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MPI
RESEARCH

DECHLORANE PLUS: ORAL REPEAT DOSE TOXICITY AND REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY IN RATS

TESTING FACILITY

MPI Research, Inc.
54943 North Main Street
Mattawan, MI 49071-9399 U.S.A.

STUDY NUMBER

(To be determined)

STUDY DIRECTOR

Raymond E. Schroeder, M.S., D.A.B.T.®

SPONSOR

Occidental Chemical Corporation
5005 LBJ Freeway
Dallas, TX 75244

December 13, 2006

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1. INTRODUCTION

The study Sponsor, Occidental Chemical Corporation, is undertaking this study in response to the High Production Volume (HPV) Challenge program initiated by the U.S. Environmental Protection Agency (EPA). This study is composed of two phases that will be undertaken concurrently. One phase, the developmental and reproductive toxicity screening phase will be generally consistent with the OECD Guideline 422 although additional toxicity endpoints are included in the study design. The other phase of this study, 28-day repeat dose toxicity, is being conducted because of EPA's concern over the potential lack of creditability of a previously conducted 90-day subchronic toxicity.

1.1. Study Number

To be determined at authorization

1.2. Study Title

Dechlorane Plus: Oral Repeat Dose Toxicity and Reproduction/Developmental Toxicity Screening Study in Rats

1.3. Sponsor

Occidental Chemical Corporation
5005 LBF Freeway
Dallas, TX 75244

1.4. Sponsor Representative

Debbie Schober

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1.5. Study Monitor

William J. Brock, Ph.D., DABT, Fellow ATS
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Fax: 301-926-4792
Email: billbrock@comcast.net

1.6. Objective

The objective of this study was two fold: 1) to evaluate for possible adverse effects of the test article following repeated oral dosing over a 28-day period to include neurologic potential (Repeat Dose Toxicity Phase), and 2) evaluate male and female reproductive performance (gonadal function, mating behavior, conception, development of the conceptus, parturition, and early neonatal development) (Reproductive and Developmental Toxicity Phase).

1.7. Regulatory Guideline

This protocol generally meets or exceeds draft Guideline 422, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, the Organization

of Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, revised January 1994.

1.8. Good Laboratory Practice

This nonclinical laboratory study will be conducted in accordance with the United States Environmental Protection Agency (EPA), Toxic Substance Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR Part 792, effective September 18, 1989, and OECD Principles of GLP ENV/MC/CHEM(98)17.

1.9. Testing Facility

MPI Research, Inc.
54943 North Main Street
Mattawan, MI 49071-9399 U.S.A.

MPI Research is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

1.10. Computer Systems

The following are the proposed computer systems to be used during the conduct of this study. The actual systems used will be documented in the final report.

MPI Research Computer Systems	
Provantis™:	Client-server, Oracle-based system primarily used for toxicology studies.
Niagara Framework® Software System:	Environmental monitoring, alarming, and reporting application.
Dispense:	Automates the test article control processes.
MotorMonitor™:	Measures locomotor activity.
MPI Archiving System (MArcS):	In-house developed application for automated storage and retrieval information for archiveable materials (e.g. lab books, study data, wet tissues, slides, etc.).
MPI Reporting System (MPIRS):	In-house developed reporting system running SAS® programs primarily reporting on Provantis™ data.
Master Schedule:	Maintains the master schedule for the company.
SAS®:	The SAS® System is an integrated system of software products that enables a user to perform data entry, retrieval, data management, reporting, graphics, statistical analysis, and applications development.
Microsoft® Office 2003 Professional:	Bundle of integrated productivity tools including word and data processing and communications software. Contains the utilities Microsoft® Access, Excel, InfoPath, Outlook, PowerPoint, Publisher, and Word.

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Dechlorane Plus: Oral Repeat Dose Toxicity and Reproduction/Developmental Toxicity Screening Study in Rats

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Occidental Chemical Corporation
5005 LBF Freeway
Dallas, TX 75244

1.4. Sponsor Representative

Debbie Schober

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1.7. Regulatory Guideline

This protocol generally meets or exceeds draft Guideline 422, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, the Organization

1.11. Personnel

1.11.1. Study Director

Raymond E. Schroeder, M.S., D.A.B.T.®

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1.12. Proposed Study Schedule

Study Initiation Date:	Date Study Director signs Study Approval-Initiation Line in this protocol
Experimental Starting Date (OECD):	To be added by amendment
Experimental Start Date:	To be added by amendment
Experimental Termination Date:	To be added by amendment
Experimental Completion Date (OECD):	To be added by amendment.
Draft Report Mail Date:	To be added by amendment

1.13. Quality Assurance

This study will be subjected to periodic inspections and the draft and final reports will be reviewed by the Quality Assurance Department of MPI Research in accordance with MPI Research's Standard Operating Procedures. Study quality assurance inspection records will be made available to the Sponsor Representatives during visits to MPI Research.

1.14. Alteration of Design

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, MPI Research will honor such change. However, written authorization will be obtained thereafter. All protocol amendments and justifications will be documented, signed, and dated by the Study Director and Sponsor. The protocol and all amendments will be issued to the Sponsor as well as at MPI Research.

1.15. Declaration of Intent

This study may be submitted to the United States Environmental Protection Agency (EPA) and an Organization for Economic Cooperation and Development (OECD) member country.

2. TEST AND CONTROL ARTICLES

2.1. Description of Test Article

2.1.1. Identity

Dechlorane Plus (DechPlus)

A description, lot number, storage conditions, expiration date, safe handling procedures, physical properties, as well as other relevant information will be documented in the study data.

2.1.2. Test Article Properties

The Sponsor will provide documentation on the strength, purity, composition, stability, and other pertinent information on each batch of test article, unless otherwise noted. If the Sponsor does not supply the above information (e.g. certificate of analysis), this deviation will be listed as a GLP deviation in the final report.

2.2. Test Article Preparation

The test article will be used as received from the Sponsor and no adjustment will be made for purity. The test article will be mixed with an appropriate vehicle to achieve the desired concentrations. The vehicle and method of preparation will be determined based upon physical characteristics of the test article and size of batches required. Fresh suspensions will be prepared for each concentration weekly, unless the stability analysis results indicate otherwise.

