FMISO TOXICOLOGY STUDIES – TWO FINAL REPORTS

Cancer Imaging Program
Division of Cancer Treatment and Diagnosis
National Cancer Institute
6130 Executive Blvd. MSC 7412, Suite 6000
Bethesda, MD 20892-7412

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I. Integrated Summary of Toxicology Studies Reported

The results of two recently completed fluoromisonidazole (FMISO) toxicology studies: 1) the 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment; and 2) the Bacterial Reverse Mutation Assay, conducted for the Cancer Imaging Program, National Cancer Institute are provided here.

The objective of the repeat dose toxicology study was to assess the toxicity, including micronucleus assessment, of FMISO administered by intravenous injection at dosage levels of 39 and 153 µg/kg/day to 2 groups of 5 male and 5 female Sprague-Dawley (CD® IGS) rats once daily for 14 consecutive days. There were 2 control groups. Animals were observed twice daily for mortality and moribundity, and clinical examinations were performed at least once daily immediately after dosing. There were no signs of toxicity at the doses tested on this study; no clinical observations noted; and no test article-related changes in body weights or feed consumption. Changes in clinical pathology parameters were not test article-related nor adverse, and there were no test article-related organ weight changes. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for intravenous administration of FMISO to rats for 14 consecutive days was greater than 153 µg/kg/day.

The Bacterial Reverse Mutation Assay tested fluoromisonidazole 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. In the first phase, for the initial toxicity-mutation assay, the dose levels tested were 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Neither precipitate nor appreciable toxicity was observed. Based on the findings, the maximum dose plated in the confirmatory mutagenicity assay was 3.75 µg per plate. In the second phase, for the confirmatory mutagenicity assay, the dose levels tested were 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate; again, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Neither precipitate nor appreciable toxicity was observed. Fluoromisonidazole was concluded to be negative in the Bacterial Reverse Mutation Assay.

Both studies were conducted in accordance with U.S. FDA Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies, 21 CFR Part 58. The test article characterization and test article formulation stability analyses were the responsibility of the Sponsor. Certificates of Analysis for the test article were provided by the suppliers. A direct impact statement was included for the genotoxicity report that while BioReliance could not confirm if the test article characterization and stability analyses were conducted with the GLP regulations, this had no adverse impact on the integrity of the data or the validity of the study conclusion.

II. Study Summaries

A. Repeat Dose Toxicology Study: 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

B. Genotoxicity Study: Bacterial Reverse Mutation Assay

A. Repeat Dose Toxicology Study Summary

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

Performed at: RTI International
Pharmacology and Toxicology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

Study Director: Brenda Faiola, Ph.D., DABT

GLP Compliance: This study was conducted in compliance with U.S. FDA Good Laboratory Practice for Nonclinical Laboratory Studies, 21 CFR Part 58, and AAALAC accreditation standards. RTI, through the administration of a quality assurance program, assesses compliance of all phases of toxicological studies with existing regulations (21 CFR Part 58). The IND Sponsor is responsible for GLP compliance of test article characterization and test article dose formulation stability analyses. The RTI Animal Research Facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Summary: Fluoromisonidazole (FMISO) in vehicle (0.9% sodium chloride for injection, USP:absolute ethanol, USP; approximately 95%:5%, v:v) was administered at dosage levels of 39 and 153 µg/kg/day to 2 groups (Groups 2 and 3, respectively) of 5 male and 5 female CD® IGS rats each by intravenous injection once daily for 14 consecutive days. A concurrent control group (Group 1) received this same vehicle on a comparable regimen as the test article-treated groups. An additional group (Group 4) of male rats was administered cyclophosphamide (positive control article for the micronucleus assay) in sterile water for injection, USP at a dosage level of 30 mg/kg by a single intraperitoneal injection on the last day of dosing. The dosing volume was 2.0 mL/kg for Groups 1 through 3 and 5.0 mL/kg for Group 4. Animals were observed twice daily for mortality and moribundity. Clinical examinations were performed at least once daily immediately after 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 (hematology and serum chemistry) parameters were evaluated using terminal samples collected from all animals in Groups 1 – 3 at necropsy. Complete necropsies were conducted on all animals in Groups 1 – 3, the day after the final dose was administered, and selected organs were weighed and/or retained in fixative. Selected tissues were examined microscopically from all animals in Groups 1 and 3. Bone marrow smear slides were prepared from all animals in Groups 1 – 4 for evaluation of micronucleus induction. 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. Changes in clinical pathology parameters were not test article-related nor adverse. There were no test article-related 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 for rats of the age and strain used on this study. Therefore, based on the results of this study, the no-observed-adverse-effect level (NOAEL) for intravenous administration of FMISO to rats for 14 consecutive days was greater than 153 µg/kg/day.

B. Genotoxicity Study Summary

Study Title: Bacterial Reverse Mutation Assay

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

Study Director: Valentine O. Wagner, III, M.S.

GLP Compliance: This study was conducted in compliance with U.S. FDA Good Laboratory Practice for Nonclinical Laboratory Studies, 21 CFR Part 58, in all material aspects with the following exceptions: BioReliance could not confirm if the test article characterization and stability analyses of the formulated test article were conducted in compliance with the GLP regulation, as this was the responsibility of the IND Sponsor and manufacturer. The BioReliance Study Director's Impact Statement concluded that since the established specifications were met and the standard stock solution was acceptable over the period of dosing, there was no adverse impact on the integrity of the data or the validity of the study conclusion.

Summary: The test article, fluoromisonidazole, 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.

The solvent, 95%:5% (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP, was selected based on the Sponsor's request, solubility of the test article and compatibility with the target cells.

In the initial toxicity-mutation assay, the maximum dose tested was 3.75 µg per plate; this dose was achieved by diluting the Sponsor-provided standard stock solution at a concentration of 1.0 mg/mL to 0.075 mg/mL for use as the top concentration in dosing the assay and using a 50 µL plating aliquot. The dose levels tested were 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. 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 3.75 µg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. Neither precipitate nor appreciable toxicity was observed.

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

III. Attachments

Attachment 1: Final Study Report: 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment (Date of Report: June 13, 2011)

FINAL REPORT

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

Prepared for:

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

Prepared by:

RTI International*
Pharmacology and Toxicology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

Final Report Date: June 13, 2011

BOA No.: 28XS246

Task Order No.: 2

PoP: July 23, 2010 to June 30, 2011

RTI Project No.: 0211886.002

RTI Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

SIGNATURE PAGE

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

Brenda Faiola 13 June 2011

Brenda Faiola, Ph.D., DABT
Study Director
RTI International

Date

Approved by:

13 JUN 2011

Hernan Navarro, Ph.D.
Senior Director, Discovery Sciences
Test Facility Management

Date

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Study Director Statement

This study was conducted in accordance with the standard operating procedures of RTI International, the study protocol and amendments as approved by the Sponsor, and US Food and Drug Administration Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies, 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 including strength, purity, stability, identity, and uniformity. The Sponsor also holds responsibility for GLP compliance of test article dose formulation stability analyses. Certificates of Analysis for the test article and positive control article were provided by the suppliers (ABX Advanced Biochemical Compounds and Sigma-Aldrich, respectively). The RTI Animal Research Facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

The study was conducted on the dates shown below:

Study initiation date (date the Study Director signed the protocol)	18 October 2010
Experimental start date (date of collection of first study-specific data)	02 November 2010
Date of first dose	09 November 2010
Date of final dose	22 November 2010
Experimental completion date (latest date that a contributing scientist report was signed by the PI)	04 May 2011
Study completion	Date the Study Director signed this report

The objectives set forth in the study protocol were achieved, and as nothing occurred to affect adversely the quality or integrity of the study, I consider the data generated to be valid.

Brenda Faiola — 13 June 2011

Brenda Faiola, Ph.D., DABT
Sr. Research Toxicologist
Study Director

Date

Quality Assurance Statement

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

Sponsor: SAIC

Study Code: Rt10-FMIS

Protocol Number: RTI-1111

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.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Audit	October 15, 2010	October 15, 2010
Protocol Amendment Audit	October 19, 2010	October 19, 2010
Formulation Inspection	October 19 & 20, 2010	October 20, 2010
Protocol Amendment 2 Audit	October 26, 2010	October 26, 2010
Protocol Amendment 3 Audit	October 28, 2010	October 28, 2010
Quarantine Inspection	November 2, 2010	November 2, 2010
Formulation Inspection	November 4, 2010	November 4, 2010
Dosing Inspection	November 9, 2010	November 9, 2010
Protocol Amendment 4 Audit	November 11, 2010	November 11, 2010
Protocol Amendment 5 Audit	November 17, 2010	November 18, 2010
Necropsy Inspection	November 23, 2010	November 23, 2010
Protocol Amendment 6	December 6, 2010	December 6, 2010
Chemistry Data & Report Audit	March 3 & 4, 2011	March 14, 2011
Data Audit	March 21-23, April 5 & 7, 2011	April 11, 2011
Data Audit	April 14 & 15, 2011	April 15, 2011
Final Report Audit	May 18, 19, 26, 27, 2011	May 27, 2011

Prepared by:

Leslie Macdonald	6-10-11
Leslie Macdonald Quality Assurance Specialist	Date

Reviewed by:

Benjamin Rauscher	6/10/11
Ben Rauscher Quality Assurance Specialist	Date

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 generated by the test facility and the test sites, with the exception of BioReliance, 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 for all costs associated with their storage beyond one year from the issuance of the final report.

For the micronucleus assay conducted at BioReliance, all raw data, the protocol, amendments, all reports and correspondence as applicable to this portion of the study will be maintained by the BioReliance RQA unit headquartered at: BioReliance, 14920 Broschart Rd., Rockville, MD 20850, with the exception of the stained slides which will be shipped back to the Testing Facility, RTI, for archival with the RTI-1111 study records as noted above. For items archived at BioReliance, 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, and all study materials returned to the Sponsor or destroyed will first be copied onto electronic media and the electronic copy will be maintained in the BioReliance archives for a minimum of 10 years.

Key Study Personnel

Brenda Faiola, PhD, DABT	Study Director
Alyssa McIntyre, DVM, DACLAM	Laboratory Animal Sciences Director and Veterinarian
Jay G. Henson, BS	Study Coordinator
Donna B. Browning, BS	Materials Handling Facility Manager
Brian F. Thomas, PhD	Analytical Chemistry and Pharmaceutics
Leslie L. Macdonald, BS	QAU Specialist
Henry Wall, DVM, PhD	Principal Investigator, Histopathology Experimental Pathology Laboratories, Inc. 615 Davis Drive Suite 500 Durham, NC 27713
Douglas Neptun	Principal Investigator, Clinical Pathology Antech Diagnostics GLP 507 Airport Blvd., Suite 113 Morrisville, NC 27560
Ljubica Krsmanovic, PhD	Principal Investigator, Micronucleus Assay BioReliance Corporation 9630 Medical Center Drive Rockville, MD 20850
G. Craig Hill, PhD	Sponsor Representative SAIC-Frederick, Inc. CIP/DCTD/NCI/NIH 6130 Executive Boulevard, Room 3005 Bethesda, MD 20892-7412

1.0 Summary

Fluoromisonidazole (FMISO) in vehicle (0.9% sodium chloride for injection, USP:absolute ethanol, USP; approximately 95%:5%, v:v) was administered at dosage levels of 39 and 153 µg/kg/day to 2 groups (Groups 2 and 3, respectively) of 5 male and 5 female CD® IGS rats each by intravenous injection once daily for 14 consecutive days. A concurrent control group (Group 1) received this same vehicle on a comparable regimen as the test article-treated groups. An additional group (Group 4) of male rats was administered cyclophosphamide (positive control article for the micronucleus assay) in sterile water for injection, USP at a dosage level of 30 mg/kg by a single intraperitoneal injection on the last day of dosing. The dosing volume was 2.0 mL/kg for Groups 1 through 3 and 5.0 mL/kg for Group 4. Animals were observed twice daily for mortality and moribundity. Clinical examinations were performed at least once daily immediately after 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 (hematology and serum chemistry) parameters were evaluated using terminal samples collected from all animals in Groups 1 - 3 at necropsy. Complete necropsies were conducted on all animals in Groups 1 – 3, the day after the final dose was administered, and selected organs were weighed and/or retained in fixative. Selected tissues were examined microscopically from all animals in Groups 1 and 3. Bone marrow smear slides were prepared from all animals in Groups 1 – 4 for evaluation of micronucleus induction.

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. Changes in clinical pathology parameters were not test article-related nor adverse. There were no test article-related 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 for rats of the age and strain used on this study. Therefore, based on the results of this study, the no-observed-adverse-effect level (NOAEL) for intravenous administration of FMISO to rats for 14 consecutive days was greater than 153 µg/kg/day.

2.0 Study Objective

The purpose of this study was to assess the toxicity, including micronucleus induction, of fluoromisonidazole (FMISO) when administered by intravenous injection to Sprague-Dawley (CD® IGS) rats for 14 consecutive days.

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:	Fluoromisonidazole
Chemical Name:	1*H*-Imidazole-1-ethanol, α-(fluoromethyl)-2-nitro-
Synomyms:	1-Fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol; FMISO
CAS No.:	13551-89-8
Chemical Formula:	$C_6H_8FN_3O_3$
Lot Number:	20100401
Supplier:	ABX Advanced Biochemical Compounds H.-Gläser-Str. 10-14 D-01454 Radeberg Germany Telephone: +49-3528-40 41 60
Purity:	>97% by ^{1}H-NMR according to the Certificate of Analysis provided by the Supplier.
Storage Conditions:	Desiccated, frozen (approximately -20 ± 5°C), protected from light under argon or nitrogen atmosphere.
Stability:	Long-term stability not determined. Short-term (<7days) storage at higher temperatures (<25°C) does not affect product quality. Retest Date: April 2012.

3.2 Positive Control Article (for Micronucleus Assessment)

Sponsor Designation:	Cytoxan (positive control article)
Name:	Cyclophosphamide monohydrate

Supplier:	Sigma Aldrich, Inc. 3050 Spruce Street Saint Louis, MO 63103 USA Telephone: 800-325-5832
CAS No.:	6055-19-2
Product No.:	C0768
Lot No.:	079K1569
Purity:	100.5% by HPLC according to the Certificate of Analysis provided by the Supplier.
Stability:	Approximately 3 years (Retest Date: July 2012)
Storage Conditions:	Refrigerated (approximately 2-8°C)

3.3 Vehicle

The vehicle for administration to the control group (Group 1) and for preparation of the test article dosing formulations was 0.9% sodium chloride for injection, USP (Baxter Healthcare Corporation; Lot No. C806307):absolute ethanol, USP (Sigma-Aldrich; Lot No. 09496HM) (approximately 95%:5%, v:v). The vehicle for the positive control article was sterile water for injection, USP (Baxter Healthcare Corporation; Lot No. C805432). The expiration date (if available) and handling procedures, as well as other pertinent information, for these vehicles were documented in the study records.

3.4 Dose Preparation

Test article formulations were prepared once by diluting a 1 mg/mL standard stock solution. Adjustments were not be made for purity of the test article. The standard stock solution was stored in aliquots at approximately 0° to -20°C and was to have expired after 6 months at these conditions, based on the available stability information provided by the Sponsor. Vehicle and diluted test article formulations were stored refrigerated at approximately 2° to 8°C and were to have expired after 1 month at these conditions, based on the available stability information provided by the Sponsor. Details of the dose preparation method were included in the study file. The vehicle and test article formulations stored refrigerated were allowed to warm by storing at room temperature for at least 30 minutes prior to administration to the test system. The positive control article was formulated once, on the day of use.

3.5 Dose Analysis

A sample was collected from each vehicle and test article dose formulation on the date of preparation (i.e., date of dilution from the standard stock solution). The samples were analyzed for concentration by the RTI ACP group prior to being released for use on study. Concentrations of test article were determined by a validated high performance liquid chromatography with ultraviolet detection (HPLC/UV) method. The standard for acceptable concentration was that the mean of the analyzed samples must be within ± 15% of nominal. Homogeneity evaluation was not performed as the formulations were solutions.

The positive control article formulation was not analyzed for stability, homogeneity, or concentration.

3.6 Test System

Species and Strain. CD® IGS [Crl:CD(SD)] rat.

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

Animal Receipt and Acclimation. Nineteen (19) male and 17 female rats were received on November 2, 2010. Each animal was examined by RTI technical staff on the day of receipt. Each animal was observed for clinical signs and weighed twice during the acclimation period (upon receipt and on Day -1, prior to randomization). All animals were checked for viability twice daily during the acclimation period. All animals were examined by the veterinarian to assess general health status prior to release for use on study.

Age. The animals were approximately 50 to 51 days old upon receipt and approximately 57 to 58 days old at initiation of dosing (Study Day 0).

Weight. For toxicology group animals (Groups 1-3), male body weights ranged from 263.3 g to 296.8 g and female body weights ranged from 182.7 g to 209.2 g on the initial day of dosing (Study Day 0). Although many of the males were above the protocol-specified weight range at initiation of dosing, this deviation was considered not to have influenced the outcome of the study.

Number/Gender. Fifteen males and 15 females (5/sex) were randomized to the toxicology groups (Groups 1-3). Two males were randomized to the cyclophosphamide positive control group (Group 4). An additional 2 males and 2 females were available to serve as replacements if needed (these animals were not used on study).

Method of Identification. Each rat was uniquely identified by a microchip transponder (BioMedic Data Systems, Inc., Seaford, DE).

Animal Welfare. Nestlets (Ancare Corp., Bellmore, NY, USA) 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 (P.J. Murphy Forest Products, Montville, NJ) was used throughout the study. 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. Purina Certified Pelleted Rodent Diet® (No. 5002, PMI Feeds, Inc., St. Louis, MO) was available *ad libitum*. Feed lots used during the study are documented in the study records. 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. Available information on the diet does not indicate the presence of any substance at a concentration likely to have influenced the outcome of the study.

Water. Municipal tap water from the Durham, NC water system was available *ad libitum* throughout the study. Municipal water supplying the facility is regularly sampled for contaminants according to standard operating procedures. Analysis of the drinking water for chemical composition and possible contamination is also provided by the supplier once per year. Available information on the water does not indicate the presence of any substance at a concentration likely to have influenced the outcome of the study.

3.8 Environment

Environmental conditions were continuously monitored, controlled, and recorded by an automated system (Siebe/Barber-Colman Network 8000 System, with Signal® Software Version 4.4.1; Siebe Environmental Controls [SEC]/Barber-Colman Company, Loves Park, IL). Target conditions for temperature and humidity in the animal room were 64-79°F and 30-70%, respectively (NRC, 1996). Although the temperature was outside the indicated range on one

occasion, this deviation was minor and of short duration and was considered not to have influenced the health of the animals and/or the outcome of the study. Lighting controlled by light timers provided illumination for a 12-hour light (0600 hours to 1800 hours)/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. In addition, a significant historical database is available for comparative evaluation. 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 monitor for 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 vehicle and positive control group, were considered the minimum number of groups necessary to provide a range of effects and allow for appropriate data interpretation.

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 with an expected clinical use pattern that will involve 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 required to support human exposure of this same duration. The daily dose of the high dose (153 µg/kg) in rats is 100 times the maximum human dose on a surface area basis. 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.

3.10 Randomization to Study

Based on pretreatment procedures (e.g., body weight and clinical observation data), no animals were excluded from randomization to study groups by the Study Director. Animals were randomized to treatment groups by sex using stratified randomization using the Provantis 8™ (Instem LSS Ltd., Staffordshire, United Kingdom) 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	Dosing Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[1]	0	0	2.0	5	5
2	Fluoromisonidazole	39 μg/kg/day	19.5 μg/mL	2.0	5	5
3	Fluoromisonidazole	153 μg/kg/day	76.5 μg/mL	2.0	5	5
4	Cyclophosphamide[2]	30 mg/kg	6.0 mg/mL	5.0	2	0

[1] Vehicle = 95:5 (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP

[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, two 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).

Clinical Observations. Observations for clinical signs of toxicity were made once daily for each animal in Groups 1-3 immediately after dosing (Days 0 to 13) and prior to scheduled necropsy (Day 14). Observations included (but were 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. If clinical signs were noted at times other than immediately after dosing, these observations were also entered into the automated data capture system (Provantis 8™). Clinical observations were not recorded for Group 4 animals since there were no observations that suggested the general well being of the animals were compromised.

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 at the time of scheduled necropsy from Groups 1-3 via cardiac puncture following exposure to CO_2. Animals were fasted overnight prior to blood collection. Blood for hematology assessments (approximately 2 mL) was collected into tubes containing K_3EDTA as the anticoagulant. Blood for serum chemistry assessments (up to 3.5 mL) was collected into tubes with no anticoagulant, allowed to clot at room temperature, 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 to -80°C until submitted for analysis. All samples were submitted to Antech Diagnostics GLP (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)	Inorganic phosphate (PO_4)
Albumin/globulin (A/G Ratio)	Potassium (K)
Alkaline phosphates (ALP)	Serum alanine transaminase (ALT)
Blood urea nitrogen (BUN)	Serum aspartate transaminase (AST)
Calcium (Ca)	Serum glucose (GLUC)
Chloride (Cl)	Sodium (Na)
Cholesterol (CHOL)	Total bilirubin (TBIL)
Creatinine (CRE)	Total protein (TP)
Gamma-glutamyltransferase (GGT)	Triglycerides (TG)
Globulin (GLOB; calculated)	

3.14 Anatomic Pathology

Necropsy. A complete necropsy was conducted on Groups 1-3 on Day 14. Animals were fasted overnight prior to necropsy. Animals were euthanized by CO_2 asphyxiation and a terminal body weight was collected. Animals were exsanguinated via cardiac puncture. 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	Oviducts[6]
Aorta	Pancreas
Brain	Parathyroid glands
Bone (right femur with epiphyseal plate of head)	Pituitary gland
Sternum with bone marrow[3]	Prostate gland
Intestine, cecum	Intestine, rectum
Cervix	Salivary gland (mandibular)
Intestine, colon	Nerve, sciatic
Intestine, duodenum	Seminal vesicles
Eartag or transponder (animal Identification)[5]	Skeletal muscle (quadriceps femoris)
Epididymides	Skin (abdominal)
Esophagus	Spinal cord (thoracolumnar junction; entire cord if neurologic abnormalities present)
Eyes, with optic nerves[1]	Spleen
Gross lesions (including tissue masses and abnormal regional lymph nodes)	Stomach (fundic area)
Heart	Testes[1]
Intestine, ileum	Thymus
Injection site (of final IV dose on Day 13)[4]	Thyroid glands
Intestine, jejunum	Tongue
Kidneys	Trachea
Liver (right medial lobe and left lateral lobe)	Ureters
Lungs[2]	Urinary bladder[2]
Lymph node (mandibular and mesenteric)	Uterus (body)
Mammary gland (to include nipple and surrounding tissue)	Vagina
Ovaries	

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

[2] Infused with formalin to ensure fixation.

[3] The entire sternum was excised intact and placed in fixative for subsequent histologic processing and microscopic evaluation of bone marrow.

[4] The site was marked by encircling it using a permanent marker.

[5] Not examined microscopically

[6] Listed separately to allow for entry of finding(s) that may be noted at necropsy, as well as histologically if a portion of the oviduct was present in the section of either the ovaries or uterus that were examined microscopically; the entire oviduct from ovary to uterus was not excised whole and trimmed specifically.

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

Adrenals	Prostate gland
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus and cervix
Pituitary	

Paired organs (adrenals, kidneys, ovaries, and testes) were weighed together. The pituitary and thyroid/parathyroids were weighed following fixation. The thyroid/parathyroid weight was collected in Provantis™ 8 as "thyroid (fixed)".

3.15 Histopathology

Fixed tissues were sent to Experimental Pathology Laboratories, Inc. (EPL) in Durham, NC for processing. The prepared slides and associated documentation were subsequently transferred to EPL in Sterling, VA. Microscopic examination of hematoxylin-eosin stained paraffin sections was performed on the tissues listed in Section 3.14 for all animals in Groups 1 and 3. Deborah A. Banas, D.V.M., M.S., DABT, DACVP, was the study pathologist.

3.16 Micronucleus Assessment

On Study Day 14 (approximately 18-25.5 hours after the last dose administration), two bone marrow smear slides from the left femur were prepared from all toxicology animals (Groups 1-3) and from the two positive control males (Group 4) for *in vivo* clastogenicity/aneugenicity assessments (micronuclei determination). Details of the bone marrow smear procedure were included in the study records. Although the timing of the bone marrow slide preparation was greater than the protocol-specified range post for several animals, this deviation was minor and was considered not to have influenced the outcome of the study. 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

Provantis™ 8 automated data collection system was used for collection of all body weights (including quarantine), feed weights, clinical observations, organs weights, and gross necropsy findings. Provantis™ 8 also calculated the volume of dosing solution to be administered to each animal on each day, based on the most recent body weight and was used to record when each animal was dosed. The following types of data were analyzed separately at each time point (when applicable) using the Tables and Statistics module of Provantis™ 8:

- 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
- Organ weights, both absolute and adjusted for terminal body weight

For continuous data, Levene's Test (Levene, 1960) was applied to test for homogeneity of variances between the groups. If Levene's test was not significant at the $p<0.05$ level, the data were subjected to a one-way analysis of variance (ANOVA) followed by Dunnett's test if the overall ANOVA was significant. If the Levene's test was significant, then a Kruskal-Wallis (Kruskal and Wallis, 1952) test was used for the overall test and pairwise comparisons were performed using a nonparametric Dunnett's test.

For clinical pathology data, results were entered into the ClinAxys v2.2 computer system by the subcontractor, Antech Diagnostics GLP. Statistical evaluation of the clinical pathology data was performed by the subcontractor using SigmaStat software. The data was analyzed for normality followed by an ANOVA ($p<0.05$) and, if significant, a comparison of groups by Holm-Sidak. If the test for normality failed, the ANOVA was based on Kruskal-Wallis ANOVA on Ranks ($p<0.05$) and, if significant, Dunn's comparison of groups was used. Values are reported as means, standard deviation (SD) and number of samples (n). The SigmaStat program was not fully validated.

For the assessment of micronucleus induction potential, a statistical evaluation of the data was performed by the subcontractor, BioReliance Corp., using Kastenbaum-Bowman tables for a significance level of $p\leq0.05$.

4.0 Results and Discussion

4.1 Dose Formulations

Data: Appendix 1

Results of the analyses of dosing formulations are summarized below:

	Mean Concentration, µg/mL (% of Target)		
Date of Preparation	Group 1 (0 µg/mL)	Group 2 (19.5 µg/mL)	Group 3 (76.5 µg/mL)
November 4, 2010	Not Detected	19.4 (99.5%)	76.4 (99.9%)

Both test article dose formulations were within ± 10% of the nominal concentration and there was no test article detected in the vehicle formulation. The relative standard deviation (RSD) for each replicate determination was ≤ 10%. 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

Summary Data: Table 1

Individual Data: Appendix 5 – Tables 1, 2

All animals survived to the scheduled necropsy.

4.3 Clinical Observations

Summary Data: Tables 2, 3

Individual Data: Appendix 5 – Tables 1, 2

There were no test article-related clinical observations. All clinical findings in the test article-treated groups were limited to single animals or single occurrences, were not noted in a dose-related manner, and/or were common findings for laboratory rats of this age and strain.

4.4 Body Weights

Summary Data: Tables 4, 5, 6, 7

Individual Data: Appendix 5 – Tables 3, 4

There were no test article-related effects on body weights and body weight changes. There were no statistically significant differences during the treatment period when the control and test article-treated groups were compared.

4.5 Feed Consumption

Summary Data: Tables 8, 9, 10 ,11

Individual Data: Appendix 5 – Tables 5, 6

There were no test article-related effects on feed consumption. There were no statistically significant differences when the control and test article-treated groups were compared.

4.6 Clinical Pathology

Summary Data: Appendix 2, Section V

Individual Data: Appendix 2, Section VI

Hematology. There were no test article-related effects on hematology parameters. Statistically significant decreased mean absolute lymphocyte count was noted in the males given 39 µg/kg/day compared with the control group. This lymphopenia was not observed in a dose-related manner and therefore was not attributed to test article administration but rather to sample collection stress. Statistically significant decreased mean absolute and/or percent monocyte counts were noted in the males given 39 and 153 µg/kg/day compared with the control group. These changes were not considered adverse since relative changes in monocytes are not considered to be biologically significant, as there are no known direct relationships of peripheral circulating monocytes and toxic processes directly related to mature monocytes. In addition, monocytopenias are not considered biologically significant. It should be noted that the control group consisted of two samples due to clotting of the other three samples. The low number of samples in the control group may have contributed to the statistically significant differences in monocyte counts.

Serum Chemistry. There were no test article-related effects on serum chemistry parameters. Statistically significant decreased mean potassium and phosphorus were noted in the males given 39 and 153 µg/kg/day. Mean alanine transaminase (ALT) and aspartate transaminase (AST) were also decreased in the males given 39 (not statistically significant) and 153 µg/kg/day (statistically significant). These apparent clinical chemistry changes were not considered test article-related but rather attributed to an increase in the mean values for these parameters in the vehicle control group, due to serum hemolysis noted for one male in the control group. The hemolysis was likely *in vitro* hemolysis since no concurrent anemia was present in the control group. Mean glucose was statistically significantly increased in the females given 39 and 153 µg/kg/day. The increase in glucose was mild and likely the result of sample collection stress, not test article administration.

4.7 Anatomic Pathology

4.7.1 Macroscopic Examination

Summary Data: Tables 12, 13
Individual Data: Appendix 5 – Tables 7, 8

There were no test article-related macroscopic findings at the scheduled necropsy. All macroscopic findings noted were considered to be spontaneous and/or incidental in nature and unrelated to test article administration.

4.7.2 Organ Weights

Summary Data: Tables 14, 15
Individual Data: Appendix 5 – Tables 7, 8

There were no test article-related effects on organ weights. Statistically significant, decreased mean absolute brain weight and increased relative thyroid weight were noted in the males given 39 µg/kg/day compared with the control group. These differences were not considered the result of test article administration since the changes were not dose related.

4.7.3 Microscopic Examination

Summary Data: Tables 16, 17
Individual Data: Appendix 3

There were no test article-related microscopic findings. A variety of spontaneous lesions and incidental findings occurred in both test article-treated and vehicle-treated control rats. These findings were the usual number and type commonly seen in rats of this age and strain.

4.8 Micronucleus Assessment

Summary Data: Appendix 4, Table 1

Individual Data: Appendix 4, Table 2

The test article did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes (PCEs) at dose levels of 39 or 153 µg/kg/day compared with the vehicle control group. The positive control group did show 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. Therefore, the test article was concluded to have no genotoxic effect on rat bone marrow when intravenously administered for 14 consecutive days.

5.0 Conclusion

The objective of this study was to assess the toxicity of fluoromisonidazole (FMISO) when administered by intravenous injection to Sprague-Dawley CD® IGS 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 test article-related clinical observations were noted during the study. There were no test article-related changes in body weights or feed consumption. There were no test article-related changes in clinical pathology parameters and organ weights. 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. There was no test article-related effect on micronucleus induction. Therefore, based on the results of this study, the NOAEL for intravenous administration of FMISO to rats for 14 consecutive days was greater than 153 µg/kg/day.

6.0 References

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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.

- **Section 7.4** states that the animals will weigh approximately 225 to 275 grams for males and 175 to 225 grams at the initiation of dosing (Study Day 0) and that animals outside this range may be used at the discretion of the Study Director. Eleven of the 15 males weighed more than 275 grams on Day 0 and were included on study by the Study Director.
- **Section 7.10** states that target conditions for temperature and humidity in the animal room will be 64-79°C and 30-70%, respectively. The relative humidity on November 2, 2010 (Study Day -7) was 71.44% at 1100 and returned to within the protocol-specified range by 1200.
- **Section 9.6.2** states that at the time of necropsy, the mammary gland from all toxicology group animals (Group 1-3) will be collected and placed in 10% neutral-buffered formalin. At necropsy, the mammary gland was collected for the male and female toxicology group animals; however, gross findings (if any were present) for this tissue were inadvertently not recorded for the males.
- **Section 9.6.4** states that two bone marrow smear slides from the left femur will be prepared from all animals (Groups 1-4) for micronucleus assessment on Study Day 14 at approximately 18-24 hours after the last dose. On Study Day 14, necropsy start times (and thus bone marrow smear collection times) for 12 animals were outside the protocol-specified range (up to 85 minutes late). In addition, bone marrow smears for the cyclophosphamide positive control males were collected and documented on a paper form that did not have an entry for time of collection; these smears were collected approximately one hour late based on staff recollection.

