Final Report
I-7070.401

INDUCTION OF HEPATIC MICROSOMAL ENZYMES IN RATS BY B213

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Final Report for
Lorillard Research Center
420 English St.
Greensboro, N.C. 27420

January 5, 1988

By
Microbiological Associates Inc.
5221 River Rd.
Bethesda, MD. 20816

QUALITY ASSURANCE STATEMENT

Study Title: INDUCTION OF HEPATIC MICROSOMAL ENZYMES IN RATS BY B213

Study Number: I7070.401

Study Director: RAYMOND M. DAVID, Ph.D., D.A.B.T.

Initiation Date: 87/08/10

Review Completed Date: 88/01/22

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice regulations (21CFR58), the U.S. EPA GLPs (40CFR792 and 40CFR160), and the OECD guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of the study.

INSPECT ON 87/08/10 - 87/08/10,TO STUDY DIR 87/08/10,TO MGMT 87/08/10

PHASES: PROTOCOL REVIEW

INSPECT ON 88/01/17 - 88/01/18,TO STUDY DIR 88/01/18,TO MGMT 88/01/22

PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Albert Chisholm 01/22/88

Quality Assurance
RA/QA Department

Date

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ABBREVIATIONS

7EC - 7-Ethoxycoumarin O-deethylase

ETR - 7-Ethoxyresorufin O-deethylase

MTD - Maximum tolerated dose

PNAS - para-Nitroanisole O-demethylase

S9 - Supernatant from 9000xg centrifugation

I. DATA PAGE

Test Article Identity: B213

Initiation Date of Dosing for Range Finder Study: August 10, 1987

Termination Date: August 20, 1987

Review Date: See Review Completed Date, Page 2

MBA Study Number: I-7070.401

MBA Notebook Number: 7070.401

Archives Location: 5221 River Road, Bethesda, Maryland 20816

Sponsor: Lorillard Research Center
420 English St.
Greensboro, NC 27420

Authorized Representative: J. Daniel Heck, Ph.D.
Thomas A. Vollmuth, Ph.D.

Testing Facility: Microbiological Associates Inc.
5221 River Road
Bethesda, Maryland 20816

Technical Staff: David R. Dansie
Lloyd G. Campbell
Guillermo Martinez
Calvin V. Dove

Study Director: Raymond M. David, Ph.D.,
Diplomate of the American
Board of Toxicology

Date 1/5/88

88979003

MICROBIOLOGICAL ASSOCIATES INC.

II. INTRODUCTION

The Cytochromes P-450 are a group of hemoproteins which are associated with the microsomal or lipid portion of a cell. Their designation as P-450 resulted from the observed maximum of the reduced hemoprotein-carbon monoxide complex at 450 nm in a difference spectrum (Omura & Sato, 1964). These hemoproteins also have enzymic activity, and can metabolize relatively lipophilic substrates to forms which are more water soluble. Such reactions are a normal function of the body and result in the formation of many important hormones. A great variety of non-physiologic compounds (xenobiotics) can also be metabolized by these enzymes, e.g. alkyl halides, aromatic hydrocarbons, aliphatic amines, etc. Such broad substrate specificity is enhanced by the fact that the relative population of isozymes can be altered by the presence of a potential substrate. Such alterations may increase the amount of only one specific isozyme that metabolizes that substrate, or all P-450 activity can be increased uniformly. In either case, the phenomenon of induction can have important consequences not only to the xenobiotic substrate that induced the activity, but also to physiologic substrates.

Of the number of compounds that have been shown to induce P-450, most have tended to fall into one of two categories based on their similarity to two classic inducers: phenobarbital and methylcholanthrene (Snyder and Remmer, 1979). Phenobarbital (PB) induces general P-450 activity, while methylcholanthrene (MC) induces a different type of P-450 called P_1-450 or P-448, named because of the shift in spectral maximum from 450 to 448 nm. Both MC and PB induced forms of P-450 can be induced by certain chlorinated biphenyls such as Aroclor 1254 (Parkinson et al., 1983).

A number of reactions have been used to assay P-450 activity. Three primary assays can be employed to examine P-450 and P-448 activity.

1) p-Nitroanisole O-demethylation - The demethylation of p-nitroanisole has been used as a marker for P-450 activity in the liver (Thurman et al., 1977).

2) 7-Ethoxycoumarin O-deethylation - The O-deethylation of 7EC has been shown to be a sensitive marker for P-448 and P-450 induction (Greenlee and Poland, 1978).

