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VINYL CHLORIDE COMBINED INHALATION TWO-GENERATION REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDY IN CD RATS

STUDY NO.: 96-4080

ISSUE NO.: 3

SUBMITTED TO: Chemical Manufacturers Association
Chemstar Department

ATTENTION: Robert Venezia, Ph.D.

DATE: October 3, 1996

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1 INTRODUCTION:

1.1	**STUDY NO.:**	96-4080
1.2	**ISSUE NO.:**	3
1.3	**STUDY TITLE:**	Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats
1.4	**TEST MATERIAL:**	Vinyl Chloride
1.5	**SPONSOR:**	Chemical Manufacturers Association Chemstar Department 1300 Wilson Boulevard Arlington, Virginia 22209
1.6	**SPONSOR REPRESENTATIVE:**	Robert A. Venezia, Ph.D. Phone - 703-741-5639 Fax - 703-741-6091
1.7	**TESTING FACILITY:**	Huntingdon Life Sciences Mettlers Road P.O. Box 2360 East Millstone, NJ 08875-2360

1.8 **PURPOSE:**

The objectives of the combined inhalation two-generation reproduction and developmental toxicity study outlined in this protocol are to evaluate the effects of the test material on parental toxicity, reproductive capability, in utero development, and neonatal growth and survival in rats.

2 STUDY PERSONNEL:

Study Director:	Raymond E. Schroeder, M.S., DABT
Alternate:	Dean E. Rodwell, M.S. Vice-President of Toxicology
Director of Toxicology:	Carol S. Auletta, B.A., DABT
Inhalation Toxicologist:	Gary M. Hoffman, B.A., DABT
Supervisor Rodent Toxicology:	Ellen Whiting, AALAS LAT
Primary Technician:	*
Vice President, Pathology:	Ward R. Richter, D.V.M., M.S., ACVP
Study Pathologist:	*
Director, Quality Assurance & Regulatory Affairs:	Michael Caulfield

Additional personnel will be documented in the project file and presented in the final report.

3 REGULATORY REFERENCES:

3.1 TEST GUIDELINE:

This study is designed to meet or exceed the guideline requirements of the following:

- EPA (Environmental Protection Agency: TSCA Test Guidelines (EPA, 1985);
- Organization for Economic Co-operation and Development (OECD, Guidelines for testing of chemicals, Section 4: Health Effects, (OECD, 1981); and
- European Economic Community (EEC), Methods for the Determination of Toxicity (EEC, 1988).

*to be determined

3.2 GOOD LABORATORY PRACTICES:

This study will be conducted in compliance with the following:

- FDA (Food and Drug Administration) Good Laboratory Practice (GLP) Regulations for Nonclinical Studies (FDA, 1988 - Part 58 of 21 CFR);
- EPA TSCA Good Laboratory Practice Standards (EPA,1990 - Part 160 of 40 CFR):
- OECD Good Laboratory Practice Procedures (OECD,1982 - Annex 2 C [81] 30 [FINAL]);
- Standard operating procedures of the Testing Facility

3.3 FACILITIES MANAGEMENT/ANIMAL HUSBANDRY:

Currently acceptable practices of good animal husbandry will be followed, e.g., Guide for the Care and Use of Laboratory Animals; DHHS Publication No. (NIH) 86-23, Revised 1985. Huntingdon Life Sciences (East Millstone, NJ) is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

3.4 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all appropriate parts of the Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991. The Sponsor should make particular note of the following:

1. The Sponsor's signature on this protocol documents for the study described, there are no generally accepted non-animal alternatives and the study does not unnecessarily duplicate previous experiments.
2. All procedures used in this study have been designed to avoid discomfort, distress and pain to the animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
3. Any aspects of this study which cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics or anesthetics unless the withholding of these agents is justified for scientific reasons, in writing by the Sponsor and the Study Director, in which case the procedure will continue for the minimum time necessary.

3.4 ANIMAL WELFARE ACT COMPLIANCE:

4. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanatized as deemed appropriate by the Testing Facility's veterinary staff and the Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

5. Methods of euthanasia used during this study are in conformance with the above referenced regulations.

4 QUALITY ASSURANCE MONITORING:

The Huntingdon Life Sciences Quality Assurance Unit (East Millstone, NJ) will monitor the facilities, equipment, personnel, methods, practices, records and controls used in this study to assure that they are in conformance with this protocol, company standard operating procedures, and the appropriate Good Laboratory Practice regulations.

5 ALTERATION OF DESIGN:

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such changes will be honored by the Testing Facility and will be followed by a written verification. All protocol modifications will be signed by the Study Director and a Sponsor representative. Any modifications potentially affecting animal welfare will also be signed by two members of the Institutional Animal Care and Use Committee prior to the modification's implementation.

6 PROPOSED STUDY DATES:

Two-generation Reproduction Study:

Initiation date:	Date Study Director Signs protocol - See Section 18.2.
Receipt of test animals:	*(to be addressed by amendment)
Initiation of exposures (P_1): (Experimental start date)	*
Termination of exposures (P_1):	*
Selection of the P_2 parental animals:	*
Termination of exposures (F_2):	*
Necropsy	
P_1:	*
P_2:	*
Experimental Termination (Date of last data collection):	*
Submission of draft final report:	*

Developmental Toxicity Study:

Receipt of test animals:	*
Initiation of mating:	*
Initiation of exposures (first Day 6 gestation):	*
Termination of exposures (last Day 19 gestation):	*
Experimental termination: (date of last data collection)	*
Submission of draft final report:	*
Study completion date:	Date final report is signed by Study Director.

7 EXPERIMENTAL DESIGN:

7.1 Two-generation Reproduction Study:

Group	Exposure Level (ppm)[a]	Number of Animals							
		Mated Adults				Microscopic Pathology Adult Generations[b]			
		P_1		P_2		P_1		P_2	
		M	F	M	F	M	F	M	F
I (control)	0[c]	30	30	30	30	30	30	30	30
II	10	30	30	30	30	A.R.	A.R.	A.R.	A.R.
III	100	30	30	30	30	A.R.	A.R.	A.R.	A.R.
IV	1100	30	30	30	30	30	30	30	30

[a] Whole body exposure 5 days/week, 6 hrs/day during the premating periods (P_1, F_1) and 7 days/week, 6 hrs/day for all other periods. Animals will be exposed in a glass and stainless steel inhalation chamber.
[b] Histologic examinations will be performed for tissues listed in Appendix A.
[c] Control animals will be chamber-housed and sham-treated with clean room air for a comparable period of time as the test animals.

Key: A.R. = As Required: 1) tissues that demonstrate treatment-related histologic changes in Group IV (additional cost); 2) gross lesions from animals found dead or euthanatized in a moribund condition during the study (additional cost); and 3) gross lesions terminal animals (additional cost);

M = Male; F = Female.

7.2 Developmental Toxicity Study:

The developmental toxicity study will be performed with a unique population of animals during the exposure period for the P_1 parental animals in the two-generation reproduction study. This will be during the mating, gestation and lactation periods for the F_1 pregnancies when animals are being exposed 7 days/week, 6 hrs/day. Animals in the developmental toxicity study will be exposed in the chamber along with the animals in the two-generation reproduction study.