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 Male and Female Animals

(Page 1 of 1)
Clinical Observations - Cumulative Mortality
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Week Number	0 ug/kg/day		39 ug/kg/day		153 ug/kg/day		30 mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
0	0	0	0	0	0	0	0	NA
1	0	0	0	0	0	0	0	NA
2*	5	5	5	5	5	5	2	NA

* = Week 2 Scheduled Euthanasia

Table 2. Summary of Male Clinical Observations Clinical Observations - Severities by Period (With Animal Count)

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Clinical Sign	Severity	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	m	ANIMALS ALIVE		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		ANIMALS NORMAL		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
2	m	ANIMALS ALIVE		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		ANIMALS NORMAL		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
3	m	ANIMALS ALIVE		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		ANIMALS NORMAL		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
4	m	ANIMALS ALIVE		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		ANIMALS NORMAL		.	.	.	.	.	.	.	.	.	.	.	.	.	.	0
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cycloph

Table 3. Summary of Female Clinical Observations

(Page 1 of 1)
Clinical Observations - Severities by Period (with Animal Count)
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Clinical Sign	Severity	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	f	ANIMALS ALIVE		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		ANIMALS NORMAL		5	5	5	5	5	5	5	5	5	5	5	4	5	5	5
		Diarrhea	Present	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
2	f	ANIMALS ALIVE		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		ANIMALS NORMAL		5	5	5	5	5	5	5	5	5	5	4	4	4	4	4
		Sore(s) on Body	Present	.	.	.	.	.	.	.	.	.	.	1	1	1	1	1
			TOTAL	.	.	.	.	.	.	.	.	.	.	1	1	1	1	1
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
3	f	ANIMALS ALIVE		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		ANIMALS NORMAL		5	5	5	3	4	4	4	4	4	4	4	4	4	4	4
		Sore(s) on Body	Present	.	.	.	2	1	1	1	1	1	1	1	1	1	1	1
			TOTAL	.	.	.	2	1	1	1	1	1	1	1	1	1	1	1
		Scheduled Removal (Terminal)	Present	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
			TOTAL	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 4. Summary and Statistical Analysis of Male Body Weights

(Page 1 of 1)
Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Bodyweight (g)

Sex: Male Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
-7	Mean	204.84	204.94	206.54
	SEM	0.88	2.98	2.27
	N	5	5	5
-1	Mean	272.20	271.88	272.94
	SEM	4.37	4.94	5.09
	N	5	5	5
0	Mean	282.60	277.48	280.48
	SEM	4.02	5.60	5.42
	N	5	5	5
6	Mean	339.22	322.94	334.46
	SEM	5.83	8.78	7.92
	N	5	5	5
13	Mean	388.90	362.98	379.72
	SEM	9.79	11.37	14.46
	N	5	5	5
14[1]	Mean	364.28	342.32	353.78
	SEM	8.36	8.98	12.50
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)
[1] Fasted weight taken after euthanasia

Table 5. Summary and Statistical Analysis of Female Body Weights

(Page 1 of 1)
Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Bodyweight (g)

Sex: Female Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
-7	Mean	160.62	158.70	159.62
	SEM	2.09	3.77	2.14
	N	5	5	5
-1	Mean	196.20	196.44	194.16
	SEM	3.39	3.41	3.07
	N	5	5	5
0	Mean	197.06	197.96	194.04
	SEM	4.65	2.60	3.17
	N	5	5	5
6	Mean	205.72	212.78	207.44
	SEM	6.75	2.50	1.67
	N	5	5	5
13	Mean	216.56	229.12	221.24
	SEM	8.71	1.87	3.93
	N	5	5	5
14[1]	Mean	204.90	212.70	204.64
	SEM	8.30	3.58	3.83
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)
[1] Fasted weight taken after euthanasia

Table 6. Summary and Statistical Analysis of Male Body Weight Changes

Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter

RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Absolute Weight Gain (g)

Sex: Male Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
-7 → -1	Mean	67.36	66.94	66.40
	SEM	4.85	2.33	4.91
	N	5	5	5
-1 → 0	Mean	10.40	5.60 **	7.54
	SEM	0.84	0.93	0.79
	N	5	5	5
0 → 6	Mean	56.62	45.46	53.98
	SEM	2.80	4.54	3.21
	N	5	5	5
6 → 13	Mean	49.68	40.04	45.26
	SEM	4.26	3.89	6.55
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)

Table 7. Summary and Statistical Analysis of Female Body Weight Changes

Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter

RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Absolute Weight Gain (g)

Sex: Female Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
-7 → -1	Mean	35.58	37.74	34.54
	SEM	1.51	2.46	4.19
	N	5	5	5
-1 → 0	Mean	0.86	1.52	-0.12
	SEM	1.57	1.42	1.53
	N	5	5	5
0 → 6	Mean	8.66	14.82	13.40
	SEM	3.53	1.63	1.67
	N	5	5	5
6 → 13	Mean	10.84	16.34	13.80
	SEM	2.41	2.11	3.27
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)

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

Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter

RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Food Mean Daily Consumption (g/day)

Sex: Male Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
0 → 6	Mean	29.27	28.11	29.59
	SEM	0.77	1.10	0.95
	N	5	5	5
6 → 13	Mean	31.09	28.05	29.78
	SEM	0.94	0.96	2.09
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)

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

Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter

RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Food Mean Daily Consumption (g/day)

Sex: Female Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
0 → 6	Mean	17.12	18.56	17.69
	SEM	0.76	0.47	0.55
	N	5	5	5
6 → 13	Mean	17.24	19.22	19.38
	SEM	0.81	0.62	1.08
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)

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

Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter

RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Food g/kg/day

Sex: Male Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
0 → 6	Mean	94.124	93.498	96.204
	SEM	1.700	1.552	1.888
	N	5	5	5
6 → 13	Mean	85.393	81.756	82.992
	SEM	1.847	1.147	3.360
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)

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

(Page 1 of 1)
Generalized Results ╦ Group Summary by Time ╦ Fixed Parameter
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Food g/kg/day

Sex: Female Day(s) Relative to Start Date		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
0 → 6	Mean	84.874	90.504	88.081
	SEM	1.773	3.178	1.919
	N	5	5	5
6 → 13	Mean	81.646	87.046	90.347
	SEM	2.248	3.050	4.596
	N	5	5	5

Statistical Test: Generalized Anova/Ancova Test Transformation: Identity (No Transformation)

Table 12. Summary of Male Macroscopic Necropsy Findings

(Page 1 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	MALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
adrenal glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
aorta;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
bone marrow, sternum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
bone marrow smear;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
bone, femur;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
brain;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
epididymides;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
esophagus;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 12. Summary of Male Macroscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	MALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
eyes with optic nerves;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
heart;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
injection site;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, cecum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, colon;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, duodenum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, ileum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, jejunum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 12. Summary of Male Macroscopic Necropsy Findings

(Page 3 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	MALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
intestine, rectum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
kidneys;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	4	5
dilation; right	0	1	0
liver;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
lungs;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
lymph node, mesenteric;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
lymph node, mandibular;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
mammary glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	0	0	0
skeletal muscle, quadriceps femoris;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 12. Summary of Male Macroscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	MALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
nerve, sciatic;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
parathyroid glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
pituitary gland;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
prostate gland;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
salivary gland, mandibular;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
seminal vesicles;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
skin;			
Submitted	(0)	(1)	(0)
No Visible Lesions	0	0	0
crust; brown	0	1	0
skin, abdominal;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 12. Summary of Male Macroscopic Necropsy Findings

(Page 5 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	MALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
spinal cord;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
spleen;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
stomach, fundic;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
testes;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
thymus;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
thyroid glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
tongue;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
trachea;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 12. Summary of Male Macroscopic Necropsy Findings

(Page 6 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	MALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
ureters;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
urinary bladder;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 13. Summary of Female Macroscopic Necropsy Findings

(Page 1 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	FEMALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
adrenal glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
aorta;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
bone marrow, sternum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
bone marrow smear;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
bone, femur;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
brain;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
cervix;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
esophagus;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 13. Summary of Female Macroscopic Necropsy Findings

(Page 2 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Removal Reason: Killed Terminal	FEMALES		
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
eyes with optic nerves;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
heart;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
injection site;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, cecum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, colon;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, duodenum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, ileum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
intestine, jejunum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 13. Summary of Female Macroscopic Necropsy Findings

(Page 3 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Removal Reason: Killed Terminal	FEMALES		
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
intestine, rectum;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
kidneys;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
liver;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
lungs;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
lymph node, mesenteric;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
lymph node, mandibular;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
mammary glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
skeletal muscle, quadriceps femoris;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 13. Summary of Female Macroscopic Necropsy Findings

(Page 4 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Removal Reason: Killed Terminal	FEMALES		
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
nerve, sciatic;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
ovaries;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
oviducts;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
pancreas;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
parathyroid glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
pituitary gland;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
salivary gland, mandibular;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
skin;			
Submitted	(1)	(1)	(0)
No Visible Lesions	0	0	0
crust; brown; dorsal; multiple	1	0	0

Table 13. Summary of Female Macroscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Removal Reason: Killed Terminal	FEMALES		
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
skin; (continued)			
crust; dorsal	0	1	0
skin, abdominal;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
spinal cord;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
spleen;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
stomach, fundic;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
thymus;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
thyroid glands;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
tongue;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 13. Summary of Female Macroscopic Necropsy Findings

(Page 6 of 6)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

	FEMALES		
Removal Reason: Killed Terminal			
	39 ug/kg/day	153 ug/kg/day	0 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
trachea;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
ureters;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
urinary bladder;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
uterus;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5
vagina;			
Submitted	(5)	(5)	(5)
No Visible Lesions	5	5	5

Table 14. Summary and Statistical Analysis of Male Organ Weights and Relative Organ Weights

(Page 1 of 3)
Generalized Results ╤ Group Summary by Parameter ╤ Fixed Time
RT1O-FMIS - 14-Day Intravenous Repeat
Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Day 14 Scheduled Necropsy

Sex: Male		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
Terminal BW (g)	Mean	364.28	342.32	353.78
	SEM	8.36	8.98	12.50
	N	5	5	5
Adrenal Glands Wt (g)	Mean	0.08290	0.07652	0.08144
	SEM	0.00249	0.00745	0.00584
	N	5	5	5
Adrenals Wt Ratio (%)[1]	Mean	0.02278	0.02228	0.02316
	SEM	0.00070	0.00194	0.00192
	N	5	5	5
Brain Wt (whole) (g)	Mean	2.14246	2.02252 *	2.07680
	SEM	0.02563	0.04154	0.01414
	N	5	5	5
Brain Wt Ratio (%)[1]	Mean	0.58963	0.59312	0.58983
	SEM	0.01745	0.02400	0.02034
	N	5	5	5
Heart Weight (g)	Mean	1.44604	1.43292	1.40524
	SEM	0.08596	0.03665	0.05513
	N	5	5	5
Heart Ratio (%)[1]	Mean	0.39743	0.41898	0.39872
	SEM	0.02424	0.00860	0.01858
	N	5	5	5
Kidney Wt (pair) (g)	Mean	3.40608	3.15440	3.35948
	SEM	0.06470	0.08656	0.13751
	N	5	5	5
Kidney Ratio (%)[1]	Mean	0.93786	0.92284	0.94976
	SEM	0.03494	0.02670	0.02164
	N	5	5	5
Liver Weight (g)	Mean	13.68558	12.43994	12.96392
	SEM	0.46924	0.69564	0.61956
	N	5	5	5
Liver Ratio (%)[1]	Mean	3.75698	3.62314	3.66291
	SEM	0.09787	0.10888	0.11378
	N	5	5	5

* - Statistical Test: Dunnett 2 Sided p < 0.05]

[1] Relative organ weight = organ weight/terminal body weight × 100

Table 14. Summary and Statistical Analysis of Male Organ Weights and Relative Organ Weights

(Page 2 of 3)
Generalized Results ╦ Group Summary by Parameter ╦ Fixed Time
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Day 14 Scheduled Necropsy

		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
Pituitary Wt (fixed) (g)	Mean	0.01240	0.01328	0.01364
	SEM	0.00094	0.00053	0.00095
	N	4	5	5
Pituitary Ratio (fix) (%)[1]	Mean	0.00340	0.00388	0.00385
	SEM	0.00030	0.00014	0.00021
	N	4	5	5
Prostate Weight (g)	Mean	1.20804	1.03674	1.02326
	SEM	0.03913	0.06671	0.06779
	N	5	5	5
Prostate Ratio (%)[1]	Mean	0.33193	0.30502	0.28862
	SEM	0.01048	0.02552	0.01216
	N	5	5	5
Spleen Weight (g)	Mean	0.74374	0.73524	0.71838
	SEM	0.01456	0.02055	0.03747
	N	5	5	5
Spleen Ratio (%)[1]	Mean	0.20462	0.21476	0.20348
	SEM	0.00624	0.00153	0.01043
	N	5	5	5
Testis Wt (paired) (g)	Mean	3.25404	3.15234	3.20488
	SEM	0.06779	0.10473	0.10413
	N	5	5	5
Testis Ratio (%)[1]	Mean	0.89634	0.92168	0.91205
	SEM	0.03542	0.02766	0.05093
	N	5	5	5
Thymus Weight (g)	Mean	0.66216	0.65764	0.52530
	SEM	0.02808	0.06973	0.02807
	N	5	5	5
Thymus Ratio (%)[1]	Mean	0.18272	0.19225	0.14834
	SEM	0.01123	0.02054	0.00528
	N	5	5	5
Thyroid Wt (fixed) (g)	Mean	0.01634	0.01898	0.01542
	SEM	0.00065	0.00058	0.00149
	N	5	5	5

* - Statistical Test: Dunnett 2 Sided p < 0.05]

[1] Relative organ weight = organ weight/terminal body weight × 100

Table 14. Summary and Statistical Analysis of Male Organ Weights and Relative Organ Weights

(Page 3 of 3)

Generalized Results ╦ Group Summary by Parameter ╦ Fixed Time
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Day 14 Scheduled Necropsy

		0 ug/kg/day FMISO		39 ug/kg/day FMISO		153 ug/kg/day FMISO
Thyroid Ratio (fix) (%)[1]	Mean	0.00450		0.00556	*	0.00433
	SEM	0.00023		0.00024		0.00032
	N	5		5		5

* - Statistical Test: Dunnett 2 Sided p < 0.05]

[1] Relative organ weight = organ weight/terminal body weight × 100

Table 15. Summary and Statistical Analysis of Female Organ Weights and Relative Organ Weights

(Page 1 of 3)
Generalized Results ╦ Group Summary by Parameter ╦ Fixed Time
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Day 14 Scheduled Necropsy

		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
Terminal BW (g)	Mean	204.90	212.70	204.64
	SEM	8.30	3.58	3.83
	N	5	5	5
Adrenal Glands Wt (g)	Mean	0.07802	0.07035	0.08404
	SEM	0.00551	0.00702	0.00654
	N	5	4	5
Adrenals Wt Ratio (%)[1]	Mean	0.03835	0.03306	0.04115
	SEM	0.00311	0.00376	0.00332
	N	5	4	5
Brain Wt (whole) (g)	Mean	1.87542	1.93620	1.89178
	SEM	0.05012	0.02776	0.04310
	N	5	5	5
Brain Wt Ratio (%)[1]	Mean	0.91765	0.91073	0.92468
	SEM	0.01723	0.01188	0.01579
	N	5	5	5
Heart Weight (g)	Mean	0.81690	0.85730	0.86884
	SEM	0.06239	0.04720	0.05097
	N	5	5	5
Heart Ratio (%)[1]	Mean	0.39650	0.40225	0.42481
	SEM	0.01475	0.01633	0.02422
	N	5	5	5
Kidney Wt (pair) (g)	Mean	1.91896	2.02162	1.97804
	SEM	0.10691	0.08851	0.07164
	N	5	5	5
Kidney Ratio (%)[1]	Mean	0.93429	0.95020	0.96689
	SEM	0.01687	0.03735	0.03212
	N	5	5	5
Liver Weight (g)	Mean	7.44592	7.72016	7.29724
	SEM	0.47621	0.29851	0.36161
	N	5	5	5
Liver Ratio (%)[1]	Mean	3.62201	3.62474	3.55838
	SEM	0.09887	0.07856	0.11302
	N	5	5	5

* - Statistical Test: Dunnett 2 Sided p < 0.05]

[1] Relative organ weight = organ weight/terminal body weight × 100

Table 15. Summary and Statistical Analysis of Female Organ Weights and Relative Organ Weights

(Page 2 of 3)
Generalized Results ╦ Group Summary by Parameter ╦ Fixed Time
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Day 14 Scheduled Necropsy

		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
Ovary Wt (pair) (g)	Mean	0.12920	0.13826	0.13432
	SEM	0.00967	0.01657	0.01801
	N	5	5	5
Ovary Ratio (%)[1]	Mean	0.06383	0.06551	0.06572
	SEM	0.00627	0.00856	0.00869
	N	5	5	5
Pituitary Wt (fixed) (g)	Mean	0.01562	0.01464	0.01374
	SEM	0.00066	0.00104	0.00079
	N	5	5	5
Pituitary Ratio (fix) (%)[1]	Mean	0.00763	0.00689	0.00672
	SEM	0.00014	0.00051	0.00042
	N	5	5	5
Spleen Weight (g)	Mean	0.53726	0.52264	0.53532
	SEM	0.03520	0.03609	0.03549
	N	5	5	5
Spleen Ratio (%)[1]	Mean	0.26152	0.24531	0.26098
	SEM	0.00908	0.01458	0.01432
	N	5	5	5
Thymus Weight (g)	Mean	0.48842	0.54898	0.48782
	SEM	0.04406	0.04519	0.04084
	N	5	5	5
Thymus Ratio (%)[1]	Mean	0.23750	0.25801	0.23743
	SEM	0.01720	0.02064	0.01632
	N	5	5	5
Thyroid Wt (fixed) (g)	Mean	0.01548	0.01408	0.01340
	SEM	0.00139	0.00060	0.00095
	N	4	5	5
Thyroid Ratio (fix) (%)[1]	Mean	0.00768	0.00663	0.00654
	SEM	0.00103	0.00030	0.00043
	N	4	5	5
UterusCervix Weight (g)	Mean	0.69994	0.92926	0.65622
	SEM	0.08270	0.12833	0.03662
	N	5	5	5

* - Statistical Test: Dunnett 2 Sided p < 0.05]

[1] Relative organ weight = organ weight/terminal body weight × 100

Table 15. Summary and Statistical Analysis of Female Organ Weights and Relative Organ Weights

(Page 3 of 3)

Generalized Results ╥ Group Summary by Parameter ╥ Fixed Time
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Day 14 Scheduled Necropsy

Sex: Female		0 ug/kg/day FMISO	39 ug/kg/day FMISO	153 ug/kg/day FMISO
UterusCervix Ratio (%)[1]	Mean	0.34128	0.43854	0.32017
	SEM	0.03823	0.06328	0.01475
	N	5	5	5

* - Statistical Test: Dunnett 2 Sided p < 0.05]

[1] Relative organ weight = organ weight/terminal body weight × 100

Table 16. Summary of Male Microscopic Necropsy Findings

(Page 1 of 7
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
adrenal glands;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	4	0	4	0
vacuolation; cortical cells	1	0	1	0
aorta;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
bone marrow, sternum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
bone, femur;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
bone, sternum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
brain;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
epididymides;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
esophagus;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0

Table 16. Summary of Male Microscopic Necropsy Findings

(Page 2 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
eyes;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
heart;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	3	0	4	0
infiltration; mononuclear cell; focal	2	0	1	0
injection site;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	2	0	2	0
hemorrhage; adjacent	2	0	3	0
inflammation, subacute; adjacent	3	0	3	0
intestine, cecum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
intestine, colon;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
intestine, duodenum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
intestine, ileum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0

Table 16. Summary of Male Microscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
intestine, jejunum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
intestine, rectum;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
kidneys;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	1	0	2	0
dilatation; pelvis; unilateral	0	0	1	0
dilatation; tubule; unilateral	2	0	0	0
infarction; unilateral; focal	0	0	1	0
regeneration; tubule; epithelium	2	0	1	0
infiltration; mononuclear cell	4	0	2	0
liver;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	0	0	0	0
hyperplasia; bile duct	3	0	2	0
inflammation, chronic; multifocal	5	0	5	0
necrosis; coagulative; focal	1	0	0	0
vacuolation; hepatocyte; centrilobular	2	0	2	0
lungs;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	1	0	0	0
foamy alveolar macrophages; multifocal	0	0	1	0
hair embolus	1	0	1	0
inflammation; interstitium; multifocal	1	0	2	0
inflammation; granulomatous; focal	1	0	1	0

Table 16. Summary of Male Microscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
lungs; (continued)				
mineralization; vascular	3	0	3	0
lymph node, mesenteric;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
lymph node, mandibular;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	3	0	4	0
plasmacytosis	2	0	1	0
mammary glands;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
skeletal muscle, quadriceps femoris;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
nerve, optic;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
nerve, sciatic;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
pancreas;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	4	0	5	0
atrophy; acinar cell; focal	1	0	0	0

Table 16. Summary of Male Microscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
parathyroid glands;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	4	0	5	0
pituitary gland;				
Examined	(4)	(0)	(5)	(0)
Within Normal Limits	4	0	5	0
Not Examined: MISSING	1	0	0	0
prostate gland;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	4	0	4	0
infiltration; mononuclear cell	1	0	1	0
salivary gland, mandibular;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
seminal vesicles;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
skin;				
Examined	(0)	(0)	(1)	(0)
Within Normal Limits	0	0	0	0
crust formation	0	0	1	0
erosion; focal	0	0	1	0
inflammation, subacute	0	0	1	0
skin, abdominal;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0

Table 16. Summary of Male Microscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
spinal cord;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
spleen;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
stomach, fundic;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
testes;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
thymus;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	4	0
atrophy	0	0	1	0
thyroid glands;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
tongue;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
trachea;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	4	0	5	0

Table 16. Summary of Male Microscopic Necropsy Findings

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	MALES			
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day	30 mg/kg
Number of Animals on Study :	5	5	5	2
Number of Animals Completed:	(5)	(5)	(5)	(2)
trachea; (continued)				
dilatation; mucosal glands	1	0	0	0
ureters;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0
urinary bladder;				
Examined	(5)	(0)	(5)	(0)
Within Normal Limits	5	0	5	0

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 1 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
adrenal glands;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
aorta;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
bone marrow, sternum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
bone, femur;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
bone, sternum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
brain;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
cervix;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
esophagus;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 2 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
eyes;			
Examined	(5)	(0)	(5)
Within Normal Limits	3	0	4
dysplasia; retina	2	0	1
heart;			
Examined	(5)	(0)	(5)
Within Normal Limits	4	0	4
infiltration; mononuclear cell; focal	1	0	1
injection site;			
Examined	(5)	(0)	(4)
Within Normal Limits	4	0	1
Not Examined: MISSING	0	0	1
hemorrhage; adjacent	1	0	2
inflammation, subacute; adjacent	1	0	2
intestine, cecum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
intestine, colon;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
intestine, duodenum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
intestine, ileum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 3 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
intestine, jejunum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
intestine, rectum;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
kidneys;			
Examined	(5)	(0)	(5)
Within Normal Limits	2	0	1
inflammation; interstitium; unilateral; focal	1	0	1
mineralization; tubule	2	0	4
infiltration; mononuclear cell	1	0	0
liver;			
Examined	(5)	(0)	(5)
Within Normal Limits	0	0	0
hyperplasia; bile duct	3	0	2
inflammation, chronic; multifocal	5	0	5
vacuolation; hepatocyte; periportal	0	0	1
lungs;			
Examined	(5)	(0)	(5)
Within Normal Limits	4	0	4
inflammation; interstitium; multifocal	1	0	1
mineralization; vascular	0	0	1
lymph node, mesenteric;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 4 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
lymph node, mandibular;			
Examined	(5)	(0)	(5)
Within Normal Limits	2	0	3
plasmacytosis	3	0	2
mammary glands;			
Examined	(5)	(0)	(4)
Within Normal Limits	5	0	4
Not Examined: MISSING	0	0	1
skeletal muscle, quadriceps femoris;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	4
infiltration; mononuclear cell; focal	0	0	1
nerve, optic;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
nerve, sciatic;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
ovaries;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
oviducts;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 5 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
pancreas;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
parathyroid glands;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
pituitary gland;			
Examined	(5)	(0)	(5)
Within Normal Limits	4	0	5
cyst	1	0	0
salivary gland, mandibular;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
skin;			
Examined	(0)	(0)	(1)
Within Normal Limits	0	0	0
crust formation	0	0	1
erosion; focal	0	0	1
inflammation, subacute	0	0	1
cyst; subcutaneous	0	0	1
skin, abdominal;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
spinal cord;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 6 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
spleen;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
stomach, fundic;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
thymus;			
Examined	(5)	(0)	(5)
Within Normal Limits	4	0	5
atrophy	1	0	0
thyroid glands;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
tongue;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
trachea;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5
ureters;			
Examined	(4)	(0)	(5)
Within Normal Limits	4	0	5
Not Examined: MISSING	1	0	0
urinary bladder;			
Examined	(4)	(0)	(5)

Table 17. Summary of Female Microscopic Necropsy Findings

(Page 7 of 7)
Pathology - Intergroup Comparison of Gross/Histo Pathology Observations
RT1O-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES		
Removal Reason: Killed Terminal	0 ug/kg/day	39 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5
Number of Animals Completed:	(5)	(5)	(5)
urinary bladder; (continued)			
Within Normal Limits	4	0	5
Not Examined: MISSING	1	0	0
uterus;			
Examined	(5)	(0)	(5)
Within Normal Limits	4	0	5
dilation; lumen	1	0	0
vagina;			
Examined	(5)	(0)	(5)
Within Normal Limits	5	0	5

Appendix 1

Analysis of Dosing Formulations (RTI International)

ANALYTICAL CHEMISTRY REPORT

RTI Project No.: 0211886.002.002
RTI Protocol No.: RTI-1111
RTI Study Code.: Rt10-FMIS

Fluoromisonidazole in 0.9 % Sodium Chloride for Injection, USP: Absolute Ethanol, USP (95:5, v:v) Formulation Results

Formulation Date: November 4, 2010

Prepared By:

4/14/11
Richard C. Daw, MChem. Date
Chemist
Analytical Chemistry and Pharmaceutics

Approved By:

4/20/2011
Brian F. Thomas, Ph.D. Date
Senior Director
Analytical Chemistry and Pharmaceutics

20 April 2011
Brenda Faiola, Ph.D. DABT Date
Study Director
Pharmacology and Toxicology

SUMMARY

One set of dose formulation samples was prepared and analyzed for concentration verification for RTI Project 0211886.002, Study No. RTI-1111. Information on the dose formulation preparation procedures and the stability of the dose formulations was the responsibility of, and provided to RTI, by the Sponsor.

The formulation analyses reported here were performed following the validated analytical method "Analysis of Fluoromisonidazole in 0.9% Sodium Chloride for Injection, USP:Absolute Ethanol, USP (95:5, v:v) Formulations" (RTI Analytical Method AM-0211886-002) to verify the FMISO concentration in 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v) formulations prepared on November 4, 2010. All dose formulations analyzed were found to be within ± 10% of the nominal concentration.

ANALYTICAL METHOD

The validated analytical method "Analysis of Fluoromisonidazole in 0.9% Sodium Chloride for Injection, USP:Absolute Ethanol, USP (95:5, v:v) Formulations "(RTI Analytical Method AM-0211886-002) used to analyze study samples is described briefly below.

On the day of dose formulation preparation, one sample of each concentration (0, 19.5, and 76.5 μg/mL) was collected for analysis. Each dose formulation analysis sample was mixed thoroughly and approximate 1 mL aliquots from the middle of the sample were transferred in triplicate to separate 2-mL amber glass vials. The triplicate aliquots were analyzed on a high performance liquid chromatograph (HPLC) with a PDA detector (Table 1) along with a series of vehicle standards used to generate a calibration curve. Vehicle standards were prepared by diluting an approximately 1 mg/mL FMISO standard stock solution in sterile water for injection, USP: absolute ethanol (95:5, v:v; prepared on October 20, 2010 and stored frozen at approximately -20°C) with 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v) to make a 100 μg/mL intermediate vehicle stock solution which also served as the highest concentration standard for the calibration curve. The intermediate vehicle stock solution was diluted with blank vehicle to prepare duplicate vehicle standards at six lower concentrations in order to create a calibration curve which encompassed the concentration range of the dose formulations (10-100 μg/mL). Test article concentrations were calculated using a least squares linear regression equation that fit the relationship between the nominal concentrations of vehicle standard and the detector response. The dose formulation sample concentrations were determined in μg/mL.

Table 1 HPLC System

Instrumentation				
Instrument:	Waters 2695 Alliance HPLC			
Detector:	Waters 2996 Photodiode Array Detector			
Column:	Thermo Fisher Aquasil C_{18} 2.1 x 150-mm (5-µm)			
Data System:	Waters Empower 2, Build 2154			
Conditions				
Mobile Phase Flow Rate	0.3 mL/min			
Column Heater:	30°C			
Wavelength Detected:	230-400 nm, extracted 325 nm.			
Gradient Program:	Time (min)	%A	%B	
	-	100	0	
	12	100	0	
Mobile Phase:	A: 10 mM formic acid in water:methanol (95:5, v:v) B: water:methanol (80:20, v:v)			
Injection Volume:	10 µL			

FORMULATION ANALYSIS

The formulation analyses were performed following the analytical method described above to verify the FMISO concentration in 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v) formulations mixed on November 4, 2010. On the formulation date, three formulations were tested for concentration; the nominal concentration of these three formulations were 0 (vehicle), 19.5, and 76.5 µg/mL. For concentration verification, the found concentration for each formulation was evaluated for accuracy and the triplicate determinations were evaluated for precision. Analytical results are presented in Attachment 1.

Note: Values presented in this report have been rounded to the correct number of significant digits based upon the accuracy of the initial laboratory observations; however, all mathematical and statistical computations within a single mode of calculation have been performed on non-rounded values in order to minimize error in the final result due to rounding. Thus, some values and summary statistics may not be accurately reproduced using the rounded intermediate values which appear here.

CONCLUSION

Both test article dose formulations analyzed were found to be within ± 10% of the nominal concentration and no test article was detected in the vehicle formulation. The relative standard deviation (RSD) for each replicate determination was ≤ 10%.

ATTACHMENT 1

Dose Formulation Analysis Final Results Report

FORMULATION ANALYSIS FINAL REPORT

(Concentration Verification)

Study Project No.: 0211886.002.002
Study Code: Rt10-FMIS
Test Article: Fluoromisonidazole
Formulation Date: 11/4/10
Vehicle: 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v)
Analysis Date: 11/4/10

Rx Code/ Color Code	RTI Log Number	Sample Description	Nominal Conc.[a]	Found Conc.[a,b]	Percent of Nominal[c]	Mean Found Concentration[a,b,d]
66861/Red	13253-16A	Analysis sample	0	ND	ND	ND
				ND	ND	
				ND	ND	
45531/Blue	13253-16B	Analysis sample	19.5	19.4	99.5	19.4 ± 0.0376 (0.19% RSD)
				19.4	99.6	
				19.4	99.3	
24355/Yellow	13253-16C	Analysis sample	76.5	76.4	99.9	76.4 ± 0.0171 (0.022% RSD)
				76.4	99.9	
				76.4	99.9	

[a]Concentration unit: µg/mL
[b]Each formulation sample was analyzed in triplicate.
[c]Percent of Nominal: 100 + [((FMISO Found Conc. – FMISO Nominal Conc.)/FMISO Nominal Conc.) x 100]
[d]Mean found concentration ± standard deviation and % RSD of n=3 results shown. Found concentration was determined with the linear regression equation (non-weighted):
y = 73374.18x – 1067.298; r = 1.0000 for calibration range from 10.0 µg/mL to 100 µg/mL
ND = Not Detected.

Appendix 2

Clinical Pathology
(Antech Diagnostics GLP)

ANTECH
DIAGNOSTICS

Report of Clinical Pathology Results

for
14-Day Intravenous Repeated Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

RTI International Study RTI-1111

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

For

RTI International
Pharmacology and Toxicology
3040 Cornwallis Road
Research Triangle Park, NC 27709

Written and approved by:

Doug Neptun, Laboratory Director — Date: 12/22/10

Janice Andrews, DVM, Clinical Pathologist — Date: 12/22/10

ANTECH DIAGNOSTICS GLP
507 AIRPORT BLVD · SUITE 113 · MORRISVILLE, NC 27560
Ph. 919-277-0822 · Fax 919-277-0825

Good Laboratory Practices Statement

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

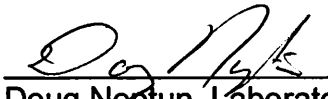
Study Number: RTI-1111

Timeperiod	Collection Date	Date Samples were received (at Antech Diagnostics)	Date Samples were analyzed
Day 14	11/23/10	11/23/10	11/23/10 & 11/24/10

Study Activities at Antech Diagnostics:
Start Date: 11/23/10 Completion Date of Analysis: 11/24/10

As Principal Investigator, I confirm that the clinical pathology portion of this study performed at Antech Diagnostics was in compliance with the Nonclinical Laboratory Studies Good Laboratory Practice Regulations issued by the U.S. Food and Drug Administration (FDA), Title 21 of the Code of Federal Regulations (CFR) Part 58.

Exception: SigmaStat program is not fully validated.



Doug Neptun, Laboratory Director 12/22/10 Date

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507 AIRPORT BLVD. · SUITE 113 · MORRISVILLE, NC 27560
Ph. 919-277-0822 Fax 919-277-0825

Quality Assurance Statement (QAS)

CONFIDENTIAL

The study listed below has been inspected and the raw data and report(s) have been audited by the Quality Assurance (QA) Unit of Antech Diagnostics GLP in accordance with the Food and Drug Administration (FDA) Good Laboratory Practices (GLP) standards of GLP for non-clinical laboratory studies and Antech Standard Operating Procedures. The reported results accurately reflect the raw data of the study.

To:	RTI International
Study Director:	Brenda Faiola, Ph.D. (and Study Management)
Principle Investigator (PI):	Doug Neptun (and Scott Moroff, DVM)
From Quality Assurance Auditor:	Katie Powell
Protocol referenced:	14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment (RTI-1111)
Timeperiod(s), material audited, inspection date(s):	Day 14, Study Data, December 13, 2010 Interpretative Report: Draft, December 14, 2010 Interpretative Report: Draft v2, December 21, 2010 Interpretative Report: Final, December 22, 2010
Date the Audit Report was issued:	December 22, 2010
Study Director and Study Management notified (Dates sent):	Audit Report (December 22, 2010) QAS (December 22, 2010)

ANTECH DIAGNOSTICS GLP

Quality Assurance Statement (QAS)

CONFIDENTIAL

PI and PI Management notified
of Audit Report
(Date sent for PI response): December 22, 2010

Printed Name: Katie Powell

Signature: Katie Powell Date: 12/22/10

Title: Senior QA Auditor

TABLE OF CONTENTS

I. Introduction

Male and female rats, (CD® IGS)[Crl:CD(SD)], were dosed with vehicle [95:5 (v:v) 0.9% sodium chloride for injection USP: absolute ethanol, USP], 39 µg/kg/day or 153 µg/kg/day Fluoromisonidazole by IV injection for 14 days All surviving animals were evaluated for clinical pathology toxicologic effects at the terminal sacrifice (Day 14).

II. Study Design and Methods

Male and female rats in three groups (5/sex/dose group) were subjected to clinical pathology analyses.

Based on the protocol, blood was to be collected by cardiac puncture from fasted rats at terminal sacrifice. Blood for hematology was to be collected into K_3EDTA-containing tubes. Blood for serum chemistry assessments was to be collected into serum separator tubes without an anticoagulant, allowed to clot, separated by cold centrifugation, and resultant serum transferred to a cyrovial. Blood and serum samples were transported to Antech Diagnostics on wet ice and dry ice, respectively, on the day of collection. All samples were evaluated for complete blood count (CBC) with differential and clinical chemistry analytes as per the study protocol. Results were entered into the ClinAxys v2.2 computer system.

CBC consisted of a total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count, leukocyte differential: (neutrophils [NEU], lymphocytes [LYM], monocytes [MON], eosinophils [EOS], basophils [BAS] and large unstained cells [LUC]), reticulocytes (RET% and RET) and RBC morphology. These analyses were performed by the Siemens Advia 120 automated hematology system (Norwood, MA).

