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SUMMARY REPORT

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Toxicology, Carcinogenicity and Reproductive Effects of Single
and Multiple Exposures to Vinyl Chloride in Rats and Mice

I. INTRODUCTION.

The background information on vinyl chloride monomer (VCM) with technical details is reviewed in "Scientific and Technical Assessment Report on Vinyl Chloride and Polyvinyl Chloride", US Environmental Protection Agency, EPA-600/6-76-004, June 1975.

VCM studies in rats and mice reported herein were conducted at the Chemical Systems Laboratory (CSL) under interagency agreement with Consumer Products Safety Commission (CPSC).

II. EXPERIMENTAL PROCEDURES.

A. Exposure Procedures for Toxicology and Carcinogenicity.

1. Doses.

Male and female rats (Fischer 344) and mice (A/J or ICR) were totally exposed in one of the following ways:

- a. One single one-hour exposure at 0, 50, 500, 5,000 or 50,000 ppm. (Fischer 344 rats, ICR mice) (Appendix, Table I)
- b. Ten one-hour exposures at 500 ppm (one-hour per day, five days per week for two weeks). (Fischer 344, A/J Mice) (Appendix, Table II)
- c. One-hundred one-hour exposures at 50 ppm (one-hour per day, five days per week for 20 weeks). (Fischer 344 rats, A/J Mice)

2. Exposure and Sampling Techniques.

The exposures were conducted in 1000 liter, stainless steel dynamic flow chambers of the Rochester type. Construction insured laminar flow and uniform exposure of the test animals.

During one-hour exposure periods four 0.5 cc gas samples were collected at selected times for concentration analysis.

A Hewlett-Packard series 5830A Reporting Gas Chromatograph equipped with a dual-flame ionization detector was used for VCM analysis.

an 18-month sacrifice. The latter is minimum suggested by the National Cancer Institute for cancer bioassays in small rodents. For the multiple dose studies (500 ppm x 10 exposures; 50 ppm x 100 exposures) 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.

b. Electron Microscopical Studies.

For the electron microscopical studies the following animals were selected at random:

Three groups consisting of five males and five female Fischer 344 rats from each of the single one-hour (50, 500, 5000 and 50,000 ppm) exposures with equal numbers of their corresponding control group. The three groups were sacrificed at 8, 16 and 24 months, respectively, after the exposure.

Three groups consisting of five males and five female Fischer 344 rats from each of the multiple one-hour exposures (10 x 500 pp., 100 x 50 ppm) with equal numbers of their control group. The three groups were sacrificed at the end of 16 and 24 months, respectively, after the final exposure.

One group of five male and five female Sprague/Dawley/Wistar rats from the parenteral generation of the reproduction group (49 x 50 ppm, 49 x 500 ppm) with equal numbers of their control group. This group was sacrificed 24 months after the final exposure.

The rats were anesthetized with pentobarbital and their livers were perfused via the vena cava with chilled electron microscopical fixative consisting of 4% formaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer² (E.M. McDowell & B.F. Trump, Histological Fixatives Suitable for Diagnostic Light and Electron Microscopy, Arch. Path. Lab. Med. 100:405-415, 1976).

After mincing the tissues were post-fixed in 1% phosphate buffered

ataxia were noted. At 59 minutes tremors were seen. Females showed some hyperactivity at 40 minutes and 25% showed respiratory difficulty and ataxia after 55 minutes. No other effects were noted.

There were no remarkable signs of toxicity during the ten repeated exposures of mice and rats at 500 ppm of VCM, nor in rats during the 49 exposures at 500 ppm, nor in either species during the 100 exposures at 50 ppm.

2. Observations After Exposure

There were no consistent or dose-related differences between control and exposed (single or multiple) mice or rats in: death rate, (Appendix, Fig I IX, inclusive) toxic signs, gain in body weight. (Appendix, Figs X-XVIII inclusive)

B. Gross Pathology

There was a suggestion of a higher frequency of masses in the lungs and livers of mice and rats exposed once or repeatedly at the higher dose levels. (500, 5,000, 50,000 ppm)

C. Light Microscopic Pathology

1. Mice (single exposure)

Histological examination at 8 and 18 months in ICR mice exposed once to concentrations of 50, 500, 5000 or 50000 ppm showed the following changes attributable to VCM. (Appendix, Table IV-VI, inclusive) The development of adenomas increased with exposure to higher dose levels of vinyl chloride:

control - 12/120 or 10%

50,000 ppm - 45/137 or 32.8%

5,000 ppm - 24/143 or 16.8%

500 ppm - 18/139 or 12.9%

50 ppm - 14/139 or 10.1%

Progression to carcinoma was minimal:

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4. Rats (single exposures)

Except for aggravation of latent pulmonary changes, particularly bronchopneumonia, changes attributable to VCM were not apparent in any of the tissues evaluated microscopically at 8, 16 or 24 months from Fischer 344 rats exposed to concentrations of 50, 500, 5000 or 50,000 ppm. (Appendix , Tables XI-XIV, inclusive)

5. Rats (multiple exposures)

No changes attributable to VCM were apparent in any of the tissues evaluated microscopically at 8, 16 or 24 months from Fischer 344 rats exposed ten times at 500 ppm or 100 times at 50 ppm. (Appendix, Tables XV-XVII, inclusive)

6. Rats (49 exposures from the reproductive study)

The following histological observations were made 24 months post exposure in Sprague-Dawley/Wistar rats which had been exposed 49 times at 50 ppm or 500 ppm of VCM. (Appendix, Table XVII)

Neoplastic and non-neoplastic lesions were observed in approximately equal frequency in control and test animals.

The only lesions that occurred in higher frequency in the VCM exposed animal than in control rats were eosinophilic cell alterations presented as foci and/or areas. The appearance of these foci was related to dosage. The nature of these lesions is controversial. Some pathologists feel that basophilic lesions have greater significance with respect to neoplasm development than do other cellular alterations. Others feel that all of these cellular alterations may be part of a spectrum capable of progressing to the formation of neoplastic nodules.

7. Summary of Light Microscopy Studies

The carcinogenic, or possible related changes (eosinophilic foci) attributable to exposure of VCM in rats and mice are summarized in Table 1.

D. Electron Microscopical Results.

1. General.

In general, these studies indicate that exposure to vinyl chloride increased organelle turnover (Fig. XIX) as well as loss of volume control (bleb formation) and increased lysosomal activity in the liver of rats (Figure XIX). These alterations progressively decreased as recovery time after exposure increased.

Hepatocellular carcinoma was seen in one male Fischer rat which had received ten 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 the vinyl chloride exposure. Thus, it appears that exposure to vinyl chloride did not produce cancer in rats in any of these single or multiple exposures.

General conclusions concerning these various segments of the electron microscopical results follow.

2. General Conclusion of 8-Month Recovery to Single Exposure 50, 500, 5000, 50,000 ppm-hr) of Vinyl Chloride in Fischer Rats.

a. Alterations from control (Fig. XVIII) occurred in all treated animals at each concentration.

b. Changes were less severe in female animals.

c. Hepatocytic alteration included lipid accumulation (Fig. XIX), increased dense bodies (Fig. XX), lipofuscin granules (Fig. XX) and residual bodies. These are an indication of cytoplasmic sublethal injury.

d. Alteration was incremental with increasing exposure concentration.

e. Most severe changes involved cellular necrosis with subsequent phagocytosis by Kupffer cells, (Figure XXI) (seen in single and multiple exposures).

6. General Conclusion to 24-Month Recovery from Single Dose (50, 500, 5000, 50,000 ppm) Vinyl Chloride in Fischer Rats.

a. Age-related changes including formation of cleft-like spaces (possibly related to hemoglobin/hemosiderin), inflammatory infiltrate (pericholangitis) and some collagen accumulation were seen in controls (Fig. XXIV) and treated animals in this group.

b. In this group hepatocytic injury - not encountered in controls - was observed. At 5000 ppm one female showed evidence of lymphosarcoma.

7. General Conclusion to 24-Month Recovery After Multiple Doses (50 ppm X 10) of Vinyl Chloride in Fischer Rats.

a. Controls and treated animals showed changes similar to those seen after 24 months recovery to single exposure.

b. These changes were regarded as age-related and non-specific (Fig. X).

8. General Conclusion to 49 Exposures for 1 Hr at Daily Intervals Followed by 24 Month of Recovery (50 and 500 ppm) (Sprague/Dawley/Wistar Rats).

a. Age-related non-specific change was encountered in all groups.

b. Most advanced changes were related to 500 ppm group of male rats in which cell swelling and platelet aggregation were seen.

