THE DOW CHEMICAL COMPANY

BENNETT BUILDING
2030 DOW CENTER
MIDLAND, MICHIGAN 48640

RALL

October 25, 1974

HCK 10/29/74

Mr. K. D. Johnson
Manufacturing Chemists' Assoc.
1825 Connecticut Ave., N.W.
Washington, D.C. 20009

Mr. H. L. Kusnetz
Shell Oil Company
Room 1574, One Shell Plaza
Houston, TX 77002

Three protocols are attached. The first summarizes briefly the current study at IBT. The second summarizes the study the committee developed Sept. 18, 1974 and which it agreed should be discussed with the Rall Committee. The third is a proposal I put together since I don't like proposal #2.

I see no reason to omit hamsters and I would like to delay the start of more mice until we have metabolic data. My proposal accomplishes both.

I'll be calling you next week.

Sincerely,

Ted R. Torkelson

Theodore R. Torkelson
Corporate Medical Department

kjf

encl.

CURRENT PROTOCOL

MANUFACTURING CHEMISTS' ASSOCIATION, INC.

CHRONIC VAPOR INHALATION TOXICITY STUDY WITH VINYL CHLORIDE

INDUSTRIAL BIO-TEST LABORATORIES
DECATUR, ILLINOIS

I. Outline of Investigation

A.	Type and Length:	9-month vapor inhalation in White Mice + 9 months observation. 12-month vapor inhalation in Albino Rats + 12 months observation. 12-month vapor inhalation in Hamsters + 12 months observation.
B.	Number of Animals:	800 Mice. 900 Rats. 800 Hamsters.
C.	Exposure Schedule:	Seven hours per day. Five days per week.
D.	Test Materials:	Vinyl Chloride (Ethylene derived).
E.	Organization:	See Table I.
F.	Dose Levels:	See Table I.
G.	Exposures Started: Exposures Terminated: Terminal Autopsy:	Sept. 1973; mice, rats & hamsters. June 1974; mice. Sept. 1974; rats & hamsters. March 1975; mice*. Sept. 1975; rats & hamsters.

*NOTE: Excess mortality made it necessary to sacrifice all TE-II and TE-III mice after 11 months on experiment.

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5-0543

TABLE I

CHRONIC VAPOR INHALATION TOXICITY STUDY

Organization of Groups

Test Material		Group	Number of Animals					
			Mice		Rats		Hamsters	
			Males	Females	Males	Females	Males	Females
None		Control	100	100	100*	100*	100	100
Vinyl Chloride (Ethylene derived)	TE-I	Low Level - 50 ppm	100	100	100*	100*	100	100
	TE-II	Intermediate Level - 200 ppm	100	100	100*	100*	100	100
	TE-III	High Level - 2500 ppm	100	100	100*	100*	100	100
	TE-IV	High Level - 2500 ppm with food	-	-	-	100	-	-

*Each of these rat groups were reduced by sacrifice of 5 rats for cytogentic and histopathological examination at the end of the 12th month of exposure.

II. Chamber Parameters

Each group of animals will be exposed in a specially constructed stainless steel and plexiglass inhalation chamber having a capacity of approximately 9.3 M^3, allowing animal loading of less than 2% when the animals reach maturity. Flow rate through the chamber will be at least 1.53 M^3/min. providing a theoretical air change every six minutes. The chamber supply air will be filtered and maintained at 40% to 60% relative humidity and 70° to 75°F. Food will be removed from all animal cages during exposure except Group TE IV rats.

III. Animal Parameters

A. Clinical Observations

All animals will be observed daily for lesions and behavioral changes attributable to the test material. The time of appearance and location of all tumors that occur among both control and test animals will be recorded. Mortality records will be kept on all groups of animals. All animals which die during the study will be necropsied and their tissues processed in accordance with the methods given in the Anatomic Pathology Section. Care will be exercised to minimize loss of tissues through cannibalism or autolysis. Animals in a moribund state will be sacrificed *in extremis* when death is imminent.

