28.2 expected, $p < 0.01$) was observed among a cohort of 4806 males employed at a synthetic chemicals plant since its startup in 1942. Upon review of pathologic material, the excess was found to be limited to adenocarcinoma and large cell undifferentiated lung cancer. Many of the workers had been exposed to vinyl chloride, as well as to chlorinated solvents, poly(vinyl chloride) (PVC) dust, acrylates and acrylonitrile. To evaluate the association between lung cancer and occupational chemical exposures, detailed work histories for each cohort member were combined with exposure ratings for each of 19 chemicals for each job for each calendar year since 1942. A serially additive expected dose model was then constructed which compared the doses of the chemicals observed for the lung cancer cases to the doses expected based on subcohorts without lung cancer individually matched to the cases. PVC dust appeared to be the most likely etiologic agent ($p = 0.037$). Time trends of PVC dust exposure indicated a potential latent period of 5-16 years before death.

Introduction

Our previously published (*1*) retrospective cohort mortality study of workers exposed to vinyl chloride monomer (VCM) at a synthetic chemicals plant demonstrated an excess risk of death from respiratory system cancer in addition to the already recognized association between VCM exposure and angiosarcoma of the liver (*2-10*). Preliminary review of the lung cancer cases indicated they were all adenocarcinomas or large cell undifferentiated tumors—an unusual histologic distribution.

Although lung tumors have been induced experimentally by exposure to VCM (*2*), the epidemiologic data are suggestive but equivocal. Two cohort studies found no excess lung cancer risk (*4-6*). In six other studies, elevated lung cancer risks were found overall or among subcohorts; however, few of these excesses were statistically significant due to moderate excess risks and/or small numbers of cases (*7-9*, *11-13*).

Because of the uncertainty in the literature regarding the association between VCM exposure and lung cancer and because of the diversity of occupational chemical exposures experienced by the lung cancer cases in our previous study (*1*), we decided to evaluate our cohort further to determine whether or not lung cancer was associated with VCM or with other chemical exposures. Thus, the following research had three objectives: (1) by using a retrospective cohort design, to determine if our previously published excess lung cancer risk among employees exposed to VCM at this synthetic plastics plant also existed for the total plant population (2) by using a case-comparand study, to determine whether an excess lung cancer risk of a particular histologic type was in force at the plant and (3) by using a serially additive expected dose model, to test whether one or more particular

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chemicals used at the plant were associated with either the excess risk of all or of a specific histologic type of lung cancer.

Methods and Results

Retrospective Cohort Study

The population at risk for the retrospective cohort study consisted of the 4806 males ever employed at this plant from its opening in 1942 until December 31, 1973. In the absence of individual data on race, everyone was assumed to be white because the company indicated that less than 2% of the population had been nonwhite. Date of birth and detailed work histories at this plant of all jobs and dates worked by each individual were coded. By follow-up of all study members from the first date hired at the plant through December 31, 1973, it was determined that 4174 were alive and 559 had died. The 73 (1.5%) persons lost to follow-up were considered alive throughout the study. The 16 (3%) deceased persons for whom no death certificates could be located were assumed dead, cause of death unknown. A modified life table analysis (NIOSH) was used to obtain person years at risk of dying by five year age and calendar time periods. United States white male death rates specific for five year age and calendar intervals were used to calculate the expected deaths and standardized mortality ratios (SMR's). SMR's were tested for statistical significance using the Poisson distribution (one-sided).

The cohort was young; by December 31, 1973, only 30% of the cohort, if alive, would have been over 54 years of age. However, 63% of the cohort had been hired before 1954 and thus had the opportunity to achieve 20 years' latency.

Two separate analyses of the cohort were made. Initially, all members were considered at risk from their first date of employment at the plant. This analysis yielded 556 observed and 550 expected deaths (Table 1). Risk of death due to malignant neoplasms of the central nervous system (SMR = 209) and respiratory system (SMR = 149) were both significantly elevated. A second "over ten-year latency" analysis was carried out by beginning person-years at risk only after an individual had achieved the tenth anniversary of his first date of employment at the plant. Results similar to those in the first cohort analysis were found but with slightly higher SMR's. Respiratory system cancer had an SMR of 156 based on 39 observed cases. Because our previously published analysis (*1*) of just the presumably VCM-exposed workers at this plant also found an SMR of 156 after 10 years latency, these results indicate an excess lung cancer risk not solely due to VCM exposure.