E. Multigeneration Study in Rats (Sprague/Dawley/Wistar).

No consistent changes attributable to VCM were found in F_0 parents which were exposed to 50 ppm or 500 ppm of the vinyl chloride monomer one hour per day, five days per week for ten weeks before mating and evaluated for numbers of matings, percentages of pregnancies, fertility and lactation indices. The F_1 , F_2 , and F_3 offspring were evaluated for litter size, percent of stillborn pups, post-natal growth, viability, survivability and reproduction anomalies. (Appendix, Table XIX - XXV, inclusive)

Electron microscopical examination of the parent rats which were held for 24 months after the 49th exposure revealed age related changes, cell swelling, and platelet aggregation but no tumors, ~~were seen~~ (Fig. XXV).

The possibility is recognized that "no effect" doses may exert carcinogenic effects which are too weak to be detected with the numbers of animals used in routine testing.

The concept of lifetime accumulative, non-tumorigenic and tumorigenic doses of radiation has been adopted. The Federal Radiation Council¹⁸ stated that for occupationally exposed personnel the accumulated dose of radiation to the whole body, head, trunk, active blood forming organs, gonads or lens of the eye shall not exceed:

1. In any calendar quarter, 1.25 roentgen equivalent mammal (rem)
2. Total lifetime dose of 5 (N-18) rem where N equals the present age in years.

B. Vinyl Chloride and Carcinogenicity.

Maltoni described a dose-response relationship for the carcinogenic effect of vinyl chloride in animals. The neoplastic response was affected by the length of exposure.^{19,20}

Lee et al.,²¹ noted that the incidence and severity of tumors increased with the concentration of VC and the length of exposure.

The above statements indicate that the total dose (concentration X exposure time) may be of importance in the carcinogenicity of vinyl chloride. Total inhaled dose can be approximated by the Haber concept.²² In its simplest form this concept states that the total inhaled dose, Ct (mg min/cu m) is the product of C (concentration in mg/cu m) X t (time in minutes).

The concentration can be expressed also in part 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 when these data are available. However, the simplified Ct approximation of total inhaled dosage is sometimes useful.

28,800,000 ppm-hrs.

A. Caputo, P.L. Viola and A. Bigotti²⁴ exposed rats to vinyl chloride four hours per day, five days per week for 12 months. The concentrations were 20,000; 10,000; 5,000; 2,000; 500; or 50 ppm. The total dose (Ct) for the 50 ppm was 48,000 ppm-hrs. No tumors were produced at this level. The total dose for 500 ppm was 480,000 ppm-hrs. Tumors did occur at the latter dose appearing as early as eight months. The total Ct in the 500 ppm study in eight months was 320,000 ppm-hrs.

Tumors appeared in the rabbits after nine months at 10,000 ppm. Thus the lowest total dose was 7,200,000 ppm-hrs.

M.L. Keplinger et al.,²⁵ exposed rats, hamsters and mice to vinyl chloride. Only the data on mice was sufficiently complete for examination of total dose effects. The animals were exposed seven hours per day, five days per week for eight months. The lowest Ct was 56,000 ppm-hrs. This Ct and all higher ones did produce tumors in mice.

Consumer Products Safety Commission Studies (Tables 1 and 2)

Table 2

Species	Results (carcinogenesis)
ICR Mice	50 and 500 ppm-hrs - negative 5000 ppm-hrs - borderline positive 50,000 ppm-hrs - positive
A/J Mice	5000 ppm-hrs - positive
Fischer Rats	50, 500, 5,000 and 50,000 ppm-hrs - negative 24,500 ppm-hrs = eosinophilic foci, no cancers

These calculations indicate strongly positive carcinogenic effects may not appear in rats until the total dose (Ct) of VCM reaches or exceeds

As an additional consideration in the total dose concept, Cts of 5000 ppm-hr produced increased incidences of adenomas in mice when the exposures were at 5000 ppm for one day, 500 ppm for 10 days or 50 ppm for 100 days. The increased incidence of adenomas produced by the single exposure of mice at 5000 ppm is of borderline significance. However, there is the indication that even single exposure of sufficient magnitude may be carcinogenic in sensitive species.

The dose-response relationship and the "no effect" dose concept have been described for other carcinogens.

It is difficult to relate these animal studies to man. Data on vinyl chloride exposure in plants have been limited. However, acute dizziness, headache, nausea and chronic liver damage have been seen in vinyl chloride workers. It is assumed that peak exposure levels of several thousand parts per million were experienced at times. Air monitoring of one group of plants during 1950-59 indicate that time-weighted (8-hr) average exposure were 120-385 ppm. (This would give daily Cts of 960-3080 ppm-hrs). Peak exposures probably exceeded 1000 ppm. This may not have been typical of all polyvinyl chloride plants.⁽¹⁾

Data on vinyl chloride in ambient air are limited also. Atmospheric measurements in the vicinity of production plants indicate that concentrations are below 1 ppm. One peak grab sample of 33 ppm has been reported at 0.5 kilometer from the center of one plant.⁽¹⁾

The time-weighted threshold limit value of the American Conference of Government Industrial Hygienists for 1977 is 200 ppm. There is a notice of intended change ²⁶. The Environmental Protection Agency ²⁷ has established the following emission limits for vinyl chloride: (1) formation and purification processes is 10 ppm, (2) emissions from equipment preceding and including the

IV. CONCLUSIONS.

A. Except at the highest concentration, 50,000 ppm, where possibly anesthetic-type effects were seen vinyl chloride produced no pharmac-toxic (excluding pathology) signs in mice or rats during or after exposure.

B. No reproductive or teratogenic changes attributable to vinyl chloride were found.

C. Vinyl chloride seemed to produce pneumonitis in mice and to aggravate bronchopneumonia in rats.

D. Vinyl chloride produced eosinophilic changes in rats but no frank (light microscopy) carcinogenesis.

E. Electron microscopical studies revealed some hepatocellular changes but no carcinogenesis related to the vinyl chloride exposures at Ct's of 50,000 ppm-hrs or less.

F. The findings from these Consumer Products Safety Commission tests are in agreement with results of previous studies.^{1,4,5,6,9,10,11,13,15,16,17}

1. The carcinogenic effectiveness of VCM depends upon concentration and exposure time, Ct.^{4,5,6}

2. There were VCM doses which were not carcinogenic and there appeared to be a total accumulated dose above which tumors were produced.^{13,15,16,17} Similar effects have been noted with other chemicals.

3. Tumors were not seen in mice at Ct's of 500 ppm-hrs nor in rats at 50,000 ppm-hrs or less.^{4,5,6,9,10,11}

4. Carcinogenic effects of VCM were seen in mice at total doses (Cts) of 5,000 ppm-hrs and above.^{6,11}

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26. Threshold Limit Values for Chemical Substances and Physical Agents in The Workroom Environment with Intended Changes for 1977, American Conference of Governmental Industrial Hygienist.
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APPENDIX

Table II. Exposure Schedule for Animals Exposed Repeatedly to VCM

Species	Sex	Dose	Exposure periods	Exposure dates		Exposure Group size(s)		Age	
				From	To	Start	End	Start	End
		ppm	days						wks
Fischer Rat	M	50	100	8/27/75	1/26/76	90	88	21	41
		500	10	7/7/75	7/18/75	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. control	100(c) 10(c)	-	-	50	50	21	41
						50	50	14	16
	F	Neg. control	100(c) 10(c)	-	-	50	47	21	41
						50	50	14	16
A/J Mous	M	Neg. control	100(c) 10(c)	-	-	40	39	15	35
						50	50	8	10
	F	Neg. control	100(c) 10(c)	-	-	50	50	15	35
						50	50	8	10

NOTE: (c) control for corresponding dose above.

