

AN ACUTE ORAL TOXICITY STUDY IN RATS WITH ADVANTRA Z™

AMENDED FINAL REPORT

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SUMMARY

The single-dose oral toxicity of Advantra Z™ was evaluated in Sprague-Dawley rats. A limit test was performed in which one group of five male and five female rats received a single oral administration of the test article at a dose of 10000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. The most notable clinical abnormalities observed during the study included rough coat, decreased activity, congested breathing, dark material around the facial area, decreased defecation, salivation, soft stools and urine/fecal stain. These clinical abnormalities were observed during the first few days after dosing, but then disappeared, with the exception of residual slight hair loss which was still present in one animal for 14 days and in another after 7 days. A slight body weight loss was noted for one female rat during the study day 7-14 body weight interval. However, since the animals were young adults and still growing, body weight gain was noted for all other animals during the test period. No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute oral LD50 of Advantra Z™ was estimated to be greater than 10000 mg/kg in the rat.

I. INTRODUCTION

This study was performed to assess the short-term toxicity of Advantra Z™ in Sprague-Dawley rats when administered by gavage as a single oral dose. This study is intended to provide information on the potential health hazards of the test article with respect to oral exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on February 28, 1997 (GLP initiation date). The in-life phase of the study was initiated with test article administration on March 13, 1997 and concluded with terminal euthanasia on March 27, 1997.

II. MATERIALS AND METHODS

A. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Advantra Z™ Lot No.: 96307-006	S97.001.3443	Brown powder	February 24, 1997	None provided

The test article was stored at room temperature. The Sponsor is responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 21 CFR 58.105.

B. Retention Sample

The Sponsor was responsible for maintaining a retention sample of the test article.

C. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

D. Method of Test Article Preparation

The test article was mixed with deionized water to produce the appropriate concentration for dose administration.

E. Animals and Animal Husbandry

1. Description, Identification and Housing

Young adult, Sprague-Dawley CrI:CD®BR VAF/Plus® rats were received at SLI from Charles River Laboratories, Inc., Portage, Michigan. The animals were approximately 8-13 weeks of age at experimental initiation. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

2. Environment

The animal room temperature and relative humidity ranges were 68-74°F and 30-53%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during fasting). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI

4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted annually by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR, Part 141).

5. Acclimation

Upon receipt the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant.

III. EXPERIMENTAL DESIGN AND PROCEDURES

A. Dosing

On day-1, the animals chosen for the limit test were weighed and fasted overnight. On day 0, the test article was administered orally as a single dose using a ball tipped stainless steel gavage needle attached to a syringe at the following level:

Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (mg/mL)	No. of Animals	
			Males	Females
10000	20	500	5	5

Individual doses were calculated based on the animal's fasted (day 0) body weight. Animals were returned to ad libitum feeding after dosing.

B. Clinical Observations

Limit test animals were observed for clinical abnormalities two times on study day 0 (postdose) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

C. Body Weights

Individual body weights were obtained for the limit test animals prior to fasting (day -1), prior to dosing on day 0 and on days 7 and 14.

D. Gross Necropsy

All limit test animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

E. Protocol Deviations

No protocol deviations occurred during this study.

IV. ANALYSIS OF DATA

Data from the limit test were analyzed and an LD50 value estimated as follows:

- < 50% Mortality: LD50 was estimated as greater than the administered dose.
- = 50% Mortality: LD50 was estimated as equal to the administered dose.
- > 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females for each limit level administered.

V. MAINTENANCE OF RAW DATA AND RECORDS

All original paper data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

VI. RESULTS

A. Mortality

Individual Data: Table 1

No mortality occurred during the limit test

B. Clinical Observations Individual Data: Table 1

The most notable clinical abnormalities observed during the study included rough coat, decreased activity, congested breathing, dark material around the facial area, decreased defecation, salivation, soft stools and urine/fecal stain. These clinical abnormalities were observed during the first few days after dosing, but then disappeared, with the exception of residual slight hair loss which was still present in one animal for 14 days and in another after 7 days.

C. Body Weight Data Individual Data: Table 2

A slight body weight loss was noted for one female rat during the study day 7-14 body weight interval. Normal body weight gain resumed for all other animals during the test period.

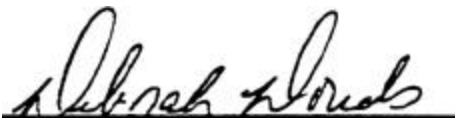
D. Gross Necropsy

Individual Data: Table 3

No significant gross internal findings were observed at necropsy on study day 14.

VII. CONCLUSION

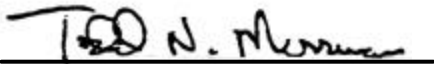
Under the conditions of this test, the acute oral LD50 of Advantra Z™ was estimated to be greater than 10000 mg/kg in the rat



Deborah A. Douds, M.S.
Study Director

Date 11/19/97

VIII. REPORT REVIEW



Todd N. Merriman, B.S., LATG
Manager of Subchronic Toxicology

Date 11/19/97



Kimberly L Bonnette, M.S., LATG
Manager of Acute Toxicology

Date 11/19/97

IX. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.