B. Body Weights

Individual body weights will be recorded once before exposure and after 1, 2, 3, and 4 weeks of testing.

Thereafter, mean group body weights will be determined monthly up to the 12-month point of study.

C. Clinical Pathology

Hemoglobin, hematocrit, total erythrocyte and total leukocyte counts will be performed at 18 and 24 months on 30 (15 male and 15 female) rats of the control and each test group. Differential leukocyte counts will be performed on all animals having high total leukocyte counts.

D. Anatomic Pathology

1. Methods of Sacrifice

Upon completion of the study, all survivors will be sacrificed by exsanguination following carbon dioxide anesthesia.

2. Gross Pathology

Complete necropsies will be performed on all animals which die or are sacrificed and all macroscopic lesions will be recorded. The lungs will be inflated with formalin fixative.

3. Histopathology

Representative specimens of the following organs and tissues will be taken from all animals at time of sacrifice and fixed in 10.0% neutral buffered formalin:

Adrenal Glands
All Gross Lesions
Bone (femur, tarsal and metatarsal, including long bones of all four limbs)
Bone Marrow (sternal)
Brain
Both Ears (external auditory canal with ceruminal (Zymbal's) glands)
Esophagus
Eye
Gonads (testes and ovaries)
Kidneys
Large Intestine (caecum and colon)
Liver
Lungs
Lymph Nodes (tracheobronchial, cervical and mesenteric)
Optic Nerve
Pancreas
Parathyroid Gland
Pituitary Gland
Prostate
Salivary Gland
Seminal Vesicles
Skin
Small Intestine (duodenum, jejunum and ileum)
Spleen
Stomach
Thymus
Thyroid Gland
Trachea
Urinary Bladder
Uterus

The above tissues, from animals of the control, TE-II, and TE-III will be processed by conventional methods, embedded in paraplast, sectioned (4-6 μ), stained with hematoxylin and eosin, and evaluated by light microscopy. If drug-related lesions are detected in tissues from the high or intermediate dose (TE-II or TE-III) animals, affected tissues from the TE-I group animals will be processed and examined in the same manner as the above.

Only major organs (liver, kidney, spleen, heart, lungs) and neoplasms from animals which die and are found in an advanced state of autolysis will be processed for histopathological evaluation.

IV. Reports

Quarterly summaries of mortalities, clinical observations, clinicopathologic and anatomopathologic findings will be prepared. Upon completion of the study, a complete report will be prepared and issued.

October 23, 1974

PROPOSED PROTOCOL A

MANUFACTURING CHEMISTS' ASSOCIATION, INC.

CHRONIC VAPOR INHALATION TOXICITY STUDY WITH VINYL CHLORIDE

I. Outline of Investigation

A. Type and Length*: 9-month vapor inhalation exposure in White Mice plus 9 months observation. 12-month vapor inhalation in Albino Rats plus 12 months observation.

B. Number of Animals: 5∞ Mice
5∞ Rats

C. Exposure Duration: Seven hours per day. Five days per week.

D. Test Materials: Vinyl Chloride

E. Organization: See Table I

F. Dose Levels: See Table I

G. Proposed Schedule:

Group	Exposures Start	Exposures Stop	Termination Autopsy
Mice: Control, TE-I, TE-II, TE-IV	Dec. 1974	Sept. 1975	June 19
Rats: Control, TE-I, TE-II, TE-IV	Dec. 1974	Dec. 1975	Dec. 19

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5-0549

TABLE I

CHRONIC VAPOR INHALATION TOXICITY STUDY VINYL CHLORIDE

Organization of Groups

	Group	Number of Animals			
		Mice		Rats	
		Males	Females	Males	Females
	Control				
TE-I	Low Level - 2 ppm				
TE-II	Intermediate Level - 5 ppm				
TE-III	High Level - 10 ppm				
	Total				

II. Chamber Parameters

Each group of animals will be exposed in a specially constructed stainless steel and plexiglass inhalation chamber having a capacity of approximately 9.3 M^3, allowing animal loading of less than 2% by volume when the animals reach maturity. Flow rate through the chamber will be at least 1.53 M^3/min. providing a theoretical air change every six minutes. The chamber supply air will be filtered and maintained at 40% to 60% relative humidity and 70° to 75°F. Mice are to be individually caged; rats will be caged in groups. Food and water will be provided ad libitum.