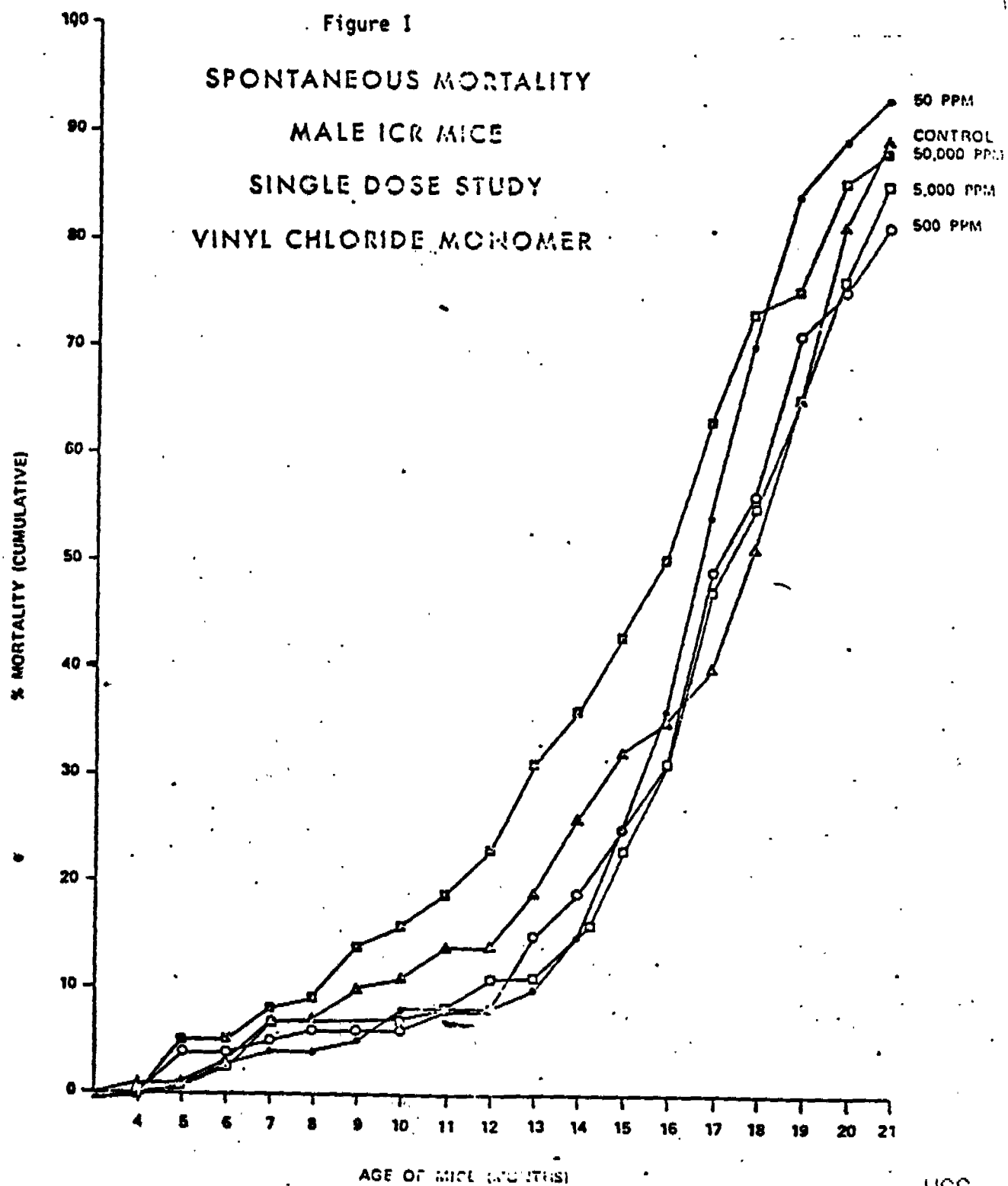


Figure III
 SPONTANEOUS MORTALITY
 MALE FISCHER RATS
 SINGLE DOSE STUDY
 VINYL CHLORIDE MONOMER

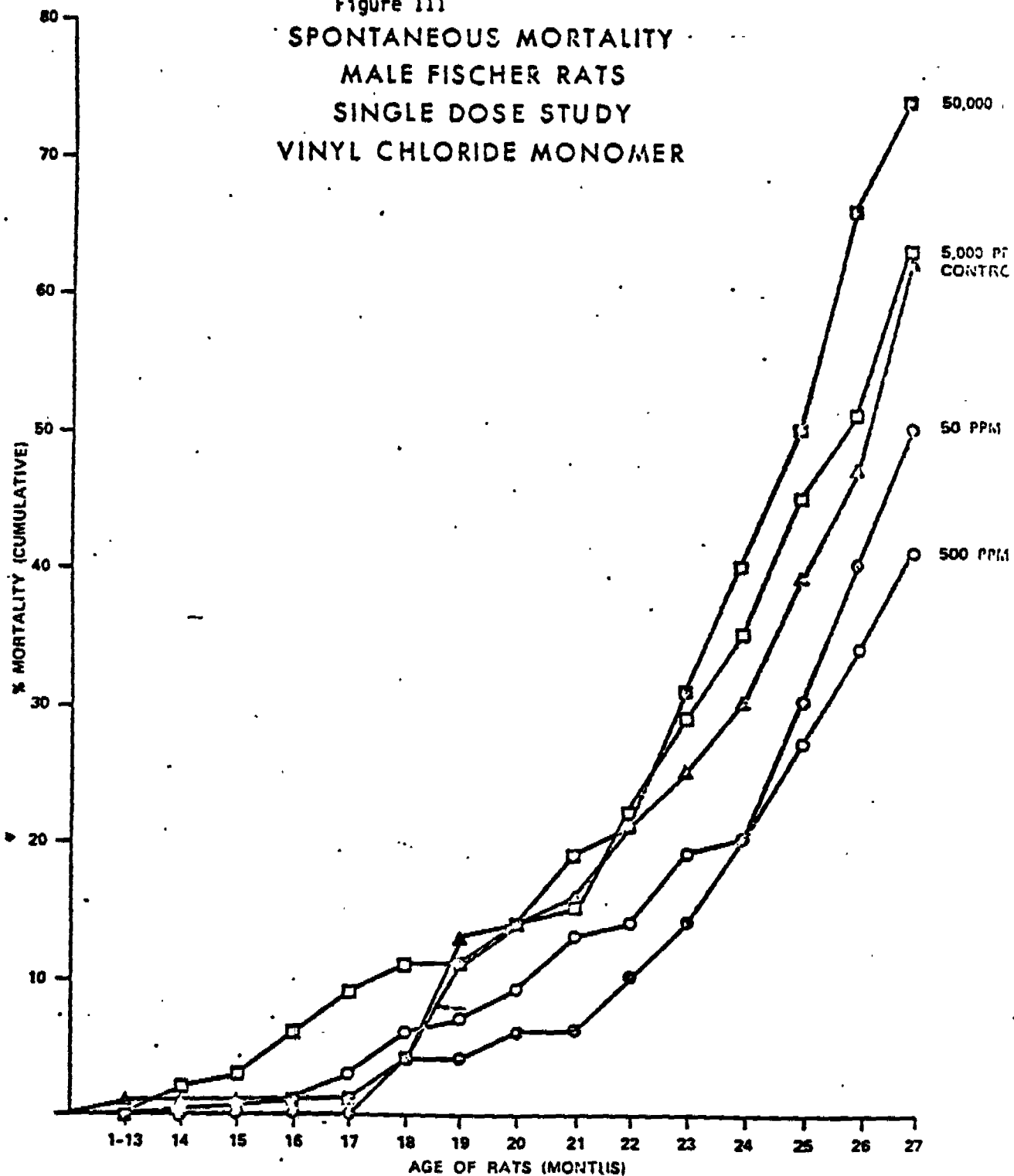


Figure V
 SPONTANEOUS MORTALITY
 AJ MICE
 10 DAY STUDY
 VINYL CHLORIDE MONOMER