Group	Exposure Levels (ppm)	Treatment Schedule 6 hrs/day	Number of Animals				
			Mated	Sacrificed[a] Gestation Day 20	Proportion of Gestation Day 20 Fetuses/Litter Malformation/Variation Evaluations		
					External	Soft Tissue	Skeletal
I (chamber - housed, sham air control)	0	Gestation Days 6-19	25	A.S.	All	½	½
II	10	Gestation Days 6-19	25	A.S.	All	½	½
III	100	Gestation Days 6-19	25	A.S.	All	½	½
IV	1100	Gestation Days 6-19	25	A.S.	All	½	½

[a] Gross postmortem examination, liver and kidneys will be weighed and preserved along with gross lesions. Microscopic examinations of these tissues will not be conducted unless deemed necessary to interpret other observations made during the study or requested by the Sponsor (additional cost).
A.S. = All Survivors

8 TEST MATERIAL:

8.1 TEST MATERIAL: Vinyl Chloride

Description, lot number, storage, expiration date and handling procedures, as well as other pertinent information will be documented in the study data. Properties of the test material are presented in the Appendix B.

8.2 IDENTIFICATION OF TEST MATERIAL:

Unless otherwise noted, the identity, strength, purity, composition, stability, and method of synthesis, fabrication and/or derivation of each batch of the test material will be documented by the Sponsor before its use in the study.

8.3 ANALYSIS OF THE TEST MATERIAL:

The purity of the lot of test material used on study will be determined at the start of study, approximately 6-months into the study and at termination. These analyses will be performed by the analytical laboratory of the Testing Facility.

8.4 ARCHIVAL SAMPLES:

A small sample from the lot of test material used on study will be taken and stored in the Archives of the Testing Facility at East Millstone, NJ.

8.5 UNUSED TEST MATERIAL:

At completion of the study unused test material in the one ton pressurized cylinder will be returned to the supplier (The GEON Company, Pedricktown, NJ 08067).

9 TEST ANIMALS: Albino Rats (Outbred) VAF/Plus®

9.1 STRAIN:

Sprague Dawley - derived (CD®)
[Crl: CD® BR]

9.2 SUPPLIER:

Charles River Laboratories
Portage, Michigan

9.3 JUSTIFICATION FOR TEST SYSTEM SELECTION:

The rat is a rodent animal model commonly utilized in reproduction and developmental toxicity studies as recommended in the referenced guidelines. In addition, a historical control data base with this strain of animal and supplier facility is available for comparative evaluation.

9.4 ANIMAL REQUIREMENTS/SPECIFICATIONS:

9.4.1 Number:

9.4.1.1 Two-generation Reproduction Study (P_1):

	Total	Males	Females
Placed on test	240	120	120

9.4.1.2 Developmental Toxicity Study:

Placed on test - 100 mated females (25/group).

9.4.2 Age:

9.4.2.1 Two-generation Reproduction Study (P_1):

Males and females: approximately four weeks at receipt; approximately six weeks (males will be 160 - 210 grams and females will be 125 - 175 grams at initiation of treatment). Animals outside this weight range will be used at the discretion of the Study Director.

9.4.2.2 Developmental Toxicity Study:

Females: eight weeks at receipt and at least 10 weeks (200-275 grams) at initiation of mating. Animals outside this weight range at mating will be used at the discretion of the Study Director. Females will be nulliparous and non-pregnant.

Males: in-house breeding colony used only for mating.

9.5 ACCLIMATION PERIOD:

Approximately two weeks; all animals will be checked for viability twice daily. Prior to assignment to study all animals will be examined to ascertain suitability for study.

9.6 ANIMAL HUSBANDRY:

9.6.1 Housing:

9.6.1.1 Two-generation Reproduction Study (P_1):

Animals will be housed in suspended, stainless steel cages with wire mesh fronts and floors. For the first week of acclimation, animals will be housed two/sex/cage. Thereafter, animals will be housed individually except as follows:

Mating: one male and one female co-housed nightly.
Lactation: dam with litter.
Postweaning: two littermates/sex until selection for P_2 parental generation.

During exposures, parental animals will be housed individually in suspended stainless steel wire mesh cages. Neonates will be housed 1-2/cage (littermates) until the formal initiation of the premating treatment period.

9.6.1.2 Developmental Toxicity Study:

Animals will be housed in suspended, stainless steel cages with wire mesh fronts and floors except during mating when females will be co-housed overnight with a male. During exposures, animals will be housed in suspended stainless steel wire mesh cages.

9.6.2 Food:

Certified Rodent Diet, No. 5002; (Meal) (PMI Feeds, Inc., St. Louis, MO). Each animal's cage will be fitted to retain a glass feeder cup with a stainless steel lid. Feed will be available *ad libitum* during non-exposure periods except on the evening prior to necropsy when parental animals (P_1 and P_2) will be fasted. During exposures, animals will not have access to feed.

9.6.3 Water:

Facility water supply (Elizabethtown Water Company, Westfield, NJ); without restriction during exposures and non-exposure periods, via an automated water delivery system to individual animal cages.

9.6.4 Bedding Material - Two-generation Reproduction Study:

Hardwood shaving bedding (Lab Aspen Shavings, North Eastern Products Corporation, Warrensburg, NY) will be provided for each mated female on Day 20 of gestation. Fresh bedding will be provided as needed to Day 14 of lactation.

9.6.5 Feed Analysis:

Analytical certification of batches of feed used during the study which are provided by the manufacturer, will be maintained on file at the Testing Facility. There are no known contaminants in the feed which are expected to interfere with the results of this study.

9.6.6 Water Analysis:

Monthly water analyses, provided by the supplier, will be maintained on file at the Testing Facility. Biannual chemical and microbiological analyses of water samples collected from representative rooms in this facility will be conducted to assure that water being provided meets standards specified under the EPA National Primary Drinking Water Regulations (40 CFR Part 141). Results will be maintained on file. There are no known contaminants in the water which are expected to interfere with the results of this study.

9.6.7 Bedding Analyses:

Analyses for each batch of bedding used on study provided by the supplier, will be maintained at the Testing Facility. There are no known contaminants in the bedding which are expected to interfere with the results of this study.

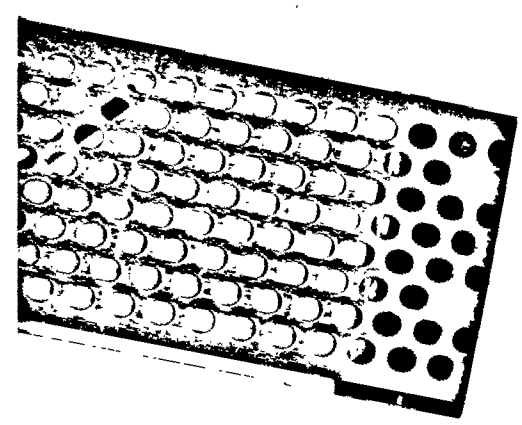
9.6.8 Veterinary Care:

Animals will be monitored by the technical staff for any conditions requiring possible veterinary care. If any such conditions are identified, a staff veterinarian will be notified for an examination and evaluation. Any medical veterinary intervention will be made only with approval of the staff veterinarian and the Study Director. The Sponsor will be consulted whenever possible. However, in emergency situations, decisions will be made as needed and the Sponsor will be advised as soon as possible.

ns:

.rk cycle daily.

orded twice daily. Temperature in the animal room
.mbers will be maintained in the range of approxi-
to the maximum extent possible.



animal quarters will be monitored and recorded once
ired humidity range in the animal room is 40-70% and
exposure chambers during exposures. Humidity will be
this range to the maximum extent possible.