Table 1. Observed and expected deaths among chemical plant worker cohort.

Cause (ICDA–7 Code)	Observed	Expected	SMR
All causes	556[a]	550.2	101
All malignant neoplasms (140–205)	109	92.5	118
Digestive system (150–159)	24	25.6	94
Respiratory system (160–164)	42	28.2	149[b]
Central nervous system (193)	9	4.3	209[c]
Lymphatic and hematologic (200-205)	9	11.4	79
Other cancers	25	23.0	109

[a]Five persons, including three of the original 559 deaths, were not included in the cohort analysis because of missing work histories.
[b]$p < 0.01$.
[c]$p < 0.05$.

Case-Comparand Study

The second objective of this study was to determine whether an excess risk existed for a particular histologic type of lung cancer. Because no historical data exist on histology-specific lung cancer incidence or mortality rates and because histologic classification is somewhat variable between pathologists and over time, a case-comparand design was chosen to accomplish the second objective.

Medical records and pathology reports were obtained on all deceased members of the cohort whose death certificates mentioned cancer or respiratory disease. For 45 cohort members, primary or unspecified lung cancer was reported on at least one of these three records. A few persons who died after December 31, 1973, the cohort ending date, were included. Of the 45 cases, 42 were born in Kentucky or an adjacent state. Histologic material was requested. The worker case group consisted of the 27 of the 45 deceased individuals for whom histologic specimens were available.

As a comparison group, the lung cancer cases (comparands) most closely preceding and succeeding the chemical plant worker case in the chronologically ordered hospital pathology logs were selected that matched in age at diagnosis, sex, race, and county of residence. For four cases, only one matched comparand could be found.

Histologic material was reviewed by a panel of pathologists unaware of the employment histories (case versus comparand status) of the deceased. The histologic type distributions, according to the

Veterans' Administration classification scheme (*14*), were compared between the cases and comparands. The results of the majority opinion of the pathology panel are listed in Table 2. The panel found a significantly ($p < 0.05$, chi-square) higher percentage of large cell undifferentiated (type 4) cancers among the worker cases than among the community comparands (30% vs. 10%).

By using the SMR for respiratory system cancer of 149 in the retrospective cohort study and the observed and expected histologic distribution of lung cancer deaths based on the case-comparand study, Table 3 demonstrates the calculation of histologic specific lung cancer SMR's (column C). If the 27 cases for which histologic specimens were available are considered representative of all 45 lung cancer cases, it appears the excess lung cancer risk appears limited to types 3 and 4, adenocarcinoma and large cell undifferentiated, with the greater risk due to the latter. Approximately 13.5 of the 18 excess lung cancers among the plant workers would be due to adenocarcinoma and large cell undifferentiated carcinoma.[c]

It appears that there is a histology-specific excess lung cancer risk among workers at this plant and that this differential distribution of cell types is not an artifact of the geographic region nor of the pathologist's techniques. The fact that this risk occurs for adenocarcinomas and for large cell undifferentiated lung cancers makes it very unlikely that it is due to cigarette smoking.

Table 2. Case and matched comparand histologic distributions.

Histologic type	Veterans Administration classification code	Frequency	
		Worker cases	Community comparand cases
Epidermoid	1	6 (22%)	15 (30%)
Small cell undifferentiated	2	6 (22%)	15 (30%)
Adenocarcinoma	3	7 (26%)	14 (28%)
Large cell undifferentiated	4	8 (30%)	5 (10%)
Other	–	0	1 (2%)
Total		27 (100%)	50 (100%)

Serially Additive Expected Dose Model

The third objective was to test whether any presumed occupational chemical exposures were associated with the excess lung cancer risk at the plant. It was decided to analyze the previously mentioned cohort data in a serially additive expected dose (SAED) model (*10*), obtaining for each lung cancer case the observed and expected doses of each chemical, conditional on certain characteristics. The purpose of the SAED model is to compare the observed exposure of each case in a study with the exposures of fellow workers close to the case in year of birth, and in age at commencement of work at the company. If the total work force in the plant under study is referred to as the cohort, then each case can be thought of as belonging to a subcohort of workers with approximately the same year of birth and age at commencement of work in the plant. In each year that a case worked at the plant, his exposure can be compared with that of the other members of his subcohort who were working in that year.