III. Animal Parameters

A. Clinical Observations:

All animals will be observed daily for lesions and behavioral changes attributable to the test material. The time of appearance and location of all tumors that occur among both control and test animals will be recorded. Mortality records will be kept on all groups of animals. All animals which die during the study will be necropsied and their tissues processed in accordance with the methods given in the Anatomic Pathology Section. Care will be exercised to minimize loss of tissues through cannibalism or autolysis. Animals in a moribund state will be sacrificed in extremis when death is imminent.

B. Body Weights

Individual body weights will be recorded once before exposure and after 1, 2, 3, and 4 weeks of testing. Thereafter, mean group body weights will be determined monthly up to the 12-month point of the study.

C. Clinical Pathology

Hemoglobin, hematocrit, total erythrocyte and total leukocyte counts will be performed at 18 and 24 months on 30 (15 male and 15 female) rats of the control and each test group. Differential leukocyte counts will be performed on all animals having high total leukocyte counts.

D. Anatomic Pathology

1. Methods of Sacrifice

Upon completion of the study, all survivors will be sacrificed by exsanguination following carbon dioxide anesthesia.

2. Gross Pathology

Complete necropsies will be performed on all animals which die or are sacrificed and all macroscopic lesions will be recorded. The lungs will be inflated with formalin fixative.

3. Histopathology

Representative specimens of the following organs and tissues will be taken from all animals at time of sacrifice and fixed in 10.0% neutral buffered formalin:

Adrenal Glands
All Gross Lesions
Bone (femur, tarsal and metatarsal, including long bones of all four limbs)
Bone Marrow (sternal)
Brain
Both Eyes (external auditory canal with ceruminal (Zymbal's) glands)
Esophagus
Eye
Gonads (testes and ovaries)
Kidneys
Large Intestine (caecum and colon)
Liver
Lungs
Lymph Nodes (tracheobronchial, cervical and mesenteric)
Optic Nerve
Pancreas
Parathyroid Gland
Pituitary Gland
Prostate
Salivary Gland
Seminal Vesicles
Skin
Small Intestine (duodenum, jejunum and ileum)
Spleen
Stomach
Thymus
Thyroid Gland
Tracheo
Urinary Bladder
Uterus

The above tissues, from all animals, will be processed by conventional methods, embedded in paraplast, sectioned (4-6 μ), stained with hematoxyline and eosin, and evaluated by light microscopy.

Only major organs (liver, kidney, spleen, heart, lungs) and neoplasms from animals which die and are found in an advanced state of autolysis will be processed for histopathologic evaluation.

XV. Reports

Quarterly summaries of mortalities, clinical observations, clinicopathologic and anatomopathologic findings will be prepared. Upon completion of the study, a complete report will be prepared and issued.

October 23, 1974

PROPOSED PROTOCOL B

MANUFACTURING CHEMISTS' ASSOCIATION, INC.

CHRONIC VAPOR INHALATION TOXICITY STUDY WITH VINYL CHLORIDE

I. Outline of Investigation

A. Type and Length*: 6-month vapor inhalation in Albino rats plus 18 months observation. ?
12-month vapor inhalation in Albino Rats plus 12 months observation.
12-month vapor inhalation in Hamsters plus 12 months observation.
9-month vapor inhalation in White Mice plus 9 months observation.

