

**28-DAY TOXICITY OF**

**IN RATS**

**SPONSOR:** Toxicology & Pharmacology Branch  
Developmental Therapeutics Program  
Division of Cancer Treatment  
National Cancer Institute  
National Institutes of Health  
Bethesda, Maryland 20892

**PROJECT OFFICER:**

**CONTRACT TYPE:** Cost Plus Fixed Fee

**CONTRACT NUMBER:**

**CONTRACTOR:**

**PRINCIPAL INVESTIGATOR:**

**STUDY DIRECTOR:**

**PROPOSED IN-LIFE PHASE:**

**Start:**

**Finish:**

**I. OBJECTIVE**

The objective of this study is to determine target organ toxicity and its reversibility of in rats treated orally for 28 consecutive days.

**II. MATERIALS AND METHODS****A. Test and Control Article:**

1. **Name of Test Article:**

2. **Name of Control Article:**

3. **Characterization and Documentation of Methods of Synthesis, Fabrication or Derivation:**

a. **Test Article:**

Compound identity, strength, quality, stability and purity as well as documentation of methods of synthesis, fabrication or derivation are the responsibility of the NCI. Confirmation of identity will be done immediately upon receipt of each shipment of the compound. Sufficient quantity of drug shall be reserved for archiving from each lot and shipment used.

b. **Control Article:**

4. **Stability and Storage:**

a. **Test Article:**

b. **Control Article:**

5. **Formulation Preparation, Stability and Storage:**

a. **Test Article:**

**b. Control Article:****6. Dose Concentration and Homogeneity Analyses:**

Dose concentration analyses will be performed on each newly formulated dosing solution prior to dosing. Homogeneity analysis will not be required, since homogeneity has been previously demonstrated. Dosing solutions must be within  $\pm 10\%$  of the theoretical concentration and all results of these analyses will be submitted to the NCI Project Officer within 7 days after each dosing. An adequate quantity of each dosing mixture will be retained for possible analysis until the acceptance of the final report on this compound.

**B. Test System:****1. Species, Strain Supplier and Test System Justification:**

Fischer 344 rats supplied by DTP, DCTDC, NCI will be used in this study. This is an accepted species to support studies of compounds used or intended for use in humans.

**2. Initial Age, Sex and Weight:**

On the first day of dosing, the weight ranges of rats will be approximately 150 to 200 grams for males and approximately 125 to 175 grams for females, and the rats will be approximately 8 to 12 weeks of age.

**3. Care and Housing:**

General procedures for animal care and housing will be in accordance with DHHS Publication No. (NIH) 86-23 (Revised, 1985) and the U.S. Department of Agriculture through the Animal Welfare Act (7 USC 2131), 1985 and Animal Welfare; Standards incorporated in 9 CFR Part 3, 1991. Rats will be group housed (three per cage) by dose and separated by sex. Appropriate caging and bedding or cage board (not cedar or pine chips) will be used. No contaminants should be present in the bedding which could interfere and affect the results of the study.

**4. Diet and Water Supply:**

Diet is to be certified, commercial, dry rodent chow provided *ad libitum*. Water source will be the public supply given *ad libitum*. No contaminants will be present in the feed or water which could interfere and affect the results of the study.

**5. Quarantine:**

All rats will be quarantined for a minimum of 7 days prior to baseline measurements. No prophylactic or therapeutic treatment will be administered during the quarantine period. Only healthy animals will be placed on study.

**6. Animal Identification:**

All rats will be given a unique identification number for this study by ear tag or other approved method.

**C. Experimental Design**

**1. Randomization:**

In order to obtain groups that are comparable by weight, all rats will be randomly assigned to their respective treatment groups using a computer-based body weight stratification procedure. The individual body weights required for randomization are to be determined on day -5.

**2. Group Assignments:**

After randomization, 60 rats of each sex (15/sex/dose group) will be assigned to three dose groups and a vehicle control group (VCTL).

GROUP	DOSE (mg/kg/hr)	# of Rats Study Start <sup>a</sup>	# of Rats Sacrificed <sup>a</sup>		
			DAY 15	DAY 29	DAY 57
I (VCTL)	0.0	30	10	10	10
II	TBD	30	10	10	10
III	TBD	30	10	10	10
IV	TBD	30	10	10	10

<sup>a</sup> Equal numbers of males and females.

**3. Route of Administration and Reason for Choice:**

The test compound will be given orally because this is the intended route of administration of this compound in humans.

**4. Dosing Procedure:**

Starting on day one, each rat within each dose group will receive drug orally by gavage three times a day (every 8 hours) for 28 days. The amount of drug administered to each rat will be based on its most recent individual body weight. Rats in the vehicle control group will receive a volume of vehicle equivalent to the greatest volume administered on a mL/kg basis three times a day (every 8 hours) for 28 consecutive days. All calculations for amount of drug given to each rat will be checked by a second individual who will initial and date the verification.

In order to reduce the possibility of masking drug effects, a volume for administration will be selected and maintained relatively constant for all rats. This can be done by making separate concentrations for rats given various dose levels, and varying the volume within the appropriate concentration to accommodate individual body weights.

**5. Measurements:****a. Clinical Signs:**

All rats will be observed at least once daily until study day 57 or more often as clinical signs warrant.