2.3. Test Article Analysis

2.3.1. Homogeneity

Prior to initiation of test article administration, the test article will be prepared employing the same methods and batch sizes to be used during the study. These preparations will be at the low and high concentrations used in the study. Exact 1.0 mL samples will be taken while the preparations are stirring. Three homogeneity sample sets will be taken for a total of 18 samples. A homogeneity sample set will consist of 2 samples from each position (top, middle, and bottom) of the batch. One homogeneity sample set from each concentration will be analyzed for test article concentration. The remaining two sets of samples will be maintained as backup. All samples will be stored frozen (approximately -20°C) pending analyses or final disposition. Backup samples will be discarded at the discretion of the Study Director.

2.3.2. Stability Analysis

Appropriately sized portions of the batch preparations mixed for homogeneity analysis will be stored refrigerated (approximately 2-8°C) for up to 14 days post-preparation to determine the stability of the test article in the vehicle. Triplicate samples (exactly 1.0 mL each) will be collected at 7 and 14 days. One sample from each dosing concentration will be analyzed for test article concentration and the remaining samples will be stored frozen (approximately -20°C) and maintained as backup. Samples delivered to the analytical laboratory will be maintained at stability storage conditions pending analysis. Any unused samples or batch portions stored for purposes of stability evaluation will be discarded at the discretion of the Study Director.

2.3.3. Concentration Analysis

Triplicate samples (exactly 1.0 mL each) will be collected from each concentration and control article. One sample from each dosing concentration and control article will be analyzed for test article concentration for the first four weeks and last week of study. The remaining samples will be maintained as backup. All concentration samples will be stored frozen (approximately -20°C) pending analyses or final disposition. Backup samples will be discarded at the discretion of the Study director.

2.3.4. Analyses

All analytical work will be conducted by Sponsor's designated laboratory using an analytical method developed and validated by that laboratory. The work performed in conjunction with this study will be conducted in compliance with GLPs and subject to review by the Quality Assurance Unit (QAU) of that laboratory. The findings of their QAU will be submitted to the MPI Research Study Director and Management. A final report, including a Quality Assurance Statement, will be prepared and submitted to MPI Research for inclusion as an appendix in the main study final report. If the analyses are not conducted by GLPs, this deviation will be listed as a GLP deviation in the final report.

2.4. Reserve Sample

A reserve sample from each batch of test article used in this study will be taken and archived at MPI Research. If multiple studies are conducted with the same test article, a common reserve sample may be taken and labeled appropriately.

2.5. Test Article Disposition

Any remaining test article will be shipped to a Sponsor-designated location after completion of the study. The Sponsor will be notified prior to such shipment. Alternatively, MPI Research will dispose of the test article after completion of the study.

2.6. Description of Vehicle

2.6.1. Identity

Vehicle Name: To be determinated

A description, lot number, storage conditions, expiration date, safe handling procedures, physical properties, as well as other relevant information will be documented in the study data.

2.6.2. Vehicle/Control Article Properties

Documentation of the strength, purity, composition, stability, and other pertinent information on each batch of vehicle/control article, will be limited to that information listed on the label of this commercially available material, unless otherwise noted.

3. TEST SYSTEM

3.1. Species

Rat

3.2. Strain

CD® [Crl:CD®(SD)]

3.3. Source

Charles River Laboratories

3.4. Justification of Test System

The current state of scientific knowledge and the applicable guidelines cited previously in this protocol do not provide acceptable alternatives, *in vitro* or otherwise, to the use of live animals to accomplish the purpose of this study. "The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species".[1] "Whole animals are essential in research and testing because they best reflect the dynamic interactions between the various cells, tissues, and organs comprising the human body".[2]

The rat is the usual rodent model[3] used for evaluating the toxicity of various classes of chemicals and for which there is a large historical database.

3.5. Expected Age

3.5.1. Repeat Dose Toxicity Phase

The test animals in the will be approximately 6 weeks of age at arrival and at least 8 weeks at the start of treatment.

3.5.2. Developmental and Reproductive Toxicity Phase

The test animals will be approximately 8 weeks at receipt and at least 10 weeks at the start of treatment.

3.6. Expected Body Weight

3.6.1. Repeat Dose Toxicity Phase

The males will weigh approximately 130 to 210 g and females will weigh approximately 100 to 150 g at arrival, as measured within 3 days of arrival. The actual range may vary but will be documented in the data.

3.6.2. Developmental and Reproductive Toxicity Phase

The males will weigh approximately 160 to 230 g and females will weigh approximately 150 to 190 g at arrival, as measured within 3 days of arrival. The actual range may vary but will be documented in the study records.

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[1] "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training", Federal Register, Vol. 50, No. 97, May 20, 1985

[2] "Position Statement on the Use of Animals in Research", NIH Guide 22: No. 8, Feb.26, 1993

[3] Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies, U.S. F.D.A. Center for Drug Evaluation and Research (CDER), January 2006.

3.7. Number of Animals

3.7.1. Number Ordered

Males: 135
Females: 135

3.7.2. Number on Study

Males: 120
Females: 120

Females will be nulliparous and nonpregnant.

3.7.3. Justification for Number on Study

This study was designed to use the fewest number of animals possible, consistent with the objective of the study, the scientific needs of the Sponsor, contemporary scientific standards, and in consideration of applicable regulatory requirements cited previously in this protocol. This study is designed to use the smallest number of animals possible that will allow sufficient group sizes for meaningful statistical analysis of data.

3.7.4. Selection for Study

All animals placed on study will have body weights that fall within ±20% of the mean body weight for each sex. If not enough animals fall within this weight range to satisfy the number of animals required to go on study, the Study Director will be notified to ascertain the appropriate action to be taken.

Animals considered suitable for study will be weighed prior to treatment. After the appropriate number of animals with the highest and lowest body weights has been excluded, the remaining required number of animals on study will be randomized, by sex, into treatment groups using a simple randomization procedure.

3.7.5. Method of Identification

Each animal will be assigned an animal number to be used in Provantis™ and will be implanted with a microchip bearing a unique identification number. Pups will be identified by tattoo on approximately Lactation Day (LD) 0. The individual animal number, implant number, and the MPI Research study number will comprise a unique identification for each animal. The animal cage will be identified by the study number, animal number, group number, and sex.

3.8. Husbandry

3.8.1. Acclimation

All animals will be permitted an acclimation period of at least 2 weeks. During this acclimation period, all animals will be observed daily for any clinical signs of disease, and all animals will be given a detailed clinical examination prior to selection for study. All animals with any evidence of disease or physical abnormalities will not be selected for study. Animals considered suitable for study will be randomly selected and divided into groups as described above.

