Toxicology Summary:

Study: Title: 14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment.

Performed at: **RTI International**
Health Science Unit
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194
Sponsor: **Clinical Monitoring Research Program, SAIC Frederick**
Study Director: **Jay G. Henson, B.S.**

Fluoroestradiol in the vehicle (15% ethanol:85% saline) was administered by intravenous injection once daily for 14 consecutive days to 2 groups (Groups 2 and 3) of Sparague-Dawley CD®(SD)IGS BR rats. An additional group (Group 4) was administered cyclophosphamide (positive control for micronucleus test) by an intraperitoneal injection on the last day of dosing. Fluoroestradiol dosage levels were 13 and 51 µg/kg for Group 2 and 3, respective. The positive control was administered at 30 mg/kg (Group 4). A concurrent control group (Group 1) received the vehicle on a comparable regimen as the test article groups. The dosage volume was 2.0 mL/kg for Groups 1-3 and 5.0 mL/kg for Group 4. Groups 1-3 each consisted of 5 animals/sex, and Group 4 consisted of 2 males. Animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily at the time of dosing individual body weights and feed consumption were recorded at selected intervals. At the end of the dosing period, all animals were humanely euthanized. Clinical pathology evaluations were performed on all animals in Groups 1-3 at necropsy. Complete necropsies were conducted on all animals in Groups 1-3 at necropsy. Complete necropsies were conducted on all animals in Groups 1-3, and selected organs were weighed. Selected tissues were examined microscopically from all animals in Groups 1 and 3. Bone marrow smear slides were prepared from all animals for micronuclei determination.

There were no signs of toxicity at the doses tested on this study. No adverse clinical observations were noted during the study. There were no test article-related changes in body weights or feed consumption. Clinical pathology parameters were unaffected by test article administration, and there were no toxicologically relevant organ weight changes. All macroscopic and microscopic findings observed were considered spontaneous and/or incidental in nature and unrelated to test article administration, as they were consistent with normal background lesion in clinically normal rats of the age and strain used on this study. Therefore, based on the results of this study, the no-observed-effect level (NOEL) for intravenous administration of fluoroestradiol to rats for 14 consecutive days was greater than 51 µg/kg/day.

GLP Statement

Toxicology studies, including single dose and multiple dose toxicity studies, immunotoxicity studies, irritation studies, reproductive and developmental toxicity studies and genotoxicity studies were generally in compliance with U.S. Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58, effective June 20,1979. A cardiovascular and renal safety study in dogs also was conducted in compliance with U.S. GLP requirements. Some of the genetic toxicity studies were repeated in Japan and Europe and these also were conducted in accordance with GLP requirements.

Toxicology Summary:

Study: Title: In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{-/-}$ Mouse Lymphoma Assay).

Performed at: **BioReliance**
9630 Medical Center Drive
Rockville, MD 20850

Sponsor: **RTI International**
Study Director: **Valentine O. Wagner, III, M.S.**

The test article, Fluoroestradiol, was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9. The preliminary toxicity assay established the concentration range for the mutagenesis assays. The mutagenesis assays were used to evaluate the mutagenic potential of the test article.

Ethanol was selected by the sponsor as the solvent for the test article was soluble in ethanol at approximately 1.0 mg/mL, the maximum concentration prepared for the preliminary toxicity assay.

In the preliminary toxicity assay, the maximum concentration of Fluorestradiol in treatment medium was 8.0 ng/mL. No visible precipitate was present at any concentration in treatment medium. Selection of concentrations for the mutation assay was based on reduction of suspension growth relative to the solvent control and maximum concentration requested by the sponsor. No substantial toxicity, i.e., suspension growth of $\leq$ 50% of the solvent control, was observed at any concentrations with or without S9 activation.

Based on the results of the preliminary toxicity assay, the concentrations treated in the initial mutagenesis assay ranged from 0.15 to 8.0 ng/mL for both the non-activated and S9-activated cultures with a 4-hour exposure. No visible precipitate was present at any concentration in treatment medium. The concentrations chosen for cloning were 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL with and without S9 activation. No cloned cultures exhibited mutant frequencies $\geq$ 90 mutants per 10^6 clonable cells over that of the solvent control. There was no concentration-related increase in mutant frequency.

Based on the results of the preliminary toxicity assay, the concentrations treated in the extended treatment assay ranged from 0.15 to 8.0 ng/mL ng/mL for non-activated cultures with a 24-hour exposure. No visible precipitate was present at any concentrations in treatment medium. The concentrations chosen for cloning were 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL. No cloned cultures exhibited mutant

frequencies $\geq$ 90 mutants per 10^6 clonable cells over that of the solvent control. There was no concentration-related increase in mutant frequency.

The trifluorothymidine-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS and DMBA positive controls yielded the expected increase in small colonies (verifying the adequacy of the methods used to detect small colony mutants) and large colonies.

Under the conditions of this study, test article Fluoroestradiol was concluded to be negative in the L5178Y/$TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

GLP Statement

Toxicology studies, including single dose and multiple dose toxicity studies, immunotoxicity studies, irritation studies, reproductive and developmental toxicity studies and genotoxicity studies were generally in compliance with U.S. Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58, effective June 20,1979. A cardiovascular and renal safety study in dogs also was conducted in compliance with U.S. GLP requirements. Some of the genetic toxicity studies were repeated in Japan and Europe and these also were conducted in accordance with GLP requirements.

Toxicology Summary:

Study: Title: Bacterial Reverse Mutation Assay.

Performed at: **BioReliance**
9630 Medical Center Drive
Rockville, MD 20850

Sponsor: **RTI International**
Study Director: **Valentine O. Wagner, III, M.S.**

The test article, Fluoroestradiol, was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia Coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article.

Ethanol (EtOH) was selected as the solvent of choice based on the request of the Sponsor and compatibility with the target cells.

In the initial toxicity-mutation assay, the maximum dose tested was 1.25 µg per plate; this dose was achieved using a concentration of 0.025 mg/mL and 50 µL plating aliquot. The dose levels tested were 0.00050, 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50 and 1.25 µg per plate. The test article formed soluble and clear solutions in ethanol from 0.000010 to 0.025 mg/mL. In the initial toxicity-mutation assay, no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 1.25 µg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed. The dose levels tested were 0.015, 0.050, 0.15, 0.50 and 1.25 µg per plate. Neither precipitate nor appreciable toxicity was observed.

Under the conditions of this study, test article Fluorestradiol was concluded to be negative in the Bacterial Reverse Mutation Assay.

GLP Statement

Toxicology studies, including single dose and multiple dose toxicity studies, immunotoxicity studies, irritation studies, reproductive and developmental toxicity studies and genotoxicity studies were generally in compliance with U.S. Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58, effective June 20,1979. A cardiovascular and renal safety study in dogs also was conducted in compliance with U.S. GLP requirements. Some of the genetic toxicity studies were repeated in Japan and Europe and these also were conducted in accordance with GLP requirements.

Toxicology Summary:

Study: Title: Effects of 16alpha-Fluorestradiol on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells.

Performed at: Chan Test Corporation
14656 Neo Parkway
Cleveland, OH 44128
Sponsor: Center for Life Science and Toxicology
Study Director: Lisa M. Shyjka, BA

The objective of this study was to examine the in vitro effects of 16alpha-Fluoroestradiol on the hERG (human ether-a'-go-go-related gene) channel current (a surrogate for $I_{Kr,}$ the rapidly activating, delayed rectifier cardiac potassium current). 16alpha-Fluoroestradiol inhibited hERG current by (Mean $\pm$ SEM, n=3) 1.4 $\pm$ 0.2% at 8 ng/mL versus 0.3 $\pm$ 0.1% in control. hERG inhibition at 8 ng/mL was statistically significant ($P < 0.05$) when compared to vehicle control values. The IC_{50} for the inhibitory effect of 16alpha Fluoroestradiol on hERG potassium current could not be determined due to solubility limitations of 16alpha-Fluoroestradiol in HB-PS + 0.3% ethanol, but it is estimated to be greater than 8 ng/mL.

The positive control (60 nM terfenadine) inhibited hERG potassium current by (Mean $\pm$ SD; n= 2) 80.4$\pm$ 0.1%. This result confirms the sensitivity of the test system to hERG inhibition.

Samples of the test article formulation solutions collected from stock preparation were analyzed for concentration verification. The results from the stock sample analysis indicated that the measured concentrations of 16alpha-Fluorestradiol at all test concentration were within $\pm$ 15% of the target concentrations (between average 91.0 to 105.7% of the targets), thereby meeting the acceptance criteria

GLP Statement

Toxicology studies, including single dose and multiple dose toxicity studies, immunotoxicity studies, irritation studies, reproductive and developmental toxicity studies and genotoxicity studies were generally in compliance with U.S. Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58, effective June 20,1979. A cardiovascular and renal safety study in dogs also was conducted in compliance with U.S. GLP requirements. Some of the genetic toxicity studies were repeated in Japan and Europe and these also were conducted in accordance with GLP requirements.

FINAL REPORT

14-Day Intravenous Repeat Dose Toxicology Study in Rats With Micronucleus Assessment

Prepared for:

Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412

Prepared by:

RTI International*
Center for Life Sciences and Toxicology
Health Sciences Unit
3040 Cornwallis Road
P.O. Box 12194
Research Triangle Park, NC 27709-2194

Study Initiation Date: November 7, 2008

Study Completion Date: June 19, 2009

RTI Project No.: 0211886.001.001

RTI Protocol No.: RTI-1059

RTI Study Code: Rt08-FES

**RTI International is a trade name of Research Triangle Institute*

SIGNATURE PAGE

Title: 14-Day Intravenous Repeat Dose Toxicology Study in Rats With Micronucleus Assessment

Jay G. Henson, B.S.
Study Director
RTI International

19 Jun 09
Date

Christina B. Myers, M.S.
Data Specialist
RTI International

6-19-09
Date

Irma M. Grossi, Ph.D.
Senior Director, Preclinical Pharmaceutical Services
RTI International

19 June 2009
Date

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Good Laboratory Practice Statement

This study was conducted in compliance with the FDA Good Laboratory Practices (GLP) regulations and AAALAC accreditation standards. The toxicology laboratories at RTI are operated in compliance with FDA GLP regulations (21 CFR Part 58). Through the administration of a quality assurance program by the Quality Assurance Unit, RTI assesses compliance of all phases of toxicological studies with existing regulations (21 CRF Part 58). The Sponsor holds responsibilities for GLP compliance of test article characterization, test article strength, purity, stability, identity, and uniformity. Certificates of Analysis for the test article and positive control article were provided by the Sponsor and Sigma-Aldrich, respectively (Appendix 6, attachment to the protocol); RTI cannot confirm if the characterization analyses were conducted according to Good Laboratory Practices. The Sponsor was also responsible for GLP compliance of test article formulation analyses. Concentration analyses for the test article formulations were performed under the direction of Dr. Jeanne Link at the University of Washington. Again, GLP compliance cannot be confirmed by RTI. The RTI Animal Research Facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Jay G. Henson, B.S.
Study Director
RTI International

19 Jun 09
Date

RTI INTERNATIONAL

Quality Assurance Statement

Study Title: 14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

Sponsor: Clinical Monitoring Research Program, SAIC Frederick

Study Code: Rt08-FES

Protocol Number: RTI-1059

This study was audited by the Regulatory and Quality Assurance (RQA) - Quality Assurance Unit and the results of the inspections and audits were reported to the Study Director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Audit	November 5,6, 2008	November 6, 2008
Randomization Process Inspection	November 14, 2008	November 14, 2008
Protocol Amendment 1	November 17, 2008	November 17, 2008
Dosing	November 17, 2008	November 17, 2008
Sample Preparation	November 17, 2008	November 21, 2008
Necropsy	December 1, 2008	December 1, 2008
Protocol Amendment 2	January 9, 2009	January 9, 2009
Data Audit	January 7, 12-15, 19, 21, 26 & Feb 2&3, 2009	February 3, 2009
Report Audit	May 28,29 & June 15, 2009	June 15, 2009

Prepared by:

Leslie Macdonald

Leslie Macdonald
Quality Assurance Specialist

6-18-09
Date

Reviewed by:

Carla F. Gibbons

Carla F. Gibbons
Assistant Manager, Quality Assurance

6-18-09
Date

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Storage, Retrieval, and Retention of Records

This study was monitored for compliance with the Food and Drug Administration's (FDA) GLP regulations (21 CFR Part 58) for conduct of nonclinical studies. Records of the study data, pertinent to the conduct of this study, are retained in labeled binders and maintained under the direction of RTI. Data stored on magnetic media are also maintained by RTI. All data documenting experimental details, study procedures, and observations were recorded and maintained as raw data. At the completion of the study, all raw data, correspondence, documentation, records, reports, preserved specimens, and retained and archived samples will be maintained in the archives of RTI for a period of one year after submission of this signed final report. The Sponsor will be responsible for the final disposition of these materials, and also responsible for all costs associated with their storage beyond one year from the issuance of the final report.

Laboratory Personnel

This study was conducted at RTI international (Research Triangle Park, NC) under contract to the Clinical Monitoring Research Program (CMRP), SAIC Frederick. Kimberly D. Ehman, Ph.D., served as the Study Director from project initiation until January 16, 2009, at which time she left RTI's employment. Upon her departure, RTI management assigned Jay G. Henson, B.S., as Study Director to complete the study. The Sponsor's Representative was Paula M. Jacobs, Ph.D. In addition, the following personnel worked on this study:

Nicole M. Barbarish, B.S.	Technical Supervisor
David E. Miller, B.S., RLATG	Animal Facility Manager
Crystal L. Thomas, D.V.M.	Veterinary Services
Donna B. Browning, B.S.	Dose Formulations Manager
Melissa C. Marr, B.A., RLATG	Necropsy Support
Christina B. Myers, M.S.	Statistical Analysis

1.0 Abstract

Fluoroestradiol in the vehicle (15% ethanol:85% saline) was administered by intravenous injection once daily for 14 consecutive days to 2 groups (Groups 2 and 3) of Sprague-Dawley CD®(SD)IGS BR rats. An additional group (Group 4) was administered cyclophosphamide (positive control for micronucleus test) by an intraperitoneal injection on the last day of dosing. Fluoroestradiol dosage levels were 13 and 51 µg/kg for Groups 2 and 3, respectively. The positive control was administered at 30 mg/kg (Group 4). A concurrent control group (Group 1) received the vehicle on a comparable regimen as the test article groups. The dosage volume was 2.0 mL/kg for Groups 1-3 and 5.0 mL/kg for Group 4. Groups 1-3 each consisted of 5 animals/sex, and Group 4 consisted of 2 males. Animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily at the time of dosing. Individual body weights and feed consumption were recorded at selected intervals. At the end of the dosing period, all animals were humanely euthanized. Clinical pathology evaluations were performed on all animals in Groups 1-3 at necropsy. Complete necropsies were conducted on all animals in Groups 1-3, and selected organs were weighed. Selected tissues were examined microscopically from all animals in Groups 1 and 3. Bone marrow smear slides were prepared from all animals for micronuclei determination.

There were no signs of toxicity at the doses tested on this study. No adverse clinical observations were noted during the study. There were no test article-related changes in body weights or feed consumption. Clinical pathology parameters were unaffected by test article administration, and there were no toxicologically relevant organ weight changes. All macroscopic and microscopic findings observed were considered spontaneous and/or incidental in nature and unrelated to test article administration, as they were consistent with normal background lesions in clinically normal rats of the age and strain used on this study. Therefore, based on the results of this study, the no-observed-effect level (NOEL) for intravenous administration of fluoroestradiol to rats for 14 consecutive days was greater than 51 µg/kg/day.

2.0 Study Objective and Introduction

The objective of this study was to assess the toxicity of fluoroestradiol when administered by intravenous injection to Sprague-Dawley CD®(SD)IGS BR rats for

14 consecutive days. In addition, bone marrow samples were collected from all animals for micronucleus assessment.

3.0 Materials and Methods

3.1 Test Article

Unless otherwise noted, the identity, purity, composition, stability, and method of synthesis of each batch of test article were the responsibility of the Sponsor. This documentation is maintained by the Sponsor/Supplier and was provided to RTI for inclusion in the study records.

Sponsor Designation:	Fluoroestradiol (in ethanol/water as provided to RTI)
Name:	Estra-1,3,5 (10)-triene-3,17-diol, 16-fluoro-, (16a,17beta)
CAS No.:	92817-10-2
Purity:	Certificate of Analysis (Appendix 6, attachment to protocol)
Disposition:	Returned to Sponsor following study completion

3.2 Positive Control Article

Sponsor Designation:	Cytoxan (positive control article)
Name:	Cyclophosphamide monohydrate
Supplier:	Sigma Aldrich
CAS No.:	6055-19-2
Lot No.:	068K1131
Purity:	98.0% by HPLC (Appendix 6, attachment to protocol)
Storage Conditions:	Refrigerated

3.3 Vehicle

The vehicle for administration to the control group (Group 1) and for preparation of the test article dosing formulations was 15% ethanol:85% saline.

3.4 Dose Preparation

Test article formulations were prepared on the first day of the study and stored refrigerated (2 to 8°C). The positive control article was prepared on the day of use (Study Day 13). Details of the dose preparation method were included in the study records. The formulations were brought to room temperature prior to administration.

3.5 Dose Analysis

Approximately 1-3-mL samples were collected from each dose formulation on the first day of dosing (Study Day 0) and on the last day of dosing (Study Day 13). The samples were shipped (overnight) in clear borosilicate vials on ice packs to Dr. Jeanne Link (the University of Washington, Seattle, WA) for stability and concentration analyses. The positive control article formulation was not analyzed for stability, homogeneity, or concentration. The analytical results were provided to RTI (Appendix 1).

3.6 Test System

Species and Strain. Sprague-Dawley CD®(SD)IGS BR rat.

Source. Charles River Laboratories, Inc. (Raleigh, NC).

Animal Receipt and Acclimation. Animals were acclimated for at least 5 days (see Section 7.0) following receipt. All animals were checked for viability twice daily during the quarantine period. Prior to study assignment, all animals were examined by the study veterinarian to ascertain suitability for study.

Age. Approximately 5 weeks old at receipt; approximately 6 weeks old at initiation of dosing.

Weight. 110 to 182 grams at first dose.

Number/Gender. Seventeen males and 15 females; 5/sex were assigned to the toxicology groups (Groups 1-3). The 2 remaining males were assigned to the cyclophosphamide positive control group (Group 4).

Method of Identification. Each animal was uniquely identified by an eartag with an RTI number. This number, plus a study number, comprised the unique identification for each animal.

Animal Welfare. Nestlets were provided to all animals for environmental enrichment.

3.7 Husbandry

Housing. All animals were housed individually in appropriately sized solid-bottom polycarbonate cages suspended from stainless steel, self-watering racks. Hardwood Sani-Chips® cage litter was used in all cages. Current acceptable practices of good animal husbandry were followed, e.g., *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996). Animals were monitored by the technical staff for any conditions requiring possible veterinary care.

Diet. PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 (pellet) was available *ad libitum*. Each lot utilized was identified and recorded. Rodent diet was stored at approximately 60-70°F, and the period of use did not exceed six months from the milling date. Each lot was analyzed by the manufacturer to assure specifications were met, and a copy of the results was maintained in the study records.

Water. Municipal tap water from the Durham, NC water system was available *ad libitum* throughout the study. Analysis of the drinking water for chemical composition and possible contamination was provided by the supplier and maintained in the study records.

3.8 Environment

Environmental conditions were continuously monitored, controlled, and recorded by an automated system. Target conditions for temperature and humidity in the animal room were 64-79°F and 30-70%, respectively (NRC, 1996). Lighting controlled by light timers provided illumination for a 12-hour light/12-hour dark photoperiod. The ventilation rate was set at a minimum of 10 air changes per hour.

3.9 Justification of the Test System and Treatment Regimen

The rat is an animal model commonly utilized in toxicity studies. The CD® (SD) rat was chosen because of the knowledge of this strain's general pathology and response to a wide variety of drugs. The number of animals on study was considered to be the minimum necessary for statistical, regulatory, and scientific reasons. The purpose of this study was to assess the toxicity of the test article. Historical control data indicated that clinical laboratory data, organ weight data, and microscopic examination of tissues vary among individual animals. The number of animals/sex/group for this study was selected based on this variability. The two test article-treated groups receiving low and high multiples of the proposed human dose, and a negative and positive control group, were considered the minimum number of groups necessary to provide a range of effects and allow for extrapolation of results to humans.

For test articles (like medical imaging agents) whose clinical use is expected to involve only a single dose, "expanded acute" studies, in which rodents undergo an extensive toxicology evaluation following a single administration of test article, are generally sufficient. Acute toxicity study designs are generally less likely to identify potentially serious, late-appearing toxicities. For this reason, repeat-dose administration studies are performed only with test

articles whose expected clinical use pattern will involved only a single or a few doses. Additionally, medical imaging agents may be required to monitor therapy in humans. Consequently, animals were dosed for 14 consecutive days and detailed toxicological evaluations performed throughout the dosing period. Because the test article will be administered to humans intravenously, the same route of administration was used in this study. This two-week preclinical study is expected to support human exposure of this duration. Based upon prior observations and the extremely low dose of the test article that is used in diagnostic imaging, this 14-day rat exposure is equivalent to a cumulative of 1400-fold greater administered dose of test article than would be the maximum experienced in human studies.

3.10 Assignment to Study

Animals were assigned to treatment groups by sex using stratified randomization using the Toxicology Analysis System Customized (TASC) computer program designed to provide uniform mean body weights across dose groups based on the last body weight taken during the acclimation period. The following table presents the study group assignment:

Group Number	Treatment	Dose	Dosing Concentration (µg/mL)	Dosing Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[1]	0	0	2.0	5	5
2	Fluoroestradiol	13 µg/kg	6.5	2.0	5	5
3	Fluoroestradiol	51 µg/kg	25.5	2.0	5	5
4	Cyclophosphamide[2]	30 mg/kg	6.0 mg/mL	5.0	2	0

[1] Vehicle = 15% ethanol:85% saline

[2] Positive control for micronucleus assay. Cyclophosphamide was administered intraperitoneally as a single dose to 2 males on Study Day 13.

3.11 Administration

The vehicle and test article formulations (Groups 1-3) were administered daily for 14 consecutive days (until the day prior to necropsy; Study Days 0-13) as an intravenous bolus dose via a lateral tail vein using appropriately sized needles and syringes. For micronucleus assessment, 2 males (Group 4) were administered cyclophosphamide (positive control) as an intraperitoneal injection on Study Day 13. Doses were calculated using the most recent body weights.

3.12 Parameters Evaluated

Viability Observations. Cage-side viability checks for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon, not less than 6 hours apart; refer to Section 7.0 for deviation).

Clinical Observations. Observations for clinical signs of toxicity were made once daily for each animal at the time of dosing. Observations included (but not limited to) changes in the skin, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems function; somatomotor activity and behavior patterns. Clinical observations were not recorded for Group 4 animals.

Body Weights. Body weights for Groups 1-3 were recorded twice pretest (upon receipt and prior to group assignment) and weekly during study conduct (Study Days 0, 6, and 13). Body weights for Group 4 animals were recorded twice pretest (upon receipt and prior to group assignment) and on Study Day 13.

Feed Consumption. Feed consumption was measured (weighed) weekly for Groups 1-3 throughout study conduct (Study Days 0-6 and 6-13).

3.13 Clinical Pathology

Clinical pathology blood samples (hematology and serum chemistry) were collected from Groups 1-3 via the vena cava at the time of scheduled necropsy. Animals were fasted overnight prior to blood collection. Blood for hematology assessments (approximately 0.5 mL) was collected into tubes containing EDTA as the anticoagulant. Blood for serum chemistry assessments (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Whole blood samples were stored on wet ice or refrigerated, and serum samples were stored on dry ice or frozen at approximately -70°C until submitted for analysis. All samples were submitted to Antech Diagnostics (Morrisville, NC) for analysis:

The following hematology parameters were evaluated:

Erythrocyte count (RBC)	Mean corpuscular hemoglobin concentration (MCHC)
Differential leukocyte count	Mean corpuscular volume (MCV)
Hematocrit (HTC)	Platelet count (PLT)
Hemoglobin (HGB)	Reticulocyte count (RETIC)
Mean corpuscular hemoglobin (MCH)	Total leukocyte count (WBC)

The following serum chemistry parameters were evaluated:

- Albumin (ALB)
- Albumin/globulin (A/G Ratio)
- Alkaline phosphates (ALP)
- Blood urea nitrogen (BUN)
- Calcium (Ca)
- Chloride (Cl)
- Cholesterol (CHOL)
- Creatinine (CRE)
- Gamma-glutamyltransferase (GGT)
- Globulin (GLOB)
- Inorganic phosphate (PO_4)
- Potassium (K)
- Serum alanine transaminase (ALT)
- Serum aspartate transaminase (AST)
- Serum glucose (GLUC)
- Sodium (Na)
- Total bilirubin (TBIL)
- Total protein (TP)
- Triglycerides (TG)

3.14 Anatomic Pathology

Necropsy. A complete necropsy was conducted on Groups 1-3. Animals were fasted overnight prior to necropsy. A final body weight was collected for all animals prior to euthanasia. Animals were euthanized by CO_2 asphyxiation, followed by exsanguination. Necropsies included examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera. At the time of necropsy, the following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):

- Adrenal glands (see Section 7.0)
- Aorta
- Brain
- Bone (femur with epiphyseal plate of head)
- Bone marrow (sternum)
- Cecum
- Colon
- Duodenum
- Eartag (animal ID)
- Epididymides
- Oviducts
- Pancreas
- Pituitary (see Section 7.0)
- Prostate
- Rectum
- Salivary gland (mandibular)
- Sciatic nerve
- Seminal vesicles
- Skeletal muscle
- Skin (ventral abdomen)

Esophagus	Spinal cord (thoracolumnar junction; entire cord if neurologic abnormalities present)
Eyes, with optic nerve[1]	Spleen
Gross lesions (including tissue masses and abnormal regional lymph nodes)	Stomach (fundic area)
Heart	Testes[1]
Ileum	Thymus
Injection site(s)	Thyroid and parathyroid glands
Jejunum	Tongue
Kidney	Trachea
Liver (right medial lobe and left lateral lobe)	Ureter
Lungs	Urinary bladder
Lymph node (mandibular and mesenteric)	Uterus (body) with cervix
Mammary gland (to include nipple and surrounding tissue; see Section 7.0)	Vagina
Ovaries	

[1]Modified Davidson's solution initially, followed by 10% neutral-buffered formalin.

The organs indicated below were weighed from Groups 1-3 euthanized at the scheduled necropsy:

Adrenals	Prostate
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus with oviducts
Pituitary	

Paired organs were weighed together. The pituitary and thyroid/parathyroids were weighed following fixation. Organ weights were not taken from animals found dead.

3.15 Histopathology

Microscopic examination of hematoxylin-eosin stained paraffin sections was performed on the tissues listed under "Necropsy" above for all animals in Groups 1 and 3. Fixed tissues were sent to Pathology Associates (Charles River Laboratories, Durham, NC) for processing and histopathological assessments. Michael P. Jokinen, D.V.M., DACVP, was the study pathologist.

3.16 Micronucleus Assessment

On Study Day 14 (approximately 18-24 hours after the last dose administration), 2 bone marrow smear slides from the femur were prepared from all toxicology animals (Groups 1-3) and from the 2 non-fasted positive control animals (Group 4) for *in vivo* clastogenicity/aneugenicity assessments (micronuclei determination). Details of the bone marrow smear procedure were included in the study records. Prepared bone marrow smears were shipped to BioReliance (Rockville, MD) for micronuclei slide staining and scoring. Ljubica Krsmanovic, Ph.D., was the principal investigator for the micronucleus assessment.

3.17 Data Analysis

The Toxicology Analysis System Customized (TASC) automated data collection system was used for collection of all body weights, feed weights, clinical observations, organs weights, and gross necropsy findings. TASC calculated the volume of dosing solution to be administered to each animal on each day, based on the most recent body weight. TASC also recorded when each animal was dosed. The following types of data were analyzed separately at each time point:

- Body weights and weight gain over specified (i.e., weekly) study periods
- Feed consumption over specified (i.e., weekly) study period
- Hematology and serum chemistry (Antech Diagnostics GLP, Morrisville, NC)
- Organ weights, both absolute and adjusted for terminal body weight

For categorical data, the proportion of animals was analyzed using Fisher's Exact Test (Steel and Torrie, 1980) for each treated group versus the control. For continuous data, Levene's Test (Levene, 1960) was applied to test for homogeneity of variances between the groups. Using tests dependent on the outcome of Levene's Test, an overall test of significance was run. If the overall test was significant ($p<0.05$), treated groups were then compared to the control group, incorporating adjustments for multiple comparisons where necessary.

4.0 Results and Discussion

4.1 Dose Formulations

All dosing formulations were within 10% of target (Appendix 1). Based on these results, the analyzed dosing formulations were found to contain the amount of test article prescribed in the protocol.

4.2 Viability Observations

All animals survived to the scheduled necropsy (Table 1).

4.3 Clinical Observations

There were no clinical observations noted during the study. All animals were considered normal throughout the 14-day dosing period (Tables 2 and 3).

4.4 Body Weights

Body weights were unaffected by test article administration. There were no statistically significant differences when the control and test article-treated groups were compared (Tables 4-7).

4.5 Feed Consumption

There were no test article-related effects on feed consumption. Statistically significant ($p<0.05$) higher mean feed consumption (g/day and g/kg/day) was noted for the 51 µg/kg group females during Study Days 0 to 6 compared with the control group. This increase in feed consumption was not considered noteworthy due to the small magnitude of change and the lack of a correlating effect on body weight. There were no other statistically significant differences from the control group (Tables 8-11).

4.6 Clinical Pathology

Hematology. There were no effects on hematology parameters that were considered test article-related. Statistically significant ($p<0.05$ or $p<0.01$) higher mean absolute eosinophil counts were noted in the 13 and 51 µg/kg group males compared with the control group. These slight changes were not considered test article-related since similar changes were not observed in percent eosinophil counts in males or absolute or percent eosinophils in females (Tables 12 and 13; Appendix 2).

Serum Chemistry. There were no effects on serum chemistry parameters that were considered test article-related. Statistically significant ($p<0.01$) lower mean sodium was noted in the 51 µg/kg group females compared with the control group. This slight change (-1%) however, was not considered to be of sufficient magnitude to be toxicologically relevant. There were no other statistically significant changes in serum chemistry parameters (Tables 12 and 13; Appendix 2).

4.7 Anatomic Pathology

Macroscopic Examination. There were no macroscopic findings at the scheduled necropsy that were considered test article-related. One 51 µg/kg group female was noted with an enlarged thymus and one 51 µg/kg group female was noted with an enlarged mandibular lymph node. These findings were considered to be spontaneous and/or incidental in nature and unrelated to test article administration (Tables 14 and 15).

Organ Weights. There were no effects on organ weights that were considered test article-related. Statistically significant ($p<0.05$ or $p<0.01$) higher mean absolute and relative pituitary weights were noted in the 13 and 51 µg/kg/day group males compared with the control group. These differences were attributed to a low control group value and were not considered test article related. Higher ($p>0.01$) mean relative kidney weights were observed in the 51 µg/kg/day group males compared with the control group. This difference was not considered test article related since mean absolute kidney weight in the 51 µg/kg/day group males was comparable to the control group and there were no remarkable body weight changes for this group. There were no other statistically significant changes in the organ weight data (Tables 16 and 17).

Microscopic Examination. No test article-related microscopic findings were noted. Minimal hemorrhage, consisting of small numbers of free red cells in the tissue surrounding the tail vein, and/or minimal fibrosis, consisting of slightly increased amounts of fibrous tissue around the tail vein, were seen at the administration site in 4 of 10 control group and 2 of 10 51 µg/kg/day group animals. The hemorrhage and fibrosis were considered secondary to mechanical trauma associated with the intravenous injection. A variety of other microscopic findings were noted in a number of tissues. These findings all were common background changes and occurred either sporadically or with similar incidences across treatment groups; all were considered unrelated to administration of test article (Tables 18 and 19; Appendix 3).

Micronucleus Assessment. The test article did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes (PCEs) at dose levels of 13 or 51 µg/kg/day relative to the control group (Group 1). Therefore, the test article was concluded to have no potential to induce chromosome damage in PCEs under the conditions of this study. The positive control (Group 4) did induce a statistically significant increase in the incidence of PCEs compared with the control group (Group 1), indicating that all criteria for the test were valid (Appendix 4).

5.0 Conclusions

The objective of this study was to assess the toxicity of fluoroestradiol when administered by intravenous injection to Sprague-Dawley CD®(SD)IGS BR rats for 14 consecutive days, including the effect on micronucleus assessment.

There were no signs of toxicity at the doses tested on this study. No clinical observations were noted during the study. There were no test article-related changes in body weights or feed consumption. Clinical pathology parameters were unaffected by test article administration, and there were no toxicologically relevant organ weight changes. All macroscopic and microscopic findings observed were consistent with normal background lesions in clinically normal rats of the age and strain used on this study and were considered spontaneous and/or incidental in nature and unrelated to test article administration. Therefore, based on the results of this study, the NOEL for intravenous administration of fluoroestradiol to rats for 14 consecutive days was greater than 51 µg/kg/day.

6.0 References

Levene, H. Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, I. Olkin, et. al., eds. Stanford University Press, Stanford, CA, 1960, pp. 278-292.

National Research Council. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission of Life Sciences, National Academy Press: Washington, DC. Revised 1996.

Steel, R.G.D.; Torrie, J.H. Principles and Procedures of Statistics, A Biometrical Approach, 2nd ed.; McGraw-Hill Book Company: New York, 1980; pp 504-506.

7.0 Protocol Deviations

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 7.11** states that the animals will be acclimated for at least six days following receipt. One female replacement animal, received on November 12, 2008, only received a five-day acclimation period.
- **Protocol Section 9.1** states that cageside viability checks for mortality and general condition will be made at least twice daily (once in the morning and once in the afternoon, not less than six hours apart). The afternoon mortality check was inadvertently not performed on November 15, 2008.
- **Protocol Section 9.6.1** states that the adrenal gland will be collected and examined for all animals at necropsy. The adrenal gland was lost at necropsy for control group male no. 7.
- **Protocol Section 9.6.1** states that the mammary gland will be collected and examined for females only at necropsy. The mammary gland was collected for all animals and evaluated macroscopically/microscopically.
- **Protocol Section 9.6.1** did not require that the pituitary gland be collected and examined at necropsy due to a typographical omission. The pituitary gland was collected from all animals at the time of necropsy, weighed and examined microscopically as intended.
- **Protocol Section 9.6.1** states that the pituitary gland will be collected and examined for all animals at necropsy. The adrenal gland was lost at necropsy for 51 µg/kg group female no. 24.

These deviations did not negatively impact the quality or integrity of the data, nor the outcome of the study.

Table 1. Summary of the Fate of the Male and Female Animals (page 1 of 1)

RX Code	77366	92088	54823	64670
Color Code	Red	Blue	Yellow	Green
Dose	0 ug/kg	13 ug/kg	51 ug/kg	30 mg/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol	Cyclophosphamide
Number of **Males** on Study	5	5	5	2
Dosing Period	0	0	0	0
Scheduled Sacrifice	5	5	5	2
Number of **Females** on Study	5	5	5	0
Dosing Period	0	0	0	
Scheduled Sacrifice	5	5	5	

Table 2. Summary of the Male Clinical Observations (page 1 of 1)

		DAY OF STUDY															
	GROUP#[a]	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL
# OF ANIMALS EXAMINED	I	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	II	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	III	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	
Normal																	
WITHIN NORMAL LIMITS	I	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5
	II	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5
	III	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
Dead																	
Scheduled Sacrifice	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Miscellaneous																	
Feed Removed for Fasting of Animal	I	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5
	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Group I is 0 ug/kg of Fluoroestradiol, II is 13 ug/kg of Fluoroestradiol, III is 51 ug/kg of Fluoroestradiol and IV is 30 mg/kg of Cyclophosphimide.

Table 3. Summary of the Female Clinical Observations (page 1 of 1)

		DAY OF STUDY															
	GROUP#[a]	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL
# OF ANIMALS EXAMINED	I	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	II	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	III	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
Normal																	
WITHIN NORMAL LIMITS	I	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5
	II	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5
	III	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5
Dead																	
Scheduled Sacrifice	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
Miscellaneous																	
Feed Removed for Fasting of Animal	I	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	5

[a]Group I is 0 ug/kg of Fluoroestradiol, II is 13 ug/kg of Fluoroestradiol, and III is 51 ug/kg of Fluoroestradiol.

Table 4. Summary and Statistical Analysis of the Male Body Weights (page 1 of 1)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Body Weight (g):				
DAY 0	MEAN	170.0 ***d***	171.3	165.7
	S.E.	3.9	4.3	4.4
	N	5	5	5
DAY 6	MEAN	223.5 ***d***	223.5	215.5
	S.E.	7.4	5.2	5.2
	N	5	5	5
DAY 13	MEAN	265.4 ***d***	279.1	274.0
	S.E.	15.6	8.5	6.6
	N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 5. Summary and Statistical Analysis of the Female Body Weights (page 1 of 1)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Body Weight (g):				
DAY 0	MEAN	128.7 ***d***	129.5	131.6
	S.E.	5.9	3.9	4.2
	N	5	5	5
DAY 6	MEAN	151.2 ***d***	152.1	159.0
	S.E.	7.3	4.6	5.8
	N	5	5	5
DAY 13	MEAN	175.3 ***d***	175.8	184.2
	S.E.	9.3	6.3	6.3
	N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 6. Summary and Statistical Analysis of the Male Body Weight Changes (page 1 of 1)

RX Code			77366	92088	54823
Color Code			Red	Blue	Yellow
Dose			0 ug/kg	13 ug/kg	51 ug/kg
Chemical			Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Body Weight Change (g):					
DAY	0 TO 6	MEAN	53.5 ***d***	52.2	49.7
		S.E.	3.9	1.9	1.5
		N	5	5	5
DAY	6 TO 13	MEAN	41.9 ***d***	55.6	58.5
		S.E.	16.8	3.9	3.2
		N	5	5	5
DAY	0 TO 13	MEAN	95.4 ***d***	107.8	108.2
		S.E.	16.6	5.7	2.7
		N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 7. Summary and Statistical Analysis of the Female Body Weight Changes (page 1 of 1)

RX Code			77366	92088	54823
Color Code			Red	Blue	Yellow
Dose			0 ug/kg	13 ug/kg	51 ug/kg
Chemical			Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Body Weight Change (g):					
DAY	0 TO 6	MEAN	22.5 ***d***	22.6	27.4
		S.E.	3.2	1.7	2.8
		N	5	5	5
DAY	6 TO 13	MEAN	24.2 ***d***	23.7	25.2
		S.E.	2.5	2.1	2.2
		N	5	5	5
DAY	0 TO 13	MEAN	46.7 ***d***	46.4	52.5
		S.E.	4.5	3.5	4.6
		N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 8. Summary and Statistical Analysis of the Male Feed Consumption (g/day) (page 1 of 1)

			RX Code	77366	92088	54823
			Color Code	Red	Blue	Yellow
			Dose	0 ug/kg	13 ug/kg	51 ug/kg
			Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Feed Consumption (g/day):						
DAY	0 to 6	MEAN		22.1 ***d***	21.4	20.8
		S.E.		0.7	0.6	0.6
		N		5	5	5
DAY	6 to 13	MEAN		23.5 ***d***	23.8	23.7
		S.E.		2.2	0.9	0.5
		N		5	5	5
DAY	0 to 13	MEAN		22.9 ***d***	22.7	22.4
		S.E.		1.3	0.7	0.5
		N		5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 9. Summary and Statistical Analysis of the Female Feed Consumption (g/day) (page 1 of 1)

RX Code			77366	92088	54823
Color Code			Red	Blue	Yellow
Dose			0 ug/kg	13 ug/kg	51 ug/kg
Chemical			Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Feed Consumption (g/day):					
DAY	0 to 6	MEAN	15.6 ***d***	15.8	17.6*
		S.E.	0.7	0.2	0.6
		N	5	5	5
DAY	6 to 13	MEAN	16.6 ***d***	16.9	18.1
		S.E.	0.7	0.3	0.5
		N	5	5	5
DAY	0 to 13	MEAN	16.1 ***d***	16.4	17.9
		S.E.	0.7	0.2	0.5
		N	5	5	5

Statistical key: ***d***=Dunnett's Test * = p<0.05

Table 10. Summary and Statistical Analysis of the Male Feed Consumption (g/kg/day) (page 1 of 1)

			RX Code	77366	92088	54823
			Color Code	Red	Blue	Yellow
			Dose	0 ug/kg	13 ug/kg	51 ug/kg
			Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Feed Consumption (g/kg/day):						
DAY	0 to 6		MEAN	112.4 ***d***	108.7	109.4
			S.E.	1.9	2.5	4.1
			N	5	5	5
DAY	6 to 13		MEAN	95.7 ***d***	94.7	97.1
			S.E.	6.8	1.5	2.8
			N	5	5	5
DAY	0 to 13		MEAN	104.0 ***d***	101.2	102.7
			S.E.	3.7	1.7	3.4
			N	5	5	5

Statistical key: ***d***=Dunnett's Test

Table 11. Summary and Statistical Analysis of the Female Feed Consumption (g/kg/day) (page 1 of 1)

			RX Code	77366	92088	54823
			Color Code	Red	Blue	Yellow
			Dose	0 ug/kg	13 ug/kg	51 ug/kg
			Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Feed Consumption (g/kg/day):						
DAY	0 to 6	MEAN		112.0 ***d***	112.4	121.0*
		S.E.		2.3	2.8	1.4
		N		5	5	5
DAY	6 to 13	MEAN		101.6 ***d***	103.9	105.8
		S.E.		2.0	4.2	0.9
		N		5	5	5
DAY	0 to 13	MEAN		106.5 ***d***	108.0	113.0
		S.E.		1.9	3.6	1.0
		N		5	5	5

Statistical key: ***d***=Dunnett's Test * = p<0.05

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 1 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY:			
Number of Males	5	5	5
White Blood Cells ($x10^3/uL$)[a]	13.47 ***d*** ± 1.85 N=5	16.98 ± 0.57 N=5	14.81 ± 1.82 N=5
Red Blood Cells ($x10^6/uL$)[a]	7.54 ***d*** ± 0.10 N=5	7.31 ± 0.23 N=5	7.39 ± 0.11 N=5
Hemoglobin (g/dL)[a]	15.0 ***d*** ± 0.3 N=5	14.9 ± 0.3 N=5	14.7 ± 0.3 N=5
Hematocrit (%)[a]	51.1 ***d*** ± 0.7 N=5	50.8 ± 1.5 N=5	51.1 ± 1.0 N=5
Mean Corpuscular Volume (fL)[a]	67.8 ***d*** ± 1.0 N=5	69.6 ± 1.0 N=5	69.3 ± 0.8 N=5
Mean Corpuscular Hemoglobin (pg)[a]	20.0 ***d*** ± 0.2 N=5	20.5 ± 0.3 N=5	19.9 ± 0.2 N=5
Mean Corpuscular Hemoglobin Concentration (g/dL)[a]	29.5 ***d*** ± 0.4 N=5	29.4 ± 0.3 N=5	28.7 ± 0.1 N=5

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 2 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY (continued):			
Platelets ($x10^3$/uL)[a]	1289 ***d*** ± 82 N=5	1223 ± 171 N=5	1484 ± 95 N=5
Percent Neutrophils[a]	11.3 ***d*** ± 0.9 N=5	11.2 ± 0.9 N=5	9.7 ± 1.5 N=5
Neutrophils ($x10^3$/uL)[a]	1.48 ***d*** ± 0.17 N=5	1.89 ± 0.16 N=5	1.45 ± 0.32 N=5
Percent Lymphocytes[a]	85.2 ***d*** ± 1.0 N=5	85.0 ± 0.8 N=5	86.3 ± 1.5 N=5
Lymphocytes ($x10^3$/uL)[a]	11.50 ***d*** ± 1.64 N=5	14.44 ± 0.47 N=5	12.78 ± 1.60 N=5
Percent Monocytes[a]	1.4 ***d*** ± 0.1 N=5	1.4 ± 0.2 N=5	1.6 ± 0.2 N=5
Monocytes ($x10^3$/uL)[a]	0.19 ***d*** ± 0.04 N=5	0.23 ± 0.04 N=5	0.23 ± 0.03 N=5

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 3 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY (continued):			
Percent Eosinophils[a]	0.6 ***d*** ± 0.1 N=5	0.7 ± 0.1 N=5	0.8 ± 0.1 N=5
Eosinophils (x10^3/uL)[a]	0.07 ***r*** ± 0.01 N=5	0.11* ± 0.01 N=5	0.11** ± 0.004 N=5
Percent Basophils[a]	0.7 ***d*** ± 0.1 N=5	0.8 ± 0.1 N=5	0.9 ± 0.04 N=5
Basophils (x10^3/uL)[a]	0.09 ***d*** ± 0.02 N=5	0.15 ± 0.02 N=5	0.13 ± 0.02 N=5
Percent Large Unstained Cells[a]	0.8 ***d*** ± 0.1 N=5	0.9 ± 0.1 N=5	0.7 ± 0.1 N=5
Large Unstained Cells (x10^3/uL)[a]	0.12 ***d*** ± 0.03 N=5	0.16 ± 0.02 N=5	0.11 ± 0.02 N=5
Percent Reticulocytes[a]	3.61 ***d*** ± 0.81 N=5	4.15 ± 0.18 N=5	4.25 ± 0.17 N=5

Statistical key: ***d***=Dunnett's Test ***r***=Robust regression test * = p<0.05 ** = p<0.01

[a]Reported as the mean ± S.E.M.

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 4 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY (continued):			
Reticulocytes ($x10^9/L$)[a]	270.3 ***d*** ± 60.3 N=5	301.8 ± 9.6 N=5	313.1 ± 8.6 N=5
Number of Samples with Hypochromasia	4	4	5
Number of Samples with Clumped Platelets	0	1	1

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 5 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
CLINICAL CHEMISTRY:			
Number of Males[b]	5	5	5[c]
Urea Nitrogen (mg/dL)[a]	17.4 ***r*** ± 0.7 N=5	16.0 ± 1.9 N=5	15.0 ± 1.1 N=4
Creatinine (mg/dL)[a]	0.3 ***d*** ± 0.02 N=5	0.3 ± 0.03 N=5	0.3 ± 0.0 N=4
Glucose (mg/dL)[a]	123 ***d*** ± 36 N=5	128 ± 20 N=5	148 ± 29 N=4
Sodium (mmol/L)[a]	146 ***d*** ± 1 N=5	146 ± 0.4 N=5	148 ± 1 N=4
Potassium (mmol/L)[a]	7.6 ***d*** ± 0.4 N=5	7.7 ± 0.3 N=5	7.5 ± 0.3 N=4
Chloride (mmol/L)[a]	99 ***d*** ± 1 N=5	100 ± 0.4 N=5	100 ± 0.4 N=4

Statistical key: ***d***=Dunnett's Test ***r***=Robust regression test

[a]Reported as the mean ± S.E.M.
[b]Samples from 5 males in the 0 ug/kg group, 3 males in the 13 ug/kg group and 4 males in the 51 ug/kg group were hemolyzed.
[c]One sample was unacceptable for analysis.

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 6 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
CLINICAL CHEMISTRY (continued):			
Calcium (mg/dL)[a]	11.1 ***d*** ± 0.3 N=5	11.3 ± 0.2 N=5	11.3 ± 0.1 N=4
Inorganic Phosphorus (mg/dL)[a]	13.2 ***d*** ± 0.7 N=5	13.7 ± 0.9 N=5	13.6 ± 0.4 N=4
Alkaline Phosphatase (U/L)[a]	268 ***d*** ± 43 N=5	318 ± 22 N=5	317 ± 13 N=4
Alanine Aminotransferase (U/L)[a]	33 ***d*** ± 3 N=5	35 ± 3 N=5	36 ± 2 N=4
Aspartate Aminotransferase (U/L)[a]	88 ***d*** ± 9 N=5	76 ± 7 N=5	78 ± 6 N=4
Gamma-glutamyl Transferase (U/L)[a]	0 ***d*** ± 0 N=5	0 ± 0 N=5	0 ± 0 N=4
Total Bilirubin (mg/dL)[a]	0.2 ***d*** ± 0.02 N=5	0.2 ± 0.02 N=5	0.2 ± 0.03 N=4

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 12. Summary and Statistical Analysis of the Male Hematology and Clinical Chemistry (page 7 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
CLINICAL CHEMISTRY (continued):			
Total Protein (g/dL)[a]	6.1 ***d*** ± 0.2 N=5	6.1 ± 0.1 N=5	6.2 ± 0.1 N=4
Albumin (g/dL)[a]	3.2 ***d*** ± 0.1 N=5	3.2 ± 0.1 N=5	3.5 ± 0.1 N=4
Globulin (g/dL)[a]	2.9 ***d*** ± 0.1 N=5	2.9 ± 0.1 N=5	3.0 ± 0.1 N=4
Albumin/Globulin Ratio[a]	1.10 ***d*** ± 0.05 N=5	1.13 ± 0.03 N=5	1.11 ± 0.04 N=4
Cholesterol (mg/dL)[a]	68 ***d*** ± 4 N=5	72 ± 8 N=5	49 ± 8 N=4
Triglycerides (mg/dL)[a]	58 ***d*** ± 9 N=5	61 ± 6 N=5	66 ± 7 N=4

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 1 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY:			
Number of Females	5	5	5
White Blood Cells ($x10^3/uL$)[a]	11.13 ***d*** ± 0.69 N=5	12.21 ± 1.47 N=5	11.09 ± 0.93 N=5
Red Blood Cells ($x10^6/uL$)[a]	7.47 ***d*** ± 0.13 N=5	7.74 ± 0.17 N=5	7.78 ± 0.15 N=5
Hemoglobin (g/dL)[a]	14.8 ***d*** ± 0.2 N=5	15.3 ± 0.3 N=5	15.3 ± 0.3 N=5
Hematocrit (%)[a]	49.7 ***d*** ± 0.8 N=5	51.1 ± 1.2 N=5	51.8 ± 1.1 N=5
Mean Corpuscular Volume (fL)[a]	66.6 ***d*** ± 0.4 N=5	66.0 ± 0.3 N=5	66.6 ± 0.7 N=5
Mean Corpuscular Hemoglobin (pg)[a]	19.8 ***d*** ± 0.3 N=5	19.8 ± 0.1 N=5	19.6 ± 0.2 N=5
Mean Corpuscular Hemoglobin Concentration (g/dL)[a]	29.8 ***d*** ± 0.3 N=5	30.0 ± 0.1 N=5	29.5 ± 0.2 N=5

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 2 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY (continued):			
Platelets ($x10^3$/uL)[a]	1424 ***d*** ± 125 N=5	1454 ± 109 N=5	1385 ± 76 N=5
Percent Neutrophils[a]	9.1 ***d*** ± 1.7 N=5	8.6 ± 1.7 N=5	5.4 ± 0.9 N=5
Neutrophils ($x10^3$/uL)[a]	0.98 ***d*** ± 0.16 N=5	1.03 ± 0.20 N=5	0.58 ± 0.08 N=5
Percent Lymphocytes[a]	87.1 ***d*** ± 1.7 N=5	87.1 ± 1.3 N=5	90.7 ± 1.4 N=5
Lymphocytes ($x10^3$/uL)[a]	9.73 ***d*** ± 0.76 N=5	10.64 ± 1.33 N=5	10.07 ± 0.91 N=5
Percent Monocytes[a]	1.0 ***d*** ± 0.1 N=5	1.4 ± 0.1 N=5	1.2 ± 0.1 N=5
Monocytes ($x10^3$/uL)[a]	0.11 ***d*** ± 0.01 N=5	0.16 ± 0.02 N=5	0.14 ± 0.02 N=5

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 3 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY (continued):			
Percent Eosinophils[a]	0.7 ***d*** ± 0.1 N=5	1.0 ± 0.3 N=5	0.8 ± 0.1 N=5
Eosinophils (x10^3/uL)[a]	0.08 ***d*** ± 0.01 N=5	0.14 ± 0.05 N=5	0.09 ± 0.02 N=5
Percent Basophils[a]	0.8 ***r*** ± 0.1 N=5	0.9 ± 0.1 N=5	0.7 ± 0.1 N=5
Basophils (x10^3/uL)[a]	0.09 ***d*** ± 0.01 N=5	0.11 ± 0.01 N=5	0.08 ± 0.01 N=5
Percent Large Unstained Cells[a]	1.4 ***d*** ± 0.2 N=5	1.1 ± 0.1 N=5	1.1 ± 0.5 N=5
Large Unstained Cells (x10^3/uL)[a]	0.16 ***d*** ± 0.04 N=5	0.13 ± 0.02 N=5	0.12 ± 0.04 N=5
Percent Reticulocytes[a]	2.41 ***d*** ± 0.16 N=5	2.45 ± 0.07 N=5	2.10 ± 0.17 N=5

Statistical key: ***d***=Dunnett's Test ***r***=Robust regression test

[a]Reported as the mean ± S.E.M.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 4 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
HEMATOLOGY (continued):			
Reticulocytes ($x10^9/L$)[a]	181.0 ***d*** ± 14.6 N=5	189.5 ± 6.4 N=5	162.2 ± 11.3 N=5
Number of Samples with Hypochromasia	1	0	2
Number of Samples with Clumped Platelets	0	0	2

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 5 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
CLINICAL CHEMISTRY:			
Number of Females[b]	5	5	5
Urea Nitrogen (mg/dL)[a]	16.4 ***d*** ± 0.7 N=5	17.2 ± 0.7 N=5	16.0 ± 1.3 N=5
Creatinine (mg/dL)[a]	0.3 ***d*** ± 0.02 N=5	0.3 ± 0.02 N=5	0.3 ± 0.02 N=5
Glucose (mg/dL)[a]	135 ***d*** ± 27 N=5	161 ± 16 N=5	150 ± 23 N=5
Sodium (mmol/L)[a]	146 ***r*** ± 0.3 N=5	145 ± 1 N=5	145** ± 0.2 N=5
Potassium (mmol/L)[a]	7.6 ***d*** ± 0.5 N=5	8.8 ± 0.7 N=5	8.9 ± 0.9 N=5
Chloride (mmol/L)[a]	101 ***d*** ± 1 N=5	102 ± 1 N=5	100 ± 1 N=5

Statistical key: ***d***=Dunnett's Test ***r***=Robust regression test ** = p<0.01

[a]Reported as the mean ± S.E.M.

[b]Samples from 4 females in the 0 ug/kg group, 3 females in the 13 ug/kg group and 5 females in the 51 ug/kg group were hemolyzed.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 6 of 7)

RX Code Color Code Dose Chemical	77366 Red 0 ug/kg Fluoroestradiol	92088 Blue 13 ug/kg Fluoroestradiol	54823 Yellow 51 ug/kg Fluoroestradiol
CLINICAL CHEMISTRY (continued):			
Calcium (mg/dL)[a]	11.4 ***d*** ± 0.1 N=5	11.3 ± 0.2 N=5	11.6 ± 0.1 N=5
Inorganic Phosphorus (mg/dL)[a]	13.0 ***d*** ± 0.9 N=5	12.9 ± 0.8 N=5	13.7 ± 1.1 N=5
Alkaline Phosphatase (U/L)[a]	238 ***d*** ± 30 N=5	234 ± 26 N=5	235 ± 33 N=5
Alanine Aminotransferase (U/L)[a]	25 ***d*** ± 2 N=5	26 ± 1 N=5	31 ± 3 N=5
Aspartate Aminotransferase (U/L)[a]	92 ***d*** ± 12 N=5	87 ± 11 N=5	80 ± 7 N=5
Gamma-glutamyl Transferase (U/L)[a]	0 ***d*** ± 0 N=5	0 ± 0 N=5	0 ± 0 N=5
Total Bilirubin (mg/dL)[a]	0.1 ***d*** ± 0.02 N=5	0.2 ± 0.02 N=5	0.1 ± 0.02 N=5

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 13. Summary and Statistical Analysis of the Female Hematology and Clinical Chemistry (page 7 of 7)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
CLINICAL CHEMISTRY (continued):			
Total Protein (g/dL)[a]	6.5 ***d*** ± 0.1 N=5	6.3 ± 0.1 N=5	6.3 ± 0.2 N=5
Albumin (g/dL)[a]	3.6 ***d*** ± 0.1 N=5	3.4 ± 0.04 N=5	3.5 ± 0.1 N=5
Globulin (g/dL)[a]	2.9 ***d*** ± 0.04 N=5	2.9 ± 0.1 N=5	2.8 ± 0.1 N=5
Albumin/Globulin Ratio[a]	1.22 ***d*** ± 0.03 N=5	1.15 ± 0.03 N=5	1.28 ± 0.08 N=5
Cholesterol (mg/dL)[a]	83 ***d*** ± 5 N=5	85 ± 8 N=5	78 ± 4 N=5
Triglycerides (mg/dL)[a]	57 ***d*** ± 10 N=5	50 ± 3 N=5	60 ± 5 N=5

Statistical key: ***d***=Dunnett's Test

[a]Reported as the mean ± S.E.M.

