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PRIMEDICA ARGUS RES LAB		
Document Title		
INITIAL SUBMISSION: ORAL (GAVAGE) DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF 10001573.01 IN RATS, WITH ATTACHMENTS & COVER LETTER DATED 10/03/00 (SANITIZED)		
Chemical Category		
CYCLOHEXYL ACETAL ETHOXYLATE (CONFIDENTIAL)		

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2000 OCT 10 PM 12: 07

Procter & Gamble

The Procter & Gamble Company
Ivorydale Technical Center
5299 Spring Grove Avenue, Cincinnati, Ohio 45217-1087

October 3, 2000

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Document Processing Center (TS-790)
Attention: Section 8(e) Coordinator
Office of Toxic Substances
U.S. Environmental Protection Agency
1200 Pennsylvania Ave..
Washington, D. C. 20460

MR 40064

RE: TSCA Section 8(e) Submission

RE: -- Cyclohexyl acetal ethoxylate

ATTN.: TSCA Section 8(e) Coordinator

This submission of information is made as required by TSCA Section 8(e) regulations and guidance.

The enclosed information does not, however, scientifically support the conclusion that the test material poses a substantial risk of injury to people or the environment.

This communication outlines preliminary findings from a dose-setting pre-study designed to identify doses which result in toxic effects and conducted to identify appropriate dose levels for a definitive, larger scale developmental toxicity study. Findings of maternal toxicity and fetal toxicity for the administration of high doses of the test material to female rats are described below. These findings of maternal and fetal toxicity are expected in this type of study and do not alter the safety profile of the test material.

There were two deaths during the study which were considered related to the test material. Both were in the high dose group. One rat, 19265, was found dead on day 10 of gestation. Another animal, rat 19258 was killed on day 12 of gestation. This rat was considered moribund and was killed for humane reasons as specified in the protocol. The description of the clinical signs and observations made at necropsy are included in attachment 3.

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Another rat in the high dose group, rat 19264, was found dead on day six but this death appeared to be the result of intubation error. Observation and conclusions from the test facility are in attachment 4.

Both animals whose death on study was related to the test substance displayed adverse clinical signs prior to death. These clinical signs included excess salivation, urine stained abdominal fur, and red perioral staining.

Clinical findings were also noted in other animals on study. These included excess salivation and the appearance of a red perioral substance in the 100, 400 and 750 mg/kg/day group. Also noted was ungroomed coat and urine stained abdominal fur in the 400 and 750 mg/kg/day groups with muscle flaccidity and ptosis in the 750 mg/kg/day group. These observations were not limited to the two animals which died on study but also were noted for other animals in the 100, 400 and 750 mg/kg/day dose groups. The frequency of these observations appeared to increase with increasing dose. These

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results are described in the unaudited draft summary provided by the test facility at the end of the in-life portion of the range finding study (attachment 5).

There was a dose-dependent reduction in maternal body weight gain which corresponded to a concomitant reduction in feed consumption. Body weight gains were reduced throughout the study in the 100, 400 and 750 mg/kg/day groups. Animals in the 10 mg/kg/day group gained weight comparably to the control animals.

Fetal body weights were reduced approximately 34% in the 750 mg/kg/day group and approximately 8% in the 400 mg/kg/day group. Fetal weights in the 10 and 100 mg/kg/day dose group animals were comparable to controls. The statistical analyses of the fetal body weights are included in attachment 6.

No other test related observation were noted. The liter averages for corpora lutea, implantation sites, litter size, average number of live fetuses, early and late resorptions, percent resorbed conspectuses and percent live male fetuses were comparable among for four dose groups and the control.

All observations are within the spectrum of effects expected for this test substance. These effects were generated under the exaggerated conditions of a range finding study design with the intention of generating adverse effects. There was no evidence of teratology from this range finding study and nothing in these observations changes the safety profile of the test substance.