3.8.2. Housing

The animals will be individually housed in suspended, stainless steel, wire-mesh type cages, except during pairing, near parturition, and during lactation. During pairing for the developmental/reproductive phase, animals (male and female) will be housed 2/cage in extended suspended stainless steel, wire-mesh type cages. On approximately GD 20 and throughout lactation, females will be individually housed with litters in plastic cages containing wood chip bedding.

3.8.3. Environmental Conditions

Fluorescent lighting will be provided via an automatic timer for approximately 12 hours per day. On occasion, the dark cycle may be interrupted intermittently due to study-related activities. Temperature and humidity will be monitored and recorded daily and maintained to the maximum extent possible between 64 to 79° F and 30 to 70%, respectively.

3.8.4. Diet and Drinking Water

3.8.4.1. Basal Diet

The basal diet will be meal Lab Diet® Certified Rodent Diet #5002, PMI Nutrition International, Inc. This diet will be available *ad libitum* unless designated otherwise. Each lot number used will be identified in the study records.

3.8.4.2. Basal Diet Contaminants

The Study Director is not aware of any potential contaminants likely to be present in the certified diet that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier will be conducted.

3.8.4.3. Water

Tap water will be supplied *ad libitum* to all animals via an automatic water system unless otherwise indicated.

3.8.4.4. Water Contaminants

The drinking water used will be monitored for specified contaminants at periodic intervals according to MPI Research Standard Operating Procedures. The Study Director is not aware of any potential contaminants likely to be present in the water that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

4. STUDY DESIGN

4.1. Repeat Dose Toxicity Phase

Group	Dose Level mg/kg/day	Number of Animals: Initial M	Initial F	Clinical Pathology[a] M	Clinical Pathology[a] F	Functional Observational Battery M	Functional Observational Battery F	Necropsy/ Microscopic Pathology[b] M	Necropsy/ Microscopic Pathology[b] F
1	0	10	10	5	5	5	5	5	5
2	750	10	10	5	5	5	5	A.R.[c]	A.R.[c]
3	1500	10	10	5	5	5	5	A.R.[c]	A.R.[c]
4	5000	10	10	5	5	5	5	5	5

[a] Hematology will be performed on 5 randomly-selected animals/sex/group, and the remaining animals will be used for serum chemistries. These evaluations will be performed at terminal euthanasia.
[b] Tissues will be collected and preserved from all animals. Microscopic evaluations will be conducted for the five animals evaluated for serum chemistries. In addition, the testes and epididymides will be evaluated in all males and the ovaries will be evaluated in all females.
[c] A.R. = As Required: 1) Target tissues identified based on Group 4 evaluations, 2) Tissues in all animals found dead or euthanized in a moribund condition.

4.2. Developmental and Reproductive Toxicity Phase

Group	Dose Level mg/kg/day	Number of Animals: Initial Males	Initial Females	Developmental Component Females	Reproductive/Lactation Component Females	Necropsy - Males
5	0	20	20	10	10	20
6	750	20	20	10	10	20
7	1500	20	20	10	10	20
8	5000	20	20	10	10	20

5. TEST AND CONTROL ARTICLE ADMINISTRATION

5.1. Route of Administration

The test and control articles will be administered by oral gavage as a single dose daily.

5.2. Justification for Route of Administration

The oral route is one of the potential routes of human exposure to this test article.

5.3. Frequency of Administration

The test and control articles will be administered once per day.

5.4. Duration of Administration

5.4.1. Repeat Dose Toxicity Phase

The test and control articles will be administered for 28 days. The animals will be dosed up to the day prior to scheduled necropsy.

5.4.2. Developmental and Reproductive Toxicity Phase

The test article will be administered for at least 42 consecutive days to males (14 days premating, 14 days of mating, and 14 days after completion of the mating period). Females will be treated for up to 53 days dependent on reproductive performance and gestation length (14 days premating, 14 days of mating, and 25 days [GD 0-LD 3]).

5.5. Dose Levels

0 (control article), 750, 1500 and 5000 mg/kg/day; the same dose levels will be used for both phases of study.

5.6. Dose Volume

The expected dose volume for this study will be 10 mL per kg per dose

5.7. Justification of Dose Levels

The acute oral toxicity of DechPlus was examined in male rats. In that study, rats were treated with a single oral dose of 10-3160 mg/kg as 1% or 10% suspension in corn oil. No mortality occurred at any doses, and no toxicologically significant gross pathology findings were noted. The oral LD_{50} was considered to be greater than 3160 mg/kg. In a separate study, rats were treated with a single oral dose of DechPlus at a dose of 1500, 3000, 6000, 12,500 or 25,000 mg/kg. No mortalities or effects on body weights were observed over a 14-day observation period. The oral LD_{50} was considered to be greater than 25,000 mg/kg.

In a 90-day toxicity study, male and female rats were administered DechPlus in the diet at concentrations of 10,000, 30,000 or 100,000 ppm; the equivalent oral doses for males were approximately 522, 1567 and 5200 mg/kg, respectively, and for females 654, 1960 and 6500 mg/kg, respectively. Other than increased absolute and relative liver weights of rats treated with the high dietary concentration of DechPlus, no other toxicologically significant finding was observed. This study was criticized by the EPA since it had been conducted prior to implementation of GLPs and was conducted at Industrial Bio-test Laboratories in the mid-1970s.

Rats were administered a single oral dose of either 1 or 113 mg/kg radiolabeled DechPlus by gavage to examine the absorption, tissue distribution and elimination of the test article. About 93-98% of the dose was excreted in feces unchanged within 4 days. Less than 0.1% of the radiolabel was excreted in urine and only 0.004% was excreted in expired air. Radiolabel plasma levels peaked at 10 hours after dose administration and the radiolabel was at least 65% DechPlus; metabolites were no tidentified. Tissue residues did not increase proportionally with dose; a 113-fold increase in dose resulted in an 8-fold decrease in the percentage of applied dose excreted in urine and remaining in the carcass. Within 4 days of dosing, only 26% of the radiolabel remaining in the carcass was DechPlus. Ovaries and liver contained the highest levels of DechPlus.

Based on the above data, dose levels expected to be used in this study are 750, 1500 and 5000 mg/kg.

5.8. Test and Control Article Administration

The test and control articles will be administered via gavage. The control animals will receive the control article at the same volume as the test article. Individual doses will be based on the most recent body weights.