Clinical chemistry testing was performed using Olympus reagents and the Olympus 640e clinical chemistry analyzer (Center Valley, PA). Tests performed by the Olympus included: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), calcium (CA), cholesterol (CHOL), chloride (CL), creatinine (CREA), gamma-glutamyltransferase (GGT), glucose (GLU), potassium (K), sodium (NA), phosphorus (PHOS), total bilirubin (TBIL), total protein (TP) and triglycerides (TRIG). The globulin (GLOB) and albumin/globulin ratio (A/G) were calculated.

Statistical evaluation of the data was performed using SigmaStat software. The data was analyzed for normality followed by an ANOVA ($p<0.05$) and, if significant, a comparison of groups by Holm-Sidak. If the test for normality failed, the ANOVA was based on Kruskal-Wallis ANOVA on Ranks ($p<0.05$) and, if significant, Dunn's

comparison of groups was used. Values are reported as means, standard deviation (SD) and number of samples (n). The SigmaStat program was not fully validated.

III. Results

Discussion of results refers to a comparison of the test article-treated group mean to the vehicle control group mean. Unless otherwise stated, the difference is a statistically significant difference.

Male Rats

Male rats given 39 µg/kg/day had a decrease in the absolute lymphocyte count. A non-test article-related sample collection stress lymphopenia in this group is the likely cause of this decrease. There was a decrease in percent monocytes in the males given 153 µg/kg/day and a decrease in the absolute monocyte count in both the males given 39 µg/kg/day and the 153 µg/kg/day. The monocytopenia observed is not considered adverse. Relative changes in monocytes are not considered to be biologically significant since there are no known direct relationships of peripheral circulating monocytes and toxic processes directly related to mature monocytes. Monocytopenias are not considered biologically significant. Note that the vehicle control group consisted of two samples due to three samples being clotted and thus could not be analyzed. The low n of the control group may have contributed to the statistical flagging of the monocyte counts as well.

Serum potassium and phosphorus were decreased in the males given 39 µg/kg/day and 153 µg/kg/day. ALT and AST activities were decreased in the males given 39 µg/kg/day (not statistically significant) and 153 µg/kg/day. These decreases in potassium, phosphorus, AST and ALT were not considered test article-related but rather attributed to an increase in the mean values for these parameters in the vehicle control group due to serum hemolysis noted in the sample from Animal #1 in that group.

Female Rats

Female rats had no changes in the hematology parameters at Day 14. Glucose was increased in the females given 39 µg/kg/day and 153 µg/kg/day.

IV. Conclusions

The lymphopenia observed in the male rats in the 39 µg/kg/day group is not considered test article-related due to a lack of dose response and is not adverse due to a likely sample collection stress (steroid) lymphopenia. The monocytopenia observed in the males rats given 39 ug/kg/day and 153 ug/kg/day is not considered test article-related, biologically significant. The clinical chemistry changes in the male rats are most likely due to an artifactual increase in the AST, ALT, potassium and phosphorus control values caused by hemolysis in one male rat in the vehicle group. The hemolysis is likely in vitro hemolysis in that no concurrent anemia is present within this control male group. The increased glucose observed in the females is mild and

may be attributed to sample collection stress and thus not test article-related. No other significant hematologic/biochemical abnormalities are present.

V. Summary Tables

Table – Hematology

Table – Clinical Chemistry

Abbreviations found in Summary Tables

Test abbreviations are found in text:
QNS – Quantity Not Sufficient for analysis
g – grams
mg – milligrams
mL – milliliter
uL – microliter
dL – deciliter
fL – fentoliters
pg – picoprams
sec – seconds

mmol/L – milimoles/Liter
U/L – Units/Liter

1 –M to 3-M Groups of Males
1 –F to 3-F Groups of Females
SD – Standard Deviation
n – Number of samples in group

Antech Diagnostics GLP
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Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1M					
1	Mean	15.48	7.46	15.0	46.6
	SD	4.087	0.099	0.99	2.40
	n	2	2	2	2
2M					
2	Mean	10.82	7.37	14.7	45.4
	SD	1.755	0.232	0.44	1.23
	n	5	5	5	5
3M					
3	Mean	12.61	7.70	15.3	47.0
	SD	1.585	0.152	0.45	1.64
	n	5	5	5	5

Group		MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1M					
1	Mean	62.5	20.2	32.3	1269
	SD	2.40	1.06	0.49	96.2
	n	2	2	2	2
2M					
2	Mean	61.6	19.9	32.3	1175
	SD	1.56	0.58	0.36	188.6
	n	5	5	5	5
3M					
3	Mean	61.0	19.9	32.6	1229
	SD	1.22	0.30	0.48	94.2
	n	5	5	5	5

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1M					
1	Mean	7.8	1.23	87.2	13.48
	SD	0.85	0.453	0.85	3.422
	n	2	2	2	2
2M					
2	Mean	11.5	1.22	83.5	9.07 *
	SD	3.16	0.203	2.76	1.618
	n	5	5	5	5
3M					
3	Mean	8.6	1.08	87.3	11.01
	SD	1.92	0.259	2.02	1.421
	n	5	5	5	5

Group		MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1M					
1	Mean	2.3	0.34	0.7	0.11
	SD	0.64	0.007	0.07	0.035
	n	2	2	2	2
2M					
2	Mean	2.0	0.21 *	0.9	0.10
	SD	0.18	0.041	0.26	0.044
	n	5	5	5	5
3M					
3	Mean	1.5 *	0.20 *	0.7	0.09
	SD	0.15	0.036	0.20	0.030
	n	5	5	5	5

* - Statistically different from Control p<0.05

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1M					
1	Mean	0.7	0.11	1.4	0.23
	SD	0.14	0.007	0.64	0.163
	n	2	2	2	2
2M					
2	Mean	0.7	0.08	1.4	0.15
	SD	0.24	0.037	0.42	0.063
	n	5	5	5	5
3M					
3	Mean	0.6	0.07	1.3	0.17
	SD	0.07	0.022	0.27	0.046
	n	5	5	5	5

Group		RET% %	RET 10^9/L
1M			
1	Mean	3.46	257.5
	SD	0.205	12.02
	n	2	2
2M			
2	Mean	2.98	219.1
	SD	0.606	40.83
	n	5	5
3M			
3	Mean	2.93	225.3
	SD	0.328	22.20
	n	5	5

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1F					
1	Mean	8.86	7.65	14.9	44.2
	SD	4.331	0.370	0.54	1.13
	n	5	5	5	5
2F					
2	Mean	10.65	7.26	14.5	42.9
	SD	0.917	0.396	0.64	1.35
	n	3	3	3	3
3F					
3	Mean	12.78	7.88	15.4	46.0
	SD	2.620	0.264	0.44	1.74
	n	5	5	5	5

Group		MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1F					
1	Mean	57.9	19.5	33.6	1244
	SD	2.43	0.97	0.48	307.0
	n	5	5	5	5
2F					
2	Mean	59.1	20.0	33.8	1004
	SD	1.32	0.60	0.78	359.5
	n	3	3	3	3
3F					
3	Mean	58.4	19.6	33.6	1085
	SD	1.63	0.46	0.40	93.9
	n	5	5	5	5

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1F					
1	Mean	8.1	0.70	87.7	7.79
	SD	3.19	0.408	3.48	3.860
	n	5	5	5	5
2F					
2	Mean	8.5	0.93	87.4	9.28
	SD	4.31	0.560	4.65	0.303
	n	3	3	3	3
3F					
3	Mean	6.8	0.88	89.2	11.38
	SD	1.46	0.341	1.30	2.214
	n	5	5	5	5

Group		MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1F					
1	Mean	1.3	0.11	0.7	0.06
	SD	0.61	0.038	0.22	0.022
	n	5	5	5	5
2F					
2	Mean	1.2	0.12	0.9	0.10
	SD	0.55	0.057	0.32	0.042
	n	3	3	3	3
3F					
3	Mean	1.4	0.18	0.8	0.10
	SD	0.25	0.049	0.29	0.042
	n	5	5	5	5

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1F					
1	Mean	0.7	0.06	1.5	0.14
	SD	0.23	0.038	0.68	0.089
	n	5	5	5	5
2F					
2	Mean	0.7	0.07	1.4	0.15
	SD	0.06	0.010	0.56	0.055
	n	3	3	3	3
3F					
3	Mean	0.6	0.08	1.3	0.16
	SD	0.13	0.033	0.30	0.033
	n	5	5	5	5

Group		RET% %	RET 10^9/L
1F			
1	Mean	1.80	136.8
	SD	0.455	31.41
	n	5	5
2F			
2	Mean	2.21	160.4
	SD	0.200	21.47
	n	3	3
3F			
3	Mean	1.99	156.2
	SD	0.424	30.09
	n	5	5

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Test Codes and Descriptions

Code	Description
BAS	Absolute Basophils
BAS%	% Basophils
EOS	Absolute Eosinophils
EOS%	% Eosinophils
HB	Hemoglobin
HCT	Hematocrit
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

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		BUN mg/dl	CREA mg/dl	GLU mg/dl	NA mmol/L
1M					
1	Mean	20	0.4	209	147
	SD	2.7	0.00	40.8	2.2
	n	5	4	4	4
2M					
2	Mean	18	0.3	175	149
	SD	1.9	0.05	47.8	1.6
	n	5	5	5	5
3M					
3	Mean	17	0.3	189	149
	SD	2.2	0.05	16.3	0.7
	n	5	5	5	5

Group		K mmol/L	CL mmol/L	ALP U/L	ALT U/L
1M					
1	Mean	7.3	99	258	180
	SD	0.74	1.5	31.5	269.0
	n	4	4	5	5
2M					
2	Mean	6.1 *	98	260	44
	SD	0.24	0.5	57.9	9.1
	n	5	5	5	5
3M					
3	Mean	5.9 *	99	237	37 *
	SD	0.34	1.3	38.1	8.5
	n	5	5	5	5

* - Statistically different from Control $p<0.05$

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		AST U/L	TBIL mg/dl	GGT U/L	TPRO g/dl
1M					
1	Mean	417	0.2	0	6.6
	SD	594.5	0.06	0.0	0.42
	n	5	4	4	4
2M					
2	Mean	108	0.1	0	6.5
	SD	46.4	0.00	0.0	0.20
	n	5	5	5	5
3M					
3	Mean	75 *	0.1	0	6.8
	SD	6.6	0.00	0.0	0.51
	n	5	5	5	5

Group		ALB g/dl	GLOB g/dL	A\G	CA mg/dl
1M					
1	Mean	3.8	2.9	1.26	12.2
	SD	0.31	0.21	0.050	0.54
	n	5	4	4	4
2M					
2	Mean	3.6	2.9	1.24	12.5
	SD	0.16	0.12	0.079	0.42
	n	5	5	5	5
3M					
3	Mean	3.7	3.0	1.24	12.5
	SD	0.11	0.43	0.136	0.38
	n	5	5	5	5

* - Statistically different from Control $p<0.05$

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1M				
1	Mean	12.0	72	71
	SD	0.64	11.7	28.1
	n	4	4	4
2M				
2	Mean	11.1 *	60	63
	SD	0.44	13.3	12.2
	n	5	5	5
3M				
3	Mean	10.8 *	67	74
	SD	0.44	8.9	16.0
	n	5	5	5

* - Statistically different from Control $p<0.05$

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		BUN mg/dl	CREA mg/dl	GLU mg/dl	NA mmol/L
1F					
1	Mean	18	0.4	142	148
	SD	3.1	0.08	22.5	0.8
	n	4	4	4	4
2F					
2	Mean	16	0.4	221 *	146
	SD	3.1	0.00	47.8	1.2
	n	5	5	5	4
3F					
3	Mean	16	0.4	201 *	147
	SD	3.4	0.05	35.6	1.1
	n	5	5	5	5

Group		K mmol/L	CL mmol/L	ALP U/L	ALT U/L
1F					
1	Mean	6.1	99	126	41
	SD	0.06	1.3	26.6	8.2
	n	4	4	5	5
2F					
2	Mean	6.8	101	175	38
	SD	0.68	1.4	53.9	11.8
	n	4	4	5	5
3F					
3	Mean	7.0	100	135	29
	SD	0.60	1.5	31.5	4.2
	n	5	5	5	5

* - Statistically different from Control $p<0.05$

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		AST U/L	TBIL mg/dl	GGT U/L	TPRO g/dl
1F					
1	Mean	111	0.1	0	6.7
	SD	61.4	0.00	0.5	0.64
	n	5	4	4	4
2F					
2	Mean	107	0.1	0	6.6
	SD	37.0	0.00	0.0	0.36
	n	5	4	4	5
3F					
3	Mean	80	0.1	0	6.6
	SD	14.1	0.00	0.0	0.33
	n	5	5	5	5

Group		ALB g/dl	GLOB g/dL	A\G	CA mg/dl
1F					
1	Mean	3.8	2.9	1.30	12.3
	SD	0.29	0.43	0.142	0.24
	n	5	4	4	4
2F					
2	Mean	3.6	2.9	1.25	12.4
	SD	0.13	0.26	0.088	0.42
	n	5	5	5	5
3F					
3	Mean	3.7	2.9	1.27	12.6
	SD	0.12	0.24	0.083	0.31
	n	5	5	5	5

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Study: RTI-1111 Species: RAT

Time point: DAY 14

Group		PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1F				
1	Mean	9.7	82	50
	SD	0.78	6.6	7.1
	n	4	4	4
2F				
2	Mean	10.2	90	47
	SD	0.75	19.5	12.5
	n	5	5	5
3F				
3	Mean	10.6	99	37
	SD	1.10	24.4	7.3
	n	5	5	5

Antech Diagnostics GLP
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Study: RTI-1111 Species: RAT

Time point: DAY 14

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
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

VI. INDIVIDUAL RESULTS

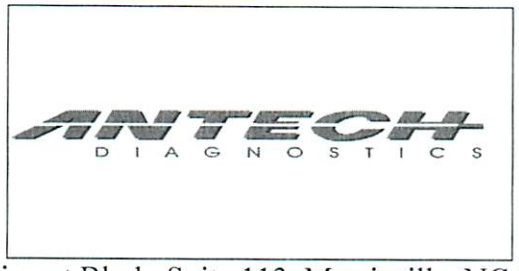
Individual Results as Attachments

Male Hematology

Female Hematology

Male Clinical Chemistry

Female Clinical Chemistry



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

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

Study: ATI-1111 Time Period: DAY 14

Signed: C Long Date: 11/29/10

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

Group	Animal Number	WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1M	1	CLOT	CLOT	CLOT	CLOT
1	3	18.37	7.53	15.7	48.3
	5	CLOT	CLOT	CLOT	CLOT
	7	12.59	7.39	14.3	44.9
	9	CLOT	CLOT	CLOT	CLOT
2M	11	12.82	7.38	15.1	46.5
2	13	10.52	7.74	15.0	46.2
	15	10.29	7.11	14.2	43.6
	17	8.33	7.37	14.2	44.7
	19	12.14	7.27	14.9	46.1
3M	21	13.72	7.71	15.6	47.2
3	23	9.89	7.54	14.7	44.6
	25	13.73	7.92	15.7	48.4
	27	12.71	7.76	15.5	48.5
	29	13.00	7.58	14.9	46.1

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
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Study: RTI-1111 Species: RAT

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Group	Animal Number	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1M	1	CLOT	CLOT	CLOT	CLOT
1	3	64.2	20.9	32.6	1201
	5	CLOT	CLOT	CLOT	CLOT
	7	60.8	19.4	31.9	1337
	9	CLOT	CLOT	CLOT	CLOT
2M	11	63.0	20.5	32.5	994
2	13	59.7	19.4	32.5	1377
	15	61.3	20.0	32.6	961
	17	60.7	19.3	31.7	1224
	19	63.4	20.5	32.3	1318
3M	21	61.3	20.3	33.1	1173
3	23	59.1	19.5	33.1	1130
	25	61.1	19.9	32.5	1236
	27	62.5	20.0	32.0	1379
	29	60.8	19.7	32.4	1226

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Study: RTI-1111 Species: RAT

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Group	Animal Number	NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1M	1	CLOT	CLOT	CLOT	CLOT
1	3	8.4	1.55	86.6	15.90
	5	CLOT	CLOT	CLOT	CLOT
	7	7.2	0.91	87.8	11.06
	9	CLOT	CLOT	CLOT	CLOT
2M	11	10.8	1.39	83.1	10.66
2	13	8.5	0.90	86.5	9.11
	15	11.5	1.18	83.4	8.59
	17	16.8	1.40	79.3	6.61
	19	10.0	1.21	85.4	10.36
3M	21	9.1	1.25	86.7	11.89
3	23	8.9	0.88	87.5	8.65
	25	7.1	0.97	88.2	12.10
	27	11.3	1.44	84.4	10.73
	29	6.4	0.84	89.9	11.69

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Group	Animal Number	MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1M	1	CLOT	CLOT	CLOT	CLOT
1	3	1.8	0.34	0.7	0.13
	5	CLOT	CLOT	CLOT	CLOT
	7	2.7	0.33	0.6	0.08
	9	CLOT	CLOT	CLOT	CLOT
2M	11	1.8	0.24	1.3	0.17
2	13	1.8	0.19	0.8	0.09
	15	2.2	0.23	0.8	0.08
	17	1.9	0.15	0.6	0.05
	19	2.1	0.25	0.8	0.09
3M	21	1.8	0.25	0.7	0.09
3	23	1.5	0.15	0.5	0.05
	25	1.5	0.20	1.0	0.13
	27	1.4	0.18	0.7	0.09
	29	1.5	0.20	0.5	0.07

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Group	Animal Number	BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1M	1	CLOT	CLOT	CLOT	CLOT
1	3	0.6	0.11	1.8	0.34
	5	CLOT	CLOT	CLOT	CLOT
	7	0.8	0.10	0.9	0.11
	9	CLOT	CLOT	CLOT	CLOT
2M	11	1.0	0.13	1.9	0.24
2	13	0.6	0.06	1.8	0.19
	15	0.9	0.09	1.2	0.12
	17	0.4	0.03	1.0	0.08
	19	0.7	0.08	1.1	0.13
3M	21	0.6	0.08	1.1	0.16
3	23	0.5	0.04	1.1	0.11
	25	0.7	0.10	1.6	0.23
	27	0.6	0.08	1.5	0.19
	29	0.6	0.07	1.0	0.14

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Group	Animal Number	RET% %	RET 10^9/L	COM
1M	1	CLOT	CLOT	CLOT
1	3	3.31	249.0	
	5	CLOT	CLOT	
	7	3.60	266.0	
	9	CLOT	CLOT	
2M	11	3.02	222.6	
2	13	2.76	213.7	
	15	3.74	266.1	
	17	2.11	155.3	
	19	3.27	237.7	
3M	21	2.57	197.7	
3	23	3.34	251.9	
	25	2.62	207.3	
	27	3.03	235.2	
	29	3.09	234.2	

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Group	Animal Number	ANIS	POLK	HYPO	HYPR
1M	1	CLOT	CLOT	CLOT	CLOT
1	3				
	5	CLOT	CLOT	CLOT	CLOT
	7				
	9	CLOT	CLOT	CLOT	CLOT
2M	11				
2	13				
	15				
	17				
	19				
3M	21				
3	23				
	25				
	27				
	29				

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Group	Animal Number	MIC	MAC	HJB	CPLT
1M	1	CLOT	CLOT	CLOT	CLOT
1	3				
	5	CLOT	CLOT	CLOT	CLOT
	7				
	9	CLOT	CLOT	CLOT	CLOT
2M	11				
2	13				
	15				
	17				
	19				
3M	21				
3	23				
	25				
	27				
	29				

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Group	Animal Number	LPLT	ATYP
1M	1	CLOT	CLOT
1	3		
	5	CLOT	CLOT
	7		
	9	CLOT	CLOT
2M	11		
2	13		
	15		
	17		
	19		
3M	21		
3	23		
	25		
	27		
	29		

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Group	Animal Number	WBC 10^3/uL	RBC 10^6/uL	HB g/dL	HCT %
1F	2	5.58	7.22	14.7	44.0
1	4	6.49	7.80	15.4	45.2
	6	6.64	7.81	15.5	45.5
	8	16.17	8.11	14.4	43.8
	10	9.41	7.33	14.4	42.7
2F	12	10.01	6.88	13.8	41.5
2	14	CLOT	CLOT	CLOT	CLOT
	16	11.70	7.23	14.9	43.0
	18	CLOT	CLOT	CLOT	CLOT
	20	10.24	7.67	14.9	44.2
3F	22	16.65	7.83	15.4	45.9
3	24	10.65	8.01	16.1	48.6
	26	13.10	8.24	15.6	46.7
	28	10.06	7.54	15.0	44.6
	30	13.45	7.76	15.1	44.3

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Group	Animal Number	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/uL
1F	2	60.9	20.3	33.4	899
1	4	57.9	19.7	34.0	1738
	6	58.3	19.9	34.1	1194
	8	54.1	17.8	32.9	1137
	10	58.2	19.6	33.6	1252
2F	12	60.3	20.0	33.2	1326
2	14	CLOT	CLOT	CLOT	CLOT
	16	59.4	20.6	34.7	616
	18	CLOT	CLOT	CLOT	CLOT
	20	57.7	19.4	33.6	1069
3F	22	58.6	19.6	33.5	1229
3	24	60.7	20.1	33.2	982
	26	56.7	18.9	33.3	1088
	28	59.1	19.9	33.7	1025
	30	57.0	19.5	34.2	1102

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Group	Animal Number	NEU% %	NEU 10^3/uL	LYM% %	LYM 10^3/uL
1F	2	9.9	0.55	87.5	4.88
1	4	12.2	0.79	82.1	5.33
	6	5.0	0.33	89.7	5.96
	8	8.4	1.36	87.8	14.20
	10	4.8	0.45	91.3	8.60
2F	12	6.3	0.63	89.9	9.00
2	14	CLOT	CLOT	CLOT	CLOT
	16	13.5	1.58	82.0	9.60
	18	CLOT	CLOT	CLOT	CLOT
	20	5.8	0.59	90.2	9.23
3F	22	8.6	1.43	87.4	14.56
3	24	5.0	0.54	90.6	9.65
	26	5.6	0.73	90.3	11.82
	28	7.5	0.75	88.8	8.93
	30	7.2	0.97	88.7	11.93

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Group	Animal Number	MON% %	MON 10^3/uL	EOS% %	EOS 10^3/uL
1F	2	1.2	0.07	0.7	0.04
1	4	2.0	0.13	1.1	0.07
	6	1.9	0.13	0.7	0.04
	8	0.8	0.14	0.5	0.09
	10	0.7	0.06	0.7	0.07
2F	12	0.6	0.06	0.7	0.07
2	14	CLOT	CLOT	CLOT	CLOT
	16	1.2	0.14	1.3	0.15
	18	CLOT	CLOT	CLOT	CLOT
	20	1.7	0.17	0.8	0.09
3F	22	1.4	0.24	0.7	0.11
3	24	1.5	0.16	0.6	0.06
	26	1.0	0.14	1.3	0.17
	28	1.4	0.14	0.7	0.08
	30	1.7	0.23	0.6	0.09

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Group	Animal Number	BAS% %	BAS 10^3/uL	LUC% %	LUC 10^3/uL
1F	2	0.3	0.02	0.3	0.02
1	4	0.6	0.04	2.0	0.13
	6	0.9	0.06	1.8	0.12
	8	0.7	0.12	1.7	0.27
	10	0.8	0.08	1.6	0.15
2F	12	0.6	0.06	2.0	0.20
2	14	CLOT	CLOT	CLOT	CLOT
	16	0.7	0.08	1.3	0.15
	18	CLOT	CLOT	CLOT	CLOT
	20	0.7	0.07	0.9	0.09
3F	22	0.8	0.13	1.1	0.19
3	24	0.5	0.05	1.8	0.19
	26	0.7	0.09	1.2	0.15
	28	0.5	0.05	1.1	0.11
	30	0.6	0.08	1.1	0.15

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Group	Animal Number	RET% %	RET 10^9/L
1F	2	2.07	149.5
1	4	1.27	98.9
	6	1.39	108.5
	8	1.92	155.7
	10	2.34	171.3
2F	12	2.20	151.4
2	14	CLOT	CLOT
	16	2.01	144.9
	18	CLOT	CLOT
	20	2.41	184.9
3F	22	1.53	119.9
3	24	2.23	178.7
	26	1.61	132.4
	28	2.54	191.2
	30	2.05	158.9

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Group	Animal Number	ANIS	POLK	HYPO	HYPR
1F	2				
1	4				
	6				
	8				
	10				
2F	12				
2	14	CLOT	CLOT	CLOT	CLOT
	16				
	18	CLOT	CLOT	CLOT	CLOT
	20				
3F	22				
3	24				
	26				
	28				
	30				

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

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Group	Animal Number	LPLT	ATYP
1F	2		
1	4		
	6		
	8		
	10		
2F	12		
2	14	CLOT	CLOT
	16		
	18	CLOT	CLOT
	20		
3F	22		
3	24		
	26		
	28		
	30		

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Codes and Descriptions for Result Comments

Code	Description
CLOT	Specimen Clotted

Doug Neptun
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Antech Diagnostics GLP
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Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

Test Codes and Descriptions

Code	Description
ANIS	Anisocytosis
ATYP	Atypical Lymphs
BAS	Absolute Basophils
BAS%	% Basophils
COM	Comment
CPLT	Clumped Platelets
EOS	Absolute Eosinophils
EOS%	% Eosinophils
HB	Hemoglobin
HCT	Hematocrit
HJB	Howell-Jolly Bodies
HYPO	Hypochromasia
HYPR	Hyperchromasia
LPLT	Large Platelets
LUC	Absolute Large Unstained Cells
LUC%	% Large Unstained Cells
LYM	Absolute Lymphocytes
LYM%	% Lymphocytes
MAC	Macrocytosis
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Co
MCV	Mean Corpuscular Volume
MIC	Microcytosis
MON	Absolute Monocytes
MON%	% Monocytes
NEU	Absolute Neutrophils
NEU%	% Neutrophils
PLT	Platelet Count
POLK	Poikilocytosis
RBC	Red Blood Cell Count
RET	Absolute Reticulocyte
RET%	% Reticulocyte
WBC	White Blood Cell Count

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Group	Animal Number	COM	BUN mg/dl	CREA mg/dl	GLU mg/dl
1M	1	HEM	23	0.4	268
1	3		22	0.4	204
	5		18	0.4	189
	7		18	0.4	176
	9	HEM	17	QNS	QNS
2M	11		17	0.4	250
2	13		21	0.4	131
	15		18	0.3	165
	17		16	0.3	140
	19		18	0.3	191
3M	21		17	0.3	191
3	23		16	0.3	165
	25		17	0.4	184
	27		20	0.4	208
	29		14	0.3	199

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Group	Animal Number	NA mmol/L	K mmol/L	CL mmol/L	ALP U/L
1M	1	144	8.3	98	236
1	3	147	7.3	98	298
	5	149	6.5	101	254
	7	148	7.2	98	222
	9	QNS	QNS	QNS	282
2M	11	149	5.7	98	260
2	13	151	6.3	99	321
	15	148	6.1	99	193
	17	150	6.3	98	314
	19	147	6.1	98	212
3M	21	150	5.4	99	210
3	23	148	6.0	100	194
	25	149	5.7	97	265
	27	149	5.9	98	286
	29	149	6.3	100	230

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Group	Animal Number	ALT U/L	AST U/L	TBIL mg/dl	GGT U/L
1M	1	661	1476	0.2	0
1	3	69	131	0.2	0
	5	73	233	0.1	0
	7	44	85	0.1	0
	9	54	159	QNS	QNS
2M	11	56	114	0.1	0
2	13	42	186	0.1	0
	15	38	84	0.1	0
	17	51	88	0.1	0
	19	34	69	0.1	0
3M	21	30	80	0.1	0
3	23	38	69	0.1	0
	25	48	66	0.1	0
	27	41	80	0.1	0
	29	27	78	0.1	0

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Group	Animal Number	TPRO g/dl	ALB g/dl	GLOB g/dL	A\G
1M	1	6.8	3.7	3.1	1.19
1	3	7.1	4.0	3.1	1.29
	5	6.2	3.5	2.7	1.30
	7	6.3	3.5	2.8	1.25
	9	QNS	4.2	QNS	QNS
2M	11	6.6	3.6	3.0	1.20
2	13	6.4	3.7	2.7	1.37
	15	6.3	3.4	2.9	1.17
	17	6.8	3.8	3.0	1.27
	19	6.4	3.5	2.9	1.21
3M	21	6.7	3.8	2.9	1.31
3	23	6.2	3.6	2.6	1.38
	25	7.0	3.8	3.2	1.19
	27	7.5	3.8	3.7	1.03
	29	6.4	3.6	2.8	1.29

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Group	Animal Number	CA mg/dl	PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1M	1	11.6	12.9	79	101
1	3	12.7	11.5	60	43
	5	11.9	12.0	65	51
	7	12.6	11.6	85	88
	9	QNS	QNS	QNS	QNS
2M	11	13.0	11.6	79	72
2	13	11.9	11.0	53	66
	15	12.3	11.5	66	76
	17	12.7	10.5	59	50
	19	12.6	11.1	44	50
3M	21	12.4	11.5	53	70
3	23	11.9	10.3	72	51
	25	12.7	10.8	70	95
	27	12.9	10.9	76	80
	29	12.4	10.6	64	73

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Group	Animal Number	COM	BUN mg/dl	CREA mg/dl	GLU mg/dl
1F	2		21	0.4	119
1	4		19	0.5	144
	6		16	0.3	133
	8		14	0.4	172
	10		QNS	QNS	QNS
2F	12		16	0.4	177
2	14		14	0.4	292
	16		21	0.4	201
	18		13	0.4	248
	20		17	0.4	189
3F	22		16	0.3	245
3	24		15	0.4	233
	26		14	0.3	168
	28		21	0.4	173
	30		12	0.4	186

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111

Species: RAT

Time point: DAY 14

Printed: 11/29/2010

Group	Animal Number	NA mmol/L	K mmol/L	CL mmol/L	ALP U/L
1F	2	149	6.1	98	132
1	4	147	6.1	99	93
	6	148	6.0	99	109
	8	148	6.0	101	163
	10	QNS	QNS	QNS	133
2F	12	147	5.8	102	271
2	14	145	6.9	101	158
	16	QNS	QNS	QNS	142
	18	147	6.9	102	147
	20	145	7.4	99	159
3F	22	147	6.6	100	92
3	24	146	8.0	99	123
	26	147	6.9	99	141
	28	149	6.5	102	179
	30	148	7.1	102	138

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

Group	Animal Number	ALT U/L	AST U/L	TBIL mg/dl	GGT U/L
1F	2	51	96	0.1	0
1	4	33	83	0.1	1
	6	32	74	0.1	0
	8	46	82	0.1	0
	10	42	220	QNS	QNS
2F	12	32	96	0.1	0
2	14	31	94	0.1	0
	16	43	170	QNS	QNS
	18	56	100	0.1	0
	20	27	73	0.1	0
3F	22	32	86	0.1	0
3	24	26	89	0.1	0
	26	29	64	0.1	0
	28	35	67	0.1	0
	30	25	96	0.1	0

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

Group	Animal Number	TPRO g/dl	ALB g/dl	GLOB g/dL	A\G
1F	2	6.2	3.7	2.5	1.48
1	4	7.6	4.1	3.5	1.17
	6	6.3	3.6	2.7	1.33
	8	6.6	3.6	3.0	1.20
	10	QNS	4.2	QNS	QNS
2F	12	6.7	3.7	3.0	1.23
2	14	6.1	3.5	2.6	1.35
	16	6.7	3.8	2.9	1.31
	18	7.0	3.7	3.3	1.12
	20	6.3	3.5	2.8	1.25
3F	22	6.5	3.7	2.8	1.32
3	24	7.1	3.8	3.3	1.15
	26	6.7	3.7	3.0	1.23
	28	6.2	3.5	2.7	1.30
	30	6.6	3.8	2.8	1.36

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

Group	Animal Number	CA mg/dl	PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1F	2	12.2	9.5	90	42
1	4	12.5	9.0	76	54
	6	12.0	9.4	84	47
	8	12.5	10.8	77	58
	10	QNS	QNS	QNS	QNS
2F	12	12.1	9.5	69	42
2	14	12.2	10.7	86	38
	16	12.9	11.2	103	69
	18	12.7	10.1	117	41
	20	11.9	9.5	77	46
3F	22	12.7	11.0	89	33
3	24	13.1	11.7	80	49
	26	12.4	9.2	138	38
	28	12.3	9.6	106	35
	30	12.6	11.3	80	30

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

Codes and Descriptions for Result Comments

Code	Description
QNS	Quantity Not Sufficient

Doug Neptun
Laboratory Director

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

Study: RTI-1111 Species: RAT

Time point: DAY 14 Printed: 11/29/2010

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

Doug Neptun
Laboratory Director

Appendix 3

Histopathology Report
(Experimental Pathology Laboratories, Inc.)

14-DAY INTRAVENOUS REPEAT DOSE TOXICOLOGY STUDY OF FLUOROMISONIDAZOLE IN RATS WITH MICRONUCLEUS ASSESSMENT

RTI PROJECT NO.: 0211886.002
RTI MASTER PROTOCOL NO.: RTI-1111
RTI STUDY CODE: Rt10-FMIS
EPL PROJECT NO.: 229-171

FINAL PATHOLOGY REPORT

Submitted by:

Experimental Pathology Laboratories, Inc.
P.O. Box 169
Sterling, VA 20167-0169

Submitted to:

RTI International
P.O. Box 12194
Research Triangle Park, NC 27709

February 25, 2011

14-DAY INTRAVENOUS REPEAT DOSE TOXICOLOGY STUDY OF FLUOROMISONIDAZOLE IN RATS WITH MICRONUCLEUS ASSESSMENT

RTI PROJECT NO.: 0211886.002
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FINAL PATHOLOGY REPORT

TABLE OF CONTENTS

14-DAY INTRAVENOUS REPEAT DOSE TOXICOLOGY STUDY OF FLUOROMISONIDAZOLE IN RATS WITH MICRONUCLEUS ASSESSMENT

RTI PROJECT NO.: 0211886.002
RTI MASTER PROTOCOL NO.: RTI-1111
RTI STUDY CODE: Rt10-FMIS
EPL PROJECT NO.: 229-171

FINAL PATHOLOGY REPORT

INTRODUCTION

The purpose of this study was to assess the toxicity, including micronucleus induction, of Fluoromisonidazole (FMISO) when administered as intravenous injection to Sprague-Dawley (CD®IGS) rats for 14 consecutive days. To help achieve this objective, microscopic examinations were performed on selected tissues. This report presents the results and conclusions from those examinations.