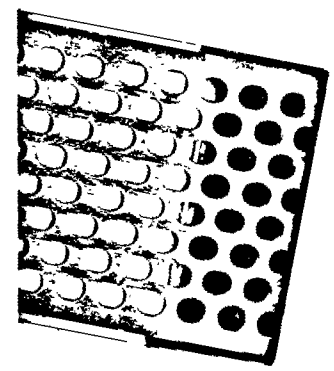
TUDY:

tion Reproduction Study (P_1) :

als than required for the study will be purchased and accli-
imals considered suitable for study on the basis of pretest
xaminations, body weight data and any other pretest evalua-
be randomly assigned to control or treated groups in an
to equalize mean group body weights. Individual weights of
placed on test shall not exceed ± 20% of the mean weight
sex. Disposition of all animals not used in the study will be
ned in the study file.

opmental Toxicity Study:

females than required for the study will be purchased and accli-
d. Animals considered suitable for study on the basis of pretest
sical examinations will included into the mating phase of the study.
ales which mate will be assigned to groups daily in such a way as
most nearly equalize both the Day 0 mean body weights between
ups and the distribution of animal into groups.



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9.8 ANIMAL IDENTIFICATION:

9.8.1 Two-generation Reproduction Study:

Each animal will be assigned a temporary identification number upon receipt. After selection for study (P_1 generation), each animal will be ear-tagged with a number assigned by the Testing Facility. This number plus the study number will comprise the unique identification for each study animal. Following the selection procedure (post-weaning) to identify the P_2 parental animals from the F_1 offspring, these selected animals will be ear-tagged with a unique number. If the tag is lost, it will be replaced or the animal will be tail tattooed for identification. Each animal's cage will be provided with a card which will be color-coded for dose level identification and will contain the study number and animal number.

9.8.2 Developmental Toxicity Study:

Each female will be assigned a temporary identification number upon receipt. Mated females sorted into study groups will be ear-tagged with a number assigned by the Testing Facility that will be unique from the identification numbers used in the two-generation study. This number plus the study number will comprise the unique identification for each study animal. Females will be eartagged on Day 0 of gestation as they are sorted into groups.

10 MATING, GESTATION AND LACTATION PROCEDURES:

10.1 MATING PROCEDURE:

10.1.1 Two-generation Reproduction Study (P_1, P_2):

After animals have been exposed to the test substance for the appropriate length of time (premating treatment period), one male and one female of equivalent dose levels will be caged together nightly (same animals) until a sign of mating (microscopic observation of sperm in the vaginal smear and/or a copulation plug in the vagina) is observed or for 14 consecutive days. The day evidence of mating is observed will be defined as Day 0 of gestation. If mating has not occurred after this interval, the animals will be separated without further opportunity for mating. During mating of the F_1 generation cohabitation of male and female littermates will be avoided.

10.1.2 Developmental Toxicity Study:

Females selected for mating will be placed with breeder males nightly in a 1:1 ratio. Vaginal smears will be taken early in the morning following nightly interval of co-housing and females will be considered to have mated if sperm is observed microscopically in the vaginal smear and/or a vaginal plug is observed. The day on which evidence of mating is observed will be defined as Day 0 of gestation.

10.2 PARTURITION AND LACTATION - TWO-GENERATION REPRODUCTION STUDY (P_1 and P_2):

On Day 20 of gestation, several days prior to expected parturition, each mated female's cage will be fitted to retain a stainless steel floor pan and bedding material provided. Examination for signs of parturition will be made twice daily (morning and afternoon). Evidence of difficult or prolonged parturition, if observed, will be recorded. The day on which all pups have been delivered will be defined as Day 0 of lactation. For females which were caged with males but exhibited no evidence of mating, preparations for undetermined pregnancies (i.e., floor pan, bedding material) will be made when the first animals mated reach their day 20 of gestation.

10.3 SELECTION OF P_2 PARENTAL GENERATION:

At weaning of each F_1 litter, two pups/sex/litter will be chosen at random to become a pool of animals from which the P_2 parental generation will be selected. Pups as chosen will be housed two littermates of the same sex/cage. After weaning of the last F_1 litter, 30 F_1 pups/sex/group will be selected from this pool of animals to become the P_2 parental animals. In this selection procedure, each litter will contribute at least one pup/sex, when possible, so as to maximize the representation of animals from different litters in each group.

After the last litter is weaned, the 30 F_1 pups/sex/group designated for the P_2 parental generation will formally initiate the premating treatment period. Thus, there may be a maximum of two weeks difference in age for the P_2 animals within each treatment group at initiation of the premating growth period. During this postweaning period, the pool of F_1 pups will be chambered and exposed (6 hrs/day) at the treatment level of the dam.

In the selection procedure, grossly malformed or obviously-diseased animals within the litters will be excluded from the pool of animals eligible for selection if, in the judgement of the Study Director, their condition may adversely affect long-term survival. Runts, if otherwise normal, will not be excluded from the selection procedure.

11 TEST MATERIAL ADMINISTRATION:

11.1 ROUTE OF ADMINISTRATION:

Inhalation (whole body).

11.2 JUSTIFICATION FOR ROUTE OF ADMINISTRATION:

The inhalation route is one of the potential routes of human exposure to this test material.

11.3 FREQUENCY OF ADMINISTRATION:

11.3.1 Two-generation Reproduction Study:

P_1 and P_2 generation animals will be exposed 6 hrs/day, five days/week for at least 10 weeks prior to mating (designated the premating treatment period). Males will continue to be exposed daily, 6 hrs/day during the 14-day mating period and postmating period until sacrificed. Females will continue to be treated daily, 6 hrs/day during mating and mated females will continue to be treated, 6 hrs/day during the ensuing gestation period through to Day 20. Treatment of females will discontinue at this point to allowed them to deliver (Day 0 of lactation) and treatment will resume on Day 4 of lactation. Females will continue to be treated (6 hrs/day) thereafter for the remainder of lactation (litters weaned on lactation Day 25) and postweaning period until sacrificed. Unmated females will continue to be treated daily (6 hrs/day) until sacrificed.

11.3.2 Developmental Toxicity Study:

Females will be treated 6 hrs/day over the Day 6-19 gestation interval. The exposure interval for these animals will coincide with the exposures for the mating/gestation/lactation periods for the F_1 litters in the two-generation reproduction study.

11.4 INHALATION EXPOSURE GENERATION PROCEDURE:

The test material will be administered as a gas in the breathing air of the animals. The test atmospheres will be generated by an appropriate procedure to be developed at the Testing Facility. The generation method will be described in the final report and maintained with the data package for this study.

