

Huntingdon Life Sciences

HUNTINGDON LIFE SCIENCES

STUDIES TO EVALUATE THE FORMATION AND REPAIR OF DNA ADDUCTS INDUCED
IN ADULT AND NEONATAL CD RATS BY VINYL CHLORIDE

STUDY NO.: 96-4092

ISSUE NO.: 3

SUBMITTED TO: Chemical Manufacturers Association
Chemstar Department

ATTENTION: Hasmukh C. Shah, Ph.D.

DATE: 17 February 1997

1 INTRODUCTION:

- 1.1 STUDY NO.: 96-4092
- 1.2 ISSUE NO.: 3
- 1.3 STUDY TITLE: Studies to Evaluate the Formation and Repair of DNA Adducts Induced in Adult and Neonatal CD Rats by Vinyl Chloride
- 1.4 TEST MATERIAL: Vinyl Chloride
- 1.5 SPONSOR: Chemical Manufacturers Association
Chemstar Department
1300 Wilson Boulevard
Arlington, Virginia 22209
- 1.6 SPONSOR REPRESENTATIVE: Hasmukh C. Shah, Ph.D.
Phone - 703-741-5637
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:

Several studies involving adult or neonatal animals will be performed to evaluate the formation and repair of DNA adducts induced by vinyl chloride. These studies will provide an improved understanding of the mechanisms of vinyl chloride carcinogenesis. These studies will also provide information relevant to cancer risk assessment of vinyl chloride.

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
Vice President, Pathology:	Ward R. Richter, D.V.M., M.S., ACVP

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

3 REGULATORY REFERENCES:

3.1 TEST GUIDELINE:

These studies are not designed to meet or exceed any particular guidelines.

3.2 GOOD LABORATORY PRACTICES:

These studies will not be conducted in compliance with GLP guidelines.

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; Institute of Laboratory Animal Resources, National Research Council, Revised 1996. 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:

3.4 ANIMAL WELFARE ACT COMPLIANCE:

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.
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 and company standard operating procedures.

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:

Study No. 1 - Adult Rats Exposed to Vinyl Chloride for 1 Week

Initiation of exposures:	17 May 1997
Termination of exposures:	22 May 1997
Necropsy	21/22 May 1997

Study No. 2 - Weanling Rats Exposed to Vinyl Chloride for 1 Week

Initiation of exposures:	8 June 1997
Termination of exposures:	13 June 1997
Necropsy	12/13 June 1997

Study No. 3 - Adult Rats Exposed to Vinyl Chloride for 4 Weeks

Initiation of exposures:	5 May 1997
Termination of exposures:	5 June 1997
Necropsy	4/5/6/11 June 1997

Study No. 4 - Adult Rats Exposed to [¹³C₂]-Vinyl Chloride for 1 Week

Initiation of exposures:	15 May 1997
Termination of exposures:	20 May 1997
Necropsy	19/20 May 1997

7 EXPERIMENTAL DESIGN:

Study type	Exposure Levels (ppm)	No. of Animals ^a	Treatment Schedule	Necropsy Schedule
1 - Adult Male Rats	0, 10, 100, 1100 ^b	32 (8/group)	5 days, 6 hrs/day	All animals following exposure Day 5
2 - Weanling Male Rats	0, 10, 100, 1100 ^b	32 (8/group)	5 days, 6 hrs/day	All animals following exposure Day 5
3 - Adult Male Rats	0, 10, 100, 1100 ^b	64 (16/group)	5 days/week, four weeks, 6 hrs/day	8 animals/group following last exposure (Week 4) and 8 animals/group following one week recovery
4 - Adult Male Rats	10, 100, 1100 ^c	24 (8/group)	5 days, 6 hrs/day	All animals following exposure Day 5

^a 1-2 "extra" animals will be exposed 6 hrs/day at each dose level in each of these studies to provide whole tissues at terminal sacrifice. No additional data will be collected for these animals and they will not be perfused.

^b Whole body exposures conducted during the performance of a vinyl chloride two-generation reproduction study (Huntingdon Life Sciences Study No. 96-4080).

^c Nose-only exposure with [¹³C₂] - vinyl chloride.

8 TEST MATERIAL:

8.1 TEST MATERIAL (Studies 1, 2 and 3): Vinyl Chloride

Description, lot number, storage, expiration date and handling procedures, as well as other pertinent information will be documented in the study data.

8.2 IDENTIFICATION OF TEST MATERIAL (Studies 1, 2 and 3):

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 (Studies 1, 2 and 3):

The purity of the lot of test material used on study will be determined from periodic assessments of the test material over the course of the study. These analyses will be performed at the Testing Facility.