6. ANTEMORTEM STUDY EVALUATIONS

6.1. Repeat Dose Toxicity Phase

6.1.1. Cageside Observations

All animals will be observed at least twice a day for morbidity, mortality, injury, and availability of food and water. Any animals in poor health will be identified for further monitoring and possible euthanasia.

6.1.2. Detailed Clinical Examinations

A detailed clinical examination of each animal will be performed daily during the study. These examinations will be conducted 60±5 minutes post-dosing. Observations will include, but will not be limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, bizarre behavior, and palpation of tissue masses.

6.1.3. Neurobehavioral Examinations

A detailed neurobehavioral observation of each animal will be performed 1-2 hours postdose once each study week. Observations will be carefully recorded, and an effort will be made to ensure that variations in the test conditions are minimal. Animals are observed one at a time by a trained technician. The ranking scales provided below are to be used for assessment of signs of autonomic function, general motor activity, gait, posture, neuromuscular function, response to handling, the presence of tonic and clonic movements, stereotypy, and bizarre behavior.

1) The animal is observed in the home cage for level of activity and scored by the following scale:

0 – Quiet or asleep
1 – Alert with routine movement (eating, sniffing at front of cage or similar movement)
2 – Increased movement

2) The animal is removed from the home cage and placed into a 16 x 16 x 12 inch box with 5.3 x 5.3 inch grid. The animal is observed for one minute and the number of grids entered with both forefeet is counted and recorded. The animal is initially placed in a corner of the box. While in the grid box the animal is observed for tremors, convulsions, stereotypy, gait, abnormality, bizarre behavior, response to a sensory stimulus, and is scored as follows:

Tremors:
0 – Not present
1 – Present

Convulsions:

0 – Not present
1 – Present

Stereotypy:
0 – Not present
1 – Constant sniffing and periodic licking on the wall or floor
2 – Constant licking on the wall or floor
3 – Constant licking and periodic biting/gnawing of the wall or floor
4 – Constant biting/gnawing of the wall or floor
5 – Biting/gnawing of the body
6 – Self-mutilation

Gait:
0 – Normal
1 – Abnormal

Bizarre Behavior:
0 – Not Present
1 – Present (Note: A description of behavior is documented in the comments section, such as retropulsion or writhing)

3) The final observation made while the animal is in the grid box is the response to sensory stimulus. A clicker is placed approximately 5 cm above the animal's back and a click is sounded suddenly. The animal's response is scored as follows:

0 – Responded
1 – No response
2 – Exaggerated response

4) Prior to the animal's return to the cage, the forefeet are placed on the front of the cage, or on any surface where the animal is able to demonstrate the ability to grip, to evaluate grip response and is scored as follows:
0 – Gripped caging
1 – Did not grip caging.

After evaluating an animal, the grid box will be cleaned using tap water and a paper towel.

6.1.4. Body Weights

Body weights will be measured and recorded within 3 days of arrival, at least once prior to randomization, at the start of treatment, at 3- and 4-day intervals weekly during the study, and at termination.

6.1.5. Food Consumption

Food consumption will be measured and recorded weekly during the study.

6.1.6. Neurobehavioral Evaluations

6.1.6.1. Functional Observational Battery (FOB) Evaluations

FOB evaluations will be conducted pretest and on one day during the last week of test article administration for five randomly selected male and females per group (the same animals when

possible for each interval). FOB evaluations will be conducted blind to treatment group and prior to dosing. Each animal will be observed for a minimum of 3 minutes in a black plexiglass, open-field observation box measuring 20 in. by 20 in. by 8 in. Parameters evaluated will be based on those outlined in Moser[4,5]. The following evaluations will be conducted:

1) Assessment of signs of autonomic function:

Ranking of the degree of lacrimation and salivation, with a range of severity scores from none to severe.

Presence or absence of piloerection and exophthalmus.

Measurement of urination and defecation including polyuria and diarrhea.

Pupillary function as indicated by constriction of the pupil in response to light.

Degree of palpebral closure, e.g. ptosis.

2) Description, incidence and severity of convulsions, tremors, abnormal motor movements, both in the home cage and the open field.

3) Ranking of the subject's reactivity to general stimuli such as removal from the cage or handling, with a range of severity scores from no reaction to hyperreactivity.

4) Ranking of the subject's general level of activity during observations of the unperturbed subject in the open field, with a range of severity scores from unresponsive to hyperreactivity.

5) Descriptions and incidence of posture abnormalities observed in the home cage and incidence of gait abnormalities observed in the open field.

6) Ranking of any gait abnormalities, with a range of severity scores from none to severe.

7) Forelimb and hindlimb grip strength measured using the procedure described by Meyer[6].

8) Quantitative measure of landing foot (hindfoot) splay as described by Edwards and Parker[7].

9) Sensorimotor responses to stimuli of different modalities will be used to detect gross sensory deficits. Pain perception will be assessed by a ranking or measure of the reaction to a tail pinch and measuring the latency to a nociceptive (thermal) response when the subject is placed on a heated (52 °C) surface, as described by Ankier[8]. The response to a mechanically produced "click" will be ranked to assess audition.

10) Body weight.

11) Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance,

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[4] Moser VC, McCormick JP, Creason JP, MacPhail RC. Comparison of chlordimeform and carbaryl using a functional observational battery. Fund Appl Toxicol 1988;11:189-206.

[5] Moser VC, Ross JF. US EPA/AIHC Training video and reference manual for a functional observational battery. US Environmental Protection Agency 1996; Washington, D.C.

[6] Meyer OA, Tilson HA, Byrd WC, Riley MT. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav Toxicol 1979;1:233-236.

[7] Edwards PM, Parker VH. A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol 1977;40:589-591.

[8] Ankier SI. New hot plate tests to quantify anti-nociceptive and narcotic antagonist activities. Eur J Pharmacol 1974;27:1-4.

red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

12) Other measures to be recorded are count of rearing activity in the open field, ranking of air righting, body temperature measured rectally, spontaneous or excessive vocalizations, alterations in rate and ease of respiration (e.g. rales or dyspnea), and sensorimotor responses to visual (approaching blunt object) and proprioceptive (touch on rump with blunt object) stimuli.

6.1.6.2. Motor Activity

Motor activity will be evaluated for the same five animals/sex/group used for the FOB evaluations and conducted pretest and one day during the last week of study. Motor activity will be evaluated before dosing. The activity of each animal will be assessed using a Hamilton Kinder Motor Monitor System equipped with an electronic analyzer-recorder.