The whole-body exposure chambers will have a volume of approximately 6000 liters (6 m^3). Each chamber will be operated at a minimum flow rate

ATION EXPOSURE GENERATION PROCEDURE:

0 liters per minute. The final airflow will be set to provide at least one ange in 5.0 minutes (12 air changes/hour) and a T_{99} equilibrium time proximately 23 minutes. This chamber size and airflow rate are dered adequate to maintain the oxygen level above 19% and the animal ng factor below 5%.

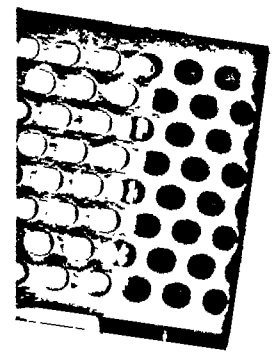
MBER MONITORING:

ominal exposure concentration will be calculated, if possible. The flow air through the chamber will be monitored using appropriate calibrated uipment. The test material consumed during the exposure will be divided the total volume of air passing through the chamber (volumetric flow rate nes total exposure time) to give the nominal concentration.

uring each test material exposure, measurements of airborne concentrations vill be made at least hourly using a MIRAN infrared spectrophotometer. Also prior to initiating of animal exposures, additional samples will be taken to determine the distribution of the test material in the exposure chamber.

During each week of exposure, particle size determinations will be performed using an appropriate instrument to characterize the aerodynamic particle size distribution of any aerosol present.

If more than the normal amount of trialing is required because of test material generation or monitoring problems (two weeks or 150 technician hours), the Sponsor will be consulted prior to additional trialing (additional cost).



11.5 CHAMBER MONITORING:

The minimum frequency of chamber activity is summarized below:

Activity	Frequency/chamber/day
Analytical Test Material Concentration	6 times (hourly)
Temperature	7 times
Relative Humidity	7 times
Airflow Rate	7 times
Nominal Test Material Concentration (excluding the control chamber)	once
Rotation Pattern of Exposure Cages	once
Loading/Unloading Verification	once

11.6 EXPOSURE CONCENTRATIONS:

For both the two-generation reproduction and developmental toxicity studies the targeted exposure concentrations of vinyl chloride will be 0, 10, 100 and 1100 ppm. These exposure levels correspond to oral equivalent doses of approximately 0, 9.2, 92 and 1012 mg/kg/day assuming ventilation rates of 1 L/min/kg, 100 percent absorption and a 6 hr/day exposure. The high concentration of vinyl chloride was selected based on the oral equivalent of the limit dose of 1000 mg/kg body weight/day. The high exposure level of 1100 ppm is also expected to produce effects on the liver and other organ systems (ATSDR, 1993). The middle and low exposure levels were selected to provide a dose response for the observed effects and a no-observed-effect level, respectively.

12 EXPERIMENTAL EVALUATIONS:

12.1 OBSERVATIONS:

12.1.1 Viability Checks - Two-generation reproduction study and developmental toxicity study:

Observations for mortality, general appearance and signs of severe toxic or pharmacologic effects as well as availability of feed and water will be made at least twice daily (morning and afternoon). Animals in extremely poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia. All animals found dead will be submitted for a gross macroscopic examination. Parental animals found dead after normal working hours will be refrigerated until a necropsy can be performed.

12.1.2 Physical Examinations:

12.1.2.1 Two-Generation Reproduction Study:

Each animal will be removed from its cage and examined at least once pretest (P_1), at the study start (first day of treatment for the P_1 and formal initiation of the premating period for the P_2) and weekly thereafter during the study period (P_1 and P_2). Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, and palpation for tissue masses. These examinations will be performed postexposure when animals are being transferred to their home cages.

12.1.2.2 Developmental Toxicity Study:

Mated animals will be given a detailed physical examination on Days 0 and 6-20 (daily) of gestation. Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, and palpation for tissue masses. During the test period, these examinations will be performed postexposure when animals are being transferred to their home cages.

12.2 BODY WEIGHTS:

12.2.1 Two-generation Reproduction Study:

Males:

P_1: at the time of sorting into groups, on the day treatment initiates, weekly throughout the study and at termination.
P_2: at the formal initiation of the premating treatment period and weekly throughout the study and at termination.

Females:

P_1: at the time of sorting into groups.
P_1 and P_2: on the day treatment initiates (formal initiation of the premating period for the P_2), weekly during the premating growth periods; gestation - Days 0, 7, 14 and 20; and lactation: Days 0, 4, 7, 14, 21 and 25 (P_1 only); and at termination.

12.2.2 Developmental Toxicity Study:

Females will be weighed on Days 0, 6, 9, 12, 15 and 20 of gestation.

12.3 FOOD CONSUMPTION:

12.3.1 Two-generation Reproduction Study:

Males:

P_1: one week pretest.
P_1 and P_2: weekly during the premating treatment period and from termination of mating through to sacrifice (postmating period). Food consumption will not be recorded during the mating periods.

Females:

P_1: one week pretest.
P_1 and P_2: weekly during the premating period; during gestation - Days 0-7, 7-14 and 14-20; during lactation - Days 1, 4, 7, 10 and 14. Food consumption will not be recorded during the mating period or postweaning period prior to termination.

12.3.2 Developmental Toxicity Study:

Food consumption will be recorded for the following intervals during Gestation: Days 0-6, 6-9, 9-12, 12-15 and 15-20.

12.4 ESTROUS CYCLE DETERMINATIONS - TWO-GENERATION REPRODUCTION STUDY:

Estrous cycle length and normality will be evaluated daily by vaginal lavage on the first 15 P_1 and P_2 females per group for the three week period prior to mating and continuing throughout the mating period or until the female is confirmed mated.

12.5 LITTER EVALUATIONS (F_1, F_2) - TWO-GENERATION REPRODUCTION STUDY:

12.5.1 Observations:

Litters will be observed as soon as possible after delivery for the number of live and dead pups and pup abnormalities (Day 0 of lactation). Thereafter, litters will be observed twice daily (morning, afternoon) for the presence of dead pups. These pups and pups euthanized in a moribund condition will be examined to the extent possible for defects and/or cause of death and preserved in 10% neutral buffered formalin. Litter size will be recorded on Days 0, 4, 7, 14, 21 (F_1 and F_2) and 25 (F_1 only) of lactation.

12.5.2 Culling:

On Day 4 of lactation, each litter with more than eight pups will be culled to that number with sex distribution equalized (four/sex) when possible. Pups will be culled using a random number table. Preferential culling of runts will not be performed. Culled pups will be examined grossly for abnormalities and following euthanasia by an overdose of inhaled carbon dioxide (CO_2) will have sex confirmed by internal inspection of the gonads. Only culled pups with abnormalities will be preserved in 10% neutral buffered formalin.

12.5.3 Physical Examinations:

Each pup will be given a gross physical examination on Days 0, 4, 7, 14, 21 and 24 (F_1 pups only).

12.5.4 Pup Body Weights and Sexing Data:

Individual pup weights and pup sexing data will be recorded on Days 0, 4 (presented for both pre- and postcull intervals), 7, 14, 21 and 25 (F_1 pups only) of lactation.

12.5.5 Physical Maturation Landmarks - Selected P_2 Animals:

All F_1 weanlings selected to become the P_2 parental animals (30 sex/group) will be observed daily for vaginal opening beginning on postnatal day 30 or preputial separation beginning on postnatal day 35. If a treatment-related effect is seen in F_1 sex ratios, age of vaginal opening or preputial separation, then ano-genital distance will be measured on postnatal day 4 for all F_2 pups.