Company personnel compiled estimated exposures on a scale of 0 to 5 (5 being the highest exposure) for each of 19 chemicals (Table 4). Each job was assigned an exposure rank for each calendar year of the study (Table 5). These exposure data were then linked with work histories identifying the jobs each worker had in the plant and the calendar time involved. The analytical method is based on estimating exposure dose by multiplying the exposure level by the number of days worked at that level. These "doses" are accumulated over a calendar year for a case to yield the observed dose and for

Table 3. Histologic specific lung cancer risk among plant personnel.

	Histologic distribution		Histologic specific SMR $C = 149\ (A/B)$	Excess cases among those pathologically reviewed $(D)^a$	Total excess cases $(E)^b$
	Observed (A)	Expected (B)			
Total	100%	98%[c]	149	8.9[c]	14.8[c]
Epidermoid	22.2%	30%	110	0.6	0.9
Small cell	22.2%	30%	110	0.6	0.9
Adenocarcinoma	25.9%	28%	138	1.9	3.2
Large cell	29.6%	10%	441	6.2	10.3

[a] $D = (27\ A) - (27\ B \times 100/149)$.
[b] $E = (45\ A) - (45\ B \times 100/149)$.
[c] Specific cell types do not add up to total because of "other" type lung cancer among comparands.

his subcohort to yield an expected dose for each year that a case works. The methodology has previously been described in detail elsewhere (*10*).

In addition to testing the total-dose hypothesis, the SAED analysis facilitates examination of the observed and expected doses for each year of exposure before death. Exposures to 19 chemicals were assessed; seven of the chemicals were, however, excluded from the formal analysis because so few persons were exposed to them at levels 3, 4 and 5 (Table 6).

The SAED model analysis resulted in the observed minus expected cumulative dose differences per case in Table 7. The differences for PVC dust are striking in comparison with the other exposures. For the large cell undifferentiated cancer and adenocarcinomas combined and for large cell undifferentiated cancer by itself, the differences in exposure to PVC dust are three to four times as large as those estimated for vinylidene chloride, the

Table 4. Exposure ratings used to classify jobs.

Rating	Exposure
0	No exposure
1	Minimal exposure to low levels (chemical in building—not handled, low vapor pressure and dust level, probably works on different floor)
2	Moderate exposure (works around the chemical, but exposure is minimal)
3	Works in areas where subject to occasional high excursions (normally exposure is minimal but occasional spills, leaks, or dust exposure may occur)
4	Works in areas where level is high (exposure levels in the area are frequently high; might consider that some risk is involved if chemical is very toxic)
5	Intimate contact, skin or high inhalation (such as poly cleaners in earlier years handling slurry)

Table 5. Chemical exposure ratings specific for job identification number and calendar year for a given chemical.

Chemical #1, job identification number	Exposure ratings					
	1942	1943	1944	1945	1946	1973
1	0	0	0	2	2	2
2	5	5	5	5	5	5
3	2	2	1	1	0	0
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
84	4	4	4	1	1	1

Table 6. Frequency of job categories having at least one year of exposure greater than specific exposures levels.[a]

	No. at each exposure level				
	1	2	3	4	5
Acrylic acid	13	4	3	0	0
Acrylamides	14	4	4	0	0
Acrylonitrile	35	25	13	6	1
Acetylene	20	17	10	3	1
Acrylates	39	30	21	8	2
Bisphenol A	7	2	0	0	0
Butadiene	23	15	9	5	1
Caprylyl chloride	25	9	6	6	3
Chlorinated solvents	30	18	7	4	4
Chloroethyl vinyl ether	22	6	4	1	0
Diethyl maleate	14	6	0	0	0
Mercuric chloride	15	7	5	3	3
Methanol	30	21	14	2	2
Phenol	2	1	0	0	0
Toluene	2	0	0	0	0
Vinyl chloride	55	40	29	21	4
Vinylidene chloride	20	14	8	5	2
Vinyl acetate	26	18	10	0	0
PVC dust	56	32	19	9	6

[a]E.g., for acrylic acid, employees could have worked in 13 different job categories that had exposure levels of one or higher in at least one calendar year between 1942 and 1973.

Table 7. Observed minus expected cumulative dose differences per lung cancer case.