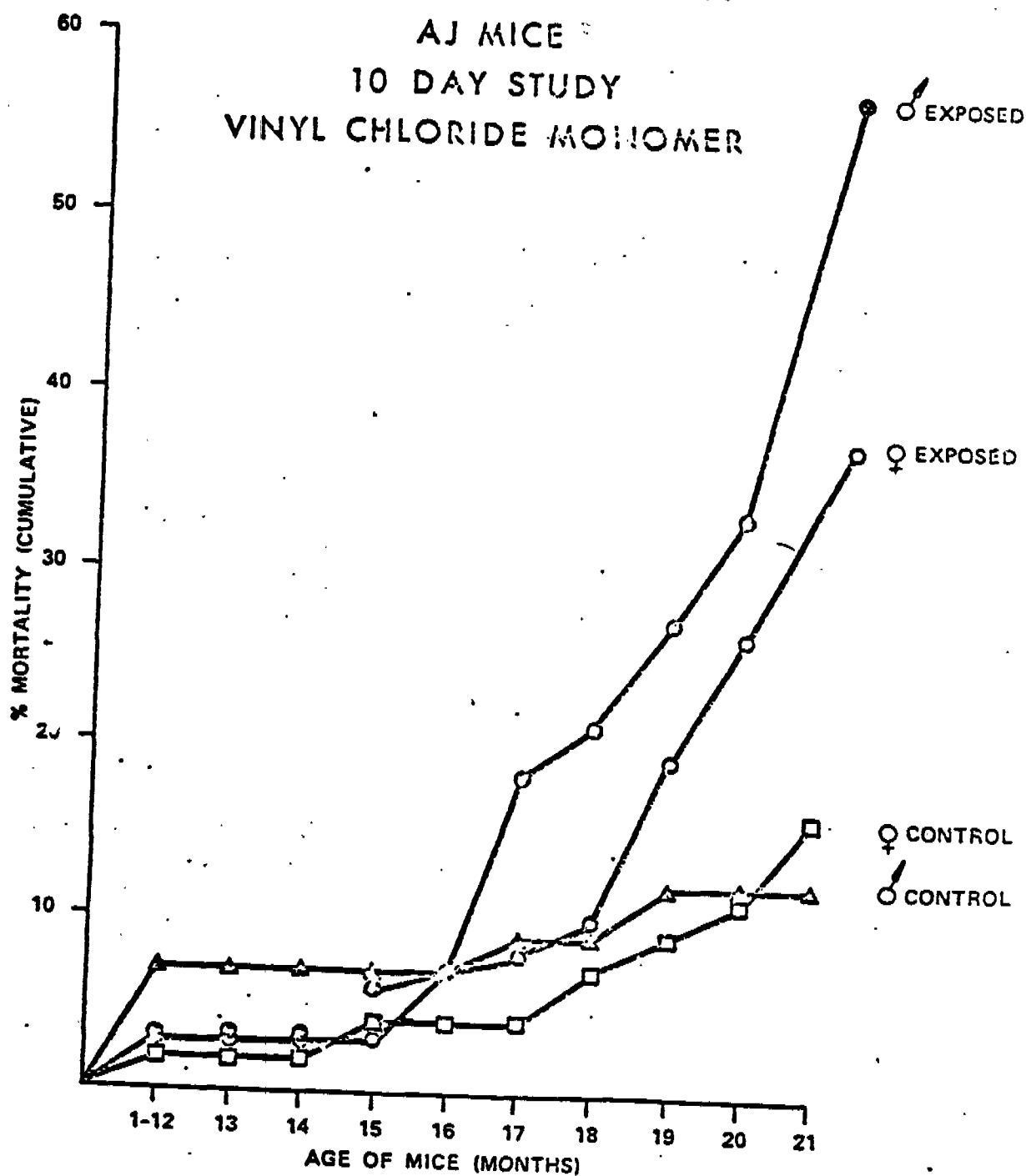


Figure VII
 SPONTANEOUS MORTALITY
 AJ MICE
 100 DAY STUDY
 VINYL CHLORIDE MONOMER

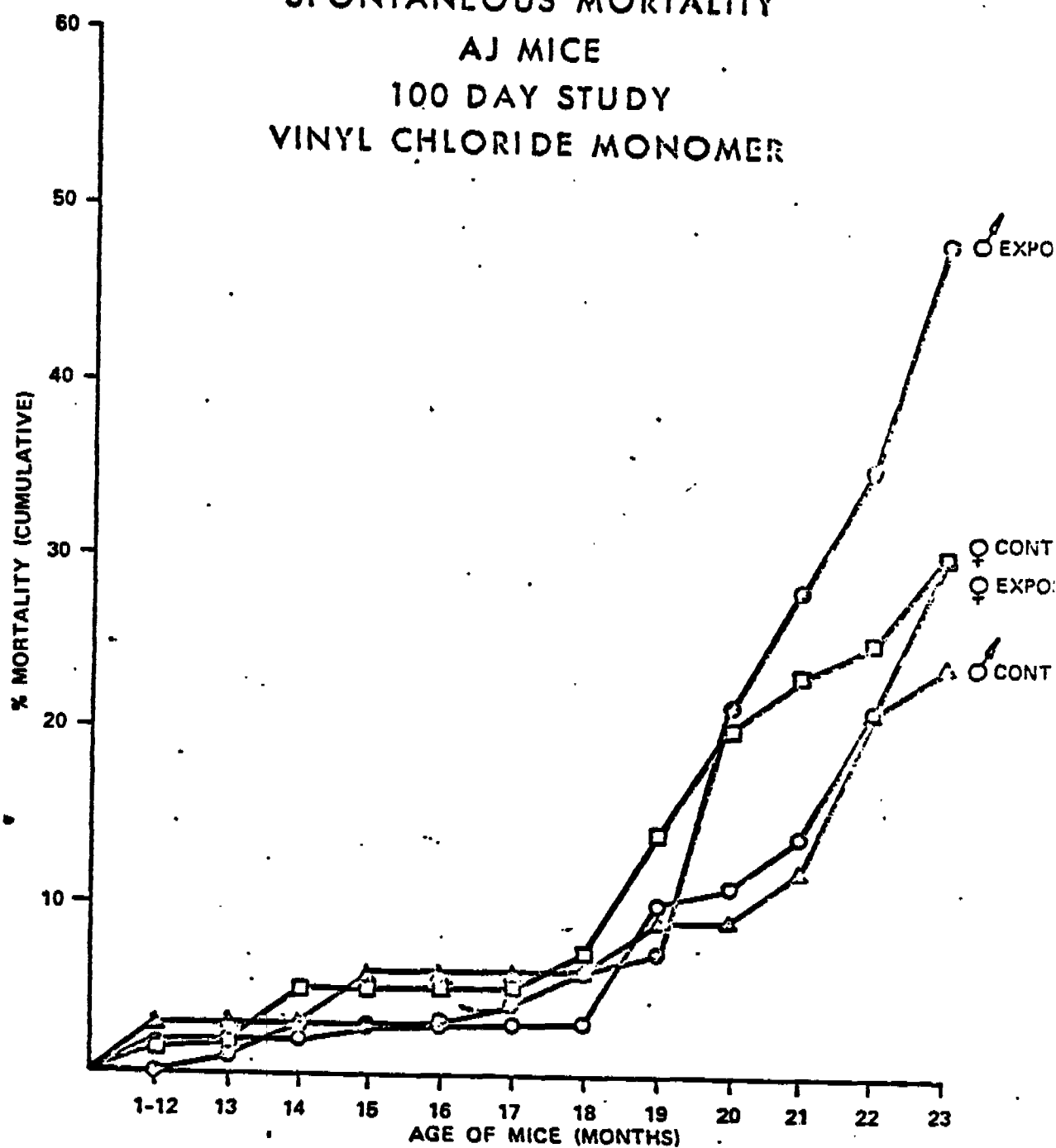
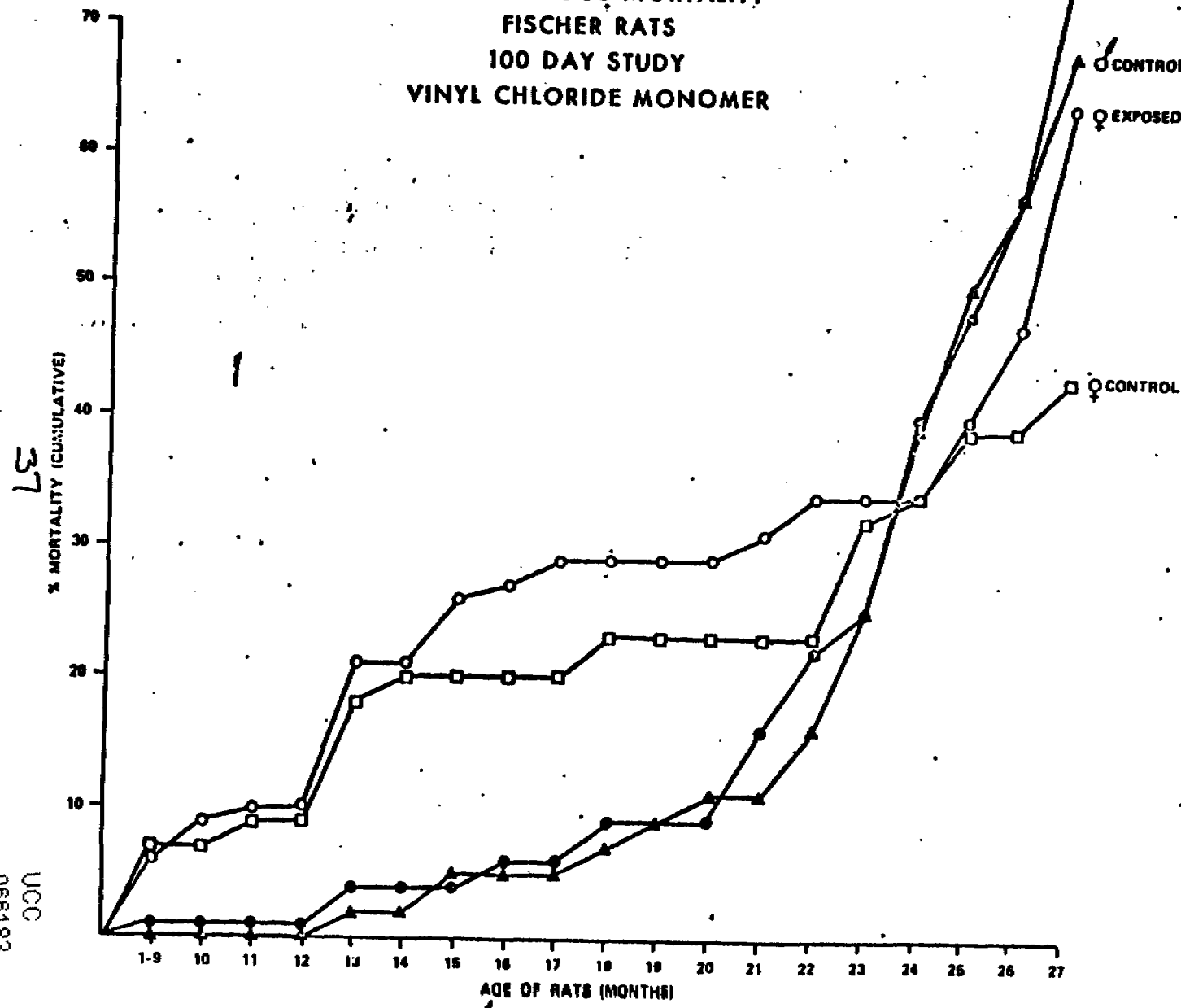
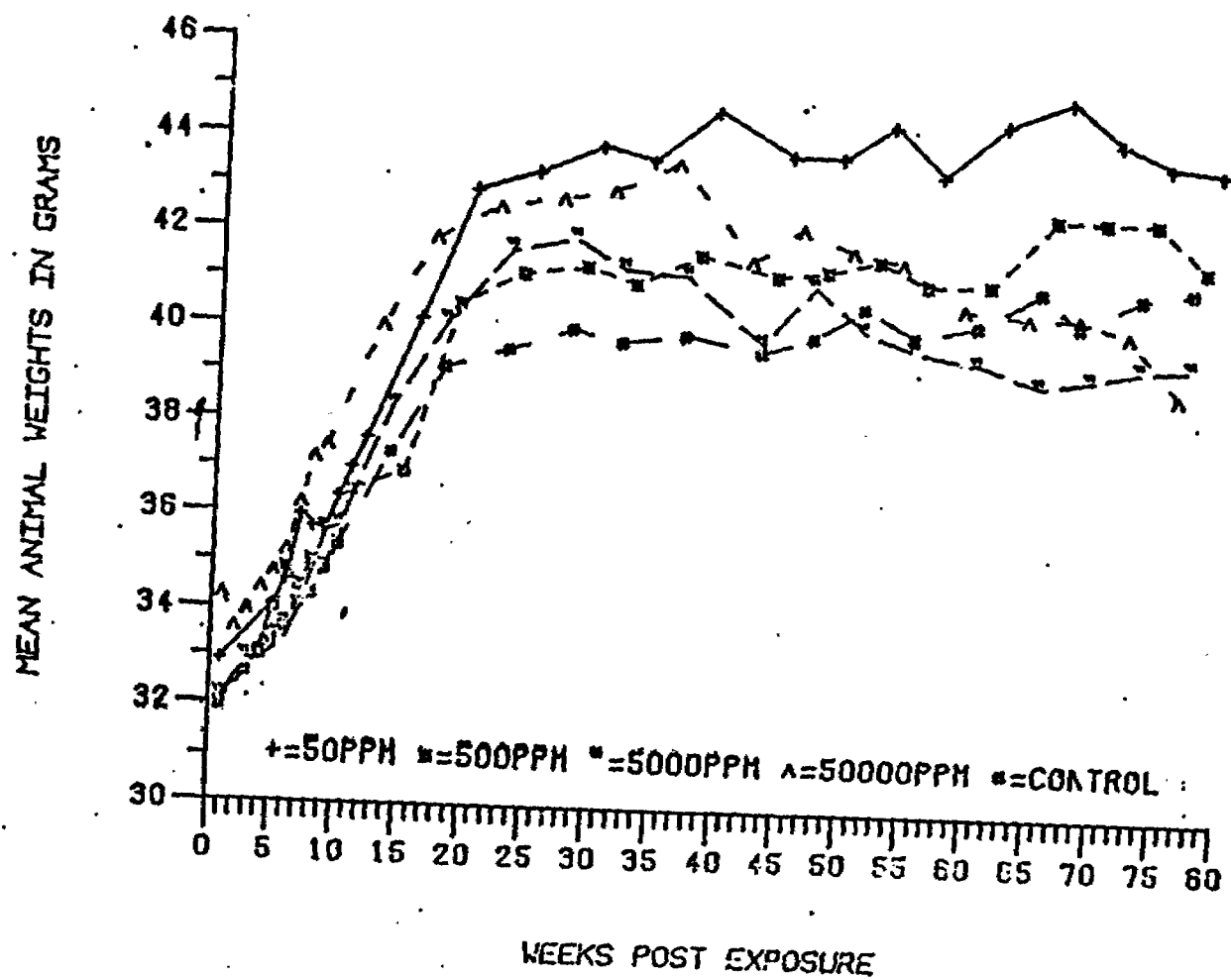