We have handled and will continue to handle this material with appropriate caution in our work environment in keeping with our standard procedures for handling all chemical substances. We will communicate appropriate hazard information for the test substance by both labels and MSDS.

We are requesting that the specific chemical identity of the test material be treated as confidential information in this submission. We have not publicly disclosed any business interest or plans regarding the test substance. Measures to protect confidential information include "need to know" internal restrictions within the Company, confidential disclosure agreements with potential suppliers, and confidentiality restrictions imposed upon information shared with the agency. Security at our technical centers is excellent and knowledge of R&D activities pertaining to this material has been carefully restricted to employees who have a need to know. Disclosure of the specific chemical identity of the test material would provide competitors with valuable knowledge which is not otherwise available and which could significantly reduce the competitive advantage normally associated with the development and commercialization of new products.

Please note that we have circled the information which we regard as legally confidential. This circled information has been deleted from a second, public display version of this submission. In the event of a proposed disclosure, notice should be given to J. C. McGregor at 513/622-5420.

If you wish further information, please contact me.

Very truly yours,

THE PROCTER AND GAMBLE COMPANY

C. D. Hassall, Ph.D.
Director
Professional & Regulatory Sciences
Telephone: 513/983-4097

A 06

Attachment 2

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

PROTOCOL 916-045P

SPONSOR'S STUDY NUMBER: 999-51613

STUDY TITLE: Oral (Gavage) Dosage-Range Developmental Toxicity Study of 10001573.01 in Rats

PURPOSE: The purpose of this study is to provide information for the selection of dosages to be used in the developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of 10001573.01 administered orally via gavage to Crl:CD®(SD)IGS BR VAF/Plus® presumed pregnant female rats.

TESTING FACILITY:
Primedica Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1297
USA
Telephone: (215) 443-8710
Telefax: (215) 443-8587

STUDY DIRECTOR:
Raymond G. York, Ph.D., DABT
Associate Director of Research
raymond.york@primedica.com

SPONSOR:
N.V. Procter & Gamble Eurocor, S.A.
Temselaan 55 & 100
B 1853, Strombeek-Bever
BELGIUM

STUDY MONITOR:
Petra Kem, Ph.D.
Toxicologist
Telephone: 011-32-2-456-4614
Telefax: 011-32-2-456-2850

ALTERNATE SPONSOR CONTACT:
Pauline M. McNamee, Ph.D., A.I.M.L.S.
Principal Scientist
Telephone: 011-44-1784-495120
Telefax: 011-44-1784-495043

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REGULATORY CITATIONS:

U.S. Environmental Protection Agency (1998). Health Effects Test Guidelines; Prenatal Developmental Toxicity Study. Office of Prevention, Pesticides and Toxic Substances (OPPTS) 870.3700, August, 1998

U.S. Environmental Protection Agency (1997). Toxic Substances Control Act (TSCA) Test Guidelines; Final Rule. Prenatal Developmental Toxicity, 799.9370 (cross-referenced to OPPTS 870.3700). *Federal Register*, August 15, 1997.

Organisation for Economic Co-operation and Development (1981). *OECD Guidelines for Testing of Chemicals.* Section 4, No. 414: Teratogenicity, adopted 12 May 1981.

Japanese Ministry of Agriculture, Forestry and Fisheries (1985). *Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration.* 59 NohSan No. 4200.

U.S. Environmental Protection Agency. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 792.

U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160.

Organization for Economic Cooperation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97)186/Final].

Japanese Ministry of Agriculture, Forestry and Fisheries (1984). *Good Laboratory Practice Standards.* 59 NohSan No. 3850.

REGULATORY COMPLIANCE:

This study will be conducted in compliance with the Good Laboratory Practice (GLP) regulations cited above.

All changes or revisions of this protocol shall be documented, signed by the Study Director and the Sponsor, dated and maintained with the protocol.

The Testing Facility's Quality Assurance Unit (QAU) will audit the protocol, the raw data and the report, and will inspect critical phases of those portions of the study conducted at the Testing Facility in accordance with the Standard Operating Procedures of Argus Research Laboratories, Inc.