Chemical	All lung cancers	Pathologically reviewed cases	Adenocarcinoma and large cell	Large cell
Acrylonitrile	-652	-402	-446	-128
Acetylene	-353	-289	291	-167
Acrylates	-322	-251	-83	-339
Butadiene	-330	-207	-406	-622
Caprylyl chloride	-547	-258	-270	-1139
Chlorinated solvents	-868	-672	-150	749
Mercuric chloride	-507	-425	-211	-62
Methanol	-430	-618	-456	-1078
Vinyl chloride monomer	-1428	-795	26	907
Vinylidene chloride	-341	210	804	1525
Vinyl acetate	-707	-480	-217	328
PVC dust	763	2448	3225	4526

next most evident chemical. The significance levels of the larger of these differences are found in Table 8 for the total cumulative doses and also for cumulative doses until 10 years before death. Statistical significance was observed only for exposure to PVC dust.

Latency analysis for PVC dust (Fig. 1) shows a peak for the difference between observed minus expected dose during the period 5 to 16 years before death. As the diagnosis of the disease of interest becomes more narrowly defined as a pathologically homogeneous entity, the dose difference per case increases, yet the implied latent period stays constant.

Discussion

The first objective of this study was to determine if the excess lung cancer risk among employees exposed to VCM also existed for the total plant population, including those persons not exposed to VCM. The retrospective cohort mortality study showed that respiratory system cancers occurred approximately 50% more frequently among the employees of the entire plant than would have been expected based on age, sex, race, and calendar year specific United States death rates. A similar excess had been previously shown to exist among the subcohort of employees exposed to VCM (*1*). Thus, the excess lung cancer risk at the plant appeared to be independent of VCM exposure unless one hypothesized that it was due to extremely low levels of VCM that could have permeated throughout the plant.

Potentially confounding variables are age, sex, calendar year, race/ethnicity, migration, urban residence, cigarette smoking and socioeconomic status (SES). In the retrospective cohort study, age and calendar year cannot confound because they are adjusted in the results. All persons in the study are male and 98% are presumed white. White male specific rates were used for comparison to control those potential confounders by subject category restriction. Ethnicity and migration (both inter- and intracountry) effects are considered minimal for all three study designs because 42 of the 45 cases were born in Kentucky or an adjacent state. Urban residence is mainly associated with an excess of epidermoid and small cell undifferentiated lung cancer (*15*) rather than the histologic types of lung cancer found in excess in this study. Excess lung cancer risk is associated with low socioeconomic status (*16*, *17*), measured either by education (relative risk of 1.2 for persons with fewer than eight years of school) or by occupation (relative risk of 1.3 for laborers). Since the cohort consists of a mixture of socioeconomic status levels, there is probably insignificant confounding due to SES at the plant considered as a whole. Furthermore, recent infor-

Table 8. Probability value of paired *t*-tests of cumulative differences between observed and expected doses for lung cancer cases.

	Total (all years)[a]	10 or more years before death
All cases (N = 45)		
PVC dust	0.185	0.253
All pathologically reviewed cases (N = 27)		
PVC dust	0.026[b]	0.047[b]
Adenocarcinoma and large cell (N = 15)		
PVC dust	0.037[b]	0.061
Vinylidene chloride	0.267	0.333
Large cell (N = 8)		
PVC dust	0.068	0.177
Chlorinated solvents	0.360	0.435
Vinylidene chloride	0.201	0.322

[a]Twelve chemicals were tested for each lung cancer group, thus, under an assumption of independence of tests, one would expect one test to be significant at the 0.08 level.
[b]$p < 0.05$.