Figure IX
SPONTANEOUS MORTALITY
FISCHER RATS
100 DAY STUDY
VINYL CHLORIDE MONOMER



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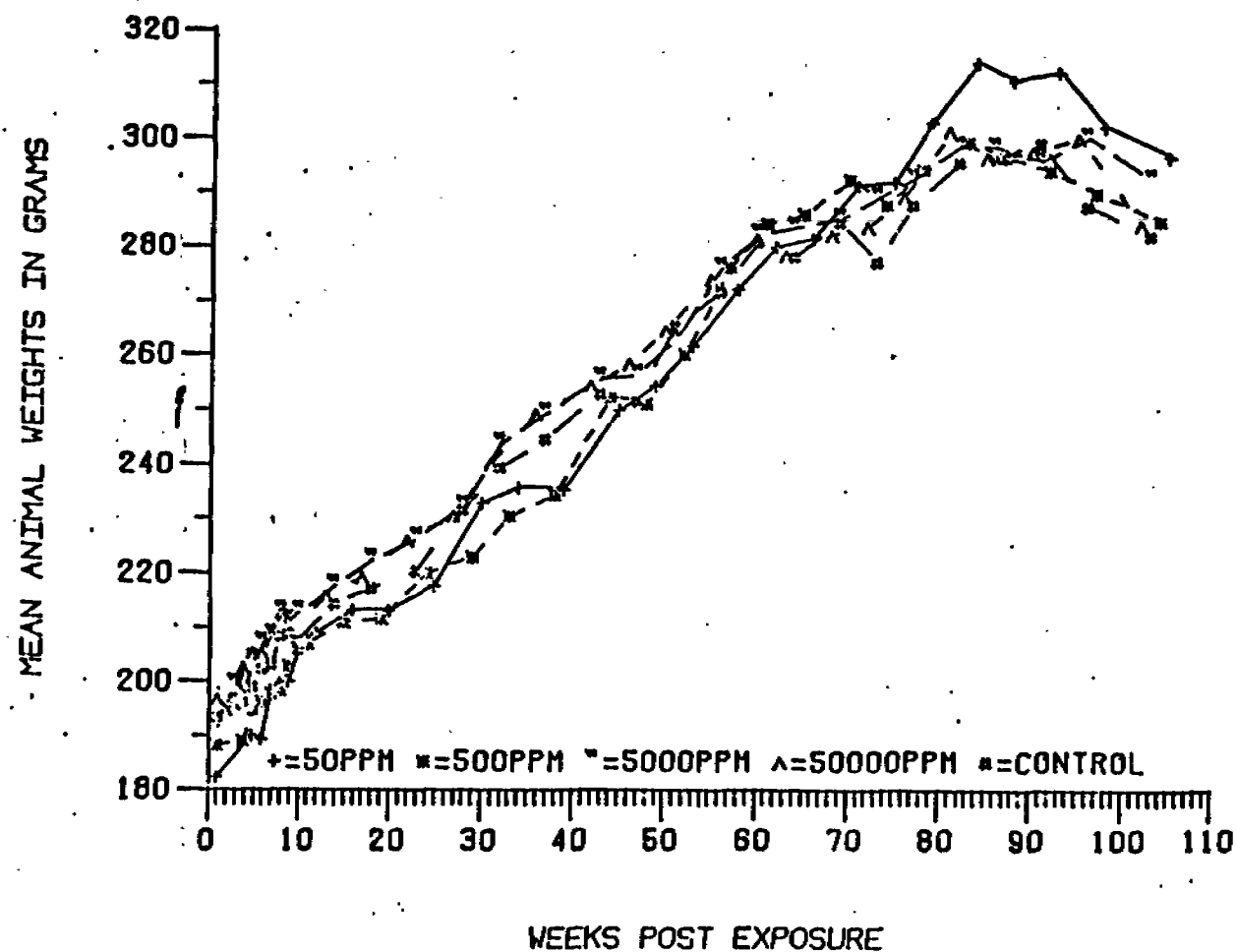
Figure XI
GROWTH CURVES OF FEMALE ICR MICE EXPOSED ONCE TO VCM