B. Number of Animals: 1400 Rats
800 Hamsters
800 Mice

C. Exposure Duration: Seven hours per day.
Five days per week.

D. Test Materials: Vinyl Chloride

E. Organization: See Table I.

F. Dose Levels: See Table I.

G. Proposed Schedule:

Group	Exposures Start	Exposures Stop	Terminal Autopsy
Rats: Control, TE-I-6, TE-II-6, TE-III-6.	Dec. 1974	June 1975	Dec. 1976
TE-I-12, TE-II-12, TE-III-12	Dec. 1974	Dec. 1975	Dec. 1976
Hamsters: TE-I-12, TE-II-12, TE-III-12	Dec. 1974	Dec. 1975	Dec. 1976
Mice: TE-I-9, TE-II-9, TE-III-9	June 1975	Mar. 1976	Dec. 1976

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TABLE I

CHRONIC VAPOR INHALATION TOXICITY STUDY VINYL CHLORIDE

Proposal B
Organization of Groups

Test Material	Group	Number of Animals					
		Rats		Hamsters		Mice	
		Males	Females	Males	Females	Males	Females
	Control	100	100	100	100	100	100
TE-I	Low Level - 2 ppm	100	100	100	100	100	100
TE-II	Intermediate Level - 5 ppm	100	100	100	100	100	100
TE-III	High Level - 10 ppm	100	100	100	100	100	100
TE-I-6	Low Level - 2 ppm	100	100				
TE-II-6	Intermediate Level - 5 ppm	100	100				
TE-III-6	High Level - 10 ppm	100	100				
	TOTAL	700	700	400	400	400	400

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II. Chamber Parameters

Each group of animals will be exposed in a specially constructed stainless steel and plexiglass inhalation chamber having a capacity of approximately 9.2 M^3, allowing animal loading of less than 2% by volume when the animals reach maturity. Flow rate through the chamber will be at least 1.53 M^3/min. providing a theoretical air change every six minutes. The chamber supply air will be filtered and maintained at 40% to 60% relative humidity and 70° to 75°F. Mice are to be individually caged; rats will be caged in groups. Food and water will be provided *ad libitum*.

All rats and hamsters will be started simultaneously. After 6 months of exposure groups TE-I-6, TE-II-6 and TE-III-6 will be removed from exposure and kept for 18 months observation. The exposures of the mice will then commence. Therefore, all terminal autopsies will take place simultaneously 24 months after the start of the experiment.

III. Animal Parameters

A. Clinical Observations:

All animals will be observed daily for lesions and behavioral changes attributable to the test material. The time of appearance and location of all tumors that occur among both control and test animals will be recorded. Mortality records will be kept on all groups of animals. All animals which die during the study will be necropsied and their tissues processed in accordance with the methods given in the Anatomic Pathology Section. Care will be exercised to minimize loss of tissues through cannibalism or autolysis. Animals in a moribund state will be sacrificed *in extremis* when death is imminent.

B. Body Weights

Individual body weights will be recorded once before exposure and after 1, 2, 3, and 4 weeks of testing. Thereafter, mean group body weights will be determined monthly up to the 12-month point of the study.

C. Clinical Pathology

Hemoglobin, hematocrit, total erythrocyte and total leukocyte counts will be performed at 18 and 24 months on 30 (15 male and 15 female) rats of the control and each test group. Differential leukocyte counts will be performed on all animals having high total leukocyte counts.

D. Anatomic Pathology

1. Methods of Sacrifice

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Esophagus
Eye
Gonads (testes and ovaries)
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Large Intestine (caecum and colon)
Liver
Lungs
Lymph Nodes (tracheobronchial, cervical and mesenteric)
Optic Nerve
Pancreas
Parathyroid Gland
Pituitary Gland
Prostate
Salivary Gland
Seminal Vesicles
Skin
Small Intestine (duodenum, jejunum and ileum)
Spleen
Stomach
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Thyroid Gland
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Urinary Bladder
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