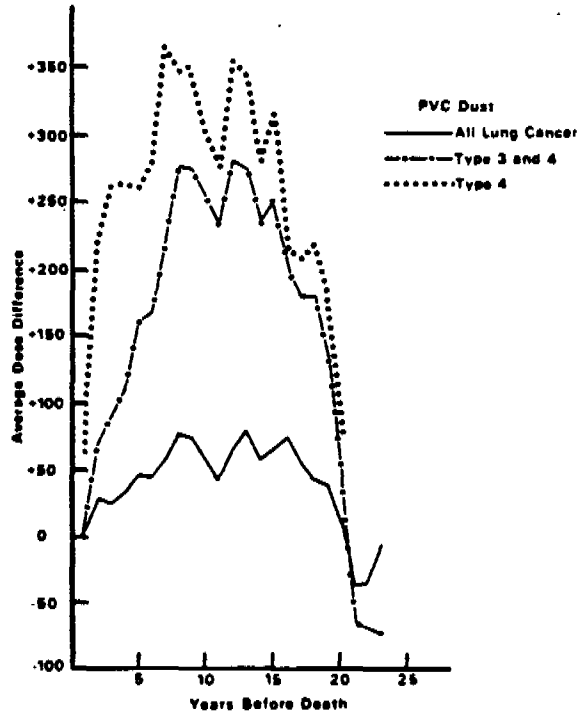

FIGURE 1. Observed minus expected dose differences per case among lung cancer cases for PVC dust.

mation indicates that these risk gradients may be partially due to smoking patterns (*18*, *19*), which would affect epidermoid and small cell undifferentiated lung cancers.

The second objective of this research was to determine whether an excess lung cancer risk of a particular histologic type existed. The case-comparand study showed that there was an excess of adenocarcinomas and large cell undifferentiated lung cancers among the cases occurring among plant employees compared to other lung cancer cases from the same community. Because the pathologists reviewed both sets of slides without knowing which were those of plant employees, it is unlikely diagnostic biases occurred.

Histologic specimens were more difficult to find among the cases which died earlier and which were older on the date of death. However, community comparands were matched with the cases on age and date of diagnosis and had to have specimens available themselves; hence a biased ascertainment by histology between cases and comparands would be unlikely. The choice of community comparands makes it unlikely that a community wide pollutant was responsible for the excess risk at the plant for types 3 and 4 lung cancer. Consequently, it can be inferred from Table 3 that the excess lung cancer risk in the cohort was limited to adenocarcinoma and large cell undifferentiated cancer. Adenocarcinomas, accounting for a minor proportion of this excess risk, have been shown to be weakly, if at all, related to cigarette smoking (*14*, *20-22*)

Large cell undifferentiated carcinoma of the lung is the only major histologic type that has appeared to be unrelated to cigarette smoking. Thus, cigarette smoking was probably not a major confounding variable. However, the role of smoking as a promoter or cocarcinogen cannot be ruled out. Nevertheless, the conclusion of this phase of the investigation was that an excess risk occurred among plant employees for types 3 and 4 lung cancer, especially type 4.

The third objective was to test whether one or more particular chemicals used at the plant were responsible for either the excess risk of all lung cancer or of the types 3 and 4 or just type 4 lung cancer. The SAED model was specifically developed for this objective.

The major hypothesis was tested for each chemical in the SAED model by using the one-sided *t*-test of the observed minus expected cumulative doses over all years before death and ten or more years before death (Table 8). PVC dust was the only chemical that was statistically significant. These results suggest that an excess of types 3 and 4 lung cancer exists at this plant and is related to PVC dust exposure.

The suggestion of PVC dust being a lung carcinogen is biologically plausible. Almost all PVC particles produced by the emulsion system, one of the systems at this plant, are in the respirable range (*23*). These particles could settle in the lungs and conceivably by themselves cause lung cancer. In fact, one case of supposedly PVC dust-induced pneumoconiosis has been found in a worker (*24*), and pulmonary granulomas (*25*, *26*) have been induced in animals exposed to PVC dust. In a large proportional mortality study of 4341 deaths that occurred among PVC fabricators, persons expected to be exposed to VCM and PVC dust demonstrated a slight (PMR = 117) excess lung cancer risk (*27*).

VCM gas is easily inhaled, and possibly would be in contact with tissue only a short time before being either exhaled or absorbed into the bloodstream. However, VCM becomes entrapped in the PVC dust and can be released slowly over time. Thus, PVC dust particles in the lung may slowly release VCM to small adjacent areas of the tissue, prolonging the contact time of that chemical to tissue. If this latter hypothesis is true, then it begs the question of why almost all of the liver angiosarcoma cases occurred among polymer reactor cleaners who received extremely high VCM doses while the lung cancer cases occurred frequently among the less heavily exposed workers. In fact, there was no relationship between VCM and lung cancer in this analysis; yet, one would expect the lung to be the major route of entry for VCM regardless of the cancer site.

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according to the ILO U/C Classification. All these cases were found among PVC exposed employees. The pattern of radiologic abnormalities described is very similar to that reported in the case in which the lung biopsy revealed fibrosis and granulomatous reaction, with inclusion of PVC particles.