Should any portion of the study be conducted by a subcontractor or by the Sponsor, the QAU for that facility will conduct critical phase inspections and audit respective results and reports for that study portion according to the SOPs of that facility. Such critical phase inspection reports and report audits will be submitted by that facility to the Study Director. The dates of the inspections and report submissions will be incorporated into a QAU Statement generated by that facility and provided to the Testing Facility for inclusion in the final report.

The final report will include a compliance statement signed by the Study Director that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study. Should significant deviations from GLP regulations occur, each will be described in detail, together with how the deviation might affect the quality or integrity of the study.

SCHEMATIC OF STUDY DESIGN AND STUDY SCHEDULE:

See ATTACHMENT 1 to the protocol.

TEST SUBSTANCE AND VEHICLE:

Identification:

Test Substance:

10001573.01 (where ".01" indicates the lot identification of the test substance).

Information on the identity, composition, method of synthesis, strength and purity of the test substance will be included in the Analytico Analytical Contributing Scientist Report, provided to the Study Director by the Sponsor for inclusion in the final report.

Documentation of the method of synthesis is on file with the Sponsor.

Vehicle:

Corn Oil, Mazola® (Lot identification will be documented in the raw data).

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the vehicle that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

Safety Precautions:

Gloves, mask, appropriate eye protection and uniform/lab coat to be worn during formulation preparation and dosage. The Material Safety Data Sheet is attached to the protocol (ATTACHMENT 2).

Storage:

Bulk Test Substance:	Room temperature, protected from light.
Vehicle:	Room temperature.
Prepared Formulations:	Room temperature, protected from light.

All test substance shipments to the Testing Facility should be addressed to the attention of Julian Gulbinski, Manager of Formulations, at the previously cited address and telephone number.

Shipments should include information concerning storage conditions and shipping cartons should be labeled appropriately. The recipient should be notified in advance of shipment.

FORMULATION:

Frequency of Preparation:

Formulations (solutions) will be prepared weekly at the Testing Facility.

Detailed preparation procedures are attached to this protocol (ATTACHMENT 3).

Adjustment for Purity:

The test substance will be considered 98.6% pure for the purpose of dosage calculations. Dose calculations will be adjusted for purity.

Testing Facility Reserve Samples:

The Testing Facility will reserve a (10 mL) sample of each lot of bulk test substance and vehicle used during the course of the study. Samples will be stored under the previously cited conditions.

ANALYSES:

The Sponsor is responsible for stability of the bulk test substance and by signature of this protocol indicates that the test substance is stable under the conditions of this study. Stability data are on file with the sponsor.

Samples additional to those described below may be taken if deemed necessary during the course of the study.

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Stability of Prepared Test Substance Formulations:

The Sponsor is responsible for stability of the test substance in formulation with the vehicle at the concentrations and conditions of the this study. Information supporting the stability of the prepared test substance formulations will be included in the Analytico Analytical Contributing Scientist Report provided to the Study Director by the Sponsor for inclusion in the final report.

Stability of the dose formulations will be determined concurrently with this study. Triplicate stability samples (approximately 5 mL) will be taken from the middle of the low and the high dose level test article formulations on the first day of preparation. Duplicate samples will be shipped for analysis; the remaining samples will be retained at the Testing Facility as backup samples. The samples will be stored at room temperature and protected from light until analyses to establish stability are performed on days 3 (72 hours post-preparation), 8 and 10 (day of preparation is considered day 0).

Homogeneity of Prepared Test Substance Formulations:

The Sponsor is responsible for documentation of the homogeneity of the test substance in the vehicle under the conditions of the study. Information supporting the homogeneity of the test substance in the vehicle will be included in the Analytico Analytical Contributing Scientist Report provided to the Study Director by the Sponsor for inclusion in the final report.