Table 14. Summary of the Male Macroscopic Necropsy Findings (page 1 of 1)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
MALES	N	5	5	5
NO REMARKABLE OBSERVATIONS		5	5	5

Table 15. Summary of the Female Macroscopic Necropsy Findings (page 1 of 1)

RX Code / Color Code / Dose / Chemical		77366 Red 0 ug/kg Fluoroestradiol	92088 Blue 13 ug/kg Fluoroestradiol	54823 Yellow 51 ug/kg Fluoroestradiol
FEMALES	N	5	5	5
Thymus	N	0	0	1
Enlarged, 17x15x2 mm, White	N	0	0	1
	%	0.0	0.0	20.0
Mandibular Lymph	N	0	0	1
Enlarged, 3x3x3 mm, White	N	0	0	1
	%	0.0	0.0	20.0
NO REMARKABLE OBSERVATIONS		5	5	4

Table 16. Summary and Statistical Analysis of the Male Organ Weights and Relative Organ Weights (page 1 of 4)

	RX Code	77366	92088	54823
	Color Code	Red	Blue	Yellow
	Dose	0 ug/kg	13 ug/kg	51 ug/kg
	Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Organ Weight (g):				
FINAL BODY WEIGHT g	MEAN	247.3 ***d***	253.5	245.4
	S.E.	11.1	7.8	4.6
	N	5	5	5
Adrenal (pair) g	MEAN	0.0532 ***d***	0.0676	0.0691
	S.E.	0.0047	0.0067	0.0043
	N	4	5	5
Brain g	MEAN	1.8765 ***d***	1.8706	1.9361
	S.E.	0.0448	0.0229	0.0500
	N	5	5	5
Heart g	MEAN	0.9881 ***d***	1.0044	0.9837
	S.E.	0.0732	0.0498	0.0400
	N	5	5	5
Kidney (pair) g	MEAN	2.3879 ***d***	2.5744	2.7286
	S.E.	0.0668	0.0901	0.1075
	N	5	5	5
Liver g	MEAN	9.4059 ***d***	10.057	9.9083
	S.E.	0.7301	0.5220	0.5395
	N	5	5	5
Prostate g	MEAN	0.5071 ***d***	0.4087	0.5985
	S.E.	0.1450	0.0610	0.1311
	N	5	5	5
Spleen g	MEAN	0.6484 ***d***	0.7799	0.6509
	S.E.	0.0624	0.0274	0.0211
	N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 16. Summary and Statistical Analysis of the Male Organ Weights and Relative Organ Weights (page 2 of 4)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Organ Weight (g):				
Testes (pair) g	MEAN	2.6526 ***d***	2.7593	2.5735
	S.E.	0.0799	0.1220	0.0251
	N	5	5	5
Thymus g	MEAN	0.5961 ***d***	0.7784	0.6804
	S.E.	0.0535	0.0500	0.0685
	N	5	5	5
Pituitary (fixed) g	MEAN	0.0084 ***d***	0.0117**	0.0109*
	S.E.	0.0007	0.0008	0.0005
	N	5	5	5
Thyroid (fixed) g	MEAN	0.0214 ***d***	0.0223	0.0198
	S.E.	0.0017	0.0016	0.0015
	N	5	5	5

Statistical key: ***d***=Dunnett's Test * = p<0.05 ** = p<0.01

Table 16. Summary and Statistical Analysis of the Male Organ Weights and Relative Organ Weights (page 3 of 4)

	RX Code	77366	92088	54823
	Color Code	Red	Blue	Yellow
	Dose	0 ug/kg	13 ug/kg	51 ug/kg
	Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Relative Organ Weight (% final body weight):				
FINAL BODY WEIGHT g	MEAN	247.3 ***d***	253.5	245.4
	S.E.	11.1	7.8	4.6
	N	5	5	5
Adrenal (pair) Ratio	MEAN	0.0226 ***d***	0.0265	0.0282
	S.E.	0.0023	0.0020	0.0019
	N	4	5	5
Brain Ratio	MEAN	0.7643 ***d***	0.7403	0.7885
	S.E.	0.0367	0.0225	0.0079
	N	5	5	5
Heart Ratio	MEAN	0.3977 ***d***	0.3959	0.4006
	S.E.	0.0115	0.0133	0.0126
	N	5	5	5
Kidney (pair) Ratio	MEAN	0.9682 ***d***	1.0166	1.1103**
	S.E.	0.0162	0.0313	0.0245
	N	5	5	5
Liver Ratio	MEAN	3.7864 ***d***	3.9589	4.0273
	S.E.	0.1678	0.1162	0.1454
	N	5	5	5
Prostate Ratio	MEAN	0.2052 ***d***	0.1614	0.2413
	S.E.	0.0607	0.0243	0.0492
	N	5	5	5
Spleen Ratio	MEAN	0.2601 ***d***	0.3082*	0.2652
	S.E.	0.0134	0.0111	0.0067
	N	5	5	5

Statistical key: ***d***=Dunnett's Test * = p<0.05 ** = p<0.01

Table 16. Summary and Statistical Analysis of the Male Organ Weights and Relative Organ Weights (page 4 of 4)

	RX Code	77366	92088	54823
	Color Code	Red	Blue	Yellow
	Dose	0 ug/kg	13 ug/kg	51 ug/kg
	Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Relative Organ Weight (% final body weight):				
Testes (pair) Ratio	MEAN	1.0755 ***d***	1.0935	1.0498
	S.E.	0.0211	0.0617	0.0180
	N	5	5	5
Thymus Ratio	MEAN	0.2420 ***d***	0.3084	0.2770
	S.E.	0.0214	0.0229	0.0273
	N	5	5	5
Pituitary (fixed) Ratio	MEAN	0.0034 ***d***	0.0046**	0.0044**
	S.E.	0.0001	0.0003	0.0002
	N	5	5	5
Thyroid (fixed) Ratio	MEAN	0.0087 ***d***	0.0088	0.0080
	S.E.	0.0008	0.0006	0.0005
	N	5	5	5

Statistical key: ***d***=Dunnett's Test ** = p<0.01

Table 17. Summary and Statistical Analysis of the Female Organ Weights and Relative Organ Weights (page 1 of 4)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Organ Weight (g):				
FINAL BODY WEIGHT g	MEAN	157.2 ***d***	158.8	165.7
	S.E.	7.9	5.9	6.0
	N	5	5	5
Adrenal (pair) g	MEAN	0.0584 ***d***	0.0632	0.0687
	S.E.	0.0052	0.0049	0.0035
	N	5	5	5
Brain g	MEAN	1.6943 ***d***	1.7333	1.7767
	S.E.	0.0556	0.0459	0.0459
	N	5	5	5
Heart g	MEAN	0.7101 ***d***	0.6586	0.7161
	S.E.	0.0341	0.0199	0.0288
	N	5	5	5
Kidney (pair) g	MEAN	1.6289 ***d***	1.6784	1.7775
	S.E.	0.1014	0.0621	0.1032
	N	5	5	5
Liver g	MEAN	6.2953 ***d***	6.3742	6.8837
	S.E.	0.3004	0.2421	0.2502
	N	5	5	5
Ovary (pair) g	MEAN	0.0852 ***d***	0.1057	0.0992
	S.E.	0.0093	0.0142	0.0102
	N	5	5	5
Spleen g	MEAN	0.4296 ***d***	0.4448	0.4388
	S.E.	0.0315	0.0330	0.0258
	N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 17. Summary and Statistical Analysis of the Female Organ Weights and Relative Organ Weights (page 2 of 4)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Organ Weight (g):				
Thymus g	MEAN	0.5310 ***d***	0.5356	0.6123
	S.E.	0.0332	0.0186	0.0407
	N	5	5	5
Uterus g	MEAN	0.5400 ***d***	0.4100	0.4074
	S.E.	0.0711	0.0564	0.0136
	N	5	5	5
Pituitary (fixed) g	MEAN	0.0099 ***d***	0.0101	0.0093
	S.E.	0.0006	0.0014	0.0007
	N	5	5	4[a]
Thyroid (fixed) g	MEAN	0.0159 ***d***	0.0167	0.0150
	S.E.	0.0015	0.0013	0.0002
	N	5	5	5

[a]Decrease in N is due to one pituitary not being present in the formalin cup at time of weighing the fixed tissue.

Statistical key: ***d***=Dunnett's Test

Table 17. Summary and Statistical Analysis of the Female Organ Weights and Relative Organ Weights (page 3 of 4)

	RX Code	77366	92088	54823
	Color Code	Red	Blue	Yellow
	Dose	0 ug/kg	13 ug/kg	51 ug/kg
	Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Relative Organ Weight (% final body weight):				
FINAL BODY WEIGHT g	MEAN	157.2 ***d***	158.8	165.7
	S.E.	7.9	5.9	6.0
	N	5	5	5
Adrenal (pair) Ratio	MEAN	0.0369 ***d***	0.0401	0.0415
	S.E.	0.0019	0.0037	0.0015
	N	5	5	5
Brain Ratio	MEAN	1.0829 ***d***	1.0957	1.0786
	S.E.	0.0326	0.0369	0.0486
	N	5	5	5
Heart Ratio	MEAN	0.4535 ***d***	0.4154	0.4324
	S.E.	0.0187	0.0052	0.0090
	N	5	5	5
Kidney (pair) Ratio	MEAN	1.0349 ***d***	1.0582	1.0713
	S.E.	0.0316	0.0239	0.0355
	N	5	5	5
Liver Ratio	MEAN	4.0105 ***d***	4.0219	4.1599
	S.E.	0.0738	0.1281	0.0968
	N	5	5	5
Ovary (pair) Ratio	MEAN	0.0542 ***d***	0.0668	0.0597
	S.E.	0.0053	0.0093	0.0053
	N	5	5	5
Spleen Ratio	MEAN	0.2730 ***d***	0.2805	0.2674
	S.E.	0.0123	0.0185	0.0226
	N	5	5	5

Statistical key: ***d***=Dunnett’s Test

Table 17. Summary and Statistical Analysis of the Female Organ Weights and Relative Organ Weights (page 4 of 4)

RX Code		77366	92088	54823
Color Code		Red	Blue	Yellow
Dose		0 ug/kg	13 ug/kg	51 ug/kg
Chemical		Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Relative Organ Weight (% final body weight):				
Thymus Ratio	MEAN	0.3393 ***d***	0.3386	0.3704
	S.E.	0.0192	0.0138	0.0237
	N	5	5	5
Uterus Ratio	MEAN	0.3444 ***d***	0.2636	0.2465
	S.E.	0.0463	0.0445	0.0075
	N	5	5	5
Pituitary (fixed) Ratio	MEAN	0.0063 ***d***	0.0064	0.0057
	S.E.	0.0003	0.0008	0.0003
	N	5	5	4[a]
Thyroid (fixed) Ratio	MEAN	0.0102 ***d***	0.0106	0.0091
	S.E.	0.0011	0.0010	0.0003
	N	5	5	5

[a]Decrease in N is due to one pituitary not being present in the formalin cup at time of weighing the fixed tissue.

Statistical key: ***d***=Dunnett's Test

Table 18. Summary of the Male Microscopic Necropsy Findings (page 1 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Adrenal Glands			
Number Examined	5	0	5
Within Normal Limits	5		5
Aorta			
Number Examined	5	0	5
Within Normal Limits	5		5
Bone Marrow, Sternum			
Number Examined	5	0	5
Within Normal Limits	5		5
Bone, Femur			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Brain			
Number Examined	5	0	5
Within Normal Limits	5		5
Epididymis			
Number Examined	5	0	5
Within Normal Limits	5		5
Esophagus			
Number Examined	5	0	5
Within Normal Limits	5		5
Eye			
Number Examined	5	0	5
Within Normal Limits	5		5
Heart			
Number Examined	5	0	5
Within Normal Limits	5		5

Table 18. Summary of the Male Microscopic Necropsy Findings (page 2 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Injection Site			
Number Examined	5	0	5
Within Normal Limits	3		3
Fibrosis, minimal	0		2
Hemorrhage, minimal	1		0
Inflammation; chronic, minimal	1		0
Intestine, Cecum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Colon			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Duodenum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Ileum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Jejunum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Rectum			
Number Examined	5	0	5
Within Normal Limits	5		5
Kidney			
Number Examined	5	0	5
Within Normal Limits	2		4
Cast; proteinaceous, minimal	1		1
Mineralization, minimal	0		1
Nephropathy, minimal	1		0
Cyst	1		0

Table 18. Summary of the Male Microscopic Necropsy Findings (page 3 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Liver			
Number Examined	5	0	5
Within Normal Limits	1		1
Infiltration, Mixed Cell, minimal	4		4
Lung			
Number Examined	5	0	5
Within Normal Limits	1		3
Hemorrhage, minimal	3		2
Hemorrhage, mild	1		0
Inflammation; chronic-active, minimal	0		1
Lymph Node, Mesenteric			
Number Examined	5	0	5
Within Normal Limits	5		5
Lymph Node, Mandibular			
Number Examined	4	0	4
Within Normal Limits	2		4
Not Examined: Not found at trimming	1		1
Hyperplasia; lymphoid, minimal	1		0
Hyperplasia; lymphoid, mild	1		0
Mammary Gland			
Number Examined	5	0	3
Within Normal Limits	5		3
Not Examined: Not found at trimming	0		1
Not Examined: Not present on slide	0		1
Skeletal Muscle			
Number Examined	5	0	3
Within Normal Limits	5		3
Not Examined: Not found at trimming	0		2
Nerve, Optic			
Number Examined	4	0	5
Within Normal Limits	4		5
Not Examined: Not present on slide	1		0

Table 18. Summary of the Male Microscopic Necropsy Findings (page 4 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Nerve, Sciatic			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Pancreas			
Number Examined	5	0	5
Within Normal Limits	5		5
Parathyroid Gland			
Number Examined	2	0	3
Within Normal Limits	2		3
Not Examined: Not present on slide	3		2
Pituitary Gland			
Number Examined	5	0	5
Within Normal Limits	5		5
Prostate Gland			
Number Examined	4	0	5
Within Normal Limits	4		5
Not Examined: Not found at trimming	1		0
Salivary Gland			
Number Examined	4	0	4
Within Normal Limits	4		4
Not Examined: Not found at trimming	1		1
Seminal Vesicle			
Number Examined	5	0	5
Within Normal Limits	5		5
Skin			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Spinal Cord			
Number Examined	5	0	5
Within Normal Limits	5		5

Table 18. Summary of the Male Microscopic Necropsy Findings (page 5 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Spleen			
Number Examined	5	0	5
Within Normal Limits	5		5
Stomach			
Number Examined	5	0	5
Within Normal Limits	5		5
Testis			
Number Examined	5	0	5
Within Normal Limits	5		5
Thymus			
Number Examined	5	0	5
Within Normal Limits	3		5
Hemorrhage, minimal	2		0
Thyroid Gland			
Number Examined	5	0	5
Within Normal Limits	4		5
Ultimobranchial Cyst	1		0
Tongue			
Number Examined	5	0	5
Within Normal Limits	5		5
Trachea			
Number Examined	5	0	5
Within Normal Limits	5		5
Ureter			
Number Examined	5	0	5
Within Normal Limits	5		5
Urinary Bladder			
Number Examined	5	0	5
Within Normal Limits	5		5

Table 19. Summary of the Female Microscopic Necropsy Findings (page 1 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Adrenal Glands			
Number Examined	5	0	5
Within Normal Limits	5		5
Aorta			
Number Examined	5	0	5
Within Normal Limits	5		5
Bone Marrow, Sternum			
Number Examined	5	0	5
Within Normal Limits	5		5
Bone, Femur			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Brain			
Number Examined	5	0	5
Within Normal Limits	5		5
Cervix			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Esophagus			
Number Examined	5	0	5
Within Normal Limits	5		5
Eye			
Number Examined	5	0	5
Within Normal Limits	4		3
Dysplasia; retinal, minimal	1		1
Hyperkeratosis; corneal, minimal	0		1
Heart			
Number Examined	5	0	5
Within Normal Limits	5		5

Table 19. Summary of the Female Microscopic Necropsy Findings (page 2 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Injection Site			
Number Examined	5	0	5
Within Normal Limits	2		5
Fibrosis, minimal	3		0
Hemorrhage, minimal	1		0
Intestine, Cecum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Colon			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Duodenum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Ileum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Jejunum			
Number Examined	5	0	5
Within Normal Limits	5		5
Intestine, Rectum			
Number Examined	5	0	5
Within Normal Limits	5		5
Kidney			
Number Examined	5	0	5
Within Normal Limits	0		1
Mineralization, minimal	1		0
Nephropathy, minimal	4		3
Cyst	1		1

Table 19. Summary of the Female Microscopic Necropsy Findings (page 3 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Liver			
Number Examined	5	0	5
Within Normal Limits	1		1
Infiltration, Mixed Cell, minimal	3		4
Infiltration, Mixed Cell, mild	1		0
Lung			
Number Examined	5	0	5
Within Normal Limits	1		1
Hemorrhage, minimal	2		2
Inflammation; chronic-active, minimal	2		3
Infiltration, Mixed Cell, minimal	2		0
Lymph Node, Mesenteric			
Number Examined	5	0	5
Within Normal Limits	5		5
Lymph Node, Mandibular			
Number Examined	5	0	5
Within Normal Limits	5		4
Hyperplasia; lymphoid, minimal	0		1
Mammary Gland			
Number Examined	5	0	5
Within Normal Limits	5		5
Skeletal Muscle			
Number Examined	4	0	5
Within Normal Limits	4		5
Not Examined: Not found at trimming	1		0
Nerve, Optic			
Number Examined	5	0	5
Within Normal Limits	5		5
Nerve, Sciatic			
Number Examined	4	0	5
Within Normal Limits	4		5
Not Examined: Not found at trimming	1		0

Table 19. Summary of the Female Microscopic Necropsy Findings (page 4 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Ovary			
Number Examined	5	0	5
Within Normal Limits	4		4
Cyst	0		1
Mineralization, minimal	1		0
Oviduct			
Number Examined	4	0	5
Within Normal Limits	4		5
Not Examined: Not found at trimming	1		0
Pancreas			
Number Examined	5	0	5
Within Normal Limits	5		5
Parathyroid Gland			
Number Examined	3	0	5
Within Normal Limits	3		5
Not Examined: Not present on slide	2		0
Pituitary Gland			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Salivary Gland			
Number Examined	5	0	5
Within Normal Limits	5		5
Skin			
Number Examined	5	0	5
Within Normal Limits	5		5
Spinal Cord			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1
Spleen			
Number Examined	5	0	5
Within Normal Limits	5		5

Table 19. Summary of the Female Microscopic Necropsy Findings (page 5 of 5)

RX Code	77366	92088	54823
Color Code	Red	Blue	Yellow
Dose	0 ug/kg	13 ug/kg	51 ug/kg
Chemical	Fluoroestradiol	Fluoroestradiol	Fluoroestradiol
Stomach			
Number Examined	5	0	5
Within Normal Limits	5		5
Thymus			
Number Examined	5	0	5
Within Normal Limits	5		4
Hyperplasia; lymphoid, mild	0		1
Thyroid Gland			
Number Examined	5	0	5
Within Normal Limits	3		1
Ultimobranchial Cyst	1		4
Ectopic Thymus	1		0
Tongue			
Number Examined	5	0	5
Within Normal Limits	5		5
Trachea			
Number Examined	5	0	5
Within Normal Limits	5		5
Ureter			
Number Examined	5	0	5
Within Normal Limits	5		5
Urinary Bladder			
Number Examined	5	0	5
Within Normal Limits	5		3
Not Examined: Not found at trimming	0		2
Uterus			
Number Examined	5	0	5
Within Normal Limits	5		5
Vagina			
Number Examined	5	0	4
Within Normal Limits	5		4
Not Examined: Not found at trimming	0		1

APPENDIX 1

Analysis of Dosing Formulations

University of Washington
PET Radiochemistry

Certificate of Analysis
Certificate No. RC-001

Study Number: 0211886.001.001 "14 day Repeat Dose Study"

The following samples were analyzed following good laboratory practices following established protocol NCI-Q319 for analysis of fluoroestradiol by HPLC with adaptations for sample matrix and increased concentrations as validated for the FES toxicity study. These measures were made using UV absorbance detection at 280 nm.

Sample No.	Matrix	Date Sample Prepared	Nominal Concentration (µg/mL)	Sample Storage
110708-A-02-1-A	15% ethanol/ 85% Saline	11/17/08	0	refrigerator 2-8°C
110708-A-02-2-A	15% ethanol/ 85% Saline	11/17/08	6.5	refrigerator 2-8°C
110708-A-02-3-A	15% ethanol/ 85% Saline	11/17/08	25.5	refrigerator 2-8°C
110708-A-02-1	15% ethanol/ 85% Saline	11/17/08	0	refrigerator 2-8°C
110708-A-02-2	15% ethanol/ 85% Saline	11/17/08	6.5	refrigerator 2-8°C
110708-A-02-3	15% ethanol/ 85% Saline	11/17/08	25.5	refrigerator 2-8°C

Sample No.	Date Received	Date Analyzed	Nominal Concentration (µg/mL)	Measured Concentration (µg/mL)
110708-A-02-1-A	11/18/08	11/19/08	0	ND
110708-A-02-2-A	11/18/08	11/19/08	6.5	6.7 ± 0.1
110708-A-02-3-A	11/18/08	11/19/08	25.5	27.5 ± 0.2
110708-A-02-1	12/2/08	12/2/08, 12/3/08	0	ND
110708-A-02-2	12/2/08	12/2/08, 12/3/08	6.5	6.7 ± 0.1
110708-A-02-3	12/2/08	12/2/08, 12/3/08	25.5	25.7 ± 0.2

Procedural variations: None.

Analysis performed by: Jeanne M Link DATE: 4/10/09

Jeanne Meyers Link, PhD
Analytical and Radio-Chemist

Molecular Imaging Center * UW Medical Center, NW045 *
1959 NE Pacific Street, Box 356004 * Seattle, WA 98195-6004

APPENDIX 2

Clinical Pathology Report

This is a Certified Copy of a KP 2/3/09

Certified Copy

12/3/08

507 Airport Blvd., Suite 113, Morrisville, NC 27560

The attached reports are FINAL reports. These reports are electronically signed.

Study: RTI-1059 Time Period: Tech

Signed: [signature] Date: 12-3-08

This is a Certified Copy of a
Certified Copy
KA 2/3/0
P1-27 12/3/08

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1M	1	16.47	7.65	15.4	52.3
1	3	10.74	7.40	15.2	52.7
	5	10.38	7.74	15.5	50.3
	7	19.25	7.21	14.1	49.0
	9	10.50	7.69	15.0	51.1
2M	11	16.55	8.02	16.0	56.6
2	13	17.23	7.33	14.7	50.0
	15	17.00	6.83	14.5	48.2
	17	18.82	7.56	14.9	50.3
	19	15.31	6.79	14.4	49.1
3M	21	17.32	7.16	14.6	51.2
3	23	11.03	7.06	13.8	47.9
	25	10.06	7.61	15.2	52.8
	27	19.35	7.58	15.4	53.3
	29	16.29	7.52	14.4	50.5

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1M	1	68.4	20.2	29.5	1453
1	3	71.1	20.5	28.8	1359
	5	65.0	20.1	30.9	1014
	7	68.0	19.6	28.8	1421
	9	66.5	19.5	29.3	1199
2M	11	70.5	20.0	28.3	1335
2	13	68.3	20.1	29.4	1270
	15	70.5	21.3	30.1	565
	17	66.6	19.8	29.7	1390
	19	72.3	21.2	29.3	1554
3M	21	71.5	20.4	28.5	1360
3	23	67.9	19.6	28.9	1496
	25	69.4	19.9	28.7	1226
	27	70.3	20.3	28.9	1789
	29	67.2	19.2	28.5	1550

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1M	1	8.2	1.35	88.5	14.58
1	3	13.5	1.45	83.2	8.94
	5	12.7	1.32	83.4	8.65
	7	11.1	2.13	84.8	16.32
	9	11.1	1.17	86.0	9.03
2M	11	10.7	1.76	86.2	14.27
2	13	10.8	1.85	85.6	14.76
	15	14.5	2.47	81.9	13.93
	17	10.2	1.92	85.2	16.02
	19	9.6	1.47	86.3	13.22
3M	21	15.0	2.59	81.6	14.12
3	23	8.3	0.92	88.0	9.71
	25	8.5	0.85	86.4	8.69
	27	6.3	1.21	90.4	17.49
	29	10.4	1.70	85.3	13.89

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507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1M	1	1.2	0.19	0.4	0.07
1	3	1.4	0.15	0.7	0.08
	5	1.3	0.13	1.0	0.10
	7	1.9	0.36	0.4	0.07
	9	1.4	0.14	0.3	0.04
2M	11	0.7	0.11	0.9	0.14
2	13	1.8	0.31	0.4	0.07
	15	1.2	0.21	0.8	0.13
	17	1.8	0.33	0.7	0.12
	19	1.3	0.21	0.5	0.08
3M	21	1.1	0.19	0.7	0.12
3	23	1.5	0.16	1.0	0.11
	25	2.3	0.24	1.0	0.10
	27	1.1	0.22	0.6	0.12
	29	1.9	0.32	0.7	0.11

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Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1M	1	0.8	0.12	1.0	0.16
1	3	0.4	0.05	0.7	0.08
	5	0.9	0.10	0.8	0.08
	7	0.7	0.14	1.1	0.22
	9	0.6	0.06	0.6	0.06
2M	11	0.8	0.13	0.8	0.13
2	13	0.7	0.12	0.7	0.13
	15	0.8	0.13	0.7	0.13
	17	1.1	0.22	1.1	0.21
	19	0.8	0.13	1.4	0.21
3M	21	1.0	0.18	0.7	0.12
3	23	0.8	0.09	0.5	0.05
	25	0.9	0.09	0.8	0.08
	27	0.8	0.16	0.8	0.15
	29	0.8	0.13	0.9	0.14

Doug Neptun
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Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	RET% %	RET 10^9/L
1M	1	4.76	364.5
1	3	4.51	333.9
	5	0.44	34.3
	7	4.62	333.2
	9	3.71	285.5
2M	11	3.75	300.4
2	13	4.57	335.0
	15	4.14	282.9
	17	3.75	283.4
	19	4.52	307.2
3M	21	4.82	345.1
3	23	4.39	310.0
	25	3.89	296.0
	27	4.14	313.8
	29	4.00	300.7

Doug Neptun
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Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	HYPO	CPLT
1M	1	++	
1	3	++	
	5		
	7	+++	
	9	+	
2M	11	+++	
2	13	++	+
	15	+	
	17	+	
	19		
3M	21	+++	
3	23	++	
	25	++	+
	27	++	
	29	++	

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1F	2	8.83	7.82	14.9	51.2
1	4	11.23	7.54	15.0	51.1
	6	11.79	7.42	15.3	49.9
	8	13.02	7.00	13.9	46.8
	10	10.80	7.57	15.0	49.7
2F	12	11.75	8.15	16.1	54.4
2	14	8.86	8.06	16.0	53.3
	16	17.72	7.61	15.2	50.4
	18	11.35	7.24	14.3	47.8
	20	11.37	7.64	15.0	49.5
3F	22	9.80	8.13	15.8	54.0
3	24	10.35	7.56	15.5	51.9
	26	12.47	7.88	15.5	53.2
	28	8.87	7.30	14.2	47.7
	30	13.94	8.03	15.3	52.2

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1F	2	65.4	19.0	29.1	1531
1	4	67.7	19.8	29.3	1835
	6	67.2	20.6	30.6	1360
	8	66.9	19.9	29.7	1091
	10	65.7	19.8	30.1	1301
2F	12	66.8	19.8	29.6	1702
2	14	66.1	19.8	30.0	1370
	16	66.2	19.9	30.1	1675
	18	65.9	19.7	29.9	1415
	20	64.8	19.6	30.3	1108
3F	22	66.4	19.4	29.2	1267
3	24	68.6	20.5	29.9	1268
	26	67.5	19.6	29.1	1680
	28	65.3	19.5	29.8	1357
	30	65.0	19.1	29.4	1351

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1F	2	11.9	1.05	83.6	7.38
1	4	13.2	1.48	84.1	9.45
	6	6.2	0.74	90.3	10.64
	8	4.1	0.53	91.7	11.94
	10	10.0	1.09	85.4	9.22
2F	12	15.1	1.77	81.9	9.62
2	14	8.1	0.71	87.5	7.75
	16	6.5	1.15	88.4	15.66
	18	6.4	0.72	89.5	10.16
	20	6.9	0.78	88.0	10.01
3F	22	4.8	0.47	92.4	9.05
3	24	6.3	0.65	90.2	9.33
	26	2.6	0.32	94.4	11.77
	28	7.9	0.70	86.3	7.65
	30	5.6	0.78	90.0	12.55

Doug Neptun
Laboratory Director

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507 Airport Blvd, Suite 113
Morrisville, NC 17560

Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1F	2	1.3	0.12	0.6	0.06
1	4	0.9	0.11	0.4	0.05
	6	0.7	0.08	1.0	0.12
	8	0.7	0.09	0.6	0.08
	10	1.4	0.15	0.8	0.09
2F	12	1.1	0.12	0.7	0.08
2	14	1.3	0.12	0.5	0.04
	16	1.3	0.23	1.9	0.33
	18	1.4	0.16	0.8	0.10
	20	1.7	0.19	1.3	0.15
3F	22	1.1	0.10	0.7	0.07
3	24	1.2	0.13	0.8	0.08
	26	1.1	0.14	0.6	0.07
	28	1.3	0.12	0.7	0.07
	30	1.5	0.21	1.3	0.18

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1F	2	1.2	0.11	1.3	0.12
1	4	0.5	0.06	0.8	0.09
	6	0.6	0.07	1.2	0.14
	8	0.6	0.08	2.3	0.30
	10	1.1	0.12	1.3	0.14
2F	12	0.8	0.09	0.6	0.07
2	14	1.2	0.11	1.5	0.13
	16	0.8	0.14	1.2	0.21
	18	0.8	0.10	1.0	0.11
	20	0.9	0.11	1.2	0.13
3F	22	0.5	0.05	0.4	0.04
3	24	0.9	0.10	0.6	0.06
	26	0.7	0.09	0.7	0.08
	28	0.5	0.04	3.2	0.29
	30	0.8	0.11	0.8	0.12

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	RET% %	RET 10^9/L
1F	2	2.74	214.2
1	4	2.78	209.5
	6	2.39	177.4
	8	1.92	134.2
	10	2.24	169.5
2F	12	2.47	201.4
2	14	2.28	183.8
	16	2.63	200.5
	18	2.31	167.2
	20	2.55	194.7
3F	22	1.84	149.2
3	24	2.68	202.7
	26	2.08	163.9
	28	2.20	160.6
	30	1.68	134.7

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	HYPO	CPLT
1F	2	+	
1	4		
	6		
	8		
	10		
2F	12		
2	14		
	16		
	18		
	20		
3F	22	++	+
3	24		
	26	+	
	28		
	30		+

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Study: RTI-1059 Species: RAT

Time point: TERM Printed: 12/03/2008

Test Codes and Descriptions

Code	Description
BAS	Absolute Basophils
BAS%	% Basophils
EOS	Absolute Eosinophils
EOS%	% Eosinophils
HB	Hemoglobin
HCT	Hematocrit
HYPO	Hypochromasia
LUC	Absolute Large Unstained Cells
LUC%	% Large Unstained Cells
LYM	Absolute Lymphocytes
LYM%	% Lymphocytes
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Co
MCV	Mean Corpuscular Volume
MON	Absolute Monocytes
MON%	% Monocytes
NEU	Absolute Neutrophils
NEU%	% Neutrophils
PLT	Platelet Count
RBC	Red Blood Cell Count
RET	Absolute Reticulocyte
RET%	% Reticulocyte
WBC	White Blood Cell Count
CPLT	Clumped Platelets

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Species: RAT

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Group	Animal Number	COM	BUN mg/dl	CREA mg/dl	GLU mg/dl
1M	1	HEM	19	0.4	76
1	3	HEM	17	0.4	72
	5	HEM	19	0.3	106
	7	HEM	15	0.3	97
	9	HEM	17	0.3	264
2M	11	HEM	20	0.4	150
2	13	HEM	15	0.3	85
	15	HEM	21	0.3	188
	17		11	0.3	86
	19		13	0.2	133
3M	21	HEM	15	0.3	111
3	23		13	0.3	88
	25	HEM	14	0.3	183
	27	HEM	18	0.3	210
	29	HEM	UAA	UAA	UAA

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Study: RTI-1059

Species: RAT

Time point: TERM

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Group	Animal Number	NA mmol/L	K mmol/L	CL mmol/L	ALP U/L
1M	1	147	7.4	99	336
1	3	145	7.8	97	387
	5	147	6.7	100	139
	7	144	9.0	101	229
	9	148	7.1	99	247
2M	11	147	7.1	100	279
2	13	147	8.5	101	394
	15	147	8.3	99	325
	17	145	7.0	101	268
	19	146	7.6	100	326
3M	21	148	7.5	100	299
3	23	149	7.9	101	348
	25	148	6.8	99	326
	27	145	7.9	100	294
	29	UAA	UAA	UAA	UAA

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Study: RTI-1059 Species: RAT

Time point: TERM Printed: 12/03/2008

Group	Animal Number	ALT U/L	AST U/L	TBIL mg/dl	GGT U/L
1M	1	35	120	0.2	0
1	3	37	86	0.2	0
	5	28	84	0.2	0
	7	41	84	0.1	0
	9	26	68	0.2	0
2M	11	30	97	0.2	0
2	13	33	79	0.2	0
	15	45	81	0.2	0
	17	32	66	0.1	0
	19	36	57	0.2	0
3M	21	35	93	0.2	0
3	23	41	85	0.1	0
	25	37	69	0.1	0
	27	31	66	0.2	0
	29	UAA	UAA	UAA	UAA

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	TPRO g/dl	ALB g/dl	GLOB g/dL	A\G
1M	1	6.3	3.2	3.1	1.03
1	3	6.7	3.4	3.3	1.03
	5	5.4	3.0	2.4	1.25
	7	5.9	3.0	2.9	1.03
	9	6.3	3.4	2.9	1.17
2M	11	6.4	3.5	2.9	1.21
2	13	6.2	3.4	2.8	1.21
	15	6.3	3.3	3.0	1.10
	17	5.7	3.0	2.7	1.11
	19	5.9	3.0	2.9	1.03
3M	21	6.6	3.3	3.3	1.00
3	23	6.1	3.3	2.8	1.18
	25	6.2	3.3	2.9	1.14
	27	5.9	3.1	2.8	1.11
	29	UAA	UAA	UAA	UAA

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Laboratory Director

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	CA mg/dl	PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1M	1	10.6	12.8	72	55
1	3	11.5	15.5	77	84
	5	10.2	12.5	75	30
	7	11.2	13.5	65	72
	9	11.9	11.5	53	50
2M	11	11.5	15.6	63	54
2	13	11.4	15.6	58	75
	15	11.6	13.6	61	61
	17	10.7	11.1	76	71
	19	11.4	12.4	101	42
3M	21	11.0	14.7	72	49
3	23	11.3	13.5	38	85
	25	11.5	12.9	35	65
	27	11.5	13.1	52	64
	29	UAA	UAA	UAA	UAA

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Morrisville, NC 17560

Study: RTI-1059 Species: RAT

Time point: TERM Printed: 12/03/2008

Group	Animal Number	COM	BUN mg/dl	CREA mg/dl	GLU mg/dl
1F	2	HEM	16	0.4	52
1	4	HEM	16	0.4	182
	6	HEM	19	0.3	117
	8	HEM	15	0.3	203
	10		16	0.3	120
2F	12	HEM	20	0.4	150
2	14	HEM	16	0.3	119
	16	HEM	17	0.3	153
	18		17	0.3	220
	20		16	0.3	163
3F	22	HEM	21	0.4	90
3	24	HEM	15	0.3	111
	26	HEM	14	0.3	203
	28	HEM	14	0.3	143
	30	HEM	16	0.3	202

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	NA mmol/L	K mmol/L	CL mmol/L	ALP U/L
1F	2	146	9.2	103	280
1	4	146	7.8	100	334
	6	145	7.0	98	205
	8	146	6.2	101	179
	10	147	7.8	102	190
2F	12	143	11.0	102	252
2	14	144	10.0	102	312
	16	142	7.5	100	209
	18	146	7.8	101	153
	20	148	7.5	103	246
3F	22	144	11.0	101	363
3	24	145	6.9	98	185
	26	144	11.0	101	233
	28	145	8.2	100	206
	30	145	7.4	100	188

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Time point: TERM

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Group	Animal Number	ALT U/L	AST U/L	TBIL mg/dl	GGT U/L
1F	2	28	121	0.2	0
1	4	25	100	0.1	0
	6	30	112	0.2	0
	8	21	67	0.1	0
	10	19	58	0.1	0
2F	12	30	113	0.2	0
2	14	26	113	0.2	0
	16	27	75	0.2	0
	18	26	67	0.1	0
	20	22	66	0.1	0
3F	22	37	95	0.2	0
3	24	29	94	0.2	0
	26	41	83	0.1	0
	28	29	64	0.1	0
	30	21	63	0.1	0

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	TPRO g/dl	ALB g/dl	GLOB g/dL	A\G
1F	2	6.7	3.8	2.9	1.31
1	4	6.6	3.6	3.0	1.20
	6	6.6	3.7	2.9	1.28
	8	6.5	3.5	3.0	1.17
	10	6.0	3.2	2.8	1.14
2F	12	6.8	3.5	3.3	1.06
2	14	6.3	3.3	3.0	1.10
	16	6.1	3.4	2.7	1.26
	18	6.2	3.3	2.9	1.14
	20	6.1	3.3	2.8	1.18
3F	22	5.9	3.6	2.3	1.57
3	24	6.9	3.8	3.1	1.23
	26	5.7	3.1	2.6	1.19
	28	6.4	3.6	2.8	1.29
	30	6.4	3.4	3.0	1.13

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Species: RAT

Time point: TERM

Printed: 12/03/2008

Group	Animal Number	CA mg/dl	PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1F	2	10.9	15.1	75	57
1	4	11.3	14.7	75	46
	6	11.6	12.8	98	49
	8	11.7	10.8	91	39
	10	11.3	11.4	77	94
2F	12	11.5	15.3	93	40
2	14	10.7	14.2	71	49
	16	11.1	12.1	64	56
	18	11.7	12.1	110	51
	20	11.4	10.6	87	52
3F	22	11.4	17.1	80	66
3	24	11.3	12.8	93	75
	26	11.9	15.4	74	53
	28	11.4	11.4	67	46
	30	12.0	11.7	77	59

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Codes and Descriptions for Result Comments

Code	Description
UAA	Unacceptable for Analysis

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Study: RTI-1059

Species: RAT

Time point: TERM

Printed: 12/03/2008

Test Codes and Descriptions

Code	Description
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
A\G	A/G Ratio
BUN	Urea Nitrogen
CA	Calcium
CHOL	Cholesterol
CL	Chloride
COM	Comment
CREA	Creatinine
GGT	Gamma-glutamyl Transferase
GLOB	Globulin
GLU	Glucose
K	Potassium
NA	Sodium
PHOS	Inorganic Phosphorus
TBIL	Total Bilirubin
TPRO	Total Protein
TRIG	Triglyceride

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Quality Assurance Statement (QAS)

CONFIDENTIAL

To:	RTI International
Study Director:	Kimberly Ehman, Ph.D. (and Study Director Management)
From Quality Assurance Auditor:	John Murphy
Protocol referenced:	14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment (RTI-1059)
Regulations followed:	FDA 21 CFR Part 58; effective 20 June 1979
Timeperiod(s), material audited, inspection date(s):	Terminal, Study Data, December 18, 2008
Date the Audit Report was issued:	December 18, 2008
Study Director and Study Director Management notified (Dates sent):	Audit Report, December 19, 2008 QAS, December 19, 2008

Printed Name: John Murphy

Signature: John Murphy Date: Dec 19, 2008

Title: QA auditor

APPENDIX 3

Histopathology Report

FINAL REPORT

Study Phase: Pathology

Test Site Phase Reference Number 08-91

Testing Facility Study Number 0211886.001.001

14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

TEST SITE:
Charles River Laboratories
Pathology Associates - North Carolina
4025 Stirrup Creek Drive, Suite 150
Durham, NC 27703

TESTING FACILITY:
RTI International
Center for Life Sciences and Toxicology
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

SPONSOR:
Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-4910

May 27, 2009

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LIST OF DATA TABLES

LIST OF APPENDICES

1. COMPLIANCE STATEMENT

This study was conducted in compliance with the Food and Drug Administration's (FDA) Good Laboratory Practices (GLP) regulations (21 CFR Part 58). Three protocol deviations occurred during the histopathology phase of the study and are attached as Appendix 2.

2. QUALITY ASSURANCE STATEMENT

Study Title: 14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

The histopathology portion of this study has been inspected and audited by the Quality Assurance Unit (QAU) as required by the Good Laboratory Practices Regulations promulgated by the U.S. Food and Drug Administration, found in Title 21 of CFR, Section 58. This report is an accurate reflection of the recorded data. The following is a record of inspections and audits conducted by the QAU.

Date of Inspection	Phase Inspected	Date Reported to Principal Investigator and PAI Management	Date Reported to Study Director and Test Facility Management
16-Jan-2009	Staining and Coverslipping	16-Jan-2009	26-Jan-2009
29 & 30-Jan-2009	Data and Draft Report	30-Jan-2009	02-Feb-2009
27-May-2009	Final Report	27-May-2009	27-May-2009

Jeanne deWard, B.S., LATG, RQAP-GLP
Quality Assurance Manager
Charles River Pathology Associates – North Carolina

27 May 2009
Date

3. RESPONSIBLE PERSONNEL

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4. SUMMARY

According to the protocol, thirty two rats were divided into four groups with 5 rats/sex/group assigned to Groups 1-3 and 2 males assigned to Group 4. Rats in Groups 1-3 were administered either test article (fluoroestradiol) or vehicle (15% ethanol/85% saline) once daily via intravenous bolus in the lateral tail vein for 14 consecutive days. The first day of dosing was designated as Study Day 0. The two males assigned to Group 4 received cyclophosphamide via intraperitoneal injection on Study Day 13 only and served as a positive control for the micronucleus assay. All rats were scheduled for euthanasia on Study Day 14 and no early deaths occurred during the conduct of the study. At study termination all animals were humanely euthanized, subjected to a complete necropsy examination, and protocol specified tissues were collected in fixative. As per the protocol, only animals in Groups 1 (control) and 3 (high dose fluoroestradiol) were examined microscopically by the undersigned board certified veterinary pathologist.

No gross or microscopic findings considered to be related to test article administration were observed. A very few gross changes were seen but all were considered to be incidental background changes unrelated to the test article. Minimal hemorrhage or fibrosis was seen at the injection site in a few control and treated animals and was considered to be secondary to mechanical trauma resulting from intravenous injection. A few other microscopic findings occurred in a variety of tissues but all were considered to be incidental findings.

5. INTRODUCTION

This report presents the histopathology findings in rats assigned to a study entitled, "14-Day Intravenous Repeat Dose Toxicology Study in rats with Micronucleus Assessment" (Study Number 0211886.001.001). The purpose of the study was to assess the toxicity of fluoroestradiol when administered by intravenous injection to rats for 14 consecutive days.

The study was sponsored by Clinical Monitoring Research Program, SAIC Frederick, 6130 Executive Boulevard, EPN, Room 6070, Bethesda, Maryland. Paula Jacobs, PhD served as the Sponsor's Representative.

The in-life procedures, necropsy examination and tissue collection were conducted at RTI International, Center for Life Sciences and Toxicology, 3040 Cornwallis Road, Research Triangle Park, NC, 27709, where Kimberly D. Ehman, PhD served as the Study Director. The fixed tissues were shipped from RTI International to Charles River Laboratories, Pathology Associates, North Carolina for tissue trimming, slide preparation and microscopic evaluation. The microscopic slide evaluation was conducted by Micheal P. Jokinen, DVM, DACVP at Charles River Laboratories, Pathology Associates (PAI), North Carolina.

An electronic copy of this report (in PDF format) was created. It is a representation of the printed pathology report; however, only the signed printed copy of the pathology report is considered raw data.

6. MATERIALS AND METHODS

According to the protocol, thirty two rats were divided into four groups with 5 rats/sex/group in Groups 1-3, and 2 males assigned to Group 4. Rats in Group 1-3 were administered either test article (fluoroestradiol) or vehicle (15% ethanol/85% saline) once daily via intravenous bolus in the lateral tail vein for 14 consecutive days. The first day of dosing was designated as Study Day 0. The two males assigned to Group 4 received cyclophosphamide via intraperitoneal injection on Study Day 13 only and served as a positive control for the micronucleus assay. The study dosing details are provided in the table titled *Summary of Study Design* below.

Summary of Study Design

Dosage Group	Treatment	Dose	Dosing Concentration (µg/mL)	Dosing Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle	0	0	2.0	5	5
2	Fluoroestradiol	13 µg/kg	6.5	2.0	5	5
3	Fluoroestradiol	51 µg/kg	25.5	2.0	5	5
4	Cyclophosphamide	30 mg/kg	6.0 mg/mL	5.0	2	0

On Study Day 14, all rats were humanely euthanized by carbon dioxide asphyxiation followed by exsanguination. A complete necropsy examination was performed on all animals including examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities (see table titled *Tissues Examined and Collected at Necropsy* below). All tissues/organs from the examined animals, as well as all gross lesions from all animals, were preserved in 10% neutral buffered formalin (10% NBF).

Tissues Examined and Collected at Necropsy

Adrenal glands
Aorta
Brain
Bone (femur with epiphyseal plate of head)
Bone marrow (sternum)
Cecum
Colon
Duodenum
Epididymides
Esophagus
Eyes, with optic nerve[1]
Gross lesions (including tissue masses and abnormal regional lymph nodes)
Heart
Ileum
Injection site(s)
Jejunum
Kidney
Liver (right medial lobe and left lateral lobe)
Lungs[2]
Lymph node (mandibular and mesenteric)
Mammary gland (females only; to include nipple and surrounding tissue)
Ovaries
Oviducts
Pancreas
Pituitary gland[3]
Prostate
Rectum
Salivary gland (mandibular)
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin (ventral abdomen)
Spinal Cord (thoracolumbar junction; entire cord if neurologic abnormalities present)
Spleen
Stomach (fundic area)
Testes[1]
Thymus
Thyroid and parathyroid glands
Tongue
Trachea
Ureter
Urinary Bladder[2]
Uterus (body) with cervix
Vagina

[1] Modified Davidson's solution initially, followed by 10% neutral-buffered formalin.
[2]Possibly infused with formalin to ensure fixation.
[3]Pituitary gland was added to the tissue list through a protocol deviation included in Appendix 2.

As per the protocol, all required tissues, as well as gross lesions, from Group 1 (control) and 3 (high dose fluoroestradiol) animals only, were trimmed, processed, embedded in paraffin, sectioned, mounted on glass slides and stained with hematoxylin and eosin (H&E). The resulting glass slides were examined by a board-certified (DACVP) veterinary pathologist and all findings were entered into a validated pathology computer program (Provantis™ NT 2000, Data Management System). Tabulated gross and histopathology data are presented at the end of this report. In tables listing the findings for individual animals, morphologies (histopathology findings) were more fully described with topographical "locators" and "modifiers".

Following issuance of the Final Report, the study materials will be returned to the Testing Facility for archiving.

7. RESULTS

7.1. Early Deaths

No early deaths occurred during the conduct of the study. All animals survived to the terminal sacrifice on Study Day 14.

7.2. Gross Pathology

Gross pathology observations for the animals included in this report (Groups 1 and 3 animals) are listed in Table 1 *Pathology – Individual Gross Pathology Observations.* There were very few gross findings and all that were seen were considered to be incidental findings.

7.3. Histopathology

The intergroup comparison of histopathology observations for the animals included in this report (Groups 1 and 3 animals) is presented in Table 2 *Pathology - Intergroup Comparison of Gross/Histopathology Observations*. The individual animal data for the animals included in this report (Groups 1 and 3 animals) is presented in Table 3 *Pathology – Individual Animal Data (Concise Edition)*. The quality of tissue fixation, processing and staining was judged to be acceptable for evaluation by the study pathologist. A few tissues were unavailable for evaluation; the absence of these tissues had no effect on the overall evaluation the study.

No test article-associated microscopic findings were noted. Minimal hemorrhage, consisting of small numbers of free red cells in the tissue surrounding the tail vein, and minimal fibrosis, consisting of slightly increased amounts of fibrous tissue around the tail vein, were seen at the administration site in a few Group 1 (control) and a few Group 3 (high dose) animals. The hemorrhage and fibrosis were considered secondary to mechanical trauma associated with the intravenous injection. A variety of other microscopic findings were noted in a number of tissues. These findings all were common background changes and occurred either sporadically or with similar incidences across treatment groups; all were considered unrelated to administration of test article.

8. CONCLUSIONS

Thirty two Sprague Dawley rats were divided into four groups with 5 rats/sex/group assigned to Groups 1-3 and two males assigned to Group 4. Rats were administered either test article (fluoroestradiol) or vehicle (15% ethanol/85% saline) once daily via intravenous bolus in the lateral tail vein for 14 consecutive days. The two males assigned to Group 4 received cyclophosphamide via intraperitoneal injection on Study Day 13 only and served as a positive control for the micronucleus assay. No early deaths occurred during the conduct of the study. At study termination all animals were humanely euthanized, subjected to a complete necropsy examination, and protocol specified tissues were collected in fixative. All tissues, including gross lesions, from Group 1 (control) and Group 3 (high dose fluoroestradiol) animals were examined microscopically by the undersigned board certified veterinary pathologist.

No gross or microscopic findings considered to be related to test article administration were observed. A few gross and microscopic findings were observed in a variety of tissues in control and treated animals and all were considered to be incidental findings.

9. SIGNATURE

Micheal P. Jokinen, DVM, DACVP
Study Pathologist
Charles River Pathology Associates – North Carolina

27-MAY-2009
Date

Table 1
Pathology - Individual Gross Pathology Observations

Group: 1 Dose: 0 Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
1	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
3	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
5	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
7	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
9	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions

Group: 1 Dose: 0 Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
2	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
4	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
6	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
8	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
10	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions

Group: 3 Dose: 51 ug/kg Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
21	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
23	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
25	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
27	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
29	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions

Group: 3 Dose: 51 ug/kg Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
22	TERMINAL EUTHANASIA	14	(2)	LYMPH NODE, MANDIBULAR; Enlarged; white (TGL): 3 X 3 X 3 mm THYMUS; Enlarged; white (TGL): 17 X 15 X 2 mm Any remaining protocol required tissues, which have been examined, have no visible lesions
24	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
26	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
28	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions
30	TERMINAL EUTHANASIA	14	(2)	No Visible Lesions

Table 2
Pathology - Intergroup Comparison of Gross/Histopathology Observations

Note:
The number of animals on study refers to the number of animals examined microscopically.
The term “Completed” in the header of the Intergroup Comparison of Gross/Histopathology Observations table indicates that all protocol required activities were performed.
In the table, the dash (“-”) indicates that the reproductive organ is not applicable for the listed group

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
ADRENAL GLAND;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
AORTA;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
BONE MARROW, STERNUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
BONE, FEMUR;				
Examined	(5)	(4)	(5)	(4)
Within Normal Limits	5	4	5	4
Not Examined: NOT FOUND AT TRIMMING	0	1	0	1
BRAIN;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
CERVIX;				
Examined	(-)	(-)	(5)	(4)
Within Normal Limits	-	-	5	4
Not Examined: NOT FOUND AT TRIMMING	-	-	0	1
EPIDIDYMIS;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	5	5	-	-
ESOPHAGUS;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
EYE;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	4	3
Dysplasia; retinal	(0)	(0)	(1)	(1)
minimal	0	0	1	1

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
EYE; (continued)				
Hyperkeratosis; corneal	(0)	(0)	(0)	(1)
minimal	0	0	0	1
HEART;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
INJECTION SITE;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	3	3	2	5
Fibrosis	(0)	(2)	(3)	(0)
minimal	0	2	3	0
Hemorrhage	(1)	(0)	(1)	(0)
minimal	1	0	1	0
Inflammation; chronic	(1)	(0)	(0)	(0)
minimal	1	0	0	0
INTESTINE, CECUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
INTESTINE, COLON;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
INTESTINE, DUODENUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
INTESTINE, ILEUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
INTESTINE, JEJUNUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
INTESTINE, RECTUM;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
KIDNEY;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	2	4	0	1
Cast; proteinaceous	(1)	(1)	(0)	(0)
minimal	1	1	0	0
Mineralization	(0)	(1)	(1)	(0)
minimal	0	1	1	0
Nephropathy	(1)	(0)	(4)	(3)
minimal	1	0	4	3
Cyst	1	0	1	1
LIVER;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	1	1	1	1
Infiltration, Mixed Cell	(4)	(4)	(4)	(4)
minimal	4	4	3	4
mild	0	0	1	0
LUNG;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	1	3	1	1
Hemorrhage	(4)	(2)	(2)	(2)
minimal	3	2	2	2
mild	1	0	0	0
Inflammation; chronic-active	(0)	(1)	(2)	(3)
minimal	0	1	2	3
Infiltration, Mixed Cell	(0)	(0)	(2)	(0)
minimal	0	0	2	0
LYMPH NODE, MESENTERIC;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
LYMPH NODE, MANDIBULAR;				
Examined	(4)	(4)	(5)	(5)

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
LYMPH NODE, MANDIBULAR; (continued)				
Within Normal Limits	2	4	5	4
Not Examined: NOT FOUND AT TRIMMING	1	1	0	0
Hyperplasia; lymphoid	(2)	(0)	(0)	(1)
minimal	1	0	0	1
mild	1	0	0	0
MAMMARY GLAND;				
Examined	(5)	(3)	(5)	(5)
Within Normal Limits	5	3	5	5
Not Examined: NOT FOUND AT TRIMMING	0	1	0	0
Not Examined: NOT PRESENT ON SLIDE	0	1	0	0
SKELETAL MUSCLE;				
Examined	(5)	(3)	(4)	(5)
Within Normal Limits	5	3	4	5
Not Examined: NOT FOUND AT TRIMMING	0	2	1	0
NERVE, OPTIC;				
Examined	(4)	(5)	(5)	(5)
Within Normal Limits	4	5	5	5
Not Examined: NOT PRESENT ON SLIDE	1	0	0	0
NERVE, SCIATIC;				
Examined	(5)	(4)	(4)	(5)
Within Normal Limits	5	4	4	5
Not Examined: NOT FOUND AT TRIMMING	0	1	1	0
OVARY;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	4	4
Cyst	-	-	0	1
Mineralization	(-)	(-)	(1)	(0)
minimal	-	-	1	0
OVIDUCT;				
Examined	(-)	(-)	(4)	(5)
Within Normal Limits	-	-	4	5
Not Examined: NOT FOUND AT TRIMMING	-	-	1	0

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
PANCREAS;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
PARATHYROID GLAND;				
Examined	(2)	(3)	(3)	(5)
Within Normal Limits	2	3	3	5
Not Examined: NOT PRESENT ON SLIDE	3	2	2	0
PITUITARY GLAND;				
Examined	(5)	(5)	(5)	(4)
Within Normal Limits	5	5	5	4
Not Examined: NOT FOUND AT TRIMMING	0	0	0	1
PROSTATE GLAND;				
Examined	(4)	(5)	(-)	(-)
Within Normal Limits	4	5	-	-
Not Examined: NOT FOUND AT TRIMMING	1	0	-	-
SALIVARY GLAND;				
Examined	(4)	(4)	(5)	(5)
Within Normal Limits	4	4	5	5
Not Examined: NOT FOUND AT TRIMMING	1	1	0	0
SEMINAL VESICLE;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	5	5	-	-
SKIN;				
Examined	(5)	(4)	(5)	(5)
Within Normal Limits	5	4	5	5
Not Examined: NOT FOUND AT TRIMMING	0	1	0	0
SPINAL CORD;				
Examined	(5)	(5)	(5)	(4)
Within Normal Limits	5	5	5	4
Not Examined: NOT FOUND AT TRIMMING	0	0	0	1

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
SPLEEN;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
STOMACH;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
TESTIS;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	5	5	-	-
THYMUS;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	3	5	5	4
Hemorrhage	(2)	(0)	(0)	(0)
minimal	2	0	0	0
Hyperplasia; lymphoid	(0)	(0)	(0)	(1)
mild	0	0	0	1
THYROID GLAND;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	4	5	3	1
Ultimobranchial Cyst	1	0	1	4
Ectopic Thymus	0	0	1	0
TONGUE;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
TRACHEA;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
URETER;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Observations: Neo-Plastic and Non Neo-Plastic	---- MALES ----		--- FEMALES ---	
Removal Reasons: All of those SELECTED	0	51 ug/kg	0	51 ug/kg
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
URINARY BLADDER;				
Examined	(5)	(5)	(5)	(3)
Within Normal Limits	5	5	5	3
Not Examined: NOT FOUND AT TRIMMING	0	0	0	2
UTERUS;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	5	5
VAGINA;				
Examined	(-)	(-)	(5)	(4)
Within Normal Limits	-	-	5	4
Not Examined: NOT FOUND AT TRIMMING	-	-	0	1

Table 3
Pathology - Individual Animal Data (Concise Edition)

Note:
The term “None” in the Individual Animal Data Report as it applies to terminal body weight indicates that no data was entered.
The term “pathologist” refers to the last person who entered or manipulated the data.