8.4 TEST MATERIAL (Study No. 4): [$^{13}\text{C}_2$]-Vinyl Chloride

Test material for this study will be supplied by Cambridge Isotope Laboratories. The Supplier will be responsible for the characterization. Unless otherwise noted, the identity, strength, purity, composition, stability, and method of synthesis, fabrication and/or derivation for the batch of test material used in Study 4 will be documented by the Supplier before its use.

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

9.1 STUDY NO. 1, ADULT RATS EXPOSED TO VINYL CHLORIDE FOR 1 WEEK:

9.1.1 Species/Strain:

Albino Rats/Sprague Dawley - derived (CD[®])
[CrI: CD[®] BR]

9.1.2 Supplier:

Charles River Laboratories
Portage, Michigan

9.1.3 Animal requirements/specifications:

9.1.3.1 Number:

Eight per group for a total of 32 animals on study. Approximately 40 animals will be ordered and acclimated. The unselected study animals will be designated "extra" animals and be distributed equally among the control and three treatment groups (1-2/group). These "extra" animals will be exposed the same as study animals but will be used only to provide tissues/organs at terminal sacrifice. No other data will be collected for these animals.

9.1.3.2 Sex and Age:

Males approximately 11 weeks at receipt and 12 weeks (approximately 450-550 grams) at initiation of treatment. Animals outside this weight range will be used at the discretion of the study director.

9.1.3.3 Acclimation period:

At least one week; all animals will be observed twice daily. Prior to assignment to study all animals will be examined to determine suitability for use.

**9.2 STUDY NO. 2, WEANLING RATS EXPOSED TO VINYL CHLORIDE FOR
1 WEEK:**

9.2.1 Species/Strain:

Albino Rat/Sprague Dawley - derived (CD®)
[CrI: CD® BR]

9.2.2 Supplied by:

Charles River Laboratories
Portage, Michigan

9.2.3 Animal requirements/specifications:

9.2.3.1 Number:

Lactating Females: Eight females with litters of eight male pups each (Lactation Day 7 or thereabouts) will be ordered. The pups weaned at Day 25 from these females will be used to provide animals for this study. Each study group will contain eight male pups for a total of 32 animals on study. Forty pups will be identified for use in this study. The unselected study animals will be designated "extra" animals and be distributed equally among the control and three treatment groups (1-2/group). These "extra" animals will be exposed the same as study animals but will be used only to provide tissues/organs at terminal sacrifice. No other data will be collected for these animals.

9.2.3.2 Sex and Age:

Male pups at Postnatal Day 26 or 27 (weaned on Postnatal Day 25) will be used on study. Animals will be approximately 70-100 grams at initiation of treatment. Animals outside this weight range will be used at the discretion of the study director.

9.2.3.3 Acclimation:

Pups will be weaned at the Testing Facility and will have been with the dam in this laboratory for approximately two weeks prior to use on study.

9.3 STUDY NO. 3, ADULT RATS EXPOSED TO VINYL CHLORIDE FOR 4 WEEKS:

9.3.1 Species/Strain:

Albino Rat/Sprague Dawley - derived (CD®)
[CrI: CD® BR]

9.3.2 Supplier:

Charles River Laboratories
Portage, Michigan

9.3.3 Animal requirements/specifications:

9.3.3.1 Number:

Sixteen animals per group for a total of 64 animals on study. More animals than required for study will be purchased and acclimated. Approximately 80 animals will be identified for use in this study. The unselected study animals (about 16 animals total) will be designated "extra" animals and be distributed equally among the four study groups (approximately 4/group). These "extra" animals will be exposed the same as study animals but will be used only to provide tissues/organs at terminal sacrifice. No other data will be collected for these animals.

9.3.3.2 Sex and Age:

Males approximately 11 weeks at receipt and 12 weeks (approximately 450-550 grams) at initiation of treatment. Animals outside this weight range will be used at the discretion of the study director.

9.3.3.3 Acclimation:

At least one week; all animals will be observed twice daily. Prior to assignment to study all animals will be examined to determine suitability for use.

9.4 STUDY 4, ADULT RATS EXPOSED TO [$^{13}\text{C}_2$] - VINYL CHLORIDE FOR 1 WEEK:

9.4.1 Species/Strain:

Albino Rat/Sprague Dawley - derived (CD[®])
[CrI: CD[®] BR]

9.4.2 Supplier:

Charles River Laboratories
Portage, Michigan

9.4.3 Animal requirements/specifications:

9.4.3.1 Number:

Eight animals per group for a total of 24 animals on study. More animals then required for study will be purchased and acclimated. Approximately 30 animals will be identified for use in this study. The unselected study animals (about 6 animals total) will be designated "extra" animals and be distributed equally among the three study groups. These "extra" animals will be exposed the same as study animals but will be used only to provide tissues/organs at terminal sacrifice. No other data will be collected for these animals.