Homogeneity of the dose formulations will be determined concurrently with this study. Additional homogeneity analyses will be conducted concurrent to the study. Triplicate samples (approximately 5 mL) will be taken from the top, middle and bottom of the low and high dose level test article formulations on the first day of preparation. Duplicate samples will be shipped for analysis; the remaining samples will be retained at the Testing Facility as backup samples.

Concentration of Prepared Test Substance Formulations

Concentration of prepared test substance formulations will be verified during the course of this study. Triplicate samples (5 mL each) will be taken from each concentration level on the first and last days of preparation. Duplicate samples will be shipped for analysis using the current validated method. The results of these analyses will be included in the Analytico Analytical Contributing Scientist Report provided to the Study Director by the Sponsor for inclusion in the final report. The remaining samples will be retained at the Testing Facility until need for evaluation or until the report is finalized, at which time the Sponsor will be contacted to determine disposition.

Shipping Instructions:

Samples to be analyzed will be shipped (ambient conditions) to:

Mrs. R. Van de Ruit
Analytico Research, B.V.
Bergschot 69-71
4817 PA BREDA
The Netherlands
Telephone: 01131-765737575
Telefax: 01131-765737777

The recipient and Mrs. Greet Debaere (N.V. Procter & Gamble Eurocor, S.A., Telephone: 011-32-24562503, Telefax: 011-32-2-4562380) will be notified in advance of sa.nple shipment.

DISPOSITION:

Prepared formulations will be discarded at the Testing Facility. All remaining bulk test substance will be returned to:

Cindy Baetens
N.V. Procter & Gamble Eurocor, S.A.
1853 Strombek-Bever, Belgium
Telephone: 011-32-2-4562874
Telefax: 011-32-2-4562850

The recipient will be notified in advance of shipment.

TEST SYSTEM:

Species/Strain and Reason for Selection:

The Crl:CD®(SD)IGS BR VAF/Plus® rat was selected as the Test System because: 1) this strain of rat has been demonstrated to be sensitive to developmental toxins and has been widely used throughout industry for nonclinical studies of developmental toxicity (embryo-fetal toxicity/teratogenicity); and 2) historical data and experience exist at the Testing Facility[1-3].

Number:

Initial population acclimated:	60 virgin female rats.
Population selected for study:	40 mated female rats (8 per dosage group).

Body Weight and Age:

Female rats will be ordered to have body weights of 200 g to 225 g each at receipt, at which time they will be expected to be at least 60 days of age. Actual body weights recorded the day after receipt will be documented in the raw data.

Sex:

Female rats will be given the test substance and/or vehicle. Male rats of the same source and strain will be used only as breeders and are not considered part of the Test System.

Source:

Charles River Laboratories, Inc.

The rats will be shipped in filtered cartons by air freight and/or truck from Charles River Laboratories, Inc., to the Testing Facility.

Identification:

Rats are permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101). Male rats are given unique permanent identification numbers upon assignment to the Testing Facility's breeder male rat population. Female rats are assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study on the basis of day 0 of presumed gestation body weights.

ANIMAL HUSBANDRY:

All cage sizes and housing conditions are in compliance with the *Guide for the Care and Use of Laboratory Animals*[4].

Housing:

The rats will be individually housed in stainless-steel wire-bottomed cages except during the cohabitation period. During cohabitation, each pair of rats will be housed in the male rat's cage. No nesting materials will be supplied because the female rats will be sacrificed before parturition is expected.

Room Air, Temperature and Humidity:

The animal room is independently supplied with at least ten changes per hour of 100% fresh air that has been passed through 99.97% HEPA filters. Room temperature will be maintained at 64°F to 79°F (18°C to 26°C) and monitored constantly. Room humidity will also be monitored constantly and maintained at 30% to 70%.

Light:

An automatically-controlled 12-hour light:12-hour dark fluorescent light cycle will be maintained. Each dark period will begin at 1900 hours EST.

Diet:

Rats will be given Certified Rodent Diet® #5002 (PMI Nutrition International) available *ad libitum* from individual feeders.