Animal No.: 1 Group: 1 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Inflammation; chronic; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LYMPH NODE, MANDIBULAR;
Hyperplasia; lymphoid; minimal

THYMUS;
Hemorrhage; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	KIDNEY	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	SKELETAL MUSCLE
NERVE, OPTIC	NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND
SEMINAL VESICLE	SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS	THYROID GLAND
TONGUE	TRACHEA	URETER	URINARY BLADDER			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 2 Group: 1 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Fibrosis; minimal

KIDNEY;
Nephropathy; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND	NERVE, OPTIC
OVARY	OVIDUCT	PANCREAS	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD
SPLEEN	STOMACH	THYMUS	THYROID GLAND	TONGUE	TRACHEA	URETER
URINARY BLADDER	UTERUS	VAGINA				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 2 Group: 1 Sex: Female (continued)

The following tissues have not been examined:

SKELETAL MUSCLE; NOT FOUND AT TRIMMING
NERVE, SCIATIC; NOT FOUND AT TRIMMING
PARATHYROID GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 3 Group: 1 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Nephropathy; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND
SKELETAL MUSCLE	NERVE, SCIATIC	PANCREAS	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE
SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS	THYMUS	THYROID GLAND
TONGUE	TRACHEA	URETER	URINARY BLADDER			

The following tissues have not been examined:

NERVE, OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 4 Group: 1 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

EYE;
Dysplasia; retinal; minimal

KIDNEY;
Mineralization; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal

THYROID GLAND;
Ultimobranchial Cyst

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND	SKELETAL MUSCLE
NERVE, OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PITUITARY GLAND	SALIVARY GLAND	SKIN
SPINAL CORD	SPLEEN	STOMACH	THYMUS	TONGUE	TRACHEA	URETER
URINARY BLADDER	UTERUS	VAGINA				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 4 Group: 1 Sex: Female (continued)

The following tissues have not been examined:

OVIDUCT; NOT FOUND AT TRIMMING
PARATHYROID GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 5 Group: 1 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Cast; proteinaceous; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND
SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	PANCREAS	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND
SEMINAL VESICLE	SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS	THYMUS
THYROID GLAND	TONGUE	TRACHEA	URETER	URINARY BLADDER		

The following tissues have not been examined:

PARATHYROID GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 6 Group: 1 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Fibrosis; minimal
Hemorrhage; minimal

KIDNEY;
Nephropathy; minimal
Cyst

LIVER;
Infiltration, Mixed Cell; minimal

THYROID GLAND;
Ectopic Thymus

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND
SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	OVARY	OVIDUCT	PANCREAS	PARATHYROID GLAND
PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD	SPLEEN	STOMACH	THYMUS
TONGUE	TRACHEA	URETER	URINARY BLADDER	UTERUS	VAGINA	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 7 Group: 1 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations:

ADRENAL GLAND;
One of pair was available for evaluation

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Cyst

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal

LYMPH NODE, MANDIBULAR;
Hyperplasia; lymphoid; mild

THYMUS;
Hemorrhage; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		MAMMARY GLAND	SKELETAL MUSCLE	NERVE, OPTIC
NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE
SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS	THYROID GLAND	TONGUE
TRACHEA	URETER	URINARY BLADDER				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 8 Group: 1 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Fibrosis; minimal

KIDNEY;
Nephropathy; minimal

LUNG;
Inflammation; chronic-active; minimal
Infiltration, Mixed Cell; minimal

OVARY;
Mineralization; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LIVER	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND
SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	OVIDUCT	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND
SALIVARY GLAND	SKIN	SPINAL CORD	SPLEEN	STOMACH	THYMUS	THYROID GLAND
TONGUE	TRACHEA	URETER	URINARY BLADDER	UTERUS	VAGINA	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 9 Group: 1 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Hemorrhage; minimal

LUNG;
Hemorrhage; minimal

THYROID GLAND;
Ultimobranchial Cyst

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	KIDNEY	LIVER	LYMPH NODE, MESENTERIC		MAMMARY GLAND	SKELETAL MUSCLE
NERVE, OPTIC	NERVE, SCIATIC	PANCREAS	PITUITARY GLAND	SEMINAL VESICLE	SKIN	SPINAL CORD
SPLEEN	STOMACH	TESTIS	THYMUS	TONGUE	TRACHEA	URETER
URINARY BLADDER						

The following tissues have not been examined:

LYMPH NODE, MANDIBULAR; NOT FOUND AT TRIMMING
PARATHYROID GLAND; NOT PRESENT ON SLIDE
PROSTATE GLAND; NOT FOUND AT TRIMMING
SALIVARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 10 Group: 1 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: 15% ethanol/85% saline Dose: 0 Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Nephropathy; minimal

LIVER;
Infiltration, Mixed Cell; mild

LUNG;
Inflammation; chronic-active; minimal
Infiltration, Mixed Cell; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND
SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	OVARY	OVIDUCT	PANCREAS	PARATHYROID GLAND
PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD	SPLEEN	STOMACH	THYMUS
THYROID GLAND	TONGUE	TRACHEA	URETER	URINARY BLADDER	UTERUS	VAGINA

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 21 Group: 3 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Fibrosis; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	KIDNEY	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR
SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND	PROSTATE GLAND
SALIVARY GLAND	SEMINAL VESICLE	SPINAL CORD	SPLEEN	STOMACH	TESTIS	THYMUS
THYROID GLAND	TONGUE	TRACHEA	URETER	URINARY BLADDER		

The following tissues have not been examined:

MAMMARY GLAND; NOT FOUND AT TRIMMING
SKIN; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 22 Group: 3 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations:	Correlated with:
ADRENAL GLAND;	
.	
LYMPH NODE, MANDIBULAR;	
Enlarged; white (TGL): 3 X 3 X 3 mm	NO CORRELATION; No Correlating Lesion (H)
THYMUS;	
Enlarged; white (TGL): 17 X 15 X 2 mm	THYMUS; Hyperplasia; lymphoid; mild (H)

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

ADRENAL GLAND;
One of pair was available for evaluation

KIDNEY;
Cyst

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal

NO CORRELATION;	
No Correlating Lesion	LYMPH NODE, MANDIBULAR; Enlarged; white (G)
THYMUS;	
Hyperplasia; lymphoid; mild	THYMUS; Enlarged; white (G)

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 22 Group: 3 Sex: Female (continued)

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BRAIN	CERVIX	ESOPHAGUS	EYE
HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND	SKELETAL MUSCLE
NERVE, OPTIC	NERVE, SCIATIC	OVARY	OVIDUCT	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND
SALIVARY GLAND	SKIN	SPINAL CORD	SPLEEN	STOMACH	THYROID GLAND	TONGUE
TRACHEA	URETER	URINARY BLADDER	UTERUS	VAGINA		

The following tissues have not been examined:

BONE, FEMUR; NOT FOUND AT TRIMMING

Animal No.: 23 Group: 3 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BRAIN	EPIDIDYMIS	ESOPHAGUS	EYE
HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	KIDNEY	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND
NERVE, OPTIC	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE
SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS	THYMUS	THYROID GLAND
TONGUE	TRACHEA	URETER	URINARY BLADDER			

The following tissues have not been examined:

BONE, FEMUR; NOT FOUND AT TRIMMING
SKELETAL MUSCLE; NOT FOUND AT TRIMMING
NERVE, SCIATIC; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 24 Group: 3 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

EYE;
Hyperkeratosis; corneal; minimal

KIDNEY;
Nephropathy; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Inflammation; chronic-active; minimal

LYMPH NODE, MANDIBULAR;
Hyperplasia; lymphoid; minimal

OVARY;
Cyst

THYROID GLAND;
Ultimobranchial Cyst

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		MAMMARY GLAND	SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC

Animal No.: 24 Group: 3 Sex: Female (continued)

The following tissues were within normal limits: (continued)

OVIDUCT	PANCREAS	PARATHYROID GLAND	SALIVARY GLAND	SKIN	SPINAL CORD	SPLEEN
STOMACH	THYMUS	TONGUE	TRACHEA	URETER	UTERUS	VAGINA

The following tissues have not been examined:

PITUITARY GLAND; NOT FOUND AT TRIMMING
URINARY BLADDER; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 25 Group: 3 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LIVER;
Infiltration, Mixed Cell; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR
SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND	PROSTATE GLAND
SALIVARY GLAND	SEMINAL VESICLE	SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS
THYMUS	THYROID GLAND	TONGUE	TRACHEA	URETER	URINARY BLADDER	

The following tissues have not been examined:

MAMMARY GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 26 Group: 3 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Nephropathy; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal
Inflammation; chronic-active; minimal

THYROID GLAND;
Ultimobranchial Cyst

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	ESOPHAGUS	EYE
HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND	SKELETAL MUSCLE
NERVE, OPTIC	NERVE, SCIATIC	OVARY	OVIDUCT	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND
SALIVARY GLAND	SKIN	SPLEEN	STOMACH	THYMUS	TONGUE	TRACHEA
URETER	UTERUS					

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 26 Group: 3 Sex: Female (continued)

The following tissues have not been examined:

CERVIX; NOT FOUND AT TRIMMING
SPINAL CORD; NOT FOUND AT TRIMMING
URINARY BLADDER; NOT FOUND AT TRIMMING
VAGINA; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 27 Group: 3 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Cast; proteinaceous; minimal
Mineralization; minimal

LIVER;
Infiltration, Mixed Cell; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR	
MAMMARY GLAND	SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	PANCREAS	PITUITARY GLAND	PROSTATE GLAND
SALIVARY GLAND	SEMINAL VESICLE	SKIN	SPINAL CORD	SPLEEN	STOMACH	TESTIS
THYMUS	THYROID GLAND	TONGUE	TRACHEA	URETER	URINARY BLADDER	

The following tissues have not been examined:

PARATHYROID GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 28 Group: 3 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

THYROID GLAND;
Ultimobranchial Cyst

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
EYE	HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LIVER	LUNG	LYMPH NODE, MESENTERIC	
LYMPH NODE, MANDIBULAR		MAMMARY GLAND	SKELETAL MUSCLE	NERVE, OPTIC	NERVE, SCIATIC	OVARY
OVIDUCT	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD
SPLEEN	STOMACH	THYMUS	TONGUE	TRACHEA	URETER	URINARY BLADDER
UTERUS	VAGINA					

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 29 Group: 3 Sex: Male Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

INJECTION SITE;
Fibrosis; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Hemorrhage; minimal
Inflammation; chronic-active; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	EPIDIDYMIS	ESOPHAGUS
EYE	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	KIDNEY	LYMPH NODE, MESENTERIC		MAMMARY GLAND	NERVE, OPTIC	NERVE, SCIATIC
PANCREAS	PITUITARY GLAND	PROSTATE GLAND	SEMINAL VESICLE	SKIN	SPINAL CORD	SPLEEN
STOMACH	TESTIS	THYMUS	THYROID GLAND	TONGUE	TRACHEA	URETER
URINARY BLADDER						

The following tissues have not been examined:

LYMPH NODE, MANDIBULAR; NOT FOUND AT TRIMMING
SKELETAL MUSCLE; NOT FOUND AT TRIMMING
PARATHYROID GLAND; NOT PRESENT ON SLIDE
SALIVARY GLAND; NOT FOUND AT TRIMMING
Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 30 Group: 3 Sex: Female Species: Rat Strain: Sprague Dawley

Test Material: Fluoroestradiol Dose: 51 ug/kg Route: Intravenous Bolus Study Type: Toxicity
Date of Death : 01DEC2008 Study Day No. (Week): 14 (2) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 01DEC2008 ** NECROPSY COMPLETE **

Pathologist: Crystal L. Johnson ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

EYE;
Dysplasia; retinal; minimal

KIDNEY;
Nephropathy; minimal

LIVER;
Infiltration, Mixed Cell; minimal

LUNG;
Inflammation; chronic-active; minimal

THYROID GLAND;
Ultimobranchial Cyst

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW, STERNUM	BONE, FEMUR	BRAIN	CERVIX	ESOPHAGUS
HEART	INJECTION SITE	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LYMPH NODE, MESENTERIC		LYMPH NODE, MANDIBULAR		MAMMARY GLAND	SKELETAL MUSCLE
NERVE, OPTIC	NERVE, SCIATIC	OVARY	OVIDUCT	PANCREAS	PARATHYROID GLAND	PITUITARY GLAND
SALIVARY GLAND	SKIN	SPINAL CORD	SPLEEN	STOMACH	THYMUS	TONGUE
TRACHEA	URETER	URINARY BLADDER	UTERUS	VAGINA		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Appendix 1
Abbreviations and Glossary

Appendix 1
Abbreviations and Glossary

ASCP	American Society for Clinical Pathology
BS	Bachelor of Science
DACVP	Diplomate, American College of Veterinary Pathologists
DVM	Doctor of Veterinary Medicine
LATG	Laboratory Animal Technologist
TGL	Trackable Gross Lesion
HTL	Histotechnologist
PhD	Doctor of Philosophy
RTI	Research Triangle Institute
RQAP-GLP	Registered Quality Assurance Professional – Good Laboratory Practices

Protocol Terminology with Abbreviations and Terms Used in Provantis™ Tables:

Protocol Tissue	**Provantis™ NT 2000 Tissue Nomenclature**
Adrenal glands	ADRENAL GLAND
Bone (femur with epiphyseal plate of head)	BONE, FEMUR
Bone marrow (sternum)	BONE MARROW, STERNUM
Cecum	INTESTINE, CECUM
Colon	INTESTINE, COLON
Duodenum	INTESTINE, DUODENUM
Epididymides	EPIDIDYMIS
Eyes, with optic nerve	EYE and NERVE, OPTIC
Ileum	INTESTINE, ILEUM
Injection site(s)	INJECTION SITE
Jejunum	INTESTINE, JEJUNUM
Liver (right medial lobe and left lateral lobe)	LIVER
Lungs	LUNG
Lymph node (mandibular and mesenteric)	LYMPH NODE, MANDIBULAR and LYMPH NODE, MESENTERIC
Mammary gland (females only; to include nipple and surrounding tissue)	MAMMARY GLAND
Ovaries	OVARY
Oviducts	OVIDUCT

Rectum	INTESTINE, RECTUM
Prostate	PROSTATE GLAND
Salivary gland (mandibular)	SALIVARY GLAND, MANDIBULAR
Sciatic nerve	NERVE, SCIATIC
Seminal vesicles	SEMINAL VESICLE
Skin (ventral abdomen)	SKIN
Spinal cord (thoracolumbar junction; entire cord if neurologic abnormalities present)	SPINAL CORD
Stomach (fundic area)	STOMACH
Testes	TESTIS
Thyroid and parathyroid glands	THYROID GLAND and PARATHYROID GLAND
Uterus (body) with cervix	UTERUS and CERVIX

Appendix 2
Protocol Deviations

RTI INTERNATIONAL

CENTER FOR LIFE SCIENCES AND TOXICOLOGY DEVIATION REPORT

Principal Investigator / Study Director: K. Ehman

Project Number: 0211886.001.001

Master Protocol Number: 1059

Study Code: Rt08-FES

Deviation Type: Protocol √ SOP

Sponsor Notification: Yes No √

Original Document and Specifications: Protocol. The pituitary gland will be weighed from all toxicology group animals (Groups 1-3) and evaluated microscopically from Groups 1 and 3.

Deviation Summary and Reason: The pituitary gland was inadvertently omitted from the necropsy tissue list (Section 9.6.1). However, the pituitary was collected from all animals at the time of necropsy since it was noted as an organ to be weighed (Section 9.6.2).

Impact: This deviation did not negatively impact the quality or integrity of the study since the pituitary gland was collected and retained as intended and there were no remarkable lesions noted.

Read and understood by responsible person(s):

Signature, 12/16/08 Date

Signature, Date

Reported by: Signature, 12/16/08 Date

Approved by: Principal Investigator/Study Director Signature, 12/16/08 Date

CLST Management Signature, 16 Dec 08 Date

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STUDY DEVIATION RECORD

STUDY NO.: 0211886.001.001	DATE(S) OF DEVIATION: January, 2009
STUDY TITLE:	14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment
STUDY DIRECTOR:	Kimberly D. Ehman

☐ GLP DEVIATION ☒ PROTOCOL DEVIATION ☐ SOP DEVIATION

PROTOCOL SECTION:	9.6. Anatomic Pathology	SOP NO.:	SECTION:

DEVIATION DESCRIPTION: The protocol specifies that mammary gland is to be evaluated for females only; mammary gland from males was also evaluated.

CAUSE: Mammary gland tissue was present on slide 18 with skin; therefore the pathologist evaluated it.

Initiated by: Micheal P. Jokinen 30-JAN-2009

Micheal P. Jokinen, DVM, DACVP
Study Pathologist Signature/Date

TO BE COMPLETED BY THE STUDY DIRECTOR:

☒ This deviation has no impact on the study. ☐ This deviation has an impact on the study.

Comments:

(Attach additional pages if needed)

Approved by: Jay G. Henson 03Feb2009

Jay G. Henson, B.S.
Study Director Signature/Date

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STUDY DEVIATION RECORD

STUDY NO.: 0211886.001.001	DATE(S) OF DEVIATION: January, 2009
STUDY TITLE:	14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment
STUDY DIRECTOR:	Kimberly D. Ehman

☐ GLP DEVIATION ☒ PROTOCOL DEVIATION ☐ SOP DEVIATION

PROTOCOL SECTION:	9.6. Anatomic Pathology	SOP NO.:	SECTION:

DEVIATION DESCRIPTION: A recut was requested for male mammary gland tissue; mammary gland is not a protocol required tissue for males.

CAUSE: The QC technician requested the recut in error.

Initiated by: April Conklin 30-Jan-2009

April Conklin, BS, HTL (ASCP)[cm]
Histology Laboratory Supervisor/Date

TO BE COMPLETED BY THE STUDY DIRECTOR:

☒ This deviation has no impact on the study. ☐ This deviation has an impact on the study.

Comments:

(Attach additional pages if needed)

Approved by: Jay G Henson 03Feb2009

Jay G. Henson, B.S.
Study Director Signature/Date

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APPENDIX 4

Micronucleus Report

PRINCIPAL INVESTIGATOR'S REPORT

Study Title

14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

In support of RTI Project Number 0211886.001

Test Article

Fluoroestradiol

Authors

Ljubica Krsmanovic, Ph.D.
Kathyayini Divi, M.S.

Study Completion Date

08 June 2009

Test Site

BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AC19NA.129GLP.BTL

Testing Facility

RTI International
Center for Life Science and Technology
Post Office Box 12194
3040 Cornwallis road
Research Triangle Park, NC 27709-2194

Sponsor

Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412

1.0 STATEMENT OF COMPLIANCE

Microscopic evaluation of bone marrow smears and analysis of data were performed by BioReliance under the study number AC19NA.129GLP.BTL, as a part of the RTI Project Number 0211886.001 (RTI Master Protocol Number RTI-1059, RTI Study Code Rt08-FES), in compliance with the OECD Guideline 474 (Genetic Toxicology: Mammalian erythrocytes Micronucleus Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998 and with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996 and 1997).

Ljubicia Krsmanovic, Ph.D.
Principal Investigator
BioReliance

08 June 2009
Date

BioReliance Management

08 JUN 2009
Date

BioReliance®

Quality Assurance Statement

Delegated Study Phase Information

Number: AC19NA.129GLP.BTL
Protocol Title: In Vivo Micronucleus Scoring of Sponsor Provided Slides

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this delegated phase was performed in accordance with the regulation(s) listed below and conducted according to the client study plan/protocol and relevant Standard Operating Procedures.

OECD Principles of Good Laboratory Practices (C(97)186/Final)

Inspections

Quality Assurance performed the inspections(s) below for this delegated phase.

Insp. Dates (From/To)		Phase Inspected	To Principal Investigator	To Test Site Management	To Study Director & Facility Management
12-Feb-200	12-Feb-2009	Data and Draft Reporting	12-Feb-2009	12-Feb-2009	20-Feb-2009
05-Jun-2009	08-Jun-2009	Final Reporting	08-Jun-2009	08-Jun-2009	08-Jun-2009

The Final Report and data phase inspection identified above represents the delegated phase of this study only. It describes the methods and procedures used in the delegated phase and attests that the reported results accurately reflect the raw data of the delegated phase.

E-signature

Test Site Quality Assurance: Allison Schaefer 08-Jun-2009 3:34 p GMT

Reason for signature: QA Approval

Printed by:Allison Schaefer
Printed on:8-Jun-09

3.0 STUDY INFORMATION

Sponsor:	Clinical Monitoring Research Program, SAIC Frederick 6130 Executive Boulevard EPN, Room 6070
Authorized Representative:	Paula M. Jacobs, Ph.D.
Testing Facility:	RTI International Center for Life Sciences and Technology Post Office Box 12194 3040 Cornwallis Road Research Triangle Park, NC 27709-2194
Study Director at RTI International:	Jay G. Henson, B.S.
RTI Project Number:	0211886.001
RTI Master Protocol Number:	RTI-1059
RTI Study Code:	Rt08-FES
Test Site:	BioReliance 9630 Medical Center Drive Rockville, MD 20850
BioReliance Study Number:	AC19NA.129GLP.BTL
Principal Investigator:	Ljubica Krsmanovic, Ph.D.
Test Article Name:	Fluoroestradiol
Material Received at BioReliance:	Bone marrow slides
Storage Conditions:	Ambient (15 to 30°C); protected from exposure to light without desiccant
Receipt/Login:	05 December 2008
Study Initiation:	09 January 2009
Experimental Start Date:	10 January 2009
Experimental Completion Date:	12 January 2009

4.0 TABLE OF CONTENTS

Page

5.0 SUMMARY

The objective of this portion of the study was to evaluate the clastogenic/aneugenic (genotoxic) potential of Fluoroestradiol when intravenously administered to male and female Sprague Dawley CD® (SD) IGS BR rats for 14 days. The bone marrow was analyzed for the presence of micronucleated polychromatic erythrocytes (MN-PCEs), which served as a biomarker/parameter of Fluoroestradiol induced gentoxicity.

Male and female Sprague Dawley CD® (SD) IGS BR rats were exposed to the test article at the testing facility (RTI International, NC). The study design is presented in Section 6.1 of this report. At the testing facility, animals were intravenously dosed for 14 days with either the vehicle or Fluoroestradiol at 13 or 51µg/kg, and then were euthanized. At the time of euthanasia, bone marrow smears (slides) were prepared from all animals in duplicate. The Test Site (BioReliance) received 32 bone marrow slides. Staining of slides, microscopic evaluation and reporting of the results were performed by the Test Site. The bone marrow smears were stained and 2000 polychromatic erythrocytes (PCEs) per each animal were microscopically evaluated for the presence of micronucleated polychromatic erythrocytes (MN-PCEs). A statistical analysis of data was performed using Kastenbaum-Bowman Tables (binomial distribution, $p \leq 0.05$).

The microscopic evaluation and analysis of data indicated the following:

- No appreciable reductions in the PCEs/ECs ratio in the bone marrow were observed in the male or female test article groups relative to the respective/concurrent negative control groups suggesting that the test article did not inhibit erythropoiesis.

- No statistically significant increase in the incidence of MN-PCEs in the bone marrow was observed in the male or female groups at any of the Fluoroestradiol doses tested (13 or 51 µg/kg/day) relative to the respective/concurrent negative control groups.

In conclusion, under the condition of the study conduct, Fluoroestradiol at exposure levels of up to and including 51 µg/kg/day did not induce a significant increase in the incidence of MN-PCEs in bone marrow of male or female Sprague Dawley CD® (SD) IGS BR rats. Therefore, Fluoroestradiol was concluded to have no genotoxic effect in the rat bone marrow when intravenously administered for 14 consecutive days.

6.0 MATERIAL AND METHODS

6.1 Study Design

The study design, conducted at RTI International under the project number 0211886.001, was as follows:

Treatment Group	Dose Level (µg/kg/day)	Concentration (µg/mL)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1/Vehicle	0	0	2	5	5
2/Fluoroestradiol	13	6.5	2	5	5
3/Fluoroestradiol	51	25.5	2	5	5
4/Cyclophosphamide monohydrate (CP)	30 mg/kg*	15 mg/mL	2	2	0

* CP was administered only once 18-24 hours prior to bone marrow collection time.

Handling of animals, dosing procedure and observation of animals following dose administrations are presented in the report generated by RTI International.

As per the Testing Facility study protocol, animals were dosed with the vehicle (15% ethanol/85% saline) and the test article for 14 consecutive days (Study day 0 to Study Day 13). The positive control animals were dosed with Cyclophosphamide (CP, 30 mg/kg) only once, on Study Day 13. On Study Day 14 (approximately 18-24 hours after the last dose administration), all animals were euthanized and a necropsy was conducted. At the time of necropsy, two bone marrow slides (smears) from all animals were prepared.

The Testing Facility submitted to the Test Site a total of 32 bone marrow slides (one slide/animal). At the Test Site, bone marrow smears (slides) were stained with Acridine orange stain (nucleic acid specific stain) and microscopically evaluated for the presence of micronucleated polychromatic erythrocytes (MN-PCEs).

6.2 Bone Marrow Micronucleus Analysis

A total of 32 bone marrow slides were received by BioReliance on 05 December 2008 and a code number AC19NA (sample 0002) was assigned. Upon receipt and prior to scoring, the bone marrow slides were stained with Acridine orange. The stained slides were coded using a random number table by an individual not involved with the scoring process. Using a fluorescent microscope and medium magnification (400X; blue excitation filter in the range of 440-490 nm and barrier filter combination at 520 nm), an area of acceptable quality was selected such that the cells were well spread and stained. Using oil immersion (1000X), the following cell populations were evaluated and enumerated:

- **Polychromatic erythrocytes (PCEs)**

  PCEs stain orange-red. PCEs are young erythrocytes (early stage of erythropoiesis) and are the target cells for evaluation of the test article clastogenicity (genotoxicity). Two-thousand PCEs per each animal were screened (scored) for the presence of micronuclei resulting in evaluation of a total of 10,000 PCEs per treatment group.

- **Normochromatic erythrocytes (NCEs)**

  NCEs appear light green in color. NCEs are mature erythrocytes (red blood cells) and are the final cell population formed during erythropoiesis. The number of NCEs and micronucleated NCEs (MN-PCEs) in the field of 1000 total erythrocytes (ECs = PCEs + MN-PCEs + NCEs + MN-NCEs = 1000 ECs) was enumerated for each animal in order to calculate the proportion of polychromatic erythrocytes to total of 1000 erythrocytes. In addition, the incidence of MN-NCEs per 2000 PCEs was enumerated for each animal, but the results were not presented in this report or used in analysis of the test article induced clastogenic response since the primary target cells are the PCEs.

- **Micronuclei (M)**

  Micronuclei are round, fluorescent green-stained nuclear (chromosome) fragments with sharp contours and diameters commonly 1/20 to 1/5 that of an erythrocyte. Micronuclei may occur in PCEs (MN-PCEs) or NCEs (MN-NCEs).

7.0 EVALUATION OF TEST RESULTS

The incidence of micronucleated polychromatic erythrocytes (MN-PCEs) per 2000 polychromatic erythrocytes for each rat and per 10,000 PCEs per the vehicle and each test article group was determined. The incident of the MN-PCEs in the positive control was expressed as per total of 4000 PCEs. A statistical evaluation of data was performed using Kastenbaum-Bowman Tables for significant level of $p \leq 0.05$.

In order to quantify the proliferation state of the bone marrow as an indicator of bone marrow toxicity, the proportion of polychromatic erythrocytes to total erythrocytes was determined for each rat and treatment group (PCEs/ECs ratio). The proportion of polychromatic erythrocytes to total erythrocytes in the test-article treated animals should not be less than 20% of the control value.

All conclusions were based on scientific judgment. As a guide to interpretation of the data, the following were considered:

- The test article would have been considered to induce a positive response if, at least, one dose was statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables).
- Values that are statistically significant but do not exceed the range of historical negative controls (Appendix I) would have been judged as not biologically significant or relevant.
- If criteria for either a positive or negative clastogenic response were not met, the results would have been judged as equivocal.

- The test article is judged negative if no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes relative to the concurrent vehicle control values and no evidence of dose response were observed.

8.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports, generated by BioReliance, will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Testing Facility will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Testing Facility or destroyed will first be copied onto electronic media and the electronic copy will be retained in the BioReliance archives for a minimum of 10 years.

Stained slides, since not permanent, will be disposed of following issuance of the final report.

9.0 DEVIATIONS

No known deviations from the Plan of Work or assay method SOPs occurred during the conduct of the portion of the study at BioReliance.

10.0 RESULTS AND DISCUSSION

The results of bone marrow micronucleus analysis are presented in Table 1 (summary data) and Table 2 (individual data). The results indicated the following:

- No appreciable reductions in the PCEs/ECs ratio in the bone marrow were observed in the male or female test article groups relative to the respective/concurrent negative control groups.
- No statistically significant increase in the incidence of MN-PCEs in the bone marrow was observed in the male or female groups at any of the Fluoroestradiol doses tested (13 or 51 μg/kg/day) relative to the respective/concurrent negative control groups.
- CP, the positive control, induced a statistically significant increase in the incidence of MN-PCEs ($p \leq 0.05$, Kastenbaum-Bowman Tables) in the male rats relative to the negative control.
- The number of micronucleated PCEs in the vehicle control group did not exceed the historical vehicle control range. Based upon this, all criteria for a valid test were met as specified in the protocol.

11.0 CONCLUSION

In conclusion, under the condition of the study conduct, Fluoroestradiol at exposure levels of up to and including 51 µg/kg/day for 14 consecutive days did not induce a significant increase in the incidence of MN-PCEs in bone marrow of Sprague Dawley CD® (SD) IGS BR rats. Therefore, Fluroestradiol was concluded to have no genotoxic effect on rat bone marrow when intravenously administered for 14 consecutive days.

12.0 DATA TABLES

Table 1: Summary of Micronucleus Analysis in Bone Marrow of Sprague Dawley CD® (SD) IGS BR Rats After Intravenous Exposure to Fluoroestradiol for 14 Consecutive Days

Treatment (2 mL/kg)	Sex	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
Vehicle	M	5	0.538 ± 0.09	---	0.2 ± 0.27	2 / 10000
	F	5	0.501 ± 0.03	---	0.3 ± 0.27	3 / 10000
Fluoroestradiol						
13 µg/kg/day	M	5	0.488 ± 0.08	-9	0.0 ± 0.00	0 / 10000
	F	5	0.498 ± 0.08	-1	0.1 ± 0.22	1 / 10000
51 µg/kg/day	M	5	0.550 ± 0.05	2	0.3 ± 0.27	3 / 10000
	F	5	0.505 ± 0.07	1	0.1 ± 0.22	1 / 10000
Cyclophosphamide						
30 mg/kg	M	2	0.495 ± 0.13	-8	8.8 ± 0.35	*35 / 4000

*statistically significant increase; p less or equal to 0.05

Table 2: Induction of Micronucleated Polychromatic Erythrocytes in Bone Marrow of Sprague Dawley CD® (SD) IGS BR Rats After Intravenous Exposure to Fluoroestradiol for 14 Consecutive Days

Treatment (2 mL/kg)	Sex	Animal Numbers	PCE/Total Erythrocytes	Micronucleated PCE (Number/PCE scored)
Vehicle	M	1	0.642	0 / 2000
		3	0.524	0 / 2000
		5	0.395	1 / 2000
		7	0.559	0 / 2000
		9	0.571	1 / 2000
	F	2	0.501	0 / 2000
		4	0.483	1 / 2000
		6	0.461	0 / 2000
		8	0.520	1 / 2000
		10	0.540	1 / 2000
Fluoroestradiol				
13 µg/kg/day	M	11	0.414	0 / 2000
		13	0.476	0 / 2000
		15	0.617	0 / 2000
		17	0.430	0 / 2000
		19	0.501	0 / 2000
	F	12	0.407	0 / 2000
		14	0.552	0 / 2000
		16	0.501	1 / 2000
		18	0.599	0 / 2000
		20	0.430	0 / 2000
51 µg/kg/day	M	21	0.574	0 / 2000
		23	0.568	1 / 2000
		25	0.487	1 / 2000
		27	0.517	0 / 2000
		29	0.605	1 / 2000
	F	22	0.449	0 / 2000
		24	0.442	1 / 2000
		26	0.600	0 / 2000
		28	0.540	0 / 2000
		30	0.493	0 / 2000
Cyclophosphamide	M	31	0.590	17 / 2000
30 mg/kg		33	0.400	18 / 2000

13.0 APPENDICES

13.1 Appendix I: Micronucleus Test Historical Control Data

Rat Micronucleus Test Historical Control Data

2005-2007

Negative Control[1]

Parameter	Ratio of PCE/Total Erythrocytes		Number of MPCE/2000 PCE Scored/Animal		Number of MPCE/10000 PCE Scored/Group	
	Males	Females	Males	Females	Males	Females
Mean[3]	0.58	0.58	0.48	0.50	2.46	2.51
Standard Deviation	0.06	0.06	0.65	0.67	1.87	1.69
Range[4]	0.28 - 0.77	0.27 - 0.83	0 - 4	0 - 4	0 - 15	0 - 9

Positive Control[2]

Parameter	Ratio of PCE/Total Erythrocytes		Number of MPCE/2000 PCE Scored/Animal		Number of MPCE/10000 PCE Scored/ Group	
	Males	Females	Males	Females	Males	Females
Mean[3]	0.47	0.45	31.60	23.12	162.49	117.08
Standard Deviation	0.08	0.08	10.81	6.54	50.12	30.69
Range[4]	0.26 - 0.75	0.27 - 0.68	10 - 88	6 - 53	90 - 353	53 – 278

[1]Since no appreciable differences in the induction of MPCEs by different vehicles and solvents (test article carriers) and different routes of administration were observed, this table contains data from carriers and routes of administration widely used during the conduct of contract studies in the period of 2005-2007 at BioReliance.
Vehicles: water, water soluble vehicles (methylcellulose, carboxymethylcellulose, dextrose), saline, corn oil and other vehicles.
Routes of administration: intraperitoneal (IP), intravenous (IV), oral gavage (PO), subcutaneous (SC).
Bone marrow collection time: 24 and 48 hours post-dose.

[2]Positive control article: Cyclophosphamide monohydrate (CP); Doses: 40 to 50 mg/kg; Route of administration: IV, IP or PO. Bone marrow collection time: 24 hours post-dose.

[3]Average of the PCE ratio observed out of 1000 erythrocytes scored per animal for the total number of animals used during 2005-2007; average of the number of MPCE per 2000 PCE for the total number of animals used from 2005-2007; average of number of MPCE/per group (containing 5 animals per group) for total number of groups used in 2005-2007.

[4]Minimum and maximum range of PCE ratio observed out of 1000 erythrocytes scored per animal, the minimum and maximum range of MPCE observed out of 2000 PCE for the total number of animals used in 2005-2007 and the minimum and maximum range of MPCE observed out of 10000 PCE for the total number of groups used in 2005-2007.

13.2 Appendix II: Plan of Work

QA Review
[redacted]
CRH 01/30/09
Init. Date

PLAN OF WORK AMENDMENT 1

Sponsor: **Clinical Monitoring Research Program, SAIC Frederick**

Testing Facility: **RTI International**

Test Article I.D.: **fluoroestradiol**

BioReliance Study No.: **AC19NA.129GLP.BTL**

RTI Project Number: **0211886.001.001**

Study Title: **14 Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment**

---

1. **LOCATION:** Page 2, Section 3.3 Study Director

   AMENDMENT: Amend the name of the Study Director, as well as the address, phone number, fax number and e-mail address of the Study Director to read as follows:
   Jay G. Henson, BS
   Life Sciences and Toxicology
   RTI International
   3040 Cornwallis Road
   140 Hermann Building
   RTP, NC 27709
   Phone: (919) 541-7206
   E-mail: jhenson@rti.org]

   REASON FOR THE AMENDMENT: Study Director changed, as per the Testing Facility information.

2. **LOCATION:** Page 3 Section 4.4 Quality Assurance Unit of BioReliance (Lead QA)

   AMENDMENT: Amend the name of the Lead Quality Assurance person, as well as the address, phone number, fax number and e-mail address of the Lead QA to read as follows:
   Jermaine Sorrell
   BioReliance
   Rockville, MD 20850
   9630 Medical Center Drive
   Senior Quality Engineer
   Phone: (301) 610- 2257
   Fax: (301)738-1036
   E-mail: Jermaine.sorrell@bioreliance.com

REASON FOR THE AMENDMENT: To indicate reassignment of responsibilities at Test Site.

3. **LOCATION:** Page 4, Study Design Table

AMENDMENT: Amend the concentration for the positive control, Cyclophosphamide monohydrate (CP), to 15 mg/mL and a dose volume to 2 mL/kg.

REASON FOR THE AMENDMENT: To indicate correct units for CP concentration and correct CP dose volume.

APPROVALS:

Principal Investigator	30-JAN-2009 Date
Test Site Management	30 Jan 2009 Date
Study Director	2 Feb 2009 Date

QA Reviewed

LPO 18Feb09
Init. Date

PLAN OF WORK AMENDMENT 2

Sponsor:	**Clinical Monitoring Research Program, SAIC Frederick**
Testing Facility:	**RTI International**
Test Article I.D.:	**fluoroestradiol**
BioReliance Study No.:	**AC19NA.129GLP.BTL**
RTI Project Number:	**0211886.001 or 0211886.001.001**
Study Title:	**14 Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment**

1. **LOCATION:** Entire Plan of Work and Plan of Work Amendment 1

   AMENDMENT: Amend the RTI Project Number to 0211886.001 or 021186.001.001.

   REASON FOR THE AMENDMENT: To clarify that both study numbers are considered equivalent.

2. **LOCATION:** Plan of Work, Page 4, Section 6.3 Receiving of Slides

   AMENDMENT: Amend the second sentence to:

   If the slides are received in good condition, the code number (Test Site identification number AC19NA, sample 0002) will be assigned, and the slides will be stored appropriately at ambient condition.

   REASON FOR THE AMENDMENT: To indicate the correct sample number since sample 0001 had been used previously.

APPROVALS:

Ljubica Krmeomovic Principal Investigator	17-Feb-2009 Date
 Test Site Management	17-FEB-2009 Date
Jay Gibson Study Director	18Feb2009 Date

BioReliance Study Number: AC19NA.129GLP.BTL

PLAN OF WORK

Title
14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

Test Site for Micronucleus Assessment
BioReliance
9630 Medical Center Drive
Rockville, MD 20850
Study Number: AC19NA.129.BTL

Testing Facility
RTI International
Center for Life Sciences and Technology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194
RTI Project Number: 0211886.001

Study Sponsor
Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412

14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

1.0 PURPOSE

The purpose of this portion of the study is to evaluate the clastogenic/aneugenic (genotoxic) potential of the test article, fluoroestradiol, when intravenously administered to male and female Sprague Dawley CD® (SD)IGS BR rats for 14 days. The genotoxic potential will be measured by the test article ability to induce micronucleated polychromatic erythrocytes in rat bone marrow.

This portion of the study will be conducted in accordance with the study protocol (RTI International Number RTI-1059) and relevant amendments (if any), testing guidelines (Section 11.0) and Test Site standard operating procedures.

2.0 SPONSOR

2.1	Sponsor Name:	Clinical Monitoring Research Program, SAIC Frederick
2.2	Address:	6130 Executive Boulevard EPN, Room 6070 Bethesda, MD 20892-7412
2.3	Representative:	Paula M. Jacobs, Ph.D. CMRP, SAIC Frederick Project Officer

3.0 TESTING FACILITY AND KEY PERSONNEL

3.1	Name:	RTI International
3.2	Address:	Center for Life Sciences and Technology Post Office Box 12194 3040 Cornwallis Road Research Triangle Park, NC 27709-2194
3.3	Study Director:	Kimberly D. Ehman, Ph.D. Phone: 919-316-3802 Fax: 919-541-5956 E-mail: kehman@rti.org

3.4 RTI Project Number: 0211886.001.001

3.5 RTI Master Protocol No.: RTI-1059

3.6 RTI Study Code: Rt08-FES

4.0 TEST SITE AND KEY PERSONNEL

4.1 Name: Toxicology Testing Facility
BioReliance

4.2 Address: 9630 Medical Center Drive
Rockville, MD 20850

4.3 Principal Investigator: Ljubica Krsmanovic, Ph.D.
Phone: 301-610-2162
Fax: 301-738-2362
E-mail: buba.krsmanovic@bioreliance.com

4.4 Quality Assurance Unit of BioReliance (Lead QA):

Charles D. Lawrie, MS
Phone: 301-610-2737
Fax: 301-738-2362
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5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 22 December 2008

5.2 Proposed Experimental Completion Date: 09 January 2009

5.3 Proposed Draft Report Date: 19 January 2009

6.0 PLAN OF WORK

6.1 Introduction

The main study will be conducted at RTI International testing facility). The purpose of the main study is to evaluate the toxicity of the test article when intravenously given to male and female rats for 14 days. The Study design is presented in the following Table:

Group/Treatment	Dose Level	Concentration (µg/mL)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1/ Vehicle	0	0	2	5	5
2/ fluoroestradiol	13 µg/kg	6.5	2	5	5
3/ fluoroestradiol	51 µg/kg	25.5	2	5	5
4/Cyclophosphamide monohydrate (CP)	30 mg/kg	15	5	2	0

Animals will be dosed with the vehicle and the test article for 14 consecutive days (starting from Study Day 0 to Study Day 13). The positive control animals (2 male rats) will be dosed with CP only once, on Study Day 13. On Study Day 14 (approximately 18-24 hrs after the last dose administration), all animals will be euthanized and a necropsy will be conducted. At the time of necropsy, two bone marrow smears (slides) per each animal will be prepared by the Testing Facility (as per the study protocol). One slide from each animal will be shipped to the Test Site and at the Test Site, bone marrow smears will be microscopically evaluated for the presence of micronucleated polychromatic erythrocytes (biomarkers of genotoxicity).

6.2 Shipment of Slides

One set of slides will be shipped to the Test Site (BioReliance, 9630 Medical Center Drive, Rockville, MD 20850, to the attention of Albert Brew). Slides will be packed carefully to avoid breakage or damage during the shipment. Appropriate documentation will be included in the shipment.

6.3 Receiving of Slides

Upon receipt, at the Test Site, the slides will be checked for possible damage. The content of the box will be compared with the documentation for possible discrepancy. If the slides are received in good condition, the code number (Test Site identification number AC19NA, sample 0001) will be assigned, and the slides will be stored appropriately at ambient condition. If a discrepancy is observed, RTI will be contacted.

At the appropriate time, the In Vivo Cytogenetics Personnel will request the slides and slides will be transferred to the laboratory.

6.4 Staining of Slides

Bone marrow slides will be stained with acridine orange, a nucleic specific stain, according to the standard operating procedures of the Test Site.

6.5 Microscopic Evaluation of Slides

The slides will be coded using a random number table by an individual not involved with the scoring process. Using a fluorescent microscope and a medium magnification (400X; blue excitation filter in the range of 440-490 nm and barrier filter combination at 520 nm), an area of acceptable quality will be selected such that the cells are well spread and stained. Using oil immersion (1000X), if possible, 2000 polychromatic erythrocytes will be scored per animal for the presence of micronuclei. The number of micronucleated normochromatic erythrocytes in the field of 2000 polychromatic erythrocytes will also be enumerated, but will not be used to evaluate the response of the test article. The proportion of polychromatic erythrocytes to total erythrocytes will also be recorded per 1000 erythrocytes (PCEs/ECs ratio).

If deemed necessary, the second set of slides may be requested from the Testing Facility and evaluated at the Test Site.

7.0 Criteria for Determination of Valid Results

The incidence of micronucleated polychromatic erythrocytes in the vehicle (negative) control group should be comparable with the historical vehicle control data generated by the Test Site using acute dosing regimen.

The incidence of micronucleated polychromatic erythrocytes in the positive control should be comparable with the historical positive control data generated by the Test Site.

8.0 EVALUATION OF THE RESULTS

The incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes for each animal and per 10,000 PCEs per the vehicle and each test article group will be determined and presented. In the event that evaluation of 2000 PCEs/animal or 10,000 PCEs/group is not possible, the evaluation of the results will be based on the actual number of enumerated cells. The incidence of the micronucleated PCEs in the positive control will be expressed as per total of 4000 PCEs. Statistical analysis of data will be performed using the Kastenbaum-Bowman Tables which are based on the binomial distribution.

In order to quantify the test article effect on erythropoiesis, as an indicator of bone marrow toxicity, the proportion of polychromatic erythrocytes to total erythrocytes will be presented for each animal and treatment group. The proportion of polychromatic erythrocytes to total erythrocytes in test article-treated animals should not be less than 20% of the control value.

All conclusions will be based on sound scientific judgment. As a guide to interpretation of the data, the following will be considered:

- The test article will be considered to induce a positive response if, at least, one dose is statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables).
- Values that are statistically significant but do not exceed the range of historical negative or vehicle controls may be judged as not biologically significant and relevant.
- The test article will be judged negative if no statistically significant increase(s) in the incidence of micronucleated polychromatic erythrocytes in the test article group(s) above the concurrent negative (vehicle) control(s) is (are) observed.
- If criteria for either a positive or negative clastogenic response are not met, the results will be judged as equivocal.

9.0 REPORTING

A report of the results of this evaluation will be prepared by the Test Site and will accurately describe all methods used for generation and analysis of the data. Unless alternate arrangements are made, the report will be initially issued as a QA-audited draft. After receipt of the Study Director/Sponsor's comments, a final report will be issued.

The report will contain at least the following:

Results: proportion of polychromatic erythrocytes among total erythrocytes; number of micronucleated polychromatic erythrocytes per animal; mean±standard deviation of micronucleated polychromatic erythrocytes per group; dose-response relationship, where possible; statistical analyses; concurrent negative and positive control data; historical negative and positive control data with ranges, means and standard deviations.

- Discussion of results
- Conclusion
- Appendices: Historical Control Data (negative and positive controls with ranges, means and standard deviations, generated following acute dosing regimen).
- Statement of Compliance
- Quality Assurance Statement

If an electronic copy of the report or another study document is provided by BioReliance, the executed paper document is considered the official master document. If there is a discrepancy between an electronic copy and the corresponding master document, the master document will be considered the official document.

10.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports, generated by BioReliance, will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Testing Facility will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Testing Facility or destroyed will first be copied onto electronic media and the electronic copy will be retained in the BioReliance archives for a minimum of 10 years.

Stained slides, since not permanent, will be disposed of following issuance of the final report.

11.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This evaluation of the slides will comply with OECD Guideline 474 (Genetic Toxicology: Mammalian Erythrocytes Micronucleus Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998 and with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996 and 1997).

The work will be conducted in compliance with the most recent version of the United States Food and Drug Administration (U.S. FDA) Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58 and the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice, C(97)186/Final.

Alterations of the procedures may be made as the evaluation progresses. All modifications (amendments) and rationale for the change(s) will be documented, reviewed by BioReliance QA, signed, dated and approved by the Principal Investigator, Study Director and the Sponsor. All protocol amendments will be delivered to the Testing Facility and the Study Director via mail, electronic file transfer or fax transmission, as well as internally at the Test Facility, on or as close as possible to the effective date of the amendment.

Deviations from the procedures will be documented in a deviation report or a note to file will be generated. The deviation report(s) will be signed by the Study Director.

BioReliance Study Number: AC19NA.129GLP.BTL

12.0 REFERENCES

Heddle, J.A. A rapid *in vivo* test for chromosomal damage. *Mutation Res.* 1973; 18:187-190.

Heddle, J.A., M. Hite, B. Kirkhart, K. Mavournin, J.T. MacGregor, G.W. Newell, and M. Salamone. The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Res.* 1983; 123:61-118.

Hayashi, M., R.R. Tice, J.T. MacGregor, D. Anderson, D.H. Blakey, M. Kirsch-Volders, F.B. Oleson Jr., F. Pacchierotti, F. Romagna, H. Shimada, S. Sutou and B. Vannier. 1994. *In vivo* rodent erythrocyte micronucleus assay. *Mutation Res.* 1994; 312: 293-304.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. *Federal Register* 61: 18198-18202, April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. *Federal Register* 62: 16026-16030, November 21, 1997.

Kastenbaum, M.A. and K.O. Bowman. Tables for determining the statistical significance of mutation frequencies. *Mutation Res.* 1970; 9:527-549.

Mavournin, K.H., D.H. Blakey, M.C. Cimino, M.F. Salamone and J.A. Heddle. The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Res.* 1990; 239:29-80.

National Research Council, Institute of Laboratory Animal Resources Commission on Life Sciences. Guide for the Care and Use of Laboratory Animals. *National Academy Press.* Washington, D.C., 1996.

OECD Guidelines for Testing of Chemicals, Guideline 474 (Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998.

BioReliance Study Number: ~~AC19NA.129.BTL~~ ① AC19NA-129GLP-BTL

13.0 APPROVALS

Kimberly D. Ehman, Ph.D.
Study Director

11/21/08
Date

Ljubica Krsmanovic
Ljubica Krsmanovic, Ph.D.
Principal Investigator

09-JAN-2009
Date

Test Site Management

09 JAN 2009
Date

① Study number changed upon Study Director's approval of the protocol. Ljubica Krsmanovic 09-JAN-2009.

APPENDIX 5

Individual Animal Data Tables

List of Individual Animal Data Tables

Table V-1. Individual Fate of the F_0 Females (page 1 of 2)

MALES:

ANIMAL NO.	SEX	DOSE[a]	PRINT POSITION	SLOT#	DAY OF DEATH	SACRIFICE #	EARTAG#
1	M	1	1	2	1-DEC-08	0	D102
3	M	1	2	6	1-DEC-08	0	D106
5	M	1	3	8	1-DEC-08	0	D108
7	M	1	4	15	1-DEC-08	0	D115
9	M	1	5	16	1-DEC-08	0	D116
11	M	2	6	1	1-DEC-08	0	D101
13	M	2	7	4	1-DEC-08	0	D104
15	M	2	8	10	1-DEC-08	0	D110
17	M	2	9	12	1-DEC-08	0	D112
19	M	2	10	17	1-DEC-08	0	D117
21	M	3	11	3	1-DEC-08	0	D103
23	M	3	12	5	1-DEC-08	0	D105
25	M	3	13	9	1-DEC-08	0	D109
27	M	3	14	11	1-DEC-08	0	D111
29	M	3	15	14	1-DEC-08	0	D114
31	M	4	16	7	1-DEC-08	0	D107
33	M	4	17	13	1-DEC-08	0	D113

[a]Dose 1 is 0 ug/kg of Fluoroestradiol, 2 is 13 ug/kg of Fluoroestradiol, 3 is 51 ug/kg of Fluoroestradiol and 4 is 30 mg/kg of Cyclophosphamide.

Table V-1. Individual Fate of the F_0 Females (page 2 of 2)

FEMALES:

ANIMAL NO.	SEX	DOSE[a]	PRINT POSITION	SLOT#	DAY OF DEATH	SACRIFICE #	EARTAG#
2	F	1	18	19	1-DEC-08	0	D119
4	F	1	19	20	1-DEC-08	0	D120
6	F	1	20	22	1-DEC-08	0	D122
8	F	1	21	26	1-DEC-08	0	D126
10	F	1	22	28	1-DEC-08	0	D128
12	F	2	23	18	1-DEC-08	0	D118
14	F	2	24	23	1-DEC-08	0	D123
16	F	2	25	24	1-DEC-08	0	D124
18	F	2	26	25	1-DEC-08	0	D125
20	F	2	27	32	1-DEC-08	0	D132
22	F	3	28	21	1-DEC-08	0	D121
24	F	3	29	27	1-DEC-08	0	D127
26	F	3	30	29	1-DEC-08	0	D129
28	F	3	31	30	1-DEC-08	0	D130
30	F	3	32	31	1-DEC-08	0	D131

[a]Dose 1 is 0 ug/kg of Fluoroestradiol, 2 is 13 ug/kg of Fluoroestradiol and 3 is 51 ug/kg of Fluoroestradiol.

Table V-2. Individual Male Body Weights (g) (page 1 of 4)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	DAY OF STUDY 0	6	13
1	161.1	205.2	253.4
3	163.5	218.1	277.6
5	181.4	240.9	217.4
7	176.9	240.8	312.7
9	167.2	212.7	265.9
MEAN	170.0	223.5	265.4
S.E.	3.9	7.4	15.6
N	5	5	5

Table V-2. Individual Male Body Weights (g) (page 2 of 4)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

	DAY OF STUDY		
ANIMAL#	0	6	13
11	162.1	208.7	254.8
13	181.8	238.9	307.9
15	169.1	222.3	276.0
17	181.1	230.6	281.8
19	162.5	217.4	275.1
MEAN	171.3	223.5	279.1
S.E.	4.3	5.2	8.5
N	5	5	5

Table V-2. Individual Male Body Weights (g) (page 3 of 4)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	DAY OF STUDY 0	6	13
21	161.6	206.3	266.5
23	175.9	226.4	291.5
25	161.5	215.4	261.7
27	153.5	201.8	261.7
29	176.1	227.5	288.4
MEAN	165.7	215.5	274.0
S.E.	4.4	5.2	6.6
N	5	5	5

Table V-2. Individual Male Body Weights (g) (page 4 of 4)

MALES 64670 - Green - **30 mg/kg Cyclophosphamide**

	DAY OF STUDY	
ANIMAL#	0	6 13
31		296.8
33		271.1
MEAN		284.0
S.E.		12.8
N		2

Table V-3. Individual Male Feed Consumption (g/day) (page 1 of 3)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	DAY 0-6	OF STUDY 6-13	0-13
1	21.2	23.3	22.3
3	21.7	25.0	23.5
5	23.3	15.8	19.3
7	24.2	29.8	27.2
9	20.1	23.8	22.1
MEAN	22.1	23.5	22.9
S.E.	0.7	2.2	1.3
N	5	5	5

Table V-3. Individual Male Feed Consumption (g/day) (page 2 of 3)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	DAY 0-6	OF STUDY 6-13	0-13
11	19.4	20.8	20.2
13	22.8	26.0	24.5
15	22.0	24.8	23.5
17	21.0	24.3	22.8
19	22.0	23.3	22.8
MEAN	21.4	23.8	22.7
S.E.	0.6	0.9	0.7
N	5	5	5

Table V-3. Individual Male Feed Consumption (g/day) (page 3 of 3)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	DAY 0-6	OF STUDY 6-13	0-13
21	18.4	22.2	20.5
23	20.6	23.3	22.0
25	21.9	23.7	22.9
27	21.6	24.7	23.3
29	21.6	24.6	23.2
MEAN	20.8	23.7	22.4
S.E.	0.6	0.5	0.5
N	5	5	5

Table V-4. Individual Male Clinical Observations (page 1 of 10)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
1	Normal	0P	17-NOV-08 11:40	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:02	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:51	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:34	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:43	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:33	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:49	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:06	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:40	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:38	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 09:49	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:22	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:03	Scheduled Sacrifice
3	Normal	0P	17-NOV-08 11:42	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:05	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:52	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:35	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:45	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:34	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:50	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:07	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:41	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:39	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 09:51	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:22	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
5	Normal	0P	17-NOV-08 11:45	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:06	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:55	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:36	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:36	WITHIN NORMAL LIMITS

Table V-4. Individual Male Clinical Observations (page 2 of 10)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
5	(continued)			
	Normal	6P	23-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:08	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:48	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:43	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:48	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:40	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 09:51	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:22	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
7	Normal	0P	17-NOV-08 11:48	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:08	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:56	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:37	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:37	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:06	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:09	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:49	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:44	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:49	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:41	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 09:53	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
9	Normal	0P	17-NOV-08 11:52	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:11	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:57	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:39	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:48	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:40	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:53	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:10	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:50	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:45	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:54	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:43	WITHIN NORMAL LIMITS

Table V-4. Individual Male Clinical Observations (page 3 of 10)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
9	(continued)			
	Normal	13P	30-NOV-08 09:58	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice

Table V-4. Individual Male Clinical Observations (page 4 of 10)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
11	Normal	0P	17-NOV-08 11:54	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:12	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:58	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:40	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:50	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:40	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:08	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:54	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:11	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:44	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 09:59	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
13	Normal	0P	17-NOV-08 11:56	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:13	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 10:59	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:41	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:52	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:42	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:12	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:52	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:48	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:45	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:01	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
15	Normal	0P	17-NOV-08 11:59	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:15	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:00	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:43	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:53	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:43	WITHIN NORMAL LIMITS

Table V-4. Individual Male Clinical Observations (page 5 of 10)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
15	(continued)			
	Normal	6P	23-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:13	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:52	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:49	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:03	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
17	Normal	0P	17-NOV-08 12:03	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:16	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:02	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:44	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:11	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:14	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:53	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:58	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:03	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
19	Normal	0P	17-NOV-08 12:06	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:18	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:03	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:45	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:12	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:16	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:54	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:52	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:48	WITHIN NORMAL LIMITS

Table V-4. Individual Male Clinical Observations (page 6 of 10)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
19	(continued)			
	Normal	13P	30-NOV-08 10:05	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice

Table V-4. Individual Male Clinical Observations (page 7 of 10)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
21	Normal	0P	17-NOV-08 12:11	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:20	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:04	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:46	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:48	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:13	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 10:02	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:17	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:53	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:49	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:06	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
23	Normal	0P	17-NOV-08 12:13	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:21	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:09	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:48	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:50	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:14	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:18	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:54	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:02	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:50	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:07	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
25	Normal	0P	17-NOV-08 12:14	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:23	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:10	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:50	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:51	WITHIN NORMAL LIMITS

Table V-4. Individual Male Clinical Observations (page 8 of 10)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
25	(continued)			
	Normal	6P	23-NOV-08 10:15	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:19	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:08	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:23	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
27	Normal	0P	17-NOV-08 12:15	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:25	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:11	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:51	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:52	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:16	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:20	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:52	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:09	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
29	Normal	0P	17-NOV-08 12:16	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:27	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:13	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:53	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:54	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:19	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:21	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:53	WITHIN NORMAL LIMITS

Table V-4. Individual Male Clinical Observations (page 9 of 10)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
29	(continued)			
	Normal	13P	30-NOV-08 10:11	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice

Table V-4. Individual Male Clinical Observations (page 10 of 10)

MALES 64670 - Green - **30 mg/kg Cyclophosphamide**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
31	Normal	13P	30-NOV-08 11:47	WITHIN NORMAL LIMITS
	Dead	14P	1-DEC-08 08:06	Scheduled Sacrifice
33	Normal	13P	30-NOV-08 11:48	WITHIN NORMAL LIMITS
	Dead	14P	1-DEC-08 08:06	Scheduled Sacrifice

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 1 of 8)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Prostate Weight g	Prostate Ratio
1	SS	231.6	0.0642	0.0277	1.8375	0.7933	0.9245	0.3991	2.2614	0.9763	9.4170	4.0655	0.1550	0.0669
3	SS	253.6	0.0489	0.0193	1.7753	0.7000	0.9787	0.3859	2.4191	0.9539	10.3315	4.0739	0.3906	0.1540
5	SS	226.1	0.0570	0.0252	1.8339	0.8113	0.8517	0.3768	2.2831	1.0100	7.3420	3.2480	0.4021	0.1779
7	SS	287.7	LOST		1.8974	0.6594	1.2696	0.4412	2.6317	0.9146	11.5361	4.0092	0.5626	0.1955
9	SS	237.7	0.0429	0.0181	2.0383	0.8576	0.9160	0.3854	2.3442	0.9863	8.4030	3.5356	1.0254	0.4314

LOST=Tissue/organ lost during necropsy

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 2 of 8)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Prostate Weight g	Prostate Ratio
11	SS	233.9	0.0499	0.0213	1.8521	0.7920	0.9693	0.4145	2.3126	0.9889	8.3937	3.5894	0.2470	0.1056
13	SS	281.8	0.0856	0.0304	1.8886	0.6701	1.1808	0.4190	2.7156	0.9636	11.5253	4.0895	0.3436	0.1219
15	SS	251.3	0.0662	0.0263	1.8388	0.7318	0.9117	0.3629	2.6141	1.0404	10.5600	4.2028	0.6152	0.2448
17	SS	252.5	0.0794	0.0314	1.8223	0.7216	0.9193	0.3640	2.4294	0.9620	9.5657	3.7879	0.4440	0.1758
19	SS	248.3	0.0569	0.0229	1.9511	0.7859	1.0409	0.4193	2.8005	1.1281	10.2406	4.1251	0.3939	0.1587

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 3 of 8)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Prostate Weight g	Prostate Ratio
21	SS	240.8	0.0785	0.0326	1.9113	0.7937	1.0191	0.4232	2.5475	1.0579	8.9857	3.7316	0.2560	0.1063
23	SS	259.0	0.0602	0.0232	2.0673	0.7982	0.9921	0.3830	3.0065	1.1608	11.6732	4.5069	1.0324	0.3986
25	SS	237.3	0.0575	0.0242	1.7978	0.7576	0.8775	0.3698	2.5035	1.0550	8.9334	3.7648	0.6500	0.2739
27	SS	236.3	0.0741	0.0314	1.8727	0.7924	0.9217	0.3900	2.6165	1.1071	9.3049	3.9372	0.4143	0.1753
29	SS	253.7	0.0751	0.0296	2.0313	0.8008	1.1083	0.4369	2.9691	1.1705	10.6442	4.1962	0.6398	0.2522

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 4 of 8)

MALES 64670 - Green - **30 mg/kg Cyclophosphamide**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Prostate Weight g	Prostate Ratio
31	SS	284.3												
33	SS	260.2												

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 5 of 8)

MALES 77366 - Red - **0 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Testes (pair) Weight g	Testes (pair) Ratio	Thymus Weight g	Thymus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
1	SS	231.6	0.5917	0.2555	2.6443	1.1416	0.6043	0.2609	0.0069	0.0030	0.0264	0.0114
3	SS	253.6	0.6795	0.2679	2.6303	1.0372	0.7906	0.3118	0.0090	0.0035	0.0213	0.0084
5	SS	226.1	0.5638	0.2494	2.4854	1.0995	0.5467	0.2418	0.0075	0.0033	0.0158	0.0070
7	SS	287.7	0.8774	0.3049	2.9508	1.0255	0.5700	0.1981	0.0107	0.0037	0.0221	0.0077
9	SS	237.7	0.5294	0.2227	2.5521	1.0738	0.4689	0.1973	0.0078	0.0033	0.0214	0.0090

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 6 of 8)

MALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Testes (pair) Weight g	Testes (pair) Ratio	Thymus Weight g	Thymus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
11	SS	233.9	0.7086	0.3030	2.5283	1.0812	0.8136	0.3479	0.0111	0.0047	0.0235	0.0100
13	SS	281.8	0.8043	0.2854	2.4635	0.8741	0.7948	0.2820	0.0137	0.0049	0.0252	0.0089
15	SS	251.3	0.7494	0.2983	2.7413	1.0910	0.8045	0.3202	0.0090	0.0036	0.0198	0.0079
17	SS	252.5	0.7665	0.3035	3.0949	1.2256	0.5898	0.2336	0.0117	0.0046	0.0175	0.0069
19	SS	248.3	0.8709	0.3508	2.9685	1.1958	0.8891	0.3581	0.0132	0.0053	0.0257	0.0104

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 7 of 8)

MALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Testes (pair) Weight g	Testes (pair) Ratio	Thymus Weight g	Thymus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
21	SS	240.8	0.6724	0.2792	2.5414	1.0554	0.8911	0.3701	0.0114	0.0047	0.0172	0.0071
23	SS	259.0	0.7126	0.2751	2.5666	0.9909	0.6706	0.2589	0.0111	0.0043	0.0256	0.0099
25	SS	237.3	0.6498	0.2738	2.6177	1.1032	0.4970	0.2094	0.0117	0.0049	0.0180	0.0076
27	SS	236.3	0.5851	0.2476	2.5018	1.0586	0.5837	0.2470	0.0089	0.0038	0.0185	0.0078
29	SS	253.7	0.6347	0.2502	2.6401	1.0408	0.7596	0.2995	0.0113	0.0045	0.0195	0.0077

