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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.

		Lung tissue: incidence of response/number evaluated[a]		
VC exposure concn, ppm	Group	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 inhalation studies showed a baseline incidence for pulmonary adenomas which was nearly identical, i.e., at 500 ppm, 34.4%; at 50 ppm, 34.5%.

Table 9 provides an overall summary of the incidence of histologically proven neoplasms observed in both strains of mice, i.e., ICR and A/J, following single and multiple exposures to VC. The neoplastic and nonneoplastic changes observed in the liver and/or lungs of A/J mice following multiple exposures to VC at 50 and 500 ppm are shown in Tables 10 and 11. Although other neoplasms and nonneoplastic changes occurred variously in all remaining organs and tissues, the response appeared either sporadically or was shared by all test groups including controls. Relationship by incidence and severity to test exposure was not evident. Furthermore, morphologic deviations were not unlike those normally observed in aging A/J mice maintained under 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 exposure to vinyl chloride increased organelle turnover as well as loss of volume control (bleb formation) and increased lysosomal activity in the liver of rats. These alterations progressively decreased as recovery after exposure increased.

Hepatocellular carcinoma was seen in one male Fischer rat which had received 10 exposures of 500 ppm. Lymphosarcoma was noted in one female Fischer rat which had received a single exposure at 500 ppm. Since these were individual cases and since no cancers were seen at 50,000 ppm, the lymphosarcoma and the hepatocellular carcinoma 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)					Total number of neoplasms observed
		8 months	16 months	18 months	20 months	Totals	
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		Vinyl chloride, 50 ppm, 100 × 1	
	M (39)[a]	F (47)[a]	M (81)[a]	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* in ppm-hr. Factors for breathing rate and detoxication can be added. However, this simplified *Ct* 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 indicate 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 lungs 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)	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.

REFERENCES

1. Viola, P. L., Biogotti, A., and Caputo, A. Oncogenic response to rat skin, lungs and bones to vinyl chloride. Cancer Res. 31: 516-522 (1971).
2. Maltoni, C. Communication sent to OSHA, Proceedings on the Proposed Permanent Standards for Occupational Exposure to Vinyl Chloride, Occupational Safety and Health Administration, U.S. Department of Labor, Washington, D.C., 1974.
3. Creech, J. L., Jr., and Johnson, M. N. Angiosarcoma of liver in the manufacture of polyvinyl chloride. J. Occup. Med. 16: 150-151 (1974).
4. National Cancer Institute. Guidelines for carcinogen bioassay in small rodents. NCI Carcinogenesis Technical Report Series No. 1, NC1-CC-TR-1, U.S. DHEW, February 1976.
5. Maltoni, C. The value of predictive experimental bioassay in occupational and environmental carcinogenesis. An example: vinyl chloride. Ambio. 4: 18-23 (1975).
6. Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Dixon, R. L., and Woods, J. S. Carcinogenicity of vinyl chloride and vinylidene chloride. J. Toxicol. Environ. Health 4: 15-30 (1978).
7. Haber, F. Die Chemie im Kriege; Zur Geschiechte des Gaskampes. In: Funf Vortrage aus den Jahren 1920-23. Julius Springer, Berlin; cited in Prentiss, Chemicals in War, McGraw-Hill, New York, 1937.
8. Caputo, A., Viola, P. L., and Bigotti, A. Oncogenicity of vinyl chloride at low concentrations in rats and rabbits. J. Int. Res. Commun. 21: 1582 (1974).
9. Keplinger, M. L., Goode, J. W., Gordon, E. E., and Calendra, J. C. Interim results of exposure of rats, hamsters and mice to vinyl chloride. Ann. N. Y. Acad. Sci. 246: 219-224 (1975).
10. Maltoni, C., and Lefemine, G. Carcinogenicity assay of vinyl chloride. Ann. N.Y. Acad. Sci. 246: 195-218 (1975).