The same study reported the presence of less marked radiologic abnormalities, of the linear-reticular type, in a much larger proportion (32%) of the population examined; these changes were present both in VC monomer exposed and in PVC exposed employees. While the prevalence was higher in smokers than nonsmokers, 65 of the 388 x-rays with linear-reticular opacities were found in persons who had never smoked.

In another large epidemiologic study (*3*), exposure to respirable PVC dust was found to be associated in a proportion of exposed workers with the presence of small rounded opacities on the chest radiograph and a decline in mean ventilatory capacity.

The question of pulmonary effects due to vinyl chloride monomer continues to be of great interest. The multi-organ effects of vinyl chloride include the peculiar syndrome of acroosteolysis, scleroderma-like skin changes, vascular changes affecting the arteries, arterioles and capillaries of hands and fingers, liver and spleen capsular fibrosis, liver fibrosis, abnormalities of the sinusoidal vessels in the liver, and portal hypertension. Ward (*16*) investigated the immunologic status of 58 workers from a VC polymerization plant. The findings included hyperimmunoglobulinemia, cryoglobulinemia, cryofibrinogenemia, *in vivo* complement activation via the classic pathway, with C_4 and C_3 conversion and an increase in the B cell lymphocyte population. Immunofluorescent examination of skin, muscle and lung biopsy specimens revealed the presence of circulating immune complexes, with deposition on the vascular endothelium and occlusion of small vessels. Immunoglobulin, complement and fibrinogen deposition in the subintimal regions of the vessel were found in areas with subintimal fibrosis and luminal occlusion.

Grainger et al. (*17*) have accumulated evidence suggesting the following mechanism: the vinyl chloride metabolite cyclic chlorethylene epoxide, an alkylating agent with high biological activity binds to IgG producing structural conformational changes that promote aggregation of IgG molecules. The modified IgG may also become antigenic. The IgG aggregates are cryoprecipitable and may initiate complement activation. Precipitation of IgG aggregates by cold leads to complement activation, platelet aggregation and conversion of fibrinogen to fibrin with polymerization. Occlusion of small vessels results, and ischemia stimulates new collagen biosynthesis.

Similar abnormalities of the immunologic status were found in another study of 22 workers exposed to vinyl chloride, with Raynaud's syndrome and, in some cases, acroosteolysis. Latent cryoglobulinemia was detected in 18 cases, with increases of immunoglobulins, IgA and IgG (*18*).

Circulating cryoimmunoglobulins are a prominent feature of idiopathic pulmonary fibrosis (*19*) and increased IgG levels have been shown to be characteristic for bronchoalveolar lavage fluid of such patients.

Interstitial pulmonary fibrosis is a possible effect of vinyl chloride exposure. The occurrence of more dramatic and specific abnormalities in other organ systems—liver, spleen and peripheral circulation—has probably prevented more focused attention on pulmonary effects of vinyl chloride in the past.

Long-term effects of vinyl chloride include well documented carcinogenicity. Lung cancer has been found to occur with an increased incidence in several mortality studies (*20-22*). Abnormalities in sputum cytology tests have been found to be more frequent in VC/PVC-exposed workers than in other chemical industry employees and in smokers (*23*). In experiments on mice, Suzuki (*24*) has described hyperplastic changes of the alveolar lining cells and pulmonary tumors in the majority of exposed animals. The ultrastructure was thought to indicate that the tumors originated in type II alveolar cells. Alveologenic tumors were also described in several other experimental studies (*25-27*). Interestingly, other known carcinogens, such as polycyclic aromatic hydrocarbons, nitrogen mustard and chromates, produce pulmonary tumors in experimental animals similarly, originating in the type II alveolar cell.

The effects of vinyl chloride-poly(vinyl chloride) exposure on the respiratory system of exposed workers seem to indicate two patterns of nonmalignant effects: a granulomatous reaction to PVC dust, with inclusion of PVC particles in macrophages and histocytes and associated interstitial fibrosis, and an interstitial pulmonary fibrosis due to vinyl chloride monomer effects on protein molecules and the immunologic mechanisms triggered by the altered protein.

The long-term carcinogenic effect, with a significant increase in the incidence of lung cancer, also is of concern, although the magnitude of this effect has not yet been completely evaluated.