The activity chamber is 16 x 16 x 12 inches and the test period is 30 minutes (3 consecutive 10 minute intervals/animal). Movement is recorded by 16 photocell sensors each in 1 vertical and 2 horizontal planes. The activity chambers are calibrated daily prior to testing. The overhead lights in the test room are turned off, and the animals are acclimated for a minimum of 5 minutes before testing begins.

The following activity is recorded:

- Basic movements
- Fine movements
- Rearing counts
- Total distance

6.1.7. Clinical Pathology

The animals will have free access to drinking water but will be fasted overnight prior to sample collection. Blood samples (3-4 mL) will be collected via the vena cava after carbon dioxide inhalation. The order of bleeding will be alternating (one animal from each dose group, then repeating) to reduce handling and time biases.

The following clinical pathology tests will be conducted.

6.1.8. Hematology

6.1.8.1. Number of Animals

5 per sex per group (Groups 1-4) (first five animals per group)

6.1.8.2. Collection Intervals

At termination (Study Day 29)

6.1.8.3. Parameters Evaluated

- leukocyte count (total and differential)
- erythrocyte count
- hemoglobin
- hematocrit

- mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated)
- absolute and percent reticulocytes
- platelet count

6.1.9. Coagulation

6.1.9.1. Number of Animals

5 per sex per group (Groups 1-4) (same animals used for Hematology evaluations)

6.1.9.2. Collection Intervals

At termination (Study Day 29)

6.1.9.3. Parameters Evaluated

- prothrombin time
- activated partial thromboplastin time

6.1.10. Clinical Chemistry

6.1.10.1. Number of Animals

5 per sex per group (Groups 1-4) (the remaining five animals per group)

6.1.10.2. Collection Intervals

At termination

6.1.10.3. Parameters Evaluated

- alkaline phosphatase
- total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dL)
- aspartate aminotransferase
- alanine aminotransferase
- gamma glutamyl transferase
- sorbitol dehydrogenase
- urea nitrogen
- creatinine
- total protein
- albumin
- globulin and A/G (albumin/globulin) ratio (calculated)
- glucose
- total cholesterol
- electrolytes (sodium, potassium, chloride)
- calcium
- phosphorus

6.2. Developmental and Reproductive Toxicity Phase

6.2.1. Cageside Observations

All animals will be observed at least twice a day for morbidity, mortality, injury, and availability of food and water. Any animals in poor health will be identified for further monitoring and possible euthanasia.

6.2.2. Detailed Clinical Examinations

A detailed clinical examination of each animal will be performed daily during the study. These examinations will be conducted 60±5 minutes post-dosing. Observations will include, but will not be limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, bizarre behavior, and palpation of tissue masses.

6.2.3. Body Weights

Body weights will be measured and recorded within 3 days of arrival, at least once prior to randomization, at the start of treatment, and at 3- and 4-day intervals during the premating period. Males and unmated females will be weighed at 3- and 4-day intervals thereafter and mated females will be weighed on GD 0, 3, 7, 10, 14, 17 and 20. Females allowed to deliver and retain litter to LD 4 will be weighed on LD 0 and 4.

6.2.4. Food Consumption

Food consumption will be measured and recorded weekly during the premating period and mated females will have food consumption recorded at corresponding intervals with body weights during gestation. Food consumption will not be recorded during the pairing period. Food consumption will be recorded for males weekly following completion of the 14-day pairing period until terminal euthanasia.

6.3. Estrous Cyclicity

At initiation of test article administration and until evidence of copulation is observed or the cohabitation period has ended, the females will be examined daily to establish estrous cycles. Vaginal lavage will be completed daily by 10:00AM.

6.4. Breeding Procedures

After 2 weeks of treatment, the males will be randomly cohabited, 1 male to 1 female, with the corresponding females in the treatment and control groups. Mating will take place in the cage of the male.

Vaginal smears will be performed daily on females during the mating period and the presence or absence of sperm or vaginal plug will be recorded. The day on which evidence of mating is observed will be designated as GD 0. The rats will be paired a maximum of 14 days. If evidence of mating is noted, the female will be removed from the cage of the male and individually housed for the remainder of the study. After the pairing, any unmated females will be individually housed in a plastic cage with wood chip bedding.

After the mating period, the males will be individually housed in suspended, stainless-steel, wire mesh type caging and continued on treatment for approximately 2 weeks. At the end of this period, the males will be euthanized and necropsied.

6.5. Parturition and F_1 Litter Observations

Toward the end of the gestation period, all mated females not selected for GD 20 euthanasia and uterine/fetal evaluations will be allowed to give birth (F_1). The duration of gestation will be calculated (females with confirmed GD 0), and any difficulties occurring at parturition will be recorded. The day on which all pups have been delivered will be designated as LD 0. The litters will be examined as soon as possible after delivery, and the following parameters will be recorded:

- Litter size
- Number of stillborn pups
- Number of live born pups
- Number of male and female pups
- Gross abnormalities of the pups

Individual pup body weights and pup external examinations will be recorded on LD 0 and 4. Any abnormal behavior observed in the pups will be recorded daily. On LD 4, the dams and pups will be euthanized and necropsied.

7. EUTHANASIA

7.1. Moribundity

Any moribund animals, as defined by a Testing Facility Standard Operating Procedure, will be euthanized for humane reasons and to prevent the loss of tissues through autolysis. All animals euthanized *in extremis* or found dead will be subjected to a routine necropsy. Where practical, a full set of tissues as listed in the Postmortem Study Evaluations portion of this protocol will be collected and preserved in the appropriate fixative.

7.2. Method of Euthanasia

Euthanasia of the parental animals will be by carbon dioxide inhalation followed by an MPI Research SOP approved method to ensure death, e.g. exsanguination. Euthanasia of fetuses/pups (LD 0-4) will be by an intraperitoneal injection of sodium pentobarbital solution.

7.3. Final Disposition

All surviving animals placed on study will be euthanized at their scheduled necropsy or, if necessary, euthanized *in extremis*. Extra animals obtained for this study, but not placed on study, will be transferred to either an MPI Research stock or training colony, or euthanized and discarded. The final disposition of each animal will be documented in the study records.

8. POSTMORTEM STUDY EVALUATIONS

8.1. Repeat Dose Toxicity Phase

Complete necropsy examinations will be performed under procedures approved by a veterinary pathologist on all animals dying spontaneously, euthanized *in extremis*, or euthanized at scheduled necropsies. Examinations will be performed 7 days a week. Animals that are found dead after regular working hours will be refrigerated overnight and necropsies performed at the start of the next working day. At the termination of the study (Day 29) all appropriate animals will be euthanized and examined.