13 POSTMORTEM:

13.1 TWO-GENERATION REPRODUCTION STUDY:

13.1.1 GROSS POSTMORTEM EXAMINATION:

13.1.1.1 Parental animals:

Complete macroscopic postmortem examinations will be performed on all adult animals, including animals euthanatized in a moribund condition or found dead. These evaluations will be performed under the direct supervision of a veterinary pathologist. The eyes of the parental animals will be examined *in situ* by gently pressing a moistened glass slide against the cornea and observing the eyes under fluorescent light. During the gross postmortem examination all abnormal observations will be recorded. The necropsy of the parental animals will include examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. Examination of all parental females which were exposed to males, will include a count of uterine implantation scars, if present.

13.1.1.2 Weanling Pups (F_1 and F_2):

At weaning (Day 25 - F_1 and Day 21 - F_2) one pup/sex/litter/group will be selected at random for a complete gross postmortem examination. These examinations will be performed under the supervision of a veterinary pathologist. To control for variation in body weight and organ weight, all pups will be sacrificed at the same age.

13.1.2 TIME OF NECROPSY:

13.1.2.1 Moribund Animals:

Animals showing signs of severe debility, particularly if death appears imminent, will be euthanatized to prevent loss of tissues through autolysis.

13.1.2.2 Terminal Necropsy Males (P_1, P_2):

P_1 and P_2 males will be sacrificed after the last F_1 and F_2 litters, respectively, have been delivered. This will permit some evaluation of fertility (i.e., number of litters delivered) prior to sacrifice. The Sponsor will be notified and authorize the sacrifice of the P_1 and P_2 males.

13.1.2.3 Terminal Necropsy Females (P_1, P_2):

All P_1 and P_2 females, regardless of reproductive status (unmated, mated but not pregnant, or retaining litters to weaning) will be sacrificed as a group after the last litters have weaned for all groups. Parental females (P_1 and P_2) will be necropsied on day 2 of diestrus whenever possible. This will be identified by daily vaginal smearing of the females for the occurrence of at least one estrous cycle after which time females found to be in day one of diestrus will be fasted overnight and necropsied the following day. The presence of diestrus will be confirmed by vaginal smearing the morning of necropsy.

13.1.2.4 Terminal Necropsy Pups (F_1, F_2):

13.1.2.4.1 Dead and Culled Pups:

Pups found dead at birth or during the lactation period will be examined to the extent possible for defects and/or the cause of death and the presence or absence of milk in the stomach. Dead pups will not be eviscerated. Viscera will remain intact and the pup will be preserved in 10% neutral buffered formalin. Cannibalized pups will be examined to the extent possible and discarded. Culled pups will be examined for external irregularities, sexed internally to confirm external sexing and if unremarkable will be discarded. Culled pups with external irregularities will be preserved intact in 10% neutral buffered formalin.

13.1.2.4.2 Unselected F_1 Pups:

Pups selected as a pool of animals from which the P_2 parental animals will be selected but which were not identified to continue on study, will be given an external examination and if unremarkable, sacrificed and discarded. Pups with external findings will be given a gross internal examination and only abnormal tissues taken and preserved in 10% neutral buffered formalin. Remaining pups in each litter will be given a gross external examination at weaning (Day 25) and if unremarkable, sacrificed and discarded. Pups with external findings will be given a gross internal examination and only abnormal tissues taken and preserved in 10% neutral buffered formalin. Some control pups not selected into the pool of animals to become the P_2 parental generation will be used in a satellite study with vinyl chloride (see Study No. 96-408_).

13.1.2.4.3 Unselected F_2 Pups:

At weaning, unselected F_2 pups in each litter will be given a gross external examination and if unremarkable, sacrificed and discarded. Pups with external findings will be given a gross internal examination and only abnormal tissues taken and preserved in 10% neutral buffered formalin.

13.1.3 METHOD OF EUTHANASIA:

13.1.3.1 Parental Animals:

Exsanguination following anesthesia with inhaled carbon dioxide.

13.1.3.2 Weanlings:

Weanling pups selected for a complete gross postmortem examination will be sacrificed by exsanguination following anesthesia with inhaled carbon dioxide. Remaining pups will be sacrificed with an overdose of inhaled carbon dioxide.

13.1.3.3 Parental Animals:

The following organs will be weighed at terminal sacrifice from the first 15 P_1 and P_2 animals in each group:

uterus	ovaries
testes	right epididymis (total and cauda)
seminal vesicles (with coagulating glands and their fluids)	prostate
brain	liver
kidneys	lungs
adrenals	spleen
thymus	

Organ weight data will be presented as absolute values and relative to terminal body weight. Organ weights will not be recorded for animal dying spontaneously or sacrificed moribund.

13.1.3.4 Weanling Pups (F_1 and F_2):

The following organs will be weighed from the first 15 male and female pups per group selected for complete macroscopic examination at weaning:

ovaries	testes
brain	liver
kidneys	adrenals
spleen	thymus

Organ weight data will be presented as absolute values and relative to terminal body weight.

13.1.4 TISSUES PRESERVED:

13.1.4.1 Parental Animals:

Grossly abnormal tissues will be preserved for all parental animals. Tissues listed in Appendix A will be obtained at necropsy and preserved for all P_1 and P_2 parental animals.

13.1.4.2 Weanling Pups:

Grossly abnormal tissues and tissues listed in Appendix A will be preserved for all F_1 and F_2 weanling pups selected for complete gross macroscopic examination.

13.1.5 Preservatives:

All tissues - 10% neutral buffered formalin. Testes and epididymides of the parental animals will be fixed in Bouin's solution for at least 48 hrs prior to permanent storage in 10% neutral buffered formalin. Lungs will be infused with formalin to their approximate normal inspiratory volume. The nasal cavity will be flushed with formalin via the pharyngeal duct to ensure rapid fixation of the tissue.

13.1.6 MICROSCOPIC PATHOLOGY EVALUATIONS:

Slides of tissues listed in Appendix A (under Microscopic Examination) will be prepared and examined microscopically for all P_1 and P_2 animals in the control and high-dose groups. If microscopic findings indicative of an effect of test material administration are seen in high-dose animals, then examinations will be made of these tissues/organs for low- and mid-dose animals (additional cost). Additional examinations will be made only after consultation with the Sponsor and receipt of authorization from the Sponsor. Note: any abnormalities not noted during macroscopic postmortem examinations which are seen during histological processing will be recorded. Gross lesions will be examined at additional cost.

13.1.7 STAINS:

Standard stains used, hematoxylin and eosin. Special stains may be employed on selected tissues to aid in making a diagnosis at the discretion of the Study Pathologist. Special stains may be employed at the request of the Sponsor (additional cost).

13.1.8 SPERM COUNT, MOTILITY AND MORPHOLOGY ASSESSMENTS:

From the first 15 P_1 and P_2 parental males sacrificed at termination in each group, samples of sperm from the vas deferens (left) and distal cauda epididymis (left) will be collected. Motility will be assessed on sperm collected from the vas deferens and a count will be performed on the sperm sample collected from the cauda epididymis. A manual procedure developed in the Testing Facility will be used to assess motility and perform the count. Additionally, a slide of the sperm sample from the cauda epididymis will be prepared for morphological assessment if deemed necessary (Sponsor decision - additional cost item).