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-5. Individual Male Organ Weights and Organ Weight Ratios (page 8 of 8)

MALES 64670 - Green - **30 mg/kg Cyclophosphamide**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Testes (pair) Weight g	Testes (pair) Ratio	Thymus Weight g	Thymus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
31	SS	284.3										
33	SS	260.2										

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-6. Individual Male Macroscopic Necropsy Findings (page 1 of 3)

77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	ORGAN	OBSERVATION
1	SS	NO REMARKABLE OBSERVATIONS
3	SS	NO REMARKABLE OBSERVATIONS
5	SS	NO REMARKABLE OBSERVATIONS
7	SS	NO REMARKABLE OBSERVATIONS
9	SS	NO REMARKABLE OBSERVATIONS

SS=Scheduled Sacrifice

Table V-6. Individual Male Macroscopic Necropsy Findings (page 2 of 3)

92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	ORGAN	OBSERVATION
11	SS	NO REMARKABLE OBSERVATIONS
13	SS	NO REMARKABLE OBSERVATIONS
15	SS	NO REMARKABLE OBSERVATIONS
17	SS	NO REMARKABLE OBSERVATIONS
19	SS	NO REMARKABLE OBSERVATIONS

SS=Scheduled Sacrifice

Table V-6. Individual Male Macroscopic Necropsy Findings (page 3 of 3)

54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	ORGAN	OBSERVATION
21	SS	NO REMARKABLE OBSERVATIONS
23	SS	NO REMARKABLE OBSERVATIONS
25	SS	NO REMARKABLE OBSERVATIONS
27	SS	NO REMARKABLE OBSERVATIONS
29	SS	NO REMARKABLE OBSERVATIONS

SS=Scheduled Sacrifice

Table V-7. Individual Female Body Weights (g) (page 1 of 3)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	DAY OF STUDY 0	6	13
2	121.3	137.1	162.9
4	140.9	157.2	181.5
6	110.2	133.9	148.7
8	141.1	174.4	204.1
10	129.9	153.2	179.5
MEAN	128.7	151.2	175.3
S.E.	5.9	7.3	9.3
N	5	5	5

Table V-7. Individual Female Body Weights (g) (page 2 of 3)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	DAY OF STUDY 0	6	13
12	123.1	145.8	167.6
14	132.3	149.0	167.5
16	142.1	169.1	197.7
18	130.1	153.4	182.3
20	119.8	143.1	164.0
MEAN	129.5	152.1	175.8
S.E.	3.9	4.6	6.3
N	5	5	5

Table V-7. Individual Female Body Weights (g) (page 3 of 3)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

	DAY OF STUDY		
ANIMAL#	0	6	13
22	128.4	148.0	169.8
24	138.6	175.6	205.1
26	117.5	144.7	172.5
28	132.2	159.8	188.4
30	141.5	166.9	185.0
MEAN	131.6	159.0	184.2
S.E.	4.2	5.8	6.3
N	5	5	5

Table V-8. Individual Female Feed Consumption (g/day) (page 1 of 3)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	DAY 0-6	OF STUDY 6-13	0-13
2	14.3	14.6	14.5
4	15.7	17.6	16.7
6	14.5	15.3	14.9
8	18.1	18.6	18.4
10	15.6	16.7	16.2
MEAN	15.6	16.6	16.1
S.E.	0.7	0.7	0.7
N	5	5	5

Table V-8. Individual Female Feed Consumption (g/day) (page 2 of 3)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	DAY 0-6	OF STUDY 6-13	0-13
12	16.1	17.1	16.6
14	15.7	17.0	16.4
16	15.9	16.0	16.0
18	16.0	17.9	17.0
20	15.1	16.7	16.0
MEAN	15.8	16.9	16.4
S.E.	0.2	0.3	0.2
N	5	5	5

Table V-8. Individual Female Feed Consumption (g/day) (page 3 of 3)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	DAY 0-6	OF STUDY 6-13	0-13
22	17.0	17.2	17.1
24	19.4	19.9	19.7
26	16.1	17.1	16.6
28	16.9	18.1	17.6
30	18.5	18.4	18.4
MEAN	17.6	18.1	17.9
S.E.	0.6	0.5	0.5
N	5	5	5

Table V-9. Individual Female Clinical Observations (page 1 of 9)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
2	Normal	0P	17-NOV-08 12:17	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:28	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:14	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:15	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:32	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:50	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:06	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:54	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:12	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
4	Normal	0P	17-NOV-08 12:19	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:29	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:15	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:16	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:08	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:48	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:33	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:52	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:12	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
6	Normal	0P	17-NOV-08 12:20	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:31	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:16	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:17	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:57	WITHIN NORMAL LIMITS

Table V-9. Individual Female Clinical Observations (page 2 of 9)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
6	(continued)			
	Normal	6P	23-NOV-08 09:49	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:34	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:52	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:02	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:15	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
8	Normal	0P	17-NOV-08 12:21	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:32	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:17	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:19	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:58	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:50	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:35	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:53	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:02	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:02	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:16	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice
10	Normal	0P	17-NOV-08 12:22	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:34	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:19	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:20	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:37	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:54	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:58	WITHIN NORMAL LIMITS

Table V-9. Individual Female Clinical Observations (page 3 of 9)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
10	(continued)			
	Normal	13P	30-NOV-08 10:17	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:04	Scheduled Sacrifice

Table V-9. Individual Female Clinical Observations (page 4 of 9)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
12	Normal	0P	17-NOV-08 12:24	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:35	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:20	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:21	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:11	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:51	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:37	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:55	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:18	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
14	Normal	0P	17-NOV-08 12:25	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:36	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:21	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:22	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:12	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:53	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:39	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:57	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:11	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:19	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
16	Normal	0P	17-NOV-08 12:26	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:37	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:22	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:22	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:13	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:02	WITHIN NORMAL LIMITS

Table V-9. Individual Female Clinical Observations (page 5 of 9)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
16	(continued)			
	Normal	6P	23-NOV-08 09:55	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:40	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:58	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:06	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:12	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:20	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
18	Normal	0P	17-NOV-08 12:27	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:38	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:23	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:24	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:15	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:56	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:41	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 08:59	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:06	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:13	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:21	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
20	Normal	0P	17-NOV-08 12:28	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:41	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:25	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:25	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:16	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:57	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:42	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:00	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:08	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:14	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:02	WITHIN NORMAL LIMITS

Table V-9. Individual Female Clinical Observations (page 6 of 9)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
20	(continued)			
	Normal	13P	30-NOV-08 10:22	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:24	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice

Table V-9. Individual Female Clinical Observations (page 7 of 9)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
22	Normal	0P	17-NOV-08 12:29	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:42	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:26	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:26	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:17	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:06	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:58	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:43	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:01	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:08	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:15	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:03	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:23	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:25	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
24	Normal	0P	17-NOV-08 12:29	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:43	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:27	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:28	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:18	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:07	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 09:59	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:44	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:01	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:16	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:04	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:24	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:25	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:05	Scheduled Sacrifice
26	Normal	0P	17-NOV-08 12:31	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:44	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:30	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:29	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:20	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:08	WITHIN NORMAL LIMITS

Table V-9. Individual Female Clinical Observations (page 8 of 9)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
26	(continued)			
	Normal	6P	23-NOV-08 10:00	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:45	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:03	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:11	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:17	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:05	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:25	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:25	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:06	Scheduled Sacrifice
28	Normal	0P	17-NOV-08 12:32	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:46	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:31	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:31	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:21	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:09	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:01	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:46	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:04	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:11	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:12	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:17	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:06	WITHIN NORMAL LIMITS
	Normal	13P	30-NOV-08 10:26	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:25	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:06	Scheduled Sacrifice
30	Normal	0P	17-NOV-08 12:33	WITHIN NORMAL LIMITS
	Normal	1P	18-NOV-08 11:47	WITHIN NORMAL LIMITS
	Normal	2P	19-NOV-08 11:34	WITHIN NORMAL LIMITS
	Normal	3P	20-NOV-08 10:33	WITHIN NORMAL LIMITS
	Normal	4P	21-NOV-08 10:22	WITHIN NORMAL LIMITS
	Normal	5P	22-NOV-08 10:10	WITHIN NORMAL LIMITS
	Normal	6P	23-NOV-08 10:02	WITHIN NORMAL LIMITS
	Normal	7P	24-NOV-08 09:47	WITHIN NORMAL LIMITS
	Normal	8P	25-NOV-08 09:05	WITHIN NORMAL LIMITS
	Normal	9P	26-NOV-08 10:12	WITHIN NORMAL LIMITS
	Normal	10P	27-NOV-08 10:13	WITHIN NORMAL LIMITS
	Normal	11P	28-NOV-08 10:19	WITHIN NORMAL LIMITS
	Normal	12P	29-NOV-08 10:07	WITHIN NORMAL LIMITS

Table V-9. Individual Female Clinical Observations (page 9 of 9)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#	CATEGORY	DAY	DATE TIME	OBSERVATIONS
30	(continued)			
	Normal	13P	30-NOV-08 10:27	WITHIN NORMAL LIMITS
	Miscellaneous	13P	30-NOV-08 16:25	Feed Removed for Fasting of Animal
	Dead	14P	1-DEC-08 08:06	Scheduled Sacrifice

Table V-10. Individual Female Organ Weights and Organ Weight Ratios (page 1 of 6)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Ovary (pair) Weight g	Ovary (pair) Ratio
2	SS	147.7	0.0479	0.0324	1.6883	1.1431	0.7596	0.5143	1.6758	1.1346	6.1215	4.1445	0.0662	0.0448
4	SS	162.2	0.0636	0.0392	1.7586	1.0839	0.7086	0.4368	1.7214	1.0610	6.2903	3.8772	0.0898	0.0554
6	SS	135.4	0.0441	0.0326	1.4816	1.0939	0.6380	0.4711	1.2821	0.9466	5.6501	4.1717	0.0668	0.0493
8	SS	182.8	0.0693	0.0379	1.7557	0.9606	0.8098	0.4430	1.8957	1.0371	7.4210	4.0601	0.0864	0.0473
10	SS	157.8	0.0669	0.0424	1.7875	1.1330	0.6347	0.4023	1.5697	0.9949	5.9936	3.7989	0.1168	0.0740

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-10. Individual Female Organ Weights and Organ Weight Ratios (page 2 of 6)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Ovary (pair) Weight g	Ovary (pair) Ratio
12	SS	150.7	0.0568	0.0377	1.7539	1.1638	0.6133	0.4069	1.4991	0.9947	5.9441	3.9441	0.1325	0.0879
14	SS	154.2	0.0496	0.0322	1.6452	1.0666	0.6419	0.4162	1.6288	1.0560	6.8998	4.4734	0.0610	0.0395
16	SS	179.4	0.0664	0.0370	1.8881	1.0525	0.7253	0.4043	1.8808	1.0484	6.6675	3.7166	0.1305	0.0727
18	SS	163.7	0.0646	0.0395	1.6339	0.9980	0.6801	0.4154	1.7174	1.0491	6.7037	4.0949	0.0838	0.0512
20	SS	145.7	0.0787	0.0540	1.7454	1.1976	0.6325	0.4340	1.6659	1.1431	5.6558	3.8807	0.1207	0.0828

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-10. Individual Female Organ Weights and Organ Weight Ratios (page 3 of 6)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Adrenal (pair) Weight g	Adrenal (pair) Ratio	Brain Weight g	Brain Ratio	Heart Weight g	Heart Ratio	Kidney (pair) Weight g	Kidney (pair) Ratio	Liver Weight g	Liver Ratio	Ovary (pair) Weight g	Ovary (pair) Ratio
22	SS	154.5	0.0673	0.0436	1.7220	1.1146	0.6337	0.4102	1.5399	0.9968	6.8899	4.4598	0.0702	0.0454
24	SS	185.2	0.0748	0.0404	1.6529	0.8927	0.8123	0.4387	2.1523	1.1624	7.8092	4.2175	0.1286	0.0695
26	SS	151.6	0.0568	0.0375	1.7564	1.1583	0.7025	0.4633	1.7122	1.1291	6.3893	4.2135	0.1088	0.0717
28	SS	166.8	0.0767	0.0460	1.9186	1.1502	0.7034	0.4217	1.8097	1.0849	6.4988	3.8959	0.1051	0.0630
30	SS	170.2	0.0679	0.0399	1.8337	1.0772	0.7288	0.4281	1.6735	0.9831	6.8313	4.0130	0.0833	0.0489

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-10. Individual Female Organ Weights and Organ Weight Ratios (page 4 of 6)

FEMALES 77366 - Red - **0 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Thymus Weight g	Thymus Ratio	Uterus Weight g	Uterus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
2	SS	147.7	0.4477	0.3031	0.5269	0.3567	0.7734	0.5236	0.0095	0.0064	0.0212	0.0144
4	SS	162.2	0.3790	0.2336	0.4376	0.2697	0.4675	0.2882	0.0090	0.0055	0.0142	0.0088
6	SS	135.4	0.3600	0.2658	0.5204	0.3842	0.3827	0.2826	0.0085	0.0063	0.0148	0.0109
8	SS	182.8	0.5393	0.2951	0.6456	0.3532	0.6296	0.3445	0.0109	0.0060	0.0168	0.0092
10	SS	157.8	0.4221	0.2675	0.5247	0.3326	0.4468	0.2832	0.0116	0.0074	0.0124	0.0079

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-10. Individual Female Organ Weights and Organ Weight Ratios (page 5 of 6)

FEMALES 92088 - Blue - **13 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Thymus Weight g	Thymus Ratio	Uterus Weight g	Uterus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
12	SS	150.7	0.4731	0.3139	0.5367	0.3561	0.3801	0.2522	0.0130	0.0086	0.0216	0.0143
14	SS	154.2	0.4073	0.2641	0.4758	0.3085	0.4007	0.2598	0.0055	0.0036	0.0166	0.0108
16	SS	179.4	0.5498	0.3065	0.5412	0.3017	0.3063	0.1707	0.0130	0.0072	0.0157	0.0088
18	SS	163.7	0.3522	0.2151	0.5932	0.3623	0.3370	0.2059	0.0094	0.0057	0.0144	0.0088
20	SS	145.7	0.4414	0.3029	0.5309	0.3643	0.6259	0.4295	0.0098	0.0067	0.0151	0.0104

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-10. Individual Female Organ Weights and Organ Weight Ratios (page 6 of 6)

FEMALES 54823 - Yellow - **51 ug/kg Fluoroestradiol**

Animal Number		FINAL BODY WEIGHT g	Spleen Weight g	Spleen Ratio	Thymus Weight g	Thymus Ratio	Uterus Weight g	Uterus Ratio	Pituitary (fixed) Weight g	Pituitary (fixed) Ratio	Thyroid (fixed) Weight g	Thyroid (fixed) Ratio
22	SS	154.5	0.4737	0.3066	0.5798	0.3753	0.3776	0.2444	0.0083	0.0054	0.0153	0.0099
24	SS	185.2	0.4236	0.2288	0.7380	0.3986	0.4057	0.2191	NPTC		0.0158	0.0085
26	SS	151.6	0.4985	0.3287	0.6645	0.4382	0.3786	0.2497	0.0085	0.0056	0.0145	0.0096
28	SS	166.8	0.3482	0.2087	0.5010	0.3003	0.4285	0.2569	0.0089	0.0053	0.0147	0.0088
30	SS	170.2	0.4498	0.2642	0.5781	0.3396	0.4468	0.2625	0.0113	0.0066	0.0147	0.0086

NPTC=Tissue/organ not present in formalin cup

SS=Scheduled Sacrifice
RATIO = ORGAN WEIGHT/BODY WEIGHT X 100

Table V-11. Individual Female Macroscopic Necropsy Findings (page 1 of 3)

77366 - Red - **0 ug/kg Fluoroestradiol**

ANIMAL#	ORGAN	OBSERVATION
2	SS	NO REMARKABLE OBSERVATIONS
4	SS	NO REMARKABLE OBSERVATIONS
6	SS	NO REMARKABLE OBSERVATIONS
8	SS	NO REMARKABLE OBSERVATIONS
10	SS	NO REMARKABLE OBSERVATIONS

SS=Scheduled Sacrifice

Table V-11. Individual Female Macroscopic Necropsy Findings (page 2 of 3)

92088 - Blue - **13 ug/kg Fluoroestradiol**

ANIMAL#	ORGAN	OBSERVATION
12	SS	NO REMARKABLE OBSERVATIONS
14	SS	NO REMARKABLE OBSERVATIONS
16	SS	NO REMARKABLE OBSERVATIONS
18	SS	NO REMARKABLE OBSERVATIONS
20	SS	NO REMARKABLE OBSERVATIONS

SS=Scheduled Sacrifice

Table V-11. Individual Female Macroscopic Necropsy Findings (page 3 of 3)

54823 - Yellow - **51 ug/kg Fluoroestradiol**

ANIMAL#		ORGAN	OBSERVATION
22	SS	Thymus	Enlarged, 17x15x2 mm, White
		Mandibular Lymph	Enlarged, 3x3x3 mm, White
24	SS		NO REMARKABLE OBSERVATIONS
26	SS		NO REMARKABLE OBSERVATIONS
28	SS		NO REMARKABLE OBSERVATIONS
30	SS		NO REMARKABLE OBSERVATIONS

SS=Scheduled Sacrifice

APPENDIX 6

Study Protocol and Amendments

RTI Project No.: 0211886.001.001

RTI Master Protocol No.: RTI-1059

RTI Study Code: Rt08-FES

TITLE: **14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment**

SPONSOR: Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]

TESTING FACILITY: RTI International*
Center for Life Sciences and Toxicology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

*RTI International is the tradename for Research Triangle Institute

APPROVALS

RTI International

11/7/08
Kimberly D. Ehman, Ph.D. Date
Study Director
Center for Life Sciences and Toxicology

07 Nov 2008
Irma M. Grossi, Ph.D. Date
Senior Director, Life Sciences and
Toxicology and DMPK
BOA/Contract Principal Investigator

Quality Assurance Review By:

Leslie L Macdonald 11-7-08
Leslie L. Macdonald, B.S. Date
Quality Assurance Specialist
RTI Quality Assurance Unit

Sponsor

Paula Jacobs 11/12/2008
Paula M. Jacobs, Ph.D. Date
CMRP, SAIC Frederick
Project Officer

Sponsor approval received via e-mail on 11/5/08 (date) by PJ 11/7/08 (Initials/Date).

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1.0 Study Title

14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

2.0 Personnel Involved in the Study

Sponsor:	CMRP, SAIC Frederick 6130 Executive Boulevard EPN, Room 6070 Bethesda, MD 20892-7412 [FedEx: Rockville, MD 20852-4910] Telephone: 301-496-9531
Sponsor Representative:	Paula Jacobs, Ph.D. [Contractor] Clinical Monitoring Research Program SAIC Frederick National Cancer Institute at Frederick 6130 Executive Boulevard Bethesda, MD 20892-7412 Telephone: 301-435-9181 Fax: E-mail: jacobsp@mail.nih.gov
RTI Study Director:	Kimberly D. Ehman, Ph.D. P.O. Box 12194 3040 Cornwallis Road HLB-121 Research Triangle Park, NC 27709-2194 Telephone: 919-316-3802 Fax: 919-541-5956 E-mail: kehman@rti.org
Study Coordinator:	Jay G. Henson, B.S. Telephone: 919-541-7206 E-mail: jhenson@rti.org
Director, Laboratory Animal Sciences:	Crystal L. Thomas, D.V.M. Telephone: 919-541-3354 E-mail: crystalthomas@rti.org
Senior Manager, Animal Research Facility:	David E. Miller, B.S., RLATG Telephone: 919-541-7085 E-mail: dmiller@rti.org
Technical Group Supervisor:	Nicole M. Barbarish, B.S. Telephone: 919-541-7274 E-mail: nbarbarish@rti.org

Materials Handling Facility Manager: Donna B. Browning, B.S.
Telephone: 919-541-6270
E-mail: dbrowning@rti.org

Data Specialist: Christina B. Myers, M.S.
Telephone: 919-541-8822
E-mail: cbm@rti.org

Quality Assurance Specialist: Susan C. Wade, B.S.
Telephone: 919-316-3454
E-mail: swade@rti.org

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

3.0 Objective

The purpose of this study is to assess the toxicity of fluoroestradiol when administered by intravenous injection to Sprague-Dawley CD®(SD)IGS BR rats for 14 consecutive days. In addition, bone marrow samples will be collected from all animals for micronucleus assessment.

4.0 Study Schedule

Proposed Animal Receipt Date:	November 11, 2008
Proposed Experimental Start Date:	November 17, 2008
Proposed Necropsy Date:	December 1, 2008
Proposed Audited Draft Report Date:	February 23, 2009

5.0 Test Article Information

Unless otherwise noted, the identity, purity, composition, stability and method of synthesis of each batch of test and control articles are the responsibility of the Sponsor. This documentation will be maintained by the Sponsor/Supplier and will be provided to RTI for inclusion in the study records.

5.1 Identification of Test Article(s)

Sponsor Designation:	Fluoroestradiol (in ethanol/water as provided to RTI)
Name:	Estra-1,3,5 (10)-triene-3,17-diol, 16-fluoro-, (16a,17beta)
CAS No.:	92817-10-2
Safety Precautions:	A Material Safety Data Sheet (MSDS) will be maintained in the study file.
Purity:	Certificate of Analysis to be included in the study records.
Disposition:	Returned to Sponsor following study completion

The supplier, lot number, purity, stability, storage, expiration date (if available) and handling procedures, as well as other pertinent information not supplied above will be documented in the study records.

Sponsor Designation:	Cytoxan (positive control article)
Name:	Cyclophosphamide monohydrate
Supplier:	Sigma Aldrich
CAS No.:	6055-19-2
Lot No.:	068K1131
Purity:	98.0% by HPLC
Stability:	TBD
Storage Conditions:	Room temperature
Safety Precautions:	Care to be taken in handling; cyclophosphamide is a potent cytotoxic agent. A Material Safety Data Sheet (MSDS) will be maintained in the study file.
Disposition:	Appropriately disposed of following study completion

5.2 Identification of Control Article(s)

The vehicle for administration to the control group (Group 1) and for preparation of the test article dosing formulations was 15% ethanol/85% saline. The lot number, supplier, expiration date (if available) and handling procedures, as well as other pertinent information for the vehicle components will be documented in the study records.

6.0 Test Article Dose Preparation and Analysis

6.1 Dose Preparation

Test article formulations will be prepared as needed on study and stored refrigerated. Details of the dose preparation method will be included in the study file. The formulations will be brought to room temperature prior to administration.

6.2 Dose Analysis

Approximately 1-3-mL samples will be collected from each dose formulation on the first day of dosing (Study Day 0) and on the last day of dosing (Study Day 13). The samples will be shipped in amber borosilicate vials on ice packs to the University of Washington for stability and concentration analyses. Aliquots will be collected on the first day of formulation (i.e., Study Day 0) and on the last day of dosing (i.e., Study Day 13). Standards for acceptable concentration and stability will be as follows: the mean of the analyzed samples must be within ± 15% of nominal, and the change in concentration from the sample collected on Study Day 0 and the sample collected on Study Day 13 must not exceed 15%. The positive control article formulation will not be analyzed for stability, homogeneity, or concentration. The Study Director and Sponsor Representative will be notified immediately if problems of this nature occur and the resolution will be documented in the study records.

A description of the analytical methods and results will be provided to RTI and included in the final report. Samples will be shipped to:

Jeanne Link, Ph.D.
Associate Professor of Radiology
Division of Nuclear Medicine
Molecular Imaging Research Box 356004
Room NW041 UWMC
University of Washington
Seattle, WA 98195-6004
Telephone: 206-598-6256
Fax: 206-598-4192

6.3 Disposition of Samples Not Used for Dosing

Remaining formulated dose samples will be appropriately disposed of following issuance of the final report.

7.0 Test System

7.1 Species and Strain

Sprague-Dawley CD®(SD)IGS BR rat

7.2 Source

Charles River Laboratories, Inc. (documentation of the specific breeding facility will be maintained in the study file).

7.3 Age

Approximately 5 weeks old at receipt; approximately 6 to 8 weeks old at initiation of dosing. Animals outside of this range may be used at the discretion of the Study Director.

7.4 Weight

Approximately 100 to 300 grams at first dose. Animals outside of this range may be used at the discretion of the Study Director.

7.5 Number/Gender

17 males and 15 females will be purchased; 5/sex will be assigned to the toxicology groups (Groups 1-3) and 2 males will be assigned to the cyclophosphamide group (Group 4).

7.6 Method of Identification

Each animal will be ear-tagged with a RTI number. This number plus a study number will comprise the unique identification for each animal. If the ear tag is lost, it will be replaced with one of the same number.

7.7 Housing

All animals will be housed individually in appropriately sized solid-bottom polycarbonate cages suspended from stainless steel, self-watering racks. Hardwood Sani-Chips® cage litter will be used in all cages.

Current acceptable practices of good animal husbandry will be followed, e.g., *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996). RTI International is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Animals will be monitored by the technical staff for any conditions requiring possible veterinary care. If any such conditions are identified, the staff veterinarian will be notified for an examination and evaluation.

7.8 Diet

PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 (pellet) will be available *ad libitum*. Each lot utilized will be identified and recorded. Rodent diet will be stored at approximately 60-70°F, and the period of use will not exceed six months from the milling date. Each lot has been analyzed by the manufacturer to assure specifications are met and a copy of the results will be maintained in the study records. Contaminants will not be present at levels expected to interfere with the objectives of this study.

7.9 Water

Municipal tap water from the Durham, NC water system will be available *ad libitum* throughout the study. Analysis of the drinking water for chemical composition and possible contamination will be provided by the supplier and maintained in the study records. It is anticipated that contaminant levels will be below certified levels and will not affect the design, conduct or conclusions of this study.

7.10 Environmental Conditions

Environmental conditions will be continuously monitored, controlled and recorded by an automated system. Target conditions for temperature and humidity in the animal room will be 64-79°F and 30-70%, respectively (NRC, 1996). Temperature and/or humidity excursions above

or below the target ranges will be documented in the study records and the final report. Lighting controlled by light timers will provide illumination for a 12-hour light/12-hour dark photoperiod. The ventilation rate will be set at a minimum of 10 air changes per hour.

7.11 Animal Receipt and Acclimation

Animals will be acclimated for at least six days following receipt. All animals will be checked for viability twice daily during the quarantine period. Prior to study assignment, all animals will be examined to ascertain suitability for study by the study veterinarian.

7.12 Animal Welfare/Psychological Enrichment

Nestlets will be provided to all animals for environmental enrichment.

7.13 Justification for Selection of Test System

The rat is an animal model commonly utilized in toxicity studies. In addition, a significant historical database is available for comparative evaluation. The number of animals on study is considered to be the minimum necessary for statistical, regulatory and scientific reasons. The purpose of this study is to monitor for toxicity of the test article. Historical control data indicate that clinical laboratory data, organ weight data and microscopic examination of tissues vary among individual animals. The number of animals/sex/group for this study was selected based on this variability. The two test article-treated groups receiving low and high multiples of the proposed human dose, and a negative and positive control group, are considered the minimum number of groups necessary to provide a range of effects and allow for extrapolation of results to humans.

8.0 Experimental Design

8.1 Method of Group Assignment

Any animal received from the vendor may be excluded from the study, per veterinarian recommendation, due to adverse clinical observations. Animals will be assigned to treatment groups by sex using stratified randomization using a Toxicology Analysis System Customized (TASC) computer program designed to provide uniform mean body weights across dose groups based on the last body weight taken during the acclimation period.

8.2 Group Designation

The following table presents the study group assignment:

Group Number	Treatment	Dose	Dosing Concentration (µg/mL)	Dosing Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[1]	0	0	2.0	5	5
2	Fluoroestradiol	13 µg/kg	6.5	2.0	5	5
3	Fluoroestradiol	51 µg/kg	25.5	2.0	5	5
4	Cyclophosphamide[2]	30 mg/kg	6.0 mg/mL	5.0	2	0

[1] Vehicle = 15% ethanol/85% saline
[2] Positive control for micronucleus assay. Cyclophosphamide will be administered intraperitoneally as a single dose to two males on Study Day 13.

8.3 Justification of Treatment Regimen

For test articles like medical imaging agents whose clinical use is expected to involve only a single dose, "expanded acute" studies, in which rodents undergo an extensive toxicology evaluation following a single administration of test article are generally sufficient. Acute toxicity study designs are less likely to identify potentially serious, late-appearing toxicities. For this reason, repeat-dose administration studies are generally performed only with test articles whose expected clinical use pattern will involved only a single or a few doses. Additionally, medical imaging agents may be required to monitor therapy in humans; consequently animals will be dosed for 14 consecutive days and detailed toxicological evaluations performed throughout the dosing period.

Because the test article will be administered to humans intravenously, the same route of administration will be used in this study. This study is intended to support administration of the test article for up to two weeks in humans. A two-week preclinical study is required to support human exposure of this duration. Based upon prior observations and the extremely low dose of the test article that is used in diagnostic imaging, the proposed 14-day rat exposure is equivalent to a cumulative 1400-fold greater administered dose of test article than would be the maximum experienced in human studies.

8.4 Administration

The vehicle and test article formulations (Groups 1-3) will be administered daily for 14 consecutive days (until the day prior to necropsy; study days 0-13) as an intravenous bolus dose via a lateral tail vein using appropriately sized needles and syringes. For micronucleus assessment, two males (Group 4) will be administered cyclophosphamide (positive control) as an intraperitoneal injection on Study Day 13. Doses will be calculated using the most recent body weights.

9.0 Parameters to be Evaluated

The TASC automated data collection system will be used for collection of all body weights, feed weights, clinical observations, organs weights and gross necropsy findings. TASC will calculate the volume of dosing solution to be administered to each animal on each day, based on the appropriate body weight. TASC will also record when each animal is dosed.

9.1 Viability Observations

Cage side viability checks for mortality and general condition will be made at least twice daily (once in the morning and once in the afternoon, not less than six hours apart). Animals in poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia.

9.2 Clinical Observations

Clinical observations for signs of toxic effects will be made once daily for each toxicology group animal at the time of dosing. Observations will include (but not be limited to) changes in the skin, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems function; somatomotor activity and behavior patterns.

9.3 Body Weights

Body weights for toxicology group animals (Groups 1-3) will be recorded twice pretest (upon receipt and prior to group assignment) and weekly during study conduct (study days 0, 6 and 13). Body weights for Group 4 animals will be recorded twice pretest (upon receipt and prior to group assignment) and on Study Day 13.

9.4 Feed Consumption

Feed consumption will be measured (weighed) for all toxicology group animals (Groups 1-3) weekly throughout study conduct (study days 0-6 and 6-13).

9.5 Clinical Pathology

Clinical pathology blood samples (hematology and serum chemistry) will be collected from all toxicology group animals (Groups 1-3) at the time of scheduled necropsy. Animals will be fasted overnight prior to blood collection. Blood for hematology assessments (approximately 0.5 mL) will be collected into tubes containing EDTA as the anticoagulant. Blood for serum chemistry assessments (approximately 1.0 mL) will be collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Whole blood samples will be stored on wet ice or refrigerated and serum samples will be stored on dry ice or frozen at approximately -70°C until submitted for analysis. All samples will be submitted to Antech Diagnostics for analysis:

Mark Morrison
Antech Diagnostics GLP
507 Airport Blvd. Suite 113
Morrisville, NC 27560
Telephone: 919-787-9528
Cell: 919-417-2542

Results of the clinical pathology analyses will be provided to RTI and included in the final report.

9.5.1 Hematology

The following hematology parameters will be evaluated:

Erythrocyte count (RBC)	Mean corpuscular hemoglobin concentration (MCHC)
Differential leukocyte count	Mean corpuscular volume (MCV)
Hematocrit (HTC)	Platelet count (PLT)
Hemoglobin (HGB)	Reticulocyte count (RETIC)
Mean corpuscular hemoglobin (MCH)	Total leukocyte count (WBC)

9.5.2 Serum Chemistry

The following serum chemistry parameters will be evaluated:

Albumin (ALB)	Potassium (K)
Albumin/globulin (A/G Ratio)	Serum alanine transaminase (ALT)
Alkaline phosphates (ALP)	Serum aspartate transaminase (AST)
Blood urea nitrogen (BUN)	Serum glucose (GLUC)
Calcium (Ca)	Sodium (Na)
Chloride (Cl)	Total bilirubin (TBIL)
Cholesterol (CHOL)	Total protein (TP)
Creatinine (CRE)	Triglycerides (TG)
Gamma-glutamyltransferase (GGT)	
Globulin (GLOB)	
Inorganic phosphate (PO_4)	

9.6 Anatomic Pathology

9.6.1 Necropsy

A complete necropsy will be conducted on all toxicology group animals (Groups 1-3). Animals will be fasted overnight prior to necropsy. A final body weight will be collected for all animals in Groups 1-3 prior to euthanasia. Animals will be euthanized by CO_2 asphyxiation followed by exsanguination. A necropsy will be conducted on animals dying spontaneously or euthanized *in extremis*; animals found dead will be maintained in a refrigerator until necropsy. Necropsies will include examination of the external surface, all orifices, and the cranial, thoracic abdominal and pelvic cavities including viscera.

At the time of necropsy, the following tissues and organs will be collected and placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands	Oviducts
Aorta	Pancreas
Brain	Prostate
Bone (femur with epiphyseal plate of head)	Rectum
Bone marrow (sternum)	Salivary gland (mandibular)
Cecum	Sciatic nerve
Colon	Seminal vesicles
Duodenum	Skeletal muscle
Eartag (animal ID)	Skin (ventral abdomen)
Epididymides	Spinal cord (thoracolumnar junction; entire cord if neurologic abnormalities present)
Esophagus	Spleen
Eyes, with optic nerve[1]	Stomach (fundic area)
Gross lesions (including tissue masses and abnormal regional lymph nodes)	Testes[1]
Heart	Thymus
Ileum	Thyroid and parathyroid glands
Injection site(s)	Tongue
Jejunum	Trachea
Kidney	Ureter
Liver (right medial lobe and left lateral lobe)	Urinary bladder[2]
Lungs[2]	Uterus (body) with cervix
Lymph node (mandibular and mesenteric)	Vagina
Mammary gland (females only; to include nipple and surrounding tissue)	
Ovaries	

[1]Modified Davidson's solution initially, followed by 10% neutral-buffered formalin.
[2]May be infused with formalin to ensure fixation.

9.6.2 Organ Weights

The organs indicated below will be weighed from all toxicology group animals (Groups 1-3) euthanized at the scheduled necropsy:

Adrenals	Prostate
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus with oviducts
Pituitary	

Paired organs will be weighed together. The pituitary and thyroid/parathyroids will be weighed following fixation. Organs will not be weighed from animals found dead.

9.6.3 Histopathology

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues listed in Section 9.6.1 for all animals euthanized *in extremis* and for all animals in Groups 1 and 3. Microscopic examination may be extended to other groups at an additional cost if necessary. Special stains may be used at the discretion of the study pathologist to further characterize lesions and changes. Any special stains used will be documented in the individual animal's data and interpretation of the results will be included in the final report. Fixed tissues will be sent to Pathology Associates (Charles River Laboratories) for processing and histopathological assessments to the contact below:

Sharon M. Ambrose
Pathology Associates (Charles River Laboratories)
4025 Stirrup Creek Drive, Suite 150
Durham, NC 27703
Phone: 919-206-7007
Fax: 919-206-7001
E-mail: sharon.ambrose@us.crl.com

The histopathology results will be provided to RTI and included in the final report.

9.6.4 Micronucleus Assessment

On Study Day 14 (approximately 18-24 hours after the last dose administration), two bone marrow smear slides from the femur will be prepared from all animals (all groups) for *in vivo* clastogenicity/aneugenicity assessments (micronuclei determination). Details of the bone marrow smear procedure will be included in the study records. Prepared bone marrow smears will be shipped to BioReliance for micronuclei slide staining and scoring to the contact below.

Ljubica Krsmanovic, Ph.D.
BioReliance
9630 Medical Center Drive
Rockville, MD 20850
Phone: 301-610-2162
Fax: 301-738-2362
E-mail: buba.krsmanovic@bioreliance.com

The results of the micronucleus assessment will be provided to RTI and included in the final report.

10.0 Statistical Methods

The following types of data will be analyzed separately at each time point:

- Body weights and weight gain over specified (i.e., weekly) study periods
- Feed consumption over specified (i.e., weekly) study period
- Hematology and serum chemistry (Antech Diagnostics GLP, Morrisville, NC)
- Organ weights, both absolute and adjusted for terminal body weight

For categorical data, the proportion of animals will be analyzed using Fisher's Exact Test (Steel and Torrie, 1980) for each treated group versus the control. For continuous data, Levene's Test (Levene, 1960) will be applied to test for homogeneity of variances between the groups. Using tests dependent on the outcome of Levene's Test, an overall test of significance will be run. If the overall test is significant ($p<0.05$), treated groups will then be compared to the control group, incorporating adjustments for multiple comparisons where necessary.

11.0 Reporting

An audited draft final report of this study will be submitted to the Sponsor (and study representative) within 12 weeks of the completion of necropsy. The Sponsor (and study representative) shall submit comments, if any, on the draft final report to the Study Director. RTI will review and respond to any comments necessary for approval and submit two hard copies (one bound, one unbound), and one electronic copy of the final report to the Sponsor.

12.0 Study Conduct, Storage of Study Materials and Records Retention

This protocol will be the controlling document in case of discrepancies between the protocol and SOPs.

The TASC automated data collection system will be used for collection of all body weights (including quarantine), feed weights, clinical observations, organ weights, and gross necropsy findings. TASC will also calculate the volume of dosing solution to be administered to each animal on each day, based on the appropriate body weight. TASC also records when each animal is dosed. Therefore, the raw data for these measurements will be the electronic data collected in TASC unless otherwise noted in the study records.

This study will be monitored for compliance with the Food and Drug Administration's (FDA) Good Laboratory Practices (GLP) regulations (21 CFR Part 58) for conduct of nonclinical studies.

Records of the study data pertinent to the conduct of this study will be maintained in labeled binders. The data will be maintained under the direction of RTI. The data stored on magnetic media will be maintained by RTI. All data documenting experimental details, study procedures, and observations will be recorded and maintained as raw data. At the completion of the study, all raw data, correspondence, documentation, records, reports, preserved specimens, and retained and archived samples will be maintained in the archives of RTI for a period of one year after submission of the signed final report. The Sponsor is responsible for the final disposition of these materials, and also responsible for all costs associated with their storage beyond one year from the issuance of the final report.

13.0 Compliance with FDA Regulations

This study will be conducted in compliance with the FDA GLP regulations and AAALAC accreditation standards. The toxicology laboratories at RTI are operated in compliance with FDA GLP regulations (21 CFR Part 58). RTI, through administration of a quality assurance program by the Quality Assurance Unit, assesses compliance of all phases of toxicological studies with existing regulations (21 CRF Part 58). The Sponsor is responsible for GLP compliance of test article characterization, as well as strength, purity, stability, identity, and uniformity. RTI is responsible for the dose formulations and auditing of chemistry phases of the study. The RTI Animal Research Facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

14.0 Study Changes

If after the study is underway it becomes necessary to change the approved protocol, agreement to make this change will be made between the Study Director and the Sponsor (or study representative). As soon as practical, the change and reasons for it will be formally approved by the Study Director and Sponsor (or study representative) in writing and amended to the study protocol. All study change documents will be maintained in the study file.

15.0 References

Levene, H. Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, I. Olkin, et. al., eds. Stanford University Press, Stanford, CA, 1960, pp. 278-292.

National Research Council. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission of Life Sciences, National Academy Press: Washington, DC. Revised 1996.

Steel, R.G.D.; Torrie, J.H. Principles and Procedures of Statistics, A Biometrical Approach, 2nd ed.; McGraw-Hill Book Company: New York, 1980; pp 504-506.

ATTACHMENT

1. PRODUCT AND COMPANY INFORMATION

Product Name:	16a-Fluoroestradiol
Product Catalog Number:	191
Use of the product:	Reference standard for 16a-[^{18}F]Fluoroestradiol
Manufacturer/Supplier:	ABX advanced biochemical compounds Biomedizinische Forschungsreagenzien GmbH
Address:	Heinrich-Gläser-Str. 10-14, D-01454 Radeberg, Germany
Phone/Fax Number:	+49-3528-404160 / +49-3528-404165

2. COMPOSITION / INFORMATION ON INGREDIENTS

Name:	Estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16a,17ß)
Synonymes:	16a-Fluoro-13ß-methyl-1,3,5(10)-gonatriene-3,17ßdiol; 16a-Fluoro-17ß-estradiol; 16a-Fluoroestradiol
CAS-RN:	[92817-10-2]
Molecular Weight:	290.37 g/mol
Molecular Formula:	$C_{18}H_{23}FO_2$
EC-No. (EINECS/ELINCS):	not listed
RTECS No:	no entry
R-Phrases:	R20/21/22

3. HAZARDS INFORMATION

Main Hazards
Potentially harmful by inhalation, in contact with skin and if swallowed!
May cause irritation of eyes, skin, mucous membranes and upper respiratory tract.
Caution, substance not yet fully tested!

Health Effects – Eyes
Dust may cause conjunctival irritation.

Health Effects – Skin
Material may cause irritation.

Health Effects – Ingestion
Swallowing may have the following effects: nausea, vomiting, diarrhoea.

Health Effects – Inhalation
Exposure to dust may have the following effects: irritation of nose, throat and respiratory tract.

Users should note that this material is not included in EINECS (the European Inventory of Existing commercial Chemical Substances) and, as such, is a new substance and is subject to the Notification of New Substances regulations. In accordance with those regulations, the phrase "Caution, substance not yet fully tested" is used on the label.

4. FIRST AID MEASURES

In case of eye contact
Flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

In case of skin contact
Flush with copious amounts of water for at least 15 minutes. Wash skin with soap and water. Remove contaminated clothes and shoes. Call a physician.

In case of ingestion
Wash out mouth with water provided person is conscious. Call a physician immediately.

In case of inhalation
Remove person from exposure and allow to rest in fresh air. If not breathing give artifical respiration. If breathing is difficult, give oxygen. Seek medical attention if symptoms persist.

5. FIRE FIGHTING MEASURES

Extinguishing Media
Water spray, carbon dioxide, dry chemical powder or appropriate foam.

Special Hazards of Product
May emit toxic fumes under fire conditions/thermal decomposition. Do not inhale combustion and/or explosion gases.

Protective Equipment for Firefighting
Wear self-contained breathing apparatus and protective clothing and safety goggles to prevent contact with skin and eyes.

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions
Wear appropriate protective clothing. Wear self-contained breathing apparatus or respiratory protection. Wear safety goggles and heavy rubber gloves.

Environmental Precautions
Prevent the material from entering drains or water courses.

Spillages
Avoid raising dust. Use appropriate protective equipment and methods to clean up spilled substance promptly. Sweep up spill, place in a bag or suitable container and hold for waste disposal. Dispose all waste in dispose of in accordance with local and national regulations. Ventilate and wash area after pickup with acetone and/or alcohol, then soap and water.

7. HANDLING AND STORAGE

Handling
Avoid inhaling dust. Avoid contact with skin, eyes and clothing. Avoid prolonged or repeated exposure. Do not eat, drink, smoke or apply cosmetics whilst using this material. Mechanical ventilation and respiratory protection are recommended. Wear protective gloves, protective clothing and protective eyewear. Follow safe laboratory practices.

Storage and Stability
Store dessicated at –20 ± 5 °C. Protect from light and moisture. Keep container tightly closed. Long-term stability not determined. Usage within 12 months after purchase is recommended.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Ventilation
Mechanical ventilation (fume hood) recommended.

Personal Protection

Respiratory protection:	Avoid inhaling dust. Handle in an efficient fume hood.
Eye protection:	Avoid eye contact. Wear chemical safety goggles.
Skin protection:	Avoid skin contact. Wear protecting gloves (rubber or nitrile recommended) and clothing. Wash hands thoroughly after handling.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	Solid
Colour:	Colourless
Odour:	Odourless
Boiling Range/Point (°C):	No data
Melting Range/Point (°C):	180 – 210 °C
Flash Point (PMCC) (°C):	Not flammable
Explosion Limits (%):	No data
Flammability:	Not classified as flammable.
Auto-flammability (°C)	Not auto-flammable.
Explosive Properties:	Not classified as explosive.
Oxidizing Properties:	Not classifed as oxidizing
Density (20 °C):	No data
Solubility in Water (g/ml):	Insoluble

10. STABILITY AND REACTIVITY

Stability
Long-term stability not determined. Usage within 12 months after purchase is recommended.

Incompatibilites
Strong oxidizing agents, water, light.

Hazardous Combustion or Decomposition Products
Thermal decomposition may produce carbon monoxide, carbon dioxide, hydrogen fluoride gas and nitrogen oxides.

Hazardous Polymerisation
Will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Effects
There is no toxicity data available for this material. It is expected to be harmful by inhalation and ingestion and a potential skin and eye irritant. To our knowledge, the health hazards have not yet been thoroughly investigated.

General Remarks
16a-Fluoroestradiol is similar to Estradiol, which acts at the estrogen receptor.

Chronic Effects - Carcinogenicity
Not listed by NTP, IARC or OSHA

12. ECOLOGICAL INFORMATION

Ecotoxicity
No specific information is available.
Do not discharge product into the environment without control.

13. DISPOSAL CONSIDERATIONS

Contact a licensed professional waste disposal service.
Dispose of in accordance with local and national regulations.

14. TRANSPORT INFORMATION

No hazardous material as defined by the transport regulations (ADR/RID, IMDG-Code, ICAO-TI/IATA-DGR).

15. REGULATORY INFORMATION

Labelling Information
This chemical substance is not classified in the Annex I of Directive 67/548/EEC.
We recommend classification and R/S phrases as follows:

Harmful.

Risk phrases:	R20/21/22	Harmful by inhalation, in contact with skin and if swallowed.
Safety phrases:	S3/7	Keep container tightly closed in a cool place.
	S20/21	When using do not eat, drink or smoke.
	S22	Do not breathe dust.
	S24/25	Avoid contact with skin and eyes.
	S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.

Additional Information
CAUTION - SUBSTANCE NOT YET FULLY TESTED.

According to article 14 of the Council Directive 67/548/EEC (European Union) all compounds with not completely known properties, which are not to be classified according to § 5 Chemikaliengesetz have to be labelled with the phrase given above. Additionally, all properties already known must be denoted as usual (symbol of hazard; R-/S-phrases).

16. OTHER INFORMATION

MSDS first issued: 12. December 2005

This material is sold for research purposes only . It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. It must be recognised that the physical and chemical properties of any product may not be fully understood and that new, possibly hazardous products may arise from reactions between chemicals. The information given in this data sheet is based on our present knowledge and shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. ABX advanced biochemical compounds - Biomedizinische Forschungsreagenzien GmbH shall not be held liable for any damage resulting from handling, contact or use of the above product.

Reference Sources

HEALSAFE (Health and Safety Science Abstracts), Cambridge Scientific Abstracts, USA.
CSNB (Chemical Safety NewsBase), The Royal Society of Chemistry, UK.
CHEMLIST File (Regulated Chemicals Listing) Chemical Abstracts Service, USA.
BIOSIS Previews/RN® (BIOSIS®)Thomas Scientific, USA.
EINECS (European Inventory of Existing commercial Chemical Substances)
HSDB (Hazardous Substances Data Bank) National Library of Medicine's Toxicology Information Program, USA.
IARC (International Agency for Research on Cancer) France
IPA (International Pharmaceutical Abstracts) American Society of Health-System Pharmacists, USA.
MEDLINE (MEDlars onLINE) U.S. National Library of Medicine (NLM), USA.
MSDS-CCOHS (Material Safety Data Sheets from the CCOHS) Canadian Centre for Occupational Health and Safety, Canada.
MSDS-OHS File (Material Safety Data Sheets - OHS) MDL Information Systems, Inc., USA.
NIOSHTIC National Institute for Occupational Safety and Health (NIOSH)
NTP (National Toxicology Program) National Institutes of Health, U.S. Department of Health and Human Services.
OSHA (Occupational Health and Safety Administration) U.S. Department of Labor.
RTECS File (Registry of Toxic Effects of Chemical Substances) MDL Information Systems, Inc.
TOXCENTER (Toxicology Center), Chemical Abstracts Service, USA.
ULIDAT File (Umweltliteraturdatenbank), Bundesumweltamt, Germany.

16alpha-Fluoroestradiol

Product no. 191.XXXX

For research purposes only. Not for human use or consumption.

Product description

16alpha-Fluoroestradiol; synonyms: 16alpha-fluoro-17beta-estradiol, FES; mol. wt. 290.37; $C_{18}H_{23}FO_2$; [92817-10-2]; BRN 3554942; chemical name: estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16alpha, 17beta). Colorless crystals, soluble in acetonitrile and chloroform.

Applications

16alpha-Fluoroestradiol may be used as a reference standard in the radiosynthesis of [^{18}F]Fluoroestradiol.

Presentation

Product 191.XXXX is available in 2 ml dark glass vials (DIN 2R), packed under argon atmosphere. Vials are sealed with teflon-faced rubber stoppers and tear-off crimp caps. Bulk chemicals in quantities ≥ 100 mg are available in dark glass screw cap vials, flushed with argon atmosphere. The content of 16alpha-Fluoroestradiol in mg is defined by the four digit number replacing XXXX in the product number. Weighing error is ± 5 %, but in maximum 0.5 mg.

Storage and stability

Store the product desiccated at –20 ± 5 °C, protected from light. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

Toxicology/Hazards

Handle with care, avoid inhalation, ingestion, eye or skin contact, no toxicological data available.

Certificate of analysis

Lot No.: 260801		Product No.: 191.XXXX	
Parameter	Method	Specification	Result
Appearance	organoleptic	colorless crystals	conforms
Melting pt	capillary	180-210 °C	187.3-188.1 °C
Identity	^{1}H-NMR ^{19}F-NMR	conforms conforms	conforms conforms
Purity	HPLC	> 90 %	> 98 %

No further analytical data available

Manufacturing Date: Aug. 2006

ABX advanced biochemical compounds
Biomedizinische Forschungsreagenzien GmbH

Quality Control date: 09-Nov-06

Dr. B. Schmitt

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereof!
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in light circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

References

1) Stalford A. C. *et al.*: The metabolism of 16-fluoroestradiols in vivo: chemical strategies for restricting the oxidative biotransformations of an estrogen-receptor imaging agent. *Steroids.* **1997**, *62*, 750-761.
2) Römer J. *et al.*: Further ^{13}C NMR spectroscopic proof of 16alpha-F configuration in 16-fluoroestradiol derivatives. *Forschungszent. Rossendorf, [Ber.] FZR* **1997**, *165*, 192-193.
3) Mankoff D. A. *et al.*: [^{18}F]Fluoroestradiol Radiation Dosimetry in Human PET Studies. *J. Nucl. Med.* **2001**, *42*, 679-684.

16alpha-Fluoroestradiol

NEW: Product no. 1910.XXXX, OLD: Product no. 191.XXXX Lot. 260801

For research purposes only. Not for human use or consumption.

VALID ONLY IN CONNECTION WITH ORIGINAL CERTIFICATE OF ANALYSIS

Retest certificate of analysis

The following parameters included in the original certificate of analysis have been retested to confirm the stability of the product or are newly introduced in the quality control of the product as they may be considered to be suitable for detection of indicators of decay and guarantee that the product still is in compliance with the original specification:

Lot No.: 260801		Product No.: 191.XXXX	
Retest-Parameter	Method	Retest specification	Result
Appearance	organoleptic	no change in color	conforms
Purity	^{1}H-NMR ^{19}F-NMR	no change in spectrum no change in spectrum	conforms Conforms

Further testing was not considered to be necessary because of absence of significant changes in parameters tested.

Date of retest: **15. Oct. 2008**
Expiry Date: **15. Oct. 2009**

Storage and stability

Store the product desiccated at -20 °C, protected from light. Product is at least one more year stable at -20 °C. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

ABX advanced biochemical compounds **Biomedizinische Forschungsreagenzien GmbH**

Quality Control date: 17-Oct-08

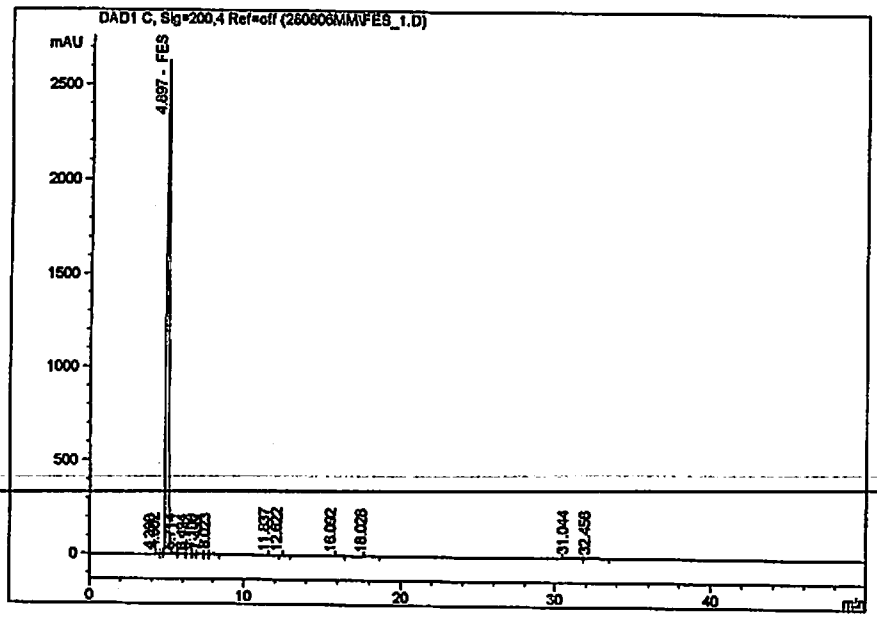
Dr. Bernd Feist

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereof.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

HPLC

HPLC-Analyse bei:DAD1 C, Sig=200,4 Ref=off



ABX advanced biochemical compounds

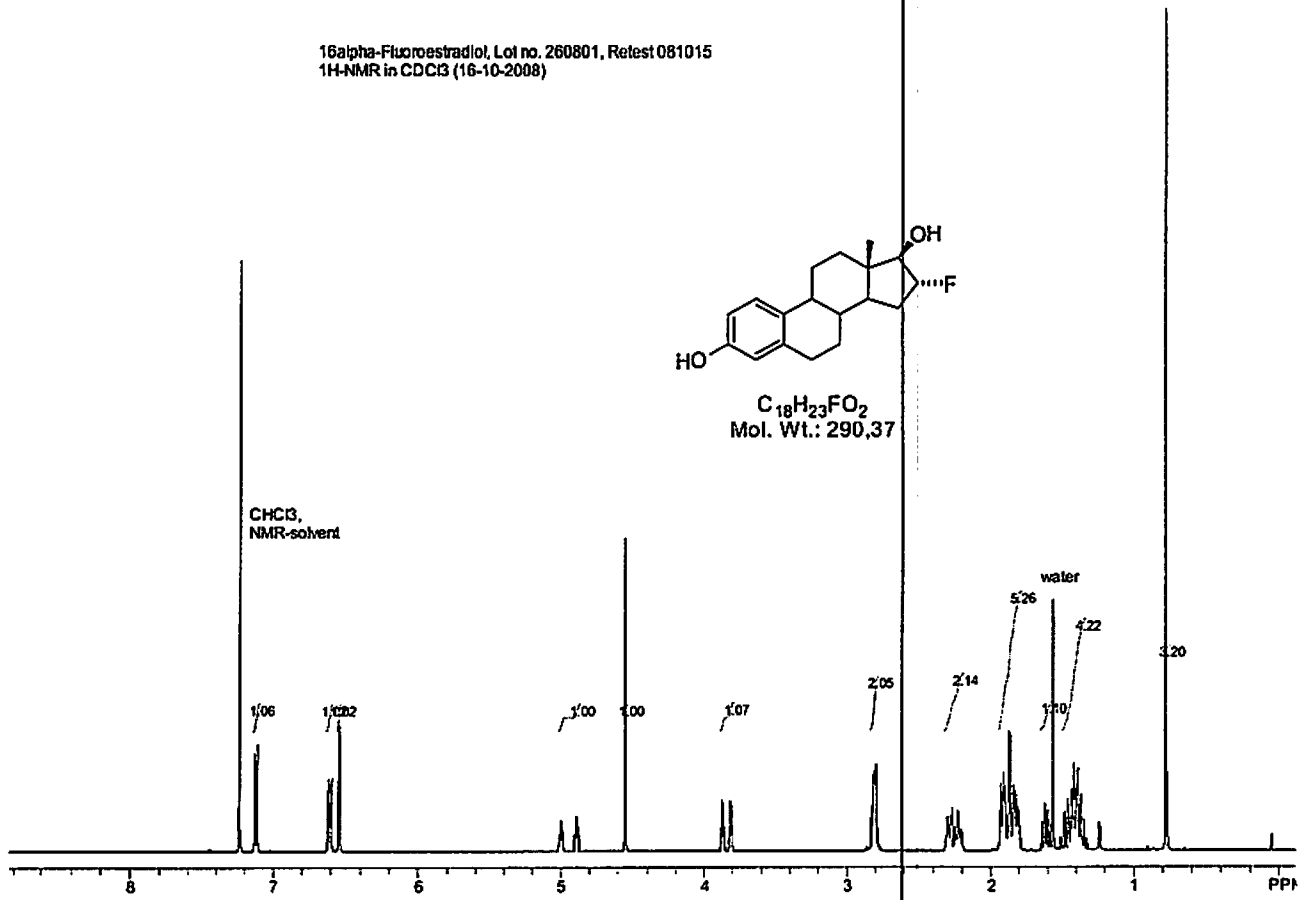
Sample Name: FES_191_260801 Vial 3

Raw data file name:D:\DATA\2006\260806MM\FES_1.D

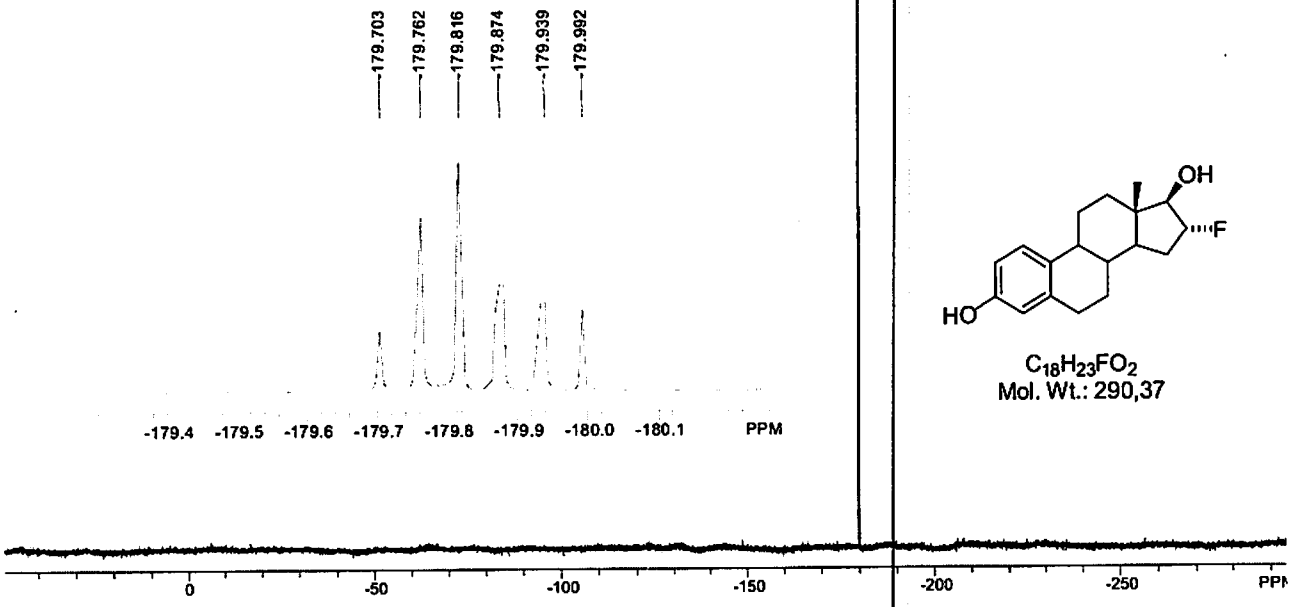
Instrument Name: ABX-HPLC-02
Injection Date: 8/28/2006 Injection Time: 10:35:24 PM
Operator: Thieme

#	Name	Ret. Time	Are	Area %
1		4.390	5.159	0.016
2		4.652	54.985	0.172
3	FES	4.897	31576.758	98.520
4		5.714	41.679	0.130
5		6.494	26.023	0.081
6		6.742	17.094	0.053
7		7.109	68.320	0.213
8		7.499	5.924	0.018
9		8.023	98.434	0.307
10		11.837	7.910	0.025
11		12.622	1.829	0.006
12		16.092	4.334	0.014
13		18.028	15.479	0.048
14	MMSE	0.000	0.000	0.000
15		31.044	72.851	0.227
16		32.456	54.456	0.170

^{1}H-NMR


16alpha-Fluoroestradiol, Lot no. 260801, Retest 081015
1H-NMR in CDCl3 (16-10-2008)
OH
F
HO
C18H23FO2
Mol. Wt.: 290,37
CHCl3,
NMR-solvent
water
1.06
1.00
1.07
2.05
2.14
5.26
4.22
3.20
8
7
6
5
4
3
2
1
PPM

16alpha-Fluoroestradiol, Lot no. 260801, Retest 081015,
19F-NMR in CDCl3 (16-10-2008)



$C_{18}H_{23}FO_2$
Mol. Wt.: 290,37

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 10/03/2008
Date Updated: 01/31/2006
Version 1.5

Section 1 - Product and Company Information

Product Name	CYCLOPHOSPHAMIDE MONOHYDRATE
Product Number	C0768
Brand	SIAL
Company	Sigma-Aldrich
Address	3050 Spruce Street SAINT LOUIS MO 63103 US
Technical Phone:	800-325-5832
Fax:	800-325-5052
Emergency Phone:	314-776-6555

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
CYCLOPHOSPHAMIDE MONOHYDRATE	6055-19-2	No

Formula: $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$

Synonyms: N,N-Bis(beta-cloraethyl) N'-O-propylenphosphorildiamid monohydratum (Romanian) * 2-(Bis(2-chloroethyl)amino)-1-oxa-3-aza-2-phosphocyclohexane 2-oxide monohydrate * 1-Bis(2-chloroethyl)amino-1-oxo-2-aza-5-oxaphosphoridine monohydrate * (Bis(chloro-2-ethyl)amino)-2-tetrahydro-3,4,5,6-oxazaphosphorine-1,3,2-oxide-2 monohydrate * Bis(2-chloroethyl)phosphoramide cyclic propanolamide ester monohydrate * N,N-Bis(beta-chloroethyl)-N',O-propylenephosphoric acid ester amide monohydrate * N,N-Bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxaphosphorin-2-amine, 2-oxide monohydrate * N,N-Bis(beta-chloroethyl)-N',O-trimethylenephosphoric acid ester diamide monohydrate * Cyclic N',O-propylene ester of N,N-bis(2-chloroethyl)phosphorodiamidic acid monohydrate * Cyclophosphamide hydrate * Cyclophosphamide monohydrate * 2-(Di(2-chloroethyl)amino)-1-oxa-3-aza-2-phosphacyclohexane-2-oxide monohydrate * N,N-Di(2-chloroethyl)amino-N,O-propylene phosphoric acid ester diamide monohydrate * Endoxan monohydrate

RTECS Number: RP6157750

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Toxic.