MATERIALS AND METHODS

STUDY DESIGN AND CONDUCT

Three groups of 10 Sprague-Dawley rats (5/sex) were given daily intravenous doses of FMISO at 0, 39, or 153 µg/kg/day for 14 days. An additional group of two male rats received 30 mg/kg of Cyclophosphamide, as a single intraperitoneal, injection as the positive control for the micronucleus assay. This study design is outlined in the table below.

Group Number	Treatment	Dose	Dosing Concentration	Dosing Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[1]	0	0	2.0	5	5
2	Fluoromisonidazole	39 (µg/kg/day)	19.5 (µg/mL)	2.0	5	5
3	Fluoromisonidazole	153 (µg/kg/day)	76.5 (µg/mL)	2.0	5	5
4	Cyclophosphamide[2]	30 (mg/kg)	6.0 (mg/mL)	5.0	2	0

[1] Vehicle – 95:5 (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP.

[2] Positive control for micronucleus assay. Cyclophosphamide will be administered by intraperitoneal injection as a single dose to two males on Study Day 13.

As part of the postmortem examination, samples of the following tissues were collected and fixed: adrenals, aorta, bone (femur with epiphyseal plate of head), bone and marrow (sternum), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, injection site (of final IV dose on day 13), jejunum, kidneys, liver, lungs, mandibular lymph node, mesenteric lymph node, mammary gland (to include nipple and surrounding tissue), skeletal muscle (quadriceps femoris), optic nerves, ovaries, oviducts, pancreas, parathyroid glands, pituitary gland, prostate, rectum, mandibular salivary gland, sciatic nerve, seminal vesicles, skin (abdominal), spinal cord (thoracolumbar junction), spleen, stomach (fundic area), testes, thymus, thyroid glands, tongue, trachea, ureters, urinary bladder, uterus, vagina, and all gross lesions.

Histology and Histopathology

Fixed tissue samples from the rats in Group 1 (control) and Group 3 (high-dose) were processed by routine methods, sectioned, mounted on slides, and stained with hematoxylin and eosin. Slides were examined by light microscopy, and histopathologic findings were recorded.

Inflammatory or degenerative lesions were graded on a scale of one to four depending on severity. Nongradable lesions such as cysts were noted as "P" for present. A few tissues were not available for examination. These few missing tissues did not affect the overall evaluation of the study.

Microscopic findings for each tissue are listed in the Histo Pathology Matrix. All findings were summarized the Pathology – Intergroup Comparison of Gross/Histo Pathology Observations.

RESULTS

No histomorphologic tissue alterations attributable to Fluoromisonidazole at 153 µg/kg/day were noted in the tissues of any of the rats after receiving the test article for 14 days.

A variety of spontaneous disease lesions and incidental findings occurred in both treated and control rats without respect to test article. These findings were the usual number and type commonly seen in rats of this age and strain.

CONCLUSIONS

Fluoromisonidazole did not produce any histopathologic findings at the highest dose level when administered intravenously for 14 days to Sprague-Dawley rats. Spontaneous disease lesions and incidental findings occurred at essentially comparable rates between control and treated rats.

Deborah A. Banas February 25, 2011

DEBORAH A. BANAS, DVM, MS, DABT, Diplomate, ACVP Date
Senior Veterinary Pathologist

DAB/cb
Attachments

QUALITY ASSURANCE FINAL CERTIFICATION

QUALITY ASSURANCE FINAL CERTIFICATION

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

Client Study: RTI Project 0211886.002; RTI Master Protocol RTI-1111; RTI Study Code: Rt10-FMIS

EPL Principal Investigator: Dr. Henry G. Wall

EPL Project Number: 229-171

EPL Pathologist: Dr. Deborah A. Banas

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

	Dates	
Area Inspected	Inspection	Reporting
EPL Project Sheets	December 1-2, 2010; January 10, 2011; February 1, 2011	December 2, 2010; January 10, 2011; February 1, 2011
Project Setup	December 7, 2010; December 8, 2010; January 6, 2011	December 7, 2010; December 8, 2010; January 6, 2011
Data Review	December 28, 2010; December 30, 2010; January 6, 2011	December 28, 2010; December 30, 2010; January 6, 2011
Draft Pathology Report	January 20, 21 & 24, 2011	January 24, 2011
Final Pathology Report	February 28, 2011	February 28, 2011

Date reported to Study Director/Management: February 2, 2011; February 28, 2011

Date of last quarterly facility inspection: February 2011

Jane J. Hollingsworth
EPL Quality Assurance Unit

28 Feb 2011
Date

APPENDIX A

PATHOLOGY – INTERGROUP COMPARISON OF GROSS/HISTO PATHOLOGY OBSERVATIONS

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
adrenal glands;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	4	4	5	5
vacuolation; cortical cells	1	1	0	0
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)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
bone, sternum;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
brain;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
cervix;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	5	5
epididymides;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	5	5	-	-
esophagus;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole
e in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
eyes;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	3	4
dysplasia; retina	0	0	2	1
heart;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	3	4	4	4
infiltration; mononuclear cell; focal	2	1	1	1
injection site;				
Examined	(5)	(5)	(5)	(4)
Within Normal Limits	2	2	4	1
Not Examined: MISSING	0	0	0	1
hemorrhage; adjacent	2	3	1	2
inflammation, subacute; adjacent	3	3	1	2
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

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole
e in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
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
kidneys;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	1	2	2	1
dilatation; pelvis; unilateral	0	1	0	0
dilatation; tubule; unilateral	2	0	0	0
infarction; unilateral; focal	0	1	0	0
inflammation; interstitium; unilateral; focal	0	0	1	1
mineralization; tubule	0	0	2	4
regeneration; tubule; epithelium	2	1	0	0
infiltration; mononuclear cell	4	2	1	0
liver;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	0	0	0	0
hyperplasia; bile duct	3	2	3	2
inflammation, chronic; multifocal	5	5	5	5
necrosis; coagulative; focal	1	0	0	0
vacuolation; hepatocyte; centrilobular	2	2	0	0
vacuolation; hepatocyte; periportal	0	0	0	1
lungs;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	1	0	4	4
foamy alveolar macrophages; multifocal	0	1	0	0
hair embolus	1	1	0	0
inflammation; interstitium; multifocal	1	2	1	1
inflammation; granulomatous; focal	1	1	0	0
mineralization; vascular	3	3	0	1
lymph node, mesenteric;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
lymph node, mandibular;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	3	4	2	3
plasmacytosis	2	1	3	2
mammary glands;				
Examined	(5)	(5)	(5)	(4)
Within Normal Limits	5	5	5	4
Not Examined: MISSING	0	0	0	1
skeletal muscle, quadriceps femoris;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	4
infiltration; mononuclear cell; focal	0	0	0	1
nerve, optic;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
nerve, sciatic;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
ovaries;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	5	5
oviducts;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	5	5
pancreas;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	4	5	5	5
atrophy; acinar cell; focal	1	0	0	0
parathyroid glands;				
Examined	(5)	(5)	(5)	(5)

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
parathyroid glands; (continued)				
Within Normal Limits	4	5	5	5
pituitary gland;				
Examined	(4)	(5)	(5)	(5)
Within Normal Limits	4	5	4	5
Not Examined: MISSING	1	0	0	0
cyst	0	0	1	0
prostate gland;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	4	4	-	-
infiltration; mononuclear cell	1	1	-	-
salivary gland, mandibular;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
seminal vesicles;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	5	5	-	-
skin;				
Examined	(0)	(1)	(0)	(1)
Within Normal Limits	0	0	0	0
crust formation	0	1	0	1
erosion; focal	0	1	0	1
inflammation, subacute	0	1	0	1
cyst; subcutaneous	0	0	0	1
skin, abdominal;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
spinal cord;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole
e in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
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, fundic;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
testes;				
Examined	(5)	(5)	(-)	(-)
Within Normal Limits	5	5	-	-
thymus;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	4	4	5
atrophy	0	1	1	0
thyroid glands;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
tongue;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5
trachea;				
Examined	(5)	(5)	(5)	(5)
Within Normal Limits	4	5	5	5
dilatation; mucosal glands	1	0	0	0
ureters;				
Examined	(5)	(5)	(4)	(5)
Within Normal Limits	5	5	4	5
Not Examined: MISSING	0	0	1	0
urinary bladder;				
Examined	(5)	(5)	(4)	(5)
Within Normal Limits	5	5	4	5

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reasons: All of those SELECTED

	MALES		FEMALES	
	0 ug/kg/day	153 ug/kg/day	0 ug/kg/day	153 ug/kg/day
Number of Animals on Study :	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)
urinary bladder; (continued)				
Not Examined: MISSING	0	0	1	0
uterus;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	4	5
dilation; lumen	-	-	1	0
vagina;				
Examined	(-)	(-)	(5)	(5)
Within Normal Limits	-	-	5	5

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

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End Of Print
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APPENDIX B

PATHOLOGY – HISTO PATHOLOGY MATRIX

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: MALE										
GROUP	1	1	1	1	1	3	3	3	3	3
REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
ANIMAL NUMBER	1	3	5	7	9	1	3	5	7	9
adrenal glands;	N	N	N	+	N	N	+	N	N	N
cortical cells; vacuolation	.	.	.	1	.	.	1	.	.	.
aorta;	N	N	N	N	N	N	N	N	N	N
bone marrow, sternum;	N	N	N	N	N	N	N	N	N	N
bone, femur;	N	N	N	N	N	N	N	N	N	N
bone, sternum;	N	N	N	N	N	N	N	N	N	N
brain;	N	N	N	N	N	N	N	N	N	N
epididymides;	N	N	N	N	N	N	N	N	N	N
esophagus;	N	N	N	N	N	N	N	N	N	N
eyes;	N	N	N	N	N	N	N	N	N	N
heart;	+	N	N	+	N	N	+	N	N	N
infiltration; mononuclear cell; focal	1	.	.	1	.	.	1	.	.	.
injection site;	N	+	+	+	N	N	+	+	N	+
adjacent; hemorrhage	.	2	2	.	.	.	3	2	.	1
adjacent; inflammation, subacute	.	1	2	1	.	.	2	1	.	1
intestine, cecum;	N	N	N	N	N	N	N	N	N	N
intestine, colon;	N	N	N	N	N	N	N	N	N	N
intestine, duodenum;	N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: MALE	GROUP	1	1	1	1	1	3	3	3	3	3
	REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
	ANIMAL	.	.	.	.	.	2	2	2	2	2
	NUMBER	1	3	5	7	9	1	3	5	7	9
intestine, ileum;		N	N	N	N	N	N	N	N	N	N
intestine, jejunum;		N	N	N	N	N	N	N	N	N	N
intestine, rectum;		N	N	N	N	N	N	N	N	N	N
kidneys;		+	N	+	+	+	N	+	+	+	N
infarction; unilateral; focal		.	.	.	.	.	.	.	2	.	.
infiltration; mononuclear cell		1	.	1	1	1	.	1	2	.	.
pelvis; dilatation; unilateral		.	.	.	.	.	.	.	.	2	.
tubule; dilatation; unilateral		.	.	.	1	1	.	.	.	.	.
tubule; epithelium; regeneration		1	.	1	.	.	.	1	.	.	.
liver;		+	+	+	+	+	+	+	+	+	+
inflammation, chronic; multifocal		2	1	1	1	1	1	1	1	1	1
necrosis; coagulative; focal		1	.	.	.	.	.	.	.	.	.
bile duct; hyperplasia		1	1	.	1	.	1	.	1	.	.
hepatocyte; centrilobular; vacuolation		1	1	.	.	.	.	.	1	.	1
lungs;		N	+	+	+	+	+	+	+	+	+
foamy alveolar macrophages; multifocal		.	.	.	.	.	.	.	1	.	.
hair embolus		.	.	.	P	.	.	P	.	.	.
inflammation; granulomatous; focal		.	.	.	2	.	.	1	.	.	.
mineralization; vascular		.	.	1	1	1	1	.	.	2	1
interstitium; inflammation; multifocal		.	1	.	.	.	.	1	2	.	.
lymph node, mesenteric;		N	N	N	N	N	N	N	N	N	N
lymph node, mandibular;		N	N	+	N	+	N	N	N	+	N
plasmacytosis		.	.	2	.	1	.	.	.	2	.
mammary glands;		N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: MALE										
GROUP	1	1	1	1	1	3	3	3	3	3
REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
ANIMAL	.	.	.	.	.	2	2	2	2	2
NUMBER	1	3	5	7	9	1	3	5	7	9
skeletal muscle, quadriceps femoris;	N	N	N	N	N	N	N	N	N	N
nerve, optic;	N	N	N	N	N	N	N	N	N	N
nerve, sciatic;	N	N	N	N	N	N	N	N	N	N
pancreas;	N	N	+	N	N	N	N	N	N	N
acinar cell; atrophy; focal	.	.	2	.	.	.	.	.	.	.
parathyroid glands;	N	N	+	N	N	N	N	N	N	N
pituitary gland;	N	N	X	N	N	N	N	N	N	N
prostate gland;	N	N	N	+	N	N	+	N	N	N
infiltration; mononuclear cell	.	.	.	1	.	.	1	.	.	.
salivary gland, mandibular;	N	N	N	N	N	N	N	N	N	N
seminal vesicles;	N	N	N	N	N	N	N	N	N	N
skin;	.	.	.	.	.	.	.	.	+	.
crust formation	.	.	.	.	.	.	.	.	P	.
erosion; focal	.	.	.	.	.	.	.	.	2	.
inflammation, subacute	.	.	.	.	.	.	.	.	2	.
skin, abdominal;	N	N	N	N	N	N	N	N	N	N
spinal cord;	N	N	N	N	N	N	N	N	N	N
spleen;	N	N	N	N	N	N	N	N	N	N
stomach, fundic;	N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: MALE										
GROUP	1	1	1	1	1	3	3	3	3	3
REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
ANIMAL	.	.	.	.	.	2	2	2	2	2
NUMBER	1	3	5	7	9	1	3	5	7	9
testes;	N	N	N	N	N	N	N	N	N	N
thymus;	N	N	N	N	N	+	N	N	N	N
atrophy	.	.	.	.	.	1	.	.	.	.
thyroid glands;	N	N	N	N	N	N	N	N	N	N
tongue;	N	N	N	N	N	N	N	N	N	N
trachea;	N	N	N	N	+	N	N	N	N	N
mucosal glands; dilatation	.	.	.	.	2	.	.	.	.	.
ureters;	N	N	N	N	N	N	N	N	N	N
urinary bladder;	N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: FEMALE	GROUP	1	1	1	1	1	3	3	3	3	3
	REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
	ANIMAL	.	.	.	.	1	2	2	2	2	3
	NUMBER	2	4	6	8	0	2	4	6	8	0
adrenal glands;		N	N	N	N	N	N	N	N	N	N
aorta;		N	N	N	N	N	N	N	N	N	N
bone marrow, sternum;		N	N	N	N	N	N	N	N	N	N
bone, femur;		N	N	N	N	N	N	N	N	N	N
bone, sternum;		N	N	N	N	N	N	N	N	N	N
brain;		N	N	N	N	N	N	N	N	N	N
cervix;		N	N	N	N	N	N	N	N	N	N
esophagus;		N	N	N	N	N	N	N	N	N	N
eyes;		+	N	+	N	N	N	+	N	N	N
retina; dysplasia		P	.	P	.	.	.	P	.	.	.
heart;		N	N	N	+	N	N	+	N	N	N
infiltration; mononuclear cell; focal		.	.	.	1	.	.	1	.	.	.
injection site;		N	+	N	N	N	+	+	N	+	X
adjacent; hemorrhage		.	2	.	.	.	.	2	.	2	.
adjacent; inflammation, subacute		.	2	.	.	.	1	2	.	.	.
intestine, cecum;		N	N	N	N	N	N	N	N	N	N
intestine, colon;		N	N	N	N	N	N	N	N	N	N
intestine, duodenum;		N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: FEMALE										
GROUP	1	1	1	1	1	3	3	3	3	3
REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
ANIMAL	.	.	.	.	1	2	2	2	2	3
NUMBER	2	4	6	8	0	2	4	6	8	0
intestine, ileum;	N	N	N	N	N	N	N	N	N	N
intestine, jejunum;	N	N	N	N	N	N	N	N	N	N
intestine, rectum;	N	N	N	N	N	N	N	N	N	N
kidneys;	+	+	N	N	+	+	N	+	+	+
infiltration; mononuclear cell	.	1	.	.	.	.	.	.	.	.
tubule; mineralization	1	1	.	.	.	1	.	1	1	1
interstitium; inflammation; unilateral; focal	.	.	.	.	1	.	.	.	1	.
liver;	+	+	+	+	+	+	+	+	+	+
inflammation, chronic; multifocal	1	2	2	1	1	1	1	1	1	1
bile duct; hyperplasia	1	.	.	1	1	1	.	.	1	.
hepatocyte; periportal; vacuolation	.	.	.	.	.	.	.	1	.	.
lungs;	N	N	N	+	N	+	N	N	N	N
mineralization; vascular	.	.	.	.	.	1	.	.	.	.
interstitium; inflammation; multifocal	.	.	.	1	.	1	.	.	.	.
lymph node, mesenteric;	N	N	N	N	N	N	N	N	N	N
lymph node, mandibular;	+	+	N	+	N	+	+	N	N	N
plasmacytosis	1	2	.	2	.	1	2	.	.	.
mammary glands;	N	N	N	N	N	N	N	N	N	X
skeletal muscle, quadriceps femoris;	N	N	N	N	N	N	+	N	N	N
infiltration; mononuclear cell; focal	.	.	.	.	.	.	1	.	.	.
nerve, optic;	N	N	N	N	N	N	N	N	N	N
nerve, sciatic;	N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: FEMALE	GROUP	1	1	1	1	3	3	3	3	3
	REMOVAL REASON	R	R	R	R	R	R	R	R	R
	ANIMAL	.	.	.	1	2	2	2	2	3
	NUMBER	2	4	6	8	0	2	4	6	8	0
ovaries;		N	N	N	N	N	N	N	N	N	N
oviducts;		N	N	N	N	N	N	N	N	N	N
pancreas;		N	N	N	N	N	N	N	N	N	N
parathyroid glands;		N	N	N	N	N	N	N	N	N	N
pituitary gland;		N	N	N	+	N	N	N	N	N	N
cyst		.	.	.	P	.	.	.	.	.	.
salivary gland, mandibular;		N	N	N	N	N	N	N	N	N	N
skin;		.	.	.	.	.	+	.	.	.	.
crust formation		.	.	.	.	.	P	.	.	.	.
erosion; focal		.	.	.	.	.	2	.	.	.	.
inflammation, subacute		.	.	.	.	.	2	.	.	.	.
cyst; subcutaneous		.	.	.	.	.	P	.	.	.	.
skin, abdominal;		N	N	N	N	N	N	N	N	N	N
spinal cord;		N	N	N	N	N	N	N	N	N	N
spleen;		N	N	N	N	N	N	N	N	N	N
stomach, fundic;		N	N	N	N	N	N	N	N	N	N
thymus;		N	N	N	+	N	N	N	N	N	N
atrophy		.	.	.	1	.	.	.	.	.	.
thyroid glands;		N	N	N	N	N	N	N	N	N	N
tongue;		N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

SEX: FEMALE										
GROUP	1	1	1	1	1	3	3	3	3	3
REMOVAL REASON	R	R	R	R	R	R	R	R	R	R
ANIMAL	.	.	.	.	1	2	2	2	2	3
NUMBER	2	4	6	8	0	2	4	6	8	0
trachea;	N	N	N	N	N	N	N	N	N	N
ureters;	N	N	N	X	N	N	N	N	N	N
urinary bladder;	N	N	N	N	X	N	N	N	N	N
uterus;	N	N	N	N	+	N	N	N	N	N
lumen; dilation	.	.	.	.	3	.	.	.	.	.
vagina;	N	N	N	N	N	N	N	N	N	N

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazol
e in Rats with Micronucleus Assessment

Key Page

Group

Code	Description
1	0 ug/kg/day
3	153 ug/kg/day

Removal Reason

Code	Description
R1	Killed Terminal

Tissue Result

Code	Description
N	N.V.L
.	Not Recorded
+	Tissue Observation Present
X	Not Examined

Grade

Code	Description
.	not recorded
1	minimal
2	mild
3	moderate
#	duplicate
P	present - no grade or classification

Pathology - Histo Pathology Matrix

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

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APPENDIX C

PATHOLOGY – CORRELATION OF FINDINGS

Pathology - Correlation of Findings

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Animal Ref.: 22 Group: 3 Sex: Female Species: Rat
Test Material: See Protocol Dose: 153 ug/kg/day
Date of Death : 11/23/2010 Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

IN-LIFE OBSERVATIONS	GROSS PATHOLOGY OBSERVATIONS	HISTO PATHOLOGY OBSERVATIONS
None	skin; crust; dorsal; minimal	skin; crust formation
		skin; erosion; focal; mild
		skin; inflammation, subacute; mild
		skin; cyst; subcutaneous

Pathology - Correlation of Findings

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole
e in Rats with Micronucleus Assessment

Animal Ref.: 27 Group: 3 Sex: Male Species: Rat
Test Material: See Protocol Dose: 153 ug/kg/day
Date of Death : 11/23/2010 Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY INCOMPLETE **

** EXAMINATION COMPLETE **

IN-LIFE OBSERVATIONS	GROSS PATHOLOGY OBSERVATIONS	HISTO PATHOLOGY OBSERVATIONS
None	kidneys; dilation; right; minimal	kidneys; pelvis; dilatation; unilateral; mild
	skin; crust; brown; minimal	skin; crust formation

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Appendix 4

Micronucleus Report (BioReliance Corporation)

PRINCIPAL INVESTIGATOR'S REPORT

Study Title

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

In support of RTI Project Number 0211886.002

Test Article

Fluoromisonidazole

Authors

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

Final Report Date

04 May 2011

Test Site

BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AD13SN.129GLP.BTL

Testing Facility

RTI International
Pharmacology and Toxicology
Post Office Box 12194
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

Sponsor

Clinical Monitoring Research Program, SAIC Frederick, Inc.
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 AD13SN.129GLP.BTL, as a part of the RTI Project Number 0211886.002 (RTI Master Protocol Number RTI-1111, RTI Study Code Rt10-FMIS), in compliance with the US Food and Drug Administration Good Laboratory Practices (GLP) 21 CFR Part 58.

Ljubicia Krsmanovic, Ph.D.
Principal Investigator
BioReliance

04 May 2011
Date

BioReliance Management

04 May 2011
Date

BioReliance®

Quality Assurance Statement

Delegated Study Phase Information

Number: AD13SN.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.

US FDA Good Laboratory Practices 21CFR 58

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
16-Dec-2010	16-Dec-2010	Observation of Test System	08-Apr-2011	08-Apr-2011	08-Apr-2011
24-Mar-2011	25-Mar-2011	Data and Draft Reporting	28-Mar-2011	28-Mar-2011	08-Apr-2011
02-May-2011	03-May-2011	Final Reporting	03-May-2011	03-May-2011	04-May-2011

Inspections listed above are study specific unless denoted with an * (process based inspections).

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: Ellen Burns 04-May-2011 8:06 pm GMT

Reason for signature: QA Approval

Printed by:Ellen Burns
Printed on:4-May-11

3.0 STUDY INFORMATION

Sponsor:	Clinical Monitoring Research Program, SAIC Frederick, Inc. 6130 Executive Boulevard EPN, Room 6070 Bethesda, MD 20892-7412
Sponsor Representative:	G. Craig Hill, Ph.D. [Contractor] SAIC-Frederick, Inc. CIP/DCTD/NCI/NIH Bethesda, MD 20892-7412
Study Director at RTI International:	Brenda Faiola, Ph.D., DABT P.O. Box 12194 3040 Cornwallis Road HLB-121 Research Triangle Park, NC 27709-2194
Testing Facility:	RTI International Pharmacology and Toxicology P.O. Box 12194 3040 Cornwallis Road Research Triangle Park, NC 27709-2194
RTI Project Number:	0211886.002
RTI Master Protocol Number:	RTI-1111
RTI Study Code:	Rt10-FMIS
Test Site:	BioReliance 9630 Medical Center Drive Rockville, MD 20850
BioReliance Study Number:	AD13SN.129GLP.BTL
Principal Investigator:	Ljubica Krsmanovic, Ph.D.
Test Article Name:	Fluoromisonidazole
Material Received at BioReliance:	Bone marrow slides
Storage Conditions:	Ambient (15 to 30°C); protected from exposure to light without desiccant
Receipt/Login:	30 November 2010
Study Initiation:	18 October 2010
Experimental Start/Completion Date (Microscopic Evaluation of Slides):	07 January 2011/08 January 2011

4.0 TABLE OF CONTENTS

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5.0 INTODUCTION

The overall objective of the in vivo study was to assess the toxicity, including micronucleus induction, of fluoromisonidazole (FMISO) when administered by intravenous injection to Sprague-Dawley (CD®IGS) rats for 14 consecutive days. The objective of this portion of the study was to analyze the bone marrow for the presence of micronucleated polychromatic erythrocytes (MPCEs) in order to assess the genotoxic potential of fluoromisonidazole.

6.0 MATERIAL AND METHODS

6.1 Study Design

The in vivo study was conducted at the testing facility (RTI International) under the RTI master Protocol No.: RTI-1111. Details of the study design, handling of animals, preparation of dosing formulations, dosing of animals, observations of animals and necropsy procedures are presented in that study protocol and study report generated by RTI International.

At the testing facility, following euthanasia, two bone marrow slides from the left femur of each animal (Groups 1-4) were prepared. One slide/animal was shipped to the test site (BioReliance) to the attention of the Principal Investigator. The test site received 32 bone marrow slides. Staining of slides, microscopic evaluation and reporting of the results were performed at the test site. The bone marrow smears were stained with acridine orange and 2000 polychromatic erythrocytes (PCEs) per animal were microscopically evaluated for the presence of micronucleated polychromatic erythrocytes (MPCEs). A statistical analysis of the data was performed using Kastenbaum-Bowman Tables (binomial distribution, $p \leq 0.05$).

Treatment Group (2 mL/kg/day)	Dose Level (µg/kg/day)	Number of Animals Used in the Study		No of Slides Received at the Test Site	
		Males	Females	Males	Females
1/Vehicle*	0	5	5	5	5
2/Fluoromisonidazole	39	5	5	5	5
3/Fluoromisonidazole	153	5	5	5	5
4/Cyclophosphamide monohydrate (CP)**	30 mg/kg**	2	-	2	0
Total Number of Slides Received:				17	15

***** 95:5% (v/v) 0.9% sodium chloride for injection, USP: absolute ethanol, USP.

**CP was administered intraperitoneally only once, at a dose volume of 5 mL/kg, approximately 24-25 hours prior to bone marrow collection time.

6.2 Bone Marrow Micronucleus Analysis

A total of 32 bone marrow slides were received by BioReliance on 30 November 2010 and a code number AD13SN (sample 0001) was assigned.

6.2.1 Staining with Acridine Orange

Upon receipt and prior to scoring, slides were stained with 12.5% Acridine orange solution (batch number 31, expiration date: 21 December 2010) for 1-2 minutes. The slides were then rinsed several times in a buffer consisting of 0.2M monobasic sodium phosphate and 0.2M disodium phosphate in deionized water. Stained slides were stored prior to scoring at 2-8°C.

6.2.2 Microscopic Evaluation

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 (ECs) in the early stage of erythropoiesis and are the target cells for the evaluation of 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 sex and per treatment group for Groups 1-3 and 4,000 PCEs for Group 4.

- **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 (MNCEs) in the field of 1000 total erythrocytes (ECs = PCEs + MPCEs + NCEs + MNCEs) was enumerated for each animal in order to calculate the proportion of polychromatic erythrocytes to total of 1000 erythrocytes.

- **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 (MPCEs) or NCEs (MNCEs).

7.0 EVALUATION OF TEST RESULTS

The incidence of micronucleated polychromatic erythrocytes (MPCEs) per 2000 polychromatic erythrocytes (PCEs) for each rat and per 10,000 PCEs per sex for the vehicle-treated and each test article-treated group was determined. The incidence of the MPCEs in the positive control group was pre-determined from a total of 4000 PCEs (2000 per rat). A statistical evaluation of the data was performed using binomial distribution and Kastenbaum-Bowman Tables for a significance 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 each sex and for each treatment group (PCEs/ total ECs ratio). A PCE/Total EC ratio in the test-article treated animals that is more than 20% of the vehicle control group value indicates bone marrow toxicity.

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

- The test article would have been considered to induce a positive genotoxic response if at least one dose was statistically significantly increased relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables).
- Values that were statistically significant but did not exceed the range of historical negative controls (Appendix I) would have been considered as not biologically significant or adverse and therefore, the test article would not have been considered to induce a positive genotoxic response.
- The test article would not have been considered to induce a positive genotoxic response if there were no statistically significantly increases at any dose level relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables).
- If criteria were not met, the results would have been judged as equivocal.
- In this study, the test article was considered not to have induced a positive genotoxic response, because there were no statistically significant increases in the incidence of MPCEs relative to the concurrent vehicle control values and no evidence of a dose response.

8.0 Records and Archives

All raw data, the protocol, amendments, all reports and correspondence, generated at BioReliance, will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RQA unit headquartered at: BioReliance, 14920 Broschart Rd., 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 maintained in the BioReliance archives for a minimum of 10 years.

All stained slides will be shipped back to the Testing Facility at the finalization of this Principal Investigator's report for archival with the RTI-1111 study data.

9.0 DEVIATION

The following deviation from BioReliance's Standard Operating Procedures (SOPs) occurred during the conduct of this portion of the study.

Bone marrow slides were coded using random numbers; however, the slides were not arranged in the ascending code numbers. This constituted a deviation (Record ID# 87534) from the

BioReliance SOPs. Since all slides were evaluated successfully and acceptance criteria were met, the Principal Investigator deemed this deviation did not have impact on the outcome of the study or integrity of the data.

10.0 RESULTS AND DISCUSSION

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

- No reductions in the ratio of polychromatic erythrocytes to total erythrocytes in the bone marrow were observed in the male or female test article-treated groups relative to the respective/concurrent negative control (vehicle-treated) groups, suggesting that the test article did not inhibit erythropoiesis or induce bone marrow toxicity.
- No statistically significant increases in the incidence of MPCEs in the bone marrow were observed in the male or female groups at either of the fluoromisonidazole doses tested (39 or 153 µg/kg/day) relative to the respective/concurrent negative control groups.
- CP, the positive control, induced a statistically significant increase in the incidence of MPCEs ($p \leq 0.05$, Kastenbaum-Bowman Tables) in male rats relative to the respective/ concurrent negative control group and induced bone marrow toxicity as anticipated.
- The incidence of MPCEs in the vehicle control groups did not exceed the historical vehicle control range.

Based on these results, all criteria for a valid test were met as specified in the protocol.

11.0 CONCLUSION

Under the conditions of the study conduct, fluoromisonidazole at dosage levels of up to and including 153 µg/kg/day for 14 consecutive days did not induce a significant increase in the incidence of micronucleated PCEs in the bone marrow of male and female Sprague Dawley CD® IGS rats. Therefore, fluoromisonidazole 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 CD® IGS [Crl:CD(SD)] Rats After Intravenous Exposure to Fluoromisonidazole for 14 Consecutive Days

Treatment (2 mL/kg/day)	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 [v]						
	M	5	0.599 ± 0.07	---	0.1 ± 0.22	1 / 10000
	F	5	0.563 ± 0.04	---	0.0 ± 0.00	0 / 10000
Fluoromisonidazole						
39 µg/kg/day	M	5	0.621 ± 0.09	4	0.2 ± 0.45	2 / 10000
	F	5	0.622 ± 0.07	10	0.1 ± 0.22	1 / 10000
153 µg/kg/day	M	5	0.631 ± 0.08	5	0.0 ± 0.00	0 / 10000
	F	5	0.585 ± 0.09	4	0.2 ± 0.27	2 / 10000
Cyclophosphamide**						
30 mg/kg	M	2	0.333 ± 0.08	-44	6.8 ± 1.06	*27 / 4000

[v]***** 95:5% (v/v) 0.9% sodium chloride for injection, USP: absolute ethanol, USP.
*Statistically significant increase compared to the respective vehicle control group
**Animals were dosed only once approximately 24-25 hours prior to bone marrow collection time, intraperitoneally at a volume of 5 mL/kg

Table 2: Induction of Micronucleated Polychromatic Erythrocytes in Bone Marrow of CD® IGS [Crl:CD(SD)] Rats After Intravenous Exposure to Fluoromisonidazole for 14 Consecutive Days

Treatment	Sex	Animal Number	PCE/Total Erythrocytes	Micronucleated PCE (Number/PCE scored)
Vehicle[v]				
	M	1	0.586	0 / 2000
		3	0.555	0 / 2000
		5	0.527	0 / 2000
		7	0.628	1 / 2000
		9	0.701	0 / 2000
	F	2	0.545	0 / 2000
		4	0.570	0 / 2000
		6	0.539	0 / 2000
		8	0.537	0 / 2000
		10	0.625	0 / 2000
Fluoromisonidazole 39 µg/kg/day	M	11	0.655	2 / 2000
		13	0.735	0 / 2000
		15	0.510	0 / 2000
		17	0.566	0 / 2000
		19	0.639	0 / 2000
	F	12	0.728	0 / 2000
		14	0.543	0 / 2000
		16	0.622	1 / 2000
		18	0.611	0 / 2000
		20	0.607	0 / 2000
153 µg/kg/day	M	21	0.610	0 / 2000
		23	0.716	0 / 2000
		25	0.564	0 / 2000
		27	0.708	0 / 2000
		29	0.555	0 / 2000
	F	22	0.675	1 / 2000
		24	0.687	0 / 2000
		26	0.540	0 / 2000
		28	0.516	0 / 2000
		30	0.507	1 / 2000
Cyclophosphamide 30 mg/kg	M	31	0.277	15 / 2000
		33	0.388	12 / 2000

[v]* 95:5% (v/v) 0.9% sodium chloride for injection, USP: absolute ethanol, USP.

