

OCT 29 1974



THE DOW CHEMICAL COMPANY

BENNETT BUILDING
2030 DOW CENTER
MIDLAND, MICHIGAN 48640

October 25, 1974

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,

Theodore R. Torkelson

Theodore R. Torkelson
Corporate Medical Department

kjf

encl.

CMA 102172

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. | <u>Type and Length:</u> | 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. | <u>Number of Animals:</u> | 800 Mice.
900 Rats.
800 Hamsters. |
| C. | <u>Exposure Schedule:</u> | seven hours per day.
Five days per week. |
| D. | <u>Test Materials:</u> | Vinyl Chloride (Ethylene Dichloride). |
| E. | <u>Organization:</u> | See Table I. |
| F. | <u>Dose Levels:</u> | See Table I. |
| G. | <u>Exposures Started:</u> | Sept. 1973; mice, rats & hamsters. |
| | <u>Exposures Terminated:</u> | June 1974; mice.
Sept. 1974; rats & hamsters. |
| | <u>Terminal Autopsy:</u> | 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.

TABLE I
CHRONIC VAPOR INHALATION TOXICITY STUDY
Organization of Groups

<u>Test Material</u>	<u>Group</u>	<u>Number of Animals</u>					
		<u>Mice</u>		<u>Rats</u>		<u>Hamsters</u>	
		<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
None Vinyl Chloride (Ethylene derived)	Control	100	100	100*	100*	100	100
	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 cytogenetic 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 $1.53 \text{ M}^3/\text{min}$. 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
 Tracheo
 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: 500 Mice
500 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:

<u>Group</u>	<u>Exposures Start</u>	<u>Exposures Stop</u>	<u>Terminal Autopsy</u>
<u>Mice</u> : Control, I, II, III	Dec. 1974	Sept. 1975	June 197
<u>Rats</u> : Control, I, II, III	Dec. 1974	Dec. 1975	Dec. 197

TABLE I
CHRONIC VAPOR INHALATION TOXICITY STUDY VINYL CHLORIDE
Organization of Groups

<u>Group</u>		Number of Animals			
		<u>Mice</u>		<u>Rats</u>	
		<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
	Control	125	125	125	125
I	Low Level - 1.5 ppm	125	125	125	125
II	Intermediate Level - 5 ppm	125	125	125	125
III	High Level - 15 ppm	125	125	125	125
	Total	500	500	500	500

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 $1.53 \text{ M}^3/\text{min.}$ 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.

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 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:

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

TABLE I
CHRONIC VAPOR INHALATION TOXICITY STUDY VINYL CHLORIDE

Proposal B
Organization of Groups

Group		Number of Animals					
		Rats		Hamsters		Mice	
		Males	Females	Males	Females	Males	Females
	Control	100	100	100	100	100	100
I	Low Level - 1.5 ppm	100	100	100	100	100	100
II	Intermediate Level - 5 ppm	100	100	100	100	100	100
III	High Level - 15 ppm	100	100	100	100	100	100
I-6	Low Level - 1.5 ppm	100	100				
II-6	Intermediate Level - 5 ppm	100	100				
III-6	High Level - 15 ppm	100	100				
TOTAL		700	700	400	400	400	400

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 $1.53 \text{ M}^3/\text{min}$. 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

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Esophagus
Eye
Gonads (testes and ovaries)
Kidneys
Large Intestine (caecum and colon)
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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.

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Five days per week.
- D. Test Materials: Vinyl Chloride
- E. Organization: See Table I
- F. Dose Levels: See Table I
- G. Proposed Schedule:

<u>Group</u>	<u>Exposures Start</u>	<u>Exposures Stop</u>	<u>Terminal Autopsy</u>
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		<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
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Total		500	500	500	500

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Gonads (testes and ovaries)
Kidneys
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Seminal Vesicles
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Trachea
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October 23, 1974

PROPOSED PROTOCOL B

MANUFACTURING CHEMISTS' ASSOCIATION, INC.

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TABLE I
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Proposal B
Organization of Groups

Group		Number of Animals					
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		Males	Females	Males	Females	Males	Females
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II	Intermediate Level - 5 ppm	100	100	100	100	100	100
III	High Level - 15 ppm	100	100	100	100	100	100
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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.

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Bone Marrow (sternal)
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Both Eyes (external auditory canal with ceruminal (Zymbal's) glands)
Esophagus
Eye
Gonads (testes and ovaries)
Kidneys
Large Intestine (caecum and colon)
Liver
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