May cause cancer. Toxic if swallowed. May cause heritable genetic damage. May cause harm to the unborn child.

Calif. Prop. 65 carcinogen & developmental hazard. Target organ(s): Bone marrow. Bladder.

HMIS RATING
HEALTH: 2*
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING
HEALTH: 2
FLAMMABILITY: 0
REACTIVITY: 0

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

DERMAL EXPOSURE
In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT
235 °F 113 °C Method: closed cup

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL
Evacuate area.

PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Wear disposable coveralls and discard them after use.

METHODS FOR CLEANING UP

Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING

User Exposure: Do not breathe dust. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure.

STORAGE

Suitable: Keep tightly closed.
Store at 2-8°C

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS

Use only in a chemical fume hood. Safety shower and eye bath.

PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator.
Hand: Compatible chemical-resistant gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES

Wash contaminated clothing before reuse. Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance	Physical State: Solid Color: White Form: Crystalline

Property	Value	At Temperature or Pressure
Molecular Weight	279.1 AMU	
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	49.0 - 51.0 °C	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	

Evaporation Rate	N/A
Viscosity	N/A
Surface Tension	N/A
Partition Coefficient	N/A
Decomposition Temp.	N/A
Flash Point	235 °F 113 °C Method: closed cup
Explosion Limits	N/A
Flammability	N/A
Autoignition Temp	N/A
Refractive Index	N/A
Optical Rotation	N/A
Miscellaneous Data	N/A
Solubility	Solvent: 0.1 g/ml H2O clear, colorless

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.
Materials to Avoid: Strong oxidizing agents, Strong acids, Strong bases.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Nitrogen oxides, Hydrogen chloride gas, Phosphorous oxides.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: Toxic if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Bladder. Bone marrow.

TOXICITY DATA

Oral
Rat
94 mg/kg
LD50
Remarks: Kidney, Ureter, Bladder:Urine volume increased.
Behavioral:Ataxia. Blood: Hemorrhage.

Intraperitoneal
Rat
121 MG/KG
LD50

Oral
Mouse
350 mg/kg
LD50
Remarks: Kidney, Ureter, Bladder:Urine volume increased.

Behavioral:Ataxia. Blood: Hemorrhage.

Intravenous
Mouse
275 MG/KG
LD50

Oral
Dog
44 mg/kg
LD50
Remarks: Behavioral:Somnolence (general depressed activity). Behavioral:Ataxia. Gastrointestinal:Nausea or vomiting.

Intravenous
Dog
40 MG/KG
LD50

Intravenous
Rabbit
130 MG/KG
LD50

Intravenous
Guinea pig
400 MG/KG
LD50

CHRONIC EXPOSURE - CARCINOGEN
Result: This is or contains a component that has been reported to be carcinogenic based on its IARC, OSHA, ACGIH, NTP, or EPA classification.

IARC CARCINOGEN LIST
Rating: Group 1 Group 1

NTP CARCINOGEN LIST
Rating: Clear evidence.
Species: Mouse/rat
Route: Intraperitoneal

CHRONIC EXPOSURE - TERATOGEN
Result: May cause congenital malformation in the fetus.

Species: Mouse
Dose: 40 MG/KG
Route of Application: Intraperitoneal
Exposure Time: (14D PREG)
Result: Specific Developmental Abnormalities: Blood and lymphatic system (including spleen and marrow). Specific Developmental Abnormalities: Hepatobiliary system.

Species: Rat
Dose: 27500 UG/KG
Route of Application: Intravenous
Exposure Time: (7-17D PREG)
Result: Specific Developmental Abnormalities: Musculoskeletal system. Specific Developmental Abnormalities: Central nervous system. Specific Developmental Abnormalities: Cardiovascular (circulatory) system.

CHRONIC EXPOSURE - MUTAGEN
Result: May alter genetic material.

Species: Human
Dose: 5 MG/L
Cell Type: lymphocyte
Mutation test: Cytogenetic analysis

Species: Rat
Route: Intraperitoneal
Dose: 200 MG/KG
Mutation test: Unscheduled DNA synthesis

Species: Mouse
Route: Oral
Dose: 15 MG/KG
Mutation test: Micronucleus test

Species: Mouse
Route: Intraperitoneal
Dose: 12500 UG/KG
Mutation test: Micronucleus test

Species: Mouse
Route: Intraperitoneal
Dose: 100 MG/KG
Mutation test: DNA damage

Species: Mouse
Route: Intraperitoneal
Dose: 25 MG/KG
Mutation test: Other mutation test systems

Species: Mouse
Route: Intraperitoneal
Dose: 25 MG/KG
Mutation test: Cytogenetic analysis

Species: Mouse
Route: Intraperitoneal
Dose: 50 UMOL/KG
Mutation test: Sister chromatid exchange

Species: Mouse
Route: Intraperitoneal
Dose: 100 MG/KG
Exposure Time: 5D
Mutation test: sperm

CHRONIC EXPOSURE - REPRODUCTIVE HAZARD

Species: Rat
Dose: 321 MG/KG
Route of Application: Oral
Exposure Time: (9W MALE)
Result: Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Rat
Dose: 88 MG/KG
Route of Application: Oral

Exposure Time: (9W MALE)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Rat
Dose: 5 MG/KG
Route of Application: Intravenous
Exposure Time: (11D PREG)
Result: Specific Developmental Abnormalities: Craniofacial (including nose and tongue). Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea). Specific Developmental Abnormalities: Musculoskeletal system.

Species: Rat
Dose: 27500 UG/KG
Route of Application: Intravenous
Exposure Time: (7-17D PREG)
Result: Maternal Effects: Ovaries, fallopian tubes. Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord). Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

Species: Rat
Dose: 27500 UG/KG
Route of Application: Intravenous
Exposure Time: (7-17D PREG)
Result: Effects on Newborn: Growth statistics (e.g., reduced weight gain). Effects on Newborn: Behavioral.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Observe all federal, state, and local environmental regulations. (DN)Requires special label: "Contains a substance which is regulated by Dannish work environmental law due to the risk of carcinogenic properties."

Section 14 - Transport Information

DOT
Proper Shipping Name: Organophosphorus compound, toxic, solid, n.o.s.
UN#: 3464
Class: 6.1
Packing Group: Packing Group III
Hazard Label: Toxic substances.
PIH: Not PIH

IATA
Proper Shipping Name: Organophosphorus compound, toxic, solid, n.o.s.
IATA UN Number: 3464
Hazard Class: 6.1
Packing Group: III

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: T
Indication of Danger: Toxic.
R: 45-25
Risk Statements: May cause cancer. Toxic if swallowed.
S: 53-45
Safety Statements: Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Toxic.
Risk Statements: May cause cancer. Toxic if swallowed. May cause heritable genetic damage. May cause harm to the unborn child.
Safety Statements: Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
US Statements: Calif. Prop. 65 carcinogen & developmental hazard. Target organ(s): Bone marrow. Bladder.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

UNITED STATES - STATE REGULATORY INFORMATION

CALIFORNIA PROP - 65
California Prop - 65: This product is or contains chemical(s) known to the state of California to cause developmental toxicity. This product is or contains chemical(s) known to the state of California to cause cancer.

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

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sigma-aldrich.com

Certificate of Analysis

Product Name	Cyclophosphamide monohydrate, bulk package
Product Number	C0768
Product Brand	Sigma-Aldrich
CAS Number	6055-19-2
Molecular Formula	$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$
Molecular Weight	279.10
Storage Temp	2-8°C

TEST	**LOT 068K1131 RESULTS**
APPEARANCE	WHITE POWDER
SOLUBILITY	CONFORMS
WATER BY KARL FISCHER	7.5%
PROTON NMR SPECTRUM	CONFORMS
ASSAY	98.0% BY HPLC
PRODUCT CROSS REFERENCE INFORMATION	REPLACEMENT FOR ALDRICH #218707
RECOMMENDED RETEST	AUGUST 2011
QC RELEASE DATE	AUGUST 2008

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri USA

RTI Project No.: 0211886.001.001

RTI Master Protocol No.: RTI-1059

RTI Study Code: Rt08-FES

AMENDMENT 1

TITLE: **14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment**

SPONSOR: Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]

TESTING FACILITY: RTI International*
Center for Life Sciences and Toxicology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

*RTI International is the tradename for Research Triangle Institute

APPROVALS

RTI International

11/19/08

Kimberly D. Ehman, Ph.D. Date
Study Director
Center for Life Sciences and Toxicology

19 Nov 08

Irma M. Grossi, Ph.D. Date
Senior Director, Life Sciences and
Toxicology and DMPK
BOA/Contract Principal Investigator

Sponsor

11/21/2008

Paula M. Jacobs, Ph.D. Date
CMRP, SAIC Frederick
Project Officer

Quality Assurance Review By:

11-19-08

Leslie L. Macdonald, B.S. Date
Quality Assurance Specialist
RTI Quality Assurance Unit

Sponsor approval received via e-mail on 11/14/08 (date) by KE 11/19/08 (Initials/Date).

The protocol, as signed by the Study Director on November 7, 2008, is amended as follows. Changes are in ***bold italics*** for clarification.

1. Location of protocol change: Section 2.0, Personnel Involved in the Study (p. 6)

Quality Assurance Specialist:	***Leslie L. Macdonald, B.S.*** ***Telephone: 919-485-2692*** ***E-mail: lmacdonald@rti.org***

Rationale: The quality assurance specialist had been changed to Leslie Macdonald prior to signing the protocol. The QA specialist was inadvertently not changed to Leslie on this particular page.

2. Location of protocol change: Section 5.1, Identification of Test Article(s) (p. 7)

Stability:	TBD
Storage Conditions:	***Refrigerated***
Safety Precautions:	Care to be taken in handling; cyclophosphamide is a potent cytotoxic agent. A Material Safety Data Sheet (MSDS) will be maintained in the study file.

Rationale: The neat compound should be stored under refrigerated conditions; however, it was incorrectly noted as room temperature in the protocol.

3. Location of protocol change: Section 6.2, Dose Analysis (p. 8)

Approximately 1-3-mL samples will be collected from each dose formulation on the first day of dosing (Study Day 0) and on the last day of dosing (Study Day 13). The samples will be shipped in ~~amber~~ clear borosilicate vials on ice packs to the University of Washington for stability and concentration analyses. Aliquots will be collected on the first day of formulation (i.e., Study Day 0) and on the last day of dosing (i.e., Study Day 13). Standards for acceptable concentration and stability will be as follows: the mean of the analyzed samples must be within ± 15% of nominal, and the change in concentration from the sample collected on Study Day 0 and the sample collected on Study Day 13 must not exceed 15%. The positive control article formulation will not be analyzed for stability, homogeneity, or concentration. The Study Director and Sponsor Representative will be notified immediately if problems of this nature occur and the resolution will be documented in the study records.

Rationale: The dose formulations will be stored and shipped in clear vials at the request of the analytical chemist, Dr. Link. Clear vials had been agreed upon by both the Sponsor and the Study Director; however, the word "amber" was inadvertently added.

RTI Project No.: 0211886.001.001

RTI Master Protocol No.: RTI-1059

RTI Study Code: Rt08-FES

AMENDMENT 2

TITLE: 14-Day Intravenous Repeat Dose Toxicology Study in Rats with Micronucleus Assessment

SPONSOR: Clinical Monitoring Research Program, SAIC Frederick
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]

TESTING FACILITY: RTI International*
Center for Life Sciences and Toxicology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

RTI International is the tradename for Research Triangle Institute

APPROVALS

RTI International

Jay G. Henson, B.S. Date
Study Director
Center for Life Sciences and Toxicology

12 Jan 2009
Irma M. Grossi, Ph.D. Date
Senior Director, Life Sciences and
Toxicology and DMPK
BOA/Contract Principal Investigator

Quality Assurance Review By:

1-12-09
Leslie L. Macdonald, B.S. Date
Quality Assurance Specialist
RTI Quality Assurance Unit

Sponsor

1/13/2009
Paula M. Jacobs, Ph.D. Date
CMRP, SAIC Frederick
Project Officer

Sponsor approval received via e-mail on 1/9/09 (date) by JGH (Initials/Date).

The protocol, as signed by the Study Director on November 7, 2008 and amended by her signature (Amendment 1) on November 19, 2008, is further amended as follows. Changes are in ***bold italics*** for clarification.

1. Location of protocol change: Signature page (p. 2)

APPROVALS

RTI International

Jay G. Henson, B.S. Date
Study Director
Center for Life Sciences and Toxicology

Irma M. Grossi, Ph.D. Date
Senior Director, Life Sciences and
Toxicology and DMPK
BOA/Contract Principal Investigator

Sponsor

Paula M. Jacobs, Ph.D. Date
CMRP, SAIC Frederick
Project Officer

2. Location of protocol change: Section 2.0, Personnel Involved in the Study (p. 5)

RTI Study Director:

Jay G. Henson, B.S.
P.O. Box 12194
3040 Cornwallis Road
HLB-140
Research Triangle Park, NC 27709-2194
Telephone: 919-541-7206
Fax: 919-541-5956
E-mail: jhensoni@rti.org

Rationale: The current Study Director, Kimberly D. Ehman, will be leaving RTI's employment effective January 16, 2009. Jay G. Henson will take over completion of this study.

FINAL REPORT

Study Title

In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Test Article

Fluoroestradiol

Author

Jane J. Clarke, M.S.

Study Completion Date

11 June 2009

Testing Facility

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AC19NA.704.BTL

Sponsor Project Number

211886.001

Sponsor

RTI International
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

STATEMENT OF COMPLIANCE

Study AC19NA.704.BTL was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58 in all material aspects with the following exception:

> Analyses to determine the uniformity or concentration of the test article dosing formulations were performed by Molecular Imaging Research but not in full compliance with the above regulations. The stability of the test article mixtures was not determined.

Jane J. Clarke, M.S.
Study Director

11 June 2009
Date

BioReliance Study Management

11 JUN 2009
Date

Quality Assurance Statement

Study Information

Number: AC19NA.704.BTL

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practices 21CFR 58

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
09-Feb-2009	09-Feb-2009	Observation of Test System	09-Feb-2009	09-Feb-2009
05-Mar-2009	09-Mar-2009	Data and Draft Reporting	09-Mar-2009	09-Mar-2009
09-Jun-2009	09-Jun-2009	Final Reporting	09-Jun-2009	09-Jun-2009

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: Allison Schaefer 11-Jun-2009 8:25 pm GMT

Reason for signature: QA Approval

Printed by:Allison Schaefer

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Sponsor:	**RTI International** **3040 Cornwallis Rd.** **Research Triangle Park, NC 27709**
Authorized Representative:	**Jay G. Henson, BS**
Testing Facility:	**BioReliance** **9630 Medical Center Drive** **Rockville, MD 20850**
Test Article I.D.:	**Fluoroestradiol**
Test Article Lot No.:	**260801**
Test Article Purity:	**>98% (Provided by Sponsor)**
Molecular Weight:	**290.37 (Provided by Sponsor)**
Sponsor Project No.:	**211886.001**
BioReliance Study No.:	**AC19NA.704.BTL**
Test Article Description:	**Colorless crystals**
Storage Conditions:	**-15 to -40°C; under Argon, protected from light and moisture**
Test Article Receipt/Login:	**24 October 2008**
Study Initiation:	**09 January 2009**
Experimental Start Date:	**13 January 2009**
Experimental Completion Date:	**09 February 2009**
Principal Investigator:	**Jeanne Link, Ph.D.**
Analytical Laboratory:	**University of Washington** **Box 356004, Room NW041 UWMC** **Seattle, WA 98195-6004**

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SUMMARY

The test article, Fluoroestradiol, was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9. The preliminary toxicity assay established the concentration range for the mutagenesis assays. The mutagenesis assays were used to evaluate the mutagenic potential of the test article.

Ethanol was selected by the sponsor as the solvent for the test article. The test article was soluble in Ethanol at approximately 1.0 mg/mL, the maximum concentration prepared for the preliminary toxicity assay.

In the preliminary toxicity assay, the maximum concentration of Fluoroestradiol in treatment medium was 8.0 ng/mL. No visible precipitate was present at any concentration in treatment medium. Selection of concentrations for the mutation assay was based on reduction of suspension growth relative to the solvent control and the maximum concentration requested by the sponsor. No substantial toxicity, i.e., suspension growth of ≤ 50% of the solvent control, was observed at any concentrations with or without S9 activation.

Based on the results of the preliminary toxicity assay, the concentrations treated in the initial mutagenesis assay ranged from 0.15 to 8.0 ng/mL ng/mL for both the non-activated and S9-activated cultures with a 4-hour exposure. No visible precipitate was present at any concentrations in treatment medium. The concentrations chosen for cloning were 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL with and without S9 activation. No cloned cultures exhibited mutant frequencies ≥ 90 mutants per 10^6 clonable cells over that of the solvent control. There was no concentration-related increase in mutant frequency.

Based on the results of the preliminary toxicity assay, the concentrations treated in the extended treatment assay ranged from 0.15 to 8.0 ng/mL ng/mL for non-activated cultures with a 24-hour exposure. No visible precipitate was present at any concentrations in treatment medium. The concentrations chosen for cloning were 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL. No cloned cultures exhibited mutant frequencies ≥ 90 mutants per 10^6 clonable cells over that of the solvent control. There was no concentration-related increase in mutant frequency.

The trifluorothymidine-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS and DMBA positive controls yielded the expected increase in small colonies (verifying the adequacy of the methods used to detect small colony mutants) and large colonies.

Under the conditions of this study, test article Fluoroestradiol was concluded to be negative in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

PURPOSE

The purpose of this study was to evaluate the genotoxic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells and sizing of the resulting colonies according to the protocol in Appendix I.

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, Fluoroestradiol, was received by BioReliance on 24 October 2008 and was assigned the code number AC19NA. The test article was described by the Sponsor as colorless crystals, which should be stored at -5 to -40°C. Its purity was given as >98%. An expiration date of 15 October 2009 was provided. Upon receipt, the test article was described as colorless crystals and was stored at -15 to -40°C, under Argon, protected from light and moisture.

The Sponsor has determined the identity, strength, purity, and composition or other characteristics to define the test article and the stability of the test article. A copy of the Certificate of Analysis is included in Appendix III. Based on the expiration date in the Certificate of Analysis, the test article is considered stable for the purpose of this study through 15 October 2009.

The vehicle (solvent) used to deliver Fluoroestradiol to the test system was Ethanol (CAS 64-17-5), lot #B0514580, and expiration date 31 March 2011, obtained from Acros. The test article dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light. Two 2 mL aliquots of the most concentrated dosing formulation and the vehicle from the mutation assay were shipped to the University of Washington (Seattle, WA) for chemical analysis on cool packs. The University of Washington determined the concentration of the test article dosing preparations. A copy of the Dosing Formulation Certificate of Analysis is included in Appendix IV.

Methyl methanesulfonate (MMS), CAS 66-27-3, lot #06823KH, expiration date 04 June 2011, supplied by Aldrich Chemical Company was diluted in water lot #1391332, expiration date January 2009, from Gibco and used as the positive control for the non-activated test system at stock concentrations of 1500 and 2000 µg/mL for the 4-hour exposure and 500 and 750 µg/mL for the 24-hour exposure. 7,12-Dimethyl-benz(a)anthracene (7,12-DMBA), CAS 57-97-6, lot #055K1360, expiration date 09 September 2010, supplied by Sigma Chemical Company was diluted in DMSO lot #48086822, expiration date 14 January 2011, from EMD Chemicals and used at stock concentrations of 100 and 125 µg/mL as the positive control for the S9-activated test system.

The negative and positive control articles have been characterized as per the Certificates of Analysis on file with the testing facility. The stability of the negative and positive control articles and their respective mixtures was demonstrated by acceptable results that met the criteria for a valid test. Historical control data are presented in Appendix II.

MATERIALS AND METHODS

Test System

L5178Y cells, clone 3.7.2C, were obtained from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, NC on 14 August 1995. Each lot of cryopreserved cells was tested using the agar culture and Hoechst staining procedures and found to be free of mycoplasma contamination. Prior to use in the assay, L5178Y cells were cleansed of spontaneous $TK^{-/-}$ cells by culturing in a restrictive medium (Clive and Spector, 1975).

Metabolic Activation System

Aroclor 1254-induced rat liver S9 lot 2313, was purchased by BioReliance from Moltox (Boone, NC) and stored at $\leq -60^{o}C$ until used. Each lot of S9 was assayed for sterility and its ability to metabolize at least two pro-mutagens to forms mutagenic to *Salmonella typhimurium* TA100. The Record of Analysis is on file with the testing facility.

Immediately prior to use, the S9 was mixed with the cofactors and Fischer's Medium for Leukemic Cells of Mice with 0.1% Pluronics (F_0P) to contain 25 µL S9, 6.0 mg nicotinamide adenine dinucleotide phosphate (NADP), 11.25 mg DL-isocitric acid, and 975 µL F_0P per mL S9-activation mixture and kept on ice until used. The cofactor/F_0P mixture was adjusted to pH 7.0 and filter-sterilized prior to the addition of S9.

Preliminary Toxicity Assay

The preliminary toxicity assay was used to establish the optimal concentrations for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.001 to 8.0 ng/mL in both the absence and presence of S9 activation with a 4-hour exposure and without activation with a 24-hour exposure. The osmolality of the solvent control and the highest soluble concentration in treatment medium were determined.

For the 4-hour exposure, cell population density was determined 24 and 48 hours after the exposure to the test article; the cultures were adjusted to $3x10^5$ cells/mL after 24 hours only. For the 24-hour exposure, cell population density was determined 24, 48, and 72 hours after the exposure to the test article. The cell population was adjusted to 3×10^5 cells/mL immediately after test article removal and 24 hours after test article removal. Cultures with less than $3x10^5$ cells/mL were not adjusted. Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures after 48 hours.

Mutagenesis Assays

The initial mutagenesis assay (with and without S9 activation with a 4-hour exposure) and extended treatment assay (without activation with a 24-hour exposure) were used to evaluate the mutagenic potential of the test article. L5178Y mouse lymphoma cells were exposed to the solvent alone and eight concentrations of test article in duplicate in both the absence and presence of S9. Positive controls, with and without S9 activation, were tested concurrently.

Treatment of the Target Cells

The mutagenesis assay was performed according to a protocol described by Clive and Spector (1975). Treatment was carried out in conical tubes by combining 6 x 10^6 L5178Y/TK$^{+/-}$ cells, F_0P medium or S9 activation mixture, and 50 µL dosing solution of test article in solvent or solvent alone in a total volume of 10 mL. The positive controls were treated with 100 µg/mL MMS (at final concentrations in treatment medium of 15 and 20 µg/mL with a 4-hour exposure or 5.0 and 7.5 µg/ml with a 24-hour exposure) or 7,12-DMBA (at final concentrations in treatment medium of 1.0 and 1.25 µg/mL). Treatment tubes were gassed with 5±1% CO_2 in air, capped tightly, and incubated with mechanical mixing for 4 or 24 hours at 37±1°C. The preparation and addition of the test article dosing solutions were carried out under amber lighting and the cells were incubated in the dark during the exposure period. After the treatment period, the cells were washed twice with F_0P or F_0P supplemented with 10% horse serum, 2 mM L-glutamine, 100 U penicillin/mL and 100 µg streptomycin/mL ($F_{10}P$). After the second wash, the cells were resuspended in 20 mL $F_{10}P$, gassed with 5±1% CO_2 in air and placed on the roller drum apparatus at 37±1°C.

Expression of the Mutant Phenotype

For expression of the mutant phenotype, the cultures were counted using an electronic cell counter and adjusted to 3x10^5 cells/mL at approximately 24 and 48 hours after treatment in 20 and 10 mL total volume, respectively. For the 24-hour exposure, cultures were adjusted to 3x10^5 cells/mL in 20 mL immediately after test article removal, then at 48 and 72 hours after treatment in 20 and 10 mL total volume, respectively. Cultures with less than 3x10^5 cells/mL were not adjusted.

For expression of the TK$^{-/-}$ cells, cells were placed in cloning medium (C.M.) containing 0.23% dissolved Noble agar in F_0P plus 20% horse serum. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or VC (viable count). Each flask was prewarmed to 37±1ºC, filled with 100 mL C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was decanted. The cells were then diluted in C.M. to concentrations of 3x10^6 cells/100 mL C.M. for the TFT flask and 600 cells/100 mL C.M. for the VC flask. After the dilution, 1.0 mL of stock solution of TFT was added to the TFT flask (final concentration of 3 µg/mL) and both this flask and the VC flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed and the cell suspension was dispensed equally into each of three appropriately labeled Petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 30 minutes. The plates were then incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for 10-14 days.

Scoring Procedures

After the incubation period, the VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with ≥ 20% total relative growth (including at least one concentration with ≥ 10% but ≤ 20% total growth). The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of

approximately 0.2 to 1.1 mm. The rationale for this procedure is as follows: Mutant L5178Y $TK^{-/-}$ colonies exhibit a characteristic frequency distribution of colony sizes. The precise distribution of large and small TFT-resistant mutant colonies appears to be the characteristic mutagenic "finger-print" of carcinogens in the L5178Y $TK^{+/-}$ system (Clive *et al*., 1979; DeMarini *et al*., 1989). Clive *et al*. (1979) and Hozier *et al*. (1981) have presented evidence to substantiate the hypothesis that the small colony variants carry chromosome aberrations associated with chromosome 11, the chromosome on which the TK locus is located in the mouse. They suggested that large colony mutants received localized damage, possibly in the form of a point mutation or small deletion within the TK locus, while small colony mutants received damage to collateral loci concordant with the loss of TK activity.

Criteria for a Valid Test

The following criteria must be met for the mutagenesis assay to be considered valid:

Negative Controls

The average spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 35 to 140 TFT-resistant mutants per 10^6 surviving cells. Low spontaneous mutant frequencies, i.e., 20 to 34 mutants per 10^6 surviving cells, are considered acceptable if small colony recovery is demonstrated (Mitchell *et al*., 1997). The average cloning efficiency of the solvent (or vehicle) controls must be between 65% and 120% and the total suspension growth between 8-32 for the 4-hour exposure and 20-180 for the 24-hour exposure (Moore, *et al*., 2002; 2006; 2007).

Positive Controls

The mutant frequency for at least one dose of the positive controls must meet the criteria for a positive response and induce an increase in small colony mutants according to the following criteria: Induced Mutant Frequency (IMF) positive control $\geq 300 \times 10^{-6}$ mutants with 40% small colonies **or** small colony IMF for positive control $\geq 150 \times 10^{-6}$ (Moore, *et al*., 2002; 2006).

Test Article-Treated Cultures:

Cultures treated with a minimum of four concentrations of test article must be attained and their mutant frequencies reported. The highest test article concentration must produce 80% to 90% toxicity (ICH, 1996) unless limited by solubility or the maximum required concentration as described in section 7.2 of the protocol. In the case of a test article with a steep toxicity curve (no concentrations with 10-20% survival), the results may be considered acceptable if a concentration spacing of $\leq$ 2-fold is used and the highest concentration tested showed <20% survival or total kill (Sofuni *et al*., 1997). For example, the test is considered acceptable if the highest concentration cloned for mutant selection exhibits >20% survival and the next highest concentration, which is $\leq$ 2 times the cloned concentration, is too toxic to clone.

Evaluation of Results

The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 106 surviving cells) for each treatment condition was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding VC plates and multiplying by the dilution factor ($2x10^{-4}$) then multiplying by 10^6. For simplicity, this is described as: (Average # TFT colonies / average # VC colonies) x 200 in the tables. The induced mutant frequency (IMF) is defined as the mutant frequency of the treated culture minus the mutant frequency of the solvent control cultures. The International Workshop on Genotoxicity established a Global Evaluation Factor (GEF) for a positive response at an IMF of ≥ 90 mutants per 10^6 clonable cells at the Aberdeen meeting in 2003, published in Moore et al., 2006.

In evaluation of the data, increases in induced mutant frequency that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on scientific judgment; however, the following criteria are presented as a guide to interpretation of the data (Moore *et al.*, 2006):

- A result was considered positive if a concentration-related increase in induced mutant frequency was observed in the treated cultures and one or more treatment conditions with 10% or greater total growth exhibited induced mutant frequencies of ≥90 mutants per 10^6 clonable cells (based on the average mutant frequency of duplicate cultures). If the average solvent control mutant frequency was >90 mutants per 10^6 clonable cells, a doubling of mutant frequency over the background will also be required (Mitchell *et al.*, 1997).

- A result was considered negative if the treated cultures exhibited induced mutant frequencies of less than 90 mutants per 10^6 clonable cells (based on the average mutant frequency of duplicate cultures) and there was no concentration-related increase in mutant frequency.

- There are some situations in which a chemical would be considered negative when there was no culture showing between 10-20% survival: 1) There was no evidence of mutagenicity (e.g. no dose response or increase in induced mutant frequencies between 45 and 89 mutants per 10^6) in a series of data points within 100% to 20% survival *and* there was at least one negative data point between 20% and 25% survival. 2) There was no evidence of mutagenicity (e.g. no dose response or increase in induced mutant frequencies between 45 and 89 mutants per 10^6) in a series of data points between 100% to 25% survival *and* there was also a negative data point between 10% and 1% survival (Office of Food Additive Safety, 2001). In this case it would be acceptable to count the TFT colonies of cultures exhibiting <10% total growth.

Electronic Data Collection Systems

The primary computer or electronic systems used for the collection or analysis of data included but were not limited to the following:

LIMS Labware version 5, Excel 2003 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

Records and Archives

All raw data, protocol, and all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years. Raw data, the protocol and reports generated at facilities other than BioReliance will be archived per the contractual arrangements between that facility and the Sponsor.

Deviations

The following deviation from the protocol occurred during the conduct of this study: Event #26316: Both aliquots of the most concentrated dosing formulation and the vehicle were sent to the analytical lab instead of just one. The study director has determined that this deviation from the protocol had no impact on the integrity or conclusion of the study because the back-up samples would have been sent at a later date or discarded at the end of the study.

RESULTS AND DISCUSSION

Solubility

Ethanol was selected by the sponsor as the solvent for the test article. The test article was soluble in Ethanol at approximately 1.0 mg/mL, the maximum concentration prepared for the preliminary toxicity assay.

Preliminary Toxicity Assay

The results of the preliminary toxicity assay are presented in Table 1. The maximum concentration tested in the preliminary toxicity assay was 8.0 ng/mL. No visible precipitate was present at any concentration in treatment medium. The osmolality of the solvent control was 309 mmol/kg and the osmolality of the highest soluble concentration, 8.0 ng/mL, was 308 mmol/kg. Suspension growth relative to the solvent controls at 8.0 ng/mL was 102% without activation with a 4-hour exposure, 96% with S9 activation with a 4-hour exposure, and 94% without activation with a 24-hour exposure. Based on the results of the toxicity test, the concentrations treated in the mutagenesis assay ranged from 0.15 to 8.0 ng/mL for both the non-activated and S9-activated cultures with a 4-hour exposure and non-activated cultures with a 24-hour exposure.

Mutagenesis Assays

The results of the initial mutagenesis assay are presented in Tables 2 and 3. Colony size distributions for the positive and solvent control cultures are presented in Figures 1 and 2. No visible precipitate was present at any concentration in treatment medium. In the non-activated system, cultures treated with concentrations of 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL were cloned and produced a range in suspension growth from 99% to 112%. In the S9-activated system, cultures treated with the same concentrations were cloned and produced a range in suspension growth from 93% to 119%.

No cloned cultures exhibited mutant frequencies that were ≥ 90 mutants per 10^6 clonable cells over that of the solvent control. No concentration-related increase in mutant frequency was observed in the non-activated or S9-activated systems. The total growth ranged from 74% to 97% for the non-activated cultures at concentrations from 1.0 to 8.0 ng/mL and 74% to 117% for the S9-activated cultures at the same concentrations.

The results of the initial assay were negative in the absence and presence of S9 activation. Because no unique metabolic requirements were known about the test article, an extended treatment assay was performed only in the absence of S9 for a 24-hour exposure period.

The results of the extended treatment assay are presented in Table 4. Colony size distributions for the positive and solvent control cultures are presented in Figure 3. No visible precipitate was present at any concentration in treatment medium. Cultures treated with concentrations of 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL were cloned and produced a range in suspension growth from 93% to 119%.

No cloned cultures exhibited mutant frequencies that were ≥ 90 mutants per 10^6 clonable cells over that of the solvent control. No concentration-related increase in mutant frequency was observed. The total growth ranged from 85% to 118% for non-activated cultures with a 24-hour exposure at concentrations from 1.0 to 8.0 ng/mL.

The TFT-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS and DMBA positive controls yielded the expected increase in small colonies (verifying the adequacy of the methods used to detect small colony mutants) and large colonies.

Dosing Formulation Analysis

Two 2 mL aliquots of the most concentrated dosing formulation and the vehicle from the mutation assay were shipped to The University of Washington (Seattle, WA) for chemical analysis on cool packs. The University of Washington determined the concentration of the test article dosing preparations. No test article was found in the vehicle control samples. The most concentrated dosing formulation, 1.6 µg/mL, was found to be 1.9 and 2.0 µg/mL in the duplicate samples. A copy of the Dosing Formulation Certificate of Analysis is included in Appendix IV. The average of the duplicate samples was 122% higher than nominal which does not meet the criteria of ±15% of nominal. The study director has concluded that this discrepancy had no impact on the integrity or conclusion of the

study. Since the concentration tested was higher than labeled, the assay was actually more stringent than intended.

CONCLUSION

All criteria for a valid study were met as described in the protocol. Under the conditions of this study, test article Fluoroestradiol was concluded to be negative in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

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TABLE 1

PRELIMINARY TOXICITY ASSAY USING Fluoroestradiol
4-Hour Exposure

DOSE LEVEL (mg/mL)	PRECIP	CELL CONCENTRATION (cells/mL x 10^6)			SUSPENSION GROWTH	
		DAY 0	DAY 1	DAY 2	TOTAL	% OF CONTROL
4HR NON-ACTIVATED CULTURES						
SOLVENT 1		Not applicable for 4-hour exposure	1.477	1.423	23.4	**100**
SOLVENT 2			1.505	1.453	24.3	
1X10-9			1.421	1.470	23.2	**97**
3X10-9			1.403	1.462	22.8	**96**
1X10-8			1.438	1.478	23.6	**99**
3X10-8			1.419	1.461	23.0	**97**
1X10-7			1.459	1.436	23.3	**98**
3X10-7			1.428	1.480	23.5	**99**
1X10-6			1.367	1.546	23.5	**98**
3X10-6			1.563	1.414	24.6	**103**
8X10-6			1.509	1.454	24.4	**102**
4HR S9-ACTIVATED CULTURES **(Induced Rat Liver S9)**						
SOLVENT 1		Not applicable for 4-hour exposure	1.003	1.500	16.7	**100**
SOLVENT 2			1.144	1.461	18.6	
1X10-9			1.212	1.487	20.0	**113**
3X10-9			1.149	1.511	19.3	**109**
1X10-8			1.206	1.432	19.2	**109**
3X10-8			1.162	1.525	19.7	**111**
1X10-7			1.253	1.491	20.7	**118**
3X10-7			1.165	1.409	18.2	**103**
1X10-6			0.975	1.472	15.9	**90**
3X10-6			1.156	1.423	18.3	**104**
8X10-6			1.102	1.388	17.0	**96**

Solvent = Ethanol

1 and 2 are duplicate cultures

Cultures containing <0.3x10^6 cells/mL on day 0, 1, and 2 are considered to have 0% total suspension growth.

$$\text{Total suspension growth (4-hr)} = \frac{\text{Day 1 cell conc.}}{0.3\text{x}10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

TABLE 1 (continued)

PRELIMINARY TOXICITY ASSAY USING Fluoroestradiol
24-Hour exposure

DOSE LEVEL (mg/mL)	PRECIP	CELL CONCENTRATION (cells/mL x 10^6)			SUSPENSION GROWTH	
		DAY 0	DAY 1	DAY 2	TOTAL	% OF CONTROL
24HR NON-ACTIVATED CULTURES						
SOLVENT 1		0.864	0.766	1.517	37.1	**100**
SOLVENT 2		0.852	0.803	1.471	37.3	
1X10-9		0.900	0.768	1.513	38.8	**104**
3X10-9		0.899	0.740	1.434	35.4	**95**
1X10-8		0.900	0.775	1.530	39.5	**106**
3X10-8		0.886	0.751	1.450	35.7	**96**
1X10-7		0.938	0.733	1.503	38.3	**103**
3X10-7		0.914	0.750	1.479	37.6	**101**
1X10-6		0.862	0.744	1.576	37.4	**101**
3X10-6		0.850	0.780	1.506	37.0	**99**
8X10-6		0.916	0.704	1.466	35.0	**94**

Solvent = Ethanol

1 and 2 are duplicate cultures

Cultures containing <0.3x10^6 cells/mL on day 0, 1, and 2 are considered to have 0% total suspension growth.

$$\text{Total suspension growth (24-hr)} = \frac{\text{Day 0 cell conc.}}{0.3\text{x}10^6 \text{ cells/mL}} \times \frac{\text{Day 1 cell conc.}}{\text{Day 0 adjusted cell conc.}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

TABLE 2

DATA SUMMARY FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Fluoroestradiol
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

DOSE LEVEL (mg/mL)	PRECIP.	TOTAL SUSP. GROWTH	% SUSP. GROWTH	TFT COLONIES				VC COLONIES				TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% TOTAL GROWTH
				PLATE COUNTS				PLATE COUNTS						
				1	2	3	MEAN	1	2	3	MEAN			
SOLVENT 1		20.7	100	48	36	26	**37**	231	228	248	**236**	31	N/A	100
SOLVENT 2		18.7		63	53	71	**62**	252	227	238	**239**	52		
1x10-6 A		22.0	112	59	48	45	**51**	210	195	209	**205**	50	**8**	**96**
1x10-6 B		19.6	99	57	45	*	**51**	223	234	235	**231**	44	**3**	**97**
2x10-6 A		20.2	103	51	45	41	**46**	206	224	194	**208**	44	**2**	**90**
2x10-6 B		20.8	106	38	44	62	**48**	186	209	*	**198**	49	**7**	**88**
4x10-6 A		21.0	107	44	36	*	**40**	182	187	*	**185**	43	**2**	**83**
4x10-6 B		20.8	106	44	40	41	**42**	209	207	201	**206**	41	**-1**	**92**
6x10-6 A		21.9	111	41	59	66	**55**	195	186	217	**199**	56	**14**	**93**
6x10-6 B		21.0	107	51	59	67	**59**	169	*	218	**194**	61	**19**	**87**
8x10-6 A		19.5	99	45	62	41	**49**	219	185	201	**202**	49	**7**	**84**
8x10-6 B		19.6	99	40	32	46	**39**	180	191	161	**177**	44	**3**	**74**
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)														
20		12.7	65	207	217	150	**191**	57	58	50	**55**	696	**654**	**15**
15		13.6	69	203	228	265	**232**	81	74	95	**83**	557	**515**	**24**

MEAN SOLVENT TOTAL SUSPENSION GROWTH: 19.7
MEAN SOLVENT CLONING EFFICIENCY: 119%
MEAN SOLVENT MUTANT FREQUENCY: 42 *(PER 10^6 CELLS)*

Solvent = Ethanol A and B or 1 and 2 are duplicate cultures

* - Plate lost to contamination

$$\text{Mutant frequency per } 10^6 \text{ surviving cells} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$\text{Induced mutant frequency per } 10^6 \text{ surviving cells} = \text{mutant frequency} - \text{average mutant frequency of solvent controls}$$

$$\text{Total suspension growth} = \frac{\text{Day 1 cell conc.}}{0.3\text{x}10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

$$\text{\% control cloning growth (not shown)} = \frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$$

$$\text{\% total growth} = \frac{(\text{\% suspension growth})(\text{\% cloning growth})}{100}$$

TABLE 3

DATA SUMMARY FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Fluoroestradiol
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

DOSE LEVEL (mg/mL)	PRECIP.	TOTAL SUSP. GROWTH	% SUSP. GROWTH	TFT COLONIES				VC COLONIES				TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% TOTAL GROWTH
				PLATE COUNTS				PLATE COUNTS						
				1	2	3	MEAN	1	2	3	MEAN			
SOLVENT 1		16.6	100	83	57	78	**73**	202	172	165	**180**	81	N/A	100
SOLVENT 2		18.6		44	42	57	**48**	139	173	184	**165**	58		
1x10-6 A		16.4	93	51	57	55	**54**	132	*	165	**149**	73	**4**	**80**
1x10-6 B		18.9	108	67	65	88	**73**	162	173	194	**176**	83	**14**	**110**
2x10-6 A		20.6	117	53	48	54	**52**	*	160	162	**161**	64	**-5**	**109**
2x10-6 B		19.4	110	79	45	73	**66**	164	150	152	**155**	85	**15**	**99**
4x10-6 A		21.0	119	58	59	77	**65**	161	177	169	**169**	77	**7**	**117**
4x10-6 B		20.0	114	53	54	69	**59**	139	180	169	**163**	72	**3**	**107**
6x10-6 A		20.7	117	58	84	67	**70**	*	*	157	**157**	89	**19**	**107**
6x10-6 B		18.9	107	69	49	54	**57**	135	108	116	**120**	96	**27**	**74**
8x10-6 A		19.0	108	50	57	54	**54**	152	154	157	**154**	70	**0**	**97**
8x10-6 B		19.7	112	53	59	67	**60**	172	161	181	**171**	70	**0**	**111**
POSITIVE CONTROL: 7,12-dimethylbenz(a)anthracene (DMBA) (µg/mL)														
1.25		2.9	17	243	227	250	**240**	100	119	99	**106**	453	**384**	**10**
1		3.9	22	261	*	230	**246**	103	114	*	**109**	453	**383**	**14**

MEAN SOLVENT TOTAL SUSPENSION GROWTH: 17.6
MEAN SOLVENT CLONING EFFICIENCY: 86%
MEAN SOLVENT MUTANT FREQUENCY: 69 ***(PER 10^6 CELLS)***

Solvent = Ethanol A and B or 1 and 2 are duplicate cultures

* - Plate lost to contamination

$$\text{Mutant frequency per } 10^6 \text{ surviving cells} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$\text{Induced mutant frequency per } 10^6 \text{ surviving cells} = \text{mutant frequency} - \text{average mutant frequency of solvent controls}$$

$$\text{Total suspension growth} = \frac{\text{Day 1 cell conc.}}{0.3\text{x}10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

$$\text{\% control cloning growth (not shown)} = \frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$$

$$\text{\% total growth} = \frac{(\text{\% suspension growth})(\text{\% cloning growth})}{100}$$

TABLE 4

DATA SUMMARY FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Fluoroestradiol
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Extended Treatment Assay (24-hour exposure)

DOSE LEVEL (mg/mL)	PRECIP.	TOTAL SUSP. GROWTH	% SUSP. GROWTH	TFT COLONIES				VC COLONIES				TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% TOTAL GROWTH
				PLATE COUNTS				PLATE COUNTS						
				1	2	3	MEAN	1	2	3	MEAN			
SOLVENT 1		34.5	100	35	23	17	**25**	157	141	165	**154**	32	N/A	100
SOLVENT 2		33.4		20	25	40	**28**	165	178	196	**180**	32		
1x10-6 A		37.7	111	25	25	23	**24**	140	148	172	**153**	32	**0**	**102**
1x10-6 B		35.0	103	27	33	24	**28**	133	161	157	**150**	37	**5**	**93**
2x10-6 A		35.5	104	20	20	17	**19**	128	141	141	**137**	28	**-4**	**85**
2x10-6 B		31.7	93	19	28	21	**23**	***						
4x10-6 A		36.2	106	23	33	24	**27**	165	136	144	**148**	36	**4**	**94**
4x10-6 B		40.3	119	27	24	41	**31**	149	161	188	**166**	37	**5**	**118**
6x10-6 A		36.5	107	17	19	21	**19**	169	140	156	**155**	25	**-7**	**100**
6x10-6 B		35.2	104	31	40	37	**36**	145	142	178	**155**	46	**14**	**96**
8x10-6 A		36.2	106	25	32	28	**28**	161	157	164	**161**	35	**3**	**102**
8x10-6 B		38.4	113	45	52	45	**47**	164	166	169	**166**	57	**25**	**113**
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)														
7.5		19.1	56	209	194	172	**192**	170	127	137	**145**	265	**233**	**49**
5		26.6	78	160	146	174	**160**	73	87	68	**76**	421	**389**	**36**
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 34.0														
MEAN SOLVENT CLONING EFFICIENCY: 84%														
MEAN SOLVENT MUTANT FREQUENCY: 32 (PER 10^6 CELLS)														

Solvent = Ethanol A and B or 1 and 2 are duplicate cultures

*** - Culture lost to dilution error

$$\text{Mutant frequency per } 10^6 \text{ surviving cells} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$\text{Induced mutant frequency per } 10^6 \text{ surviving cells} = \text{mutant frequency} - \text{average mutant frequency of solvent controls}$$

$$\text{Total suspension growth} = \frac{\text{Day 0 cell conc.}}{0.3\text{x}10^6 \text{ cells/mL}} \times \frac{\text{Day 1 cell conc.}}{\text{Day 0 adjusted cell conc.}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

$$\text{\% control cloning growth (not shown)} = \frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$$

$$\text{\% total growth} = \frac{(\text{\% suspension growth})(\text{\% cloning growth})}{100}$$

Figure 1

Initial Assay without Activation, 4-Hour Exposure
Colony Size Distribution in the Absence of Metabolic Activation
(Non-Activated Positive Control and Control for Colony Sizing Compared with Solvent Control)

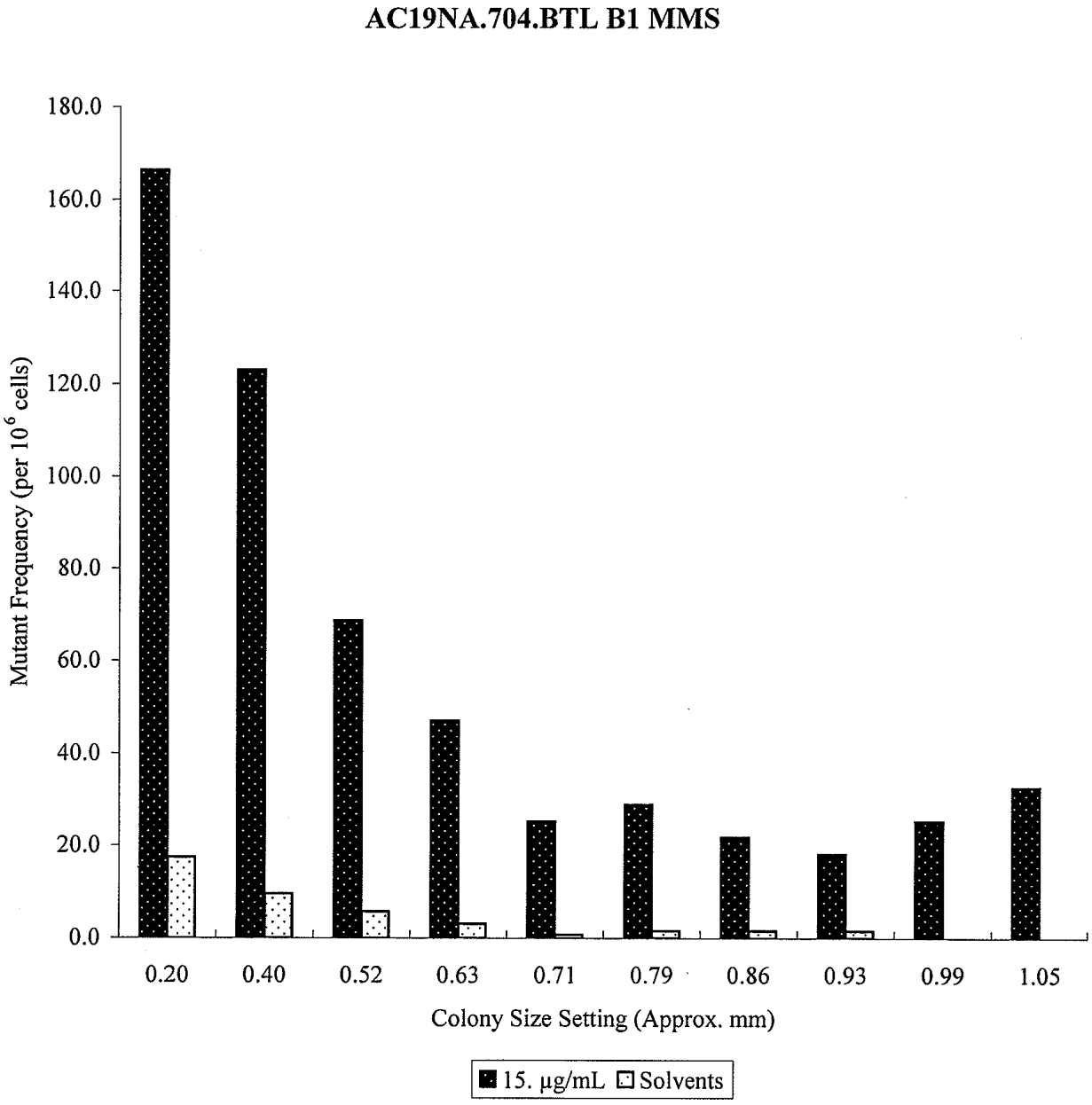


Figure 2

Initial Assay with S9 Activation, 4-Hour Exposure
Colony Size Distribution in the Presence of Metabolic Activation
(Positive Control Compared with Solvent Control)

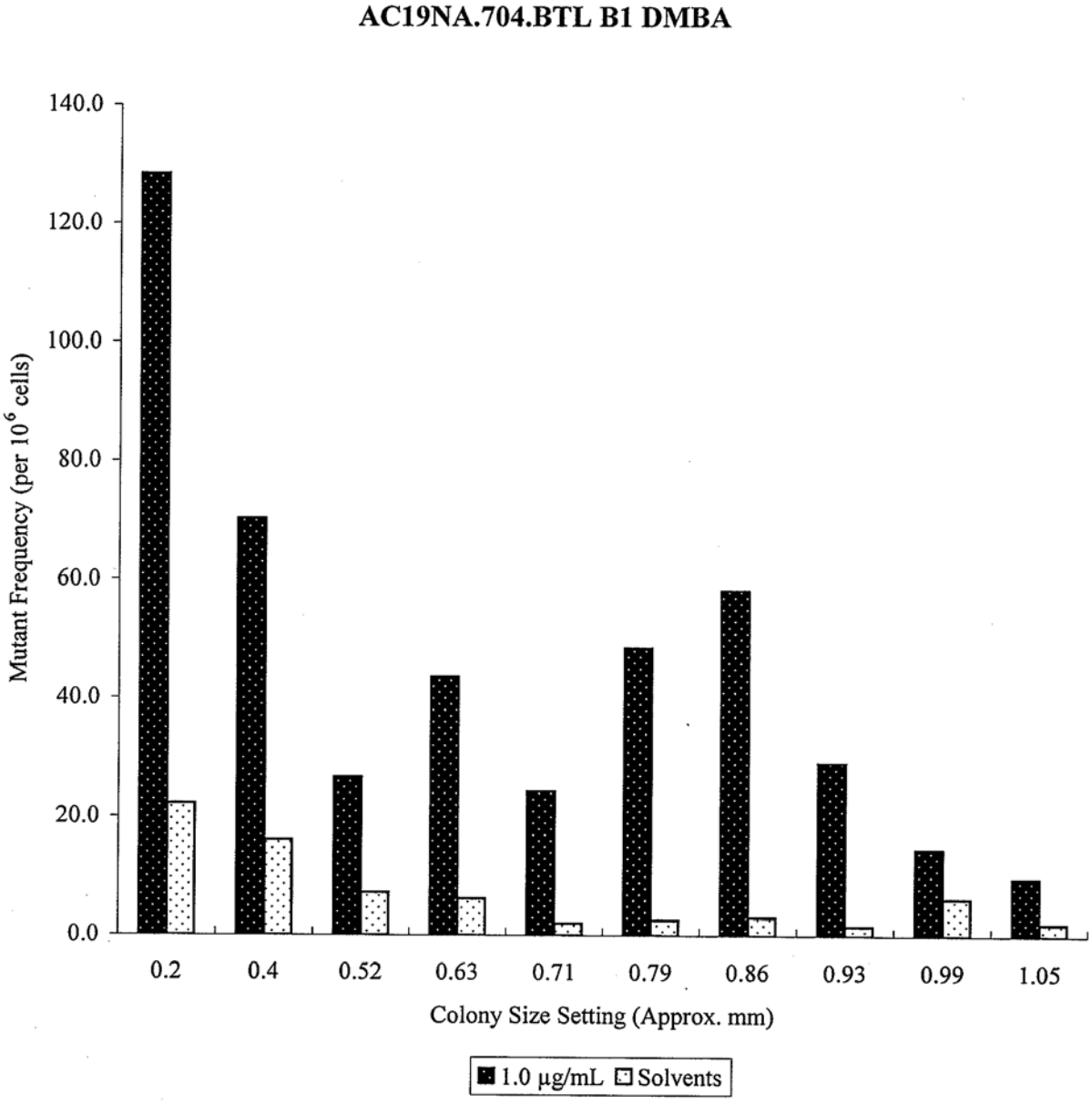
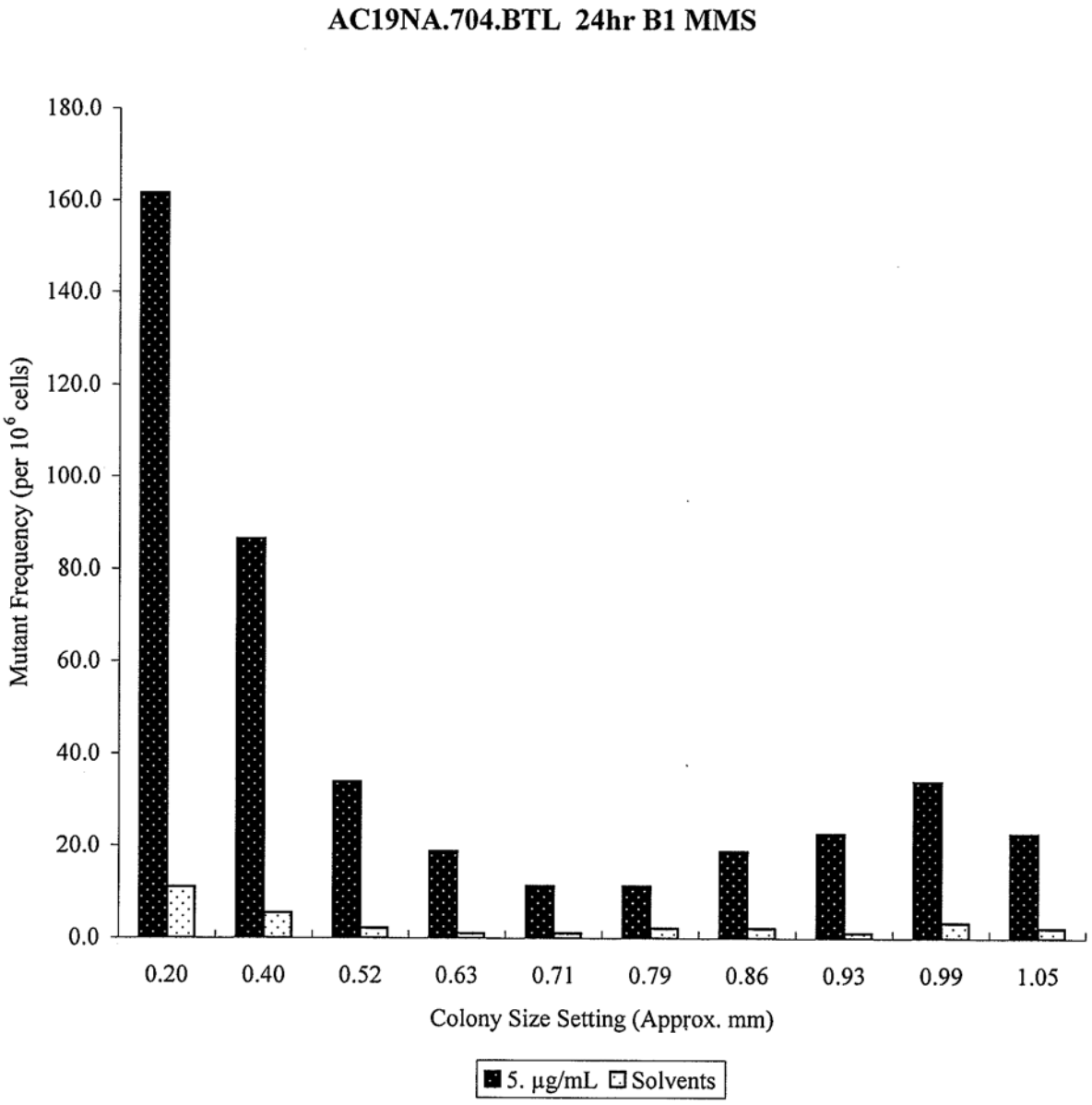


Figure 3

Extended Treatment Assay without Activation, 24-Hour Exposure
Colony Size Distribution in the Absence of Metabolic Activation
(Non-Activated Positive Control and Control for Colony Sizing Compared with Solvent Control)



APPENDIX I:

Study Protocol

QA Reviewed

CRH 01/12/09
Init. Date

Doc CRH 1/12/09

PROTOCOL AMENDMENT 1

Sponsor: **RTI International**

Test Article I.D.: **Fluoroestradiol**

BioReliance Study No.: **AC19NA.704.BTL**

Sponsor Project No.: **211886.001**

Protocol Title: ***In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)**

1. **LOCATION:** Page 1, section 2.3, Representative

AMENDMENT: Change the representative to:
Jay G. Henson, BS
(919) 541-7206
jhenson@rti.org

REASON FOR THE AMENDMENT: Kimberly Ehman has left RTI.