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Figure XIII

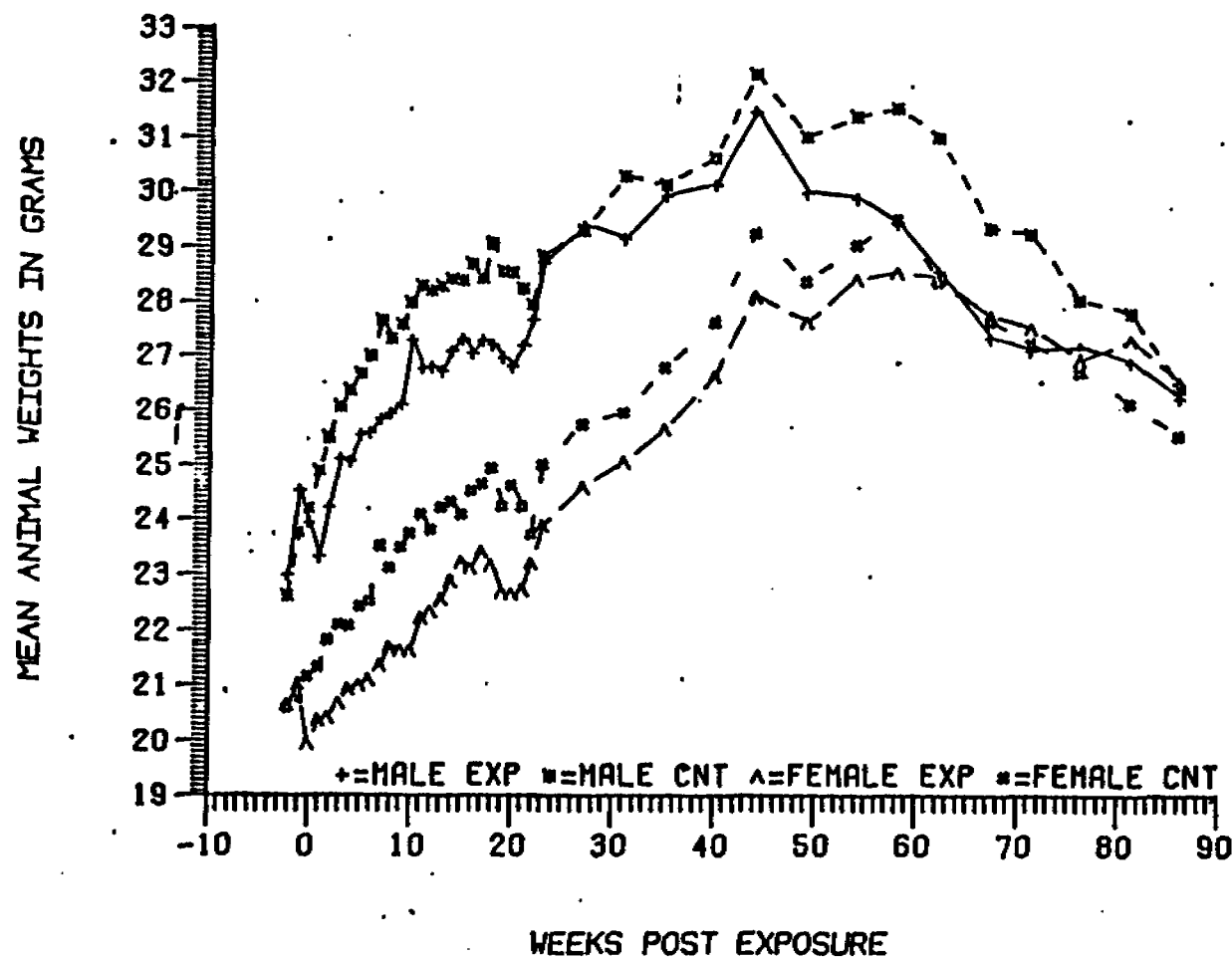
GROWTH CURVES OF FEMALE FISCHER RATS EXPOSED ONCE TO VCM



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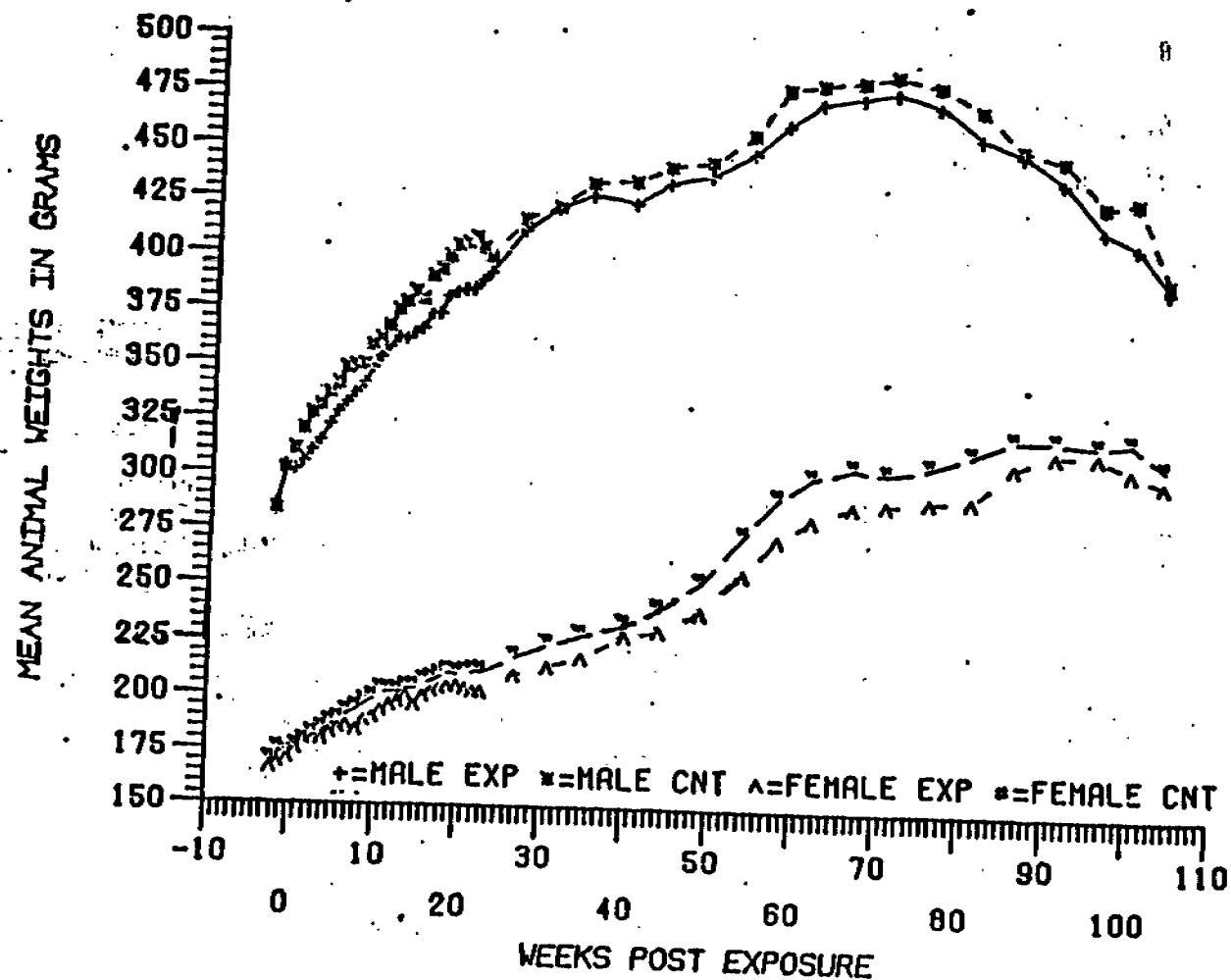
Figure XV

GROWTH CURVES OF A/J MICE EXPOSED TO VCM IN 100 DAY STUDY



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Figure XVII
GROWTH CURVES OF FISCHER RATS EXPOSED TO VCM IN 100 DAY STUDY



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Spontaneous Deaths (single initiation exposure)

