

Industrial **BIO-TEST** *Laboratories, Inc.*

1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

MANUFACTURING CHEMISTS ASSOCIATION

GROSS AND HISTOPATHOLOGIC FINDINGS
ONE-YEAR INTERIM SACRIFICE

CHRONIC VAPOR INHALATION STUDY WITH
VINYL CHLORIDE (ETHYLENE DERIVED)
IN ALBINO RATS

MAY 9, 1975

IBT NO. 663-03222

CMA 102254

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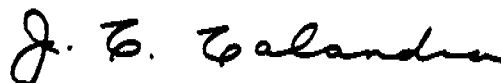
Mr. Milton Freifield
Project Manager, Vinyl Chloride Research
Manufacturing Chemists Association
1825 Connecticut Avenue, N.W.
Washington, D. C. 20009

Dear Mr. Freifield:

Re: IBT No. 663-03222 - Chronic Vapor Inhalation Study with
Vinyl Chloride (Ethylene Derived) in Albino Rats - Gross
and Histopathologic Findings - One-Year Interim Sacrifice

We are submitting herewith our laboratory report prepared
in connection with the above study.

Very truly yours,



J. C. Calandra
President

JCC:mcb

CMA 102255

REPORT TO
MANUFACTURING CHEMISTS ASSOCIATION
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ONE-YEAR INTERIM SACRIFICE
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MAY 9, 1975

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I. Introduction

At the request of the Manufacturing Chemists Association, 40 rats (5 males and 5 females) from the control and test groups were sacrificed on September 5, 1974, to conduct cytogenetic studies on bone marrow specimens obtained at time of sacrifice. The purpose of this report is to present the gross and histopathological findings in other tissues from these animals.

II. Summary

Treatment-related gross and/or histopathological findings were confined to the liver of test animals at exposure levels of 50, 200 and 2,500 ppm.

Gross pathologic findings ascribed to vinyl chloride were confined to the liver of 2 animals of the 200 ppm level (group T-II) and the liver of 5 animals of the 2,500 ppm level (group T-III). Findings consisted of solitary, dark red, nodular foci in the subcapsular region of single liver lobes. These nodules varied in size from approximately 4 mm to 4 cm in diameter and, on cross-section, they contained blood-filled cystic spaces.

Upon histopathological examination, the above lesions were identified as sites of hepatic angiosarcoma. In addition, there was focal hypertrophy and hyperplasia of hepatocytes in the liver sections from test animals at each exposure level. The incidence and relative severity of this finding could be correlated with the level of exposure to the test material. No extra-hepatic angiosarcomas were observed.

Materials and Methods

All animals, selected at random for sacrifice, were rendered unconscious in a closed chamber by exposure to carbon dioxide gas and exsanguinated, and a complete necropsy examination was performed. The organs were weighed. Representative specimens of the following organs and tissues were placed in 10% neutral buffered formalin for fixation:

Adrenal Glands	Pancreas
All Gross Lesions	Parathyroid Gland
Bone (femur and sternum)	Pituitary Gland
Bone Marrow (sternal)	Prostate Gland
Brain and Spinal Cord	Salivary Gland
Esophagus	Seminal Vesicles
Eye	Skin
Gonads (testes and ovaries)	Small Intestine (duodenum, jejunum and ileum)
Kidneys	Spleen
Large Intestine (caecum and colon)	Stomach
Liver	Thymus
Lungs	Thyroid Gland
Lymph Nodes (tracheobronchial, cervical and mesenteric)	Trachea
Optic Nerve	Urinary Bladder
	Uterus

The above tissues from all animals were processed by conventional methods, embedded in Paraplast*, sectioned at 4-6 microns, stained with Hematoxylin and Eosin and examined by light microscopy.

The gross pathologic findings are presented in Tables I through II.
The histopathological findings are presented in Tables III and IV.

The pathologist's report is presented on pages 4 through 6.

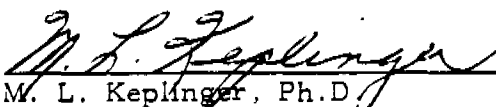
Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:


Donovan E. Gordon, D.V.M., Ph.D.
Section Head, Pathology

Report approved by:


M. L. Keplinger, Ph.D.
Manager, Toxicology

mcb

* Sherwood Medical Industries, St. Louis, Missouri 63103.