3) Ethoxyresorufin O-deethylation - Ethoxyresorufin deethylation is a sensitive marker for P-448 induction in rat liver, kidneys, and lungs (Nims et al., 1984).

III. PURPOSE

The purpose of this study is to determine if test materials induce cytochrome P-450 and/or P-448 activity in rat liver.

IV. TEST ARTICLE IDENTIFICATION AND PROPERTIES

Test Article identification:	B213
Lot number:	MP04412LP
Test Article number:	T07070A
Quantity received:	100g
Date received:	July 31, 1987
Expiration date:	January 20, 1988
Physical description:	White crystalline powder
Storage conditions:	Refrigerated (amber bottle)
Purity:	Assume 100%
Suggested solvent:	Distilled water
Solvent used:	Distilled water

V. TEST DESCRIPTION

A Range-Finding Study was conducted prior to the Main Induction Study in which 3 animals per group were given either 5000 or 2500 mg/kg of B213 for 4 consective days. The animals were observed twice daily (just prior to dosing and at least 4 hours after dosing) for mortality and clinical signs of toxicity. If mortality was observed, an attempt was made to determine the cause of death. Additional doses of 1250 and 625 mg/kg were given to naive animals because of mortality at 5000 and 2500 mg/kg.

Mortality of all three animals in group one was observed after 3 days and the study was terminated at the request of the Sponsor.

VI. METHODS

A. Animals

Female Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Raleigh, N.C. at 6 weeks of age. Animals were quarantined for 13 days. Stringent disease control procedures were followed during quarantine to assure the use of healthy animals. Rats were observed for signs of illness and cultures from the respiratory tract were examined for the presence of pathogens. In addition, sera from sentinel animals were examined for antibody titers to common rodent viruses and bacteria (Reovirus type 3, Pneumonia virus of mice, Sendai virus, Encephalomyelitis virus (GD VII), Mouse adenovirus, Toolan H-1 virus, Mycoplasma pulmonis, Kilham rat virus, Lymphocytic choriomeningitis virus, Rat coronavirus, Sialodacryoadenitis virus). The animals were judged to be healthy prior to utilization in this study and were 10 weeks old at initiation of dosing.

Animals were housed 3 or 4 per cage in an AAALAC-accredited facility under a controlled environment of 75 ± 6°F, 62 ± 18% relative humidity, and a 12 hour light/dark cycle. Rats were housed in polycarbonate autoclavable cages with filter top cage lids. Corn-cob bedding was used and animals had free access to certified laboratory rodent chow which had been analyzed for environmental contaminants. Water and food were provided ad libitum.

B. Treatment with Test Materials

Test material was administered at four dose levels. The doses for test material B213 were 5000, 2500, 1250 and 625 mg/kg. Three female rats per group were given one of four doses by gavage for 4 consecutive days prior to sacrifice. The vehicle was distilled water.

Dose Preparation:

Test material was stored according to Sponsor instructions and routine safety precautions were observed in handling. Test material was prepared in distilled water according to Sponsor instructions. Dosing solutions were prepared daily.

Volume:

The volume of liquid administered to the test animals was 10 ml/kg body weight. Daily volumes over the 4 day period were based on individual body weights taken on Day 1. Concentrations were adjusted so that a constant dosing volume was given at both dose levels. The test material was administered in a single dose by gavage, using a 16 gauge gavage needle.

Body Weights:

Animals were weighed prior to treatment on Day 1 and on Day 5 prior to

VII. RESULTS

Female Sprague-Dawley rats were quarantined for 13 days prior to utilization, during which time their health status was evaluated by observation. In addition, sentinel animals were sacrificed and cultures from the respiratory tract were examined for pathogens. Sera from sentinel rats were examined for titers to common rodent viruses (see Section VI, Methods). Animals were free of titers to rodent viruses. No pathogens were found in the cultures of the respiratory tract, and the animals were judged to be healthy prior to the initiation of the study.

A Range-Finding Study was conducted prior to the Main Induction Study in which 3 animals per group were given either 5000 or 2500 mg/kg of B213 for 4 consective days. The animals were observed twice daily (just prior to dosing and at least 4 hours after dosing) for mortality and clinical signs of toxicity. If mortality was observed, an attempt was made to determine the cause of death. Additional doses of 1250 and 625 mg/kg were given to naive animals because of mortality at 5000 and 2500 mg/kg. Body weights were taken on Days 1 and 5 and are presented in Table 1. Clinical observations and incidence of mortality are presented in Table 2. All animals were sacrificed following the final body weight measurement and were not used as part of the induction study. The study was terminated at the request of the Sponsor.

VIII. REFERENCES

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