Water:

Water will be available *ad libitum* from individual bottles attached to the cages or from an automatic watering access system. All water will be from a local source and passed through a reverse osmosis membrane before use. Chlorine will be added to the processed water as a bacteriostat; processed water is expected to contain no more than 1.2 ppm chlorine at the time of analysis. Water is analyzed monthly for possible bacterial contamination and twice annually for possible chemical contamination.

Contaminants:

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the certified diet or in the drinking water at levels that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier or those mentioned in this protocol will be conducted.

RANDOMIZATION AND COHABITATION:

Upon arrival, male and female rats will be assigned to individual housing on the basis of computer-generated random units. After acclimation of at least six days, virgin female rats will be cohabited with breeder male rats, one male rat per female rat. The cohabitation period will consist of a maximum of five days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* will be considered to be at day 0 of presumed gestation and assigned to individual housing.

Healthy mated female rats will be assigned to dosage groups based on computer-generated (weight-ordered) randomization procedures. The mean body weight for each group will not be statistically different at the 5% level (using Bartlett's Test for Homogeneity of Variances)[5].

ADMINISTRATION:

Route and Reason for Choice:

The oral (gavage) route was selected for use because: 1) in comparison with the dietary route, the exact dosage can be accurately administered; and 2) it is one possible route of human exposure.

Method and Frequency:

Female rats will be given the test substance and/or vehicle once daily on days 6 through 19 of presumed gestation. Dosages will be adjusted daily for body weight changes and given at approximately the same time each day. Formulations will be stirred continuously (magnetic stir plate with stir bar) during dosage administration.

Rationale for Dosage Selection:

Dose levels were selected by the Sponsor on the basis of previous studies conducted by the sponsor with related materials. The range of dosage levels selected for this study was suitable for the purpose of establishing dosage levels for a subsequent developmental toxicity study in rats.

Dosage Levels, Concentrations and Volumes:

Group	Number of Rats	Dosage (mg/kg/day)	Concentration (mg/mL)	Volume (mL/kg)	Argus Batch Number
I	8	0 (Vehicle)	0	3	B-916-045P-A(Day.Month.Year)
II	8	10	3.3	3	B-916-045P-B(Day.Month.Year)
III	8	100	33.3	3	B-916-045P-C(Day.Month.Year)
IV	8	400	133.3	3	B-916-045P-D(Day.Month.Year)
V	8	750	250.0	3	B-916-045P-E(Day.Month.Year)

The test substance will be considered 98.6% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS:

Viability:

All Periods: At least twice daily.

Clinical Observations and/or General Appearance:

Acclimation Period: Weekly.

Predosage Period: Day 0 of presumed gestation.

Dosage Period: Daily before dosage. Postdosage observations will be recorded approximately 60 ± 10 minutes after administration.

Day of Sacrifice: Once.

Clinical observations may be recorded more frequently than cited above, if deemed appropriate by the Study Director and/or Study Monitor.

Body Weights:

Acclimation Period:	Weekly.
Predosage Period:	Day 0 of presumed gestation.
Dosage Period:	Daily.
Day of Sacrifice:	Terminal weight.

Feed Consumption Values (recorded and tabulated):

Predosage Period:	Day 0 of presumed gestation.
Dosage Period:	Days 6, 9 12, 15, 18 and 20 of presumed gestation.
Day of Sacrifice:	Feed left recorded.

Feed consumption values may be recorded more frequently if it is necessary to replenish the feed. These intervals will not be tabulated.

Mating Performance:

Mating will be evaluated daily during the cohabitation period and confirmed by observation of spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed *in situ*.

Caesarean-Sectioning Observations:

Rats will be Caesarean-sectioned on day 20 of presumed gestation. Caesarean-sectioning and subsequent fetal observations will be conducted without knowledge of dosage group to minimize bias. The gravid uterus will be excised and weighed. The fetuses will be removed from the uterus and placed in individual containers. The rats will be examined for number and distribution of:

Corpora Lutea.