IBT No. 663-03222
Manufacturing Chemists Association

February 27, 1975

A gross and histopathological examination was conducted on a series of tissues and organs from rats of the 12-month interim sacrifice of IBT No. 663-03222.

Treatment-related gross pathologic changes were confined to the liver of 2 T-II females (Nos. 550 and 599), 1 T-III male (No. 694) and 4 T-III females (Nos. 717, 732, 735 and 742). The lesions consisted of solitary, dark red nodules within the subcapsular region of 1 liver lobe. The nodules measured approximately 4 mm to 4 cm in diameter and contained blood-filled cystic spaces. There were fibrous adhesions between the capsular surface of some of these nodules and adjacent visceral structures. In 1 animal (No. 735), there were 2 different liver lobes with solitary nodular foci similar to the above. Upon histopathologic examination of the hepatic nodules, they were found to be angiosarcomas. A small amount of hemorrhagic fluid was present in the abdominal cavity of 1 animal (No. 59) as a result of hemorrhage from the surface of the liver tumor.

Treatment-related histopathologic findings were confined to the liver of 5 T-I animals and all of the T-II and T-III animals. There was an exposure-related incidence and severity of hypertrophy and hyperplasia of hepatocytes throughout the liver sections. The hypertrophied hepatocytes had an increase in cytoplasmic mass which had a light, eosinophilic staining appearance with a few basophilic condensations. In some sections, these cells formed small

nodules with very little compression of the adjacent parenchymal cells of the liver. Some of the hyperplastic hepatocytes had prominent nuclei and nucleoli. They also formed small, discrete nodules in some areas. In addition, these cells often had a slightly increased cytoplasmic basophilia. In regard to the angiosarcomas, the early and more advanced stages of the tumor could be identified. In the early lesion, there was focal sinusoidal dilatation accompanied by hyperplastic and/or hypertrophied endothelial-like lining cells. The areas usually communicated with frank areas of angiosarcoma. The latter were characterized by the presence of blood-filled cystic sinusoidal spaces (peliotic foci) in which there were papillary projections of cords of liver cells that were covered by large, hyperchromatic to pleomorphic lining cells on the sinusoidal surface. The hepatocytes in the area adjacent to the tumor were also invaded by these tumor cells. In some areas, there was complete destruction of the enveloped liver cords and replacement by solid sheets of sarcomatous tumor cells which contained numerous mitotic figures and had anaplastic features. In most instances, the tumors were situated just beneath the capsular surface of the affected liver lobule and there was focal invasion of tumor cells into the capsule with hemorrhage into the peritoneal cavity at these sites. There were also fibrous adhesions to the adjacent visceral organs at the capsular surface of the tumors which correlated with the gross observations. In 2 animals (Nos. 663 and 668), the tumor foci were too small to be detected microscopically and they were in the early stages of development. In this study, there were no metastatic

tumor foci found in other organs, however; tumor emboli were present in some of the vessels of the larger liver tumors. All liver lesions, observed by light microscopy, summarized in Table III.

The other changes described in the liver and other tissues (Table IV) were regarded as lesions of naturally occurring diseases. The lesions were present, in most instances, among both control and test animals. The small (1-3 mm), red, subcapsular foci observed at necropsy in the liver of both control and test animals could be correlated with scattered foci of hepatocellular necrosis observed histologically in these animals.



Donovan E. Gordon, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists

TABLE I

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Individual Gross Pathologic Findings in the Liver - Males

Group	Animal Number	Gross Pathologic Findings
Control	39	1 mm dark red foci in all lobes
	54	None
	55	Few 1 mm dark red foci in all lobes
	69	Numerous 1 mm dark red foci in all lobes
	84	None
T-I	205	Few 1 mm dark red foci in all lobes
	256	1-3 mm dark red foci in all lobes
	264	None
	279	None
	299	Numerous 1-3 dark red depressed foci in all lobes
T-II	404	Numerous 1-5 mm dark red depressed foci in all lobes
	433	Few 1-3 mm dark red foci in all lobes
	459	Few 1 mm dark red foci in all lobes
	469	1 mm dark red foci in all lobes
	489	1 mm dark red foci in all lobes, slight pale discoloration
T-III	609	None
	639	Numerous 1 mm dark red foci in all lobes
	663	Granular surface with numerous 1 mm dark red foci in all lobes
	668	1 mm dark red focus in 1 lobe
	694	0.5 cm dark red subcapsular nodule in 1 lobe with capsular adhesions to spleen