13.0 APPENDICES

12.1 Appendix I: Micronucleus Test Historical Control Data

Rat Micronucleus Test Historical Control Data
2006-2009

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]	0.55	0.55	0.44	0.44	2.24	2.19
Standard Deviation	0.06	0.06	0.59	0.58	1.60	1.45
Range[4]	0.22 - 0.77	0.23 - 0.83	0 - 4	0 - 3	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]	0.43	0.41	33.78	23.45	174.58	118.24
Standard Deviation	0.07	0.07	13.27	6.53	68.80	30.59
Range[4]	0.23 - 0.75	0.19 - 0.66	10 - 97	11 - 55	92 - 472	72 – 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 2006-2009 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: 20 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 2006-2009; average of the number of MPCE per 2000 PCE for the total number of animals used from 2006-2009; average of number of MPCE/per group (containing 5-7 animals per group) for total number of groups used in 2006-2009.

[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 2006-2009 and the minimum and maximum range of MPCE observed out of 10000 PCE for the total number of groups used in 2006-2009.

FINAL REPORT AMENDMENT

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

Test Article I.D.: **Fluoromisonidazole**

BioReliance Study No.: **AD13SN.129GLP.BTL**

RTI Project Number: **0211886.002**

Report Title: **14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment**

Final Report Date: **04 May 2011**

Date of Final Report Being Amended: **31 May 2011**

1. **Part of Final Report to be Amended:** Page 5, Table of Contents

   Amendment: To update the Table of Contents to include Section number 8.0 and to reflect the correct section numbers from there onwards.

   Reason for the Amendment: Report preparation error. Amended page attached.

2. **Part of Final Report to be Amended:** Page 14, Section number.

   Amendment: Section number should read as 13.1.

   Reason for the Amendment: Report preparation error. Amended page attached.

Study Director's Statement

The changes to this report had no impact on the scientific validity or interpretation of the results of this study.

The amendment did not entail generation of new data, revision of calculations, or modification of previously submitted data and did not change the conclusion of the study. The signature below certifies that the revised pages have been reviewed and approved by the Principal Investigator.

Ljubica Krsmanovic — 31 May 2011

Ljubica Krsmanovic, Ph.D.
Principal Investigator

Date

Quality Assurance Statement

Quality Assurance performed the inspections below for this study.

Inspection Start Date	Inspection End Date	Phase Inspected	Date Reported to Principal Investigator/ Study Director	Date Reported to Principal Investigator/Study Director Management
05/26/2011	05/26/2011	Report Amendment	27 May 2011/31 May 2011	27 May 2011/31 May 2011

The Final Report Amendment for this study accurately reflects the changes made to the Final Report.

Luleayenwa Aberra-Degu — 31 may 2011

Luleayenwa Aberra-Degu
Quality Assurance

Date

4.0 TABLE OF CONTENTS

THIS PAGE REVISED

BioReliance Study No. AD13SN.129GLP.BTL

Zulica Krmpotic — Signature

31 May 2011 — Date

13.1 Appendix I: Micronucleus Test Historical Control Data

THIS PAGE REVISED

BioReliance Study No. AD13SN.129GLP.BTL

Julica Krsmanovic
Signature

31 May 2011
Date

Appendix 5

Individual Animal Data Tables

Table of Contents

Table 1. Male Clinical Observations

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	m	1	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		3	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		5	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		7	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		9	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 1. Male Clinical Observations

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	m	11	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		13	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		15	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		17	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		19	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 1. Male Clinical Observations

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	m	21	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		23	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		25	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		27	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		29	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

3

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 1. Male Clinical Observations

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	m	31	Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		33	Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 2. Female Clinical Observations

(Page 1 of 3)

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	f	2	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		4	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		6	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	.	X	X	X
			Diarrhea	.	.	.	.	.	.	.	.	.	.	.	X	.	.	.
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		8	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		10	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 2. Female Clinical Observations

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date														
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	f	12	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		14	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		16	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		18	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Sore(s) on Body	.	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		20	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 2. Female Clinical Observations

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

				Day numbers relative to Start Date													
Group	Sex	Animal	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	f	22	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Sore(s) on Body	.	.	.	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		24	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		26	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		28	No Abnormalities Detected	X	X	X	.	X	X	X	X	X	X	X	X	X	X	X
			Sore(s) on Body	.	.	.	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		30	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled Removal (Terminal)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 3. Male Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
1	m	1	207.1	272.1	284.5	334.7	389.3	362.9
		3	204.9	258.4	268.6	324.5	362.0	342.3
		5	205.1	268.3	279.8	333.0	376.3	353.3
		7	201.7	284.1	291.5	358.3	420.1	391.5
		9	205.4	278.1	288.6	345.6	396.8	371.4
		Mean	204.84	272.20	282.60	339.22	388.90	364.28
		S.D.	1.96	9.77	8.98	13.04	21.90	18.69
		N	5	5	5	5	5	5

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* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day MISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 3. Male Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
2	m	11	205.0	271.3	277.1	328.5	381.8	359.6
		13	196.1	261.3	263.3	300.4	336.5	324.2
		15	214.5	288.1	295.3	353.2	397.2	368.2
		17	206.5	276.4	283.0	316.3	347.8	330.5
		19	202.6	262.3	268.7	316.3	351.6	329.1
		Mean	204.94	271.88	277.48	322.94	362.98	342.32
		S.D.	6.66	11.04	12.51	19.64	25.43	20.07
		N	5	5	5	5	5	5

* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day MISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 3. Male Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
3	m	21	208.2	255.8	263.7	307.0	329.9	313.7
		23	198.9	272.1	278.0	340.1	391.4	363.2
		25	204.1	271.2	278.2	329.0	369.1	340.8
		27	210.2	279.2	285.7	342.3	392.6	363.2
		29	211.3	286.4	296.8	353.9	415.6	388.0
		Mean	206.54	272.94	280.48	334.46	379.72	353.78
		S.D.	5.08	11.38	12.11	17.71	32.34	27.94
		N	5	5	5	5	5	5

* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day MISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 3. Male Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
4	m	31	200.4	262.1	.	.	360.5	.
		33	208.9	275.2	.	.	374.1	.
		Mean	204.65	268.65	.	.	367.30	.
		S.D.	6.01	9.26	.	.	9.62	.
		N	2	2	0	0	2	0

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* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day MISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO Group 4 - 30 mg/kg Cyclophos

Table 4. Female Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
1	f	2	164.0	200.7	204.8	221.0	231.0	222.0
		4	157.0	187.8	182.7	186.1	190.2	182.2
		6	159.8	194.3	195.8	194.3	203.0	191.4
		8	166.7	206.8	209.2	217.9	236.6	224.6
		10	155.6	191.4	192.8	209.3	222.0	204.3
		Mean	160.62	196.20	197.06	205.72	216.56	204.90
		S.D.	4.67	7.58	10.41	15.08	19.48	18.56
		N	5	5	5	5	5	5

* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 4. Female Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
2	f	12	149.0	188.1	191.2	209.7	228.4	209.6
		14	165.5	207.0	204.0	214.8	226.1	213.2
		16	163.2	201.3	202.5	221.2	235.9	226.3
		18	165.7	194.0	199.6	211.7	225.4	206.8
		20	150.1	191.8	192.5	206.5	229.8	207.6
		Mean	158.70	196.44	197.96	212.78	229.12	212.70
		S.D.	8.42	7.62	5.82	5.59	4.18	7.99
		N	5	5	5	5	5	5

* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 4. Female Body Weights

Clinical Observations - Clinical Signs by Animal

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

			Bodyweight (g)					
			Day numbers relative to Start Date					
Group	Sex	Animal	-7	-1	0	6	13	14
3	f	22	158.3	187.5	188.9	205.0	221.0	207.3
		24	164.0	189.4	185.7	204.0	207.7	195.6
		26	155.4	204.8	203.7	213.6	225.7	209.6
		28	155.1	192.5	197.5	207.5	231.5	214.8
		30	165.3	196.6	194.4	207.1	220.3	195.9
		Mean	159.62	194.16	194.04	207.44	221.24	204.64
		S.D.	4.78	6.87	7.10	3.74	8.80	8.56
		N	5	5	5	5	5	5

* = Result to left has an associated comment or marker

Group 1 - 0 ug/kg/day FMISO Group 2 - 39 ug/kg/day FMISO Group 3 - 153 ug/kg/day FMISO

Table 5. Male Feed Consumption

Generalized Results - Animals by Time - Fixed Parameter
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Sex: Male

Food Mean Daily Consumption (g/day)

0 ug/kg/day FMISO	Day(s) Relative to Start Date	
	0 → 6	6 → 13
1	28.8	30.4
3	28.8	31.1
5	27.5	29.0
7	32.1	34.6
9	29.1	30.4
Mean	29.27	31.09
SEM	0.77	0.94
N	5	5

Table 5. Male Feed Consumption

Generalized Results - Animals by Time - Fixed Parameter
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Sex: Male

Food Mean Daily Consumption (g/day)

39 ug/kg/day FMISO	Day(s) Relative to Start Date	
	0 → 6	6 → 13
11	29.0	30.1
13	25.8	26.7
15	31.9	30.6
17	27.5	26.1
19	26.4	26.7
Mean	28.11	28.05
SEM	1.10	0.96
N	5	5

Table 5. Male Feed Consumption

Generalized Results - Animals by Time - Fixed Parameter
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Sex: Male

Food Mean Daily Consumption (g/day)

153 ug/kg/day FMISO	Day(s) Relative to Start Date	
	0 → 6	6 → 13
21	28.2	23.5
23	29.7	31.2
25	27.1	27.7
27	30.4	30.4
29	32.7	36.2
Mean	29.59	29.78
SEM	0.95	2.09
N	5	5

Table 6. Female Feed Consumption

Generalized Results - Animals by Time - Fixed Parameter

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Sex: Female

Food Mean Daily Consumption (g/day)

0 ug/kg/day FMISO	Day(s) Relative to Start Date	
	0 → 6	6 → 13
2	17.8	17.5
4	14.9	15.8
6	16.4	16.1
8	19.6	20.2
10	17.0	16.6
Mean	17.12	17.24
SEM	0.76	0.81
N	5	5

Table 6. Female Feed Consumption

Generalized Results - Animals by Time - Fixed Parameter
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Sex: Female Food Mean Daily Consumption (g/day)

39 ug/kg/day FMISO	Day(s) Relative to Start Date	
	0 → 6	6 → 13
12	20.0	20.2
14	18.5	18.2
16	17.7	19.0
18	17.5	17.7
20	19.1	21.0
Mean	18.56	19.22
SEM	0.47	0.62
N	5	5

Table 6. Female Feed Consumption

Generalized Results - Animals by Time - Fixed Parameter
RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

Sex: Female

Food Mean Daily Consumption (g/day)

153 ug/kg/day FMISO	Day(s) Relative to Start Date	
	0 → 6	6 → 13
22	16.7	22.6
24	17.3	16.3
26	19.6	20.2
28	18.2	20.0
30	16.7	17.8
Mean	17.69	19.38
SEM	0.55	1.08
N	5	5

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 1 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None Palpable Mass Details: None

Terminal Body Weight: 362.9g

Organ Weights:

		adrenal gland (paired):	0.0860g	brain :	2.1710g
heart :	1.5944g	kidney (paired) :	3.2408g	liver :	14.6431g
		pituitary gland (fixed):	0.0111g	prostate gland :	1.3099g>
spleen :	0.7697g	testis (paired) :	3.1978g	thymus :	0.6803g
thyroid (fixed) :	0.0186g				

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but no gross observations were recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 1 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: Correlated with:

heart;
infiltration; mononuclear cell; focal; minimal

kidneys;
infiltration; mononuclear cell; minimal
tubule; epithelium; regeneration; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; mild
necrosis; coagulative; focal; minimal
hepatocyte; centrilobular; vacuolation; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides
esophagus	eyes	injection site	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric		lymph node, mandibular	
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	pancreas	parathyroid glands
pituitary gland	prostate gland	salivary gland, mandibular		seminal vesicles	skin, abdominal	spinal cord
spleen	stomach, fundic	testes	thymus	thyroid glands	tongue	trachea
ureters	urinary bladder					

The following tissues have not been examined: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 1 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 3 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 342.3g

Organ Weights:

		adrenal gland (paired)	0.0843g	brain		2.1158g	
heart	1.2356g	kidney (paired)	3.6264g	liver		13.2661g	
		pituitary gland (fixed)	0.0133g	prostate gland		1.1183g	
spleen	0.7405g	testis (paired)	3.2858g	thymus		0.7386g	
thyroid (fixed)	0.0155g						

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 3 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: Correlated with:

injection site;
adjacent; hemorrhage; mild
adjacent; inflammation, subacute; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; minimal
hepatocyte; centrilobular; vacuolation; minimal

lungs;
interstitium; inflammation; multifocal; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	kidneys	lymph node, mesenteric		lymph node, mandibular	
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	pancreas	parathyroid glands
pituitary gland	prostate gland	salivary gland, mandibular		seminal vesicles	skin, abdominal	spinal cord
spleen	stomach, fundic	testes	thymus	thyroid glands	tongue	trachea
ureters	urinary bladder					

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 3 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 5 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 353.3g

Organ Weights:

		adrenal gland (paired)	0.0730g	brain		2.2298g	
heart	1.6497g	kidney (paired)	3.3731g	liver		12.2558g	
		pituitary gland (fixed)	NC	prostate gland		1.2304g	
spleen	0.7147g	testis (paired)	3.4377g	thymus		0.6408g	
thyroid (fixed)	0.0169g						

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 5 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: NC = Not Calculable

Histo Pathology Observations:		Correlated with:
injection site;	adjacent; hemorrhage; mild	
	adjacent; inflammation, subacute; mild	
kidneys;	infiltration; mononuclear cell; minimal	
	tubule; epithelium; regeneration; minimal	
liver;	inflammation, chronic; multifocal; minimal	
lungs;	mineralization; vascular; minimal	
lymph node, mandibular;	plasmacytosis; mild	
pancreas;	acinar cell; atrophy; focal; mild	
parathyroid glands;	ONE OF A PAIR PRESENT.	

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lymph node, mesenteric		mammary glands	skeletal muscle, quadriceps femoris	
nerve, optic	nerve, sciatic	prostate gland	salivary gland, mandibular		seminal vesicles	skin, abdominal

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 5 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues were within normal limits: (continued)

spinal cord spleen stomach, fundic testes thymus thyroid glands tongue

trachea ureters urinary bladder

The following tissues have not been examined:

pituitary gland; MISSING

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 7 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 391.5g

Organ Weights:

		adrenal gland (paired)	0.0854g	brain		2.0995g	
heart	1.4977g	kidney (paired)	3.3379g	liver		14.7906g	
		pituitary gland (fixed)	0.0106g	prostate gland		1.2653g	
spleen	0.7102g	testis (paired)	3.0307g	thymus		0.5687g	
thyroid (fixed)	0.0149g						

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 7 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: Correlated with:

adrenal glands;
cortical cells; vacuolation; minimal

heart;
infiltration; mononuclear cell; focal; minimal

injection site;
adjacent; inflammation, subacute; minimal

kidneys;
infiltration; mononuclear cell; minimal
tubule; dilatation; unilateral; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; minimal

lungs;
hair embolus
inflammation; granulomatous; focal; mild
mineralization; vascular; minimal

parathyroid glands;
ONE OF A PAIR PRESENT.

prostate gland;
infiltration; mononuclear cell; minimal

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 7 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues were within normal limits:

aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides	esophagus
eyes	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum	intestine, jejunum	intestine, rectum
lymph node, mesenteric		lymph node, mandibular		mammary glands	skeletal muscle, quadriceps femoris	
nerve, optic	nerve, sciatic	pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular	
seminal vesicles	skin, abdominal	spinal cord	spleen	stomach, fundic	testes	thymus
thyroid glands	tongue	trachea	ureters	urinary bladder		

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 9 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 371.4g

Organ Weights:

			adrenal gland (paired)	0.0858g	brain		2.0962g
heart		1.2528g	kidney (paired)	3.4522g	liver		13.4723g
			pituitary gland (fixed)	0.0146g	prostate gland		1.1163g
spleen		0.7836g	testis (paired)	3.3182g	thymus		0.6824g
thyroid (fixed)		0.0158g					

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None
None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 9 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations:		Correlated with:
kidneys;	infiltration; mononuclear cell; minimal tubule; dilatation; unilateral; minimal	
liver;	inflammation, chronic; multifocal; minimal	
lungs;	mineralization; vascular; minimal	
lymph node, mandibular;	plasmacytosis; minimal	
trachea;	mucosal glands; dilatation; mild	

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum
brain	epididymides	esophagus	eyes	heart
injection site	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lymph node, mesenteric	mammary gland	skeletal muscle, quadriceps femoris
nerve, optic	nerve, sciatic	pancreas	parathyroid glands	pituitary gland
prostate gland	salivary gland, mandibular	seminal vesicles	skin, abdominal	spinal cord
spleen	stomach, fundic	testes	thymus	thyroid glands
tongue	ureters	urinary bladder		

The following tissues have not been examined: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 9 Group: 1 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 11 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 359.6g

Organ Weights:

		adrenal gland (paired)	0.0928g	brain		2.0549g	
heart	1.3980g	kidney (paired)	3.0743g	liver		13.6936g	
		pituitary gland (fixed)	0.0152g	prostate gland		0.8468g	
spleen	0.7788g	testis (paired)	3.3844g	thymus		0.7822g	
thyroid (fixed)	0.0175g						

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 11 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 13 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 324.2g

Organ Weights:

			adrenal gland (paired)	0.0664g	brain		2.0783g
heart		1.3375g	kidney (paired)	2.8560g	liver		10.7888g<
			pituitary gland (fixed)	0.0122g	prostate gland		1.2330g
spleen		0.6825g	testis (paired)	2.9571g	thymus		0.6035g
thyroid (fixed)		0.0179g					

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were
inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 13 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 15 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 368.2g

Organ Weights:

		adrenal gland (paired)	0.0818g	brain		1.8994g	
heart	1.5615g	kidney (paired)	3.3458g	liver		14.4458g	
		pituitary gland (fixed)	0.0127g	prostate gland		1.0045g	
spleen	0.7865g	testis (paired)	3.2109g	thymus		0.6181g	
thyroid (fixed)	0.0195g						

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 15 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 17 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 330.5g

Organ Weights:

			adrenal gland (paired)	0.0888g	brain		1.9546g
heart		1.4340g	kidney (paired)	3.2589g	liver		11.4578g<
			pituitary gland (fixed)	0.0127g	prostate gland		1.1304g
spleen		0.7255g	testis (paired)	2.8603g	thymus		0.4463g
thyroid (fixed)		0.0193g					

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were
inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 17 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 19 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 329.1g

Organ Weights:

			adrenal gland (paired)	0.0528g	brain		2.1254g
heart		1.4336g	kidney (paired)	3.2370g	liver		11.8137g<
			pituitary gland (fixed)	0.0136g	prostate gland		0.9690g
spleen		0.7029g	testis (paired)	3.3490g	thymus		0.8381g
thyroid (fixed)		0.0207g					

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were
inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 19 Group: 2 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 21 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 313.7g

Organ Weights:

			adrenal gland (paired)	0.0707g	brain		2.0724g
heart		1.4371g	kidney (paired)	2.9354g	liver		10.6762g<
			pituitary gland (fixed)	0.0099g	prostate gland		0.8857g
spleen		0.6137g	testis (paired)	3.1228g	thymus		0.4318g
thyroid (fixed)		0.0115g					

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 21 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations:		Correlated with:
liver;	bile duct; hyperplasia; minimal inflammation, chronic; multifocal; minimal	
lungs;	mineralization; vascular; minimal	
parathyroid glands;	ONE OF A PAIR PRESENT.	
thymus;	atrophy; minimal	

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides
esophagus	eyes	heart	injection site	intestine, cecum	intestine, colon	intestine, duodenum
intestine, ileum	intestine, jejunum	intestine, rectum	kidneys	lymph node, mesenteric		lymph node, mandibular
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	pancreas	parathyroid glands
pituitary gland	prostate gland	salivary gland, mandibular		seminal vesicles	skin, abdominal	spinal cord
spleen	stomach, fundic	testes	thyroid glands	tongue	trachea	ureters
urinary bladder						

The following tissues have not been examined: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 21 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 23 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 363.2g

Organ Weights:

		adrenal gland (paired)	0.0780g	brain		2.0343g	
heart	1.4402g	kidney (paired)	3.5978g	liver		13.7994g	
		pituitary gland (fixed)	0.0145g	prostate gland		1.0316g	
spleen	0.7680g	testis (paired)	3.1595g	thymus		0.4998g	
thyroid (fixed)	0.0187g						

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 23 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: Correlated with:

adrenal glands;
cortical cells; vacuolation; minimal

heart;
infiltration; mononuclear cell; focal; minimal

injection site;
adjacent; hemorrhage; moderate
adjacent; inflammation, subacute; mild

kidneys;
infiltration; mononuclear cell; minimal
tubule; epithelium; regeneration; minimal

liver;
inflammation, chronic; multifocal; minimal

lungs;
hair embolus
inflammation; granulomatous; focal; minimal
interstitium; inflammation; multifocal; minimal

nerve, optic;
ONE OF A PAIR PRESENT.

prostate gland;
infiltration; mononuclear cell; minimal

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 23 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues were within normal limits:

aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides	esophagus
eyes	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum	intestine, jejunum	intestine, rectum
lymph node, mesenteric		lymph node, mandibular		mammary glands	skeletal muscle, quadriceps femoris	
nerve, optic	nerve, sciatic	pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular	
seminal vesicles	skin, abdominal	spinal cord	spleen	stomach, fundic	testes	thymus
thyroid glands	tongue	trachea	ureters	urinary bladder		

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 25 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 340.8g

Organ Weights:

		adrenal gland (paired):	0.1031g	brain :	2.0688g
heart :	1.3410g	kidney (paired) :	3.3923g	liver :	12.9611g
		pituitary gland (fixed):	0.0152g	prostate gland :	1.0112g
spleen :	0.8174g	testis (paired) :	3.6035g	thymus :	0.5369g
thyroid (fixed) :	0.0123g				

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 25 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations:		Correlated with:
injection site;	adjacent; hemorrhage; mild	
	adjacent; inflammation, subacute; minimal	
kidneys;	infarction; unilateral; focal; mild	
	infiltration; mononuclear cell; mild	
liver;	bile duct; hyperplasia; minimal	
	inflammation, chronic; multifocal; minimal	
	hepatocyte; centrilobular; vacuolation; minimal	
lungs;	foamy alveolar macrophages; multifocal; minimal	
	interstitium; inflammation; multifocal; mild	

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lymph node, mesenteric		lymph node, mandibular		mammary glands
skeletal muscle, quadriceps femoris	nerve, optic	nerve, sciatic	pancreas	parathyroid glands	pituitary gland	
prostate gland	salivary gland, mandibular	seminal vesicles	skin, abdominal	spinal cord	spleen	
stomach, fundic	testes	thymus	thyroid glands	tongue	trachea	ureters
urinary bladder						

The following tissues have not been examined: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 25 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 27 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 363.2g

Organ Weights:

		adrenal gland (paired)	0.0831g	brain		2.0869g	
heart	1.2390g	kidney (paired)	3.1822g	liver		14.2775g	
		pituitary gland (fixed)	0.0144g	prostate gland		0.9167g	
spleen	0.6523g	testis (paired)	3.1481g	thymus		0.5943g	
thyroid (fixed)	0.0181g						

Gross Pathology Observations:

Correlated with:

kidneys;
dilation; right; minimal (TGL)

mammary glands;
Tissue was saved and submitted for histology but gross observations were
inadvertently not recorded

skin;
crust; brown; minimal (TGL)

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

The following tissues have not been examined: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 27 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Codes Used: (TGL) = Trackable Gross Lesion

Histo Pathology Observations: Correlated with:

kidneys;
pelvis; dilatation; unilateral; mild

liver;
inflammation, chronic; multifocal; minimal

lungs;
mineralization; vascular; mild

lymph node, mandibular;
plasmacytosis; mild

skin;
crust formation
erosion; focal; mild
inflammation, subacute; mild

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides	
esophagus	eyes	heart	injection site	intestine, cecum	intestine, colon	intestine, duodenum	
intestine, ileum	intestine, jejunum	intestine, rectum	lymph node, mesenteric	mammary glands			

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 27 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues were within normal limits: (continued)

skeletal muscle, quadriceps femoris	nerve, optic	nerve, sciatic	pancreas	parathyroid glands	pituitary gland	prostate gland
salivary gland, mandibular	seminal vesicles	skin, abdominal	spinal cord	spleen	stomach, fundic	testes
thymus	thyroid glands	tongue	trachea	ureters	urinary bladder	

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 29 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: 388g

Organ Weights:

adrenal gland (paired)	0.0723g	brain	2.1216g		
heart	1.5689g	kidney (paired)	3.6897g	liver	13.1054g
pituitary gland (fixed)	0.0142g	prostate gland	1.2711g		
spleen	0.7405g	testis (paired)	2.9905g	thymus	0.5637g
thyroid (fixed)	0.0165g				

Gross Pathology Observations: Correlated with:

mammary glands;
Tissue was saved and submitted for histology but gross observations were inadvertently not recorded

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 29 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: None

Histo Pathology Observations: Correlated with:

injection site;
adjacent; hemorrhage; minimal
adjacent; inflammation, subacute; minimal

liver;
inflammation, chronic; multifocal; minimal
hepatocyte; centrilobular; vacuolation; minimal

lungs;
mineralization; vascular; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	epididymides
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	kidneys	lymph node, mesenteric		lymph node, mandibular	
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	pancreas	parathyroid glands
pituitary gland	prostate gland	salivary gland, mandibular		seminal vesicles	skin, abdominal	spinal cord
spleen	stomach, fundic	testes	thymus	thyroid glands	tongue	trachea
ureters	urinary bladder					

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 29 Group: 3 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Cause Of Death: None

Codes Used: None

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Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 31 Group: 4 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 30 mg/kg Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None Palpable Mass Details: None

Terminal Body Weight: None

Organ Weights: None

Gross Pathology Observations: Correlated with:

This animal is positive control for collection of bone marrow smear only

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 31 Group: 4 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 30 mg/kg Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 33 Group: 4 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 30 mg/kg Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations: None

Palpable Mass Details: None

Terminal Body Weight: None

Organ Weights: None

Gross Pathology Observations: Correlated with:

This animal is a positive control for collection of bone marrow smear only

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Codes Used: None

Table 7. Male Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 33 Group: 4 Sex: Male Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 30 mg/kg Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Histo Pathology Observations: None Correlated with: None

The following tissues were within normal limits: None

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Codes Used: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 2 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 222g

Organ Weights:

			adrenal gland (paired)	:	0.0700g	brain	: 1.9263g
heart	:	0.9649g	kidney (paired)	:	2.0824g<	liver	: 7.9059g<
ovary (paired)	:	0.1311g				pituitary gland (fixed)	: 0.0169g
spleen	:	0.6600g	thymus	:	0.5763g	thyroid (fixed)	: 0.0156g
uterus and cervix	:	0.6515g					

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Probable cause of death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 2 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

eyes;
retina; dysplasia

kidneys;
tubule; mineralization; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; minimal

lymph node, mandibular;
plasmacytosis; minimal

parathyroid glands;
ONE OF A PAIR PRESENT.

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	heart	injection site	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric		mammary glands	
skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts	pancreas
parathyroid glands	pituitary gland	salivary gland, mandibular		skin, abdominal	spinal cord	spleen
stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters	urinary bladder
uterus	vagina					

The following tissues have not been examined: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 2 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 4 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 182.2g

Organ Weights:

			adrenal gland (paired)	: 0.0734g		brain	: 1.7624g
heart	: 0.6792g		kidney (paired)	: 1.5945g<		liver	: 6.2642g<
ovary (paired)	: 0.1219g					pituitary gland (fixed)	: 0.0147g
spleen	: 0.4574g		thymus	: 0.3453g		thyroid (fixed)	: 0.0193g
uterus and cervix	: 0.4979g						

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Probable cause of death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 4 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

injection site;
adjacent; hemorrhage; mild
adjacent; inflammation, subacute; mild

kidneys;
infiltration; mononuclear cell; minimal
tubule; mineralization; minimal

liver;
inflammation, chronic; multifocal; mild

lymph node, mandibular;
plasmacytosis; mild

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric		mammary glands	
skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts	pancreas
parathyroid glands	pituitary gland	salivary gland, mandibular		skin, abdominal	spinal cord	spleen
stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters	urinary bladder
uterus	vagina					

The following tissues have not been examined: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 4 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 6 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 191.4g

Organ Weights:

adrenal gland (paired)	0.0734g	brain	1.7561g		
heart	0.7074g	kidney (paired)	1.7890g<	liver	6.7050g<
ovary (paired)	0.1579g			pituitary gland (fixed)	0.0138g
spleen	0.4842g	thymus	0.4591g	thyroid (fixed)	0.0141g
uterus and cervix	0.6351g				

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Probable cause of death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 6 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

eyes;
retina; dysplasia

liver;
inflammation, chronic; multifocal; mild

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	heart	injection site	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	kidneys	lungs	lymph node, mesenteric	lymph node, mandibular	
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts
pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular		skin, abdominal	spinal cord
spleen	stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters
urinary bladder	uterus	vagina				

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 6 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 8 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 224.6g

Organ Weights:

				adrenal gland (paired)	:	0.0734g	
brain	:	2.0118g					
heart	:	0.9667g		kidney (paired)	:	2.2012g<	
liver	:	8.9862g<					
ovary (paired)	:	0.0986g<					
pituitary gland (fixed)	:	0.0173g					
spleen	:	0.5577g		thymus	:	0.4751g	
thyroid (fixed)	:	0.0129g					
uterus and cervix	:	0.7167g					

Gross Pathology Observations:

Correlated with:

None

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Probable cause of death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 8 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

heart;
infiltration; mononuclear cell; focal; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; minimal

lungs;
interstitium; inflammation; multifocal; minimal

lymph node, mandibular;
plasmacytosis; mild

pituitary gland;
cyst

thymus;
atrophy; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	eyes	injection site	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	kidneys	lymph node, mesenteric		mammary glands	
skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts	pancreas
parathyroid glands	salivary gland, mandibular		skin, abdominal	spinal cord	spleen	stomach, fundic
thyroid glands	tongue	trachea	urinary bladder	uterus	vagina	

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 8 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues have not been examined:

ureters; MISSING

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 10 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 204.3g

Organ Weights:

adrenal gland (paired)	0.0999g	brain	1.9205g		
heart	0.7663g	kidney (paired)	1.9277g<	liver	7.3683g<
ovary (paired)	0.1365g			pituitary gland (fixed)	0.0154g
spleen	0.5270g	thymus	0.5863g	thyroid (fixed)	NM
uterus and cervix	0.9985g				

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Probable cause of death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 10 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: NM = Not Measured

Histo Pathology Observations: Correlated with:

kidneys;
interstitium; inflammation; unilateral; focal; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; minimal

parathyroid glands;
ONE OF A PAIR PRESENT.

uterus;
lumen; dilation; moderate

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix	
esophagus	eyes	heart	injection site	intestine, cecum	intestine, colon	intestine, duodenum	
intestine, ileum	intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric	lymph node, mandibular		
mammary glands	skeletal muscle, quadriceps femoris	nerve, optic	nerve, sciatic	ovaries	oviducts		
pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular	skin, abdominal	spinal cord		
spleen	stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters	
vagina							

The following tissues have not been examined: urinary bladder; MISSING

No observations recorded for the following protocol required tissues: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 10 Group: 1 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 0 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 12 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 209.6g

Organ Weights:

		adrenal gland (paired)	: 0.0633g		brain	:	1.8677g
heart	: 0.8538g	kidney (paired)	: 1.7476g<		liver	:	7.5407g<
ovary (paired)	: 0.1268g				pituitary gland (fixed)	:	0.0167g
spleen	: 0.4901g	thymus	: 0.5195g		thyroid (fixed)	:	0.0143g
uterus and cervix	: 1.3330g>						

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 12 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

None

The following tissues were within normal limits:

None

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 14 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 213.2g

Organ Weights:

			adrenal gland (paired)	:	0.0789g	brain	: 1.8932g
heart	:	0.8072g	kidney (paired)	:	2.1198g<	liver	: 7.4877g<
ovary (paired)	:	0.1397g				pituitary gland (fixed)	: 0.0175g
spleen	:	0.4416g	thymus	:	0.7154g	thyroid (fixed)	: 0.0153g
uterus and cervix	:	0.5721g					

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 14 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

None

The following tissues were within normal limits:

None

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 16 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 226.3g

Organ Weights:

			adrenal gland (paired)	: 0.0543g	brain	:	2.0271g
heart	: 1.0244g		kidney (paired)	: 2.1911g<	liver	:	8.9023g<
ovary (paired)	: 0.0846g<				pituitary gland (fixed)	:	0.0133g
spleen	: 0.6344g		thymus	: 0.5407g	thyroid (fixed)	:	0.0139g
uterus and cervix	: 0.8549g						

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 16 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

None

The following tissues were within normal limits:

None

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 18 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 206.8g

Organ Weights:

		adrenal gland (paired)	: 0.0849g		brain	:	1.9324g
heart	: 0.7386g	kidney (paired)	: 2.1718g<		liver	:	7.3042g<
ovary (paired)	: 0.1551g				pituitary gland (fixed)	:	0.0136g
spleen	: 0.4691g	thymus	: 0.4403g		thyroid (fixed)	:	0.0150g
uterus and cervix	: 1.0714g>						

Gross Pathology Observations:

Correlated with:

skin;
crust; brown; dorsal; multiple; mild

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 18 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

None

The following tissues were within normal limits:

None

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 20 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 207.6g

Organ Weights:

			adrenal gland (paired)	:	NM	brain	: 1.9606g
heart	: 0.8625g		kidney (paired)	:	1.8778g<	liver	: 7.3659g<
ovary (paired)	: 0.1851g					pituitary gland (fixed)	: 0.0121g
spleen	: 0.5780g		thymus	:	0.5290g	thyroid (fixed)	: 0.0119g
uterus and cervix	: 0.8149g						

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 20 Group: 2 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 39 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

NM = Not Measured

Histo Pathology Observations: Correlated with:

None

The following tissues were within normal limits:

None

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 22 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 207.3g

Organ Weights:

			adrenal gland (paired)	: 0.0960g		brain	: 1.9156g
heart	: 0.7498g		kidney (paired)	: 2.2445g<		liver	: 7.2747g<
ovary (paired)	: 0.1815g					pituitary gland (fixed)	: 0.0132g
spleen	: 0.6349g		thymus	: 0.4623g		thyroid (fixed)	: 0.0124g
uterus and cervix	: 0.7002g						

Gross Pathology Observations:

Correlated with:

skin;
crust; dorsal; minimal (TGL)

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 22 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used: (TGL) = Trackable Gross Lesion

Histo Pathology Observations:		Correlated with:
injection site;	adjacent; inflammation, subacute; minimal	
kidneys;	tubule; mineralization; minimal	
liver;	bile duct; hyperplasia; minimal inflammation, chronic; multifocal; minimal	
lungs; mineralization; vascular; minimal interstitium; inflammation; multifocal; minimal		
lymph node, mandibular; plasmacytosis; minimal		
skin; crust formation erosion; focal; mild inflammation, subacute; mild cyst; subcutaneous		

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lymph node, mesenteric		mammary glands	skeletal muscle, quadriceps femoris	
nerve, optic	nerve, sciatic	ovaries	oviducts	pancreas	parathyroid glands	pituitary gland
salivary gland, mandibular		skin, abdominal	spinal cord	spleen	stomach, fundic	thymus

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 22 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues were within normal limits: (continued)

thyroid glands tongue trachea ureters urinary bladder uterus vagina

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 24 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 195.6g

Organ Weights:

				adrenal gland (paired)	:	0.0805g	
brain	:	1.7756g					
heart	:	0.8157g		kidney (paired)	:	1.8106g<	
liver	:	6.6340g<					
ovary (paired)	:	0.1291g					
pituitary gland (fixed)	:	0.0114g					
spleen	:	0.4233g		thymus	:	0.4381g	
thyroid (fixed)	:	0.0104g					
uterus and cervix	:	0.5178g					

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Probable cause of death:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 24 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

eyes;
retina; dysplasia

heart;
infiltration; mononuclear cell; focal; minimal

injection site;
adjacent; hemorrhage; mild
adjacent; inflammation, subacute; mild

liver;
inflammation, chronic; multifocal; minimal

lymph node, mandibular;
plasmacytosis; mild

skeletal muscle, quadriceps femoris;
infiltration; mononuclear cell; focal; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum	intestine, jejunum	intestine, rectum
kidneys	lungs	lymph node, mesenteric		mammary glands	nerve, optic	nerve, sciatic
ovaries	oviducts	pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular	
skin, abdominal	spinal cord	spleen	stomach, fundic	thymus	thyroid glands	tongue
trachea	ureters	urinary bladder	uterus	vagina		

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 24 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

The following tissues have not been examined:

None

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 26 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 209.6g

Organ Weights:

			adrenal gland (paired)	:	0.0600g	brain	: 1.8373g
heart	:	1.0539g	kidney (paired)	:	1.9344g<	liver	: 7.5031g<
ovary (paired)	:	0.0718g<				pituitary gland (fixed)	: 0.0136g
spleen	:	0.5532g	thymus	:	0.5965g	thyroid (fixed)	: 0.0135g
uterus and cervix	:	0.6600g					

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 26 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

kidneys;
tubule; mineralization; minimal

liver;
inflammation, chronic; multifocal; minimal
hepatocyte; periportal; vacuolation; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix	
esophagus	eyes	heart	injection site	intestine, cecum	intestine, colon	intestine, duodenum	
intestine, ileum	intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric		lymph node, mandibular	
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts	
pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular		skin, abdominal	spinal cord	
spleen	stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters	
urinary bladder	uterus	vagina					

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 26 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 28 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 214.8g

Organ Weights:

			adrenal gland (paired)	0.0914g		brain	2.0335g
heart	0.8445g		kidney (paired)	1.9593g<		liver	8.5415g<
ovary (paired)	0.1522g					pituitary gland (fixed)	0.0142g
spleen	0.5672g		thymus	0.5656g		thyroid (fixed)	0.0158g
uterus and cervix	0.7298g						

Gross Pathology Observations:

None

Correlated with:

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 28 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

injection site;
adjacent; hemorrhage; mild

kidneys;
tubule; mineralization; minimal
interstitium; inflammation; unilateral; focal; minimal

liver;
bile duct; hyperplasia; minimal
inflammation, chronic; multifocal; minimal

nerve, optic;
ONE OF A PAIR PRESENT.