Station or Group	Spontaneous Deaths									Schedule Sacrifice								
	0-6 Months			7-12 Months			13-18 Months			8 Months			18 Months			Totals		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	Male	Female	
10	0	0	0	5	11	16	33	32	65	4	1	5	7	17	24	49	61	
0	0	2	2	4	2	6	22	27	49	2	1	3	5	13	18	33	45	
0	0	5	5	6	8	14	19	14	33	1	0	1	5	24	29	31	51	
1	0	0	0	4	15	19	9	18	27	0	0	0	2	14	16	15	47	
1)	2	0	2	0	1	1	14	41	55	0	0	0	2	12	14	18	54	
S	0	7	7	19	36	55	83	91	174	7	2	9	19	68	87			

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Table VII . Over-all Summary Incidence of Non-neoplastic Changes and Histologically-Proven Neoplasms Within The Liver and Lungs of AJ Mice Exposed to Vinyl Chloride (Multiple Inhalation Exposure)

Tissue/Response	Exposure Dose Level (PPM) Hours Exposure Sex of Animal Animals Per Group*	Incidence of Response			
		Control		Vinyl chloride	
		0		500	
		10 x 1		10 x 1	
		M	F	M	F
		46	48	78	92
	: <u>Number Evaluated</u>	<u>45</u>	<u>48</u>	<u>78</u>	<u>89</u>
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
myeloidosis				1	
angiectasis					1
hepatic cell adenoma		1			
cholangiocarcinoma				1	
	: <u>Number Evaluated</u>	<u>43</u>	<u>47</u>	<u>76</u>	<u>90</u>
edema					1
pneumonitis			2		
bronchio-alveolar hyperplasia		2	1		2
bronchio-alveolar adenoma		15	16	56	68
bronchio-alveolar carcinoma			3	12	10

total includes animals from scheduled sacrificed (8, 16, 20 month periods) and spontaneous deaths

Table VII . Over-all Summary Incidence of Non-neoplastic Changes and Histologically-Proven Neoplasms Within The Liver and Lungs of AJ Mice Exposed to Vinyl Chloride (Multiple Inhalation Exposure)

Tissue/Response	Exposure Dose Level (PPM) Hours Exposure Sex of Animal Animals Per Group*	Incidence of Response			
		Control		Vinyl chloride	
		0		500	
		10 x 1		10 x 1	
		M	F	M	F
	Animals Per Group*	46	48	78	92
	: 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
myeloidosis				1	
angiectasis					1
hepatic cell adenoma		1			
cholangiocarcinoma				1	
	: 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

total includes animals from scheduled sacrificed (8, 16, 20 month periods) and spontaneous deaths

Exposed to Vinyl Chloride Monomer (Multiple Inhalation Exposure)

Exposure Schedule	1-hr Inhalation Exposures to Vinyl Chloride Monomer									TOTALS			
	50 ppm x 100			Control			500 ppm x 10			Control			CON
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	
Spontaneous													
0 mths	0	0	0	0	0	0	0	2	2	0	0	0	0
Months	8	3	11	0	0	0	2	1	3	0	0	0	0
Months	23	20	43	3	5	8	36	27	63	2	3	5	8
ed se mths	3	1	4	0	1	1	6	6	12	0	0	0	1
mths	10	6	16	2	3	5	14	8	22	1	1	2	4
mths	19	44	63	16	11	27	29	41	70	17	21	38	32
Is	63	74	137	21	20	41	87	85	172	20	25	45	75
5/													

Tab. Table XI. Over-all Summary Incidence of Non-neoplastic Changes Within
The Lungs of Fischer Rats Exposed To Vinyl Chloride.

Single Inhalation Exposure

Tissue/Response	Exposure Dose Level (PPM) Sex of Animal Animals Per Group*	Incidence of Response									
		Control		Vinyl chloride							
		0		50,000		5,000		500		50	
		M	F	M	F	M	F	M	F	M	F
		89	74	86	87	83	93	87	100	90	91
1	<u>Number Evaluated</u>	<u>85</u>	<u>67</u>	<u>80</u>	<u>77</u>	<u>80</u>	<u>87</u>	<u>85</u>	<u>95</u>	<u>80</u>	<u>74</u>
bronchopneumonia		26	10	45	13	13	10	15	3	28	3
5	% incidence	30.6	14.9	56.2	16.9	16.3	11.5	17.6	31.6	35.0	4.1

Total includes animals from scheduled sacrifice (8, 16, and 24 month periods) and spontaneous deaths.

Sacrificed Period	Organ Tissue	50,000 ppm			5,000 ppm			500 ppm			50 ppm			0 ppm (control)		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Educated months	Lung	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Liver	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stomach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Other*	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
	Total	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
months	Lung	2	0	2	0	0	0	1	0	1	1	0	1	0	0	0
	Liver	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stomach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Other*	2	2	4	5	1	6	12	0	12	6	0	6	11	3	14
	Total	4	2	6	5	1	6	13	0	13	7	0	7	11	3	14
months	Lung	0	2	2	0	0	0	3	3	6	1	2	3	3	2	5
	Liver	1	3	4	3	2	5	8	4	12	9	3	12	9	1	10
	Kidney	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1
	Stomach	0	1	1	0	1	1	0	0	0	0	0	0	0	1	1
	Other*	20	28	48	34	49	83	70	30	100	54	22	76	47	23	70
	Total	21	34	55	37	52	89	82	37	119	64	27	91	60	27	87

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Exposed to Vinyl Chloride Monomer (Multiple Inhalation Exposures)

Office and Exposure Description	1-hr Inhalation Exposures to Vinyl Chloride Monomer												Totals		
	50 ppm x 100			Control			500 ppm x 10			Control			VCM		Control
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	M
Previous Months	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Months	0	0	0	3	0	3	0	0	0	0	0	0	0	0	3
Months	14	6	20	5	0	5	10	4	14	3	0	3	24	12	8
Months	111	51	162	52	29	81	52	29	81	33	14	47	163	80	85
Standard Office Months	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0
Months	8	10	18	4	1	5	10	1	11	0	0	0	18	11	4
Months	43	43	86	32	29	61	42	71	113	69	27	96	85	114	101
Sums	176	110	286	96	59	155	114	108	222	105	61	166	290	218	201

Sacrifice Period	Organ Tissue	Scheduled Sacrifice											
		50 ppm x 100			Control			500 ppm x 100 ¹⁰			Control		
		M	F	Total	M	F	Total	M	F	Total	M	F	Total
8 Months	Lung	0	0	0	0	0	0	0	0	0	0	0	0
	Liver	0	0	0	0	0	0	0	0	0	0	0	0
	Kidney	0	0	0	0	0	0	0	0	0	0	0	0
	Stomach	0	0	0	0	0	0	0	0	0	0	0	0
	Other*	0	0	0	0	0	0	0	1	1	0	0	0
	Total	0	0	0	0	0	0	0	1	1	0	0	0
16 Months	Lung	0	0	0	0	0	0	0	0	0	0	0	0
	Liver	0	0	0	0	0	0	0	0	0	0	0	0
	Kidney	0	0	0	0	0	0	2	0	2	0	0	0
	Stomach	0	0	0	0	0	0	0	0	0	0	0	0
	Other*	8	10	18	4	1	5	8	1	9	0	0	0
	Total	8	10	18	4	1	5	10	1	11	0	0	0
24 Months	Lung	1	0	1	1	1	2	4	2	6	3	0	3
	Liver	7	2	9	2	3	5	10	1	11	5	4	9
	Kidney	0	0	0	0	0	0	2	0	2	0	0	0
	Stomach	0	0	0	0	0	0	0	0	0	1	0	1
	Other*	35	41	76	29	25	54	26	68	94	60	23	83
	Total	43	43	86	32	29	61	42	71	113	69	27	96

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Table XXII. Percentage of Stillborn Upus in the VCM Three-Generation Study

	Control			50 ppm			500 ppm		
	Total Pups Born	Total Stillborn	% Stillborn	Total Pups Born	Total Stillborn	% Stillborn	Total Pups Born	Total Stillborn	% Stillborn
F ₁ eration	195	1	0.51	202	5	2.48	184	3	1.63
F ₂ eration	239	0	0.00	234	2	0.85	255	2	0.78
F ₃ eration	225	2	0.89	204	5	2.45	265	3	1.13

Table XXIV. Numbers, Sex, and Weights of F_2 Generation

e ps ys	Total Number of Pups						Average Weight (Grams)					
	Control		50 ppm		500 ppm		Control		50 ppm		500 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	120	119	114	118	123	130	6.38	6.13	6.55	6.16	6.52	6.18
4	118	119	114	117	122	130	9.70	9.13	9.82	9.21	10.00	9.27
7	118	119	113	116	122	130	14.38	13.85	14.57	13.45	14.72	13.97
14	118	119	113	116	122	130	27.77	27.26	28.10	26.15	28.80	27.44
21	118	119	113	116	122	130	40.89	40.57	41.54	38.67	42.93	41.20

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Table XXVI. Viability, Survival, and Lactation Indexes in a Three-Generation Study of Reproductive Performance After Exposure of the F₀ Parents to VCM Gas.