9.4.3.2 Sex and Age:

Males approximately 11 weeks at receipt and 12 weeks (approximately 450-550 grams) at initiation of treatment. Animals outside this weight range will be used at the discretion of the study director.

9.4.3.3 Acclimation:

At least one week; all animals will be observed twice daily. Prior to assignment to study all animals will be examined to determine suitability for use.

9.5 ANIMAL HUSBANDRY:

9.5.1 Housing:

Animals will be housed in suspended, stainless steel cages with wire mesh fronts and floors during exposure and non-exposure periods in Studies 1-3 and during non-exposure periods in Study 4. During exposures for Study 4, the animals will be housed in polycarbonate tubes. During periods when urine is to be collected, animals will be housed individually in stainless steel, suspended cages designed to collect urine.

9.5.2 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.

9.5.3 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 in Studies 1-3. Animals in Study No. 4 will not have access to water during the exposure period.

9.5.4 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.

9.5.5 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

9.5.5 Water Analysis:

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.

9.5.6 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.

9.5.7 Environmental Conditions:

9.5.7.1 Light/Dark Cycle:

Twelve hour light/dark cycle daily.

9.5.7.2 Temperature:

Monitored and recorded twice daily. Temperature in the animal room and exposure chambers will be maintained in the range of approximately 20-24°C to the maximum extent possible.

9.5.7.3 Humidity:

Humidity in the animal quarters will be monitored and recorded once daily. The desired humidity range in the animal quarters is 30-70% and 40-60% in the chambers during exposures. Humidity will be maintained in this range to the maximum extent possible.

9.6 SELECTION FOR STUDY:

In Studies No. 1, 3 and 4 animals will be sorted into groups so as to equalize the mean body weights between groups. A manual sorting procedure will be used. In Study No. 2 animals will be manually sorted among the groups to equalize as best possible the distribution of pups from each F₁ litter.

9.7 ANIMAL IDENTIFICATION:

Each animal will be assigned a temporary identification number upon receipt. After selection for study, each animal will be ear-tagged (metal eartag) for Studies 1-3 and by tail tattoo for Study 4 with a number assigned by the Testing Facility. This number plus the study number will comprise the unique identification for each study animal.

10 TEST MATERIAL ADMINISTRATION:

10.1 ROUTE OF ADMINISTRATION:

Studies No. 1, 2 and 3 - whole body inhalation; Study No. 4 - nose only inhalation.

10.2 JUSTIFICATION FOR ROUTE OF ADMINISTRATION:

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

10.3 FREQUENCY OF ADMINISTRATION:

10.3.1 Study No. 1:

Five consecutive days, 6 hrs/day.

10.3.2 Study No. 2:

Five consecutive days, 6 hrs/day.

10.3.3 Study No. 3:

Five consecutive days per week, 6 hrs/day, for four weeks.

10.3.4 Study No. 4:

Five consecutive days, 6 hrs/day.

10.4 INHALATION EXPOSURE GENERATION PROCEDURE:

10.4.1 Studies 1, 2 and 3:

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

10.4.1 Studies 1, 2 and 3:

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 of 1200 liters per minute. The final airflow will be set to provide at least one air change in 5.0 minutes (12 air changes/hour) and a T_{99} equilibrium time of approximately 23 minutes. This chamber size and airflow rate are considered adequate to maintain the oxygen level above 19% and the animal loading factor below 5%.

10.4.2 Study No. 4:

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 flow-past nose-only exposure chambers will be operated at a minimum flow rate of 3.0 liters per minute of house-line air. This chamber airflow rate is considered adequate to maintain the oxygen level above 19% .

10.5 CHAMBER MONITORING:

A nominal exposure concentration will be calculated, if possible. The flow of air through the chamber/nose only generation apparatus will be monitored using appropriate calibrated equipment. The test material consumed during the exposure will be divided by the total volume of air passing through the chamber (volumetric flow rate times total exposure time) to give the nominal concentration.

During each test material exposure, measurements of airborne concentrations will be made at least hourly using a MIRAN infrared spectrophotometer.

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.

10.5 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 or Tubes	once
Loading/Unloading Verification	once

10.6 EXPOSURE CONCENTRATIONS:

For all studies (except Study No. 4 which will not have a control group) 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. The middle and low exposure levels were selected to provide a dose response for the observed effects and a no-observed-effect level, respectively.