13.2 DEVELOPMENTAL TOXICITY STUDY:

13.2.1 MATERNAL TERM SACRIFICES:

Macroscopic postmortem examinations will be performed on all mated rats, including those dying spontaneously or killed in a moribund condition and on females sacrificed after aborting or premature delivery of a litter. The livers and kidneys will be weighed and preserved in 10% neutral buffered formalin for all females killed on Day 20 of gestation but microscopic examinations of these tissues will not be conducted unless deemed necessary to interpret other observations made during the study or as requested by the sponsor (additional cost item). Gross lesions identified during the macroscopic evaluations will be saved in 10% formalin. Dams showing signs of abortion or premature delivery (expulsion of concepti) will be killed (overdose of inhaled carbon dioxide) on the day such evidence is observed. Reproductive tracts will be examined and fetuses obtained 19 days or later will be given an external examination, eviscerated and processed for skeletal staining with Alizarin Red S. These fetuses will then be examined for skeletal malformations. Fetuses obtained earlier than Day 19 will be evaluated for external malformations and saved (10% neutral buffered formalin) at the discretion of the Study Director. Examination data for these fetuses (Day 19 of gestation or earlier) will not be analyzed with data for term Day 20 gestation fetuses. Data for these fetuses will be presented in a separate appendix to the final report.

13.2.2 Reproductive System:

The intact uteri (ovaries attached) will be removed from the abdominal cavity and weighed. This uterine weight data will then be used to calculate a corrected Day 20 gestation body weight for each animal. The corrected Day 20 gestation weight will be determined by

13.2.2 Reproductive System:

subtracting the gravid uterine weight from the terminal Day 20 gestation weight. Each uterine horn will then be evaluated for the number and location of the following:

- live fetuses (movement in response to touch);

- dead fetuses (lack of movement in response to touch but with no visible degeneration);

- late resorptions (recognizable dead fetus undergoing degeneration, regardless of size);

- early resorptions (evidence of implantation but no recognizable fetus);

- implantation sites (total of fetuses plus resorptions).

Ovaries: corpora lutea will be counted for each ovary.

Uteri without grossly visible implantations will be stained with ammonium sulfate (Salewski, 1964). If stained foci are present, the female will be considered pregnant for purposes of calculating pregnancy rates. The number of foci will not be used in the calculation of uterine implantation data.

13.2.3 Fetal evaluations:

13.2.3.1 External Evaluations:

All fetuses will be weighed and individually identified. Each fetus will be given a gross external examination for defects to include observation of the palate. The sex of each fetus will be noted by observation of the ano-genital distance.

13.2.3.2 Fetal Skeletal Evaluations:

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter) will be processed for Alizarin Red S staining of the skeletal structures. Prior to processing, the intact fetuses will be killed (overdose of inhaled carbon dioxide) and eviscerated (internal sex noted). Following staining, these fetuses will be evaluated for skeletal malformations and ossification variations.

13.2.3.3 **Fetal Soft Tissue Evaluations:**

The remaining fetuses in each litter will be processed for soft tissue examination using a microdissection technique similar to the procedure of Staples (1974) . The evaluations will be performed on the fresh fetal specimens soon after removal from the uterus. The fetuses designated for soft tissue evaluation will be decapitated (head placed in Bouin's solution for later evaluation). The fetal specimens will then be secured beneath a dissecting microscope and dissected so as to permit evaluation of tissues in the thoracic, abdominal and pelvic cavities. At completion of the examination, the decapitated fetal specimens will be eviscerated and processed for Alizarin Red S staining. These fetuses will not be evaluated skeletally unless authorized by the sponsor (additional cost).

Fetal heads, preserved in Bouin's solution, will be sectioned with a razor blade. The serial, transverse sections generated during this procedure will be evaluated for malformations of the palate, eyes and brain.

Fetal soft tissue and skeletal evaluations (microdissection, stained specimens and head sections) will be performed under a dissecting microscope.

13.2.4 **Resorptions:**

Late resorptions will be examined externally for malformations. Malformation data for late resorptions will be reported but not included in the analyses of malformation data for live or dead fetuses recovered on Day 20 of gestation. Only late resorptions with external malformations will be saved (10% neutral buffered formalin) for future possible examination. Early resorption will be discarded.

14 PRESERVATION OF RECORDS AND SPECIMENS:

All data documenting experimental details and study procedures and observations will be recorded and maintained as raw data.

At the completion of the study, all reports, raw data, preserved specimens and retained samples will be maintained in the Testing Facility's Archives for a period of 10 years after submission of the signed final report.

The Sponsor will be contacted in order to determine the final disposition of these materials. The Sponsor is responsible for all costs associated with the storage of these materials beyond 10 years from the issuance of the final report

14 PRESERVATION OF RECORDS AND SPECIMENS:

and for any costs associated with the shipment of these materials to the Sponsor or to any other facility designated by the Sponsor.

15 STATISTICAL EVALUATIONS:

The following items will be analyzed statistically in the final report:

15.1 CONTINUOUS DATA:

15.1.1 Two-generation Reproduction Study:

Mean body weights (all recorded intervals - premating, gestation, lactation and postmating);
Mean body weight change;
-entire premating period (males and females) - entire premating period;
-over each weighing interval during the gestation and lactation periods to include Days 0-20 of gestation and Days 0-25 (F_1 litters) or Day 0-21 (F_2) of lactation;
-males during the postmating period (weekly and over the entire period).

Mean food consumption values;
-premating growth period (weekly);
-postmating period (weekly for males);
-gestation (Days 0-7, 7-14, 14-20);
-lactation (all recorded intervals);

Organ weight data (absolute and relative to the terminal body weight);
Mean pup weights (all recorded intervals during lactation);
Mean number of pups (live, dead, total) at birth (F_1 and F_2 litters);
Mean gestation length (F_1 and F_2 litters);
Mean pup live birth indices (F_1 and F_2 litters);
Mean pup viability indices (Days 0-4) and weaning indices (Days 4-25 or 21 for the F_1 and F_2 litters, respectively);
Mean age-to-criteria for vaginal opening and preputial separation (P_2 animals);
Mean sperm count and motility data.

15.1.2 Developmental Toxicity Study:

Mean maternal body weights (all recorded intervals during gestation) and weight gain (between all weighing intervals to include Days 6-20 of gestation using both the actual and corrected Day 20 gestation weight);

15.1.2 Developmental Toxicity Study:

Mean organ weight data, absolute and relative to the corrected Day 20 gestation weight;
Mean number of corpora lutea, implants, live and dead fetuses, resorptions per pregnant female;
Mean pre- and post-implantation loss indices;
Mean fetal weights (distinguished by sex and as a composite for both sexes);

15.1.3 STATISTICAL ANALYSES CONTINUOUS DATA - MULTIPLE GROUP ANALYSES:

Data will be compared between the sham, chamber-housed control and the treated groups.