Implantation Sites.

Live and Dead Fetuses.
(A live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and that is not markedly autolyzed; dead fetuses demonstrating marked to extreme autolysis are considered to be late resorptions.)

Early and Late Resorptions.
(A conceptus is defined as a late resorption if it is grossly evident that organogenesis has occurred; if this is not the case, the conceptus is defined as an early resorption.)

Fetal Observations:

Fetal observations will be conducted without knowledge of dosage group to minimize bias.

Fetuses will be examined for sex and for gross external alterations. Late resorptions and dead fetuses will be examined for gross external alterations to the extent possible. The body weight of each fetus will be recorded. Only body weights of live fetuses will be used to determine litter fetal body weight averages. Fetuses with gross external alterations will be fixed in Bouin's solution; all other fetuses will be discarded. Representative photographs of fetal gross external alterations will be taken.

METHOD OF SACRIFICE:

Rats will be sacrificed by carbon dioxide asphyxiation. Live fetuses will be sacrificed by an intraperitoneal injection of Beuthanasia®-D Special, manufactured by Schering-Plough Animal Health.

NECROPSY:

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation. All other tissues will be discarded.

Scheduled Sacrifice:

On day 20 of presumed gestation, female rats will be Caesarean-sectioned, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. The gravid uterus will be excised and weighed. Uteri of apparently nonpregnant rats will be examined while being pressed between glass plates to confirm the absence of implantation sites.

Rats Found Dead or Moribund:

Rats that die or are sacrificed because of moribund condition, abortion or premature delivery will be examined for the cause of death or moribund condition on the day the observation is made. The rats will be examined for gross lesions. Pregnancy status and uterine contents of female rats will be recorded. Gravid uterine weights will not be recorded if precluded by autolysis. Aborted fetuses, delivered pups and/or concepti *in uteri* will be examined to the extent possible, using the methods described for term fetuses. Uteri of apparently nonpregnant rats will be examined while being pressed between glass plates to confirm the absence of implantation sites.

STATISTICAL EVALUATION:

Averages and percentages will be calculated. Litter values will be used where appropriate. Additional procedures and/or analyses may be performed if deemed appropriate and agreed by protocol amendment.

DATA ACQUISITION, VERIFICATION AND STORAGE:

Data generated during the course of this study will be recorded either by hand or using the *Primedica Argus Automated Data Collection and Management System* and the *Vivarium Temperature and Relative Humidity Monitoring System.* All data will be tabulated, summarized and/or statistically analyzed using the *Primedica Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System, Microsoft Excel* [part of Microsoft Office 97 (version SR-2)] and/or *The SAS System* (version 6.12).

Records will be reviewed by the Study Director and/or appropriate management personnel within 21 days after generation. All original records, with the exception of any analytical data and reports received from subcontracting laboratories, will be stored in the archives of the Testing Facility. Any analytical records produced by the subcontracting laboratory will be stored at the laboratory's facility. All original data will be bound and indexed. A copy of all raw data will be supplied to the Sponsor upon request. All raw data, reserve samples and the final report will be stored indefinitely at the Testing Facility. Preserved tissues will be sent according to the instructions in ATTACHMENT 4 to Pathology Associates, Inc. (PAI) after the Sponsor receives the final report.

RECORDS TO BE MAINTAINED:

All study records will be maintained, including:

Protocol and Amendments.
Test Substance, Vehicle and/or Reagent Receipt, Preparation and Use.
Animal Acquisition.
Randomization Schedules.
Mating History.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Blood Sample Collection, Processing and Shipment (if required).
Body Weights.
Feed Consumption Values.
Caesarean-Sectioning and Fetal Observations.
Gross Necropsy Observations.
Organ Weights.
Photographs (if required).
Study Maintenance (room and environmental records).
Feed and Water Analyses.
Packing and/or Shipment Lists.