	Generation	Control	Low Dose 50 ppm	High Dose 500 ppm
Viability Index	F ₁	100	100	99.4
	F ₂	99.2	99.7	99.6
	F ₃	100	100	100
Survival Index Day 21	F ₁	98.5	99.5	99.4
	F ₂	99.2	98.7	99.6
	F ₃	99.6	99.5	97.3
Lactation Index	F ₁	98.5	99.5	99.4
	F ₂	100	99.1	100
	F ₃	99.6	99.5	97.3

$$\text{Viability Index} = \frac{\text{No. of pups alive at Day 4} \times 100}{\text{No. of pups born alive}}$$

$$\text{Day 21 Survival Index} = \frac{\text{No. of pups alive at Day 21}}{\text{No. of pups born alive}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of pups alive at Day 21}}{\text{No. of pups alive at Day 4}} \times 100$$

Banerjee, B.N., Course Director, Teratology-Principles and Procedures Related to Fetal Development. The Center for Professional Advancement, Somerville, New Jersey. September 1974. Course Notes.

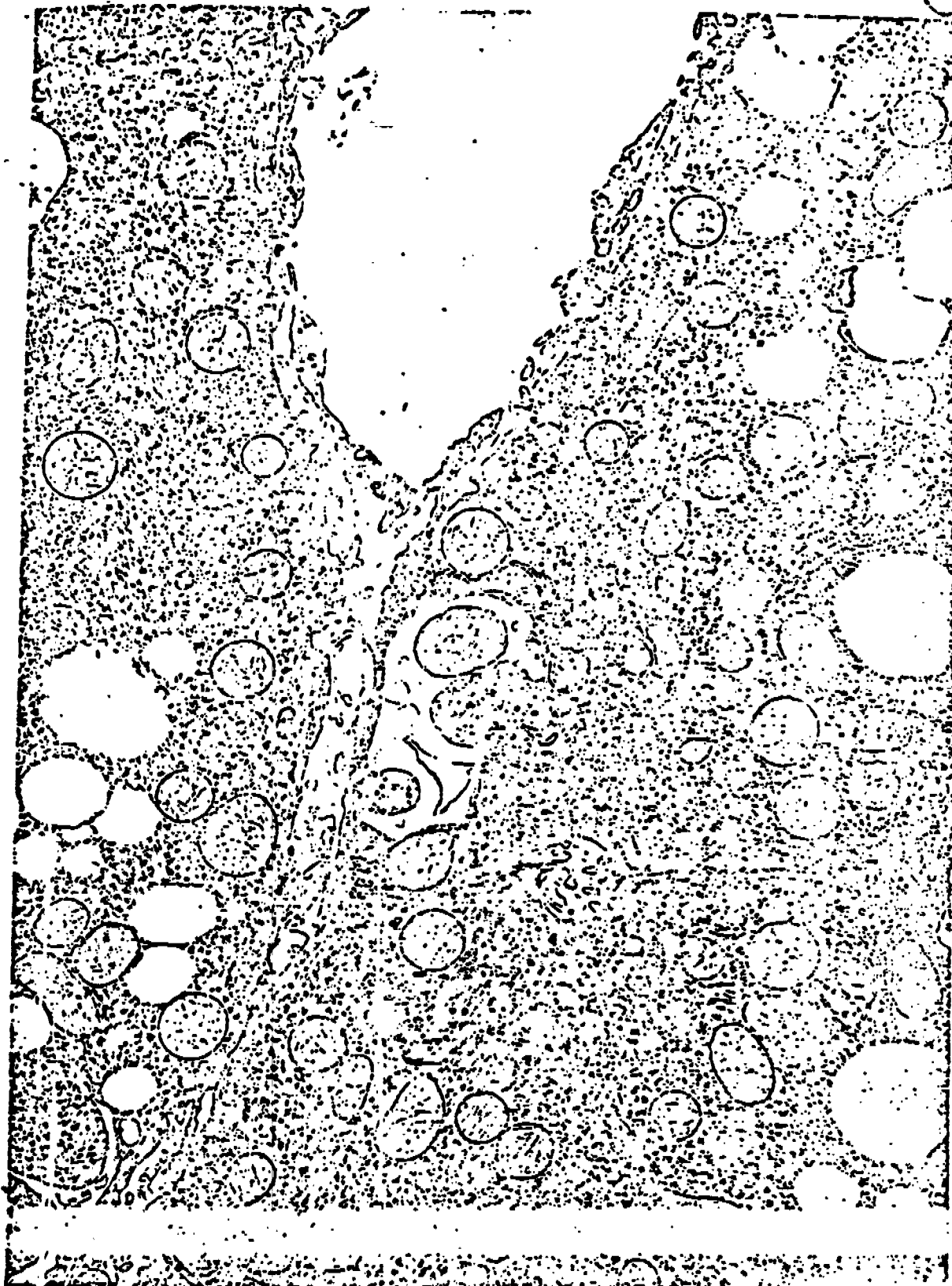
Figure XVIII
Control - 8 months

FIGURE LEGEND

Control hepatocyte morphology with normal appearing
endothelium adjacent. X5,000.

Negative #198,063

Figure XIX, Single Exposure - 8 months post-exposure



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Figure XX
Single Exposure - 8 months post-exposure



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Figure XXI

Multiple Exposure - 8 months post-exposure.



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Figure XXII
Controls - Multiple Exposure - 16 months post-exposure

FIGURE LEGEND

Hepatocyte contains numerous dense bodies and lipofuchsin granules. Cleft-like white spaces in dense bodies may represent crystalline material extracted during processing. X5,000.

Negative #197,157

75

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Figure XXIII
Multiple Exposure - 16 months post exposure

FIGURE LEGEND

Indented nucleus at center of field consistent with adenocarcinoma.
Cell at upper left is necrotic and shows mitochondria with high
amplitude swelling and flocculent densities. X5,000.

Negative #197,434

Figure XXIV
Controls - 24 months

FIGURE LEGEND

Normal appearance of hepatocyte in this control group. X5,000.

Negative #209,298

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Figure XXV

VF77 67-71

MALES

10 WEEKLY EXPOSURES TO 8PM VINYL CHLORIDE

POST EXPOSURE 106 WEEKS

General Summary of Findings:

Changes within hepatocytes included increased lipofuchsin granules and endoplasmic reticulum dilatation with a material of low electron density filling endoplasmic reticulum cisternae. In addition, bile canaliculi frequently showed distended lumens with the simplification of the plasma membrane at this site. Non-hepatocytic changes included enlarged Kupffer cells with abundant lysosomes and dense bodies and a mononuclear infiltrate into hepatic parenchyma.

Summary of Comments by Robert H. Harris at the April 4 Meeting

We have analyzed the CPSC vinyl chloride study by using methods that employ time-to-tumor data to calculate the carcinogenic potency (TD_{50}) of the compound. The TD_{50} is the daily dose (mg/kg) required to reduce the probability of being tumor-free by 50% when given over a standard life time (24 months in mice and rats). The statistical methods employed used log-likelihood techniques. To date, we have analyzed only those sites with a suggestion of a treatment-related effect based on summary data.

For the experiments employing single 1-hour administration over the dose range 50 - 50,000 ppm, vinyl chloride-caused lung alveolar/bronchiolar adenomas or carcinomas in both male and female ICR Swiss mice and liver adenomas or carcinomas in female ICR Swiss mice; in Fisher rats, vinyl chloride caused an increase in adrenal pheochromocytomas in both sexes, which can be considered only a suggestion of a carcinogenic effect since this site is known to have shown a variable response in historical controls.

In the multiple exposure experiments, exposure to 50 ppm (1 hour x 100) caused lung alveolar/bronchiolar carcinomas in female A/J mice, but not in male A/J mice or in Fisher rats of either sex. At 500 ppm (1 hour x 10), vinyl chloride produced lung alveolar/bronchiolar adenomas in both male and female A/J mice, lung alveolar/bronchiolar carcinomas in male A/J mice, but it failed to produce tumors in Fisher rats.

It was evident from our quantitative analysis, that for the same lifetime cumulative dose, vinyl chloride produced a considerably larger carcinogenic effect when administered at higher doses over short periods of time (500 ppm, 1 hour x 10) compared to lower concentrations administered over longer periods of time (50 ppm, 1 hour x 100). Furthermore, based on the experiments with ICR Swiss mice and A/J mice, vinyl chloride was more potent as a carcinogen than was indicated from previous bioassay tests published in the literature.