Statistical evaluation of equality of means will be made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. Bartlett's test will be performed to determine if groups have equal variance. If the variances are equal ($p > 0.01$), parametric procedures will be used; if not ($p < 0.01$), nonparametric procedures will be used. The parametric procedures will be the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means are indicated, Dunnett's test will be used to determine which means are significantly different from the control. If a nonparametric procedure for testing equality of means is needed, the Kruskal-Wallis test will be used, and if differences are indicated, a summed rank test (Dunn) will be used to determine which treatments differ from control.

A statistical test for trend in the dose levels will also be performed. In the parametric case (i.e., equal variance), standard regression techniques with a test for trend and lack of fit will be used. In the non-parametric case, Jonckheere's test for monotonic trend will be used.

The test for equal variance (Bartlett's) will be conducted at the 1% two-sided risk level. All other statistical tests will be conducted at the 5% and 1%, two-sided risk levels.

All ratios (pup survival indices, pre- and postimplantation loss indices) will be transformed via Bartlett's transformation followed by the arcsine transformation prior to analysis. Data will be presented untransformed.

15.1.3 STATISTICAL ANALYSES CONTINUOUS DATA - MULTIPLE GROU:P ANALYSES

References for these techniques are Snedecor, G.W., Cochran, W.G., *Statistical Methods*, 6th edition, Iowa State Univ. Press (1967); Hollander and Wolfe, *Nonparametric Statistical Methods*, John Wiley and Sons, New York (1973); Dunnett, C.W., *J. Am. Sta. Assn.* 50: 1096-1121 (1955) and *Biometrics* 20: 482 (1964).

Bartlett's Test	pp. 296-298	Snedecor & Cochran
ANOVA	pp. 277-279	Snedecor & Cochran
Dunnett's Test	pp. 1096-1121	Dunnett
	pp. 482-491	Biometrics
Kruskal-Wallis	pp. 114-116	Hollander & Wolfe
Summed Rank Test (Dunn)	p. 131	Hollander & Wolfe
Arc Sine Transformation	pp. 327-329	Snedecor & Cochran
Bartlett's transformation	p. 329	Snedecor & Cochran
Regression Analysis-Trend	pp. 135-153	Snedecor & Cochran
Lack of fit	pp. 456-459	Snedecor & Cochran
Jonckheere's Statistic	pp. 120-123	Hollander & Wolfe

15.2 INCIDENCE DATA:

15.2.1 Two-generation Reproduction Study:

Mortality rates;
Mating indices (male and female);
Pregnancy rates;
Male fertility indices;
Litter survival indices;

15.2.2 Developmental Toxicity Study:

Maternal Mortality
Pregnancy rates
Incidence of females with resorptions;
Incidence of fetuses with malformations/variations - external, soft tissue and skeletal examinations;
Incidence of litters containing fetuses with malformations/variations - external, soft-tissue and skeletal.

15.2.3 INCIDENCE DATA ANALYSIS:

Data will be compared between the sham, chamber-housed control and the treated groups.

Statistical analysis of incidence data will be performed using contingency tables. First, a standard Chi-square analysis will be performed to determine if the proportion of incidences differed between the groups tested. Next, each treatment group will be compared to the control group using a 2 x 2 Fisher Exact Test; the significance level will be corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Thirdly, Armitage's test for linear trend in the dosage groups will be performed. In keeping with standard statistical practice, if any one cell has an expected value less than 5, the Chi-square and Armitage's tests will not be reported. When this occurs, only the Fisher Exact test (corrected via Bonferroni inequality) will be performed and reported.

All tests will be reported at the 5% and 1% level of significance.

References for the techniques are Snedecor, G.W., and Cochran, W.G., *Statistical methods*, 6th ed., Iowa State University Press, Ames, Iowa (1971); Bradley, J.V., *Distribution Free Statistical Tests*, Prentice-Hall, Englewood Cliffs, New Jersey (1968); Miller, R.G., Jr., *Simultaneous Statistical Inference*, McGraw-Hill Book Co., New York (1966); Armitage, P., "Tests for Linear Trends in Proportions and Frequencies", *Biometrics*, (Sept. 1955).

Chi-square	pp. 250-253	Snedecor & Cochran
Fisher Exact Test	pp. 195-203	Bradley
Bonferroni Inequality	p. 15	Miller
Armitage's Test	pp. 375-386	Armitage

16 REFERENCES:

ATSDR, (1993). Toxicology Profile for Vinyl Chloride. U.S. Department of Health and Human Services. Public Health Services. Agency for Toxic Substances and Disease Registry.

EEC (1988). European Economic Community. Methods for the Determination of Toxicity. Official Journal of the European Communities, Vol.31, No.. L133, 30 May 1988. ISSN 0378-6978.

EPA (1985). Environmental Protection Agency Toxic Substances Control Act Test Guidelines, Final Rule. 40 CFR Part 798, 27 September 1985, pp. 39426-39433.

16 REFERENCES:

EPA (1990). Environmental Protection Agency Toxic Substances Control Act; Good Laboratory Practice Standards. 40 CFR Part 792 (1 July 1990).

FDA (1988). Food and Drug Administration Good Laboratory Practice for Nonclinical Studies. 21 CFR Part 58 (1 April 1988).

OECD (1981). Organisation for Economic Co-Operation and Development Guidelines for Testing of Chemicals, Section 4 - Health Effects, Paris.

OECD (1982). Organisation for Economic Co-Operation and Development, Principles of Good Laboratory Practice, ISBN 92-64-12367-9, Paris.

Plowchalk, D.R., Smith, B.J. and Mattison, D.R. (1993). Assessment of toxicity of the ovary using follicle quantitation and morphometrics. In Methods in Toxicology, Vol.3, Part B, Female Reproductive Toxicology. (J. J. Heindel and R.E. Chapin, Eds.). Academic Press, Inc., New York, NY.

Salewski, E. (1964). Farbemethode zum makroskopischen machweis von implantationsstellen am uterus der ratte. *Archiv. Path. Exp. Pharmakol.*, 247:367

Staples, R. E. (1974). Detection of Visceral Alterations in Mammalian Fetuses. *Teratology*, 9:37 (Abstract).

17 REPORT:

17.1 STATUS REPORT:

For the two-generation reproduction study, status reports will be issued monthly during the premating treatment period and after weaning of the F_1 and F_2 litters. In the developmental toxicity study a status report will be submitted after the last Day 20 gestation maternal sacrifices. These reports will include:

Two-generation Reproduction Study:

Mortality rates
Mean weekly body weight and weight gain data;
Mean weekly food consumption premating period;
Summary of detailed physical examinations;
Mating indices (males and females);
Male fertility indices;
Pregnancy rates;
Gestation length;

17.1 STATUS REPORT:

Number of pups at birth (live, dead and total) and number of live pups surviving during lactation;
Mean pup weights (lactation);
Individual female litter data (F_1, F_2);
Maternal gestation body weights and weight gains;
Maternal food consumption - gestation/lactation;
Summary of gross postmortem evaluations (adults, weanlings)

Developmental Toxicity Study:

Mortality rates:
Mean body weight and weight gain data during gestation;
Mean food consumption data;
Summary of detailed physical examination data;
Mean corpora lutea and uterine implantation data;
Mean number of live and dead fetuses and resorptions per pregnant female;
Mean pre- and post-implantation loss indices;
Incidence of females with resorptions;
Mean fetal weights;
Fetal external examination data;
Individual maternal Day 20 gestation sacrifice data;
Maternal organ weight data;
Summary of maternal gross postmortem examination data.