APPROVALS:

Sponsor Representative — 21 Jan 2009 Date

Study Director — 09 Jan 2009 Date

BioReliance Study Management — 09 JAN 2009 Date

QA Reviewed

Init. Date

PROTOCOL AMENDMENT 2

Sponsor: **RTI International**

Test Article I.D.: **Fluoroestradiol**

BioReliance Study No.: **AC19NA.704.BTL**

Sponsor Project No.: **211886.001**

Protocol Title: ***In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)**

1. **LOCATION:** Page 1, section 3.1, Storage Temperature

   AMENDMENT: Change temperature to -5 to -40°C.

   REASON FOR THE AMENDMENT: Error in protocol preparation.

APPROVALS:

Sponsor Representative — 3/11/09 Date

Study Director — 09 Mar 2009 Date

BioReliance Study Management — 09 MAR 2009 Date

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

1.0 PURPOSE

The purpose of this study is to evaluate the genotoxic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells and the sizing of the resulting colonies.

2.0 SPONSOR

2.1	Sponsor Name:	RTI International
2.2	Address:	3040 Cornwallis Rd Research Triangle Park, NC 27709
2.3	Representative:	Kimberly Ehman Phone: 919-316-3802 Fax: 919-541-5956 Email: kehman@rti.org
2.5	Sponsor Project #:	211886.001

3.0 TEST AND CONTROL ARTICLES

3.1	Test Article Name:		Fluoroestradiol
	Storage Temperature:		Ambient, or 4 to 8°C based on the shipping conditions of ambient or cool packs.
	Storage Parameters:		Unless otherwise indicated, all test articles will be stored in the dark and solids will be stored with desiccant.
	Purity:		An adjustment for purity or active ingredient will not be made.
	Molecular Weight:		290.37
3.2	Controls:	Negative:	Test article solvent (or vehicle)
		Positive:	Methyl methanesulfonate (MMS) 7,12-dimethylbenz(a)anthracene (DMBA)

3.3 Characterization and Stability of the Test Article

BioReliance will not perform analysis of the test article. The Sponsor will be directly responsible for determination and documentation of the analytical purity, composition and stability of the test article, and the stability and strength of the test article in the solvent (or vehicle).

3.4 Characterization of Test Article Dose Formulations at the Sponsor's Designated Laboratory

The Sponsor or their designated analytical laboratory has accepted responsibility for characterization of the test article dose formulations. BioReliance will not perform analysis of the test article or dose formulations.

3.4.1 Sampling

Upon preparation for use in the definitive study, the following samples will be collected:

If dose formulations are solutions, 2 x 2.0 mL aliquots of the vehicle and most concentrated dose formulations will be collected for concentration analysis. If necessary, alternate volumes or aliquots may be collected.

If sampled dosing formulations are suspensions, 2 x 2.0 mL aliquots from the top (T), middle (M) and bottom (B) of each test article concentration will be collected for homogeneity analysis in lieu of concentration analysis. In this case, the vehicle will be sampled from the middle portion only. If necessary, alternate volumes or aliquots may be collected. One aliquot of each sample will be sent to the Sponsor's designated analytical laboratory:

Jeanne Link, Ph.D.
Associate Professor of Radiology
Division of Nuclear Medicine
Molecular Imaging Research Box 356004
Room NW041 UWMC
University of Washington
Seattle, WA 98195-6004
Telephone: 206-598-6256
Fax: 206-598-4192
jeanne@u.washington.edu

These samples will be sent on wet ice packs on the day of preparation except as noted below. Samples prepared late in the day or on a day immediately preceding a weekend or holiday will be stored at 2-8°C and shipped on a Monday through Thursday. The second aliquot of each sample will be stored at BioReliance as a backup and will be analyzed only as needed. Unused samples will be discarded following issue of the analytical report.

3.4.2 Dose Formulation Analyses

Upon receipt and prior to analyses, the samples will be kept cold (2-8°C) at the Test Site (analytical laboratory). The samples will be analyzed for concentration and/or homogeneity. The results from samples taken from the middle portion of each concentration will serve as a confirmation of concentration of the formulation.

All analytical work will be conducted by the Analytical Laboratory (Test Site) using a validated method (developed and qualified per Method Number NCI-Q319) and under the direction of the Principal Investigator.

All unused samples will be handled as per the Standard Operating Procedures of the Test Site.

3.4.3 Acceptance Criteria

The acceptable specification for the concentration of the test article in the vehicle will be as follows:

If formulations are solutions:

- 85 to 115% of nominal with <5% relative standard deviation (RSD) of each concentration.

If formulations are suspensions:

- T-M-B samples (each) 80 to 120% of nominal with <10% RSD.

The concentration of the test article in the vehicle formulation must be lower than or equal to the Limit of Quantification of the analytical method.

In the event that a sample is outside of the acceptable specification range, the Study Director will justify the acceptability of the results or suggest re-analysis of the backup samples or retest the affected portion of the study.

In the event that formal stability of the test article in mixtures is not performed and if the analyzed sample was within the protocol specified range of target, the dose formulations will be considered stable for the purpose of this study.

3.4.4 Compliance

The work performed in conjunction with the dose formulation analyses will be conducted in compliance with the study protocol and protocol amendments, appropriate standard operating procedures of the analytical laboratory and GLPs (listed in section 12.0 of this protocol). The work will be subject to a laboratory process audit and the reports will be reviewed by the Analytical Laboratory Quality Assurance Unit (AQAU). All deviations

and AQAU audit findings at the Test Site laboratory will be reported to the Study Director and BioReliance Management.

3.4.5 Reporting

A draft report (PI-phase report) describing the work carried out by the Analytical laboratory will be provided to the BioReliance Study Director. After acceptance of the report, a copy of the final report, including a signed Test Site Quality Assurance Statement, and a Statement of GLP Compliance signed by the PI and Test Site Management will be prepared and submitted to BioReliance for inclusion in the main study final report.

3.4.6 Archiving

All raw data, documentation and reports generated as a result of sample analyses will be retained, archived or return to the Sponsor, as per the contractual agreement between the Sponsor and the Analytical Laboratory.

3.5 Test Article Retention Sample

Since the in-life portion of this study is less than four weeks in duration, BioReliance will not retain a reserve sample of the test article.

3.6 Residual Test Article and Dosing Preparations

Dosing preparations, excluding those saved for concentration or homogeneity analysis, will be disposed of following administration to the test system. Residual test article will be returned to RTI after finalization of the report.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1 Name: Toxicology Testing Facility
BioReliance

4.2 Address: 9630 Medical Center Drive
Rockville, MD 20850

4.3 Study Director: Jane J. Clarke, M.S.
Phone: (301) 610-2219
Fax: (301) 738-2362
E-mail: jane.clarke@bioreliance.com

4.4 Principal Investigator (Dose Formulation Analysis):

Jeanne Link, Ph.D.
Associate Professor of Radiology
Division of Nuclear Medicine
Molecular Imaging Research Box 356004

Room NW041 UWMC
University of Washington
Seattle, WA 98195-6004
Telephone: 206-598-6256
Fax: 206-598-4192
jeanne@u.washington.edu

4.5. Quality Assurance Unit of BioReliance (Lead QA):

Name: Jermaine Sorrell
Phone: 301-610-2257
Fax: 301-738-1036
Email: jermaine.sorrell@bioreliance.com

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 13 January 2009

5.2 Proposed Experimental Completion Date: 23 February 2009

5.3 Proposed Report Date: 09 March 2009

6.0 TEST SYSTEM

L5178Y/TK$^{+/-}$ mouse lymphoma cells are heterozygous at the normally diploid thymidine kinase (TK) locus. L5178Y/TK$^{+/-}$ cells, clone 3.7.2C, were received from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, NC or American Type Culture Collection, Manassas, VA. Each freeze lot of cells has been tested and found to be free of mycoplasma contamination. This system has been demonstrated to be sensitive to the mutagenic activity of a variety of chemicals.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The mammalian mutation assay will be performed by exposing duplicate cultures of L5178Y/TK$^{+/-}$ cells to a minimum of eight concentrations of test article as well as positive and negative (solvent) controls. Exposures will be for 4 hours in the presence and absence of an S9 activation system and 24 hours in the absence of S9 activation, if the extended treatment assay is necessary. Following a two-day expression period, with daily cell population adjustments, cultures demonstrating 0% to the first concentration showing at least 80% growth inhibition will be cloned, in triplicate, in both complete medium and selective medium containing soft agar. After a 10- to 14-day selection period, the colonies will be enumerated. The mutagenic potential of the test article will be measured by its ability to induce TK$^{+/-}$ $\rightarrow$ TK$^{-/-}$ mutations. For those test articles demonstrating a positive response, mutant colonies will be sized as an indication of mechanism of action.

7.1 Selection of Solvent

The Sponsor has indicated the test article vehicle will be ethanol (CAS 64-17-5).

7.2 Concentration Selection

In the preliminary toxicity test, L5178Y/TK$^{+/-}$ cells will be exposed to solvent alone and to at least nine concentrations of test article; the highest concentration targeted will be 8 ng/mL unless limited by workability/solubility of the test article. Higher concentrations may be tested if the ethanol concentration doesn't adversely impact the data. The pH of the treatment medium will be adjusted, if necessary, to maintain a neutral pH in the treatment medium. The osmolality of the highest soluble treatment condition at the beginning of treatment will also be measured. After a 4-hour treatment in the presence and absence of S9 activation, cells will be washed twice with F_0P (Fischer's Media for Leukemic Cells of Mice with 0.1% Pluronic F-68) or $F_{10}P$ (F_0P supplemented with 10% horse serum and 2 mM L-glutamine) and cultured in suspension for two days post-treatment, with cell concentration adjustment on the first day. After a 24-hour treatment in the absence of S9 activation, cells will be washed with F_0P or $F_{10}P$ and immediately readjusted to 3 x 10^5 cells/mL. Cells will then be cultured in suspension for an additional two days post-treatment with cell concentration adjustment on the first day.

Selection of test article concentration levels for the mutation assay will be based on reduction of suspension growth after treatment in the preliminary toxicity test. Unless specified otherwise by the Sponsor, the highest test article concentration for the mutation assay will be that concentration exhibiting approximately 100% growth inhibition, or a target of 8 ng/mL. In all cases, precipitation will be evaluated at the beginning and at the end of the treatment period using the naked eye (ICH, 1996).

7.3 Route and Frequency of Administration

Cell cultures will be treated for 4 hours by way of a vehicle compatible with the system, both in the presence and absence of metabolic activation. This technique of administration has been demonstrated to be effective in the detection of chemical mutagens in this system.

7.4 Exogenous Metabolic Activation

Immediately prior to use, Aroclor 1254-induced rat liver S9 will be thawed and mixed with a cofactor pool to contain 11.25 mg DL-isocitric acid (or 13.88 mg glucose-6-phosphate), 6 mg NADP, and 0.025 mL S9 homogenate per mL in F_0P. The cofactor mix will be adjusted to pH 7 prior to the addition of S9. Each 10 mL culture will contain 4 mL S9 mix (final S9 concentration of 1%).

7.5 Controls

No analyses will be performed on the positive control articles or the positive control dose formulations. The neat positive control articles and the vehicles used to prepare the test article and positive control formulations will be characterized by the Certificates of Analysis provided by the Supplier(s). Copies of the Certificates of Analysis will be kept on file at BioReliance.

7.5.1 Negative Control

The solvent (or vehicle) for the test article will be used as the negative control.

7.5.2 Positive Controls

Results obtained from treatment with these articles will be used to assure responsiveness of the test system but not to provide a standard for comparison with the test article.

Methyl methanesulfonate (MMS) will be used at two concentrations between 1.0 and 20 μg/mL in single cultures as the positive control for the non-activated test system and to determine that the assay is capable of detecting small colonies. For the S9-activated system, 7,12-dimethylbenz(a)anthracene (DMBA) will be used at two or three concentrations between 0.5 and 10 μg/mL in single cultures.

7.6 Preparation of Target Cells

Prior to use in the assay, L5178Y/$TK^{+/-}$ cells will be cleansed to reduce the frequency of spontaneously occurring $TK^{-/-}$ cells. Using the procedure described by Clive and Spector (1975), L5178Y cells will be cultured for 24 hours in the presence of thymidine, hypoxanthine, methotrexate and glycine to poison the $TK^{-/-}$ cells.

L5178Y/$TK^{+/-}$ cells will be prepared in 50% conditioned $F_{10}P$ and 50% F_0P.

7.7 Identification of the Test System

The treatment tubes will be identified by the study number and a code system to designate the treatment condition and test phase.

7.8 Treatment of Target Cells

Treatment will be carried out in conical tubes by combining 100 μL of dosing solution of test or control article in solvent or solvent alone, F_0P medium or S9 activation mixture with 6×10^6 L5178Y/$TK^{+/-}$ cells in a total volume of 10 mL. A minimum of eight concentrations of test article will be tested in duplicate. All pH adjustments will be performed prior to adding S9 or target cells to the treatment medium. Volumes of test article dosing solution in excess of 100 μL may be used if required to achieve the target final concentration in treatment medium. Treatment tubes will be gassed with 5±1% CO_2 in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C. The preparation and addition of the test article dosing solutions will be carried out under amber lighting and the cells will be incubated in the dark during the 4-hour exposure period.

7.9 Expression of the Mutant Phenotype

At the end of the exposure period, the cells will be washed twice with F_0P or $F_{10}P$ and collected by centrifugation. The cells will be resuspended in 20 mL $F_{10}P$, gassed with 5±1% CO_2 in air and cultured in suspension at 37±1°C for two days following treatment. For the 24-hour exposure, the cell population will be adjusted to 3×10^5 cells/mL immediately after test article removal. Cell population adjustments to 3×10^5 cells/mL will be made at 24 and 48 hours post-treatment for the 4-hr treatment cultures and at 24, 48, and 72 hours post-treatment for the 24-hr treatment cultures.

7.10 Selection of the Mutant Phenotype

For selection of the $TK^{-/-}$ (i.e., trifluorothymidine (TFT)-resistant phenotype), cells will be plated into three replicate dishes at a density of 1×10^6 cells/100mm plate in cloning medium containing 0.22% to 0.23% agar and 2-4 µg TFT/mL. For estimation of cloning efficiency at the time of selection, 200 cells/100mm plate will be plated in triplicate in cloning medium free of TFT (viable cell (VC) plate). Plates will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 for 10-14 days.

The total number of colonies per plate will be determined for the VC plates and the total relative growth calculated. The total number of colonies per TFT plate will then be determined for those cultures with ≥10% total growth (including at least one concentration with between 10% and 20% total growth, if possible). Colonies are enumerated using an automatic counter; if the automatic counter cannot be used, the colonies will be counted manually. The diameters of the TFT colonies from the positive control and solvent control cultures will be determined over a range of approximately 0.2 to 1.1 mm. In the event the test article demonstrates a positive response, the diameters of the TFT colonies for at least one concentration level of the test article (the highest positive concentration) will be determined over a range of approximately 0.2 to 1.1 mm.

7.11 Extended Treatment and/or Confirmatory Assay

Verification of a clear positive response will not be required (OECD Guideline 476; ICH, 1997). For equivocal and negative results without activation, an extended treatment assay will be performed in which cultures are continuously exposed to the test article for 24 hours without S9 activation. A preliminary toxicity assay without S9 activation using a 24-hour continuous treatment may be performed (where appropriate) to select concentrations for the extended treatment assay. The extended treatment assay may be performed concurrently with the initial assay. For equivocal results with S9 activation, a confirmatory assay may be performed using modified concentration levels or study design. For negative results with S9 activation, a confirmatory assay will not be required unless the test article is known to have specific requirements of metabolism.

7.12 Electronic Data Collection Systems

The primary computer or electronic systems used for the collection or analysis of data will include but not limited to the following:

LIMS Labware version 5, Excel 2003 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

8.1 Negative Controls

The average spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 35 to 140 TFT-resistant mutants per 10^6 surviving cells. Low spontaneous mutant frequencies, *i.e.*, 20 to 34 mutants per 10^6 surviving cells, are considered acceptable if small colony recovery is demonstrated (Mitchell *et al.*, 1997). The average cloning efficiency of the solvent (or vehicle) controls must be between 65% and 120% and the total suspension growth between 8-32 for the 4-hour exposure and 20 to 180 for the 24-hour exposure (Moore, *et al.*, 2002, 2006, and 2007).

8.2 Positive Controls

The mutant frequency for at least one dose of each positive control must meet the criteria for a positive response. The mutant frequency for at least one dose of one of the positive controls must induce an increase in small colony mutants according to the following criteria: Induced Mutant Frequency (IMF) positive control $\geq 300 \times 10^{-6}$ mutants with 40% small colonies **or** small colony IMF for positive control $\geq 150 \times 10^{-6}$ (Moore, *et al.*, 2002; 2006).

8.3 Test Article-Treated Cultures

Cultures treated with a minimum of four concentrations of test article must be attained and their mutant frequencies reported. The highest test article concentration must produce 80% to 90% toxicity (ICH, 1996) unless limited by solubility or the maximum required concentration as described in section 7.2. In the case of a test article with a steep toxicity curve (no concentrations with 10-20% survival), the results may be considered acceptable if a concentration spacing of ≤ 2-fold is used and the highest concentration tested showed <20% survival or total kill (Sofuni *et al.*, 1997). For example, the test is considered acceptable if the highest concentration cloned for mutant selection exhibits >20% survival and the next highest concentration, which is ≤ 2 times the cloned concentration, is too toxic to clone.

9.0 EVALUATION OF TEST RESULTS

The cytotoxic effects of each treatment condition are expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency for each treatment condition is calculated by dividing the mean number of colonies on the TFT-

plates by the mean number of colonies on the VC-plates and multiplying by the dilution factor (2×10^{-4}), and is expressed as TFT-resistant mutants per 10^6 surviving cells.

In evaluation of the data, increases in mutant frequencies which occur only at highly toxic concentrations (i.e., less than 10% total growth) are not considered biologically relevant. All conclusions will be based on scientific judgment; however, the following criteria are presented as a guide to interpretation of the data (Moore *et al.*, 2006):

- A result will be considered positive if a concentration-related increase in mutant frequency is observed in the treated cultures and one or more treatment conditions with 10% or greater total growth exhibit mutant frequencies of ≥90 mutants per 10^6 clonable cells over the background level (based on the average mutant frequency of duplicate cultures). If the average solvent control mutant frequency is >90 mutants per 10^6 clonable cells, a doubling of mutant frequency over the background will also be required (Mitchell *et al.*, 1997).

- A result will be considered negative if the treated cultures exhibit mutant frequencies of less than 90 mutants per 10^6 clonable cells over the background level (based on the average mutant frequency of duplicate cultures) and there is no concentration-related increase in mutant frequency.

- There are some situations in which a chemical may be considered negative when there is no culture showing between 10-20% survival: 1) There is no evidence of mutagenicity (e.g. no dose response or increase in mutant frequencies between 45 and 89 mutants per 10^6 above control) in a series of data points within 100% to 20% survival *and* there is at least one negative data point between 20% and 25% survival. 2) There is no evidence of mutagenicity (e.g. no dose response or increase in mutant frequencies between 45 and 89 mutants per 10^6 above control) in a series of data points between 100% to 25% survival *and* there is also a negative data point between 10% and 1% survival (Office of Food Additive Safety, 2001. In this case it is acceptable to count the TFT colonies of cultures exhibiting <10% total growth.

10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. Unless alternate arrangements are made, the report will be initially issued as a QA-audited draft. After receipt of the Sponsor's comments a 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 or a designated representative, the draft report will be issued as a final report. If all supporting analytical documents have not been provided to BioReliance, the report will be written based on those that are provided to BioReliance.

The report will include:

- Test substance: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.

- Solvent/vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- Cell type used, number of cultures, methods for maintenance of cell cultures
- Rationale for selection of concentrations and number of cultures
- Test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period, selective agent
- Method used to enumerate numbers of viable and mutant colonies and the number of colonies in each plate
- Concentration-response relationship, if applicable
- Distribution of the mutant colony diameter for the solvent and positive controls and, when the test article induces a positive response, for at least one concentration level of the test article (the highest positive concentration)
- Positive and solvent control historical data
- Statement of Compliance
- Quality Assurance Statement

If an electronic copy of the protocol, the report or another study document is provided by BioReliance, the executed paper document is considered the official master document. If there is a discrepancy between an electronic copy and the corresponding master document, the master document will be considered the official document. Six months after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor or a designated representative, the draft report will be issued as a final report. If all supporting analytical documents have not been provided to BioReliance, the report will be written based on those that are provided to BioReliance.

11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports, generated by BioReliance, will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied onto electronic media and the electronic copy will be retained in the BioReliance archives for a minimum of 10 years.

12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), February 1998, with the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995, Federal Register 61:18198-18202, April 24, 1996, and with the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997, Federal Register 62:16026-16030, November 21, 1997, with the exception that the maximum concentration targeted in treatment medium will be 8 ng/mL at the request of the Sponsor.

The following Good Laboratory Practices (GLP) regulations will be followed at BioReliance as requested by the Sponsor.

- US FDA Good Laboratory Practices 21 CFR Part 58

For the study, an in-process phase, the raw data, and report(s) will be inspected per the Standard Operating Procedures (SOPs) of BioReliance by the Quality Assurance Unit of BioReliance for compliance with GLPs, the SOPs of BioReliance and the study protocol. At least one, study-specific, in-process inspection will be performed for this study. A signed QA Statement will be included in the final report. This statement will list the study-specific phases inspected at BioReliance, the dates of each inspection, and the dates the results of each inspection were reported to the Study Director and the Study Director's management. In addition, a signed GLP Compliance Statement will be included in the final report. This statement will cite the GLP regulations with which this study is compliant and any exceptions to this compliance, if applicable, including the omission of characterization or stability analyses of the test article or its mixtures.

Raw data, the protocol and reports generated at locations other than BioReliance will or will not be QA audited per the contractual arrangements between that site and the Sponsor.

Alterations of this protocol may be made as the study progresses. All protocol procedural modifications and rationale for the change(s) will be documented, signed, dated and approved by the Study Director, BioReliance QA and the Sponsor. All applicable protocol amendments will be delivered to the Sponsor via mail, electronic file transfer or fax transmission, as well as internally at the Test Facility, on or as close as possible to the effective date of the amendment.

Deviations from the protocol and/or BioReliance SOPs will be documented in a deviation report or a note to file will be generated. The deviation report will be signed by the Study Director and BioReliance QA.

13.0 REFERENCES

Aaron, C.S., Bolcsfoldi, G., Glatt, H.-R., Moore, M., Nishi, Y., Stankowski, L., Theiss, J. and Thompson, E. (1994) Mammalian cell gene mutation assays working group report. Mutation Research 312:235-239.

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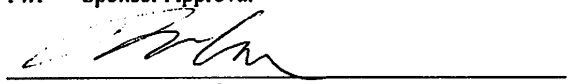
14.0 APPROVAL

14.1 Sponsor Approval



Sponsor Representative

12/22/08
Date

14.2 Study Director and Test Facility Management Approvals

Jane J. Clarke, MS
BioReliance Study Director

09 Jan 2009
Date

BioReliance Study Management

09 JAN 2009
Date

14.3 Analytical Chemist or Principal Investigator Approval

The signature of the Analytical Chemist or Principal Investigator indicates that he or she intends to conduct the delegated phase(s) of this study in accordance with this study protocol, the test site's SOP and the GLP regulations cited in §12.0.

Analytical Chemist or Principal Investigator — Date 3/1/09

APPENDIX II:

Historical Control Data

Mouse Lymphoma Historical Control Data

2006-2008

	Non-Activated (4-Hour)			Non-Activated (24-Hour)		
	Solvent Control	15 µg/mL MMS	20 µg/mL MMS	Solvent Control	5.0 µg/mL MMS	7.5 µg/mL MMS
Mean MF	47.4	440.3	614.6	38.9	337.2	517.9
SD	16.0	119.5	168.6	11.8	76.7	116.8
Maximum	116	906	1030	104	651	914
Minimum	24	65	199	22	192	192

	S9-Activated (4-Hour)			
	Solvent Control	0.75 µg/mL DMBA	1.0 µg/mL DMBA	1.25 µg/mL DMBA
Mean MF	51.6	247.1	322.8	379.5
SD	15.7	41.8	55.7	65.4
Maximum	111	358	504	536
Minimum	21	167	168	234

Solvent control: Fischer's medium, distilled water, saline, DMSO, ethanol, acetone or vehicle supplied by Sponsor. It has been demonstrated that all of the above solvents exhibit the same mutant frequency range.

MMS Methyl methanesulfonate
DMBA Dimethylbenz(a)anthracene
MF Mutant frequency per 10^6 clonable cells
SD Standard deviation

APPENDIX III:

Certificate of Analysis

16alpha-Fluoroestradiol

Product no. 191.XXXX

For research purposes only. Not for human use or consumption.

Product description

16alpha-Fluoroestradiol; synonyms: 16alpha-fluoro-17beta-estradiol, FES; mol. wt. 290.37; $C_{18}H_{23}FO_2$; [92817-10-2]; BRN 3554942; chemical name: estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16alpha, 17beta). Colorless crystals, soluble in acetonitrile and chloroform.

Applications

16alpha-Fluoroestradiol may be used as a reference standard in the radiosynthesis of [^{18}F]Fluoroestradiol.

Presentation

Product 191.XXXX is available in 2 ml dark glass vials (DIN 2R), packed under argon atmosphere. Vials are sealed with teflon-faced rubber stoppers and tear-off crimp caps. Bulk chemicals in quantities ≥ 100 mg are available in dark glass screw cap vials, flushed with argon atmosphere. The content of 16alpha-Fluoroestradiol in mg is defined by the four digit number replacing XXXX in the product number. Weighing error is ± 5 %, but in maximum 0.5 mg.

Storage and stability

Store the product desiccated at –20 ± 5 °C, protected from light. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

Toxicology/Hazards

Handle with care, avoid inhalation, ingestion, eye or skin contact, no toxicological data available.

Certificate of analysis

Lot No.: 260801		Product No.: 191.XXXX	
Parameter	Method	Specification	Result
Appearance	organoleptic	colorless crystals	conforms
Melting pt	capillary	180-210 °C	187.3-188.1 °C
Identity	^{1}H-NMR ^{19}F-NMR	conforms conforms	conforms conforms
Purity	HPLC	> 90 %	> 98 %

No further analytical data available

Manufacturing Date: Aug. 2006

ABX advanced biochemical compounds
Biomedizinische Forschungsreagenzien GmbH

Quality Control date: 09-Nov-06

Dr. B. Schmitt

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereof.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

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3) Mankoff D. A. *et al.:* [^{18}F]Fluoroestradiol Radiation Dosimetry in Human PET Studies. *J. Nucl. Med.* **2001**, *42*, 679-684.

ABX *advanced biochemical compounds* - Biomedizinische Forschungsreagenzien GmbH. -H.-Gläser-Str. 10-14 - D-01454 Radeberg Germany - E-mail: info@abx.de - Internet: http://www.abx.de. - Tel.: +49 / 3528 / 40 41 60 - Fax: +49 / 3528 / 40 41 65

16alpha-Fluoroestradiol

NEW: Product no. 1910.XXXX, OLD: Product no. 191.XXXX Lot. 260801

For research purposes only. Not for human use or consumption.

VALID ONLY IN CONNECTION WITH ORIGINAL CERTIFICATE OF ANALYSIS

Retest certificate of analysis

The following parameters included in the original certificate of analysis have been retested to confirm the stability of the product or are newly introduced in the quality control of the product as they may be considered to be suitable for detection of indicators of decay and guarantee that the product still is in compliance with the original specification:

Lot No.: 260801		Product No.: 191.XXXX	
Retest-Parameter	Method	Retest specification	Result
Appearance	organoleptic	no change in color	conforms
Purity	^{1}H-NMR ^{19}F-NMR	no change in spectrum no change in spectrum	conforms Conforms

Further testing was not considered to be necessary because of absence of significant changes in parameters tested.

Date of retest: **15. Oct. 2008**
Expiry Date: **15. Oct. 2009**

Storage and stability

Store the product desiccated at -20 °C, protected from light. Product is at least one more year stable at -20 °C. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

ABX advanced biochemical compounds **Biomedizinische Forschungsreagenzien GmbH**

Quality Control date: 17-Oct-08

Dr. Bernd Feist

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereoff.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

APPENDIX IV:

Dosing Formulation Certificate of Analysis

University of Washington
PET Radiochemistry

Certificate of Analysis
Certificate No. RC-004

Study Number: AC19NA.704.BTL "In Vitro Mouse Lymphoma Study"

The following samples were analyzed following good laboratory practices following established protocol NCI-Q319 for analysis of fluoroestradiol by HPLC with adaptations for sample matrix and increased concentrations as validated for the FES toxicity study. These measures were made using UV absorbance detection at 280 nm.

Sample No.	Matrix	Date Sample Prepared	Nominal Concentration (µg/mL)	Sample Storage
AC19NA.704.BTLB1 solvent "1"	ethanol	1/27/09	solvent	freezer -10 to -25°C
AC19NA.704.BTLB1 solvent "2"	ethanol	1/27/09	solvent	freezer -10 to -25°C
AC19NA.704.BTLB1 "3"	ethanol	1/27/09	1.6	freezer -10 to -25°C
AC19NA.704.BTLB1 "4"	ethanol	1/27/09	1.6	freezer -10 to -25°C

Sample No.	Date Received	Date Analyzed	Nominal Concentration (µg/mL)	Measured Concentration (µg/mL)
AC19NA.704.BTLB1 solvent "1"	1/28/09 (b)	1/28/09	solvent	ND
AC19NA.704.BTLB1 solvent "2"	1/28/09 (b)	1/28/09	solvent	ND
AC19NA.704.BTLB1 "3"	1/28/09 (b)	1/28/09	1.6	1.9 ± 0.2
AC19NA.704.BTLB1 "4"	1/28/09 (b)	1/28/09	1.6	2.0 ± 0.1

Procedural variations: None .

Analysis performed by: Jeanne Meyers Link DATE: May 4, 2009
Jeanne Meyers Link, PhD
Analytical and Radio-Chemist

Molecular Imaging Center * UW Medical Center, NW045 *
1959 NE Pacific Street, Box 356004 * Seattle, WA 98195-6004

FINAL REPORT

Study Title

Bacterial Reverse Mutation Assay

Test Article

Fluoroestradiol

Authors

Valentine O. Wagner, III, M.S.
Melissa R. VanDyke, B.S.

Study Completion Date

11 June 2009

Testing Facility

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AC19NA.503.BTL

Sponsor Study Number

RTI-1059

Sponsor

RTI International
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

STATEMENT OF COMPLIANCE

Study No. AC19NA.503.BTL was conducted in compliance with the US FDA Good Laboratory Practices 21 CFR Part 58 and the OECD Principles of Good Laboratory Practice (C(97)186/Final) in all material aspects with the following exception:

> Analyses to determine the uniformity or concentration of the test article dosing formulations were performed by Molecular Imaging Research but not in full compliance with the above regulations. The stability of the test article dosing formulations was not determined.

Valentine O. Wagner, III

Valentine O. Wagner, III, M.S.
Study Director

11 Jun 2009
Date

11 JUN 2009
Date

BioReliance Study Management

BioReliance

Quality Assurance Statement

Study Information

Number: AC19NA.503.BTL

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practices 21CFR 58
OECD Principles of Good Laboratory Practices (C(97)186/Final)

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
13-Jan-2009	13-Jan-2009	Administration of Test Article To Test System	13-Jan-2009	13-Jan-2009
02-Mar-2009	02-Mar-2009	Data and Draft Reporting	03-Mar-2009	03-Mar-2009
09-Jun-2009	09-Jun-2009	Final Reporting	09-Jun-2009	09-Jun-2009

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

E-signature

Quality Assurance: Allison Schaefer 11-Jun-2009 8:21 pm GMT

Reason for signature: QA Approval

Printed by:Allison Schaefer

Printed on:11-Jun-09

Bacterial Reverse Mutation Assay

STUDY INFORMATION

Sponsor:	**RTI International** **3040 Cornwallis Rd.** **Research Triangle Park, NC 27709**
Authorized Representative:	**Jay G. Henson, B.S.**
Testing Facility:	**BioReliance** **9630 Medical Center Drive** **Rockville, Maryland 20850**
Test Article I.D.:	**Fluoroestradiol**
Test Article Lot No.:	**260801**
Test Article CAS No.:	**92817-10-2**
Test Article Purity:	**> 98% (by HPLC, per Certificate of Analysis)**
BioReliance Study No.:	**AC19NA.503.BTL**
Sponsor Study Number:	**RTI-1059**
Test Article Description:	**colorless crystals**
Storage Conditions:	**-15 to -40°C, stored under argon in the dark with desiccant**
Test Article Receipt and Login:	**24 October 2008**
Study Initiation:	**09 January 2009**
Experimental Start Date:	**13 January 2009**
Experimental Completion Date:	**05 February 2009**
Laboratory Manager:	**Emily W. Dakoulas, B.S.**
Analytical Chemistry:	**Jeanne Link, Ph.D., Principal Investigator** **Molecular Imaging Research** **Room NW041 UWMC** **Box 356004** **University of Washington** **Seattle, WA 98195-6004**

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SUMMARY

The test article, Fluoroestradiol, was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article.

Ethanol (EtOH) was selected as the solvent of choice based on the request of the Sponsor and compatibility with the target cells.

In the initial toxicity-mutation assay, the maximum dose tested was 1.25 µg per plate; this dose was achieved using a concentration of 0.025 mg/mL and a 50 µL plating aliquot. The dose levels tested were 0.00050, 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50 and 1.25 µg per plate. The test article formed soluble and clear solutions in ethanol from 0.000010 to 0.025 mg/mL. In the initial toxicity-mutation assay, no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 1.25 µg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed. The dose levels tested were 0.015, 0.050, 0.15, 0.50 and 1.25 µg per plate. Neither precipitate nor appreciable toxicity was observed.

Under the conditions of this study, test article Fluoroestradiol was concluded to be negative in the Bacterial Reverse Mutation Assay.

PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9. A copy of the Historical Negative and Positive Control Values is included in Appendix I. A copy of the study protocol, and all amendments, if any, is included in Appendix II.

This study was conducted in compliance with the testing guidelines of the ICH (1996 and 1997) and OECD (1998) with the exception that the maximum concentration tested was 1.25 µg per plate at the request of the Sponsor.

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, Fluoroestradiol, was received by BioReliance on 24 October 2008 and was assigned the code number AC19NA. The test article was characterized as colorless crystals on the Certificate of Analysis. Per the protocol and Test Article Submission Form submitted by the Sponsor, the test article should be stored at -5 to -40°C under argon in the dark with desiccant. An expiration date of 15 October 2009 was provided on the test article sample label. Upon receipt, the test article was described as colorless crystals and was stored at -15 to -40°C under argon in the dark with desiccant.

ABX advanced biochemical compounds (Radeberg, Germany) has determined the identity, strength, purity and composition or other characteristics to define the test article and the stability of the test article. A copy of the Certificate of Analysis is included in Appendix IV. Based on the expiration date provided on the Certificate of Analysis, the test article was considered stable for the purposes of this study through 15 October 2009 when stored desiccated at -20°C, protected from light.

The vehicle used to deliver Fluoroestradiol to the test system was ethanol (EtOH, CAS No. 64-17-5, Lot No. B0514580, Exp. Date: March 2011), obtained from Acrōs Organics. A 1.0 mg/mL stock concentration in ethanol was prepared and provided by the Mouse Lymphoma Laboratory at BioReliance on the day of dosing. The stock formulation was stored at 2-8°C until ready for use and was, then, allowed to come to room temperature and mixed to homogeneity prior to subsequent dilution. Next, the 1.0 mg/mL concentration was diluted to a concentration of 0.025 mg/mL for use as the top dose. Subsequent test article dilutions were prepared from the 0.025 mg/mL concentration immediately before use and delivered to the test system at room temperature under yellow light.

Duplicate samples of dosing formulations (0.50 mL from the high dose and the vehicle) were collected from each assay. One set of samples was shipped on dry ice to Molecular Imaging Research (Seattle, WA) for analysis. Samples not shipped on the day of preparation were

stored at -15 to -40°C until shipment. The second set of samples was retained at -15 to -40°C at BioReliance as a backup and was discarded upon receipt of the final analytical report. A copy of the Dosing Formulation Certificate of Analysis is included in Appendix V.

The negative and positive control articles have been characterized as per the Certificates of Analysis on file with the testing facility. The stability of the negative and positive control articles and their mixtures was demonstrated by acceptable results that met the criteria for a valid test.

Positive controls plated concurrently with the initial toxicity-mutation assay and the confirmatory mutagenicity assay are listed in the following table. All positive controls were diluted in dimethyl sulfoxide (DMSO) except for sodium azide, which was diluted in water. All subdivided solutions of positive control were stored at -15 to -40°C.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535, TA1537	Rat	2-aminoanthracene (Aldrich Chemical Co., Inc.) Lot No. 12317CE Exp. Date 01-Feb-2009 CAS No. 613-13-8 Purity 99.9%	1.0
TA100			2.0
WP2 *uvr*A			10

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	None	2-nitrofluorene (Aldrich Chemical Co., Inc.) Lot No. 03926DC Exp. Date 18-Aug-2010 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Alfa Aesar) Lot No. G24R025 Exp. Date 10-Feb-2010 CAS No. 26628-22-8 Purity 99% min.	1.0
TA1537		9-aminoacridine (Sigma Chemical Co.) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (Aldrich Chemical Co., Inc.) Lot No. 126K3721 Exp. Date 07-Jan-2011 CAS No. 66-27-3 Purity 99.9%	1,000

To confirm the sterility of the test article, the highest test article dose levels used in the initial toxicity-mutation and confirmatory mutagenicity assays were plated on selective agar with an aliquot volume equal to that used in the assay. These plates were incubated under the same conditions as the assay.

MATERIALS AND METHODS

For submission to Japanese regulatory agencies, additional information is included in Appendix III.

Test System

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al.* (1975) and *Escherichia coli* WP2 *uvr*A as described by Green and Muriel (1976). *Salmonella* tester strains were received from Dr. Bruce Ames' designated distributor, Discovery Partners International, San Diego,

California. *E. coli* tester strains were received from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester strain TA1535 is reverted by mutagens that cause basepair substitutions. Tester strain TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations. Specificity of the reversion mechanism in *E. coli* is sensitive to basepair substitution mutations, rather than frameshift mutations (Green and Muriel, 1976).

Overnight cultures were prepared by inoculating from the appropriate master plate or from the appropriate frozen permanent stock into a vessel containing ~50 mL of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a resting shaker/incubator at room temperature. The shaker/incubator was programmed to begin shaking at approximately 125 rpm at 37±2°C approximately 12 hours before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of approximately 109 cells per milliliter. The actual titers were determined by viable count assays on nutrient agar plates.

Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The lot of S9 was prepared by and purchased from MolTox (Boone, NC). Upon arrival at BioReliance, the S9 was stored at -60°C or colder until used. Each bulk preparation of S9 was assayed for its ability to metabolize at least two promutagens to forms mutagenic to *Salmonella typhimurium* TA100.

The S9 mix was prepared immediately before its use and contained 10% S9, 5 mM glucose-6-phosphate, 4 mM ß-nicotinamide-adenine dinucleotide phosphate, 8 mM MgCl2 and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4. The Sham S9 mixture (Sham mix), containing 100 mM phosphate buffer at pH 7.4, was prepared immediately before its use. To confirm the sterility of the S9 and Sham mixes, a 0.5 mL aliquot of each was plated on selective agar.

Solubility

Ethanol (EtOH) was selected as the solvent of choice based on the request of the Sponsor and compatibility with the target cells. The Sponsor indicated that the test article is soluble in 15% ethanol in PBS at a maximum concentration of 25 µg/mL and anticipated that higher percentages of ethanol would result in higher soluble concentrations.

Initial Toxicity-Mutation Assay

The initial toxicity-mutation assay was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. Vehicle control, positive controls and eight dose levels of the test article were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9.

Confirmatory Mutagenicity Assay

The confirmatory mutagenicity assay was used to evaluate and confirm the mutagenic potential of the test article. Five dose levels of test article along with appropriate vehicle control and positive controls were plated with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvr*A on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. All dose levels of test article, vehicle control and positive controls were plated in triplicate.

Plating and Scoring Procedures

The test system was exposed to the test article via the plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983).

On the day of its use, minimal top agar, containing 0.8 % agar (W/V) and 0.5 % NaCl (W/V), was melted and supplemented with L-histidine, D-biotin and L-tryptophan solution to a final concentration of 50 µM each. Top agar not used with S9 or Sham mix was supplemented with 25 mL of water for each 100 mL of minimal top agar. For the preparation of media and reagents, all references to water imply sterile, deionized water produced by the Milli-Q Reagent Water System. Bottom agar was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) containing 1.5 % (W/V) agar. Nutrient bottom agar was Vogel-Bonner minimal medium E containing 1.5 % (W/V) agar and supplemented with 2.5 % (W/V) Oxoid Nutrient Broth No. 2 (dry powder). Nutrient Broth was Vogel-Bonner salt solution supplemented with 2.5 % (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

Each plate was labeled with a code system that identified the test article, test phase, dose level, tester strain and activation, as described in detail in BioReliance's Standard Operating Procedures.

One-half (0.5) milliliter of S9 or Sham mix, 100 µL of tester strain (cells seeded) and 50 µL of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45±2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test article aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated after the incubation period by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the vehicle control plate using the codes shown in the following table.

Code	Description	Characteristics
1	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies compared to the vehicle control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn over greater than or equal to 90% of the plate.
6	Obscured by Particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., greater than 3 particles on a plate with 30 revertants). These plates are counted manually.

Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

Evaluation of Results

For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated and are reported.

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article.

Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

Criteria for a Valid Test

The following criteria must be met for the initial toxicity-mutation and the confirmatory mutagenicity assays to be considered valid. All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/mL. The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

Automated Data Collection Systems

The primary computer or electronic systems used for the collection of data or analysis included but were not limited to the following:

Minicount Colony Counter (Imaging Products International), LIMS System (BioReliance), Excel 2003 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

Archives

All raw data, the protocol and all reports, generated by BioReliance, will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the

Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied onto electronic media and the electronic copy will be retained in the BioReliance archives for a minimum of 10 years.

Deviation

No known deviations from the protocol or assay-method SOPs occurred during the conduct of this study.

RESULTS AND DISCUSSION

Solubility

Ethanol (EtOH) was selected as the solvent of choice based on the request of the Sponsor and compatibility with the target cells.

Sterility Results

No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions and the S9 and Sham mixes.

Tester Strain Titer Results

	Tester Strain				
	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
	Titer Value (x 10^9 cells per mL)				
Experiment B1	1.8	1.4	1.7	1.1	4.4
Experiment B2	1.2	1.0	1.1	0.6	2.7

Initial Toxicity-Mutation Assay

The results of the initial toxicity-mutation assay are presented in Tables 1 through 10 and summarized in Table 21. These data were generated in Experiment B1. In the initial toxicity-mutation assay, the maximum dose tested was 1.25 µg per plate; this dose was achieved using a concentration of 0.025 mg/mL and a 50 µL plating aliquot. The dose levels tested were 0.00050, 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50 and 1.25 µg per plate. The test article formed soluble and clear solutions in ethanol from 0.000010 to 0.025 mg/mL. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 1.25 µg per plate.

In Experiment B1 (Initial Toxicity-Mutation Assay), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

Confirmatory Mutagenicity Assay

The results of the confirmatory mutagenicity assay are presented in Tables 11 through 20 and summarized in Table 22. These data were generated in Experiment B2. The dose levels tested were 0.015, 0.050, 0.15, 0.50 and 1.25 µg per plate. Neither precipitate nor appreciable toxicity was observed.

In Experiment B2 (Confirmatory Mutagenicity Assay), no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

Dosing Formulation Analysis

Dosing formulations were shipped to Molecular Imaging Research (Seattle, WA) for analysis. A copy of the Dosing Formulation Certificate of Analysis is included in Appendix V. Concentration analysis indicates that the actual concentrations of the most concentrated dosing formulations (25 µg/mL) were 30 and 32 µg/mL. No test article was detected in the vehicle control samples. The concentration analysis indicates that the actual concentrations of the targeted dose level were higher than expected (20 and 28% higher than nominal, respectively) and, therefore, do not meet the acceptance criteria of ±15% of the target concentration. Since the regulatory-required top dose level was exceeded in each case, the Study Director has concluded that the analytical results for the dosing formulations and the observed differences from nominal concentrations did not adversely impact the integrity of the data or the validity of the study conclusion. The test results were considered valid since the actual concentrations of the targeted dose level were higher than intended, and the assay was actually more stringent than anticipated.

CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, Fluoroestradiol did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9.

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DATA TABLES

Bacterial Mutation Assay
Table 1

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA98	Experiment No	:	B1
Activation Condition	:	None	Cells Seeded	:	1.8 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	22	1		
	2	26	1	24	3
0.00050	1	23	1		
	2	20	1	22	2
0.0015	1	22	1		
	2	17	1	20	4
0.0050	1	13	1		
	2	16	1	15	2
0.015	1	23	1		
	2	17	1	20	4
0.050	1	24	1		
	2	23	1	24	1
0.15	1	27	1		
	2	22	1	25	4
0.50	1	21	1		
	2	32	1	27	8
1.25	1	25	1		
	2	21	1	23	3
Positive Control	2-nitrofluorene 1.0 µg per plate				
	1	149	1		
	2	137	1	143	8

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 2

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA98	Experiment No	:	B1
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.8 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	31	1		
	2	33	1	32	1
0.00050	1	37	1		
	2	34	1	36	2
0.0015	1	24	1		
	2	34	1	29	7
0.0050	1	28	1		
	2	31	1	30	2
0.015	1	24	1		
	2	28	1	26	3
0.050	1	33	1		
	2	30	1	32	2
0.15	1	29	1		
	2	22	1	26	5
0.50	1	39	1		
	2	27	1	33	8
1.25	1	42	1		
	2	32	1	37	7
Positive Control	2-aminoanthracene 1.0 µg per plate				
	1	1608	1		
	2	1053	1	1331	392

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 3

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA100	Experiment No	:	B1
Activation Condition	:	None	Cells Seeded	:	1.4 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	184	1		
	2	182	1	183	1
0.00050	1	165	1		
	2	172	1	169	5
0.0015	1	150	1		
	2	162	1	156	8
0.0050	1	173	1		
	2	173	1	173	0
0.015	1	168	1		
	2	177	1	173	6
0.050	1	147	1		
	2	172	1	160	18
0.15	1	173	1		
	2	147	1	160	18
0.50	1	190	1		
	2	160	1	175	21
1.25	1	186	1		
	2	190	1	188	3
Positive Control	sodium azide 1.0 µg per plate				
	1	597	1		
	2	619	1	608	16

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 4

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA100	Experiment No	:	B1
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.4 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	177	1		
	2	183	1	180	4
0.00050	1	174	1		
	2	184	1	179	7
0.0015	1	153	1		
	2	178	1	166	18
0.0050	1	200	1		
	2	180	1	190	14
0.015	1	189	1		
	2	181	1	185	6
0.050	1	186	1		
	2	187	1	187	1
0.15	1	176	1		
	2	178	1	177	1
0.50	1	199	1		
	2	182	1	191	12
1.25	1	173	1		
	2	207	1	190	24
Positive Control	2-aminoanthracene 2.0 µg per plate				
	1	1324	1		
	2	1187	1	1256	97

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 5

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1535	Experiment No	:	B1
Activation Condition	:	None	Cells Seeded	:	1.7 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	14	1		
	2	14	1	14	0
0.00050	1	14	1		
	2	16	1	15	1
0.0015	1	13	1		
	2	18	1	16	4
0.0050	1	18	1		
	2	9	1	14	6
0.015	1	16	1		
	2	13	1	15	2
0.050	1	14	1		
	2	11	1	13	2
0.15	1	13	1		
	2	14	1	14	1
0.50	1	13	1		
	2	17	1	15	3
1.25	1	14	1		
	2	12	1	13	1
Positive Control	sodium azide 1.0 µg per plate				
	1	324	1		
	2	401	1	363	54

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 6

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1535	Experiment No	:	B1
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.7 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	15	1		
	2	11	1	13	3
0.00050	1	11	1		
	2	16	1	14	4
0.0015	1	19	1		
	2	14	1	17	4
0.0050	1	16	1		
	2	8	1	12	6
0.015	1	27	1		
	2	18	1	23	6
0.050	1	25	1		
	2	13	1	19	8
0.15	1	12	1		
	2	21	1	17	6
0.50	1	17	1		
	2	21	1	19	3
1.25	1	11	1		
	2	14	1	13	2
Positive Control	2-aminoanthracene 1.0 µg per plate				
	1	129	1		
	2	107	1	118	16

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 7

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1537	Experiment No	:	B1
Activation Condition	:	None	Cells Seeded	:	1.1 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	7	1		
	2	3	1	5	3
0.00050	1	4	1		
	2	4	1	4	0
0.0015	1	7	1		
	2	6	1	7	1
0.0050	1	9	1		
	2	5	1	7	3
0.015	1	8	1		
	2	4	1	6	3
0.050	1	4	1		
	2	12	1	8	6
0.15	1	7	1		
	2	4	1	6	2
0.50	1	1	1		
	2	6	1	4	4
1.25	1	10	1		
	2	9	1	10	1
Positive Control	9-aminoacridine 75 µg per plate				
	1	2057	1		
	2	1449	1	1753	430

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 8

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1537	Experiment No	:	B1
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.1 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	7	1		
	2	6	1	7	1
0.00050	1	6	1		
	2	9	1	8	2
0.0015	1	7	1		
	2	7	1	7	0
0.0050	1	6	1		
	2	4	1	5	1
0.015	1	7	1		
	2	6	1	7	1
0.050	1	6	1		
	2	4	1	5	1
0.15	1	3	1		
	2	8	1	6	4
0.50	1	5	1		
	2	3	1	4	1
1.25	1	1	1		
	2	10	1	6	6
Positive Control	2-aminoanthracene 1.0 µg per plate				
	1	110	1		
	2	112	1	111	1

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 9

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	WP2 *uvr*A	Experiment No	:	B1
Activation Condition	:	None	Cells Seeded	:	4.4 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	30	1		
	2	43	1	37	9
0.00050	1	44	1		
	2	44	1	44	0
0.0015	1	39	1		
	2	35	1	37	3
0.0050	1	30	1		
	2	36	1	33	4
0.015	1	49	1		
	2	52	1	51	2
0.050	1	43	1		
	2	41	1	42	1
0.15	1	37	1		
	2	35	1	36	1
0.50	1	56	1		
	2	38	1	47	13
1.25	1	46	1		
	2	38	1	42	6
Positive Control	methyl methanesulfonate 1000 µg per plate				
	1	380	1		
	2	446	1	413	47

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 10

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	WP2 *uvr*A	Experiment No	:	B1
Activation Condition	:	Rat Liver S9	Cells Seeded	:	4.4 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	13-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	48	1		
	2	66	1	57	13
0.00050	1	50	1		
	2	52	1	51	1
0.0015	1	52	1		
	2	45	1	49	5
0.0050	1	60	1		
	2	51	1	56	6
0.015	1	54	1		
	2	54	1	54	0
0.050	1	45	1		
	2	54	1	50	6
0.15	1	58	1		
	2	55	1	57	2
0.50	1	55	1		
	2	56	1	56	1
1.25	1	51	1		
	2	54	1	53	2
Positive Control	2-aminoanthracene 10 µg per plate				
	1	449	1		
	2	517	1	483	48

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 11

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA98	Experiment No	:	B2
Activation Condition	:	None	Cells Seeded	:	1.2 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	29	1		
	2	30	1		
	3	28	1	29	1
0.015	1	31	1		
	2	33	1		
	3	35	1	33	2
0.050	1	31	1		
	2	26	1		
	3	32	1	30	3
0.15	1	23	1		
	2	32	1		
	3	33	1	29	6
0.50	1	34	1		
	2	35	1		
	3	30	1	33	3
1.25	1	33	1		
	2	26	1		
	3	27	1	29	4
Positive Control	2-nitrofluorene 1.0 µg per plate				
	1	389	1		
	2	338	1		
	3	339	1	355	29

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 12

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA98	Experiment No	:	B2
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.2 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	29	1		
	2	43	1		
	3	34	1	35	7
0.015	1	38	1		
	2	40	1		
	3	43	1	40	3
0.050	1	42	1		
	2	34	1		
	3	34	1	37	5
0.15	1	47	1		
	2	31	1		
	3	41	1	40	8
0.50	1	42	1		
	2	34	1		
	3	30	1	35	6
1.25	1	32	1		
	2	33	1		
	3	42	1	36	6
Positive Control	2-aminoanthracene 1.0 µg per plate				
	1	492	1		
	2	496	1		
	3	476	1	488	11

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 13

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA100	Experiment No	:	B2
Activation Condition	:	None	Cells Seeded	:	1.0 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	180	1		
	2	180	1		
	3	186	1	182	3
0.015	1	157	1		
	2	163	1		
	3	168	1	163	6
0.050	1	192	1		
	2	171	1		
	3	154	1	172	19
0.15	1	159	1		
	2	143	1		
	3	144	1	149	9
0.50	1	159	1		
	2	169	1		
	3	143	1	157	13
1.25	1	164	1		
	2	180	1		
	3	172	1	172	8
Positive Control	sodium azide 1.0 µg per plate				
	1	670	1		
	2	725	1		
	3	751	1	715	41

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 14

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA100	Experiment No	:	B2
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.0 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	160	1		
	2	158	1		
	3	167	1	162	5
0.015	1	165	1		
	2	146	1		
	3	167	1	159	12
0.050	1	163	1		
	2	176	1		
	3	144	1	161	16
0.15	1	171	1		
	2	169	1		
	3	177	1	172	4
0.50	1	157	1		
	2	165	1		
	3	167	1	163	5
1.25	1	178	1		
	2	158	1		
	3	144	1	160	17
Positive Control	2-aminoanthracene 2.0 µg per plate				
	1	1831	1		
	2	C			
	3	1714	1	1773	83

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate
C=Contaminated

Bacterial Mutation Assay
Table 15

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1535	Experiment No	:	B2
Activation Condition	:	None	Cells Seeded	:	1.1 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	21	1		
	2	15	1		
	3	16	1	17	3
0.015	1	18	1		
	2	21	1		
	3	26	1	22	4
0.050	1	21	1		
	2	14	1		
	3	20	1	18	4
0.15	1	21	1		
	2	23	1		
	3	27	1	24	3
0.50	1	18	1		
	2	14	1		
	3	22	1	18	4
1.25	1	17	1		
	2	23	1		
	3	14	1	18	5
Positive Control	sodium azide 1.0 µg per plate				
	1	519	1		
	2	552	1		
	3	525	1	532	18

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 16

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1535	Experiment No	:	B2
Activation Condition	:	Rat Liver S9	Cells Seeded	:	1.1 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	19	1		
	2	21	1		
	3	16	1	19	3
0.015	1	23	1		
	2	20	1		
	3	22	1	22	2
0.050	1	19	1		
	2	15	1		
	3	17	1	17	2
0.15	1	21	1		
	2	18	1		
	3	14	1	18	4
0.50	1	17	1		
	2	13	1		
	3	19	1	16	3
1.25	1	15	1		
	2	27	1		
	3	C		21	8
Positive Control	2-aminoanthracene 1.0 µg per plate				
	1	164	1		
	2	167	1		
	3	154	1	162	7

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate
C=Contaminated

Bacterial Mutation Assay
Table 17

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1537	Experiment No	:	B2
Activation Condition	:	None	Cells Seeded	:	0.6 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	10	1		
	2	9	1		
	3	11	1	10	1
0.015	1	8	1		
	2	8	1		
	3	7	1	8	1
0.050	1	11	1		
	2	12	1		
	3	12	1	12	1
0.15	1	7	1		
	2	7	1		
	3	13	1	9	3
0.50	1	10	1		
	2	10	1		
	3	8	1	9	1
1.25	1	8	1		
	2	8	1		
	3	7	1	8	1
Positive Control	9-aminoacridine 75 µg per plate				
	1	1948	1		
	2	1852	1		
	3	1631	1	1810	163

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 18

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	TA1537	Experiment No	:	B2
Activation Condition	:	Rat Liver S9	Cells Seeded	:	0.6 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	10	1		
	2	5	1		
	3	7	1	7	3
0.015	1	5	1		
	2	6	1		
	3	4	1	5	1
0.050	1	5	1		
	2	10	1		
	3	9	1	8	3
0.15	1	7	1		
	2	4	1		
	3	8	1	6	2
0.50	1	9	1		
	2	8	1		
	3	10	1	9	1
1.25	1	8	1		
	2	7	1		
	3	6	1	7	1
Positive Control	2-aminoanthracene 1.0 µg per plate				
	1	79	1		
	2	97	1		
	3	79	1	85	10

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 19

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	WP2 *uvr*A	Experiment No	:	B2
Activation Condition	:	None	Cells Seeded	:	2.7 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	44	1		
	2	47	1		
	3	57	1	49	7
0.015	1	48	1		
	2	47	1		
	3	57	1	51	6
0.050	1	42	1		
	2	39	1		
	3	47	1	43	4
0.15	1	45	1		
	2	45	1		
	3	56	1	49	6
0.50	1	48	1		
	2	53	1		
	3	49	1	50	3
1.25	1	48	1		
	2	41	1		
	3	42	1	44	4
Positive Control	methyl methanesulfonate 1000 µg per plate				
	1	459	1		
	2	369	1		
	3	481	1	436	59

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Table 20

Test Article Id	:	Fluoroestradiol			
Study Number	:	AC19NA.503.BTL			
Strain	:	WP2 *uvr*A	Experiment No	:	B2
Activation Condition	:	Rat Liver S9	Cells Seeded	:	2.7 x 10^8
Vehicle	:	EtOH			
Plating Aliquot	:	50 µL	Date Plated	:	27-Jan-09

Concentration µg per plate	Plate Number	Revertants per plate	Background Code	Average Revertants	Standard Deviation
Vehicle	1	53	1		
	2	55	1		
	3	57	1	55	2
0.015	1	72	1		
	2	53	1		
	3	54	1	60	11
0.050	1	65	1		
	2	49	1		
	3	49	1	54	9
0.15	1	47	1		
	2	47	1		
	3	51	1	48	2
0.50	1	58	1		
	2	42	1		
	3	57	1	52	9
1.25	1	55	1		
	2	51	1		
	3	38	1	48	9
Positive Control	2-aminoanthracene 10 µg per plate				
	1	430	1		
	2	410	1		
	3	491	1	444	42

Background Lawn Code
1=Normal; 2=Slightly reduced; 3=Moderately reduced; 4=Extremely reduced; 5=Absent
6=Obscured by particulate; NP=Non-Interfering precipitate; IP=Interfering precipitate

Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay
Table 21

Test Article Id : Fluoroestradiol
Study Number : AC19NA.503.BTL
Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	24 ± 3	183 ± 1	14 ± 0	5 ± 3	37 ± 9
0.00050	22 ± 2	169 ± 5	15 ± 1	4 ± 0	44 ± 0
0.0015	20 ± 4	156 ± 8	16 ± 4	7 ± 1	37 ± 3
0.0050	15 ± 2	173 ± 0	14 ± 6	7 ± 3	33 ± 4
0.015	20 ± 4	173 ± 6	15 ± 2	6 ± 3	51 ± 2
0.050	24 ± 1	160 ± 18	13 ± 2	8 ± 6	42 ± 1
0.15	25 ± 4	160 ± 18	14 ± 1	6 ± 2	36 ± 1
0.50	27 ± 8	175 ± 21	15 ± 3	4 ± 4	47 ± 13
1.25	23 ± 3	188 ± 3	13 ± 1	10 ± 1	42 ± 6
Positive	143 ± 8	608 ± 16	363 ± 54	1753 ± 430	413 ± 47

Activation Condition : Rat Liver S9

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	32 ± 1	180 ± 4	13 ± 3	7 ± 1	57 ± 13
0.00050	36 ± 2	179 ± 7	14 ± 4	8 ± 2	51 ± 1
0.0015	29 ± 7	166 ± 18	17 ± 4	7 ± 0	49 ± 5
0.0050	30 ± 2	190 ± 14	12 ± 6	5 ± 1	56 ± 6
0.015	26 ± 3	185 ± 6	23 ± 6	7 ± 1	54 ± 0
0.050	32 ± 2	187 ± 1	19 ± 8	5 ± 1	50 ± 6
0.15	26 ± 5	177 ± 1	17 ± 6	6 ± 4	57 ± 2
0.50	33 ± 8	191 ± 12	19 ± 3	4 ± 1	56 ± 1
1.25	37 ± 7	190 ± 24	13 ± 2	6 ± 6	53 ± 2
Positive	1331 ± 392	1256 ± 97	118 ± 16	111 ± 1	483 ± 48

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay
Table 22

Test Article Id : Fluoroestradiol
Study Number : AC19NA.503.BTL
Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	29 ± 1	182 ± 3	17 ± 3	10 ± 1	49 ± 7
0.015	33 ± 2	163 ± 6	22 ± 4	8 ± 1	51 ± 6
0.050	30 ± 3	172 ± 19	18 ± 4	12 ± 1	43 ± 4
0.15	29 ± 6	149 ± 9	24 ± 3	9 ± 3	49 ± 6
0.50	33 ± 3	157 ± 13	18 ± 4	9 ± 1	50 ± 3
1.25	29 ± 4	172 ± 8	18 ± 5	8 ± 1	44 ± 4
Positive	355 ± 29	715 ± 41	532 ± 18	1810 ± 163	436 ± 59

Activation Condition : Rat Liver S9

Dose (µg per plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	35 ± 7	162 ± 5	19 ± 3	7 ± 3	55 ± 2
0.015	40 ± 3	159 ± 12	22 ± 2	5 ± 1	60 ± 11
0.050	37 ± 5	161 ± 16	17 ± 2	8 ± 3	54 ± 9
0.15	40 ± 8	172 ± 4	18 ± 4	6 ± 2	48 ± 2
0.50	35 ± 6	163 ± 5	16 ± 3	9 ± 1	52 ± 9
1.25	36 ± 6	160 ± 17	21 ± 8	7 ± 1	48 ± 9
Positive	488 ± 11	1773 ± 83	162 ± 7	85 ± 10	444 ± 42

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL

APPENDIX I: Historical Control Data

Historical Negative and Positive Control Values
2005 – 2007

revertants per plate

Strain	Control	Activation							
		None				Rat Liver			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA98	Neg	17	6	5	56	25	8	6	60
	Pos	189	112	30	1812	526	343	73	2669
TA100	Neg	133	30	52	250	143	32	67	263
	Pos	548	148	112	4349	698	343	232	2652
TA1535	Neg	20	7	4	53	16	5	4	45
	Pos	420	146	54	1019	104	59	18	985
TA1537	Neg	7	3	1	20	8	3	1	29
	Pos	740	356	14	2514	88	87	13	1297
WP2 *uvr*A	Neg	21	9	5	69	23	10	4	65
	Pos	214	137	28	1086	268	174	29	1178

SD=standard deviation; Min=minimum value; Max=maximum value; Neg=negative control (including but not limited to deionized water, dimethyl sulfoxide, ethanol and acetone); Pos=positive control

APPENDIX II: Study Protocol

Bacterial Reverse Mutation Assay

1.0 PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test article by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvr*A in the presence and absence of S9 activation.