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1970 through 1978. The annual incidence rate for this period was also 0.25, almost double the expected rate of 0.14 per million in the United States (*8*). There are several possible explanations for these observations. New York is more industrialized than many other states, and it is possible that the exposed cohort might be larger. Increased recognition and reporting of this entity might also be a factor. Finally, relatively few workers have been exposed for a long time to high levels of vinyl chloride monomer.

As Fox and Collier (*17*) point out, more than half of the people who have ever been exposed to VCM in the manufacture of PVC in Great Britain are currently employed in the industry; approximately 75% of men who have been employed in the industry have been employed for less than 10 years; only 8% have been employed for 20 years or longer; approximately half the persons who have been exposed to VCM have been intermittently exposed; and only 10% of those who have been constantly exposed have been exposed to high concentrations. Thus it is possible that we are only beginning to see the full impact of industrial exposure to VCM. This suggestion is supported by the observation of Monson et al. (*18*) that the relative frequency of all cancers appears to be increasing with time in vinyl chloride workers.

Other factors must also be considered in examining the frequency of ASL in our society. Working conditions in the manufacture of PVC have undoubtedly been influenced since the early 1940s. This was largely due to the recognition after 1957 that exposure to high concentrations of VCM can cause acro-osteolysis (*19*) and Raynaud-like phenomena, and in the early 1970s that high concentrations of this chemical increase the risk of dying from ASL (*6*). A reasonable assumption is that earlier workers were probably exposed to higher concentrations of VCM than those who entered this industry during the last decade. Interestingly, it has recently been suggested that the age at diagnosis and latency period for ASL might be increasing (*15*). If verified, these preliminary observations

Table 1. Angiosarcoma of the liver (ASL) in residents of New York State (excluding New York City), 1970–1978.

Case	Age	Race[a]	Sex	Year of ASL diagnosis	Exposure history[b]	Metastatic site	Other tumors		
							Type	Site	Year of diagnosis
1	60	W	M	1970	Direct, VC	Adrenal, omentum	—		
2	45	W	F	1970	Possible, VC	Kidney, scalp, dura	—		
3	45	W	M	1970	—	Bone, adrenal	—		
4	18	B	F	1970	—	Spleen, lymph nodes	—		
5	55	W	F	1971	—	Spleen, omentum, adrenals, lymph nodes	—		
6	68	W	M	1971	—	—	Lymphosarcoma	Ileum	1955
7	4/12	W	F	1971	—	Lungs, adrenals, skin	—		
8	31	O	F	1972	Possible, VC	Heart, brain, lungs, adrenals	—		
9	77	W	M	1972	—	Spleen, marrow, lymph nodes	—		
10	64	W	F	1973	Direct, AS	—	Fibromyxolipo-sarcoma	Knee	1969
							Mesenchymoma	Liver	1973
11	47	W	M	1973	Direct, ThO_2	Brain, lymph nodes	—		
12	62	W	F	1973	Possible, VC	Spleen	—		
13	63	W	M	1973	—	Spleen, pancreas, adrenals, brain, bone, lungs, lymph nodes	Transitional cell carcinoma	Bladder	1973
14	59	W	M	1973	—	—	—		
15	31	W	F	1974	Possible, VC	—	—		
16	45	W	M	1974	—	—	—		
17	62	W	F	1974	—	—	—		
18	65	W	M	1975	Direct, VC	—	—		
19	73	W	M	1975	—	—	—		
20	43	W	M	1977	—	Spleen, brain, lungs, duodenum	—		
21	54	W	F	1977	—	—	—		
22	63	W	M	1977	—	—	—		
23	83	W	F	1978	—	—	—		
24	46	W	M	1978	—	—	—		

[a]W = White; B = black, O = oriental.
[b]VC = vinyl chloride, PVC = poly(vinyl chloride), As = arsenic, ThO_2 = thorium dioxide; possible = lived within one mile of VC or PVC factory.

might be of great importance in evaluating the possibility that lower doses of VCM might be associated with ASL after a prolonged latency period.