17.2 FINAL REPORT:

One copy of a draft report will be submitted following termination of the study. After receipt and review of the Sponsor's comments, appropriate changes will be made and two copies of a signed, final report will be issued. (Additional copies will be provided at additional cost). The report will include but not be limited to the following:

17.2.1 General:

Compliance Statement;
Abstract;
Introduction;
Experimental Design;
Materials and Methods;
Discussion of study results;
Conclusion and No Observed Effect Level (NOEL) statement, if applicable;
Inhalation Exposure Report;
References for experimental methodology;
Senior personnel participating in the study;

17.2.1 General:

Quality Assurance Statement.

17.2.2 Data tabulations for parental generations (P_1, P_2):

Mortality - termination history;
Physical in-life observations (summarized and individual data presented monthly throughout the study);
Mating indices;
Pregnancy rates;
Male fertility indices;
Mean body weight data (all interval);
Mean food consumption data (all intervals);
Mean weight gain data (premating, postmating [males], gestation and lactation intervals);
Statement on estrous cycle data;
Macroscopic postmortem observations (adults, weanlings);
Microscopic pathology examinations;
Organ weight data;
Sperm assessment data.

17.2.3 Data tabulations for litters and offspring (F_1 and F_2):

Mean gestation length;
Mean number of pups (live, dead and total) at birth and live pups at Days 4, 7, 14 and weaning (Day 25 - F_1 and Day 21 - F_2);
Litter survival indices;
Pup live birth index;
Pup viability and weaning indices;
Mean pup weights (all recorded intervals during lactation);
Pup sex ratio at birth, Day 4 (pre- and postcull) and weaning;
Pup gross postmortem observations;
Individual female litter data.

17.2.4 Data tabulation for the developmental toxicity study:

Maternal mortality
Maternal body weight and weight gain data;
Maternal food consumption;
Physical observation data:
Pregnancy rates;
Mean number of corpora lutea and uterine implantations;
Mean number of live and dead fetuses;
Mean pre- and post-implantation loss indices;
Mean number of resorptions;
Incidence of females with resorptions;

17.2.4 Data tabulation for the developmental toxicity study:

Types of findings and incidence of fetuses with external, soft tissue and skeletal malformations/variations;
Incidence of litters containing fetuses with malformations/variations (external, soft tissue and skeletal)
Mean fetal weights;
Fetal sex distribution ratios;

17.2.5 Appendices:

All individual animal data (adults, pups, weanlings and fetuses) including but not limited to the following will be presented in the appendices: body weight and weight gain, food consumption, physical observation data, litter and uterine implantation data, organ weight data, sperm assessment data, estrous cycle data, gross postmortem findings, microscopic examination data, chamber exposure data.

18 SIGNATURES:

18.1 INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC):

The IACUC Protocol Review Subcommittee has reviewed this protocol and found it to be in compliance with all appropriate regulations.

BY: _____ DATE: 11 Oct 96

TITLE: Institutional Animal Care and Use Committee Member
FOR: Huntingdon Life Sciences
Institutional Animal Care and Use Committee

BY: _____ DATE: 11Oct96

TITLE: Institutional Animal Care and Use Committee Member
FOR Huntingdon Life Sciences
Institutional Animal Care and Use Committee

18.2 PROTOCOL REVIEWED AND ACCEPTED:

BY: _____ DATE: 25Oct96

Raymond E. Schroeder, M.S., DABT
TITLE: Study Director
FOR: Huntingdon Life Sciences

BY: _____ DATE: 10/16/96

Robert A. Venezia, Ph.D.
TITLE: Sponsor Representative
FOR: Chemical Manufacturers Association
Chemstar Department

APPENDIX A
Tissues Preserved/Examined Microscopically

No.[a]	Tissue	Preserved	Microscopic Examination (Groups)	
			I, IV	II, III
2	adrenals	X		
1	aorta	X		
1	auditory sebaceous glands	X		
1	bone (including joint)	X		
3	brain (cerebrum, brainstem, cerebellum)	X		
1	cecum	X		
2	coagulating glands	X	X	
1	colon	X		
1	duodenum	X		
2	epididymis	X	X	
1	esophagus	X		
2	eyes	X		
1	heart	X		
1	ileum	X		
1	jejunum	X		
2	kidneys	X	X	
2	lacrimal/Hardarian glands	X		
1	larynx	X		
2	liver	X	X	
2	lungs	X	X	
1	mammary glands	X	X	
1	mediastinal lymph nodes	X		
1	mediastinal tissues	X		
1	mesenteric lymph nodes	X		

APPENDIX A
Tissues Preserved/Examined Microscopically

No.[a]	Tissue	Preserved	Microscopic Examination (Groups)	
			I, IV	II, III
1	mesenteric tissues	X		
4	nasal tissues (turbinates)	X	X	
1	oral tissues	X		
1	ovary[b]	X	X	
2	oviducts	X	X	
1	pancreas	X		
2	parathyroid glands	X		
1	peripheral nerve	X		
1	pituitary	X	X	
1	prostate	X	X	
1	rectum	X		
2	salivary glands	X		
2	seminal vesicles	X	X	
1	skeletal muscle	X		
1	skin	X		
3	spinal cord (cervical, thoracic, lumbar)	X		
1	spleen	X		
1	stomach	X		
2	testis	X	X	
1	thymus	X		
2	thyroid gland	X		
1	tongue	X		
1	trachea	X	X	
1	urinary bladder	X		

APPENDIX A
Tissues Preserved/Examined Microscopically

No.[a]	Tissue	Preserved	Microscopic Examination (Groups)	
			I, IV	II, III
2	uterus (body/horns with cervix)	X	X	
1	vagina	X	X	
	gross lesions	X	X	

[a] Number of organs/sections preserved/examined.

[b] Only the right ovary will be routinely processed for microscopic evaluation. The left ovary will be saved for possible oocyte quantification. If deemed necessary by the Sponsor (additional cost), oocyte quantification will include evaluation of a minimum of 10 sections randomly selected from one completely sectioned ovary per female of the high-dose and control groups. Ovarian follicles will be placed into one of three categories as described by Plowchalk et al., (1993). The total number of follicles and the number of follicles in each of the three categories will be evaluated. Ovaries from the low- and mid-dose groups may be evaluated if treatment-related changes are observed in the high-dose group.

APPENDIX B

TEST MATERIAL PROPERTIES

Chemical name	Vinyl chloride
Synonyms	Monochloroethylene, VC, VCM, vinyl chloride monomer
Molecular formula	C_2H_3Cl
Molecular weight	62.5
Structures	$CH_2=CHCl$
Appearance	Colorless gas
Vapor pressure	2,530 mm Hg at 20 degrees C
Saturated atmosphere	Gas at room temperature
Vapor density	2.16
Flash point	-77.75 degrees C (open cup)
Boiling point	-13.6 degrees C
Specific gravity	0.9121
Conversion factors	1 mg/m^3 = 0.39 ppm in air 1 ppm = 2.60 mg/m^3 in air