TABLE I continued

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Individual Gross Pathologic Findings in the Liver - Females

Group	Animal Number	Gross Pathologic Findings
Control	106	1-3 mm dark red foci in all lobes
	149	Few 1 mm dark red foci in all lobes
	160	None
	178	Numerous 1 mm red foci in all lobes
	200	Few 1 mm red foci in all lobes
T-I	303	Few 1 mm dark red foci in all lobes
	349	1-3 mm dark red depressed foci in all lobes
	369	1-3 mm dark red foci in all lobes
	381	1-3 mm dark red foci in all lobes
	397	Few 1-2 mm dark red foci in all lobes
T-II	524	Few 1-3 mm dark red foci in all lobes
	550	0.5 cm dark red nodule in 1 lobe
	569	1 mm dark red foci in all lobes
	594	1-2 mm dark red foci in all lobes
	599	4 cm subcapsular nodular focus in major lobe, capsular adhesions to diaphragm, spleen and serosal surface of small intestine
T-III	717	0.5 cm dark red focus in 1 lobe with capsular adhesions to mesentery
	728	None
	732	1 cm dark red focus in 1 lobe
	735	3 cm nodule in 1 lobe; 0.5 cm dark red nodule in another lobe; focal capsular adhesions between nodule, small intestine, stomach, spleen and mesentery
	742	4 mm dark red depressions in 1 lobe, 1-5 mm dark red foci in all other lobes

TABLE II

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Summary of Gross Pathologic Findings in All Tissues Except Liver

Organ	Findings	Group: Number per Group:	Control 10/10	T-I 10/10	T-II 10/10	T-III 10/10
			Male/Female Incidence	Male/Female Incidence	Male/Female Incidence	Male/Female Incidence
Lung	Focal adhesions to diaphragm or thorax		3/0	1/0	2/0	0/0
	Few pin-point red foci in all lobes		1/0	0/0	0/0	1/0
	Focal consolidation of 1 or more lobes		0/1	0/0	0/1	1/0
	Abscesses in parenchyma		0/0	1/0	0/0	0/0
Kidneys	Unilateral hydronephrosis		0/0	1/0	0/0	0/0
	Bilateral hydronephrosis		1/0	0/0	0/0	0/0
Ovaries	Unilateral or bilateral follicular cysts		2	2	0	1
Adrenal glands	Small (1 mm) blood-filled cysts in cortex of 1 gland		0/1	0/0	0/0	0/0
	Enlarged and dark red color		0/0	0/0	0/1	0/0
Uterine horns	Hydrometra		2	0	0	0

TABLE II continued

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Summary of Gross Pathologic Findings in All Tissues Except Liver

Organ	Findings	Group:	Control	T-I	T-II	T-III
		Number per Group:	10/10	10/10	10/10	10/10
			Male/Female Incidence	Male/Female Incidence	Male/Female Incidence	Male/Female Incidence
Pituitary gland	Hemorrhagic focus		0/0	1/0	0/0	0/0
Spleen	Few adhesions to mesentery		0/0	0/0	1/0	1/0
	Enlarged (3 times normal size)		0/0	0/0	0/1	0/0
Abdominal cavity	Small amount of blood in peritoneal cavity		0/0	0/0	0/1	0/0

TABLE III
TEST MATERIAL: Vinyl Chloride (Ethylene Derived)
Chronic Vapor Inhalation Study - Albino Rats
One Year Interim Sacrifice
Summary of Liver Lesions* - Males