The animals will be examined carefully for external abnormalities including palpable masses. The skin will be reflected from a ventral midline incision, and any subcutaneous masses will be identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities will be examined for abnormalities and the organs removed, examined, and, where required, placed in fixative. The pituitary will be fixed *in situ*. The testes and epididymides will be fixed using a modified Davidson's fixative[9]. All other tissues will be fixed in neutral buffered formalin. Formalin will be infused into the lung via the trachea and into the urinary bladder.

Body weight and the organ weights identified in the following table will be recorded for all animals at scheduled necropsies, and appropriate organ weight ratios will be calculated (relative to body and brain weights). Paired organs will be weighed together. Organs will not be weighed for animals dying spontaneously or euthanized *in extremis*. A combined weight of the thyroid gland with bilateral parathyroid glands will be obtained, and the right mandibular/sublingual salivary glands will be weighed together. The thyroid/parathyroid gland and pituitary gland will be weighed following fixation.

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections will be performed on sections of tissues identified in the following table from five males and females in the control and high-dose groups (same animals evaluated for serum chemistry). If treatment related effects are seen, the affected tissue(s) from the remaining animals in these groups will be processed and evaluated microscopically and these tissues evaluated for the animals at the lower dose levels until a no effect dose level has been determined (additional cost consideration).

Organs or Tissues to be Weighed, Preserved and Microscopically Examined

Tissue	Organ Weight Taken (all animals)	Collected and Preserved (all animals)	Microscopic Examination Groups: 1,4	2, 3
Adrenal gland	X	X	X	

[9] Latendresse JR, Warbrittion AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol. 2002 Jul-Aug;30(4):524-33.

Tissue	Organ Weight Taken (all animals)	Collected and Preserved (all animals)	Microscopic Examination Groups: 1,4	2, 3
Bone marrow (sternum)[a]		X	X	
Brain (medulla/pons, cerebrum, cerebellum)	X	X	X	
Epididymis[d, e]	X	X	X	X
Heart	X	X	X	
Kidney	X	X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Liver	X	X	X	
Lung		X	X	
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Lymph node, regional (if applicable)[b]		X	X	
Nerve (sciatic)		X	X	
Prostate[d]		X	X	
Seminal vesicles (with coagulating glands attached)[d]		X	X	
Ovaries[d, e]	X	X	X	X
Peyer's patches		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, thoracic		X	X	
Spinal cord, lumbar		X	X	
Spleen	X	X	X	
Stomach		X	X	
Testes[d,e]	X	X	X	X
Thymus	X	X	X	
Thyroid (with parathyroid)[c]		X	X	

Tissue	Organ Weight Taken (all animals)	Collected and Preserved (all animals)	Microscopic Examination Groups: 1,4[f]	2, 3
Trachea		X	X	
Urinary bladder		X	X	
Uterus with oviduct[d]		X	X	
Gross lesions		X	X	

[a] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals.*
[b] *A regional lymph node drains the region where a tissue mass is located.*
[c] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*
[d] *Tissues will be collected and preserved from all animals.*
[e] *Tissues will be microscopically examined in all males and/or females.*
[f] *Microscopic evaluations conducted for five males and females per group (same animals whenpossible evaluated for serum chemistrie with the exception of the tissues evaluated for all animals (Footnote "e").*

The pathologist as needed to aid in the diagnosis of specific lesions may use special stains and techniques. If after routine sectioning, a tissue is missed, the block will be resectioned once or the tissue re-embedded for resectioning. If the tissue is still missing, the block will not be resectioned unless the missing tissue is determined to be a target organ. In this case, the tissue will be resectioned until located or until it is determined that it is not present in the block or in wet tissue. All missing tissues will be identified in the pathology portion of the final report. Tissues which are unintentionally sectioned or are present in the plane with a required tissue, though not required by protocol, will be examined and documented, if abnormal.

8.2. Developmental and Reproductive Toxicity Phase

8.2.1. Uterine and Ovarian Examinations

In the developmental toxicity component, 10 mated females (randomly selected from animals with confirmed GD 0 dates and GD 20 dates that fall within the Monday – Friday workweek) will be euthanized and immediately subjected to a cesarean section. The skin will be reflected from a ventral midline incision to examine mammary tissue and locate any subcutaneous masses. The abdominal cavity will be opened, and the uterus will be exposed. The uterus will be excised, and the gravid uterine weight will be recorded. Beginning at the distal end of the left uterine horn, the location of viable and nonviable fetuses, early and late resorptions for each uterine horn, position of the cervix, and the number of total implantations will be recorded. The number of corpora lutea on each ovary will also be recorded. The embryonic membrane of each fetus will be gently removed, and each fetus will be pulled away from the placenta, fully extending the umbilical cord. The fetuses will be removed from the uterus. The placentae will be grossly examined.

Each implant will be categorized according to the following criteria.

- Viable fetus - responds to touch
- Nonviable fetus - does not respond to touch, no signs of autolysis
- Late resorption - recognizable fetal form, but undergoing autolysis
- Early resorption - implantation site, tissue has no recognizable fetal characteristics

Uteri from females that appear nongravid will be opened and placed in 10% ammonium sulfide solution for detection of implantation sites[10]. The foci, if detected, will be considered early resorptions, and data from this female will be included in mean calculations. If no foci are seen, the female is considered to be nonpregnant.

A necropsy will be conducted on each dam. All tissues listed in the table above (Section 8.1) will be saved with the exception of the uterus. This will be discarded.

8.2.2. Fetal Evaluations

Fetuses will be individually sexed, tagged, and examined for external malformations and variations. Following completion of the external examination of all fetuses in the litter, each fetus will be euthanized via intraperitoneal injection of sodium pentobarbital solution and weighed. Approximately one-half of the fetuses in each litter will be decapitated and the head section placed in Bouin's solution. All fetuses will be processed for visceral examination using a microdissection procedure. Heads will be processed for evaluation using a razor-blade sectioning technique. Following the microdissection evaluations, the viscera from each fetus will be removed and the carcasses preserved in 95% ethanol for possible further evaluation of the skeletal structures (additional cost consideration). Fetal findings will be classified as malformations or developmental variations under criteria approved by a developmental toxicologist, unless otherwise specified.