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric		lymph node, mandibular	
mammary glands	skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts
pancreas	parathyroid glands	pituitary gland	salivary gland, mandibular		skin, abdominal	spinal cord
spleen	stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters
urinary bladder	uterus	vagina				

The following tissues have not been examined: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 28 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

No observations recorded for the following protocol required tissues:

None

Cause Of Death:

None

Codes Used:

None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 30 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Last Clinical Observations:

None

Palpable Mass Details:

None

Terminal Body Weight: 195.9g

Organ Weights:

			adrenal gland (paired)	: 0.0923g		brain	: 1.8969g
heart	: 0.8803g		kidney (paired)	: 1.9414g<		liver	: 6.5329g<
ovary (paired)	: 0.1370g					pituitary gland (fixed)	: 0.0163g
spleen	: 0.4980g		thymus	: 0.3766g		thyroid (fixed)	: 0.0149g
uterus and cervix	: 0.6733g						

Gross Pathology Observations:

Correlated with:

None

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

The following tissues have not been examined: None

No observations recorded for the following protocol required tissues: None

Probable cause of death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

---

Animal Ref.: 30 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Histo Pathology Observations: Correlated with:

kidneys;
tubule; mineralization; minimal

liver;
inflammation, chronic; multifocal; minimal

The following tissues were within normal limits:

adrenal glands	aorta	bone marrow, sternum	bone, femur	bone, sternum	brain	cervix
esophagus	eyes	heart	intestine, cecum	intestine, colon	intestine, duodenum	intestine, ileum
intestine, jejunum	intestine, rectum	lungs	lymph node, mesenteric		lymph node, mandibular	
skeletal muscle, quadriceps femoris		nerve, optic	nerve, sciatic	ovaries	oviducts	pancreas
parathyroid glands	pituitary gland	salivary gland, mandibular		skin, abdominal	spinal cord	spleen
stomach, fundic	thymus	thyroid glands	tongue	trachea	ureters	urinary bladder
uterus	vagina					

The following tissues have not been examined:

injection site; MISSING
mammary glands; MISSING

No observations recorded for the following protocol required tissues: None

Cause Of Death: None

Table 8. Female Organ Weights, Gross Pathology and Histopathology

Pathology - Individual Animal Data

RT10-FMIS - 14-Day Intravenous Repeat Dose Toxicology Study of Fluoromisonidazole in Rats with Micronucleus Assessment

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Animal Ref.: 30 Group: 3 Sex: Female Species: Rat Strain: Sprague-Dawley

Test Material: See Protocol Dose: 153 ug/kg/day Route: See Protocol Study Type: 14 Day Toxicity
Date of Death : 11/23/2010 Study Day No. (Week): 14 (2) Mode of Death: Killed Terminal
Date of Necropsy: 11/23/2010 ** NECROPSY COMPLETE **

** EXAMINATION COMPLETE **

Codes Used:

None

Appendix 6

Study Protocol and Amendments

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

SPONSOR: Clinical Monitoring Research Program
SAIC-Frederick, Inc.
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]
Telephone: 301-496-9531

TESTING FACILITY: RTI International*
Pharmacology 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

Brenda Faiola 18 Oct 2010
Brenda Faiola, Ph.D., DABT Date
Sr. Research Toxicologist, Pharmacology & Toxicology
Study Director
BOA/Contract Principal Investigator

18 Oct 2010
Hernan A. Navarro, Ph.D. Date
Senior Director, Discovery Sciences
Test Facility Management

Quality Assurance Review By:

Benjamin Rauscher
for Leslie Macdonald 10/18/2010
Leslie L. Macdonald, B.S. Date
Quality Assurance Specialist
RTI Quality Assurance Unit

Sponsor

G. Craig Hill 10/15/10 ①
G. Craig Hill, Ph.D. Date
SAIC-Frederick, Inc
Contracting Officer's Technical Representative

① For clarification, day is difficult to read. Should read "15" as per confirmation e-mail from Sponsor to Study Director sent on 10/15/10. BF 18 Oct 10

TABLE OF CONTENTS

1.0 Study Title

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

2.0 Personnel

Sponsor Representative:
G. Craig Hill, Ph.D. [Contractor]
SAIC-Frederick, Inc.
CIP/DCTD/NCI/NIH
6130 Executive Boulevard
Room 3005
Bethesda, MD 20892-7412
Telephone: 301-435-9184
Fax: 301-480-4631
E-mail: hillgc@mail.nih.gov

RTI Study Director:
Brenda Faiola, Ph.D., DABT
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: bfaiola@rti.org

Study Coordinator:
Jay G. Henson, B.S.
Telephone: 919-541-7206
E-mail: jhenson@rti.org

Director, Laboratory Animal Sciences:
Alyssa McIntyre, D.V.M.
Telephone: 919-541-3354
E-mail: amcintyre@rti.org

Manager, Animal Research Facility:
Mary Martinez, LAT
Telephone: 919-541-7085
E-mail: mmartinez@rti.org

Technical Group Supervisor:
Nicole M. Sayers, B.S.
Telephone: 919-541-7274
E-mail: nsayers@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
Analytical Chemistry	Brian F. Thomas, Ph.D. Telephone: 919-541-6552 E-mail: bft@rti.org
Quality Assurance Specialist:	Leslie L. Macdonald, B.S. Telephone: 919-485-2692 E-mail: lmacdonald@rti.org
Principal Investigator, Histopathology:	Glen E. Marrs, DVM Experimental Pathology Laboratories, Inc. Telephone: 919-998-9407 E-mail: gmarrs@epl-inc.com
Principal Investigator, Clinical Pathology:	Douglas Neptun Antech Diagnostics GLP Telephone: 919--277-0822 E-mail: doug.neptun@antechmail.com
Principal Investigator, Micronucleus Assay:	Ljubica Krsmanovic, Ph.D. BioReliance Telephone: 301-610-2162 E-mail: buba.krsmanovic@bioreliance.com

Additional personnel will be documented in the study file and presented in the final report as applicable.

3.0 Objective

The purpose of this study is to assess the toxicity, including micronucleus induction, of fluoromisonidazole (FMISO) when administered by intravenous injection to Sprague-Dawley (CD® IGS) rats for 14 consecutive days.

4.0 Study Schedule

Proposed Animal Receipt Date:	November 2010
Proposed Experimental Start Date:	November 2010
Proposed Necropsy Date:	November 2010
Proposed Audited Draft Report Date:	March 2011

5.0 Test and Control Article and Vehicle Information

Unless otherwise noted, the identity, purity, composition, stability and method of synthesis of each batch of test and control articles used in the conduct of the study are the responsibility of the

Supplier and selected by the Sponsor. This documentation will be maintained by the Supplier, and will be provided to RTI, approved by the Sponsor, and included in the study records.

5.1 Test Article

Sponsor Designation:	Fluoromisonidazole
Chemical Name:	1*H*-Imidazole-1-ethanol, α-(fluoromethyl)-2-nitro-
Synomyms:	1-Fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol; FMISO
CAS No.:	13551-89-8
Chemical Formula:	$C_6H_8FN_3O_3$
Lot Number:	20100401
Supplier:	ABX Advanced Biochemical Compounds
	H.-Gläser-Str. 10-14
	D-01454 Radeberg Germany
	Telephone: +49-3528-40 41 60
Purity:	>97% by ^{1}H-NMR according to the Certificate of Analysis provided by the Supplier.
Storage Conditions:	Desiccated, frozen (approximately -20 ± 5°C), protected from light under argon or nitrogen atmosphere.
Stability:	Long term stability not determined. Short term (<7days) storage at higher temperatures (<25°C) does not affect product quality. Retest Date: April 2012.
Safety Precautions:	Handle with care. Avoid inhalation, ingestion, and eye or skin contact.
Disposition:	Returned to Sponsor or disposed of according to RTI SOP as instructed by the Sponsor following study completion.
Reserve sample:	Since the in-life portion of the study is less than 4 weeks in duration, a reserve sample will not be retained.
Shipment to subcontractor:	Unopened vials of test article will be supplied by RTI to BioReliance (9630 Medical Center Drive, Rockville, MD 20850) for use in a bacterial reverse mutation assay which will be conducted under a separate study protocol signed by the BioReliance Study Director.

5.2 Control Article (for micronucleus assessment)

Sponsor Designation:	Cytoxan (positive control article)
Name:	Cyclophosphamide monohydrate

Supplier:	Sigma Aldrich, Inc. 3050 Spruce Street Saint Louis, MO 63103 USA Telephone: 800-325-5832
CAS No.:	6055-19-2
Product No.:	C0768
Lot No.:	079K1569
Purity:	100.5% by HPLC according to the Certificate of Analysis provided by the Supplier.
Stability:	approximately 3 years (retest date July 2012)
Storage Conditions:	Refrigerated (approximately 2-8°C)
Safety Precautions:	Care to be taken in handling; cyclophosphamide is a potent cytotoxic agent. A summary of the known hazards of cyclophosphamide is available in the material safety data sheet (MSDS) for this substance. Cyclophosphamide is a known human carcinogen and reproductive toxicant (teratogen). Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy. It cross-links DNA, causes strand breakage, and induces mutations. Use of engineering controls and appropriate PPE (including but not limited to gloves and a filtering facepiece respirator), as described in standard operating procedures (SOPs), will be used by staff working on this study.
Disposition:	Disposed of according to RTI SOP following study completion.
Reserve sample:	Since the in-life portion of the study is less than 4 weeks in duration, a reserve sample will not be retained.

5.3 Vehicle(s)

The vehicle for administration to the control group (Group 1) and for preparation of the test article dosing formulations will be 0.9% sodium chloride for injection, USP:absolute ethanol, USP (approximately 95%:5%, v:v). 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.

The vehicle for the positive control article will be sterile water for injection, USP. The control article dose formulation will be prepared on the day of use. The lot number, supplier, expiration date (if available) and handling procedures, as well as other pertinent information for the vehicle will be documented in the study records.

6.0 Test and Control Article Dose Preparation and Analysis

6.1 Dose Preparation

Information on dose formulation stability is the responsibility of the Sponsor. Based on the available stability information from the Sponsor, test article formulations will be prepared once by diluting a 1 mg/mL standard stock solution. Adjustments will not be made for the purity of the test article. The standard stock solution will be stored in 25 mL aliquots at approximately 0° to -20°C and will expire after 6 months at these conditions. Vehicle and diluted test article formulations will be stored refrigerated at approximately 2° to 8°C and will expire after 1 month at these conditions. Details of the dose preparation method will be included in the study file. The vehicle and test article formulations stored refrigerated will be allowed to warm by storing at room temperature for at least 30 minutes or by warming in a water bath set to 23°C for at least 10 minutes prior to administration to the test system.

The positive control article will be formulated once, on the day of use.

6.2 Dose Analysis

Approximately 1- to 3-mL samples will be collected from each vehicle and test article dose formulation on the date of preparation (i.e., date of dilution from the standard stock solution). The samples will be analyzed for concentration by RTI prior to being released for use on study. Concentrations of test article will be determined by a validated high performance liquid chromatography with ultraviolet detection (HPLC/UV) method. The standard for acceptable concentration will be that the mean of the analyzed samples must be within ± 15% of nominal. Homogeneity evaluation will not be performed as the formulations are solutions.

The positive control article formulation will not be analyzed for stability, homogeneity, or concentration.

6.3 Disposition of Samples Not Used for Dosing

Remaining formulated dose samples will be appropriately disposed of according to applicable RTI SOPs.

7.0 Test System

7.1 Species and Strain

CD® IGS rat [Crl:CD(SD)]

7.2 Source

Charles River Laboratories, Inc. (documentation of the specific breeding facility will be maintained in the study file).

7.3 Age

Approximately 7 weeks old at receipt; approximately 8 weeks old at initiation of dosing (Study Day 0). Animals outside of this range may be used at the discretion of the Study Director.

7.4 Weight

Approximately 225 to 275 grams for males and 175 to 225 grams for females at initiation of dosing (Study Day 0). Animals outside of this range may be used at the discretion of the Study Director.

7.5 Number/Gender

19 males and 17 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). Additional rats will be maintained to serve as replacements if needed.

7.6 Method of Identification

Each animal will be uniquely identified by ear-tag or implantable transponder.

7.7 Housing

All animals will be housed individually in appropriately sized solid-bottom polycarbonate cages suspended from stainless steel, self-watering racks or placed covered with a wire top lid on a shelf rack for use with water bottles. 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 for any conditions requiring possible veterinary care. If any such conditions are identified, the study director and staff veterinarian will be notified.

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 is conducted according to RTI SOP. 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. All animals will be examined by the veterinarian prior to release from quarantine.

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 vehicle and positive control group, are considered the minimum number of groups necessary to provide a range of effects and allow for appropriate data interpretation.

8.0 Experimental Design

8.1 Method of Group Assignment

Based on pretreatment procedures (e.g., body weight and clinical observation data), animals considered unsuitable for the study will be excluded from randomization to study groups by the Study Director. The randomization program of Provantis 8™ will be used to randomly allocate animals to groups while balancing body weights across groups. Additional rats (if available) will be retained for possible replacement if assigned animals. Any replacements will be documented in the study records.

8.2 Group Designation

The following table presents the study group assignment:

Group Number	Treatment	Dose	Dosing Concentration	Dosing Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[1]	0	0	2.0	5	5
2	Fluoromisonidazole	39 μg/kg/day	19.5 μg/ml	2.0	5	5
3	Fluoromisonidazole	153 μg/kg/day	76.5 μg/ml	2.0	5	5
4	Cyclophosphamide[2]	30 mg/kg	6.0 mg/ml	5.0	2	0

[1] Vehicle = 95:5 (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP
[2] Positive control for micronucleus assay. Cyclophosphamide will be administered by intraperitoneal injection 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. The daily dose of the high dose (153 μg/kg) in rats is 100 times the maximum human dose on a surface area basis. 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.

	Absolute Dose (μg)	Weight Dose (μg/kg)	Surface area dose[1] ($\mu g/m^2$)
60 kg human	15	0.249	9.24
250 g rat	39	153	924
Factor	2.6x	614x	100x
Cumulative dose	36.4x	8596x	1400x

[1] based on surface area of 60 kg human equal to 1.623 m^2 and of 250 g rat equal to 0.0415 m^2

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 Provantis 8™ (Instem LSS Ltd., Staffordshire, United Kingdom) automated data collection system will be used for collection of all body weights, feed weights, clinical observations, organs weights and gross necropsy findings. Provantis 8™ will calculate the volume of dosing solution to be administered to each animal on each day, based on the appropriate body weight. Provantis 8™ 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 will be made at least once daily for each toxicology group animal immediately after 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. If clinical signs are noted at times other than immediately after dosing, these observations will also be entered into the automated data capture system.

9.3 Body Weights

Body weights for toxicology group animals (Groups 1-3) will be recorded twice pretest [upon receipt and the day prior to start of dosing (i.e., Day -1)] 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 the day prior to start of dosing (i.e., Day -1)] and on Study Day 13.

9.4 Feed Consumption

Feed consumption will be measured 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 samples will be collected from all toxicology group animals (Groups 1-3) at the time of scheduled necropsy via cardiac puncture following exposure to CO_2. Animals will be fasted overnight prior to blood collection. Blood for hematology assessments (approximately 2 mL) will be collected into tubes containing K_3EDTA as the anticoagulant. Blood for serum chemistry assessments (up to 3.5 mL) will be collected into tubes with no anticoagulant, allowed to clot at room temperature, 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 then maintained frozen at approximately -70°C to -80°C until submitted for analysis. All samples will be submitted to Antech Diagnostics GLP:

Antech Diagnostics GLP
507 Airport Blvd. Suite 113
Morrisville, NC 27560
Telephone: 919-787-9528
Cell: 919-417-2542

A contributing scientist report detailing the methods and 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 (HCT)	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/calculated:

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

9.6 Anatomic Pathology

A complete necropsy will be conducted on all toxicology group animals (Groups 1-3). Animals will be fasted overnight prior to the terminal necropsy scheduled on Day 14. Animals will be euthanized by CO_2 asphyxiation and a final body weight will be collected (for all animals in Groups 1-3). Animals will be exsanguinated via cardiac puncture. A necropsy will be conducted on animals dying spontaneously or euthanized unscheduled; 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.

9.6.1 Organ Weights

The organs indicated below will be weighed from all toxicology group animals (Groups 1-3) euthanized at the scheduled necropsy on Day 14:

Adrenals[1]	Prostate
Brain	Spleen
Heart	Testes[1]
Kidneys[1]	Thymus
Liver	Thyroid with parathyroids[2]
Ovaries[1]	Uterus with oviducts
Pituitary[2]	

[1] Paired organs (adrenals, kidneys, ovaries, and testes will be weighed together.
[2] The pituitary and thyroid/parathyroids will be weighed following fixation.

Organs will not be weighed from animals found dead or euthanized unscheduled.

9.6.2 Tissue Fixation

At the time of necropsy, the following tissues and organs will be collected from all toxicology group animals (Groups 1-3) and placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands	Ovaries
Aorta	Oviducts
Brain	Pancreas
Bone (right femur with epiphyseal plate of head)	Prostate
Bone marrow (sternum)	Rectum
Cecum	Salivary gland (mandibular)
Colon	Sciatic nerve
Duodenum	Seminal vesicles
Eartag (animal ID)	Skeletal muscle (thigh)
Epididymides	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 (of final IV dose on Day 13)	Thyroid and parathyroid glands
Jejunum	Tongue
Kidney	Trachea
Liver (right medial lobe and left lateral lobe)	Ureter
Lungs[2]	Urinary bladder[2]
Lymph node (mandibular and mesenteric)	Uterus (body) with cervix
Mammary gland (females only; to include nipple and surrounding tissue)	Vagina

[1]Modified Davidson's solution initially, followed by 10% neutral-buffered formalin.
[2]Infused with formalin to ensure fixation.

9.6.3 Histopathology

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues listed in Section 9.6.2 for all animals euthanized *in extremis* and for all animals in Groups 1 and 3. Microscopic examination of target tissues will be extended to Group 2 at an additional cost to the Sponsor. Special stains, used at the discretion of the study pathologist to further characterize lesions and changes, will be at additional cost to the Sponsor. 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 Experimental Pathology Laboratories, Inc. (EPL) for processing and histopathological assessments to the contact below:

Experimental Pathology Laboratories, Inc.
615 Davis Drive
Suite 500
Durham, NC 27713
Telephone: 919-998-9407
Attention: Dr. John Seely

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 left femur will be prepared from all animals (Groups 1-4) 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 (1 slide per animal) will be shipped to BioReliance 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

Once received, the slides will be stained with acridine orange and scored according to BioReliance SOPs and methods. RTI will retain prepared bone marrow smears (1 slide per animal). If needed, these back-up slides will be shipped to BioReliance for assessment. If not needed for assessment, the slides will be archived. A contributing scientist report detailing the methods and results of the micronucleus assessment will be provided to RTI and included in the final report.

9.7 Observations for Unscheduled Deaths

	Groups 1 through 3		Group 4	
Circumstances of death	Euthanized	Found Dead	Euthanized	Found Dead
Clinical observations	yes	no	yes	no
Hematology	no	no	no	no
Serum chemistry	no	no	no	no
Terminal body weight	yes	no	no	no
Necropsy with macroscopic examination	yes	yes	no	no
Organ weights	no	no	no	no
Tissue fixation and microscopic examination	yes	yes	no	no
Micronucleus assessment	no	no	no	no

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
- 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

A data-audited draft report of this study will be submitted to the Sponsor within 12 weeks of the completion of necropsy. The Sponsor shall submit comments, if any, on the draft report to the Study Director within 45 working days. RTI will review and respond to any comments necessary for approval. The revised report will be audited and RTI will submit two hard copies (one bound, one unbound), and one electronic copy of the final signed report to the Sponsor. The statement of work and associated price included the issuance of an initial draft final report, 1 cycle of client comments and revisions, and issuance of a signed final report. Additional review cycles for draft reports or amendments/edits to the signed final report will result in the issuance of an additional work notice and additional charges 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 Provantis 8™ data collection system will be used for collection of all body weights (including quarantine), feed weights, clinical observations, organ weights, and gross necropsy findings. Provantis 8™ will also calculate the volume of dosing solution to be administered to each animal on each day, based on the appropriate body weight. Provantis 8 also records when each animal is dosed. Therefore, the raw data for these measurements will be the electronic data collected in Provantis 8™ 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 CFR 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 and in-life 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 a change will be made between the Study Director and the Sponsor. As soon as practical, the change and reasons for it will be formally document by the Study Director in an amendment to the study protocol which the Sponsor's representative will sign. 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.

U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. *Federal Register* **52 (172),** 33768-33782 (Sept 4, 1987).

U.S. Food and Drug Administration. Good Laboratory Practice Regulations for Nonclinical Laboratory Studies. Code of Federal Regulations (CFR), Title 21, Volume 1, 21CFR58 (Last Revised: April 1, 2008).

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

SPONSOR:
Clinical Monitoring Research Program
SAIC-Frederick, Inc.
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]
Telephone: 301-496-9531

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

AMENDMENT APPROVED BY:

G. Craig Hill 10/19/10
G. Craig Hill, Ph.D. Date
Contracting Officer's Technical Representative
SAIC-Frederick, Inc

Brenda Faiola 20 Oct 10
Brenda Faiola, PhD, DABT Date
Senior Research Toxicologist
Study Director
BOA/Contract Principal Investigator
RTI International

Hernan Navarro, PhD Date
Senior Director, Pharmacology & Toxicology 20 OCT 2010
RTI International

QA Review By:

Benjamin Rausch for Leslie Macdonald 10/20/10
Leslie Macdonald, B.S. Date
Quality Assurance Specialist
RTI International

**RTI International is a tradename for Research Triangle Institute*

Additions are indicated by **bold** type and deletions are indicated by ~~strikethrough~~.

Changes to Protocol:

1. Page 9, Section 6.1 Dose Preparation

Change as follows:

Information on dose formulation stability is the responsibility of the Sponsor. Based on the available stability information from the Sponsor, test article formulations will be prepared once by diluting a 1 mg/mL standard stock solution. Adjustments will not be made for the purity of the test article. The standard stock solution will be stored ~~in 25 mL aliquots~~ at approximately 0° to -20°C and will expire after 6 months at these conditions. Vehicle and diluted test article formulations will be stored refrigerated at approximately 2° to 8°C and will expire after 1 month at these conditions. Details of the dose preparation method will be included in the study file. The vehicle and test article formulations stored refrigerated will be allowed to warm by storing at room temperature for at least 30 minutes or by warming in a water bath set to 23°C for at least 10 minutes prior to administration to the test system.

The positive control article will be formulated once, on the day of use.

Reason for Change

Changed to allow flexibility in the storage volume of the standard stock solution.

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

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Telephone: 301-496-9531

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

AMENDMENT APPROVED BY:

G. Craig Hill 10/26/10
G. Craig Hill, Ph.D. Date
Contracting Officer's Technical Representative
SAIC-Frederick, Inc

Brenda Faiola 28 Oct 10
Brenda Faiola, PhD, DABT Date
Senior Research Toxicologist
Study Director
BOA/Contract Principal Investigator
RTI International

01 Nov 2010
Hernan Navarro, PhD Date
Senior Director, Pharmacology & Toxicology
RTI International

QA Review By:

Leslie Macdonald 10-28-10
Leslie Macdonald, B.S. Date
Quality Assurance Specialist
RTI International

**RTI International is a tradename for Research Triangle Institute*

Additions are indicated by **bold** type and deletions are indicated by ~~strikethrough~~.

Changes to Protocol:

1. Page 6, Section 2.0 Personnel

Change as follows:

Principal Investigator, Histopathology: ~~Glen E. Marrs~~**Henry G. Wall**, DVM**, PhD**
Experimental Pathology Laboratories, Inc.
Telephone: 919-~~998-9407~~**313-0607**
E-mail: ~~gmarrs~~**HWall**@epl-inc.com

Reason for Change

Changed to reflect staffing change for this project by the subcontractor.

2. Page 17, Section 9.6.3 Histopathology

Change as follows:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues listed in Section 9.6.2 for all animals euthanized *in extremis* and for all animals in Groups 1 and 3. Microscopic examination of target tissues will be extended to Group 2 at an additional cost to the Sponsor. Special stains, used at the discretion of the study pathologist to further characterize lesions and changes, will be at additional cost to the Sponsor. 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 **initially** be sent to Experimental Pathology Laboratories, Inc. (EPL) ~~for processing and histopathological assessments~~ to the contact below:

Experimental Pathology Laboratories, Inc.
615 Davis Drive
Suite 500
Durham, NC 27713
Telephone: 919-998-9407
Attention: ~~Dr. John Seely~~**Dr. Henry Wall**

Fixed tissues will subsequently be transferred for processing to the contact below:

Experimental Pathology Laboratories, Inc.
22866 Shaw Road
Sterling, VA 20166
Telephone: 703-471-7060
Attention: Ms. Vivian English, Laboratory Manager Histology

Histological assessment will be conducted by a board certified veterinary pathologist. The histopathology results will be provided to RTI and included in the final report.

Reason for Change

Changed to reflect resource and staffing change for this project by the subcontractor.

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

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6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]
Telephone: 301-496-9531

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

AMENDMENT APPROVED BY:

G. Craig Hill 10/28/10
G. Craig Hill, Ph.D. Date
Contracting Officer's Technical Representative
SAIC-Frederick, Inc

Brenda Faiola 02 Nov 10
Brenda Faiola, PhD, DABT Date
Senior Research Toxicologist
Study Director
BOA/Contract Principal Investigator
RTI International

02 Nov 2010
Hernan Navarro, PhD Date
Senior Director, Pharmacology & Toxicology
RTI International

QA Review By:

Leslie Macdonald 11-4-10
Leslie Macdonald, B.S. Date
Quality Assurance Specialist
RTI International

**RTI International is a tradename for Research Triangle Institute*

Additions are indicated by **bold** type and deletions are indicated by ~~strikethrough~~.

Changes to Protocol:

1. Page 13, Section 9.2 Clinical Observations

Change as follows:

Clinical observations will be made **at least twice during the pretreatment phase (upon receipt and on Day -1) on all animals,** at least once daily for each toxicology group animal **(Groups 1-3)** immediately after dosing **on Days 0-13, and at least once on Day 14 prior to scheduled necropsy**. 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. If clinical signs are noted at **other** times ~~other than immediately after dosing~~, these observations will also be entered into the automated data capture system. **Clinical signs for Group 4 animals may be recorded in the automated data capture system at the discretion of the Study Director if the general well being of the animal is compromised.**

Reason for Change

Changed to add clinical observations during the pretreatment phase of the study (as mentioned in Section 8.1) and on the day of scheduled termination, and to provide clarification for recording clinical observations for Group 4.

2. Page 16, Section 9.6.2 Tissue Fixation

Change as follows:

At the time of necropsy, the following tissues and organs will be collected from all toxicology group animals (Groups 1-3) and placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands	Ovaries
Aorta	Oviducts
Brain	Pancreas
Bone (right femur with epiphyseal plate of head)	Prostate
Sternum with Bone marrow[3] ~~(sternum)~~	Rectum
Cecum	Salivary gland (mandibular)
Colon	Sciatic nerve
Duodenum	Seminal vesicles
Eartag **or transponder** (animal ID)	Skeletal muscle (thigh)
Epididymides	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 (of final IV dose on Day 13)[4]	Thyroid and parathyroid glands
Jejunum	Tongue
Kidney	Trachea
Liver (right medial lobe and left lateral lobe)	Ureter
Lungs[2]	Urinary bladder[2]
Lymph node (mandibular and mesenteric)	Uterus (body) with cervix
Mammary gland (females only; to include nipple and surrounding tissue)	Vagina

[1]Modified Davidson's solution initially, followed by 10% neutral-buffered formalin.
[2]Infused with formalin to ensure fixation.
[3] The entire sternum will be excised intact and placed in fixative for subsequent histologic processing and microscopic evaluation of bone marrow (see Section 9.6.3).
[4] The site will be marked by encircling it using a permanent marker.

Reason for Change

Changed to provide clarification for collection of the sternum for histological assessment of the bone marrow and to clarify the collection of the animal ID (regardless of ID method) and injection site.

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

SPONSOR:
Clinical Monitoring Research Program
SAIC-Frederick, Inc.
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]
Telephone: 301-496-9531

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

AMENDMENT APPROVED BY:

G. Craig Hill 11/13/10
G. Craig Hill, Ph.D. Date
Contracting Officer's Technical Representative
SAIC-Frederick, Inc

Brenda Faiola 16 Nov 2010
Brenda Faiola, PhD, DABT Date
Senior Research Toxicologist
Study Director
BOA/Contract Principal Investigator
RTI International

16 Nov 10
Hernan Navarro, PhD Date
Senior Director, Pharmacology & Toxicology
RTI International

QA Review By:

Leslie Macdonald 11-17-10
Leslie Macdonald, B.S. Date
Quality Assurance Specialist
RTI International

**RTI International is a tradename for Research Triangle Institute*

Additions are indicated by **bold** type and deletions are indicated by ~~strikethrough~~.

Changes to Protocol:

1. Amendment 1, Item 1. (Page 9, Section 6.1 Dose Preparation)

Change as follows:

Information on dose formulation stability is the responsibility of the Sponsor. ~~Based on the available stability information from the Sponsor, t~~**T**est article formulations will be prepared ~~once~~ by diluting a ~1 mg/mL standard stock solution. Adjustments will not be made for the purity of the test article. The standard stock solution will be stored at approximately 0° to -20°C and will expire after 6 months at these conditions **based on the available stability information from the Sponsor**. Vehicle and diluted test article formulations will be stored refrigerated at approximately 2° to 8°C and will expire after 1 month at these conditions **based on the available stability information from the Sponsor**. Details of the dose preparation method will be included in the study file. The vehicle and test article formulations stored refrigerated will be allowed to warm by storing at room temperature for at least 30 minutes ~~or by warming in a water bath set to 23°C for at least 10 minutes~~ prior to administration to the test system.

The positive control article will be formulated once, on the day of use.

Reason for Change

Changed to allow for preparation of formulations more than once and to reflect the one method of dose formulation warming that will be used on study.

