PROTOCOL

MANUFACTURING CHEMISTS' ASSOCIATION, INC.

CHRONIC VAPOR INHALATION TOXICITY STUDY WITH VINYL CHLORIDE

I. Outline of Investigation

A.	Type and Length*:	12-month vapor inhalation in White Mice 12-month vapor inhalation in Albino Rats 12-month vapor inhalation in Hamsters
B.	Number of Animals:	800 Mice 800 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

*After exposure, animals will be maintained for observation and sacrifice at end of two-year period (18 months for mice).

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 - 500 ppm	100	100	100	100	100	100
	TE-III	High level - 5000 ppm	100	100	100	100	100	100

II. Chamber Parameters

Each group of animals will be exposed in a specially constructed plexiglas inhalation chamber having a capacity of approximately 6.0 M^3, allowing animal loading of less than 2% when the animals reach maturity. Flow rate through the chamber will be at least 0.60 M^3/min. providing a theoretical air change every ten minutes. The chamber supply air will be filtered and maintained at 40% to 60% relative humidity and 70° to 75°F.

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 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 (4-6μ), 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 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.

February 1, 1973