PROTOCOL 1601.403

Study Number: _____ Scheduled Initiation Date:_____

Proposed Completion Date:_____

DISTRIBUTION OF RADIOLABELED MATERIAL IN TISSUES

1.0 PURPOSE

The purpose of this study is to determine the distribution of a radioactivity following a single treatment of radiolabeled test article.

2.0 SPONSOR

2.1 Name:

2.2 Address:

2.3 Authorized Representative:

3.0 TESTING FACILITY

3.1 Name: Metabolism Section;
Microbiological Associates Inc.

3.2 Address: 5221 River Road,
Bethesda, Maryland 20816

3.3 Study Director: Raymond M. David, Ph.D., D.A.B.T.

4.0 TEST ARTICLE

4.1 Name (or Code):

4.2 Test Article Number:

4.3 Vehicle: One of the following vehicles will be used depending on the solubility of the material and the route of administration: (1) normal saline (0.9%), (2) distilled water, (3) corn oil, (4) CMC, or (5) other vehicles as recommended by the Sponsor.

4.4 Purity: Radiochemical purity should be at least 98%. Non-labeled test article should be of comparable purity. Radiolabeled material may be diluted with non-labeled to an appropriate specific activity.

5.0 TEST SCHEDULE

5.1 Date Protocol Issued: January 17, 1986

5.2 Scheduled Initiation Date:

5.3 Proposed Completion Date:

6.0 TEST SYSTEMS

87461842A

6.1 Animals:

6.1.1 Strain/Species/Sex → Sprague-Dawley Rats

6.1.2 Justification:

Sprague-Dawley rat ed because they are available commerci ndard animal model for metabolism studies.

6.1.3 Source:

Charles R 87461842B oratories,

6.1.4 Number/se

6.1.5 Age at ini t least 8 weeks

6.2 Route of Administra → injection or gavage.

87461842 Justificat:

Intravenous is used bec information can be obtained about elimina se and 2) the process of absorption is . 87461842C

Gavage is used because a) xposure and b) it includes the process of

NTAL DESIGN

Radiolabeled test article will be admin e IV injection or gavage dose to 32 male or 3 mediately after exposure, the animals will be plac cages and groups of 4 sacrificed at specified inte below) for 72 hours after the administration of the test article. Collection times will be based on the results obtained from disposition studies. However, recommended time points for sampling of excreta are 0.5, 1, 2, 4, 8, 24, 48 and 72 hours after treatment. If after consultation with the

87461842D

5.0 TEST SCHEDULE

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5.2 Scheduled Initiation Date:

5.3 Proposed Completion Date:

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87461842D

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5.3 Proposed Completion Date:

6.0 TEST SYSTEMS

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6.1.2 Justification:

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87461842D

5.0 TEST SCHEDULE

5.1 Date Protocol Issued: January 17, 1986

5.2 Scheduled Initiation Date:

5.3 Proposed Completion Date:

6.0 TEST SYSTEMS

6.1 Animals:

6.1.1 Strain/Species/Sex: Male or Female Sprague-Dawley Rats

6.1.2 Justification:

Sprague-Dawley rats have been selected because they are available commercially and are a standard animal model for metabolism studies.

6.1.3 Source:

Charles River Breeding Laboratories,

6.1.4 Number/sex: 32

6.1.5 Age at initiation of test: at least 8 weeks

6.2 Route of Administration: Intravenous injection or gavage.

6.2.1 Justification:

Intravenous injection is used because 1) information can be obtained about elimination of a known dose and 2) the process of absorption is avoided.

Gavage is used because a) it mimics human exposure and b) it includes the process of absorption.