2.0 SPONSOR

2.1	Sponsor Name:	RTI International
2.2	Address:	3040 Cornwallis Rd Research Triangle Park, NC 27709
2.3	Representative:	Jay G. Henson, B.S. Phone: 919-541-7206 Fax: 919-541-5956 Email: jhenson@rti.org
2.4	Sponsor Study No.:	RTI-1059

3.0 TEST AND CONTROL ARTICLES

3.1	Test Article Name:		Fluoroestradiol
	Storage Temperature:		–5 to –40°C.
	Storage Parameters:		The test article will be stored in the dark and with desiccant.
	Purity:		An adjustment for purity or active ingredient will not be made.
	Molecular Weight:		290.37
3.2	Controls:	Negative:	Test article vehicle
		Positive:	9-aminoacridine 2-aminoanthracene methyl methanesulfonate 2-nitrofluorene sodium azide

3.3 Characterization and Stability of the Test Article

BioReliance will not perform analysis of the test article. The Sponsor will be directly responsible for determination and documentation of the analytical purity, composition and stability of the test article, and the stability and strength of the test article in the solvent (or vehicle).

3.4 Characterization of Test Article Dose Formulations at the Sponsor's Designated Laboratory

The Sponsor or their designated analytical laboratory has accepted responsibility for characterization of the test article dose formulations. BioReliance will not perform analysis of the test article or dose formulations.

3.4.1 Sampling

Upon preparation for use in initial toxicity-mutation assay and the confirmatory mutagenicity assay, the following samples will be collected:

If dose formulations are solutions, 2 x 0.5 mL aliquots of the vehicle and most concentrated dose formulations will be collected for concentration analysis. If necessary, alternate volumes or aliquots may be collected.

If sampled dosing formulations are suspensions, 2 x 0.5 mL aliquots from the top (T), middle (M) and bottom (B) of each test article concentration will be collected for homogeneity analysis in lieu of concentration analysis. In this case, the vehicle will be sampled from the middle portion only. If necessary, alternate volumes or aliquots may be collected. One aliquot of each sample will be sent to the Sponsor's designated analytical laboratory:

Jeanne Link, Ph.D.
Associate Professor of Radiology, Division of Nuclear Medicine
Molecular Imaging Research
Room NW041 UWMC
Box 356004
University of Washington
Seattle, WA 98195-6004
Phone: (206)598-6256
Fax: (206) 598-4192
Email: jeanne@u.washington.edu

These samples will be sent on dry ice on the day of preparation except as noted below. Samples prepared late in the day or on a day immediately preceding a weekend or holiday will be stored at -5 to -40°C and shipped on a Monday through Thursday. The second aliquot of each sample will be stored at BioReliance as a backup and will be analyzed only as needed. Unused samples will be discarded following issue of the analytical report.

3.4.2 Dose Formulation Analyses

Upon receipt and prior to analyses, the samples will be kept frozen (nominally at -20°C per the test site SOP) at the Test Site (analytical laboratory). The samples will be analyzed for concentration and/or homogeneity. The results from samples taken from the middle portion of each concentration will serve as a confirmation of concentration of the formulation.

All analytical work will be conducted by the Analytical Laboratory (Test Site) using a validated method (developed and qualified per Method Number NCI-Q319) and under the direction of the Principal Investigator.

All unused samples will be handled as per the Standard Operating Procedures of the Test Site.

3.4.3 Acceptance Criteria

The acceptable specification for the concentration of the test article in the vehicle will be as follows:

If formulations are solutions:

- 85 to 115% of nominal with <5% relative standard deviation (RSD) of each concentration.

If formulations are suspensions:

- T-M-B samples (each) 80 to 120% of nominal with <10% RSD.

The concentration of the test article in the vehicle formulation must be lower than or equal to the Limit of Quantification of the analytical method.

In the event that a sample is outside of the acceptable specification range, the Study Director will justify the acceptability of the results or suggest re-analysis of the backup samples or retest the affected portion of the study.

3.4.4 Compliance

The work performed in conjunction with the dose formulation analyses will be conducted in compliance with the study protocol and protocol amendments, appropriate standard operating procedures of the analytical laboratory and GLPs (listed in section 12.0 of this protocol). The work will be subject to a laboratory process audit and the reports will be reviewed by the Analytical Laboratory Quality Assurance Unit (AQAU). All deviations and AQAU audit findings at the Test Site laboratory will be reported to the Study Director and BioReliance Management.

3.4.5 Reporting

A draft report (PI-phase report) describing the work carried out by the Analytical laboratory will be provided to the BioReliance Study Director. After acceptance of the report, a copy of the final report, including a signed Test Site Quality Assurance Statement, and a Statement of GLP Compliance signed by the PI and Test Site Management will be prepared and submitted to BioReliance for inclusion in the main study final report.

3.4.6 Archiving

All raw data, documentation and reports generated as a result of sample analyses will be retained, archived or return to the Sponsor, as per the contractual agreement between the Sponsor and the Analytical Laboratory.

3.5 Test Article Retention Sample

Since the in-life portion of this study is less than four weeks in duration, BioReliance will not retain a reserve sample of the test article.

3.6 Residual Test Article and Dosing Preparations

Dosing preparations, excluding those saved for concentration or homogeneity analysis (if any), will be disposed of following administration to the test system. Following finalization of the report, residual test article will be discarded unless otherwise indicated by the Sponsor.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Toxicology Testing Facility BioReliance
4.2	Address:	9630 Medical Center Drive Rockville, MD 20850
4.3	Study Director:	Valentine O. Wagner III, M.S. Phone: 301-610-2152 Fax: 301-738-2362 Email: skip.wagner@bioreliance.com

4.4 Principal Investigator (Dose Formulation Analysis):

Jeanne Link, Ph.D.
Associate Professor of Radiology, Division of Nuclear Medicine
Molecular Imaging Research
NW041b UWMC
Box 356004
University of Washington
Seattle, WA 98195-6004
Phone (206)598-6256
Fax (206) 598-4192
jeanne@u.washington.edu

4.5 Quality Assurance Unit of BioReliance (Lead QA):

Jermaine Sorrell
Phone: 301-610-2257
Fax: 301-738-2362
Email: jermaine.sorrell@bioreliance.com

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 13-Jan-2009

5.2 Proposed Experimental Completion Date: 17-Feb-2009

5.3 Proposed Report Date: 03-Mar-2009

6.0 TEST SYSTEM

The tester strains will include the *S. typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al.* (1975) and the *E. coli* tester strain WP2 *uvr*A as described by Green and Muriel (1976).

Histidine Mutation			Tryptophan Mutation	Additional Mutations		
*his*G46	*his*C3076	*his*D3052	*trp*E	LPS	Repair	R-factor
TA1535	TA1537	-	-	*rfa*	Δ*uvr*B	-
TA100	-	TA98	-	*rfa*	Δ*uvr*B	+R
-	-	-	WP2 *uvr*A	-	Δ*uvr*A	-

Each *S. typhimurium* tester strain contains, in addition to a mutation in the histidine operon, additional mutations that enhance sensitivity to some mutagens. The *rfa* mutation results in a cell wall deficiency that increases the permeability of the cell to certain classes of chemicals such as those containing large ring systems that would otherwise be excluded. The deletion in the *uvr*B gene results in a deficient DNA excision-repair system. Tester strains TA98 and TA100 also contain the pKM101

plasmid (carrying the R-factor). It has been suggested that the plasmid increases sensitivity to mutagens by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. TA100 is reverted by both frameshift and base substitution mutagens and TA1535 is reverted only by mutagens that cause base substitutions.

The *E. coli* tester strain has an AT base pair at the critical mutation site within the *trp*E gene (Wilcox *et al.*, 1990). Tester strain WP2 *uvr*A has a deletion in the *uvr*A gene resulting in a deficient DNA excision-repair system. Tryptophan revertants can arise due to a base change at the originally mutated site or by a base change elsewhere in the chromosome causing the original mutation to be suppressed. Thus, the specificity of the reversion mechanism is sensitive to base-pair substitution mutations (Green and Muriel, 1976).

The *S. typhimurium* tester strains were received directly from Dr. Bruce Ames, University of California, Berkeley or a vendor authorized by his laboratory. The *E. coli* tester strain was received from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (United Kingdom).

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

7.1 Solubility Determination

The Sponsor has indicated that the test article vehicle will be ethanol and that the test article is soluble in 15% ethanol in PBS at a maximum concentration of 25 µg/mL. It is anticipated that higher percentages of ethanol will result in a higher soluble concentration.

7.2 Initial Toxicity-Mutation Assay

Selection of dose levels for the confirmatory mutagenicity assay will be based upon the toxicity and precipitation profile of the test article assessed in an initial toxicity-mutation assay. The test article will be tested at a minimum of eight dose levels along with appropriate negative and positive controls with tester strains TA98, TA100, TA1535, TA1537 and WP2 *uvr*A with and without S9 activation. All dose levels of test article, negative controls and positive controls will be plated in duplicate. Unless indicated otherwise by the Sponsor, the highest dose will be the highest workable concentration in the vehicle of choice but not to exceed 1.25 µg/plate. Solubility or workability permitting, the dose levels will be 1.25, 0.50, 0.15, 0.050, 0.015, 0.0050, 0.0015 and 0.00050 µg per plate. In selecting dose levels for the confirmatory mutagenicity assay the following guidelines will be employed. Doses will be selected such that precipitate does not interfere with manual scoring. Whenever possible, the highest dose for the confirmatory mutagenicity assay will be selected to give some indication of toxicity without

exceeding 5 mg/plate. For freely soluble, nontoxic test articles, the highest dose level will be 5 mg/plate. For precipitating, nontoxic test articles, the highest dose level may be selected in an attempt to yield precipitate at only the top one or two dose levels. The Sponsor will be consulted regarding dose selection if (1) the maximum dose level is selected based on precipitation and this dose level is less than 1.25 µg/plate or (2) the maximum achievable test article dose level is less than 1.25 µg/plate and this dose level is nontoxic. The doses selected for the confirmatory mutagenicity assay will be documented in the raw data and report.

7.3 Confirmatory Mutagenicity Assay

The test article will be tested at a minimum of five dose levels along with appropriate negative and positive controls with tester strains TA98, TA100, TA1535, TA1537 and WP2 *uvr*A with and without S9 activation. All dose levels of test article, negative controls and positive controls will be plated in triplicate.

7.4 Frequency and Route of Administration

The test system will be exposed to the test article via the plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983). This test system has been shown to detect a wide range of classes of chemical mutagens (McCann *et al.*, 1975; McCann and Ames, 1976).

If the Sponsor is aware of specific metabolic requirements (e.g., azo compounds), this information will be utilized in designing the assay. Verification of a clear positive response is not required. Equivocal results will be retested in consultation with the Sponsor using an appropriate modification of the experimental design (e.g., dose levels, activation system or treatment method). This guidance is based on the OECD Guideline 471 (1998) and ICH Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (1997).

7.5 Controls

No analyses will be performed on the positive control articles or the positive control dose formulations. The neat positive control articles and the vehicles used to prepare the test article and positive control formulations will be characterized by the Certificates of Analysis provided by the Supplier(s). Copies of the Certificates of Analysis will be kept on file at BioReliance.

7.5.1 Positive Controls

The positive controls that will be plated concurrently with the assay are listed below. Results obtained from these articles will be used to assure responsiveness of the test system but not to provide a standard for comparison with the test article.

Strain	S9	Positive Control	Concentration (µg/plate)
Salmonella Strains	Rat	2-aminoanthracene	1.0 – 2.0
WP2 *uvr*A			10
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 *uvr*A		methyl methanesulfonate	1,000

7.5.2 Negative Controls

Appropriate negative controls will be plated for each tester strain with and without S9 activation. The negative control will be the vehicle alone, unless there is no historical basis for use of the selected vehicle. In the latter case, both untreated and vehicle controls will be used.

7.5.3 Sterility Controls

The most concentrated test article dilution and the Sham and S9 mixes will be checked for sterility.

7.6 Exogenous Metabolic Activation

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The S9 homogenate will be prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The S9 will be batch prepared and stored frozen at -60°C or colder until used. Each batch of S9 homogenate will be assayed for its ability to metabolize at least two promutagens to forms mutagenic to *S. typhimurium* TA100.

Immediately prior to use, the S9 will be thawed and mixed with a cofactor pool to contain 10% S9 homogenate, 5 mM glucose-6-phosphate, 4 mM β-nicotinamide-adenine dinucleotide phosphate, 8 mM $MgCl_2$ and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4. This mixture is referred to as S9 mix. Sham mix will be 100 mM phosphate buffer at pH 7.4.

7.7 Preparation of Tester Strain

Overnight cultures will be inoculated from the appropriate master plate, from the appropriate frozen stock or from a lyophilized pellet. To ensure that cultures are harvested in late log phase, the length of incubation will be controlled and monitored. At the end of the working day, each inoculated flask will be placed in a resting shaker/incubator at room temperature. The shaker/incubator will be programmed to begin shaking at approximately 125 rpm at 37±2°C approximately 12 hours before the anticipated time of harvest.

All cultures will be harvested by spectrophotometric monitoring of culture turbidity rather than by duration of incubation since overgrowth of cultures can cause loss of sensitivity to some mutagens. Cultures will be removed from incubation at a density of approximately 10^9 cells/mL.

7.8 Test System Identification

Each plate will be labeled with a code system that identifies the test article, test phase, dose level, tester strain and activation type as described in BioReliance's Standard Operating Procedures.

7.9 Test Article Preparation

The most concentrated dilution will be prepared in 100% ethanol and used on the day of preparation. **Since this formulation is being shared between labs, it may be stored at 2 to 8°C between used. The formulation will be warmed to room temperature and mixed to homogeneity before use. All test article dosing will be at room temperature under yellow light.**

7.10 Treatment of Test System

One half milliliter (0.5 mL) of S9 mix or Sham mix, 100 µL of tester strain and 50 µL of vehicle, test article dilution or positive control will be added to 2.0 mL of molten selective top agar at 45±2°C. When necessary to achieve the target concentration or eliminate toxic vehicle effects, aliquots of other than 50 µL of test article or vehicle or positive control will be plated. When plating untreated controls, the addition of test article, vehicle and positive control will be omitted. The mixture will be vortex mixed and overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay has solidified, the plates will be inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that are not counted immediately following the incubation period will be stored at 2-8°C.

7.11 Scoring

The condition of the bacterial background lawn will be evaluated for evidence of test article toxicity and precipitate. Evidence of toxicity will be scored relative to the negative control plate and recorded along with the revertant count for that plate. Toxicity will be evaluated as a decrease in the number of revertant colonies per plate and/or a thinning or disappearance of the bacterial background lawn. Precipitation will be evaluated after the incubation period by visual examination without magnification.

7.12 Tester Strain Verification

On the day of use in the initial toxicity-mutation assay and the confirmatory mutagenicity assays, all tester strain cultures will be checked for the appropriate genetic markers cited in §6.0.

7.13 Automated Data Collection Systems

The primary computer or electronic systems used for the collection of data or analysis may include but not limited to the following:

Minicount Colony Counter (Imaging Products International), LIMS System (BioReliance), Excel 2003 (Microsoft Corporation) and Kaye Lab Watch Monitoring System (Kaye GE).

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The following criteria must be met for the initial toxicity-mutation assay and the confirmatory mutagenicity assay to be considered valid:

8.1 Tester Strain Integrity

To demonstrate the presence of the *rfa* mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to crystal violet. To demonstrate the presence of the *uvr*B mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the *uvr*A mutation, all *E. coli* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the pKM101 plasmid R-factor, tester strain cultures of TA98 and TA100 must exhibit resistance to ampicillin.

8.2 Negative Controls Values

Based on historical control data, all tester strain cultures must exhibit characteristic number of spontaneous revertants per plate in the vehicle controls. The mean revertants per plate must be within the following ranges (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60. Untreated controls, when part of the design, must also be within the ranges cited above.

8.3 Tester Strain Titers

To ensure that appropriate numbers of bacteria are plated, all tester strain culture titers must be equal to or greater than $0.3x10^9$ cells per milliliter.

8.4 Positive Control Values

Each mean, positive control value must exhibit at least a 3.0-fold increase over the respective mean, negative control value (vehicle) for each tester strain.

8.5 Toxicity

A minimum of three non-toxic dose levels will be required to evaluate assay data. A dose level is considered toxic if it causes a >50% reduction in the mean number of revertants per plate relative to the mean vehicle control value (this reduction must be accompanied by an abrupt dose-dependent drop in the revertant count) or a

reduction in the background lawn. In the event that less than three non-toxic dose levels are achieved, the affected portion of the assay will be repeated with an appropriate change in dose levels.

9.0 EVALUATION OF TEST RESULTS

For a test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

9.1 Strains TA1535 and TA1537

Data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value.

9.2 Strains TA98, TA100 and WP2 *uvr*A

Data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used for generation and analysis of the data. Unless alternate arrangements are made, the report will be initially issued as a QA-audited draft. After receipt of the Sponsor's comments a final report will be issued. The report will include:

- Test article: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.
- Solvent/Vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- Strains: strains used; number of cells/mL per culture; strain characteristics.
- Test conditions: amount of test article per plate with rationale for dose selection and number of plates per concentration; media used; type and composition of metabolic activation system, including acceptability criteria; treatment procedures.

- Results: signs of toxicity; signs of precipitation; individual plate counts; the mean number of revertant colonies per plate and standard deviation; dose-response relationship, if any; statistical analysis, if any; concurrent negative and positive control data means and standard deviations.
- Discussion of results
- Conclusion
- Appendices: Historical Control Data (negative and positive controls with ranges, means and standard deviations), copy of protocol and any amendment, and, if provided by the Sponsor, copies of the analyses that characterized the test article, its stability and the stability and strength of the dosing preparations.
- Statement of Compliance
- Quality Assurance Statement

If an electronic copy of the protocol, the report or another study document is provided by BioReliance, the executed paper document is considered the official master document. If there is a discrepancy between an electronic copy and the corresponding master document, the master document will be considered the official document. Six months after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor or a designated representative, the draft report will be issued as a final report. If all supporting analytical documents have not been provided to BioReliance, the report will be written based on those that are provided to BioReliance.

11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports, generated by BioReliance, will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance Quality Assurance unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied onto electronic media and the electronic copy will be retained in the BioReliance archives for a minimum of 10 years.

12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998 and with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996 and 1997) with the exception that the maximum concentration tested will be 1.25 µg per plate at the request of the Sponsor.

The following Good Laboratory Practices (GLP) regulations will be followed at BioReliance as requested by the Sponsor.

- US FDA Good Laboratory Practices 21 CFR Part 58
- OECD Principles of Good Laboratory Practice (C(97)186/Final)

For the study, an in-process phase, the raw data, and report(s) will be inspected per the Standard Operating Procedures (SOPs) of BioReliance by the Quality Assurance Unit of BioReliance for compliance with GLPs, the SOPs of BioReliance and the study protocol. At least one, study-specific, in-process inspection will be performed for this study. A signed QA Statement will be included in the final report. This statement will list the study-specific phases inspected at BioReliance, the dates of each inspection, and the dates the results of each inspection were reported to the Study Director and the Study Director's management. In addition, a signed GLP Compliance Statement will be included in the final report. This statement will cite the GLP regulations with which this study is compliant and any exceptions to this compliance, if applicable, including the omission of characterization or stability analyses of the test article or its mixtures.

Raw data, the protocol and reports generated at locations other than BioReliance will or will not be QA audited per the contractual arrangements between that site and the Sponsor.

Alterations of this protocol may be made as the study progresses. All protocol procedural modifications and rationale for the change(s) will be documented, signed, dated and approved by the Study Director, Study Management and the Sponsor. BioReliance QA will review all protocol amendments and document this review by initials and date. All applicable protocol amendments will be delivered by physical or electronic means to the Sponsor representative, within the Test Facility, and if applicable, to the test site(s) and Study Monitor(s).

Deviations from the protocol and/or BioReliance SOPs will be documented in a deviation report or a note to file will be generated. The deviation report will be signed by the Study Director and BioReliance QA.

13.0 REFERENCES

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research 31:347-364.

Green, M.H.L., and Muriel, W.J. (1976). Mutagen testing using trp^+ reversion in *Escherichia coli*. Mutation Research 38:3-32.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for

adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202, April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. Federal Register 62:16026-16030, November 21, 1997.

McCann, J. and Ames, B.N. (1976). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion. Proc. Natl. Acad. Sci. USA 73:950-954.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. USA 72:5135-5139.

Maron, D.M. and Ames, B.N. (1983). Revised Methods for the *Salmonella* Mutagenicity Test. Mutation Research 113:173-215.

OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, published by OECD, Paris, February 1998.

Wilcox, P., Naidoo, A., Wedd, D.J. and Gatehouse, D.G. (1990). Comparison of *Salmonella typhimurium* TA102 with *Escherichia coli* WP2 tester strains. Mutagenesis 5:285-291.

14.0 APPROVALS

14.1 Sponsor Approval

Sponsor Representative — Date: 1/8/09

14.2 Study Director and Test Facility Management Approvals

Valentine O. Wagner, III — BioReliance Study Director — 09 Jan 2009 — Date

BioReliance Study Management — 9-JAN-2009 — Date

14.3 Analytical Chemist or Principal Investigator Approval

The signature of the Analytical Chemist or Principal Investigator indicates that he or she intends to conduct the delegated phase(s) of this study in accordance with this study protocol, the test site's SOP and the GLP regulations cited in §12.0.

Jeanne McGinty, Ph.D.

Analytical Chemist or Principal Investigator

Jan 13, 2009

Date

APPENDIX III: Information for Japanese Regulatory Agencies

Report of Results of Reverse-Mutation Assay in Bacteria

1. Tester Strains

(1) Procurement

Strain	Obtained from	Date obtained	Date inspected the strain lot in storage
TA98	Dr. Bruce Ames' designated distributor, Discovery Partners International, San Diego, California	13 November 2002; 16 January 2004 (TA100 only)	The genetic markers for each culture are confirmed on the day of use
TA100			
TA1535			
TA1537			
TA1538			
TA97			
TA102			
WP2 *uvr*A	National Collection of Industrial and Marine Bacteria Aberdeen, Scotland	1 July 1987	
WP2 *uvr*A (pkM101)			
WP2 (pKM101)		19 February 1993	

(2) Storage

Freezing method	Large quantity	
Storage temperature	-60°C or colder	
Composition	Bacterial suspension	1.0 mL
	DMSO	0.09 mL

2. S9 Mix

(1) Source, Storage Temperature, etc. of S9

Purchased from MolTox	Prepared on	Used in Experiment No.	
	28 October 2008 (Lot 2359)	B1 and B2	
Storage temperature	-60°C or colder	Name and model of storage apparatus	So-Low, Model PR120-9

(2) Preparation of S9

Animal used		Inducing substance	
Species, Strain	Rattus norvegicus, Sprague Dawley	Name	Aroclor 1254
Sex	Male	Administration method	intraperitoneal
Age (in weeks)	Unknown (Lot 2359)	Administration period and amount (g/kg-weight)	single dose at 0.5 gm/kg body weight, 5 days prior to sacrifice
Weight	Unknown (Lot 2359)		

3. Preparation of Test Substance Solution

Solvent used			
Name	Manufacturer	Lot No.	Grade and/or Purity (%)
Ethanol (EtOH) CAS No. 64-17-5	Acrōs Organics	B0514580	99.5%
Stability of test substance in the solvent	Unknown (See Appendix V)		
Method of suspension when test substance is difficult to dissolve	Not Applicable		

4. Conditions of Pre-culture

Nutrient broth	Name	Manufacturer	Lot No.
	Oxoid Nutrient Broth No. 2	Oxoid Ltd.	655453
Period of pre-culture	12±2 hours		
Storage time and temp. from inoculation to beginning of shaking culture	<5 hours at ambient temperature		
Storage time and temp. from end of culture to use for test	<12 hours at 2-8°C		
Model and manufacturer of shaker	New Brunswick Scientific, model G-24		
Method of shaking (shaking type, speed, etc.)	Rotary (125 rev/min.)		
Culture vessel (shape, capacity)	shape: cylinder, 200 mL		
Culture volume	50 mL		
Volume of inoculum	1 colony		

5. Agar Plate Medium

(1) Top agar

Agar	Name	BBL Select
	Manufacturer	Becton Dickinson
	Lot No.	8190321

(2) Minimum Glucose Agar

Made in-house	Agar	Name		BBL Select
		Manufacturer		Becton Dickinson
		Lot Nos.		8190321
		Batch No.	Preparation Date	Used in Experiment No.
		17305	08 January 2009	B1
		17329	22 January 2009	B2
	Volume of agar plate medium			25 mL

6. Test Results - Judgement of the results

Judgement	Negative
Reason for judgement and referential matters: No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.	

Referential matters

The vehicle and positive control values indicate that all tester strains were functioning correctly and were capable of detecting a mutagen.

APPENDIX IV: Certificate of Analysis

16alpha-Fluoroestradiol

Product no. 191.XXXX

For research purposes only. Not for human use or consumption.

Product description

16alpha-Fluoroestradiol; synonyms: 16alpha-fluoro-17beta-estradiol, FES; mol. wt. 290.37; $C_{18}H_{23}FO_2$; [92817-10-2]; BRN 3554942; chemical name: estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16alpha, 17beta). Colorless crystals, soluble in acetonitrile and chloroform.

Applications

16alpha-Fluoroestradiol may be used as a reference standard in the radiosynthesis of [^{18}F]Fluoroestradiol.

Presentation

Product 191.XXXX is available in 2 ml dark glass vials (DIN 2R), packed under argon atmosphere. Vials are sealed with teflon-faced rubber stoppers and tear-off crimp caps. Bulk chemicals in quantities ≥ 100 mg are available in dark glass screw cap vials, flushed with argon atmosphere. The content of 16alpha-Fluoroestradiol in mg is defined by the four digit number replacing XXXX in the product number. Weighing error is ± 5 %, but in maximum 0.5 mg.

Storage and stability

Store the product desiccated at –20 ± 5 °C, protected from light. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

Toxicology/Hazards

Handle with care, avoid inhalation, ingestion, eye or skin contact, no toxicological data available.

Certificate of analysis

Lot No.: 260801		Product No.: 191.XXXX	
Parameter	Method	Specification	Result
Appearance	organoleptic	colorless crystals	conforms
Melting pt	capillary	180-210 °C	187.3-188.1 °C
Identity	^{1}H-NMR ^{19}F-NMR	conforms conforms	conforms conforms
Purity	HPLC	> 90 %	> 98 %

No further analytical data available

Manufacturing Date: Aug. 2006

ABX advanced biochemical compounds
Biomedizinische Forschungsreagenzien GmbH

Quality Control date: 09-Nov-06

Dr. B. Schmitt

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereoff.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

References

1) Stalford A. C. *et al.:* The metabolism of 16-fluoroestradiols in vivo: chemical strategies for restricting the oxidative biotransformations of an estrogen-receptor imaging agent. *Steroids.* **1997**, *62*, 750-761.
2) Römer J. *et al.:* Further ^{13}C NMR spectroscopic proof of 16alpha-F configuration in 16-fluoroestradiol derivatives. *Forschungszent. Rossendorf, [Ber.] FZR* **1997**, *165*, 192-193.
3) Mankoff D. A. *et al.:* [^{18}F]Fluoroestradiol Radiation Dosimetry in Human PET Studies. *J. Nucl. Med.* **2001**, *42*, 679-684.

ABX ***advanced biochemical compounds*** - Biomedizinische Forschungsreagenzien GmbH. ·H.-Gläser-Str. 10-14 · D-01454 Radeberg Germany · E-mail: info@abx.de · Internet: http://www.abx.de. · Tel.: +49 / 3528 / 40 41 60 · Fax: +49 / 3528 / 40 41 65

16alpha-Fluoroestradiol

NEW: Product no. 1910.XXXX, OLD: Product no. 191.XXXX Lot. 260801

For research purposes only. Not for human use or consumption.

VALID ONLY IN CONNECTION WITH ORIGINAL CERTIFICATE OF ANALYSIS

Retest certificate of analysis

The following parameters included in the original certificate of analysis have been retested to confirm the stability of the product or are newly introduced in the quality control of the product as they may be considered to be suitable for detection of indicators of decay and guarantee that the product still is in compliance with the original specification:

Lot No.: 260801		Product No.: 191.XXXX	
Retest-Parameter	Method	Retest specification	Result
Appearance	organoleptic	no change in color	conforms
Purity	^{1}H-NMR ^{19}F-NMR	no change in spectrum no change in spectrum	conforms Conforms

Further testing was not considered to be necessary because of absence of significant changes in parameters tested.

Date of retest: **15. Oct. 2008**
Expiry Date: **15. Oct. 2009**

Storage and stability

Store the product desiccated at -20 °C, protected from light. Product is at least one more year stable at -20 °C. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

ABX advanced biochemical compounds **Biomedizinische Forschungsreagenzien GmbH**

Quality Control date: 17-Oct-08

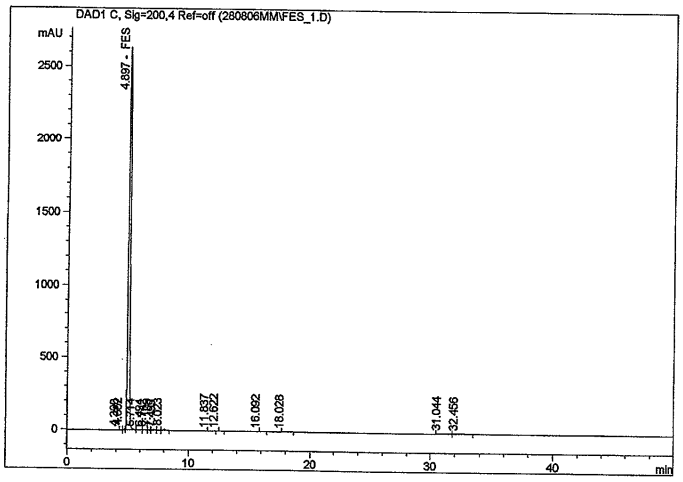
Dr. Bernd Feist

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereoff.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

HPLC

HPLC-Analyse bei:DAD1 C, Sig=200,4 Ref=off



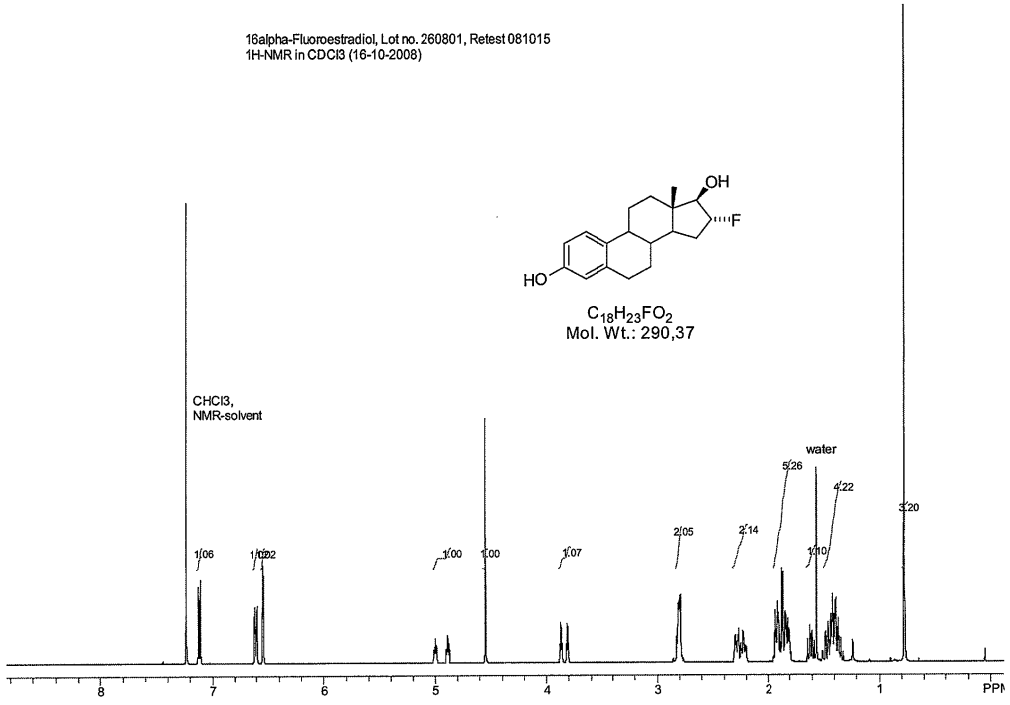
ABX advanced biochemical compounds

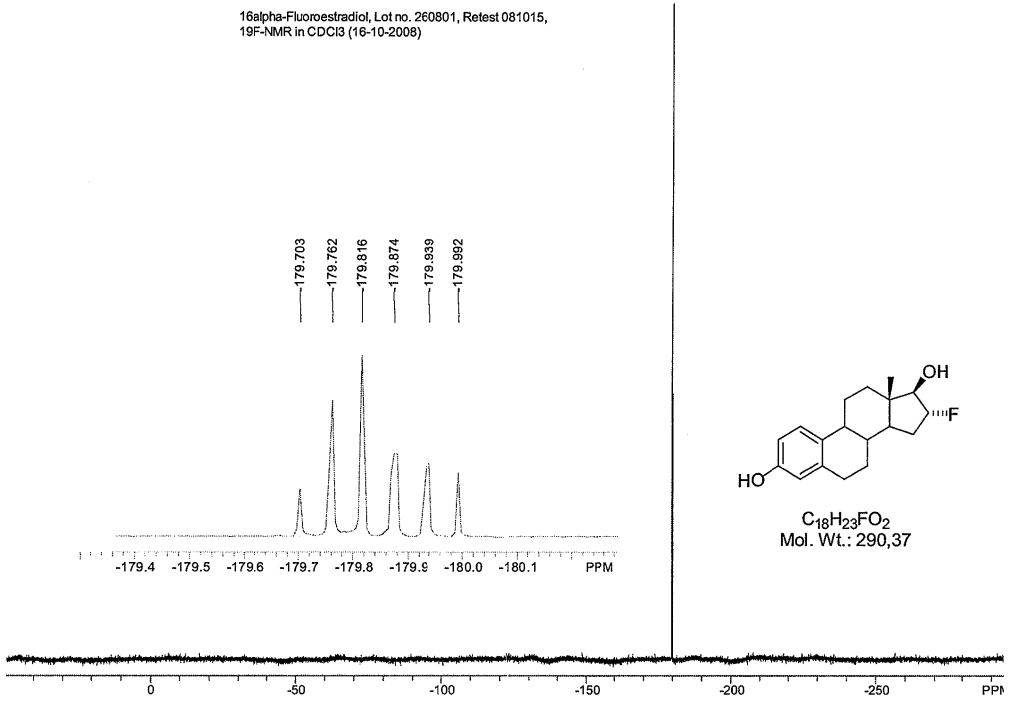
Sample Name: FES_191_260801 Vial 3

Raw data file name:D:\DATA\2006\280806MM\FES_1.D

Instrument Name: ABX-HPLC-02
Injection Date: 8/28/2006 Injection Time: 10:35:24 PM
Operator: Thieme

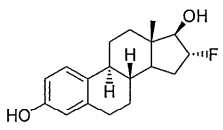
#	Name	Ret. Time	Are	Area %
1		4.390	5.159	0.016
2		4.652	54.985	0.172
3	FES	4.897	31576.758	98.520
4		5.714	41.679	0.130
5		6.494	26.023	0.081
6		6.742	17.094	0.053
7		7.109	68.320	0.213
8		7.499	5.924	0.018
9		8.023	98.434	0.307
10		11.837	7.910	0.025
11		12.622	1.829	0.006
12		16.092	4.334	0.014
13		18.028	15.479	0.048
14	MMSE	0.000	0.000	0.000
15		31.044	72.851	0.227
16		32.456	54.456	0.170


16alpha-Fluoroestradiol, Lot no. 260801, Retest 081015
1H-NMR in CDCl3 (16-10-2008)
OH
F
HO
$C_{18}H_{23}FO_2$
Mol. Wt.: 290,37
CHCl3,
NMR-solvent
water
1.06
1.22
1.00
1.00
1.07
2.05
2.14
5.26
1.10
4.22
3.20
8
7
6
5
4
3
2
1
PPM


16alpha-Fluoroestradiol, Lot no. 260801, Retest 081015,
19F-NMR in CDCl3 (16-10-2008)
179.703
179.762
179.816
179.874
179.939
179.992
-179.4 -179.5 -179.6 -179.7 -179.8 -179.9 -180.0 -180.1 PPM
0 -50 -100 -150 -200 -250 PPM
OH
F
HO
C18H23FO2
Mol. Wt.: 290,37

16a-Fluoroestradiol

Reference standard for 16a-[^{18}F]Fluoroestradiol

Product Number	**1910**
Chemical name	CA index name: Estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16a,17ß)
Synonyms	16a-Fluoro-13ß-methyl-1,3,5(10)-gonatriene-3,17ßdiol; 16a-Fluoro-17ß-estradiol; 16a-Fluoroestradiol
CAS RN	[92817-10-2]
M. F.	$C_{18}H_{23}FO_2$
Mol. Wt.	290.37
Structure	
Characteristics	Colourless crystals
Presentation	**Packaged in dark glass screw cap vials, argon flushed.**
Certificate	CoA; ^{1}H and ^{19}F NMR spectra
Purity	> 90 %
Order Number	**1910.0001: 1 mg** per vial **1910.0002: 2 mg** per vial **1910.0010: 10 mg** per vial **Please inquire for customized filling and bulk quantities**
Literature	1. Mankoff D.A. *et al.* [^{18}F]Fluoroestradiol Radiation Dosimetry in Human PET Studies. *J. Nucl. Med.* **2001**, *42*, 679-684. 2. Stalford A.C. *et al.* The metabolism of 16-fluoroestradiols in vivo: chemical strategies for restricting the oxidative biotransformations of an estrogen-receptor imaging agent. *Steroids.* **1997**, *62*, 750-761. 3. Roemer J. *et al.* Further ^{13}C NMR spectroscopic proof of 16alpha-F configuration in 16-fluoroestradiol derivatives. *Forschungszent. Rossendorf, [Ber.] FZR* **1997**, *165*, 192-193.

APPENDIX V: Dosing Formulation Analysis

University of Washington
PET Radiochemistry

Certificate of Analysis
Certificate No. RC-003

Study Number: 211886.001 "AMES Study"

The following samples were analyzed following good laboratory practices following established protocol NCI-Q319 for analysis of fluoroestradiol by HPLC with adaptations for sample matrix and increased concentrations as validated for the FES toxicity study. These measures were made using MS detection as per NCI-Q319.

Sample No.	Matrix	Date Sample Prepared	Nominal Concentration (µg/mL)	Sample Storage
AC19NA.503.BTL	ethanol vehicle	1/13/09	0	freezer ~ - 20°C
AC19NA.503.BTL	ethanol	1/13/09	25	freezer ~ - 20°C
AC19NA.503DTL-B2	ethanol	1/27/09	0	freezer ~ - 20°C
AC19NA.503DTL-B2	note leaked in shipment	1/27/09	25	freezer ~ - 20°C
AC19NA.503.BTL	ethanol*	1/27/09	25	freezer ~ - 20°C

Sample No.	Date Received	Date Analyzed	Nominal Concentration (µg/mL)	Measured Concentration (µg/mL)
AC19NA.503.BTL	1/15/09	4/10/09	0	ND
AC19NA.503.BTL	1/15/09	1/16/09	25	30 ± 2
AC19NA.503DTL-B2	1/28/09(a)	3/13/09	0	ND
AC19NA.503DTL-B2	1/28/09 (a)	Not measured	25	NM
AC19NA.503.BTL	1/30/09	1/30/09, 2/2/09	25	32 ± 3

Procedural variations: An interfering peak from the matrix at 280 nm made detection by UV invalid because The FES was not fully resolved from the interference. Thus MS was used to obtain selectivity.

Analysis performed by: Jeanne Meyers Link DATE: 4/10/09
Jeanne Meyers Link, PhD
Analytical and Radio-Chemist

Molecular Imaging Center * UW Medical Center, NW045 *
1959 NE Pacific Street, Box 356004 * Seattle, WA 98195-6004

REPORT

Effects of 16alpha-Fluoroestradiol on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

ChanTest Study Number:	080912.SUJ
Testing Facility:	ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128
Sponsor:	Center for Life Sciences and Toxicology RTI International 3040 Cornwallis Rd. Research Triangle Park, NC 27709
Study Director:	Lisa M. Shyjka, BA
Staff Scientist:	Zhixiong Lu, MS
Study Initiation Date:	04-Dec-2008
Experimental Start Date:	08-Dec-2008
Experimental End Date:	09-Dec-2008
Document Type:	Report
Document Status:	FINAL
Date:	15-Jun-2009
Number of Pages:	48

Personnel

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Testing Facility Staff Scientist	Zhixiong Lu, MS
Testing Facility Management	Glenn E. Kirsch, PhD ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128 Tel. 216-332-1665; Fax 216-332-1706 Email: gkirsch@chantest.com
Testing Facility Quality Assurance	Jennifer A. Scocos, MS, RQAP-GLP ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128 Tel. 216-332-1665; Fax 216-332-1706 Email: jscocos@chantest.com
Test Site Principal Investigator for Formulation Analysis	Jeanne Link, PhD Associate Professor of Radiology Division of Nuclear Medicine Molecular Imaging Research Box 356004 Room NW041 UWMC University of Washington Seattle, WA 98195-6004 Tel. 206-598-6256; Fax 206-598-4192
Study Monitor (Protocol Amendment 1)	Jay G. Henson, BS Life Sciences and Toxicology RTI International 3040 Cornwallis Rd. Research Triangle Park, NC 27709 Tel. 919-541-7206; Fax 919-541-5956 Email:jhenson@rti.org

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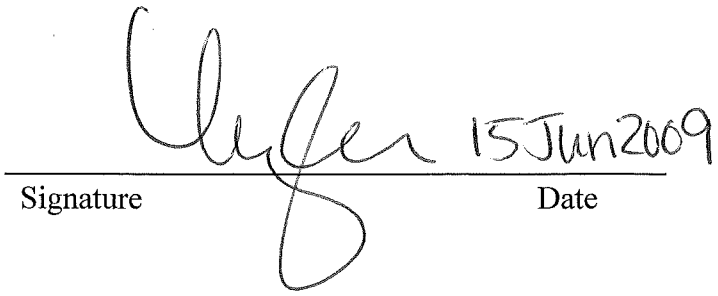
1 Signature

Study Director:
Lisa M. Shyjka, BA

ChanTest Corporation



Signature Date

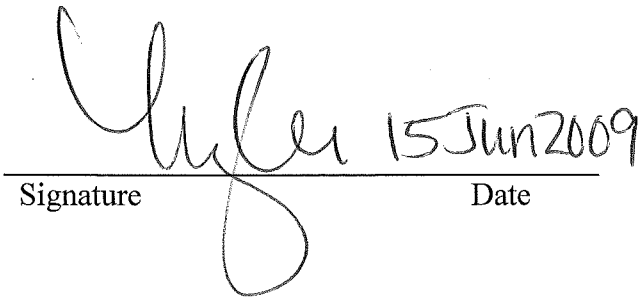
2 GLP Compliance Statement

The study complied with the most recent version of the Food and Drug Administration (FDA) Good Laboratory Practices Regulations (21 CFR Part 58), with the following exceptions:

- The positive control article formulation was not analyzed for stability, homogeneity or concentration (its potency was demonstrated by the comparison of results with ChanTest historical data).
- Sample analysis for homogeneity, stock and concentration verification was conducted under non-GLP conditions. The analytical method used was not validated in HB-PS + 0.3% Ethanol.
- Formulation stability was not determined under conditions of use.
- Test article stability and characterization was conducted non-GLP conditions.

The study complied with ICH Guidance for Industry (July 2001), "S7A Safety Pharmacology Studies for Human Pharmaceuticals" and ICH Guidance for Industry (October 2005), "S7B Nonclinical Evaluation of the Potential for Delayed Repolarization (QT Interval Prolongation) by Human Pharmaceuticals." The study was conducted in accordance with the standard operating procedures (SOPs) of ChanTest Corporation.

Lisa M. Shyjka, BA
Study Director
ChanTest Corporation



Signature Date

3 ChanTest Quality Assurance Statement

Inspection Date	Inspection Type	Date Reported to the Study Director	Date Reported to Management
05-Dec-2008	Protocol Review	05-Dec-2008	05-Dec-2008
08-Dec-2008	Test Article Preparation	08-Dec-2008	08-Dec-2008
08-Dec-2008	Data Acquisition	08-Dec-2008	09-Dec-2008
18-Dec-2008	Data	18-Dec-2008	19-Dec-2008
18-Dec-2008	Draft Report	18-Dec-2008	19-Dec-2008
16-Jan-2009	Protocol Amendment 1	16-Jan-2009	16-Jan-2009
26-May-2009	Final Report	26-May-2009	27-May-2009

The Quality Assurance Unit has reviewed the report and has determined that the results incorporated into this report accurately reflect the study raw data.

Alicia DePlatchett, BA

Quality Assurance Auditor

ChanTest Corporation

ADePlatchett 5-Jun-2009

Signature Date

4 Summary

The objective of this study was to examine the *in vitro* effects of 16alpha-Fluoroestradiol on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for I_{Kr}, the rapidly activating, delayed rectifier cardiac potassium current). 16alpha-Fluoroestradiol inhibited hERG current by (Mean ± SEM, n = 3) 1.4 ± 0.2% at 8 ng/mL versus 0.3 ± 0.1% in control. hERG inhibition at 8 ng/mL was statistically significant ($P < 0.05$) when compared to vehicle control values. The IC_{50} for the inhibitory effect of 16alpha-Fluoroestradiol on hERG potassium current could not be determined due to solubility limitations of 16alpha-Fluoroestradiol in HB-PS + 0.3% ethanol, but it is estimated to be greater than 8 ng/mL.

The positive control (60 nM terfenadine) inhibited hERG potassium current by (Mean ± SD; n = 2) 80.4 ± 0.1%. This result confirms the sensitivity of the test system to hERG inhibition.

Samples of the test article formulation solutions collected from stock preparation were analyzed for concentration verification. The results from the stock sample analysis indicated that the measured concentrations of 16alpha-Fluoroestradiol at all test concentrations were within ±15% of the target concentrations (between average 91.0 to 105.7% of the targets), thereby meeting the acceptance criteria. Although samples met acceptance criteria, it is unknown if storage conditions upon arrival at analytical site were appropriate. The samples were stored refrigerated and shipped on ice packs at ChanTest and stored frozen upon arrival at analytical site.

Samples of the test article formulation solutions collected from the outflow of the perfusion apparatus were analyzed for concentration verification. The results from the sample analysis showed that the measured concentration range for 8 ng/mL formulation was (average) 175% of the target concentration, which was outside the acceptance range (% target concentration ± 15%). Since the stability of the test article formulations in HB-PS + 0.3% ethanol under conditions of use was not determined prior to sample analysis the impact of formulation storage is unknown, therefore the nominal concentration (8 ng/mL) is reported.

The sample analysis indicates that all formulations were homogeneous at the start of day.

5 Introduction and Objective

The objective of this study was to examine the *in vitro* effects of 16alpha-Fluoroestradiol on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for I_{Kr}, the rapidly activating, delayed rectifier cardiac potassium current, Redfern et al., 2003). The concentration-response relationship of the effect of 16alpha-Fluoroestradiol on the hERG potassium channel current was evaluated at near-physiological temperature.

5.1 Sponsor Responsibilities

The Sponsor was responsible for the following information:

1. Documentation on the strength, purity, composition, physical properties, stability, and other pertinent information on the bulk test article in the form of a Certificate of Analysis and a Retest Certificate of Analysis for inclusion in the final report (Appendix B).
2. Stability, homogeneity and concentration of the formulated test article under conditions of use.
3. Amount of test article provided.

5.2 Protocol Amendments and Deviations

There was one (1) protocol amendment and one (1) protocol deviation. (Appendix A) The amendment and deviation did not have an impact on the study.

5.3 Quality Assurance Unit

The Quality Assurance Unit (QAU) at ChanTest acted as QAU and inspected the study according to ChanTest SOPs. ChanTest QAU provided inspection reports to the Study Director and Testing Facility Management. Inspection types and dates are included in Section 3 of this report.

6 Test and Control Articles

6.1 Test Article

Test Article ID:	16alpha-Fluoroestradiol
Lot Number:	260801
Form:	Non-basic steroid
Molecular Weight:	290.37 g/mol
Purity:	> 98%
Storage Conditions (bulk):	Frozen, desiccated, protected from light
Retest Date:	15-Oct-2009
Carrier:	Ethanol
Solubility:	At least 8 ng/mL in HB-PS + 0.3% Ethanol

6.1.1 Rationale for Concentration Selection

Based on sponsor correspondence, only one concentration (8 ng/mL) was evaluated. This concentration is approximately 10 times the maximum possible human concentration. 8 ng/mL was tested in three (3) cells (n = 3).

6.1.2 Test Article Disposition

Bulk test article, stock solutions, and analytical samples remaining at the end of the study will be discarded after issuance of the final report and records of disposition will be filed with the study data.

6.1.3 Test Article Carrier

Name:	Ethanol (also known as Ethyl alcohol, denatured for HPLC)
Source:	Acros Organics
Lot Number:	B0513134
Molecular Weight:	46.06 g/mol
Storage Conditions:	Room temperature, protected from light
Expiration Date:	30-June-2012
Rationale for Selection:	Previous results have shown that 0.3% ethanol does not affect channel current (data on file at ChanTest).

6.1.4 Positive Control Article and Reference Substance Carrier

Name:	DMSO
Source:	Sigma-Aldrich
Lot Number:	01533MH
Molecular Weight:	78.13 g/mol
Storage Conditions:	Room temperature
Retest Date:	31-Oct-2009
Rationale for Selection:	Previous results have shown that 0.3% DMSO does not affect channel current (data on file at ChanTest Corporation).

6.2 Vehicle Control

The vehicle consisted of a HEPES-buffered physiological saline (HB-PS) solution (composition in mM): NaCl, 137; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use), supplemented with 0.3% Ethanol. Chemicals used in vehicle preparation were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted and were of ACS reagent grade purity or higher.

Name:	HB-PS + 0.3% Ethanol
Source:	ChanTest Corporation
HB-PS Batch Numbers:	Expiration Dates:
081205CP168	19-Dec-2008
081208CP171	22-Dec-2008
Storage Conditions:	Refrigerated
Rationale for Selection:	HB-PS provides the appropriate ionic composition for *in vitro* recording.

6.3 Positive Control Article

Name:	Terfenadine
Source:	Sigma-Aldrich
Lot Number:	117K1623
Molecular Weight:	471.7 g/mol
Purity:	99.5%
Storage Conditions (bulk):	Refrigerated
Expiration Date:	31-Jan-2010
Rationale for Selection:	Previous results have shown that 60 nM terfenadine inhibits hERG potassium current by approximately 80% (data on file at ChanTest).

6.4 Reference Substance

Name:	E-4031
Source:	Sigma-Aldrich
Lot Number:	086K46162
Molecular Weight:	474.44 g/mol
Purity:	> 99.0%
Storage Conditions:	Frozen, desiccated
Expiration Date:	31-Aug-2009
Rationale for Selection:	E-4031 selectively inhibits hERG potassium current with IC_{50} = 12 nM (data on file at ChanTest Corporation). A supramaximal inhibiting concentration of 500 nM was used to eliminate the contribution of non-hERG currents.

6.5 Formulations

6.5.1 Positive Control and Reference Substance Formulations

Stock solutions of the positive control and reference substance were prepared in dimethyl sulfoxide (DMSO), aliquoted for individual use, stored frozen, and used within one month. Final positive control and reference substance concentrations were prepared fresh daily by diluting stock solutions in vehicle (final DMSO concentration, 0.3% v/v). Positive control article and reference substance formulations as set forth in this section are solutions and, therefore, homogeneous, by definition. Homogeneity, concentration, and stability analyses were not performed. Their potency for hERG inhibition is demonstrated by the comparison of results with ChanTest historical data.

6.5.2 Test Article Formulations

A 0.3 mg/mL primary stock solution of 16alpha-Fluoroestradiol was prepared in ethanol and stored refrigerated, protected from light. A secondary stock, 10 µg/mL, was also prepared and stored refrigerated, protected from light. 16alpha-Fluoroestradiol at 8 ng/mL was prepared fresh daily by diluting the secondary stock solution in vehicle final ethanol concentration, 0.3% v/v. To avoid precipitation near the solubility limit, the test article formulation was prepared at room temperature (15 - 30ºC) and vortexed for 30 seconds.

6.5.3 Preparation of Vehicle and Formulation Samples

One set of vehicle control samples (blank, 3 mL, accurately measured, in duplicate) were taken from the vehicle reservoir at the beginning of testing. One set of samples for homogeneity determination (3 mL each, accurately measured, in duplicate) were aliquoted from the top, middle, and bottom of the test article formulation reservoir in the patch clamp perfusion apparatus after preparation at the beginning of testing.

Representative test article formulation samples for concentration verification were collected on the only day of testing. Samples for concentration testing (3 mL, accurately measured, in duplicate) were aliquoted from the outflow of the perfusion apparatus.

Immediately after preparation, (1 mL, accurately measured, in duplicate) were collected from the primary and secondary stocks (in 100% ethanol) at each concentration.

The above formulation samples were stored refrigerated until completion of the electrophysiological phase of the study, at which time, one set of samples were shipped to the University of Washington for analysis. The duplicate set of samples was retained at ChanTest as backup replacements.