2. Page 9, Section 6.2 Dose Analysis

Change as follows:

~~Approximately 1- to 3-mL~~ **A** sample~~s~~ will be collected from each vehicle and test article dose formulation on the date of preparation (i.e., date of dilution from the standard stock solution). The samples will be analyzed for concentration by RTI prior to being released for use on study. Concentrations of test article will be determined by a validated high performance liquid chromatography with ultraviolet detection (HPLC/UV) method. The standard for acceptable concentration will be that the mean of the analyzed samples must be within ± 15% of nominal. Homogeneity evaluation will not be performed as the formulations are solutions.

The positive control article formulation will not be analyzed for stability, homogeneity, or concentration.

Reason for Change

Changed to allow for collection of an analytical sample of any volume necessary from each formulation.

3. Page 15, Section 9.6.1 Organ Weights

Change as follows:

The organs indicated below will be weighed from all toxicology group animals (Groups 1-3) euthanized at the scheduled necropsy on Day 14:

Adrenals[1]	Prostate **gland**
Brain	Spleen
Heart	Testes[1]
Kidneys[1]	Thymus
Liver	Thyroid with parathyroids[2]
Ovaries[1]	Uterus **and cervix** ~~with oviducts~~
Pituitary[2]	

[1] Paired organs (adrenals, kidneys, ovaries, and testes**)** will be weighed together.
[2] The pituitary and thyroid/parathyroids will be weighed following fixation.
Note: the thyroid/paratyroids weight will be collected in Provantis as "Thyroid (fixed)".

Organs will not be weighed from animals found dead or euthanized unscheduled.

Reason for Change

Changed to more closely align with the Provantis organ weight glossary terms.

4. Amendment 3, Item 2. (Page 16, Section 9.6.2 Tissue Fixation)

Change as follows:

At the time of necropsy, the following tissues and organs will be collected from all toxicology group animals (Groups 1-3) and placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands	Oviducts[6]
Aorta	Pancreas
Brain	**Parathyroid glands**
Bone (right femur with epiphyseal plate of head)	**Pituitary gland**
Sternum with Bone marrow[3]	Prostate **gland**
Intestine, Cecum	**Intestine,** Rectum
Cervix	Salivary gland (mandibular)
Intestine, Colon	~~Sciatic n~~**N**erve**, sciatic**
Intestine, Duodenum	Seminal vesicles
Eartag or transponder (animal I**dentification**~~D~~) [5]	Skeletal muscle (~~thigh~~**quadriceps femoris)**
Epididymides	Skin (~~ventral~~ abdom**inal**~~en~~)
Esophagus	Spinal cord (thoracolumnar junction; entire cord if neurologic abnormalities present)
Eyes, with optic nerve**s**[1]	Spleen
Gross lesions (including tissue masses and abnormal regional lymph nodes)	Stomach (fundic area)
Heart	Testes[1]
Intestine, Ileum	Thymus
Injection site (of final IV dose on Day 13)[4]	Thyroid ~~and parathyroid~~ glands
Intestine, Jejunum	Tongue
Kidney**s**	Trachea
Liver (right medial lobe and left lateral lobe)	Ureter**s**
Lungs[2]	Urinary bladder[2]
Lymph node (mandibular and mesenteric)	Uterus (body) ~~with cervix~~
Mammary gland (~~females only;~~ to include nipple and surrounding tissue)	Vagina
Ovaries	

[1]Modified Davidson's solution initially, followed by 10% neutral-buffered formalin.
[2]Infused with formalin to ensure fixation.
[3] The entire sternum will be excised intact and placed in fixative for subsequent histologic processing and microscopic evaluation of bone marrow (see Section 9.6.3).
[4] The site will be marked by encircling it using a permanent marker.
[5] Not examined microscopically
[6] Listed separately to allow for entry of finding(s) that may be noted at necropsy as well as histologically if a portion of the oviduct is present in the section of either the ovaries or uterus that are examined microscopically; the entire oviduct from ovary to uterus will not be excised whole and trimmed specifically**.**

Reason for Change

Changed to more closely align with the Provantis gross pathology glossary terms and to clarify procedures.

5. Amendment 2, Item 2. (Page 17, Section 9.6.3 Histopathology)

Change as follows:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues listed in Section 9.6.2 for all animals euthanized *in extremis* and for all animals in Groups 1 and 3. Microscopic examination of target tissues will be extended to Group 2 at an additional cost to the Sponsor. Special stains, used at the discretion of the study pathologist to further characterize lesions and changes, will be at additional cost to the Sponsor. 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 ~~initially~~ be sent to Experimental Pathology Laboratories, Inc. (EPL) **for processing** to the contact below:

Experimental Pathology Laboratories, Inc.
615 Davis Drive
Suite 500
Durham, NC 27713
Telephone: 919-998-9407
Attention: **Mary Parker, Manager of Histology**~~Dr. Henry Wall~~

~~Fixed tissues~~ **The prepared slides and associated documentation** will subsequently be transferred ~~for processing~~ to the contact below:

Experimental Pathology Laboratories, Inc.
~~22866 Shaw Road~~**45600 Terminal Drive**
Sterling, VA 2016~~6~~**7**
Telephone: 703-471-7060 **ext. 206**
Attention: **Kathleen Funk, DVM, PhD, DAVCP**~~Ms. Vivian English, Laboratory Manager Histology~~

Histological assessment will be conducted by a board certified veterinary pathologist. The histopathology results will be provided to RTI and included in the final report.

Reason for Change

Changed to reflect changes in resources and staffing for this project by the subcontractor.

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

SPONSOR:
Clinical Monitoring Research Program
SAIC-Frederick, Inc.
6130 Executive Boulevard
EPN, Room 6070
Bethesda, MD 20892-7412
[FedEx: Rockville, MD 20852-4910]
Telephone: 301-496-9531

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

AMENDMENT APPROVED BY:

G. Craig Hill 11/19/10
G. Craig Hill, Ph.D. Date
Contracting Officer's Technical Representative
SAIC-Frederick, Inc

Brenda Faiola 23 Nov 10
Brenda Faiola, PhD, DABT Date
Senior Research Toxicologist
Study Director
BOA/Contract Principal Investigator
RTI International

29 NOV 10
Hernan Navarro, PhD Date
Senior Director, Pharmacology & Toxicology
RTI International

QA Review By:

Leslie Macdonald 11-30-10
Leslie Macdonald, B.S. Date
Quality Assurance Specialist
RTI International

**RTI International is a tradename for Research Triangle Institute*

Additions are indicated by **bold** type and deletions are indicated by ~~strikethrough~~.

Changes to Protocol:

1. Page 7, Section 5.1 Test Article

Change as follows:

Sponsor Designation:	Fluoromisonidazole
Chemical Name:	1*H*-Imidazole-1-ethanol, α-(fluoromethyl)-2-nitro-
Synomyms:	1-Fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol; FMISO
CAS No.:	13551-89-8
Chemical Formula:	$C_6H_8FN_3O_3$
Lot Number:	20100401
Supplier:	ABX Advanced Biochemical Compounds
	H.-Gläser-Str. 10-14
	D-01454 Radeberg Germany
	Telephone: +49-3528-40 41 60
Purity:	>97% by ^{1}H-NMR according to the Certificate of Analysis provided by the Supplier.
Storage Conditions:	Desiccated, frozen (approximately -20 ± 5°C), protected from light under argon or nitrogen atmosphere.
Stability:	Long term stability not determined. Short term (<7days) storage at higher temperatures (<25°C) does not affect product quality. Retest Date: April 2012.
Safety Precautions:	Handle with care. Avoid inhalation, ingestion, and eye or skin contact.
Disposition:	Returned to Sponsor or disposed of according to RTI SOP as instructed by the Sponsor following study completion.
Reserve sample:	Since the in-life portion of the study is less than 4 weeks in duration, a reserve sample will not be retained.
~~Shipment to subcontractor:~~	~~Unopened vials of test article will be supplied by RTI to BioReliance (9630 Medical Center Drive, Rockville, MD 20850) for use in a bacterial reverse mutation assay which will be conducted under a separate study protocol signed by the BioReliance Study Director.~~

Reason for Change

The information regarding shipment of test article to another facility for use in a genotoxicity assay is not necessary in this toxicology study protocol.

PROTOCOL AMENDMENT 6	RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-1111 Page 1 of 2

RTI Project No.: 0211886.002

RTI Master Protocol No.: RTI-1111

RTI Study Code: Rt10-FMIS

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

SPONSOR:
Clinical Monitoring Research Program
SAIC-Frederick, Inc.
6130 Executive Boulevard
EPN, Room 6070
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Telephone: 301-496-9531

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

AMENDMENT APPROVED BY:

G. Craig Hill 12/8/10
G. Craig Hill, Ph.D. Date
Contracting Officer's Technical Representative
SAIC-Frederick, Inc

Brenda Faiola 15 Dec 10
Brenda Faiola, PhD, DABT Date
Senior Research Toxicologist
Study Director
BOA/Contract Principal Investigator
RTI International

16 Dec 10
Hernan Navarro, PhD Date
Senior Director, Pharmacology & Toxicology
RTI International

QA Review By:

Leslie Macdonald 12-17-10
Leslie Macdonald, B.S. Date
Quality Assurance Specialist
RTI International

**RTI International is a tradename for Research Triangle Institute*

Additions are indicated by **bold** type and deletions are indicated by ~~strikethrough~~.

Change to Protocol:

1. Amendment 4, Item 5. (Page 17, Section 9.6.3 Histopathology)

Change as follows:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues listed in Section 9.6.2 for all animals euthanized *in extremis* and for all animals in Groups 1 and 3. Microscopic examination of target tissues will be extended to Group 2 at an additional cost to the Sponsor. Special stains, used at the discretion of the study pathologist to further characterize lesions and changes, will be at additional cost to the Sponsor. 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 ~~initially~~ be sent to Experimental Pathology Laboratories, Inc. (EPL) for processing to the contact below:

Experimental Pathology Laboratories, Inc.
615 Davis Drive
Suite 500
Durham, NC 27713
Telephone: 919-998-9407
Attention: Mary Parker, Manager of Histology

The prepared slides and associated documentation will subsequently be transferred to the contact below:

Experimental Pathology Laboratories, Inc.
45600 Terminal Drive
Sterling, VA 2016**7**
Telephone: 703-471-7060 ext. 2**2**0~~6~~
Attention: **Ms. Kristi Larson** ~~Kathleen Funk, DVM, PhD, DAVCP~~

Histological assessment will be conducted by a board certified veterinary pathologist. The histopathology results will be provided to RTI and included in the final report.

Reason for Change

Changed to reflect changes in staffing for this project by the subcontractor.

Attachment 2: Final Study Report: Bacterial Reverse Mutation Assay
(Date of Report: June 17, 2011)

FINAL REPORT

Study Title

Bacterial Reverse Mutation Assay

Test Article

Fluoromisonidazole

Authors

Valentine O. Wagner, III, M.S.
Melissa R. VanDyke, B.S.

Study Completion Date

17 June 2011

Testing Facility

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AD13SN.503.BTL

Sponsor Project (Study) Number

0211886.002.003 (RTI-1114-AN)

Sponsor

RTI International
3040 Cornwallis Rd
Research Triangle Park, NC 27709

STATEMENT OF COMPLIANCE

Study No. AD13SN.503.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 exceptions:

1. The manufacturer, ABX advanced biochemical compounds (Radeberg, Germany), has determined the identity, strength, purity, composition or other characteristics to define the bulk test article and the stability of the bulk test article. However, BioReliance cannot confirm if the characterization and stability analyses were conducted in compliance with the GLP regulation cited above.

   Study Director Impact Statement: Since the test article was released for use and was used prior to the retest date for this study, the Study Director concluded that this had no adverse impact on the integrity of the data or the validity of the study conclusion.

2. The Sponsor's client (Clinical Monitoring Research Program, SAIC-Frederick, Inc.) has determined the stability of the formulated test article (i.e. the ~1 mg/mL stock solution and dilutions of the stock solution down to ~20.1 µg/mL). The Sponsor's client was responsible for the GLP compliance of these test article dose formulation stability analyses. However, BioReliance cannot confirm if the stability analyses were conducted in compliance with the GLP regulation cited above.

   Study Director Impact Statement: Since the established specifications were met and the standard stock solution was acceptable for use over the period of dosing, the Study Director concluded that this had no adverse impact on the integrity of the data or the validity of the study conclusion.

Valentine O. Wagner III — 17 Jun 2011

Valentine O. Wagner, III, M.S.
Study Director — Date

17 Jun 2011

BioReliance Study Management — Date

QUALITY ASSURANCE STATEMENT

BioReliance

Quality Assurance Statement

Study Information

Number: AD13SN.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

Inspections

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

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
14-Dec-2010	14-Dec-2010	Test System Preparation	14-Dec-2010	14-Dec-2010
11-Jan-2011	11-Jan-2011	Observation of Test System	11-Jan-2011	11-Jan-2011
02-Feb-2011	03-Feb-2011	Data and Draft Reporting	04-Feb-2011	04-Feb-2011
15-Jun-2011	15-Jun-2011	Final Reporting	15-Jun-2011	15-Jun-2011

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.

For a multisite study, test site QA Statements are located in the corresponding contributing scientist report.

E-signature

Quality Assurance: Olufunke Adefemi 17-Jun-2011 2:51 pm GMT

Reason for signature: QA Approval

Printed by:Olufunke Adefemi

Printed on:17-Jun-11

Bacterial Reverse Mutation Assay

STUDY INFORMATION

Sponsor:	**RTI International** **3040 Cornwallis Rd** **Research Triangle Park, NC 27709**
Authorized Representative:	**Brenda Faiola, Ph.D., DABT**
Testing Facility:	**BioReliance** **9630 Medical Center Drive** **Rockville, Maryland 20850**
Test Article I.D.:	**Fluoromisonidazole**
Bulk Test Article Lot No.:	**20100401**
Bulk Test Article CAS No.:	**13551-89-8**
Bulk Test Article Purity:	**> 97% (per Certificate of Analysis)**
Bulk Test Article Description:	**Yellowish solid**
Test Article Formulation Concentration:	**~1 mg/mL in 95%:5% (v:v) sterile water for injection, USP:absolute ethanol, USP (supplied as a standard stock solution, prepared by the Sponsor; BioReliance Sample 0002)**
Test Article Formulation Log/Batch No.:	**13253-21A**
Test Article Formulation Description:	**Clear, colorless liquid**
Test Article Formulation Storage Conditions:	**-15 to -40°C, stored in the dark without desiccant**
Test Article Solvent:	**95%:5% (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP**
Solvent Component 1:	**0.9% sodium chloride for injection, USP (provided by the Sponsor; BioReliance Sample 0004)**
Component 1 Lot No.:	**C806307**
Component 1 Description:	**Clear, colorless liquid**

Component 1 Storage Conditions:	**Room temperature, stored in the dark without desiccant**
Solvent Component 2:	**Absolute ethanol, USP (provided by the Sponsor; BioReliance Sample 0003)**
Component 2 Lot No.:	**09496HM**
Component 2 Purity:	**99.99% (per Certificate of Analysis)**
Component 2 Description:	**Clear, colorless liquid**
Component 2 Storage Conditions:	**Room temperature, stored in the dark without desiccant**
Sponsor Project (Study) No.:	**0211886.002.003 (RTI-1114-AN)**
BioReliance Study No.:	**AD13SN.503.BTL**
Test Article and Solvent Receipt/Login Date:	**09 December 2010**
Study Initiation Date:	**13 December 2010**
Experimental Start Date:	**14 December 2010**
Experimental Completion Date:	**11 January 2011**
Laboratory Manager:	**Emily W. Dakoulas, B.S.**
Principal Investigator, Analytical:	**Brenda Faiola, Ph.D., DABT**
Analytical Test Site:	**RTI International** **East Institute Drive** **Research Triangle Park, NC 27709**

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SUMMARY

The test article, Fluoromisonidazole, 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.

The solvent, 95%:5% (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP, was selected based on the Sponsor's request, solubility of the test article and compatibility with the target cells.

In the initial toxicity-mutation assay, the maximum dose tested was 3.75 µg per plate; this dose was achieved by diluting the Sponsor-provided standard stock solution at a concentration of 1.0 mg/mL to 0.075 mg/mL for use as the top concentration in dosing the assay and using a 50 µL plating aliquot. The dose levels tested were 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. 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 3.75 µg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. Neither precipitate nor appreciable toxicity was observed.

Under the conditions of this study, test article Fluoromisonidazole 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. Copies of the study protocol and amendment are included in Appendix II.

This study was conducted in compliance with the testing guidelines of the ICH (1996 and 1997) and OECD (1998).

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, Fluoromisonidazole, was supplied as a standard stock solution by the Sponsor at a concentration of ~1 mg/mL in 95%:5% (v:v) sterile water for injection, USP:absolute ethanol, USP. The formulated test article was received by BioReliance on 09 December 2010 and was assigned the code number AD13SN. Per protocol, the formulated test article should be stored frozen, 0 to -40°C. Upon receipt, the formulated test article was described as a clear, colorless liquid and was stored at -15 to -40°C in the dark without desiccant. Based on information provided by the Sponsor, the formulated stock solution was found to be stable through 07 June 2011 (i.e. six months from the date of preparation; see Appendix V).

ABX advanced biochemical compounds (Radeberg, Germany) has determined the identity, strength, purity, composition or other characteristics to define the bulk test article and the stability of the bulk test article. Copies of the Certificate of Analysis and retest memo are included in Appendix IV. As per the manufacturer, a retest date of April 2012 (two years from the manufacturing date) was assigned to the bulk test article. Therefore, the test article was considered stable through April 2012.

The vehicle used, by the Sponsor, to prepare the stock solution was 95%:5% (v:v) sterile water for injection, USP:absolute ethanol, USP. The materials used were as follows:

Chemical	Supplier	Lot Number	Expiration Date
Sterile water for injection, USP (CAS No. 7732-18-5)	Baxter Healthcare	C805432	June 2011
Absolute ethanol, USP (CAS No. 64-17-5)	Sigma-Aldrich	09496HM	August 2015

The vehicle used to deliver the formulated test article to the test system was 95%:5% (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP. Both the sodium chloride for injection and ethanol were provided by the Sponsor. The materials used were as follows:

Chemical	Supplier	Lot Number	Expiration Date
0.9% sodium chloride for injection, USP (CAS No. 7647-14-5)	Baxter Healthcare	C806307	December 2011
Absolute ethanol, USP (CAS No. 64-17-5)	Sigma-Aldrich	09496HM	August 2015

The vehicle and top dose level used in dosing the assay were prepared by BioReliance as follows:

1. For each 10 mL of vehicle to be prepared, 0.5 mL of ethanol and 9.5 mL of 0.9% sodium chloride for injection were dispensed into a sterile test tube.
2. The solution was mixed until homogeneous and used to prepare dilutions of the test article.
3. A vial of the Sponsor-provided stock solution at ~1 mg/mL was removed from the freezer and allowed to thaw at ambient temperature. The necessary volume was transferred by pipette into a sterile graduated cylinder, diluted with the vehicle in Step 2 above and mixed until homogeneous to form the top concentration for use in dosing the assay (0.075 mg/mL). Subsequent test article dilutions were prepared by serial dilution, using the 0.075 mg/mL formulation and vehicle, prepared in Steps 2 and 3 above.
4. Test article dilutions were prepared immediately before use and delivered to the test system at room temperature under yellow light.

Duplicate dosing formulation samples (1.0 mL from the highest dose level used for dosing and the vehicle) were collected from each assay. All samples were sent on cool packs to the Sponsor for analysis. The backup samples were stored refrigerated at RTI and were not needed for analysis. Unused samples were discarded following issuance of the final analytical report. A copy of the analytical report 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 and TA1537	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
WP2 *uvr*A			15
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Sigma Aldrich Chemical Co. or Alfa Aesar) Lot Nos. 71980 or A23U048 [a] Exp. Dates 28-Dec-2010 or 04-Dec-2012 [a] CAS No. 26628-22-8 Purity 99.8%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co.) Lot No. 106F06682 Exp. Date 28-Oct-2011 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. 76296KJ Exp. Date 02-Jun-2012 CAS No. 66-27-3 Purity 99.8%	1,000

[a] Lot 71980 of sodium azide was used in the initial assay, which was dosed on 14 December 2010. Lot A23U048 of sodium azide was used in the confirmatory assay, which was dosed on 04 January 2011.

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 from Dr. Bruce Ames' Master cultures, *E. coli* tester strains were from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland and both species of tester strain were distributed by Moltox (Boone, NC).

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, appropriate frozen permanent stock or with a lyophilized pellet into a vessel, containing ~30 to 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 shaker/incubator programmed to begin shaking at approximately 125 to 175 rpm at 37±2°C approximately 12 to 14 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 greater than or equal to $0.3x10^9$ 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 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 $MgCl_2$ 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.

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 a minimum of 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. A minimum of 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. 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 or no code	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 greater than or equal to 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 greater than or equal to 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:

Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments), 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.

Deviations

No known deviations from the protocol or assay-method SOPs occurred during the conduct of this study.

RESULTS AND DISCUSSION

Solubility

The solvent, 95%:5% (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP, was selected based on the Sponsor's request, solubility of the test article 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

Experiment	Tester Strain				
	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
	Titer Value (x 10^9 cells per mL)				
B1	0.9	0.5	0.7	0.4	1.7
B2	1.3	0.5	0.9	0.8	2.4

Initial Toxicity-Mutation Assay

The results of the initial toxicity-mutation assay are presented in Tables 1 and 2. These data were generated in Experiment B1.

In Experiment B1 (Initial Toxicity-Mutation Assay), the maximum dose tested was 3.75 µg per plate; this dose was achieved by diluting the Sponsor-provided standard stock solution at a concentration of 1.0 mg/mL to 0.075 mg/mL for use as the top concentration in dosing the assay and using a 50 µL plating aliquot. The dose levels tested were 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. 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 3.75 µg per plate.

Confirmatory Mutagenicity Assay

The results of the confirmatory mutagenicity assay are presented in Tables 3 and 4. These data were generated in Experiment B2.

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. The

dose levels tested were 0.050, 0.15, 0.50, 1.5 and 3.75 µg per plate. Neither precipitate nor appreciable toxicity was observed.

Dosing Formulation Analysis

Dosing formulations were sent to the Sponsor for analysis. A copy of the final analytical report is included in Appendix V. Concentration analysis indicates that the actual mean concentrations of the analyzed dose level (nominally 0.075 mg/mL) were 110% and 113% of target for the initial and confirmatory assays, respectively. This indicates that the regulatory-required top dose level was achieved in each case and the results support the validity of the study conclusion. No test article was detected in the vehicle control samples. Since the most concentrated dosing formulations were within 85 to 115% of target, the dosing formulations were considered stable.

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, Fluoromisonidazole 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.

REFERENCES

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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.

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Maron, D.M. and B.N. Ames (1983) Revised Methods for the *Salmonella* Mutagenicity Test, Mutation Research, 113:173-215.

Vogel, H.J. and D.M. Bonner (1956) Acetylornithinase of *E. coli*: Partial Purification and Some Properties, J. Biol. Chem., 218:97-106.

DATA TABLES

Table 1
Initial Toxicity-Mutation Assay without S9 activation

Study Number: AD13SN.503.BTL
Experiment: B1
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 12/14/2010
Evaluation Period: 12/22/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**Fluoromisonidazole**	3.75 µg	*20*	*4*	*0.8*	23^{A}, 17^{A}
		1.5 µg	*21*	*1*	*0.8*	21^{A}, 20^{A}
		0.50 µg	*16*	*7*	*0.6*	21^{A}, 11^{A}
		0.15 µg	*20*	*12*	*0.8*	28^{A}, 11^{A}
		0.050 µg	*23*	*2*	*0.9*	21^{A}, 24^{A}
		0.015 µg	*20*	*4*	*0.8*	17^{A}, 23^{A}
		0.0050 µg	*20*	*5*	*0.8*	23^{A}, 16^{A}
		0.0015 µg	*28*	*1*	*1.1*	28^{A}, 27^{A}
	Vehicle[a]	50 µL	*26*	*4*		23^{A}, 29^{A}
TA100	**Fluoromisonidazole**	3.75 µg	*136*	*37*	*1.4*	110^{A}, 162^{A}
		1.5 µg	*127*	*11*	*1.3*	134^{A}, 119^{A}
		0.50 µg	*104*	*4*	*1.0*	101^{A}, 106^{A}
		0.15 µg	*99*	*3*	*1.0*	101^{A}, 97^{A}
		0.050 µg	*107*	*8*	*1.1*	113^{A}, 101^{A}
		0.015 µg	*98*	*8*	*1.0*	92^{A}, 103^{A}
		0.0050 µg	*96*	*8*	*1.0*	90^{A}, 102^{A}
		0.0015 µg	*97*	*9*	*1.0*	103^{A}, 90^{A}
	Vehicle	50 µL	*100*	*10*		107^{A}, 93^{A}
TA1535	**Fluoromisonidazole**	3.75 µg	*12*	*0*	*1.0*	12^{A}, 12^{A}
		1.5 µg	*14*	*4*	*1.2*	16^{A}, 11^{A}
		0.50 µg	*13*	*3*	*1.1*	11^{A}, 15^{A}
		0.15 µg	*12*	*5*	*1.0*	15^{A}, 8^{A}
		0.050 µg	*9*	*5*	*0.8*	12^{A}, 5^{A}
		0.015 µg	*8*	*0*	*0.7*	8^{A}, 8^{A}
		0.0050 µg	*5*	*1*	*0.4*	4^{A}, 5^{A}
		0.0015 µg	*12*	*5*	*1.0*	15^{A}, 8^{A}
	Vehicle	50 µL	*12*	*1*		13^{A}, 11^{A}

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

[a] On all data tables, vehicle = 95%:5% (v:v) 0.9% sodium chloride for injection, USP:absolute ethanol, USP.

Table 1 cont.
Initial Toxicity-Mutation Assay without S9 activation

Study Number: AD13SN.503.BTL
Experiment: B1
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 12/14/2010
Evaluation Period: 12/22/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	**Fluoromisonidazole**	3.75 µg	*28*	*1*	*1.3*	28^{A}, 27^{A}
		1.5 µg	*21*	*6*	*1.0*	16^{A}, 25^{A}
		0.50 µg	*16*	*1*	*0.8*	15^{A}, 16^{A}
		0.15 µg	*15*	*6*	*0.7*	19^{A}, 11^{A}
		0.050 µg	*19*	*4*	*0.9*	16^{A}, 21^{A}
		0.015 µg	*12*	*0*	*0.6*	12^{A}, 12^{A}
		0.0050 µg	*16*	*13*	*0.8*	7^{A}, 25^{A}
		0.0015 µg	*18*	*13*	*0.9*	27^{A}, 8^{A}
	Vehicle	50 µL	*21*	*1*		20^{A}, 21^{A}
WP2*uvr*A	**Fluoromisonidazole**	3.75 µg	*48*	*0*	*1.4*	48^{A}, 48^{A}
		1.5 µg	*33*	*2*	*0.9*	34^{A}, 31^{A}
		0.50 µg	*32*	*9*	*0.9*	25^{A}, 38^{A}
		0.15 µg	*34*	*10*	*1.0*	41^{A}, 27^{A}
		0.050 µg	*34*	*3*	*1.0*	36^{A}, 32^{A}
		0.015 µg	*26*	*10*	*0.7*	33^{A}, 19^{A}
		0.0050 µg	*27*	*4*	*0.8*	24^{A}, 29^{A}
		0.0015 µg	*24*	*5*	*0.7*	27^{A}, 20^{A}
	Vehicle	50 µL	*35*	*4*		32^{A}, 38^{A}
TA98	**2NF**	1.0 µg	*207*	*16*	*8.0*	196^{A}, 218^{A}
TA100	**SA**	1.0 µg	*429*	*10*	*4.3*	422^{A}, 436^{A}
TA1535	**SA**	1.0 µg	*326*	*74*	*27.2*	378^{A}, 273^{A}
TA1537	**9AAD**	75 µg	*431*	*103*	*20.5*	358^{A}, 503^{A}
WP2*uvr*A	**MMS**	1000 µg	*313*	*24*	*8.9*	296^{A}, 330^{A}

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Table 2
Initial Toxicity-Mutation Assay with S9 activation

Study Number: AD13SN.503.BTL
Experiment: B1
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 12/14/2010
Evaluation Period: 12/22/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**Fluoromisonidazole**	3.75 µg	*31*	*4*	*1.1*	33^{A}, 28^{A}
		1.5 µg	*21*	*8*	*0.8*	27^{A}, 15^{A}
		0.50 µg	*40*	*4*	*1.4*	42^{A}, 37^{A}
		0.15 µg	*31*	*3*	*1.1*	33^{A}, 29^{A}
		0.050 µg	*31*	*4*	*1.1*	28^{A}, 33^{A}
		0.015 µg	*28*	*6*	*1.0*	24^{A}, 32^{A}
		0.0050 µg	*36*	*11*	*1.3*	44^{A}, 28^{A}
		0.0015 µg	*38*	*6*	*1.4*	42^{A}, 33^{A}
	Vehicle	50 µL	*28*	*6*		32^{A}, 24^{A}
TA100	**Fluoromisonidazole**	3.75 µg	*143*	*52*	*1.2*	106^{A}, 179^{A}
		1.5 µg	*165*	*3*	*1.4*	163^{A}, 167^{A}
		0.50 µg	*150*	*0*	*1.3*	150^{A}, 150^{A}
		0.15 µg	*130*	*29*	*1.1*	150^{A}, 109^{A}
		0.050 µg	*114*	*16*	*1.0*	103^{A}, 125^{A}
		0.015 µg	*111*	*6*	*1.0*	115^{A}, 107^{A}
		0.0050 µg	*116*	*15*	*1.0*	105^{A}, 126^{A}
		0.0015 µg	*121*	*6*	*1.1*	117^{A}, 125^{A}
	Vehicle	50 µL	*115*	*28*		95^{A}, 134^{A}
TA1535	**Fluoromisonidazole**	3.75 µg	*9*	*1*	*0.7*	8^{A}, 9^{A}
		1.5 µg	*8*	*4*	*0.6*	5^{A}, 11^{A}
		0.50 µg	*8*	*1*	*0.6*	7^{A}, 9^{A}
		0.15 µg	*13*	*0*	*1.0*	13^{A}, 13^{A}
		0.050 µg	*14*	*1*	*1.1*	15^{A}, 13^{A}
		0.015 µg	*8*	*1*	*0.6*	7^{A}, 8^{A}
		0.0050 µg	*8*	*1*	*0.6*	8^{A}, 7^{A}
		0.0015 µg	*12*	*1*	*0.9*	11^{A}, 13^{A}
	Vehicle	50 µL	*13*	*1*		13^{A}, 12^{A}

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Table 2 cont.
Initial Toxicity-Mutation Assay with S9 activation

Study Number: AD13SN.503.BTL
Experiment: B1
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 12/14/2010
Evaluation Period: 12/22/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	**Fluoromisonidazole**	3.75 µg	*29*	*3*	*1.5*	27[A], 31[A]
		1.5 µg	*24*	*11*	*1.3*	31[A], 16[A]
		0.50 µg	*19*	*4*	*1.0*	16[A], 21[A]
		0.15 µg	*11*	*3*	*0.6*	13[A], 9[A]
		0.050 µg	*22*	*4*	*1.2*	25[A], 19[A]
		0.015 µg	*19*	*2*	*1.0*	17[A], 20[A]
		0.0050 µg	*24*	*1*	*1.3*	23[A], 24[A]
		0.0015 µg	*26*	*2*	*1.4*	27[A], 24[A]
	Vehicle	50 µL	*19*	*8*		24[A], 13[A]
WP2*uvr*A	**Fluoromisonidazole**	3.75 µg	*45*	*7*	*1.3*	40[A], 50[A]
		1.5 µg	*43*	*1*	*1.3*	42[A], 44[A]
		0.50 µg	*33*	*6*	*1.0*	37[A], 28[A]
		0.15 µg	*31*	*4*	*0.9*	28[A], 34[A]
		0.050 µg	*32*	*1*	*0.9*	32[A], 31[A]
		0.015 µg	*26*	*8*	*0.8*	32[A], 20[A]
		0.0050 µg	*31*	*11*	*0.9*	23[A], 38[A]
		0.0015 µg	*27*	*4*	*0.8*	29[A], 24[A]
	Vehicle	50 µL	*34*	*4*		31[A], 36[A]
TA98	**2AA**	1.0 µg	*321*	*23*	*11.5*	337[A], 305[A]
TA100	**2AA**	2.0 µg	*699*	*98*	*6.1*	629[A], 768[A]
TA1535	**2AA**	1.0 µg	*149*	*32*	*11.5*	171[A], 126[A]
TA1537	**2AA**	1.0 µg	*96*	*55*	*5.1*	135[A], 57[A]
WP2*uvr*A	**2AA**	10 µg	*330*	*30*	*9.7*	309[A], 351[A]

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

Table 3
Confirmatory Mutagenicity Assay without S9 activation

Study Number: AD13SN.503.BTL
Experiment: B2
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 1/4/2011
Evaluation Period: 1/11/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**Fluoromisonidazole**	3.75 µg	*16*	*9*	*0.8*	15^{A}, 8^{A}, 25^{A}
		1.50 µg	*23*	*10*	*1.1*	15^{A}, 34^{A}, 21^{A}
		0.50 µg	*15*	*3*	*0.7*	17^{A}, 16^{A}, 11^{A}
		0.15 µg	*14*	*2*	*0.7*	16^{A}, 13^{A}, 13^{A}
		0.050 µg	*16*	*4*	*0.8*	15^{A}, 20^{A}, 12^{A}
	Vehicle	50 µL	*21*	*2*		20^{A}, 20^{A}, 23^{A}
TA100	**Fluoromisonidazole**	3.75 µg	*144*	*44*	*1.5*	143^{A}, 101^{A}, 188^{A}
		1.50 µg	*109*	*13*	*1.1*	114^{A}, 118^{A}, 94^{A}
		0.50 µg	*88*	*5*	*0.9*	85^{A}, 86^{A}, 94^{A}
		0.15 µg	*90*	*16*	*0.9*	97^{A}, 102^{A}, 72^{A}
		0.050 µg	*84*	*12*	*0.9*	81^{A}, 74^{A}, 97^{A}
	Vehicle	50 µL	*97*	*10*		86^{A}, 103^{A}, 102^{A}
TA1535	**Fluoromisonidazole**	3.75 µg	*13*	*3*	*1.6*	11^{A}, 16^{A}, 12^{A}
		1.50 µg	*5*	*2*	*0.6*	4^{A}, 4^{A}, 7^{A}
		0.50 µg	*7*	*0*	*0.9*	7^{A}, 7^{A}, 7^{A}
		0.15 µg	*9*	*7*	*1.1*	3^{A}, 17^{A}, 8^{A}
		0.050 µg	*7*	*4*	*0.9*	5^{A}, 12^{A}, 5^{A}
	Vehicle	50 µL	*8*	*1*		9^{A}, 8^{A}, 7^{A}
TA1537	**Fluoromisonidazole**	3.75 µg	*4*	*2*	*0.8*	3^{A}, 7^{A}, 3^{A}
		1.50 µg	*4*	*1*	*0.8*	4^{A}, 4^{A}, 5^{A}
		0.50 µg	*5*	*2*	*1.0*	5^{A}, 7^{A}, 4^{A}
		0.15 µg	*6*	*2*	*1.2*	8^{A}, 5^{A}, 5^{A}
		0.050 µg	*3*	*3*	*0.6*	7^{A}, 1^{A}, 1^{A}
	Vehicle	50 µL	*5*	*0*		5^{A}, 5^{A}, 5^{A}
WP2*uvr*A	**Fluoromisonidazole**	3.75 µg	*41*	*1*	*1.4*	40^{A}, 40^{A}, 42^{A}
		1.50 µg	*38*	*4*	*1.3*	40^{A}, 33^{A}, 41^{A}
		0.50 µg	*26*	*4*	*0.9*	27^{A}, 21^{A}, 29^{A}
		0.15 µg	*33*	*10*	*1.1*	44^{A}, 31^{A}, 24^{A}
		0.050 µg	*29*	*5*	*1.0*	29^{A}, 34^{A}, 24^{A}
	Vehicle	50 µL	*29*	*5*		25^{A}, 34^{A}, 29^{A}