8.2.3. Males and Females Reproductive Phase

At the termination of the study, all surviving adult animals (males, females with litters, non-pregnant females reproductive phase) will be euthanized and necropsied. The animals will be examined carefully for external abnormalities including palpable masses. The skin will be reflected from a ventral midline incision and any subcutaneous masses will be identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities will be examined for abnormalities. Special emphasis will be placed on organs of the reproductive system. Implantation sites will be counted and recorded in females.

The epididymides and testes will be weighed and the weights recorded. All tissues listed in the Table in Section 8.1 will be preserved for possible further evaluation (additional cost consideration).

8.2.4. F_1 Pups

On LD 4, pups will be euthanized and externally examined, and any abnormalities will be recorded. The carcasses will then be discarded. Dead pups found at birth or during LD 0-4 and

[10] Kopf R, Lorenz D, Salewski E. The effects of thalidomide on the fertility of rats studied in two generations. Naunyn Schmiedebergs Arch Pharmacol 1964;247:121-135.

pups euthanized *in extremis* will be examined internally for irregularities and only pups with gross lesions or malformations will be saved (10% neural buffered formalin) for possible further evaluation (additional cost consideration).

9. STATISTICS

9.1. Repeat Dose Toxicity Phase Parameters

The following is the proposed analysis plan to be used when data assumptions are met. If there are deviations to this plan due to violations of assumptions or if any other techniques are used (Sponsor consulted), they will be documented in the final report.

Table of Statistical Comparisons

Control Group	Comparison Groups
1	2, 3, 4

The above table defines the set(s) of comparisons to be used in the statistical analyses described below. If more than one set of comparisons is required, all analyses will be conducted separately on each set unless stated otherwise. Data for each sex within a set will also be analyzed separately.

The raw data will be tabulated within each time interval, and the mean and standard deviation and/or incidence counts (categorical variables) will be calculated for each endpoint by sex and group. For each endpoint, treatment groups will be compared to the control group using the analysis outlined below. Data for some endpoints, as indicated, will be transformed by either a log or rank transformation prior to conducting the specified analysis.

Endpoints	Type of Analysis
Body Weights Food Consumption Hematology (except leukocyte counts) Coagulation Clinical Chemistry Organ Weights Absolute Weights Relative to Body and Brain Weights FOB (Continuous Endpoints) Body Weight Body Temperature Defecation Urination Rearing Thermal Response Forelimb Grip Strength Hindlimb Grip Strength	Group Pair-wise Comparisons (Levene's/ANOVA-Dunnett's/Welch's)

Endpoint	Analysis
Nonviable Fetuses/dam	Descriptive Statistics
Total Number Resorptions/dam	Group Pair-wise Comparisons
Number Early Resorptions/dam	Group Pair-wise Comparisons
Number Late Resorptions/dam	Group Pair-wise Comparisons
% Preimplantation Loss	Arcsin-Square-Root Transformation
% Postimplantation Loss	Arcsin-Square-Root Transformation
Mean Fetal Body Weights	Covariate Analysis
Malformations by finding and exam type (external and visceral)-litter incidence[a]	Fisher's Exact Test
Variations by finding and exam type (external and visceral)-litter incidence[a]	Fisher's Exact Test
Total Malformations (external and visceral combined)-litter incidence[a]	Fisher's Exact Test

[a]Fetal and litter incidences will be reported, but only the litter incidences will be statistically analyzed.

9.3. Reproductive Toxicity Parameters

The following is the proposed analysis plan to be used when data assumptions are met. If there are deviations to this plan due to violations of assumptions or if any other techniques are used (Sponsor consulted), they will be documented in the final report.

Table of Statistical Comparisons

Control Group	Comparison Groups
5	6, 7, and 8

The above table defines the set(s) of comparisons to be used in the statistical analyses described below. If more than one set of comparisons is required, all analyses will be conducted separately on each set unless stated otherwise. Data for each sex within a set will be analyzed separately.

The following endpoints will be analyzed according to the table below.

Endpoint	Analysis
Parental In-life Data	
Premating Body Weights (F_1)	Group Pair-wise Comparisons
Lactation Body Weights (P)	Group Pair-wise Comparisons
Lactation Body Weight Changes (P)	Group Pair-wise Comparisons
Fertility Indices	
Gestation Length (P)	Group Pair-wise Comparisons
Copulatory Interval (F_1)	Group Pair-wise Comparisons
Male Fertility Index (F_1)	Fisher's Exact Test
Female Fertility Index (F_1)	Fisher's Exact Test
Female Mating Index (F_1)	Fisher's Exact Test

Endpoint	Analysis
Male Mating Index (F_1)	Fisher's Exact Test
Female Fecundity Index (P, F_1)	Fisher's Exact Test
Male Fecundity Index (F_1)	Fisher's Exact Test
Gestation Index (P)	Fisher's Exact Test
Estrous Cycle (mean cycle time)	Group Pair-wise Comparisons
Estrous Cycle (# cycles/period)	Group Pair-wise Comparisons
Uterine Exam	
Number Implantations/dam (P, F_1)	Group Pair-wise Comparisons
Litter	
Litter Size	Group Pair-wise Comparisons
Viable Pups	Group Pair-wise Comparisons
Pup Sex Ratio (% viable males/litter)	Arcsin-Square-Root Transformation
Stillborn Pups	Group Pair-wise Comparisons
Stillborn Index	Arcsin-Square-Root Transformation
Pup Weights	Covariate Analysis
Pup Survival (Days 0-4)	Arcsin-Square-Root Transformation

9.4. Statistical Analyses

9.4.1. Group Pair-Wise Comparisons (Levene's/ANOVA-Dunnett's/Welch's)

If sample sizes for all groups are 3 or greater, Levene's test[11] will be used to assess homogeneity of group variances for each specified endpoint (see table above) and for all collection intervals. If Levene's test is not significant ($p \geq 0.01$), a pooled estimate of the variance (Mean Square Error or MSE) will be computed from a one-way analysis of variance (ANOVA) and utilized by a Dunnett's[12] comparison of each treatment group with the control group. If Levene's test is significant ($p < 0.01$), comparisons with the control group will be made using Welch's t-test[13] with a Bonferroni correction.

In the case that sample size is less than 3 for at least one treatment group, Levene's method cannot be implemented. Groups with sample sizes less than 3 will be excluded from the analysis and control-treatment pairwise comparisons that satisfy the sample size assumption ($n \geq 3$) will be conducted using Welch's t-test with a Bonferroni correction.