The analytical samples were shipped on ice packs to:

Jeanne Link, PhD
Associate Professor of Radiology
Division of Nuclear Medicine
Molecular Imaging Research Box 356004
Room NW041 UWMC
University of Washington
Seattle, WA 98195-6004
Tel. 206-598-6256
Fax 206-598-4192

ChanTest sent notification of shipment to the Sponsor (via Email) and to the Principal Investigator (via Fax).

Disposition of any unused portion of the analytical samples sent to the test site is the responsibility of the test site.

7 Test System

Cells were maintained in tissue culture incubators per ChanTest SOP. Stocks were maintained in cryogenic storage. Cells used for electrophysiology were plated in plastic culture dishes. Each culture dish was identified by a notation of clone identity number, passage number, and date. These data were retained in the study file.

7.1 Rationale for Selection of Ion Channel and Expression Systems

The cardiac potassium channel, hERG, is responsible for a rapid delayed rectifier current (I_{Kr}) in human ventricles. This channel has been selected for evaluation because inhibition of I_{Kr} is the most common cause of cardiac action potential prolongation by non-cardiac drugs (Brown and Rampe, 2000; Weirich and Antoni, 1998; Yap and Camm, 1999). Increased action potential duration causes prolongation of the QT interval and has been associated with a dangerous ventricular arrhythmia, *torsade de pointes* (Brown and Rampe, 2000). In this assay, hERG potassium channels are expressed in a human embryonic kidney (HEK293) cell line that lacks endogenous I_{Kr}.

7.2 HEK/hERG

Organism:	*Homo sapiens*
Designation:	293
Tissue:	Kidney; Transformed with adenovirus 5 DNA; Transfected with human-ether-a-go-go cDNA
Morphology:	Epithelial
Age/Stage:	Embryo
Source-Strain:	ATCC, Manassas, VA
Source-Sub Strain:	ChanTest Corporation, Cleveland, OH

7.3 Cell Culture Procedures

HEK293 cells were stably transfected with hERG cDNA. Stable transfectants have been selected by coexpression with the G418-resistance gene incorporated into the expression plasmid. Selection pressure was maintained by including G418 in the culture medium. Cells were cultured in Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 (D-MEM/F-12) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate and 500 μg/mL G418.

8 Test Method

8.1 Treatment Groups

All experiments were performed at near-physiological temperature (33 to 35 º C). Each cell acted as its own control.

8.1.1 Positive Control Group

The positive control was applied to two (2) cells (n = 2).

8.1.2 Concentration-Response Test Group

One concentration (8 ng/mL) was evaluated. This concentration is approximately 10 times the maximum possible human concentration. This concentration was tested in three (3) cells (n = 3).

8.1.3 Vehicle Control Group

Vehicle control solution was applied in three (3) cells (n = 3). Duration of application was at least as long as the time necessary for the lowest effective test article concentration to reach steady-state inhibition (approximately 4 minutes).

8.2 Electrophysiological Procedures

Cells were transferred to the recording chamber and superfused with vehicle control solution. Micropipette solution for whole cell patch clamp recordings was composed of (mM): potassium aspartate, 130; $MgCl_2$, 5; EGTA, 5; ATP, 4; HEPES, 10; pH adjusted to

7.2 with KOH. Micropipette solution was prepared in batches (081121CP002K; expiration date 18-Dec-2008), aliquoted, stored frozen, and a fresh aliquot thawed each day. The recording was performed at a temperature of 33 to 35 º C using a combination of in-line solution pre-heater, chamber heater, and feedback temperature controller. Temperature was measured using a thermistor probe in the recording chamber. Micropipettes for patch clamp recording were made from glass capillary tubing using a P-97 micropipette puller (Sutter Instruments, Novato, CA). A commercial patch clamp amplifier was used for whole cell recordings. Before digitization, current records were low-pass filtered at one-fifth of the sampling frequency.

8.3 Experimental Procedures

8.3.1 Concentration Range

Cells stably expressing hERG were held at -80 mV. Onset and steady state inhibition of hERG potassium current due to16alpha-Fluoroestradiol was measured using a pulse pattern with fixed amplitudes (conditioning prepulse: +20 mV for 1 sec; repolarizing test ramp to –80 mV (-0.5 V/s) repeated at 5 s intervals. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition.

16alpha-Fluoroestradiol at 8 ng/mL was applied to three cells (n = 3). An inhibitory effect on hERG potassium current amplitude of 1.4 % was observed.

8.4 Electrophysiological Data Analysis

Data were stored on the ChanTest computer network (and backed-up nightly) for off-line analysis. Data acquisition and analyses were performed using the suite of pCLAMP (Ver. 8.2) programs (MDS-AT, Sunnyvale, CA), and were reviewed by the Study Director. Percent inhibition at each concentration in the test group was compared with the vehicle control group using one-way ANOVA followed by Dunnett's multiple comparison test (JMP Version 5.0.1, SAS Institute, Cary, NC). Significant inhibition was defined at the level of $P < 0.05$.

Steady state was defined by the limiting constant rate of change with time (linear time dependence). The steady state before and after test article application was used to calculate the percentage of current inhibited at each concentration.

8.5 Analytical Data Calculation

The following formulas were used in the analysis of data included in the analytical report (Certificate of Analysis) (Appendix C).

% target concentration = 100 * (measured mean concentration – nominal concentration)/nominal concentration

% Coefficient of Variance for Homogeneity of the formulation samples:

% CV = 100 * (std. dev. of concentration of replicates/mean concentration of replicates)

9 Results

9.1 Stability of the Bulk Test Article

16alpha-Fluoroestradiol was synthesized and characterized under non-GLP conditions. The stability of the test article was determined under non-GLP conditions. The Certificate of Analysis with a retest date of 15 October 2009 is presented in Appendix B.

9.2 Analysis of Formulation Samples for Homogeneity, Stability and Concentration Verification

The stock and formulation samples were analyzed at the University of Washington by mass spectrometry (MS) (Protocol Deviation 1) under non-GLP conditions using method number (protocol) NCI-Q319. The analytical report (Certificate of Analysis) is presented in Appendix C.

9.2.1 Concentration Verification of Electrophysiology Dose Stock Formulation Samples

Samples of the test article formulation solutions collected from stock preparation were analyzed for concentration verification. The results from the primary and secondary stock samples (0.3 mg/mL and 10 µg/mL) analysis indicated that the measured concentrations of 16alpha-Fluoroestradiol at all test concentrations were within ±15% of the target concentrations (between (average) 91.0 to 105.7% of the targets), thereby meeting the acceptance criteria. However, stability of the stock formulations under conditions of use was not determined prior to sample analysis. Samples were stored refrigerated at ChanTest and the stored under frozen conditions at the analytical test site Therefore, the impact is unknown.

9.2.2 Homogeneity Analysis of Electrophysiology Dose Formulation Samples

The sample analysis indicated that all formulations were homogeneous at the start of day. The analysis of the reservoir samples indicated that the dose formulation (8 ng/mL) met the % CV acceptance criteria of ≤ 15.0%.

Samples for homogeneity determination, aliquoted from the top, middle, and bottom of the dose formulation reservoir at the start of day were analyzed. The % CV value for the dose formulations was 4.7% demonstrating that the solutions were homogenous.

9.2.3 Concentration Verification of Electrophysiology Dose Formulation Samples

Samples of the test article formulation solutions collected from the outflow of the perfusion apparatus were analyzed for concentration verification. The results from the

sample analysis showed that the measured concentration range for 8 ng/mL formulation was (average) 175% of the target concentration, which was outside the acceptance range (% target concentration ± 15%). Since the stability of the test article formulations in HB-PS + 0.3% ethanol under conditions of use (room temperature and refrigerated) was not determined prior to sample analysis the impact of formulation storage is unknown, therefore the nominal concentration (8 ng/mL) is reported. However, it can be assumed that the test results could be considered valid since the concentration result was higher than 8 ng/mL, which was labeled on the sample vial; therefore, the small inhibition of hERG current seen is for concentration higher than 8 ng/mL. Regardless, this inhibition at this concentration is statistically significant.

ChanTest performed an investigation of the out-of-range results in the following areas: solution preparation, equipment, sample collection, storage, and shipment. Errors were found that could impact the results. Errors were found in the storage of the stock solution samples at the analytical test site. Stock solution samples were stored refrigerated at ChanTest and stored under frozen conditions at the analytical test site. Also, it is unclear if the validated method (filed by the sponsor IND #79005, see correspondence in raw data) was appropriate for the analysis of the test article in the matrix (HB-PS + 0.3% EtOH) and if the storage conditions were suitable for the test article stock formulations. Although a Certificate of Analysis was provided, information regarding analysis and reanalysis of the samples on different days does not seem to support the data. The above-range results of the concentration analysis may be attributed to the above mentioned errors. Therefore, based on the above mentioned information and unknown impacts that these may have, the nominal concentration is reported.

9.3 Electrophysiological Results

Table 1 provides individual data points and Table 2 provides statistics for each concentration of 16alpha-Fluoroestradiol used to construct the hERG concentration-response relation.

Typical hERG potassium current records acquired during control, after equilibration with 16alpha-Fluoroestradiol at 8 ng/mL and after equilibration with the reference substance (0.5 μM E-4031) are superimposed in Figure 1. In the control trace for this experiment, following activation of hERG potassium channels by the conditioning prepulse (+20 mV), partial repolarization evoked a large, slowly decaying outward current. In this example, 16alpha-Fluoroestradiol application reduced the amplitude of the outward tail currents by 1.2% at 8 ng/mL.

Figure 2 exemplifies the time course of the effect of 16alpha-Fluoroestradiol at 8 ng/mL on the amplitude of hERG tail currents (same experiment as Figure 1). Figure 3 presents concentration-response relationship. The IC_{50} for the inhibitory effect of 16alpha-Fluoroestradiol on hERG potassium current could not be determined but it is estimated to be greater than 8 ng/mL.

Tables 3-4 summarize the effect of application of terfenadine, the positive control. Terfenadine at 60 nM produced 80.4% inhibition of hERG potassium current.

10 Conclusions

The *in vitro* effects of 16alpha-Fluoroestradiol on ionic currents in voltage-clamped human embryonic kidney cells (HEK293) that stably express the human ether-à-go-go-related gene (hERG) were determined. One concentration of 16alpha-Fluoroestradiol (8 ng/mL) was tested. 16alpha-Fluoroestradiol inhibited hERG potassium current by (mean ± SEM, n = 3) 1.4 ± 0.2% at 8 ng/mL verses 0.3 ± 0.1% in control. The IC_{50} for the inhibitory effect of 16alpha-Fluoroestradiol on hERG potassium current could not be determined due to solubility limitations of 16alpha-Fluoroestradiol in HB-PS + 0.3% ethanol, but it is estimated to be greater than 8 ng/mL.

The positive control (60 nM terfenadine) inhibited hERG potassium current by (Mean ± SD; n = 2) 80.4 ± 0.1%. The effect of terfenadine confirms the sensitivity of the test system to hERG inhibition.

11 Archiving of Materials

The protocol (including amendments and deviations), the raw data collected by ChanTest (including but not limited to electronic electrophysiology records, data collection forms, and worksheets), and the final report shall be archived by ChanTest Corporation following ChanTest SOPs for on-site and off-site storage. ChanTest shall be responsible for the retention of such records for five years (or less, upon Sponsor request) following the study completion date, at which time the Sponsor will be contacted for final disposition. All data and the original analytical report generated by the University of Washington will be archived at the University of Washington.

12 References

Brown AM and Rampe D. (2000). Drug-induced long QT syndrome: is HERG the root of all evil? Pharmaceutical News 7, 15-20.

Kirsch GE, Trepakova ES, Brimecombe JC, Sidach SS, Erickson HD, Kochan MC, Shyjka LM, Lacerda AE, and Brown AM. (2004). Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. J Pharmacol Toxicol Methods 50, 93-101.

Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PK, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG. (2003). Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovascular Research. 58(1):32-45.

Weirich J and Antoni H. (1998). Rate-dependence of antiarrhythmic and proarrhythmic properties of class I and class III antiarrhythmic drugs. Basic Res Cardiol 93 Suppl 1, 125-132.

Yap YG and Camm AJ. (1999). Arrhythmogenic mechanisms of non-sedating antihistamines. Clin Exp. Allergy 29 Suppl 3, 174-181.

13 Tables and Figures

13.1 16alpha-Fluoroestradiol effect on hERG potassium current

Table 1: Percent Inhibition at each 16alpha-Fluoroestradiol Concentration

Cell ID (filename)	Concentration (ng/mL)	
	0	8
C 0 ZL 081208_0000_sub.abf		1.8%
D 0 ZL 081208_0000_sub.abf		1.2%
E 0 ZL 081208_0000_sub.abf		1.2%
F 0 ZL 081208_0000_sub.abf	0.3%	
A 0 ZL 081209_0000_sub.abf	0.4%	
B 0 ZL 081209_0000_sub.abf	0.1%	

Table 2: 16alpha-Fluoroestradiol Summary Statistics
Mean percent inhibition at each 16alpha-Fluoroestradiol concentration (Mean), standard deviation (SD), standard error of the mean (SEM) and number of cells (N).

Concentration (ng/mL)	Mean	SD	SEM	N
0	0.3%	0.2%	0.1%	3
8	1.4%*	0.3%	0.2%	3

* Value is statistically different than vehicle alone.

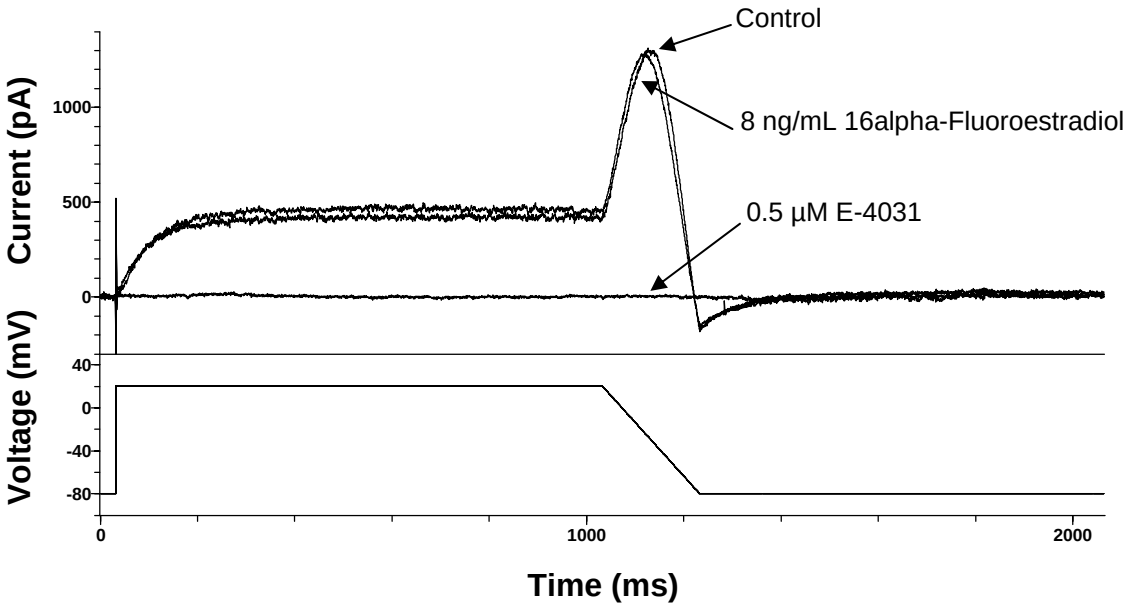


Figure 1: Typical hERG potassium current traces
Upper panel [Current (pA); Time (ms)] shows superimposed, records of hERG potassium currents obtained in a single cell during application of control, test article and reference substance. HERG potassium currents were evoked by the voltage protocol shown in the lower panel [Voltage (mV)]. Cell ID: E 0 ZL 081208_0000_sub.abf.

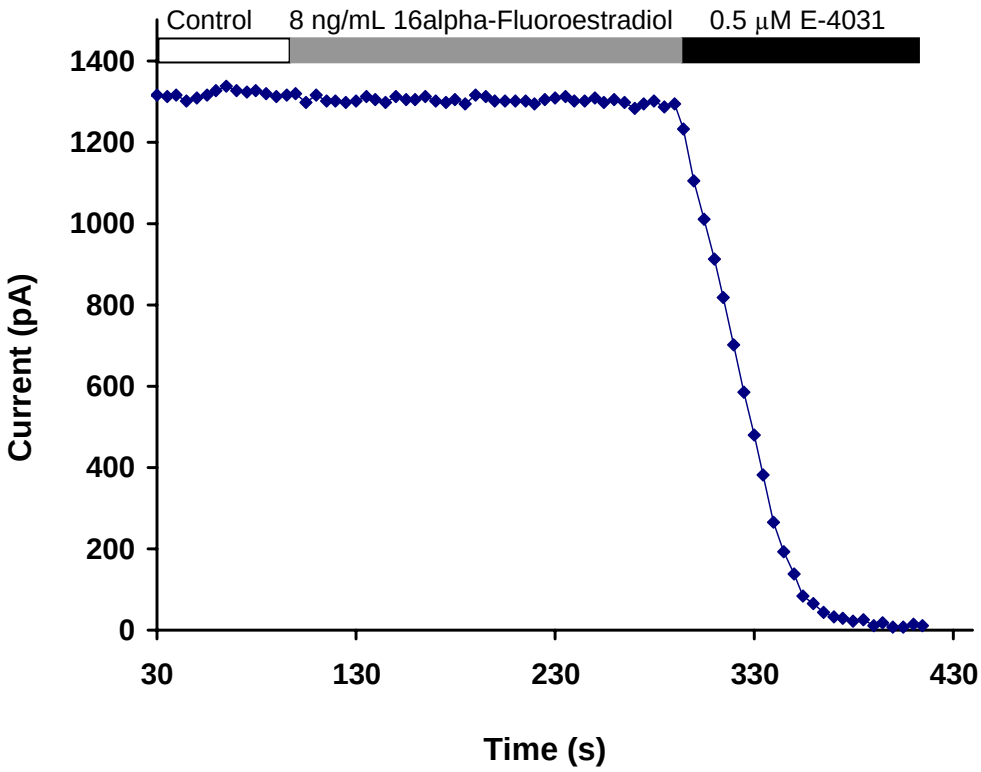


Figure 2: Typical time course of the effect of 16alpha-Fluoroestradiol on the hERG current

Peak current amplitude during application of vehicle (control), test article, and reference substance. The horizontal bars indicate the control, test article concentration and E-4031. Cell ID: E 0 ZL 081208_0000_sub.abf.

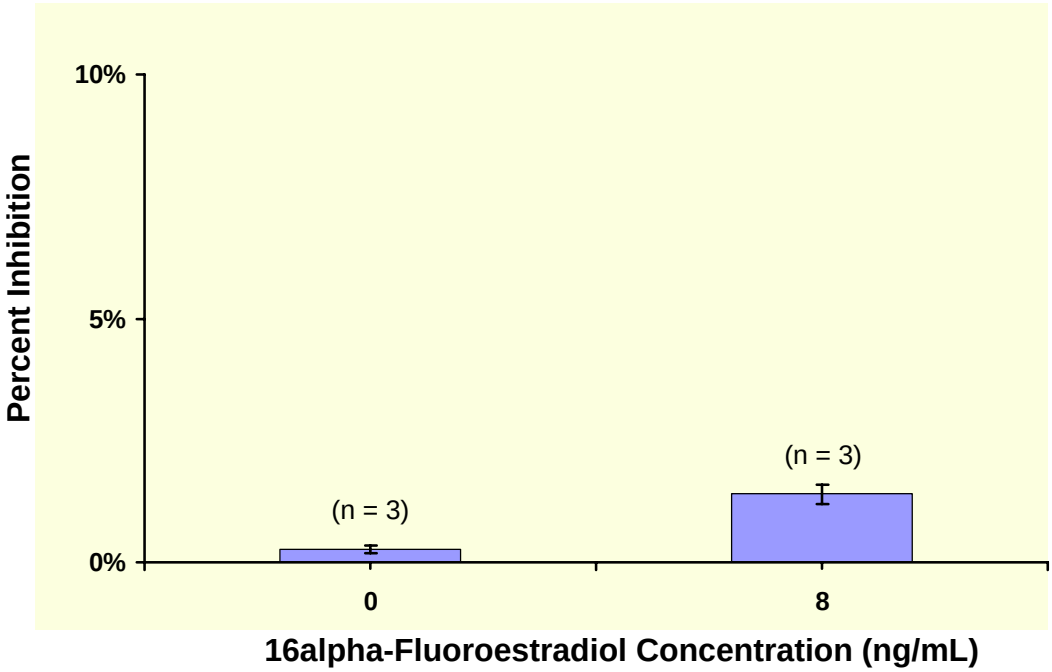


Figure 3: Concentration-response relationship
Percent inhibition of hERG potassium current after application of each concentration of 16alpha-Fluoroestradiol

13.2 Terfenadine effect on hERG potassium current

Table 3: Percent Inhibition at 60 nM Terfenadine

Cell ID (filename)	Terfenadine Concentration (nM)
	60
A 0 ZL 081208_0000_sub.abf	80.3%
B 0 ZL 081208_0000_sub.abf	80.5%

Table 4: Terfenadine Summary Statistics
Mean percent inhibition after application of 60 nM terfenadine (Mean), standard deviation (SD) and number of cells (N).

Terfenadine concentration (nM)	Mean	SD	N
60	80.4%	0.1%	2

14 Appendix A – Protocol, Protocol Amendment and Deviation

PROTOCOL

Effects of 16alpha-Fluoroestradiol on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

ChanTest Study Number:	080912.SUJ
Testing Facility:	ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128
Sponsor:	Center for Life Sciences and Toxicology RTI International 3040 Cornwallis Rd. RTP, NC 27709
Estimated Experiment Start Date:	Week of December 8, 2008
Estimated Experiment End Date:	Week of December 15, 2008
Estimated Draft Report to Sponsor Date:	Week of January 12, 2009

Note: Due to the nature of the experiments, conditions may arise to delay study completion. These conditions may include, but are not limited to: 1) cell health, 2) toxicity of test article, 3) solubility of test article, and 4) the inhibiting properties of test article. Every effort will be made to stay within the expected timeframe; however, the Sponsor will be informed if any difficulties arise.

Personnel

Testing Facility Study Director	Lisa M. Shyjka, BA ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128 Tel. 216-332-1665; Fax 216-332-1706 Email: lshyjka@chantest.com
Testing Facility Management	Glenn E. Kirsch, PhD ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128 Tel. 216-332-1665; Fax 216-332-1706 Email: gkirsch@chantest.com
Testing Facility Quality Assurance	Jennifer A. Scocos, MS, RQAP-GLP ChanTest Corporation 14656 Neo Parkway Cleveland, OH 44128 Tel. 216-332-1665; Fax 216-332-1706 Email: jscocos@chantest.com
Test Site Principal Investigator for Formulation Analysis	Jeanne Link, Ph.D. Associate Professor of Radiology Division of Nuclear Medicine Molecular Imaging Research Box 356004 Room NW041 UWMC University of Washington Seattle, WA 98195-6004 Telephone: 206-598-6256 Fax: 206-598-4192
Study Monitor	Kimberly Ehman, PhD Neurotoxicologist RTI International Life Sciences & Toxicology 3040 Cornwallis Rd., PO Box 12194 Research Triangle Park, NC 27709 Tel. 919.316.3802; Fax 919.541.5956 Email: kehman@rti.org

1 Introduction and Objective

The objective of this study is to examine the in vitro effects of 16alpha-Fluoroestradiol on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for I_{Kr}, the rapidly activating, delayed rectifier cardiac potassium current, Redfern et al., 2003). The concentration-response relationship of the effect of 16alpha-Fluoroestradiol on the hERG potassium channel current will be evaluated at near-physiological temperature in stably transfected mammalian cells that express the hERG gene.

2 Regulatory Compliance

The study will comply with the most recent version of the Food and Drug Administration (FDA) Good Laboratory Practices Regulations (21 CFR Part 58), with the following exceptions:

- The positive control article formulation will not be analyzed for stability, homogeneity, or concentration (its potency will be demonstrated by the comparison of results with ChanTest historical data).
- Sample analysis for homogeneity, stock and concentration verification will be conducted under non-GLP conditions.

The study will comply with ICH Guidance for Industry (July 2001), "S7A Safety Pharmacology Studies for Human Pharmaceuticals" and ICH Guidance for Industry (October 2005), "S7B Nonclinical Evaluation of the Potential for Delayed Repolarization (QT Interval Prolongation) by Human Pharmaceuticals." The study will be conducted in accordance with published procedures (Kirsch et al., 2004) and with the Standard Operating Procedures (SOPs) of ChanTest Corporation.

2.1 Sponsor Responsibilities

The Sponsor is responsible for the following information:

1. Documentation on the strength, purity, composition, physical properties, stability, and other pertinent information on the bulk test article in the form of a Certificate or Record of Analysis for inclusion in the final report. The Sponsor will provide a Certificate of Stability or other documentation for the bulk test article for inclusion in the final report.
2. Stability, homogeneity and concentration of the formulated test article under conditions of use.
3. Amount of test article provided.

2.2 Protocol Amendments and Deviations

Modifications of the protocol will be done in consultation with the Study Monitor and documented as protocol amendments. Amendments to the agreed upon protocol will be documented and authorized according to ChanTest SOPs. A copy will be included in the final report as an appendix. Any deviations from the protocol will be documented, authorized and evaluated by the Study Director, and included in the final report as an appendix.

2.3 Quality Assurance Unit

The Quality Assurance Unit (QAU) at ChanTest will act as lead QAU and will inspect the study according to ChanTest SOPs. ChanTest QAU will provide inspection reports to the Study Director and Testing Facility Management. Inspection types and dates will be included in the final report.

3 Test and Control Articles

3.1 Test Article

Test Article ID:	16alpha-Fluoroestradiol
Lot Number:	260801
Form:	To be documented in the final report
Molecular Weight:	290.37 g/mol
Purity:	> 98%
Storage Conditions (bulk):	Frozen, desiccated, protected from light
Carrier:	Ethanol
Solubility (mg/mL):	To be documented in the final report

3.1.1 Concentration Selection and Rationale

Concentrations will be chosen to achieve a range of approximately 10% to 90% inhibition of hERG current, within the specifications of Section 5.3 (Experimental Procedures) of this protocol and the limits imposed by the physiochemical effects of the test article (e.g. solubility or cytotoxicity).

3.1.2 Test Article Disposition

Bulk test article, stock solutions and analytical samples remaining at the end of the study will be discarded after issuance of the final report and records of disposition will be filed with the study data.

3.1.3 Test Article Carrier

Name:	Ethanol
Source:	To be documented in the final report
Lot Number:	To be documented in the final report
Molecular Weight:	To be documented in the final report
Storage Conditions:	Room temperature
Rationale for Selection:	Previous results have shown that 0.3% Ethanol does not affect channel current (data on file at ChanTest Corporation).

3.2 Vehicle Control

The vehicle will consist of a HEPES-buffered physiological saline (HB-PS) solution (composition in mM): NaCl, 137; KCl, 4.0; CaCl2, 1.8; MgCl2, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use), [supplemented with 0.3% Ethanol]. Chemicals used in vehicle preparation will be

purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted and will be of ACS reagent grade purity or higher.

Name:	HB-PS + 0.3% Ethanol
Source:	ChanTest Corporation
HB-PS Batch Number:	To be documented in the final report
Storage Conditions:	Refrigerated
Rationale for Selection:	HB-PS provides the appropriate ionic composition for in vitro recording.

3.3 Positive Control Article

Name:	Terfenadine
Source:	Sigma-Aldrich
Lot Number:	To be documented in the final report
Molecular Weight:	To be documented in the final report
Purity (%):	To be documented in the final report
Storage Conditions (bulk compound):	To be documented in the final report
Rationale for Selection:	Previous results have shown that 60 nM terfenadine inhibits hERG potassium current by approximately 80% (data on file at ChanTest Corporation)

3.3.1 Positive Control Article and Reference Substance Carrier

Name:	DMSO
Source:	Sigma-Aldrich
Lot Number:	To be documented in the final report
Molecular Weight:	To be documented in the final report
Storage Conditions:	To be documented in the final report
Rationale for Selection:	Previous results have shown that 0.3% DMSO does not affect channel current (data on file at ChanTest Corporation).

3.4 Reference Substance

Name:	E-4031
Source:	Sigma-Aldrich
Lot Number:	To be documented in the final report
Molecular Weight:	To be documented in the final report
Purity (%):	To be documented in the final report
Storage Conditions:	To be documented in the final report
Rationale for Selection:	E-4031 selectively inhibits hERG potassium current with IC50 = 12 nM (data on file at ChanTest Corporation). A supramaximal inhibiting concentration of 500 nM will be used to eliminate the contribution of non-hERG currents.

3.5 Formulations

3.5.1 Positive Control and Reference Substance Formulations

Stock solutions of the positive control and reference substance will be prepared in dimethyl sulfoxide (DMSO), aliquoted for individual use, stored frozen, and used within one month. Final positive control and reference substance concentrations will be prepared fresh daily by diluting stock solutions in vehicle (final DMSO concentration, 0.3% v/v). Positive control article and reference substance formulations are solutions and, therefore, homogeneous, by definition. Homogeneity, concentration and stability analyses will not be performed. Their potency for hERG inhibition will be demonstrated by the comparison of results with ChanTest historical data.

3.5.2 Test Article Formulations

Stock solutions of the test article will be prepared in ethanol and stored refrigerated, protected from light. Test article concentrations will be prepared fresh daily by diluting stock solutions in vehicle final ethanol concentration, 0.3% v/v. To avoid precipitation near the solubility limit, test article formulations will be prepared at room temperature (15 - 30 °C) with sonication if necessary (the time required will be noted in the raw data). A description of the method for preparing test article formulations will be included in the final report.

3.5.3 Preparation of Vehicle and Formulation Samples

One set of vehicle control samples (blank, 3 mL, accurately measured, in duplicate) will be taken from the vehicle reservoir at the beginning of testing. One set of samples for homogeneity determination (3 mL each, accurately measured, in duplicate) will be aliquoted from the top, middle, and bottom of the test article formulation reservoir in the patch clamp perfusion apparatus after preparation at the beginning of testing.

Representative test article formulation samples for concentration verification will be collected on each day of testing. Samples of each test concentration (3 mL, accurately measured, in duplicate) will be aliquoted from the outflow of the perfusion apparatus.

Immediately after preparation, (1 mL, accurately measured, in duplicate) will be collected from the primary and secondary stocks (in 100% ethanol) at each concentration.

The above formulation samples will be stored refrigerated until completion of the electrophysiological phase of the study, at which time, one set of samples will be shipped to the University of Washington, Seattle for analysis. The duplicate set of samples will be retained at ChanTest as backup replacements.

The analytical samples will be shipped on ice packs to:

Jeanne Link, Ph.D.
Associate Professor of Radiology
Division of Nuclear Medicine
Molecular Imaging Research Box 356004
Room NW041 UWMC
University of Washington
Seattle, WA 98195-6004
Telephone: 206-598-6256
Fax: 206-598-4192

ChanTest will send notification of shipment to the Sponsor and to the Principal Investigator.

Disposition of any unused portion of the analytical samples sent to the test site will be the responsibility of the test site.

3.5.4 Analysis of Formulation Samples

The Principal Investigator shall be responsible for formulation sample analysis at the University of Washington, Seattle by high performance liquid chromatography. Analysis shall include concentration verification and homogeneity of the test article formulation samples provided by ChanTest.

4 Test System

Cells will be maintained in tissue culture incubators per ChanTest SOP. Stocks will be maintained in cryogenic storage. Cells used for electrophysiology will be plated in plastic culture dishes. Each culture dish will be identified by a notation of clone identity number, passage number, and date. These data will be retained in the study file.

4.1 Rationale for Selection of Ion Channel and Expression Systems

The cardiac potassium channel, hERG, is responsible for a rapid delayed rectifier current (I_{Kr}) in human ventricles. This channel has been selected for evaluation because inhibition of I_{Kr} is the most common cause of cardiac action potential prolongation by non-cardiac drugs (Brown and Rampe, 2000; Weirich and Antoni, 1998; Yap and Camm, 1999). Increased action potential duration causes prolongation of the QT interval and has been associated with a dangerous ventricular arrhythmia, torsade de pointes (Brown and Rampe, 2000). In this assay, hERG potassium channels are expressed in a human embryonic kidney (HEK293) cell line that lacks endogenous I_{Kr}.

4.2 HEK/hERG

Organism:	Homo sapiens
Designation:	293
Tissue:	Kidney; Transformed with adenovirus 5 DNA; Transfected with human-ether-a-go-go cDNA
Morphology:	Epithelial
Age/Stage:	Embryo
Source-Strain:	ATCC, Manassas, VA
Source-Sub Strain:	ChanTest Corporation, Cleveland, OH

4.3 Cell Culture Procedures

HEK293 cells were stably transfected with hERG cDNA. Stable transfectants have been selected by coexpression with the G418-resistance gene incorporated into the expression plasmid. Selection pressure will be maintained by including G418 in the culture medium. Cells will be cultured in Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 (D MEM/F-12) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 µg/mL streptomycin sulfate and 500 µg/mL G418. Cell line documentation and cell culture records will be maintained in ChanTest facility records.

5 Test Method

5.1 Treatment Groups

All experiments will be performed at near-physiological temperature (33 to 35° C). Each cell will act as its own control.

5.1.1 Positive Control Group

The positive control will be applied to at least two (2) cells ($n \geq 2$). The performance of the test system will be considered acceptable if application of the positive control elicits a response within ± 2 standard deviations from the historic average response obtained by ChanTest. Results outside this range will require an out-of-specification investigation before proceeding with the concentration-response experiments.

5.1.2 Concentration-Response Group

One concentration (8ng/mL) will be evaluated due to solubility limitations of the test article in HB-PS + 0.3% ethanol. This concentration is approximately 10 times the maximum possible human concentration. This concentration will be tested in at least three (3) cells ($n \geq 3$).

5.1.3 Vehicle Control Group

Vehicle control solution will be applied to at least three (3) cells ($n \geq 3$). Duration of application will be at least as long as the time necessary for the lowest effective test article concentration to reach steady-state inhibition (≤ 12 minutes).

5.2 Electrophysiological Procedures

Cells will be superfused with vehicle control solution. Micropipette solution for whole cell patch clamp recordings will be composed of (mM): potassium aspartate, 130; $MgCl_2$, 5; EGTA, 5; ATP, 4; HEPES, 10; pH adjusted to 7.2 with KOH. Micropipette solution will be prepared in batches, aliquoted, stored frozen, and a fresh aliquot thawed each day. The recording will be performed at a temperature of 33 to 35 ºC using a combination of in-line solution pre-heater, chamber heater, and feedback temperature controller. Temperature will be measured using a thermistor probe in the recording chamber. Micropipettes for patch clamp recording will be made from glass capillary tubing using a P 97 micropipette puller (Sutter Instruments, Novato, CA). A commercial patch clamp amplifier will be used for whole cell recordings. Before digitization, current records will be low-pass filtered at one-fifth of the sampling frequency.

5.3 Experimental Procedures

5.3.1 Concentration Response

Cells stably expressing hERG will be held at -80 mV. Onset and steady state inhibition of hERG potassium current due to 16alpha-Fluoroestradiol will be measured using a pulse pattern (Figure 1) with fixed amplitudes (conditioning prepulse: +20 mV for 1 s; repolarizing test ramp to –80 mV (0.5 V/s) repeated at 5 s intervals). Each recording will end with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM), to assess the contribution of endogenous currents. The remaining uninhibited current will be subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition.

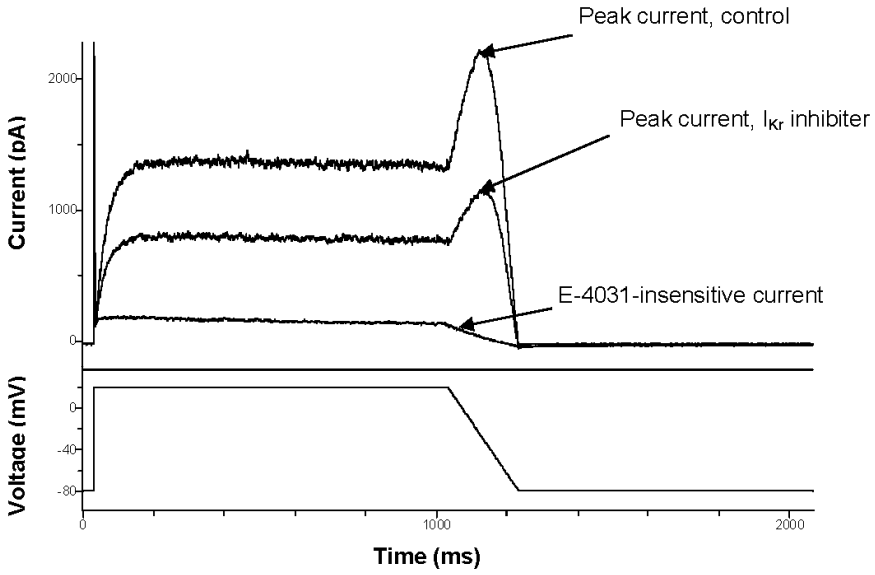


Figure 1: Typical hERG potassium current tracings recorded at 35 °C. Superimposed records in control after application of an inhibiter, and after 500 nM E-4031 application. Lower panel shows voltage stimulus (depolarizing prepulse, +20 mV; repolarizing test ramp, +20 mV to –80 mV at –0.5 V/s) repeated at 5 s intervals from a holding potential of –80 mV.

16alpha-Fluoroestradiol at 8 ng/mL will be applied to 3 cells and the effect on hERG potassium current amplitude will be monitored. If a steady state cannot be reached within 12 minutes, the response at 12 minutes will be substituted for the steady state value and a notation made in the final report. If the inhibitory response is less than 50%, then the study is complete. Peak current will be measured during the test ramp. A steady state will be maintained for at least 20 s before applying test article or positive control. Peak current will be measured until a new steady state is achieved or 12 minutes of exposure time have elapsed.

5.4 Electrophysiological Data Analysis

Data will be stored on the ChanTest computer network (and backed-up nightly) for off-line analysis. Data acquisition and analyses will be performed using the suite of pCLAMP (Version 8.2) programs (MDS-AT, Sunnyvale, CA), and will be reviewed by the Study Director. Percent inhibition at each concentration in the test group will be compared with the vehicle control group using one-way ANOVA followed by Dunnett's multiple comparison test (JMP Version 5.0.1, SAS Institute, Cary, NC). Significant inhibition will be defined at the level of $P<0.05$.

Steady state is defined by the limiting constant rate of change with time (linear time dependence). The steady state before and after test article application will be used to calculate the percentage of current inhibited at each concentration.

6 Reporting

During the course of the study, brief progress reports will be made available to the Sponsor upon request.

6.1 Analytical Report

The University of Washington, Seattle Principal Investigator shall submit to the Study Director a draft analytical report containing a summary of the analytical activities and the results of the concentration verification and homogeneity analysis of the test article formulation samples provided by ChanTest. The analytical report shall be written in English and shall contain a reference to the test method used in the analysis.

The Study Director will provide comments on the draft analytical report to the Principal Investigator in writing. After incorporation of the Study Director's comments, the Principal Investigator shall submit a final analytical report to the Study Director for inclusion in the final report.

6.2 Draft Report

An audited Draft Report of this study will be submitted to the Sponsor after review by the Study Director and QAU. The Sponsor shall submit comments on the Draft Report to the Study Director in writing. ChanTest will issue a Final Report within thirty (30) days following receipt of the Sponsor's comments. If no response to the Draft Report is received from the Sponsor within thirty (30) days, then ChanTest may issue the Final Report.

6.3 Final Report

The final report will include the effects of the test and control articles on the test system. The analytical report will be included as an appendix. The report also will include a compliance statement and a statement of quality assurance. The signed, final report shall be submitted electronically (PDF file). An unbound hardcopy and/or an electronic version on CD-ROM will be submitted upon request.

7 Archiving of Materials

The protocol (including amendments and deviations), the raw data collected by ChanTest (including but not limited to electronic electrophysiology records, data collection forms, and worksheets), and the final report shall be archived by ChanTest Corporation following ChanTest SOPs for on-site and off-site storage. ChanTest shall be responsible for the retention of such records for five years (or less, upon Sponsor request) following the study completion date, at which time the Sponsor will be contacted for final disposition. All data and the original analytical report generated by the University of Washington, Seattle will be archived at the University of Washington, Seattle.

8 Protocol Distribution

ChanTest

1. Original – Study File
2. Copy – QAU
3. Copy - Study Director
4. Copy - Staff Scientist

Additional signed copies of the final protocol will be provided to employees as required to complete the experimental and report writing phases of the study.

Sponsor

1. Copy

Test Site

1. Copy – Principal Investigator

9 References

Brown AM and Rampe D. (2000). Drug-induced long QT syndrome: is HERG the root of all evil? Pharmaceutical News 7, 15-20.

Kirsch GE, Trepakova ES, Brimecombe JC, Sidach SS, Erickson HD, Kochan MC, Shyjka LM, Lacerda AE, and Brown AM. (2004). Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. J Pharmacol Toxicol Methods 50, 93-101.

Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PK, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG. (2003). Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovascular Research. 58(1):32-45.

Weirich J and Antoni H. (1998). Rate-dependence of antiarrhythmic and proarrhythmic properties of class I and class III antiarrhythmic drugs. Basic Res Cardiol 93 Suppl 1, 125-132.

Yap YG and Camm AJ. (1999). Arrhythmogenic mechanisms of non-sedating antihistamines. Clin Exp. Allergy 29 Suppl 3, 174-181.

10 Protocol Approval

On behalf of RTI International

Kim Ehman, PhD
Study Monitor

12/3/08
Date

On behalf of the University of Washington, Seattle

Jeanne Link, PhD
Principal Investigator

12/2/08
Date

On behalf of ChanTest Corporation

Glenn E. Kirsch, PhD
Facility Management

12/4/08
Date

Lisa M. Shyjka, BA
Study Director

12/4/08
Date

Protocol Amendment No. 1

Study Title: **Effects of 16alpha-Fluoroestradiol on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells**

Modification #1	**Modification to the Final Protocol** Original: Page 2, Personnel Kimberly Ehman, PhD Neurotoxicologist RTI International Life Sciences & Toxicology 3040 Cornwallis Rd., PO Box 12194 Research Triangle Park, NC 27709 Tel. 919.316.3802; Fax 919.541.5956 Email: kehman@rti.org **Change:** Jay G. Henson, BS Life Sciences and Toxicology RTI International 3040 Cornwallis Rd. Research Triangle Park, NC 27709 Tel. 919-541-7206; Fax 919-541-5956 Email:jhenson@rti.org **Reason for Change** Personnel no longer at Sponsor site
Effective Date: 07-Jan-2009	**Impact Statement** This change will not impact study results.

Accepted for RTI International:

Jay G. Henson, BS — 08 Jan 09 — Date

Accepted for ChanTest Corporation:

Lisa Shyjka, BA — 08 Jan 09 — Date

Protocol Deviation No.1

Study Title: **Effects of 16alpha-Fluoroestradiol on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells**

Deviation from the Final Protocol
Original: Reference: Page 7, Section 3.5.4, Analysis of Formulation Samples

....University of Washington, Seattle by high performance liquid chromatography.

Deviation:

Samples were analyzed using mass spectrometry (MS)

Reason
The sample concentrations were to low to be detected under high performance liquid chromatography (HPLC).

Impact
There is no impact on the study. The correct procedure was used in sample concentration detection.

Accepted for ChanTest Corporation:

26-May-2009

Lisa M. Shyjka, BA
Study Director

Date

15 Appendix B – Test Article Certificate of Analysis

16alpha-Fluoroestradiol

Product no. 191.XXXX

For research purposes only. Not for human use or consumption.

Product description

16alpha-Fluoroestradiol; synonyms: 16alpha-fluoro-17beta-estradiol, FES; mol. wt. 290.37; $C_{18}H_{23}FO_2$; [92817-10-2]; BRN 3554942; chemical name: estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16alpha, 17beta). Colorless crystals, soluble in acetonitrile and chloroform.

Applications

16alpha-Fluoroestradiol may be used as a reference standard in the radiosynthesis of [^{18}F]Fluoroestradiol.

Presentation

Product 191.XXXX is available in 2 ml dark glass vials (DIN 2R), packed under argon atmosphere. Vials are sealed with teflon-faced rubber stoppers and tear-off crimp caps. Bulk chemicals in quantities ≥ 100 mg are available in dark glass screw cap vials, flushed with argon atmosphere. The content of 16alpha-Fluoroestradiol in mg is defined by the four digit number replacing XXXX in the product number. Weighing error is ± 5 %, but in maximum 0.5 mg.

Storage and stability

Store the product desiccated at –20 ± 5 °C, protected from light. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

Toxicology/Hazards

Handle with care, avoid inhalation, ingestion, eye or skin contact, no toxicological data available.

Certificate of analysis

Lot No.: 260801		Product No.: 191.XXXX	
Parameter	Method	Specification	Result
Appearance	organoleptic	colorless crystals	conforms
Melting pt	capillary	180-210 °C	187.3-188.1 °C
Identity	^{1}H-NMR ^{19}F-NMR	conforms conforms	conforms conforms
Purity	HPLC	> 90 %	> 98 %

No further analytical data available

Manufacturing Date: Aug. 2006

ABX advanced biochemical compounds
Biomedizinische Forschungsreagenzien GmbH

Quality Control date: 09-Nov-06

Dr. B. Schmitt

This document does not exempt you from performing the standard control upon receipt of incoming goods !

This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereof.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

References

1) Stalford A. C. *et al.:* The metabolism of 16-fluoroestradiols in vivo: chemical strategies for restricting the oxidative biotransformations of an estrogen-receptor imaging agent. *Steroids*. **1997**, *62*, 750-761.
2) Römer J. *et al.:* Further ^{13}C NMR spectroscopic proof of 16alpha-F configuration in 16-fluoroestradiol derivatives. *Forschungszent. Rossendorf, [Ber.] FZR* **1997**, *165*, 192-193.
3) Mankoff D. A. *et al.:* [^{18}F]Fluoroestradiol Radiation Dosimetry in Human PET Studies. *J. Nucl. Med.* **2001**, *42*, 679-684.

JMS-10/24/08
LNS
10-24-08

ABX *advanced biochemical compounds* - Biomedizinische Forschungsreagenzien GmbH. ·H.-Gläser-Str. 10-14 · D-01454 Radeberg
Germany · E-mail: info@abx.de · Internet: http://www.abx.de. · Tel.: +49 / 3528 / 40 41 60 · Fax: +49 / 3528 / 40 41 65

Version 2.0a, 19.Sep. 2008 ***ABX*** *advanced biochemical compounds*

16alpha-Fluoroestradiol

NEW: Product no. 1910.XXXX, OLD: Product no. 191.XXXX Lot. 260801

For research purposes only. Not for human use or consumption.

VALID ONLY IN CONNECTION WITH ORIGINAL CERTIFICATE OF ANALYSIS

Retest certificate of analysis

The following parameters included in the original certificate of analysis have been retested to confirm the stability of the product or are newly introduced in the quality control of the product as they may be considered to be suitable for detection of indicators of decay and guarantee that the product still is in compliance with the original specification:

Lot No.: 260801		Product No.: 191.XXXX	
Retest-Parameter	Method	Retest specification	Result
Appearance	organoleptic	no change in color	conforms
Purity	^{1}H-NMR ^{19}F-NMR	no change in spectrum no change in spectrum	conforms Conforms

Further testing was not considered to be necessary because of absence of significant changes in parameters tested.

Date of retest: **15. Oct. 2008**
Expiry Date: **15. Oct. 2009**

Storage and stability

Store the product desiccated at -20 °C, protected from light. Product is at least one more year stable at -20 °C. Long term stability was not determined. Short term (< 7 days) storage at higher temperatures (< 25 °C) does not affect product quality.

ABX advanced biochemical compounds **Biomedizinische Forschungsreagenzien GmbH**

Quality Control Bernd Feist date: 17-Oct-08
Dr. Bernd Feist

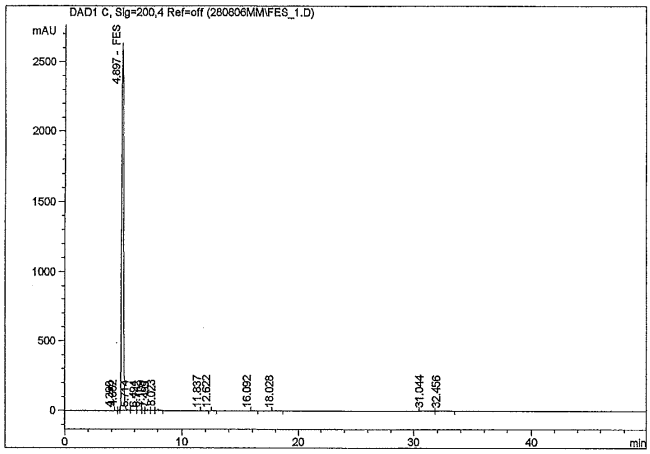
This document does not exempt you from performing the standard control upon receipt of incoming goods !
This product has been manufactured according to the regulations applicable at the site of manufacture. It is a chemical with defined specifications as declared in the certificate of analysis – which deems suitable as a starting material for the synthesis of drugs or diagnostics depending on the validated processes used for manufacture thereoff.
The quality of a potential final pharmaceutical product has to be checked by the producer, the quality of the product is only partially determined by the quality of the ingredients.
The substance is not intended and suitable to be used directly and/or unprocessed in humans.
The customer has to ensure himself that he is in compliance with all applicable legal requirements from all competent authorities for the site of use.
In particular it is emphasized that drugs/diagnostics/radiopharmaceuticals that are not registered/approved by the competent authorities might only be used in tight circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

Freigabeprotokoll_FES-Standard_260801_V1-1.doc Dr. Bettina Schmitt
ABX GmbH

19. Juli 2006
Version 1.1

HPLC

HPLC-Analyse bei:DAD1 C, Sig=200,4 Ref=off



ABX advanced biochemical compounds

Sample Name: FES_191_260801 Vial 3

Raw data file name:D:\DATA\2006\280806MM\FES_1.D

Instrument Name: ABX-HPLC-02
Injection Date: 8/28/2006 Injection Time: 10:35:24 PM
Operator: Thieme

#	Name	Ret. Time	Are	Area %
1		4.390	5.159	0.016
2		4.652	54.985	0.172
3	FES	4.897	31576.758	98.520
4		5.714	41.679	0.130
5		6.494	26.023	0.081
6		6.742	17.094	0.053
7		7.109	68.320	0.213
8		7.499	5.924	0.018
9		8.023	98.434	0.307
10		11.837	7.910	0.025
11		12.622	1.829	0.006
12		16.092	4.334	0.014
13		18.028	15.479	0.048
14	MMSE	0.000	0.000	0.000
15		31.044	72.851	0.227
16		32.456	54.456	0.170

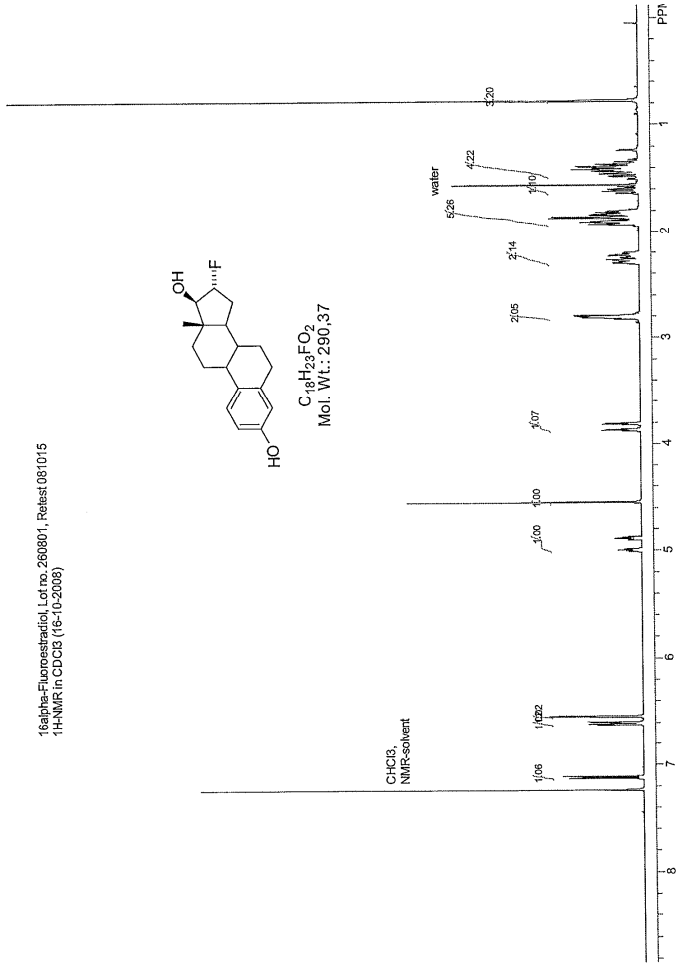
LMS 10/24/08
LNS 10-24-08

17. Sep. 2008
Version 2.0

ABX GmbH
^{1}H-NMR

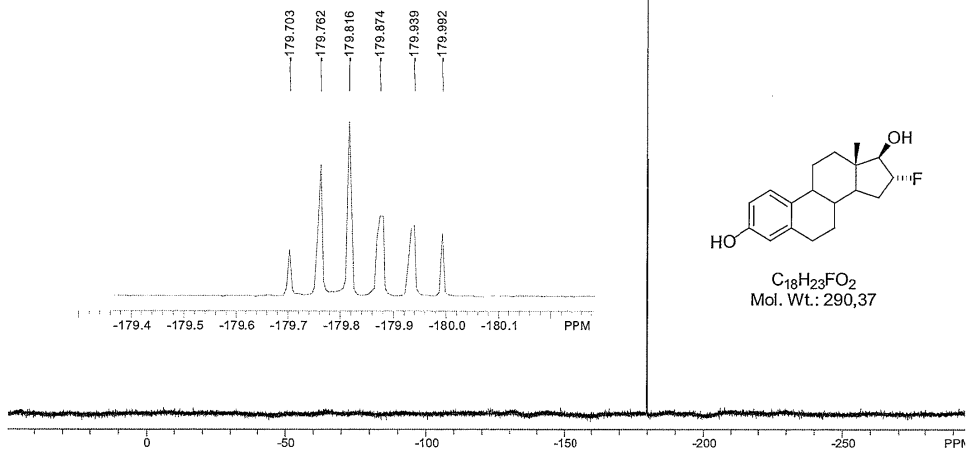
Retestprotokoll_FES-Srandard_260801_081015_V2-0.doc
Dr. Bernd Feist

16alpha-Fluoroestradiol, Lot no. 260801, Retest 081015
1H-NMR in CDCl3 (16-10-2008)



2/3

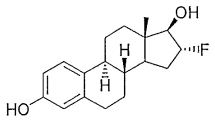
16alpha-Fluoroestradiol, Lot no. 260801, Retest 081015,
19F-NMR in CDCl3 (16-10-2008)



$C_{18}H_{23}FO_2$
Mol. Wt.: 290,37

16a-Fluoroestradiol

Reference standard for 16a-[^{18}F]Fluoroestradiol

Product Number	**1910**
Chemical name	CA index name: Estra-1,3,5(10)-triene-3,17-diol, 16-fluoro-, (16a,17ß)
Synonyms	16a-Fluoro-13ß-methyl-1,3,5(10)-gonatriene-3,17ßdiol; 16a-Fluoro-17ß-estradiol; 16a-Fluoroestradiol
CAS RN	[92817-10-2]
M. F.	$C_{18}H_{23}FO_2$
Mol. Wt.	290.37
Structure	
Characteristics	Colourless crystals
Presentation	**Packaged in dark glass screw cap vials, argon flushed.**
Certificate	CoA; ^{1}H and ^{19}F NMR spectra
Purity	> 90 %
Order Number	**1910.0001: 1 mg** per vial **1910.0002: 2 mg** per vial **1910.0010: 10 mg** per vial **Please inquire for customized filling and bulk quantities**
Literature	1. Mankoff D.A. *et al.* [^{18}F]Fluoroestradiol Radiation Dosimetry in Human PET Studies. *J. Nucl. Med.* **2001**, *42*, 679-684. 2. Stalford A.C. *et al.* The metabolism of 16-fluoroestradiols in vivo: chemical strategies for restricting the oxidative biotransformations of an estrogen-receptor imaging agent. *Steroids*. **1997**, *62*, 750-761. 3. Roemer J. *et al.* Further ^{13}C NMR spectroscopic proof of 16alpha-F configuration in 16-fluoroestradiol derivatives. *Forschungszent. Rossendorf, [Ber.] FZR* **1997**, *165*, 192-193.

LMS 10/24/08 LNS 10-24-08

16 Appendix C – Analytical Report (Certificate of Analysis)

University of Washington
PET Radiochemistry

Certificate of Analysis
Certificate No. RC-002

Study Number: 080912.SUJ "ChanTest"

The following samples were analyzed following good laboratory practices following established protocol NCI-Q319 for analysis of fluoroestradiol by HPLC with adaptations for sample matrix and increased concentrations as validated for the FES toxicity study. These measures were made using MS detection as per NCI-Q319.

Sample No.	Matrix	Date Sample Prepared	Nominal Concentration (µg/mL)	Sample Storage
1	ethanol	12/8/08	300	freezer
3	ethanol	12/8/08	10	freezer
5	HB-PS + 0.3% ethanol	12/8/08	0	refrigerator 2-8°C
7	HB-PS + 0.3% ethanol	12/8/08	0.008	refrigerator 2-8°C
9	HB-PS + 0.3% ethanol	12/8/08	0.008	refrigerator 2-8°C
11	HB-PS + 0.3% ethanol	12/8/08	0.008	refrigerator 2-8°C
13	HB-PS + 0.3% ethanol	12/8/08	0.008	refrigerator 2-8°C

Sample No.	Date Received	Date Analyzed	Nominal Concentration (µg/mL)	Measured Concentration (µg/mL)
1	12/10/08	12/12/08 and 12/19/08	300	317 ± 27
3	12/10/08	12/12/08 and 12/19/08	10	9.1 ± 0.4
5	12/10/08	12/19/08, 2/2/09. 2/3/09	0	ND
7	12/10/08	12/11/08, 12/19/08 2/2/09. 2/3/09	0.008	0.012± 0.003
9	12/10/08	12/11/08, 12/19/08 2/2/09. 2/3/09	0.008	0.012 ± 0.002
11	12/10/08	12/11/08, 12/19/08 2/2/09. 2/3/09	0.008	0.013 ± 0.002
13	12/10/08	12/11/08, 12/19/08 2/2/09. 2/3/09	0.008	0.014 ± 0.002

University of Washington
PET Radiochemistry

Study Number: 080912.SUJ "ChanTest" (continued).

Procedural variations: The Chan Test samples were near limit of detection for this method. The salt solution, HB-PS was not considered in the original validation for the toxicity testing and the sensitivity of the MS changed with time with this salt. Therefore, rather than running the samples in triplicate, standard curves were run pre- and post- each sample analysis and each sample was analyzed with full curves multiple (4 to 6) times. Some of the analyses on 12/11/08 and 12/12/08 had standard curves that were not valid. Volumes injected were increased in later sample analyses to increase sensitivity and to obtain valid analyses. The mean and standard deviation from all of the assays with valid standard curves were calculated for each sample.

Analysis performed by: _____ DATE: 4/10/09
Jeanne Meyers Link, PhD
Analytical and Radio-Chemist