7.0 EXPERIMENTAL DESIGN

Radiolabeled test article will be administered by a single IV injection or gavage dose to 32 male or 32 female rats. Immediately after exposure, the animals will be placed into individual cages and groups of 4 sacrificed at specified intervals (see below) for 72 hours after the administration of the test article. Collection times will be based on the results obtained from disposition studies. However, recommended time points for sampling of excreta are 0.5, 1, 2, 4, 8, 24, 48 and 72 hours after treatment. If after consultation with the

5.0 TEST SCHEDULE

5.1 Date Protocol Issued: January 17, 1986

5.2 Scheduled Initiation Date:

5.3 Proposed Completion Date:

6.0 TEST SYSTEMS

87461842A

6.1 Animals:

6.1.1 Strain/Species/Sex → Sprague-Dawley Rats

6.1.2 Justification:

Sprague-Dawley rat ed because they are available commerci_____, ____ ndard animal model for metabolism studies.

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Sponsor other time points are recommended, no less than 7 collection time points will be used. At each time period, animals will be sacrificed by carbon dioxide asphyxiation and blood collected. Other tissues (liver, kidneys, spleen, gonads and reproductive organs, small intestines, large intestine, thymus, brain, lungs, and heart) will be excised. Aliquots of blood, of each tissue and of the remaining carcass will be taken for determination of the levels of radioactivity.

7.1 Number of Animals:

32 rats total.

7.2 Test Material:

The Sponsor will assume responsiblility for the preparation of the dosing solutions and ship them ready-to-use. The radiolabeled compound should be prepared in a suitable vehicle and diluted with non-labeled test compound to make up a dosing solution sufficient to give each animal approximately 20 uCi. Doses will be specified by the Sponsor. Prepared solutions will be stored as required until dosing. Dosing volumes will be 1.0 ml/kg (injection) or 10.0 ml/kg (gavage) unless other dosing volumes are required because of the solubility of the compound.

8.0 METHODS

8.1 Receipt, Quarantine, Vaccination, Monitoring and Randomization:

Animals will be obtained at 6-7 weeks of age from a source monitored and known to be free of adventitious agents, and quarantined for at least 14 days. Stringent disease control procedures will be followed during quarantine to assure the use of healthy animals. Animals will be examined each working day during the quarantine period for deaths or signs of illness. Dead or ill animals will be separated from healthy ones and any unusual observations will be reported to the Study Director and recorded in the raw data book. Upon successful completion of the quarantine period, animals will be randomized according to standard operating procedures and individually identified by a unique 4 digit numbered ear tag.

8.2 Animal Care:

All animals will have free access to certified laboratory diet and water ad libitum except during exposure. Certified diet is lot numbered and dated. The nature and level of contaminants in the feed will not interfere with this study. The water source is Washington Suburban Sanitary Commission (WSSC), Potomac Plant, no additional treatment; water meets USEPA drinking water standards.

Sponsor other time points are recommended, no less than 7 collection time points will be used. At each time period, animals will be sacrificed by carbon dioxide asphyxiation and blood collected. Other tissues (liver, kidneys, spleen, gonads and reproductive organs, small intestines, large intestine, thymus, brain, lungs, and heart) will be excised. Aliquots of blood, of each tissue and of the remaining carcass will be taken for determination of the levels of radioactivity.

7.1 Number of Animals:

32 rats total.

7.2 Test Material:

The Sponsor will assume responsibli of the dosing solutions and ship them radiolabeled compound should be pre cle and diluted with non-labeled test c ing solution sufficient to give each animal approximately 20 uci. Doses will be specified by the Sponsor. Prepared solutions will be stored as required until dosing. Dosing volumes will be 1.0 ml/kg (injection) or 10.0 ml/kg (gavage) unless other dosing volumes are required because of the solubility of the compound.

87461843

8.0 METHODS

8.1 Receipt, Quarantine, Vaccination, Monitoring and Randomization:

Animals will be obtained at 6-7 weeks of age from a source monitored and known to be free of adventitious agents, and quarantined for at least 14 days. Stringent disease control procedures will be followed during quarantine to assure the use of healthy animals. Animals will be examined each working day during the quarantine period for deaths or signs of illness. Dead or ill animals will be separated from healthy ones and any unusual observations will be reported to the Study Director and recorded in the raw data book. Upon successful completion of the quarantine period, animals will be randomized according to standard operating procedures and individually identified by a unique 4 digit numbered ear tag.