Group and Animal Number	Bile Duct Proliferation	Portal to Sinusoidal Extramedullary Hematopoiesis	Necrosis of Hepatocytes	Cytoplasmic Lipid Vacuoles in Hepatocytes	Hypertrophy of Hepatocytes	Hypertrophy and Hyperplasia of Hepatocytes	Sinusoidal Dilatation Subcapsular Region Only	Throughout Section	Blood-Filled Cystic Spaces (Pelusis)	Angiosarcoma
Control										
39			2/M					1-2		
54		1-2					1			
55			1-2/M							
69			2/M					1		
84			1							
T-I										
205	1		1-2/M		1 (few)			1		
256			1-2					1		
264			1-2	1				1-2		
279		1		1-2	1 (few)		1			
299		1	1-2	4/D (1 lobe)		1	1-2			
T-II										
404	1-2	2	1			1-2		1		
433			2/M	2		1-2		1		
459			1-2			1	1-2			
469	1		2/M			1-2	1	1-2		
489	1		2/M			1-2		2		
T-III										
609	1		2	1		2/M	1			
639	1		2/M	2		1-2/M		2-3	P	P
663	1		2	1		2/M		1	P	P
668	1-2		2	1		2-3/M		2	P	P
694	1		1-2/M			2/M				

* All lesions are focal unless otherwise indicated.

Grading System and Abbreviations

1 = minimal in severity
2 = mild in severity
3 = moderate in severity
4 = marked in severity
5 = extreme in severity

P = Present, no grade
M = multifocal
D = diffuse

TABLE III continued
 TEST MATERIAL: Vinyl Chloride (Ethylene Derived)
 Chronic Vapor Inhalation Study - Albino Rats
 One-Year Interval Sacrifice
 Summary of Liver Lesions* Females

Group and Animal Number	Bile Duct Proliferation	Portal to Sinusoidal Extramedullary Hematopoiesis	Necrosis of Hepatocytes	Cytoplasmic Lipid Vacuoles in Hepatocytes	Hypertrophy of Hepatocytes	Hypertrophy and Hyperplasia of Hepatocytes	Sinusoidal Dilatation Subcapsular Region Only	Sinusoidal Dilatation Throughout Section	Blood Filled Cystic Spaces (Peliosis)	Angiosarcoma
Control										
106	1						1			
149			1-2/M							
160								1		
178			2					1		
200		1	1					1		
T-I										
403								1-2		
349					1			1-2		
369						1		1-2		
381	1					1		1-2		
397			1-2 (few)			1		1		
T-II										
524	1	1		1-2		1		1		
550	1				1			2	P	P
569	1	1				1-2		1-3		
594						1-2/M	2			
599		1-2				2/M			P	P
T-III										
717	1	1	2	1		2/M		2	P	P
728	1	1				2-3/M		1-2		
732	1			1-2		2-3/M		2	P	P
735	1-3	1-2				2-3/M		2	P	P
742		1		1		2/M		2	P	P

* All lesions are focal unless otherwise indicated

Grading System and Abbreviations

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TABLE IV

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interval Sacrifice

Incidental Histopathologic Changes in Tissues Other Than Liver - Males

Organ	Findings	Group:		Control		T I		T-II		T-III	
		Number Examined:		10		10		10		10	
				Average Incidence	Average Grade	Average Incidence	Average Grade	Average Incidence	Average Grade	Average Incidence	Average Grade
Trachea	Chronic tracheitis			2	2.0	3	1.0	2	1.8	3	1.7
Lung	Chronic focal murine pneumonia			3	1.3	5	1.3	2	1.5	5	1.3
	Focal aggregates of alveolar macrophages			2	1.0			1	1.0	2	1.0
	Acute focal bronchopneumonia					1	2.0				
Heart	Focal myocardial degeneration and fibrosis			2	1.0	2	1.0	1	1.0		
Kidney	Bilateral focal proteinaceous tubular casts			3	1.2	2	1.0	2	1.8	4	1.5
	Bilateral focal tubular nephrosis			1	1.5			2	1.8	3	1.7
	Bilateral focal interstitial lymphoid infiltrations					1	1.0			1	1.0
	Bilateral chronic focal nephritis			2	1.0	1	1.5				
	Bilateral focal mineralized microconcretions in tubules							1	1.0		
	Unilateral hydronephrosis					1	2.0				
	Bilateral hydronephrosis			1	2.5						
Urinary bladder	Proteinaceous plug in lumen			2	1.0	1	1.0	3	1.0	4	1.0
Prostate gland	Focal interstitial lymphoid infiltrations							1	1.0		
Testes	Unilateral focal testicular degeneration							1	1.0		
	Unilateral focal testicular degeneration with spermatocetes, spermatie granulomas and dystrophic mineralization of some affected tubules							1	3.5		

