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W. A. K.
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November 21, 1974

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To: Technical Task Group on Vinyl Chloride
Subject: Test Protocols for Consumer Product Safety
Commission Studies on Vinyl Chloride

Gentlemen:

Distributed herewith are copies of the subject test protocols, and Dr. Hehir's letter of November 15, 1974, soliciting review and comments. Please note the December 15 deadline for their receipt. You are free to make your comments directly to Dr. Hehir, or if you wish, MCA can forward them without identification of the sources.

Sincerely,

Kenneth D. Johnson, Ph.D.
Technical Project Manager
Vinyl Chloride Research

KDJ/mb

Enclosures

cc: Mr. A. W. Barnes
D. P. Duffield, M.D.
Dr. Tiziano Garlanda

CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, D.C. 20207

November 15, 1974

Dr. Kenneth D. Johnson
Manufacturing Chemist's Association
1825 Connecticut Avenue N.W.
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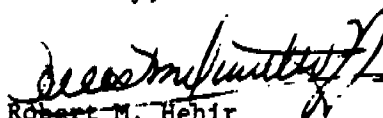
Dear Dr. Johnson:

The Consumer Product Safety Commission is about to initiate its studies on vinyl chloride. The testing protocols (copy attached) were developed by the Bureau of Biomedical Science, CPSC, and the technical staff of Edgewood Arsenal. I am submitting them for your information, review, and comment.

If you have any comments or questions about the protocols we would like to receive them in writing before December 15, 1974.

Thank you for your cooperation in this matter.

Sincerely,


Robert M. Hehir
Director
Bureau of Biomedical Science
5401 Westbard Avenue
Bethesda, Maryland 20207

Enclosure

TEST PROTOCOL FOR TOXICOLOGICAL
TESTING OF VINYL CHLORIDE MONOMER

CONSUMER PRODUCT SAFETY COMMISSION

1 NOVEMBER 1974

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STUDY I. LIFETIME CANCER STUDY IN RATS AND MICE

A. Protocol

Respective groups of 90 male and 90 female rats and mice will be exposed once to concentration levels of 50, 500, 5000, and 50,000 ppm of vinyl chloride for one hour. Twenty males and 20 females of each species will be sacrificed before exposure to establish blood chemistry and pathology base lines and 90 males and 90 females of each species will serve as negative experimental controls. No positive controls will be carried. The exposure chamber used for testing will be precalibrated for each exposure level.^{1/} Airflow will be regulated, producing stable, dynamic exposure conditions. Following calibration of the chamber, animals will be exposed to the required concentration. Continuous sampling will be performed during each exposure to determine the concentration of airborne VC.

The exposure and sacrifice schedules are as follows:

SACRIFICE SCHEDULES

Time of Sacrifice (Months)	Exposure Concentration (ppm)									
	Control		50		500		5000		50,000	
	M	F	M	F	M	F	M	F	M	F
0	20	20								
8	20	20	20	20	20	20	20	20	20	20
16	20	20	20	20	20	20	20	20	20	20
24	50	50	50	50	50	50	50	50	50	50
TOTALS	110	110	90	90	90	90	90	90	90	90

A complete gross pathological examination will be performed on each negative control and exposed animal. Light microscopic examination will be made of the following tissues: Lung and trachea, heart, liver, stomach, small intestine, large intestine, spleen, kidneys and bladder, bone marrow (sternum), adrenals, pancreas and duodenum, brain (2 sections), pituitary, spinal cord (cervical), skin, thyroid, uterus-ovaries, eye, muscle, and bone femur.

Particular emphasis will be placed on examination of brain, lung and liver tissues and the Zymbal gland in the rodent ear.

1/ NOTE: Appropriate methods of dispersion will be used. NCI and OSHA regulations will be observed throughout.¹⁰⁴

It is recognized that even though a chemical alters the structure of chromosomes, it in itself may not constitute a mutagenic effect but a cell killing effect. Therefore, following a conversation with Dr. Bruno Papirmeister of Edgewood Arsenal (he is also a member of the Environmental Mutagen Panel) we decided to investigate the possibility of performing tests specifically designed to detect effects on VC on genes. The two tests that Dr. Papirmeister mentioned were: 1) the use of the Ames strains of bacteria to shed light on either an alteration of base pairing on DNA or an effect on the repair systems of DNA by VC, and 2) the use of a mouse lymphoma cell line to determine the effect of VC on a mammalian cell system. The specifics of these tests would be elaborated upon further by Dr. Papirmeister pending acceptance and funding.