Apart from the risk of ASL associated with industrial exposure to VCM, the possibility exists that nonoccupational exposure might also be associated with ASL. Block (*20*) has suggested that this disorder might result from chronic low level exposure to this chemical. Landrigan and Heath (*21*) found cases of ASL among workers at two poly(vinyl chloride) fabrication plants and in residents living nearby. A study in New York State (*16*) found that five individuals with ASL (but no apparent occupational exposure to VCM) lived in closer proximity to polymerization and fabrication plants than their matched controls. In a study of 10 patients with ASL in Wisconsin (*22*), one patient lived near a chemical company that made plastics and resins. These observations might be fortuitous and must be tempered by the preliminary evidence suggesting that no excess of ASL is found among PVC fabricators (*11*). At present there is no conclusive evidence associating this tumor with nonoccupational exposure to VCM. However, this issue and a closely related one—that chronic low dose exposure might be associated with an increased risk of ASL—require further evaluation. To fully appreciate the potential magnitude of this issue, one must consider both residential proximity to plants and other potential sources of public exposure. Estimates have been made that 4.6 million people live within five miles of United States monomer and polymer production facilities (*23*). During the years of uncontrolled emissions, it was calculated that the average exposure level to this population was approximately 17 PPB (*23*). Household use of aerosol products in enclosed spaces, even in short 30-sec bursts, could result in air concentrations of VCM as high as 400 ppm which can persist several hours after spraying (*9*, *10*).

The Biologic Spectrum of Vinyl Chloride

Reference has already been made to the association between VCM and acro-osteolysis, Raynaud-like disorders and ASL. It is also clear that this agent can induce nonmalignant liver disease. In Russia a form of chronic hepatitis was found in approximately 25% of VCM workers (*24*). In addition, Thomas et al. (*25*) documented the occurrence of portal hypertension associated with hepatic fibrosis (Banti's syndrome) in a study of the hepatic tissues obtained from 20 workers with industrial exposure.

Of great concern is the growing evidence suggesting that the spectrum of disorders associated with VCM might include certain other neoplastic diseases, pneumoconiosis and possibly excess fetal loss. Several mortality studies have been conducted in recent years. Monson et al. (*18*) found an apparent excess of lung and brain cancers. A slight excess of digestive tract, lymphatic and hematopoietic tumors was also observed. A historical prospective mortality study of 8384 men who had at least one year of occupational exposure to vinyl chloride demonstrated that cancers of the digestive system (primarily ASL), respiratory system and brain and lymphomas occurred more often than expected in those members with the greatest estimated exposure (*26*). Similar results were observed by Waxweiler et al. (*27*) in a retrospective cohort study of workers from four plants engaged in the polymerization of vinyl chloride for at least 15 years. Although observed numbers in each of these studies are quite small, the strength of these observations lie in their consistency. In addition inhalation studies by Viola et al. (*28*), Maltoni (*29*) and Neplinger et al. (*30*) have demonstrated that this chemical induces adenomas and adenocarcinomas of the lung, neuroblastoma of the brain, lymphoma and various other tumors in a variety of animal species. Two other studies are notable for the different results obtained. A mortality study of over 4000 deaths among current and former employees of 17 PVC fabricators from 1964 through 1973 found an excess of cancers of the breast and urinary organs among white females (*31*). Fox and Collier (*17*) studied 7000 men who were at some time between 1940 and 1974 exposed to VCM and found no evidence to support the hypothesis that cancers other than those of the liver are associated with this agent.

Vinyl chloride inhalation or PVC might cause abnormalities of pulmonary function and chest x-rays (*32*, *33*). Szende et al. (*34*) was the first to describe pneumoconiosis due to PVC. Although PVC dust appears to be the major offender, Lilis et al. (*32*) showed that inhalation of this agent induced less severe respiratory function abnormalities than simultaneous inhalation of VCM and PVC. In a more recent study of 1216 PVC production workers, 20 cases of pneumoconiosis were found. Duration of exposure was about 12 years, on the average, and never less than five years (*35*). This study also suggests that pulmonary changes are directly related to PVC dust, while VCM exposure alone fails to cause these changes (*35*).

Available evidence also suggests that VCM is mutagenic (*36*), and workers appear to have an excess of chromosomal aberrations in lymphocytes

when compared to nonexposed controls (*37*). Selikoff (*38*) found that fetal death rates among wives of VCM workers ranged from seven to 14 per 100 pregnancies. Infante et al. (*39*) conducted a case-control study of the pregnancy outcome among wives of workers exposed to VCM and found a significant excess of fetal loss in the exposed group. While suggestive, these observations require verification, particularly in view of the many factors known to influence pregnancy outcome.

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