If there are only 2 groups involved, the above methodology applies and the Dunnett's test reduces to a Student's t-test[14].

[11] Milliken GA, Johnson DE. Analysis of messy data. London: Chapman and Hall: 1992.
[12] Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 1955;50:1096-1121.
[13] Welch BL. The significance of difference between two means when the population variances are unequal. Biometrika 1937;29:350-362.
[14] Steel RGD, Torrie JH. Principles and Procedures of Statistics. A biometrical approach. New York: McGraw-Hill; 1980.

Results of all pair-wise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise.

9.4.2. Arcsin-Square-Root Transformation Followed by Group-Pairwise Comparisons

Data comprised of percent values will be transformed using the arcsin of the square root[4]. The analysis described in the Group Pair-Wise Comparison paragraph will be used to analyze the transformed percentage values.

9.4.3. Fisher's Exact Test

An overall test for association between response and treatment will be done using a Fisher's exact[15] test. If this overall test is significant ($p < 0.05$) and there are more than two groups, then a follow up analysis will be done where each treatment group will be compared to the control group using a Fisher's exact test. Results will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise.

Overall testing and follow up pair-wise testing will be conducted for the control and all treatment groups that have sufficient sample size ($n \geq 3$). If all groups have an insufficient sample size ($n<3$), or if just the control group has a sample size of less than 3, no testing will be performed.

9.4.4. Covariate Analysis

For groups with sample sizes greater than or equal to 3, an analysis of covariance[16] will be conducted to compare treatment groups for each endpoint (mean of fetal bodyweights or mean of pup body weights per dam) and time period. The covariate will be litter size. If the control group does not satisfy the sample size assumption ($n<3$), no further analysis will be performed. Each treatment satisfying the sample size assumption will be compared to control using Dunnett's test under the analysis of covariance model.

The LSMEANS (Least Square Means), which are the means adjusted for values of the covariate, will be displayed on the summary table.

Results of pairwise comparisons will be reported at the 0.05 and 0.01 significance levels. Endpoints will be analyzed using two-tailed tests unless indicated otherwise.

9.4.5. Cochran Mantel Haenszel Test

For endpoints that describe categories rather than numerical measures, a test of association between response and treatment using Cochran Mantel Haenszel[17] tests will be conducted if there is sufficient variability among the groups.

Results of all pairwise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise.

[15] Zar JH. Biostatistical Analysis. 4th ed. New Jersey: Prentice Hall; 1999.
[16] Snedecor, GW, Cochran, WG. Statistical Methods, Eighth Edition. Ames (IA): Iowa State Press; 1989.
[17] Agresti A. Categorical data analysis. New York: John Wiley and Sons; 1990.

9.4.6. Descriptive Statistics

For continuous endpoints, descriptive statistics will consist of means, standard deviations, and group size for each group and time period. Incidence counts and percentages will be provided for categorical endpoints.

10. STUDY REPORTS

10.1. Progress/Status Reports

Regular progress reports will be submitted to the Sponsor throughout the study. The frequency of these reports will be determined after consultation with the Sponsor.

10.2. Final Report

After completion of the study, a comprehensive draft report containing the results of all tests, analyses, observations and measurements required by this protocol, and an interpretative summary of the study results will be submitted to the Sponsor. The report will include all items required by the applicable regulatory agency. After receipt of any Sponsor comments, 2 copies (1 bound and 1 unbound) of the final report will be issued.

Six months after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor. Any modifications or changes to the draft report requested 6 months after issuance of the draft will be performed at additional cost to the Sponsor.

11. DATA AND SPECIMEN RETENTION

All raw data, documentation, records, protocol, reserve samples, specimens (slides, blocks, and wet tissues), and the final report generated as a result of this study will be retained at MPI Research, or an approved archive facility contracted by MPI Research, for a period of 1 year following completion of the study (final report issue date). All data generated at the Sponsor's designated laboratory will be retained at that laboratory for at least the same time period. Retention of materials after the time stated above will be subject to a future contractual agreement between the Sponsor and MPI Research.

12. ANIMAL WELFARE

MPI Research is committed to complying with all applicable regulations governing the care and use of laboratory animals. Animal welfare for this study will be in compliance with the U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR Parts 1, 2 and 3). The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 1996, will be followed. This facility maintains an Animal Welfare Assurance statement with National Institutes of Health, Office of Laboratory Animal Welfare.

To ensure compliance:

A. This protocol will be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) before animal receipt or transfer.

B. The Sponsor, by his or her signature, attests that the activities specified in this protocol do not unnecessarily duplicate any previous experiment.

C. The Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animal and has signified that (select one):

__ i.) The relevant supervisory government agency currently gives no alternatives.

X ii.) The following literature searches have been performed to determine whether an alternative species could be used or another procedure to reduce any pain or distress was available and none was found.

Date: November 2, 2006 Literature Search Reference Number: 0001
Interval Searched: All years to Present
Search terms: general alternative testing methods; alternative testing methods, toxicology; general toxicology testing method alternatives
Databases searched: toxnet.nlm.nih.gov;pubmed.gov;medscape.com;caat.jhsph.edu

Date: November 2, 2006 Literature Search Reference Number: 0002
Interval Searched: All years to Present
Search terms: alternative testing methods rodents; alternative toxicology methods rodents; rodents, alternative testing; testing methods rodents
Databases searched: toxnet.nlm.nih.gov; pubmed.gov; medscape.com; caat.jhsph.edu

__ iii.) This study does not require any procedures that may cause more than slight or momentary pain or distress to the animal. Note, unknown test articles are presumed to have the potential to cause more than slight pain or distress.

D. The Study Director has determined that the withholding of agents such as analgesics or sedatives (unless specified in this protocol) is justified for scientific reasons. The purpose of the study is to provide some side effects in animals at least in the high dose under study. Regulations and standards of government agencies require that the affected animals remain on study unless in severe (or chronic) pain and or otherwise moribund.

13. SIGNATURES

13.1. Sponsor Approval

DRAFT PROTOCOL - DO NOT SIGN

Debbie Schober — Date
Sponsor Representative

William J. Brock, Ph.D., D.A.B.T.®, Fellow ATS — Date
Sponsor Representative and Study Monitor

13.2. Study Director Approval/Study Initiation

Raymond E. Schroeder, M.S., D.A.B.T.® — Date
Study Director