KEY PERSONNEL:

Executive Director of Research: Mildred S. Christian, Ph.D., Fellow, ATS
Director of Research: Alan M. Hoberman, Ph.D., DABT
Study Director and Associate Director of Research: Raymond G. York, Ph.D., DABT
Director of Laboratory Operations: John F. Barnett, B.S.
Manager of Animal Operations and Chairperson, Institutional Animal Care and Use Committee: Dena C. Lebo, V.M.D.
Director of Operations and Compliance: Barbara J. Patterson, B.A.
Director of Study Management: Valerie A. Sharper, M.S.
Consultant, Veterinary Pathology: W. Ray Brown, D.V.M., Ph.D., ACVP

REPORT:

An unaudited letter report for the purpose of dosage selection for the full study will be prepared immediately following completion of the in-life phase.

An audited report will be prepared containing all regulatory required items including: summaries of the methods, results and conclusion; table of contents; Study Director's GLP compliance statement; copy of the protocol; amendments and deviations; QAU statement; summary and individual tables; and reports of supporting data (if appropriate). The Sponsor will receive one copy of the draft report and two copies of the final report.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STATEMENT:

The procedures described in this protocol have been reviewed by the Testing Facility's Animal Care Committee. All procedures described in this protocol that involve study animals will be conducted in a manner to avoid or minimize discomfort, distress or pain to the animals.

Unless otherwise addressed in this protocol, the Testing Facility will not use any paralytics in the course of this study. Sponsor has data on file that paralytic activity is not associated or has not been previously observed with this compound.

If necessary, animals will be relieved of pain or distress by the use of appropriate sedatives, analgesics or anesthetics unless the withholding of such agent(s) is justified for scientific reasons; the withholding of such agent(s) will continue only for the necessary period of time. Use of such agent(s), if required, will be documented in the raw data.

Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure (or, if appropriate, during the procedure) after consultation with the Study Director and/or staff veterinarian. Methods and agents used for euthanasia will be in accordance with acceptable standards, unless a deviation is justified for scientific reasons. The disposition of all animals purchased for this study will be documented in the raw data.

The Sponsor's signature below documents the fact that information concerning the necessity for conducting this study and the fact that this is not an unnecessarily duplicative study may be obtained from the Sponsor. No alternative (*in vitro*) procedures were available for meeting the stated purposes of the study. Alternative methods to those employed in this study, which may cause more than slight or momentary pain or distress, have been considered but were not available or appropriate for this study.

REFERENCES:

1. Christian, M.S. and Voytek, P.E. (1982). *In Vivo Reproductive and Mutagenicity Tests.* Environmental Protection Agency, Washington, D.C. National Technical Information Service, U.S. Department of Commerce, Springfield, VA 22161.

2. Christian, M.S. (1984). Reproductive toxicity and teratology evaluations of naltrexone (Proceedings of Naltrexone Symposium, New York Academy of Sciences, November 7, 1983), J. Clin. Psychiat., 45(9):7-10.

3. Lang, P.L. (1988). *Embryo and Fetal Developmental Toxicity (Teratology) Control Data in the Charles River Crl:CD®BR Rat.* Charles River Laboratories, Inc., Wilmington, MA 01887-0630., (Data base provided by Argus Research Laboratories, Inc.)

4. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals.* National Academy Press, Washington, D.C.

5. Sokal, R.R. and Rohlf, F.J. (1969). Bartlett's test of homogeneity of variances. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 370-371.

B 06

PROTOCOL APPROVAL:

FOR THE TESTING FACILITY

Alan M. Hoberman, Ph.D., DABT
Director of Research

3-APR-00
Date

Raymond G. York, Ph.D., DABT
Associate Director of Research
Study Director

21-APR-00
Date

Dena C. Lebo, V.M.D.
Chairperson, Institutional Animal Care and Use Committee

21 Apr 00
Date

FOR THE SPONSOR

Petra Kern, Ph.D.
Study Monitor

25-APR-00
Date