11 EXPERIMENTAL EVALUATIONS:

11.1 OBSERVATIONS:

11.1.1 Viability Determinations:

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. Animals found dead after normal working hours will be refrigerated until a necropsy can be performed.

11.2 BODY WEIGHTS:

11.2.1 Study No. 1:

Animals will be weighed pretest, on the day treatment initiates and at terminal sacrifice. The "extra" animals included on study will be weighed at study start and at terminal sacrifice.

11.2.2 Study No. 2:

Animals will be weighed pretest (Day 25), on the day treatment initiates and at terminal sacrifice. The "extra" pups included on study will be weighed at study start and at terminal sacrifice.

11.2.3 Study No. 3:

Animals will be weighed pretest, on the day treatment initiates, weekly thereafter and at terminal sacrifice. The "extra" animals included on study will be weighed at study start and at terminal sacrifice.

11.2.4 Study No. 4:

Animals will be weighed pretest, on the day treatment initiates and at terminal sacrifice. The "extra" animals included on study will be weighed at study start and at terminal sacrifice.

11.3 FOOD CONSUMPTION:

Food consumption will not be recorded during these studies.

11.4 URINE COLLECTION:

11.4.1 Study No. 3:

Urine will be collected over the non-exposure interval (a period of approximately 17 hrs) on Day 1, 5, 10 and 19 from all animals in the group exclusive of the "extra" animals in each group. Urine from each animal on each day will be collected on dry ice and stored frozen (-20° F) until being transferred to Dr. Swenberg's lab at the University of North Carolina for evaluation.

11.4.2 Study No. 4:

Urine will be collected for each animal for three days prior to exposure and during the non-exposure interval (a period of approximately 17 hrs) on Days 1-4. Urine from each animal on each day will be collected on dry ice and stored frozen (-20° F) until being transferred to Dr. Swenberg's lab (University of North Carolina) for evaluation.

12 POSTMORTEM:

12.1 SCHEDULED SACRIFICE:

12.1.1 Study No. 1:

All eight rats per group and all "extra" dosed and control animals on Day 5 after the last exposure.

12.1.2 Study No. 2:

All eight rats per group and all "extra" dosed and control animals on Day 5 after the last exposure.

12.1.3 Study No. 3:

Eight rats per group and two "extra" dosed and control animals per group will be sacrificed on the last day of exposure (end of Week 4). The remaining animals in each group to include the remaining "extra" animals will be sacrificed seven days after the last exposure (following a one week period when animals will not have been exposed to vinyl chloride).

12.1.4 Study No. 4:

All 8 rats per group and the "extra" animals in each group will be sacrificed on Day 5 after the last exposure.

12.2 METHOD OF EUTHANASIA:

Studies No. 1-4: Whole body perfusion following anesthesia with pentobarbital. The "extra" animals will be sacrificed by exsanguination following anesthesia from pentobarbital.

12.3 NECROPSY:

Studies No. 1-4: The sacrifice, perfusion and necropsy of these animals will be performed under the supervision of a technical staff from Dr. James Swenberg's laboratory at University of North Carolina and all data generated during these examinations will be their responsibility and retained at his laboratory. The "extra" animals in each group within these studies will not be perfused but will be used to collect whole tissues at necropsy. The tissues will be transferred to Dr. Swenberg's laboratory at University of North Carolina.

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

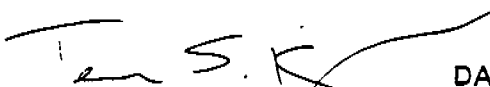
14 REPORT:

The reports for each of these studies (No. 1-4) will be prepared by Dr. James Swenberg, University of North Carolina. Copies of all in-life data generated by Huntingdon Life Sciences will be sent to Dr. Swenberg, University of North Carolina, Dept. of Environmental Sciences and Engineering, Campus Box 7400, Rosenau Hall, Chapel Hill, NC 27599-7400. The original raw data will be retained in the study file.

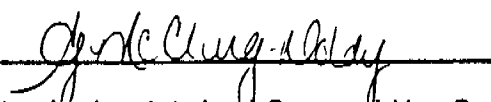
15 SIGNATURES:

15.1 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: 24 Feb 97

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

BY:  DATE: 25 Feb 97

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

15.2 PROTOCOL REVIEWED AND ACCEPTED:

BY: _____ DATE: _____

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

BY:  DATE: March 31, 1997

Hasmukh C. Shah, Ph.D.
TITLE: Sponsor Representative
FOR: Chemical Manufacturers Association
Chemstar Department