Daily observations will be made on all animals for toxic signs and weekly body weights will be made.

STUDY II. REPRODUCTION STUDIES

A. Teratology Screening Program in Rats

1. The following experimental procedure is proposed as a model protocol for testing compounds for possible teratogenic effects.

2. In-Life Methods:

a. Cohabitation: Females selected for mating will be placed with male rats on day 0 in a 2:1 ratio. They will remain with the male for four days (length of rat estrus cycle) at which time the males will be removed.

b. Assignment to Group: Each cage of females will be divided randomly into six groups explained in the following experimental design.

EXPERIMENTAL DESIGN

Group	Compound	Dose	Number of Mated Females	Remarks
I		Control	20	{ Fetuses to be examined for visceral and skeletal malformations at Day 20.
II		Low	20	
III		High	20	
IV		Control	10	{ Females allowed to whelp and fetuses examined grossly, sexed, weighed and crown-rump lengths measured.
V		Low	10	
VI		High	10	

5. Post-natal Evaluation (Offspring of Groups IV, V, VI) During Lactation Litter Observations:

Pups observed: Daily record presence of dead pups.

Pups counted: Days 0-4 (daily), 14 and 21.

Pups sexed and weighed: Males and females for each litter on days 4, 7, 14, and 21.

Dead pups: Examine externally, necropsy to maximum extent possible, check for presence of milk and weigh only if found dead on days 4, 7, 14, and 21.

Death of entire litter prior to day 21: Sacrifice dam, save mammary tissue in buffered neutral formalin. Subsequent histological processing and microscopic examination of mammary tissue will be performed only with the understanding that lactation can be classified as present or absent, but not evaluated quantitatively or as to sufficiency to support pups.

Dead or Moribund Dams: Post mortum examinations of dams. Sacrifice surviving pups, and use same procedure as for dead pups.

6. Post-Weaning Evaluation (Offspring of Groups IV, V, VI)

Wean date: Day 21 of lactation.

Post-weaning observation: 10 males and 10 females F_1 offspring randomly selected from each group will be held until two years of age for carcinogenic evaluation.

STUDY III. MULTI-GENERATION REPRODUCTION (See attached Bibliography, Appendix II)

The three generation reproduction study was described in the "Procedures for Appraisal of the Toxicity of Chemicals in Foods" by the Staff of the Division of Pharmacology, Food and Drug Administration in 1949.^{1,2,3} The same agency further described this test in 1955 and 1959.^{4,5} The report of the Science Advisory Committee to the President of the United States 1963⁶, recommended that a multi-generation test be used in toxicity studies on pesticides. The multi-generation test was described by Fitzhugh.¹ The general plan for the multi-generation test is as follows:

TEST PROTOCOL

EXPERIMENTAL DESIGN

GROUP	COMPOUND	DOSE	NO. OF MALES	NO. OF FEMALES
I		Control	15	15
II		Low	15	15
III		High	15	15

a - Extra males and females are started on treatment to give greater assurance that 10 and 10 respectively will survive until the mating period.

5. Weaning

All pups will be weaned on Day 21 of lactation. Forty-five males and 45 females will be selected randomly to be used as parents of the F₂ generation. They will be mated at 12 weeks of age. The same procedure as outlined above in 3, 4, and 5 will be followed through the F₃ generation.

6. Autopsy Procedures

Ten males and ten females from each dose group and control group will be sacrificed at day 21 of gestation. This procedure will be followed with each of the three generations. A gross necropsy will be performed to evaluate any anomalies or gross pathological changes present in the offspring.

7. Carcinogenic Evaluation of F₀ Breeders

The F₀ breeders will be held until they are 24 months of age for observation of the development of tumors. They will be autopsied at 24 months of age. A complete autopsy, both gross and microscopic, will be performed at this time.