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Table 3 cont.
Confirmatory Mutagenicity Assay without S9 activation

Study Number: AD13SN.503.BTL
Study Code: AD13SN
Experiment: B2
Date Plated: 1/4/2011
Exposure Method: Plate incorporation assay
Evaluation Period: 1/11/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**2NF**	1.0 µg	*236*	*28*	*11.2*	263^{A}, 236^{A}, 208^{A}
TA100	**SA**	1.0 µg	*576*	*32*	*5.9*	584^{A}, 603^{A}, 540^{A}
TA1535	**SA**	1.0 µg	*500*	*24*	*62.5*	473^{A}, 520^{A}, 508^{A}
TA1537	**9AAD**	75 µg	*403*	*79*	*80.6*	438^{A}, 459^{A}, 313^{A}
WP2*uvr*A	**MMS**	1000 µg	*200*	*35*	*6.9*	221^{A}, 160^{A}, 219^{A}

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Table 4
Confirmatory Mutagenicity Assay with S9 activation

Study Number: AD13SN.503.BTL
Experiment: B2
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 1/4/2011
Evaluation Period: 1/11/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**Fluoromisonidazole**	3.75 µg	*29*	*2*	*0.9*	28^{A}, 32^{A}, 28^{A}
		1.50 µg	*29*	*11*	*0.9*	34^{A}, 16^{A}, 36^{A}
		0.50 µg	*20*	*6*	*0.6*	27^{A}, 15^{A}, 17^{A}
		0.15 µg	*27*	*3*	*0.9*	28^{A}, 29^{A}, 23^{A}
		0.050 µg	*23*	*4*	*0.7*	23^{A}, 27^{A}, 19^{A}
	Vehicle	50 µL	*31*	*9*		29^{A}, 23^{A}, 40^{A}
TA100	**Fluoromisonidazole**	3.75 µg	*141*	*14*	*1.5*	158^{A}, 133^{A}, 133^{A}
		1.50 µg	*133*	*17*	*1.4*	151^{A}, 118^{A}, 131^{A}
		0.50 µg	*112*	*13*	*1.2*	127^{A}, 109^{A}, 101^{A}
		0.15 µg	*108*	*13*	*1.1*	114^{A}, 117^{A}, 94^{A}
		0.050 µg	*84*	*11*	*0.9*	84^{A}, 95^{A}, 74^{A}
	Vehicle	50 µL	*96*	*10*		102^{A}, 101^{A}, 85^{A}
TA1535	**Fluoromisonidazole**	3.75 µg	*8*	*3*	*0.7*	11^{A}, 7^{A}, 5^{A}
		1.50 µg	*12*	*1*	*1.1*	12^{A}, 13^{A}, 11^{A}
		0.50 µg	*11*	*6*	*1.0*	12^{A}, 5^{A}, 16^{A}
		0.15 µg	*15*	*7*	*1.4*	9^{A}, 23^{A}, 12^{A}
		0.050 µg	*8*	*5*	*0.7*	3^{A}, 13^{A}, 9^{A}
	Vehicle	50 µL	*11*	*4*		15^{A}, 11^{A}, 7^{A}
TA1537	**Fluoromisonidazole**	3.75 µg	*6*	*2*	*1.2*	7^{A}, 7^{A}, 4^{A}
		1.50 µg	*7*	*4*	*1.4*	8^{A}, 3^{A}, 11^{A}
		0.50 µg	*6*	*3*	*1.2*	8^{A}, 8^{A}, 3^{A}
		0.15 µg	*6*	*2*	*1.2*	4^{A}, 5^{A}, 8^{A}
		0.050 µg	*4*	*1*	*0.8*	3^{A}, 4^{A}, 4^{A}
	Vehicle	50 µL	*5*	*2*		7^{A}, 5^{A}, 4^{A}
WP2*uvr*A	**Fluoromisonidazole**	3.75 µg	*41*	*14*	*1.2*	45^{A}, 52^{A}, 25^{A}
		1.50 µg	*30*	*11*	*0.9*	42^{A}, 27^{A}, 21^{A}
		0.50 µg	*34*	*7*	*1.0*	32^{A}, 42^{A}, 28^{A}
		0.15 µg	*33*	*6*	*1.0*	38^{A}, 27^{A}, 33^{A}
		0.050 µg	*27*	*9*	*0.8*	38^{A}, 21^{A}, 23^{A}
	Vehicle	50 µL	*33*	*10*		25^{A}, 44^{A}, 31^{A}

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Table 4 cont.
Confirmatory Mutagenicity Assay with S9 activation

Study Number: AD13SN.503.BTL
Experiment: B2
Exposure Method: Plate incorporation assay

Study Code: AD13SN
Date Plated: 1/4/2011
Evaluation Period: 1/11/2011

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**2AA**	1.0 µg	*247*	*34*	*8.0*	249^{A}, 279^{A}, 212^{A}
TA100	**2AA**	2.0 µg	*396*	*33*	*4.1*	358^{A}, 411^{A}, 419^{A}
TA1535	**2AA**	1.0 µg	*60*	*6*	*5.5*	54^{A}, 62^{A}, 65^{A}
TA1537	**2AA**	1.0 µg	*30*	*10*	*6.0*	24^{A}, 41^{A}, 24^{A}
WP2*uvr*A	**2AA**	15 µg	*167*	*32*	*5.1*	150^{A}, 204^{A}, 147^{A}

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

APPENDIX I: Historical Control Data

Historical Negative and Positive Control Values
2007 – 2009

revertants per plate

Strain	Control	Activation							
		None				Rat Liver			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA98	Neg	18	7	4	57	25	8	6	69
	Pos	221	131	34	1299	526	245	49	2342
TA100	Neg	132	32	51	255	141	35	56	268
	Pos	613	153	226	1837	776	380	224	3206
TA1535	Neg	17	7	3	58	15	5	1	49
	Pos	456	159	33	1601	120	85	18	1216
TA1537	Neg	8	4	0	28	8	4	1	41
	Pos	1040	576	24	4814	102	156	13	2360
WP2 *uvr*A	Neg	28	11	6	72	31	12	5	77
	Pos	344	163	44	1178	274	131	32	1656

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

QA Reviewed

Init. Date

PROTOCOL AMENDMENT 1

Sponsor: RTI International

BioReliance Study No.: AD13SN.503.BTL; **Sponsor Project (Study) No.:** 0211886.002.003 (RTI-1114-AN)

Title: Bacterial Reverse Mutation Assay

1. Page 5, §4.5 Quality Assurance Unit of BioReliance (Lead QA)

   Change the QA lead to:

   Karen Westray
   QA Manager, Toxicology
   Phone: 301-610-2856
   Fax: 301-738-2362
   Email: Karen.westray@bioreliance.com

   Reason: Ms. Westray is the QA Lead effective 31-Jan-2011.

Approvals:

Valentine O. Wagner, III — 22 Apr 2011

Valentine O. Wagner, III, MS
BioReliance Study Director — Date

BioReliance Study Management — 22 Apr 2011

Date

QA Reviewed

MAC 22 Dec 2010
Init. Date

Received by RA/QA 14 Dec 2010

BioReliance Study Number: AD13SN.503.BTL

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:	Brenda Faiola, Ph.D., DABT Phone: 919-316-3802 Fax: 919-541-5956 Email: bfaiola@rti.com

2.4 Sponsor Project (Study) No.: 0211886.002.003 (RTI-1114-AN)

3.0 TEST AND CONTROL ARTICLES

3.1	Test Article Name:	Fluoromisonidazole (CAS No.: 13551-89-8; Lot No.: 20100401)
	Supply/Storage parameters:	The test article will be supplied by as a standard stock solution (~1 mg/mL) in 95%:5% (v:v) sterile water for injection, USP:absolute ethanol, USP. The solution will be prepared by the Sponsor and stored in polypropylene cyrovials, each containing approximately 3 mL of the solution. The vials will be stored frozen at approximately 0° to -20°C prior to shipment on dry ice to the Test Facility. This standard stock solution will expire 6 months after the date of preparation when stored under these conditions.
	Storage Temperature:	Frozen, 0 to –40°C.
	Purity:	>97%, An adjustment for purity will not be made.
	Molecular Weight:	189.4

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 and Test Article Mixtures

BioReliance will not perform analysis of the test article. The Supplier will be directly responsible for determination and documentation (in a Certificate of Analysis or equivalent document) of the analytical purity, composition and stability of the test article. The stability and strength of the test article in the solvent (or vehicle) is the responsibility of the Sponsor's Client. If there is no characterization and/or stability analysis of the test article formulation, the GLP compliance statement in the final report will cite these deficiencies as exceptions to the GLP regulations with which this study is compliant.

3.4 Characterization of Test Article Dose Formulations at the Sponsor's Designated Laboratory

The Sponsor 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 each initial toxicity-mutation assay trial and each confirmatory mutagenicity assay trial, samples will be collected from the vehicle and most concentrated formulation **used for dosing**.

- For dose formulations that are solutions, 2 x 1.0 mL aliquots will be collected for concentration analysis.
- For the vehicle, 2 x 1.0 mL aliquots will be collected to confirm the absence of test article. One set will be used for analysis the other will serve as the backup.
- If necessary, as noted by the Analytical Chemist, alternate volumes or aliquots may be collected for any of the above samples.

3.4.2 Sample Disposition

Both sets of samples (analysis and back-up) will be sent to the Sponsor at the following address:

Name:	Donna Browning
Company (Test Site):	Research Triangle Institute International

Materials Handling Facility
Address: East Institute Drive
Research Triangle Park, NC 27709
Phone: 919-541-6270
Email: dbrowning@rti.org

These samples will be placed in plastic bags and sent on a Monday through Thursday by overnight delivery on wet ice and/or cold packs on the day of preparation. The backup aliquot(s) of each sample will be stored refrigerated at RTI and will be analyzed only as needed. Unused samples will be discarded following issue of the analytical report.

The recipient will be notified by email in advance or on the day of the shipment with the following information: Tracking number, sample identity and storage requirements. An MSDS will also be provided with each shipment.

3.4.3 Dose Formulation Analyses

Upon receipt and prior to analyses, the samples will be kept refrigerated (at approximately 2-8°C) at the Test Site (analytical laboratory). Each analysis sample submitted in singlet will be analyzed in at least duplicate (with samples taken from the middle of the container) for concentration.

All analytical work will be conducted by the Analytical Laboratory (Test Site) using a validated analytical method (AM-0211886-002) under the direction of the Principal Investigator.

All unused samples will be handled as per the Standard Operating Procedures of the Test Site.

3.4.4 Acceptance Criteria

The acceptable specification for the concentration of the test article in the vehicle will be as follows:

- 85 to 115% of nominal with ≤10% relative standard deviation (RSD) of each concentration.

For a vehicle sample to be reported as free of test article, the concentration of the test article in the vehicle formulation must be below the Estimated Limit of Detection or 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.

3.4.5 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 critical phase inspection 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. The Study Director will in turn report audit findings to BioReliance Management.

3.4.6 Reporting

A draft contributing scientist 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.

3.4.7 Archiving

All raw data, documentation and reports generated as a result of sample analyses will be retained and archived at the analytical laboratory.

3.5 Test Article Retention Sample

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 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):
Name Brenda Faiola, PhD, DABT
Phone: 919-316-3802
Fax: 919-541-5956
Email: bfaiola@RTI.org

4.5 Quality Assurance Unit of BioReliance (Lead QA):
Luleayenwa Aberra-Degu
Phone: 301-610-2667
Fax: 301-738-2362
Email: Luleayenwa.aberra-degu@bioreliance.com

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 09-December-2010

5.2 Proposed Experimental Completion Date: 14-January-2011

5.3 Proposed Report Date: 28-January-2011

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 from Dr. Bruce Ames, University of California, Berkeley. The *E. coli* tester strain was from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (United Kingdom). The tester strains may also be obtained from Molecular Toxicology Inc. (Moltox) using cultures derived from the above sources.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

7.1 Solubility

The vehicle will be 95%:5% (v:v) 0.9% sodium chloride for injection, USP: absolute ethanol, USP. The Sponsor has indicated that the test article is soluble in this vehicle at 75 µg/mL. The Sponsor will provide any formulation instructions that are needed to prepare the test article formulations from the standard stock solution.

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 3.75 µg/plate. Solubility or workability permitting, the dose levels will be 3.75, 1.5, 0.50, 0.15, 0.050, 0.015, 0.005 and 0.0015 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 3.75 µg/plate. For freely soluble, nontoxic test articles, the highest dose level will be 3.75 µg/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 the maximum dose level is selected based on precipitation and this dose level is less than 3.75 µg/plate. The doses selected for the confirmatory mutagenicity assay will be documented in the raw data and report. If a retest of the initial toxicity-mutation assay is needed, a minimum of five dose levels of test article will be used in the retest.

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			10-20
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 homogenate was or will be purchased from Moltox and stored frozen at -60°C or colder until used. Each batch of S9 homogenate was or 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

Each tester strain culture will be inoculated from the appropriate master plate, from the appropriate frozen stock or with the appropriate lyophilized pellet(s). 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 shaker/incubator programmed to begin shaking at approximately 125 to 175 rpm and incubating at 37±2°C for approximately 12 to 14 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

Unless specified otherwise, test article dilutions will be prepared immediately prior to 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, 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 are not limited to the following:

Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments), 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. If one or more of these parameters are not acceptable, the affected condition(s) will be retested.

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 numbers 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 0.3×10^9 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
- Contributing scientist report of the dose formulation analysis from the test site

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 top dose level to be tested will be 3.75 µg per plate rather than 5 mg per plate.

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, 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.

US FDA Good Laboratory Practices 21 CFR Part 58

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

Brenda Faith — Sponsor Representative

22 Nov 2010 — Date

14.2 Study Director and Test Facility Management Approvals

Valentine D. Wagner, III — BioReliance Study Director — 13 Dec 2010 — Date

BioReliance Study Management — 13 Dec 2010 — 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.

Brenda Faith — 22 Nov 2010

Analytical Chemist or Principal Investigator — 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	*Salmonella* tester strains were from Dr. Bruce Ames' Master cultures, *E. coli* tester strains were from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland and both species of tester strain were distributed by Moltox (Boone, NC).	Single use lyophilized pellets used for each inoculation (QC Statement maintained in BioReliance files)	The genetic markers for each culture are confirmed on the day of use
TA100			
TA1535			
TA1537			
WP2 *uvr*A			

(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.	
	26 August 2010 (Lot 2656)	B1	
	08 December 2010 (Lot 2691)	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 (Lots 2656 and 2691)	Administration period and amount (g/kg-weight)	single dose at 0.5 gm/kg body weight, 5 days prior to sacrifice
Weight	Unknown (Lots 2656 and 2691)		

3. Preparation of Test Substance Solution

Solvent used			
Name	Manufacturer	Lot No.	Grade and/or Purity (%)
Absolute ethanol (CAS No. 64-17-5)	Sigma-Aldrich	09496HM	USP
Sodium chloride for injection (CAS No. 7467-14-5)	Baxter Healthcare	C806307	0.9%, USP
Stability of test substance in the solvent	Since the most concentrated dosing formulations were within 85 to 115% of target, the dosing formulations were considered stable (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 Nos.
	Oxoid Nutrient Broth No. 2	Oxoid Ltd.	891519 and 907248
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 or 1 to 2 pellets		

5. Agar Plate Medium

(1) Top agar

Agar	Name	BBL Select
	Manufacturer	Becton Dickinson
	Lot No.	0223574
	Name	BD Bacto
	Manufacturer	Becton Dickinson
	Lot No.	9341026

(2) Minimum Glucose Agar

Purchased from Moltox	Agar	Name		BBL Select
		Manufacturer		Becton Dickinson
		Lot No.		0223574
		Batch No.	Preparation Date	Used in Experiment No.
		36669	30 November 2010	B1
		36704	07 December 2010	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 response was observed with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9.	

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 and Retest Memo

Fluoromisonidazole

Product no. 1410.XXXX

For research purposes only. Not for human use or consumption.

Product description

Fluoromisonidazole; synonyms: FMISO; 1-Fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol; mol. wt. 189.14; $C_6H_8FN_3O_3$; [13551-89-8]; chemical name: 1*H*-Imidazole-1-ethanol, α-(fluoromethyl)-2-nitro-. Yellowish solid, soluble in DMSO.

Applications

Fluoromisonidazole may be used as reference standard for [^{18}F]Fluoromisonidazole.

Presentation

Product 1410.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 Fluoromisonidazole 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 under argon or nitrogen atmosphere. Long term stability 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. LD_{50} 620 mg/kg (mouse, i.p.).

Certificate of analysis

Lot No.: 20100401		Product No.: 1410.XXXX	
Parameter	Method	Specification	Result
Appearance	organoleptic	yellowish solid	conforms
Melting pt	capillary	122-141 °C	123-127 °C
Identity	^{1}H-NMR ^{19}F-NMR	conforms conforms	conforms conforms
Purity	^{1}H-NMR	> 95 %	> 97 %

No further analytical data available

Manufacturing Date: Apr. 2010

ABX advanced biochemical compounds
Biomedizinische Forschungsreagenzien GmbH

Production date: 13-Apr-10

Dr. M. Diekers

Quality Control date: 11-May-10

S. Anders

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 right circumstances e.g. for research purposes depending on the locally applicable legislation for the site of use.

References

1) Oh S. J. *et al.*: Fully automated synthesis of [^{18}F]fluoromisonidazole using a conventional [^{18}F]FDG module. *Nucl. Med. Biol.* **2005**, *32*, 899-905.
2) Lim J. *et al.*: An efficient radiosynthesis of [^{18}F]Fluoromisonidazole. *Appl. Radiat. Isot.* **1993**, *44*, 1085-1091.
3) Martin G. V. *et al.*: Noninvasive detection of hypoxic myocardium using [^{18}F]Fluoromisonidazole and positron emission tomography. *J. Nucl. Med.* **1992**, *33*, 2202-2208.
4) Rasey J. S. *et al.*: Radiolabeled fluoromisoindazole as an imaging agent for tumor hypoxia. *Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *17*, 985-991.

ABX *advanced biochemical compounds* - Biomedizinische Forschungsreagenzien GmbH. -H.-Glaser-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

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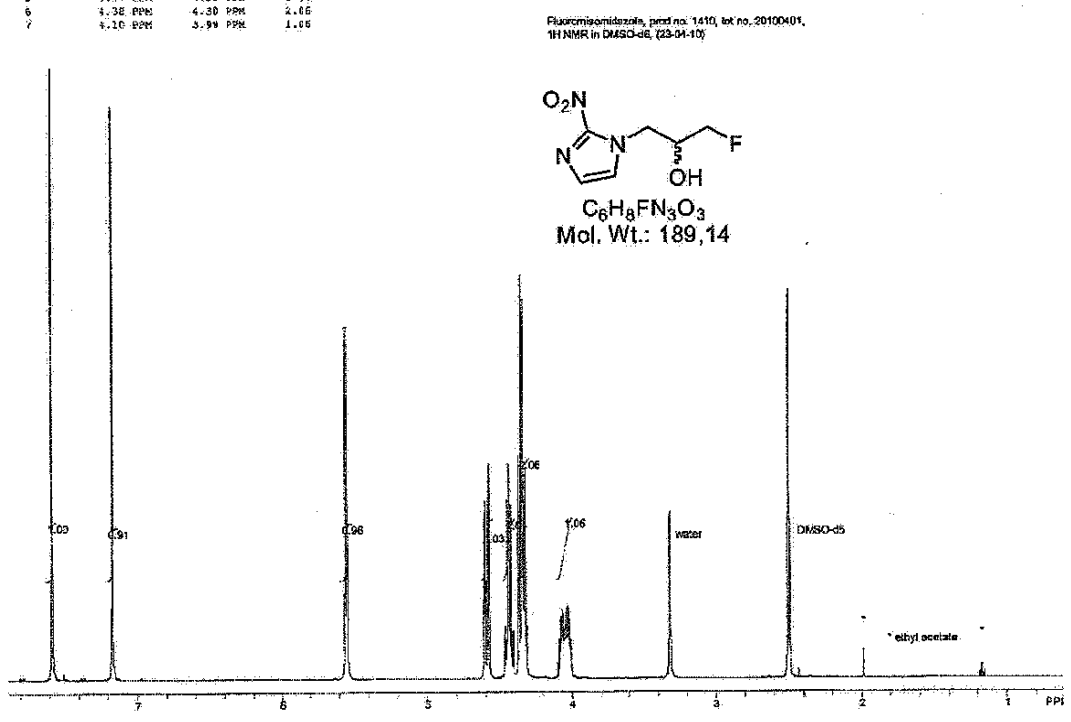
S. Anders

ABX GmbH

^{1}H-NMR

11. Mai 2010
Version 1.4

NUMBER	SHIFT	TO	VALUE
1	7.52 PPM	7.55 PPM	1.00
2	7.21 PPM	7.24 PPM	0.91
3	5.50 PPM	5.52 PPM	0.86
4	4.63 PPM	4.65 PPM	1.03
5	4.47 PPM	4.58 PPM	1.94
6	4.38 PPM	4.30 PPM	2.05
7	4.10 PPM	3.99 PPM	1.05



2/3

11. Mai 2010
Version 1.4
3/3
ABX GmbH
^{19}F-NMR
Freigabeprotokoll_FMISO_20100401_V1-4.doc
S. Anders
Fluoromisonidazole; prod.nu. 1410, lot no. 20100401,
19F-NMR in DMSO-d6, (23-04-10)
$C_6H_8FN_3O_3$
Mol. Wt.: 189,14

ABX Heinrich-Gläser-Str. 10-14 · D – 01454 Radeberg

To whom it may concern

ABX advanced biochemical compounds
Biomedizinische Forschungsreagenzien GmbH

Heinrich-Gläser-Str. 10-14
D - 01454 Radeberg
Germany

Phone: +49 / 3528 / 40 41 60
Fax: +49 / 3528 / 40 41 65
E-mail: info@abx.de
Internet: http://www.abx.de

Radeberg, 26.05.2011

Retest

This document describes the retest policy for our product FMISO reference standard, product number 1410, manufactured at ABX advanced biochemical compounds Biomedizinische Forschungsreagenzien GmbH – department Medicinal Chemistry.

We hereby state, that we perform retest after two years after manufacturing provided that the respective batch is not sold completely. We issue a retest certificate and provide a date of next retest. The date of the next restest would be one year after the first retest, provided that the batch is not yet sold completely.

Our scientific and expert knowledge as well as experience with the product as a chemical substance and retest data allow us to propose that the product can be used one year after the retest date.

Dr. Alexander Hoepping
Head of Medicinal Chemistry

Dinka Colditz
Head of Quality Control

APPENDIX V: Dosing Formulation Analysis

ANALYTICAL CHEMISTRY REPORT

RTI (Test Site) Project No.: 0211886.002.003
RTI (Test Site) Protocol No.: RTI-1114-AN
Bioreliance (Test Facility) Study No.: AD13SN.503.BTL

Dose Formulation Analysis of Fluoromisonidazole

Prepared By:

4/14/11
Richard C. Daw, M.Chem. Date
Chemist
Analytical Chemistry and Pharmaceutics

Approved By:

4/29/2011
Brian F. Thomas, Ph.D. Date
Senior Director
Analytical Chemistry and Pharmaceutics

Approved By:

20 April 2011
Brenda Faiola, Ph.D., D.A.B.T. Date
Senior Research Toxicologist
Pharmacology and Toxicology
Principal Investigator

STATEMENT BY PRINCIPAL INVESTIGATOR

This study was designed in accordance with national and international guidelines, to fulfill the requirements of regulatory authorities, for the toxicity testing of new drugs.

This study was conducted in accordance with US Food and Drug Administration Good Laboratory Practice for Nonclinical Laboratory Studies, 21 CFR Part 58.

RTI's Sponsor (Clinical Monitoring Research Program, SAIC-Frederick, Inc) holds responsibilities for GLP compliance of test article characterization, test article strength, purity, stability, identity, and uniformity. A Certificate of Analysis for the test article was provided by the supplier (ABX Advanced Biochemical Compounds). The Sponsor provided information on the stability of the test article dose formulations and was also responsible for the GLP compliance of these test article dose formulation stability analyses.

The objectives set forth in the protocol were achieved, and as nothing occurred to affect adversely the quality or integrity of the study, I consider the data generated to be valid.

Brenda Faiola 2①
Brenda Faiola, Ph.D., D.A.B.T.
Senior Research Toxicologist
Principal Investigator

20 April 2011
Date

① Starting writing date in wrong place. BF 20 April 2011

RTI INTERNATIONAL

Quality Assurance Statement

Study Title: **Dose Formulation Analysis of Fluoromisonidazole**

Sponsor: **SAIC**

Protocol Number: **RTI-1114-AN**

This report was audited by the Regulatory and Quality Assurance (RQA) – Quality Assurance Unit and the results of the audit were reported to the Principal Investigator, Study Director and Management as identified below.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Reports Sent to Principal Investigator and Management	Date Inspection/Audit Reports Sent to Study Director and Management
Protocol Audit	11/18/2010	11/18/2010	03/18/2011
Dose Analvsis	12/15/2010	12/17/2010	03/18/2011
Data and Report Audit	03/04, 03/08, 03/11, 03/14/2011	03/14/2011	03/18/2011

Prepared by:

Sandra Williams 4-14-2011

Sandra Williams
Quality Assurance Specialist

Date

Reviewed by:

Bianca Lopez 4/14/2011

Bianca Lopez
Quality Assurance Specialist

Date

SUMMARY

The Test Site (RTI) prepared and shipped the test article standard stock solution to the Test Facility (BioReliance) for use in the Bacterial Reverse Mutation Assay.

Two sets of dose formulation samples were prepared from the standard stock solution by the Test Facility, shipped to the Test Site, and analyzed for concentration verification for RTI Project 0211886.002.003, per the analytical study plan RTI-1114-AN. Information on the dose formulation preparation procedures was the responsibility of RTI. The stability of the dose formulations was the responsibility of the Sponsor and provided to RTI.

The formulation analyses were performed following the validated analytical method "Analysis of Fluoromisonidazole in 0.9% Sodium Chloride for Injection, USP:Absolute Ethanol, USP (95:5, v:v) Formulations" (RTI Analytical Method AM-0211886-002) to verify the fluoromisonidazole (FMISO) concentration in 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v) formulations prepared on December 14, 2010 and January 4, 2011 at the Test Facility. All dose formulations analyzed were found to be within ± 15% of the nominal concentration.

TEST ARTICLE STANDARD STOCK SOLUTION PREPARATION

The test article was supplied by the Test Site to the Test Facility as a standard stock solution (~1 mg/mL) in 95%:5% (v:v) sterile water for injection, USP:absolute ethanol, USP prepared on December 7, 2010. The solution was stored in polypropylene cyrovials, each containing approximately 3 mL of the solution. The vials were stored frozen at approximately 0° to -20°C at the Test Site prior to shipment on dry ice to the Test Facility on December 8, 2010. Under these conditions, the standard stock solution was acceptable for use through June 7, 2011 (6 months after the date of preparation). The Sponsor provided information on the stability of the test article dose formulations and was also responsible for the GLP compliance of these test article dose formulation stability analyses.

ANALYTICAL METHOD

The validated analytical method "Analysis of Fluoromisonidazole in 0.9% Sodium Chloride for Injection, USP:Absolute Ethanol, USP (95:5, v:v) Formulations "(RTI Analytical Method AM-0211886-002) used to analyze study samples is described briefly below.

On the day of dose formulation preparation, two 1-mL vials of each dose formulation concentration (0 and 75.0 μg/mL) were collected at the Test Facility and shipped to the Test Site for analysis. Triplicate aliquots from each dose formulation were analyzed on a high performance liquid chromatograph (HPLC) with a PDA detector (Table 1) along with a series of vehicle standards used to generate a calibration curve. Vehicle standards were prepared by diluting an approximately 1 mg/mL FMISO standard stock solution in sterile water for injection, USP: absolute ethanol (95:5, v:v; prepared on December 7, 2010 and stored frozen at

approximately -20 °C) with 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v) to make a 100 µg/mL intermediate vehicle stock solution which also served as the highest concentration standard for the calibration curve. The intermediate vehicle stock solution was diluted with blank vehicle to prepare duplicate vehicle standards at six lower concentrations in order to create a calibration curve which encompassed the concentration range of the dose formulations (10-100 µg/mL). Test article concentrations were calculated using a least squares linear regression equation that fit the relationship between the nominal concentrations of vehicle standard and the detector response. The dose formulation sample concentrations were determined in µg/mL.

Table 1 HPLC System

Instrumentation			
Instrument:	Waters 2695 Alliance HPLC		
Detector:	Waters 2996 Photodiode Array Detector		
Column:	Thermo Fisher Aquasil C_{18} 2.1 x 150-mm (5-µm)		
Data System:	Waters Empower 2, Build 2154		
Conditions			
Mobile Phase Flow Rate:	0.3 mL/min		
Column Heater:	30 °C		
Wavelength Detected:	230-400 nm, extracted 325 nm		
Gradient Program:	Time (min)	%A	%B
	-	100	0
	12	100	0
Mobile Phase:	A: 10 mM formic acid in water:methanol (95:5, v:v) B: water:methanol (80:20, v:v)		
Injection Volume:	10 µL		

FORMULATION ANALYSIS

The formulation analyses were performed following the analytical method described above to verify the FMISO concentration in 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v) formulations prepared on December 14, 2010 and January 4, 2011 at the Test Facility; the nominal concentrations of the two formulations sent from each preparation date were 0 (vehicle) and 75.0 µg/mL. On the day after the formulation dates, the formulations were received at the Test Site (shipped on cold packs) and analyzed for concentration. For concentration verification, the found concentration for each formulation was evaluated for

accuracy and the triplicate determinations were evaluated for precision. Analytical results are presented in Attachment 1.

Note: Values presented in this report have been rounded to the correct number of significant digits based upon the accuracy of the initial laboratory observations; however, all mathematical and statistical computations within a single mode of calculation have been performed on non-rounded values in order to minimize error in the final result due to rounding. Thus, some values and summary statistics may not be accurately reproduced using the rounded intermediate values which appear here.

CONCLUSION

Each test article dose formulation analyzed for each preparation date was found to be within ± 15% of the nominal concentration and no test article was detected in either of the vehicle formulations. The relative standard deviation (RSD) for each replicate determination was ≤ 10%.

ATTACHMENT 1

Dose Formulation Analysis Final Results Reports

FORMULATION ANALYSIS FINAL REPORT

(Concentration Verification)

Test Site Study No.: 0211886.002.003
Test Facility Study No: AD13SN.503.BTL
Test Article: Fluoromisonidazole
Formulation Date: 12/14/10
Vehicle: 0.9% sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v)
Analysis Date: 12/15/10

RTI Log Number	Sample Description	Nominal Conc.[a]	Found Conc.[a,b]	Percent of Nominal[c]	Mean Found Concentration[a,b,d]
121510-A-01	Analysis sample	0	ND	ND	ND
			ND	ND	
			ND	ND	
121510-A-02	Analysis sample	75.0	82.5	110	82.6 ± 0.180 (0.22% RSD)
			82.7	110	
			82.4	110	

[a]Concentration unit: µg/mL
[b]Each formulation sample was analyzed in triplicate.
[c]Percent of Nominal: 100 + [((FMISO Found Conc. – FMISO Nominal Conc.)/FMISO Nominal Conc.) x 100]
[d]Mean found concentration ± standard deviation and % RSD of n=3 results shown. Found concentration was determined with the linear regression equation (non-weighted):
y = 72280.84x + 7244.745; r = 0.9999 for calibration range from 10.0 µg/mL to 100 µg/mL
ND = Not Detected.

FORMULATION ANALYSIS FINAL REPORT

(Concentration Verification)

Test Site Study No.:	0211886.002.003	Test Facility Study No.:	AD13SN.503.BTL
Test Article:	Fluoromisonidazole	Formulation Date:	01/04/11
Vehicle:	0.9 % sodium chloride for injection, USP:absolute ethanol, USP (95:5, v:v)	Analysis Date:	01/05/11

RTI Log Number	Sample Description	Nominal Conc.[a]	Found Conc.[a,b]	Percent of Nominal[c]	Mean Found Concentration[a,b,d]
010511-A-01	Analysis sample	0	ND	ND	ND
			ND	ND	
			ND	ND	
010511-A-02	Analysis sample	75.0	84.6	113	84.9 ± 0.223 (0.26% RSD)
			85.0	113	
			85.1	113	

[a]Concentration unit: µg/mL
[b]Each formulation sample was analyzed in triplicate.
[c]Percent of Nominal: 100 + [((FMISO Found Conc. – FMISO Nominal Conc.)/FMISO Nominal Conc.) x 100]
[d]Mean found concentration ± standard deviation and % RSD of n=3 results shown. Found concentration was determined with the linear regression equation (non-weighted):
y = 71667.37x – 1033.152; r = 0.9999 for calibration range from 10.0 µg/mL to 100 µg/mL
ND = Not Detected.