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Until treatment, animals will be singly housed in an AAALAC accredited facility in "shoe box" type cages equipped with filter covers. After treatment, animals will be maintained in individual cages. The light cycle is regulated at 12 hours light/12 hours dark and the temperature and humidity will be controlled and monitored. Cages will be changed biweekly and feeders will be changed weekly.

8.3 Treatment:

The test material will be administered in a single dose by IV injection or gavage. The radiolabeled compound will be prepared in a suitable vehicle (see section 4.3) and diluted with non-labeled test compound to make up a dosing solution sufficient to give each animal 20 uCi.

8.4 Doses:

8.4.1 Recommended Dose: _____

8.5 Sampling Times:

Animals (4 per time point) will be sacrificed at _____ _____ after treatment.

8.6 Collection of Tissue Samples:

8.6.1 Sacrifice:

All animals are to sacrificed by carbon dioxide asphyxiation.

8.6.2 Blood:

Blood will be collected at sacrifice by aortic or cardiac puncture. Blood will be separated into plasma and packed cells and aliquots will be taken for determination of radioactivity.

8.6.3 Tissues:

The following tissues will be excised at sacrifice and placed into separate containers: liver, kidneys, spleen, gonads and reproductive organs, small intestines, large intestine, thymus, brain, lungs, and heart. The remaining carcass will also be weighed and placed into a container. All tissues will be frozen immediately.

8.7 Determination of Radioactivity:

8.7.1 Specific Activity:

The specific activity of the dosing solution will be determined by removing aliquots (100 ul) of the solution prior to dosing, diluting with water (1:10) and counting aliquots of the diluted sample.

8.7.2 Blood:

Duplicate aliquots of plasma and packed cells from blood taken at sacrifice will be placed in the tissue oxidizer for combustion, and subsequent scintillation counting.

8.7.3 Other Tissues and Remaining Carcass:

Aliquots of other tissues and remaining carcass will be placed in a tissue oxidizer for combustion and subsequent scintillation counting.

9.0 EVALUATION OF TEST RESULTS

The following will be described in the final report.

9.1 The percentage of radioactivity present in the heart, brain, thymus, lungs, blood, spleen, liver, kidneys, intestine, gonads and remaining carcass relative to the amount administered will be presented for individual animals. Group means and standard deviations will be presented for each sacrifice time.

9.2 A plot of the log of the radioactivity present in the blood at each time point versus the average time of colletion will be presented. Estimates of the elimination half-life will also be calculated.

9.3 All methods used in treating the animals and determining the level of radioactivity.

10.0 RECORDS AND SAMPLE ARCHIVES

10.1 Records:

10.1.1 Upon completion of the final report, all raw data and reports will be retired to the archives located at 5221 River Road, Bethesda, MD. 20816

10.1.2 The archives will be maintained by the Regulatory Affairs/Quality Assurance Unit.

10.2 Test Article:

Unused radiolabeled test article will be returned to the Sponsor.

11.0 STANDARD OPERATING PROCEDURES

All procedures not specified in this protocol will be performed in accordance with the Microbiological Associates Inc. Standard Operating Procedures Manual and in compliance with the appropriate agency Good Laboratory Practice Regulations.

Alterations of the study protocol may be made after the study has been initiated. In the event that the sponsor verbally authorizes a change in protocol, such change will be honored by Microbiological Associates Inc. However, it is then the responsibility of the sponsor to produce written verification of the verbal authorization, which will include documentation of the justification and which will result in the issuance of an official protocol amendment, to be dated and signed by both Study Director and Sponsor.

Will this study be submitted to a regulatory agency? _____
Which agency? _____

12.0 SIGNATURES

_____ _____
Sponsor Approval Date

_____ _____
Study Director Date

_____ _____
RA/QA Date