TABLE IV continued

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Incidental Histopathologic Changes in Tissues Other Than Liver Males

Organ	Findings	Group:		Control		T-I		T-II		T-III	
		Number Examined:		10		10		10		10	
				Average Incidence	Average Grade	Average Incidence	Average Grade	Average Incidence	Average Grade	Average Incidence	Average Grade
Pituitary gland	Focal cytoplasmic vacuolation of basophils Small chromophobe adenoma	2		2	1.0	2	1.0			1	1.0
						1	P				
Adrenal glands	Unilateral focus of lipomatous degeneration in cortex	2		2	1.0			1	1.5	1	1.0
Thyroid glands	Bilateral diffuse hypertrophy of follicular epithelial cells							1	2.0		
Colon	Nematode parasites in lumen (nematodiasis)	1		1	-						
Bone	Focal medial hypertrophy of arterioles in femoral marrow	1		1	3.0	1	2.0	1	1.0	1	2.0

Grading System

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- P = Present, no grade

TABLE IV continued

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Incidental Histopathologic Changes in Tissues Other Than Liver - Females

Organ	Findings	Group: Number Examined:		Control 10		T-I 10		T-II 10		T-III 10	
		Average		Average		Average		Average		Average	
		Incidence	Grade	Incidence	Grade	Incidence	Grade	Incidence	Grade	Incidence	Grade
Trachea	Chronic tracheitis	3	1.8	3	1.5	2	1.8	1	1.0		
Lung	Chronic focal murine pneumonia	5	1.2	4	1.0	4	1.6	4	1.1		
	Focal aggregates of alveolar macrophages	2	1.0					1	1.0		
	Diffuse hemorrhage and edema (agonal)	1	3.0								
	Focal suppurative bronchiolitis	1	1.0			1	2.0				
Heart	Few small foci of chronic myocarditis									1	1.0
Kidneys	Unilateral focal proteinaceous tubular casts	1	1.0			1	1.0				
	Bilateral focal proteinaceous tubular casts	1	2.0			3	2.0	1	1.0		
	Bilateral focal tubular nephrosis	1	1.5			1	1.0				
	Unilateral chronic focal nephritis	1	1.0								
	Unilateral focal mineralized microconcretions in tubules									1	1.0
	Bilateral focal mineralized microconcretions in tubules	4	1.9	3	1.0	4	1.6	4	1.9		
	Unilateral large tubular cyst in cortex									1	P
	Bilateral diffuse brown granular pigment and protein resorption droplets within cytoplasm of cortical tubular epithelial cells					1	1.5				
Spleen	Increased hemosiderin pigment	2	1.5	2	1.5	1	1.0	2	1.0		
	Diffuse increased extramedullary hematopoiesis					1	3.0	1	2.0		
Uterus	Hydrometra	1	3.0								
	Suppurative metritis with endometrial abscesses					1	2.0				
	Suppurative metritis							1	2.0		

TABLE IV continued

TEST MATERIAL: Vinyl Chloride (Ethylene Derived)

Chronic Vapor Inhalation Study - Albino Rats

One-Year Interim Sacrifice

Incidental Histopathologic Changes in Tissues Other Than Liver - Females

Organ	Findings	Group: Number Examined:		Control 10		T-I 10		T-II 10		T-III 10	
		Average		Incidence		Average		Incidence		Average	
Ovaries	Bilateral focal follicular cysts					1	P	1	P		
	Unilateral focal follicular cysts					1	P			2	P
Pituitary gland	Focal hyperplasia of chromophobe cells							2	P		
Adrenal glands	Unilateral focus of lipomatous degeneration in cortex							1	2.0	1	3.0
	Unilateral focal hematocysts in cortex	1	4.0	1	1.0	2	2.8	1	2.0		
	Bilateral focal hematocysts in cortex					1	1.0				
Pancreas	Focal atrophy of acinar cells	1	1.0					1	1.0	1	2.0
Thyroid gland	Bilateral diffuse hypertrophy of follicular epithelial cells									1	1.0
Eye	Unilateral chronic focal dacryoadenitis	1	1.0								
	Unilateral focal retinal degeneration					1	1.0				
Colon	Nematode parasites in lumen (nematodiasis)									2	P

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- 5.0 = extreme in severity
- P = Present, no grade