APPENDIX I
MUTAGENICITY TESTING OF VINYL CHLORIDE

Detection of DNA-damaging and Mutagenic Chemical Compounds In Vitro.

A. Repair Test:

This test utilizes six strains of E. coli which differ significantly in their ability to repair damage to their DNA. The test is performed as follows:

Small, known amounts of the test compound (in solution or in neat form) are added to small filter disks which are then placed in the center of petri dishes which have been seeded with the indicator bacteria. The six test strains utilized include the three repair-capable strains of E. coli (polA+, hcr+, and rec+) and three incapable strains are compared. A test compound is deemed to have DNA-damaging properties if the ratio of the diameter of growth inhibition for repair-incapable strains/diameter of growth inhibition for repair-capable strains is significantly greater than one.

B. Mutation Test:

The mutagenic activity of a chemical will be examined in Salmonella typhimurium according to procedures described by Bruce Ames coworkers^{1,2}. A set of tester strains harboring specific alterations in the histidine locus is utilized and the reverse mutation frequency to histidine independence is determined. The sensitivity of the assay is greatly enhanced by introduction of the mutations into these tester strains - elimination of excision repair capability and

frequency at the asparagine locus was found to be 1-5 mutated cells per million, a value similar to other known mutation frequencies. Mice bearing the lymphoblast ascites cells are treated 4 days after inoculation by an alternate route with the chemical in question and after 3 additional days of growth the cells are harvested and subcultured in vitro in the presence and absence of asparagine. The mutation frequency is the average of three values for each dose and is expressed as the number of colonies formed in the absence of asparagine per million viable cells, the indication of mutagenic potency being provided by a dose-response curve. Comparison of the mutation frequencies induced in vivo and in vitro exposures provides a measure of the effects of host metabolism. Thus, it is not necessary to identify and study separately all of the possible metabolites of a chemical in order to obtain evidence of mutagenic activity in a compound. In addition changes in activity attributable to different routes and/or schedules of administration are readily detected by the test.

Cytogenetic Effects in Somatic Cells

The host-mediated assay employing mammalian cells has the advantage over the corresponding bacterial assay in that it permits simultaneous analysis of induced chromosomal aberrations. In a typical protocol both the L5178Y and bone marrow cells harvested from animals 24 hours after exposure. Cells of each kind are incubated in appropriate media for 4 hours and slides are prepared from the resulting culture. For each condition, at least 200 metaphase cells are analyzed for chromosomal

BIBLIOGRAPHY

1. Ames, B. N., Lee, F. D., and Durston, W. E. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. USA 70:782, 1973.
2. Ames, B. N., Durston, W. E., Yamasaki, E., and Lee, F. D. Carcinogens are Mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Ntl. Acad. Sci. USA 70:2281, 1973.
3. Capizzi, P. L., Summers, W. P., and Papirmeister, B. Use of the Asparagine Auxotroph of the L5178Y Murine Leukemia for the Detection of Chemical Mutagens In Vitro and in the Host-Mediated Assay. Environm. Health Perspectives, Experim. Issue 6:137, Dec 1973.
4. Capizzi, R. L., Papirmeister, B., Mullins, J. M., and Chang, E. The Detection of Chemical Mutagens Using the L5178Y/ASN Murine Leukemia In Vitro and in a Host-Mediated Assay. Submitted to Cancer Research, May 1974.

VINYL CHLORIDE TEST SCHEDULE - FY 75 - FY 78

Test Phase	FY 75 (QTR)				FY 76 (QTR)				FY 77 (QTR)				FY 78 (QTR)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
I. Lifetime cancer study in rats and mice																
Biochemical determinations																
Metabolic fate of vinyl chloride																
Detection of DNA damaging & mutagenic chemical compounds <u>in vitro</u>																
Host mediated assay																
Cytogenetic effects																
II. Teratology																
III. Multigeneration																

Note: 1. Accomplished by Edgewood Arsenal or Consumer Product Safety Commission
2. With cancer evaluation
3. Two month exposure