Rat identification numbers, dose volumes, drug formulations, vehicle, clinical effects, day(s) of death, individual body weights as specified below and other pertinent information will be recorded.

**b. Body Weight:**

All surviving rats will be individually weighed on study days -5, 1, 5, 8, 12, 15, 19, 22, 26, 29, 36, 43, 50 and 57, and terminally prior to sacrifice. The rats should be weighed at approximately the same time each day.

**c. Clinical Pathology:**

Blood should be drawn from each rat for clinical pathology determinations on study days 5, 15, 25, 35 and 45 and prior to termination. Blood will be taken from the retro-orbital plexus, except prior to sacrifice when this route may be supplemented with cardiac puncture, if necessary. Blood will not be taken from the treatment site. A blood sample will be obtained prior to the necropsy of each rat sacrificed in a moribund condition.

**Hematology:**

Erythrocyte count (RBC) -  $10^6/\text{mm}^3$

Hemoglobin (HGB) - g/dL

Hematocrit (HCT) - %

Mean corpuscular volume (MCV) - fl

Mean corpuscular hemoglobin (MCH) - pg

Mean corpuscular hemoglobin concentration (MCHC) - g/dL

Platelet count (Plate) -  $10^3/\text{mm}^3$

Reticulocyte count (RETIC) - % RBC

Total leukocyte count (WBC) -  $10^3/\text{mm}^3$

Differential leukocyte count - %

Nucleated red blood cell count (nRBC) - nRBC/100 WBC

**Clinical Chemistry:**

Blood urea nitrogen (BUN) - mg/dL

Serum aspartate aminotransferase (AST) - I.U./L

Serum alanine aminotransferase (ALT) - I.U./L

Alkaline phosphatase (Alk. Phos.) - I.U./L

Serum glucose (BS) - mg/dL

Creatinine (CREAT) - mg/dL

**d. Plasma Drug Level Determination:**

Blood samples for plasma drug level determinations will be obtained from each rat treated with drug prior to sacrifice 30 minutes after dosing on days 15 and 29. An aliquot of each sample will be mixed with EDTA. The samples will be centrifuged and the plasma will be frozen at  $\leq -20^{\circ}\text{C}$  until analyzed. A blood sample for plasma drug level determination will be obtained from moribund rats prior to sacrifice during the dosing period. Analytical procedures have been previously supplied in the compound information.

**e. Necropsy Procedure:**

Five male and five female rats from each group will be sacrificed on study days 15 and 29 and the surviving rats will be sacrificed on study day 57.

Moribund rats should be terminated out of sequence with complete histopathology and clinical pathology performed as for scheduled necropsies. Rats found dead will have a complete necropsy, unless severely autolyzed.

All rats will have final body weights taken and will be bled for clinical pathology determinations prior to termination. A complete necropsy and all antemortem observations will be recorded for each rat and commented on or confirmed at necropsy. Rats which are clinically normal will also be so indicated. A pathologist will be available to examine any unusual findings.

The tissues listed below will be examined, sampled and fixed in cold, buffered neutral 10% formalin. The rat identification

will be retained with tissues taken during necropsy.

- \* Adrenal glands (2)
- \* Bone (femur)
- \* Bone marrow (femur)
- \* Brain
- \* Cecum
- \* Colon
- \* Duodenum
- \* Epididymides
- \* Esophagus
- \* Eyes (2)
- \* Gonads - Testes/ovaries (2)
- \* Gross lesions
- \* Heart
- \* Ileum
- \* Jejunum
- \* Kidneys (2)
- \* Liver
- \* Lungs (infuse with formalin)
- \* Lymph nodes (mandibular and mesenteric)
- \* Mammary gland (when present in regular abdominal skin section)
- \* Pancreas
- \* Parathyroid gland (when present in regular thyroid section)
- \* Pituitary gland
- \* Salivary glands (mandibular, sublingual, and parotid)
- \* Sciatic nerve
- \* Skeletal muscle
- \* Skin (1. ventral abdomen 2. injection site)
- \* Spinal Cord (thoracolumbar segment for routine section or spinal cord if neurological signs indicate cord involvement)
- \* Spleen
- \* Stomach (forestomach and glandular)
- \* Thymus
- \* Thyroid glands
- \* Trachea
- \* Urinary Bladder
- \* Uterine horn (2)

All fixed tissues marked with an asterisk (\*) will be embedded and put

into blocks.

**f. Microscopic Pathology:**

Sections of the above tissues marked with an asterisk (\*) will be cut approximately 5 microns thick and stained with hematoxylin and eosin.

All tissues will be examined microscopically by a pathologist. Records of gross findings for a specimen from postmortem observations shall be available to the pathologist when examining that specimen histopathologically.

All lesions will be categorized either as drug-related or nondrug related. Each lesion should be listed and coded by the most specific topographic and morphologic diagnoses, severity and distribution using the International Harmonization of Nomenclature and Diagnostic criteria (<http://www.goreni.org/>), Standardized System of Nomenclature and Diagnostic Criteria (SSNDC) Guides and the International Harmonization of Rat Nomenclature (<http://www.toxpath.org/nomen/>). Pathology code tables found at the National Toxicology Program website <http://ntp.niehs.nih.gov/?objectid=72016020-BDB7-CEBA-F3E5A7965617C1C1>, may also be used.

Reporting of Pathology data should follow as closely as possible guidelines presented in "Toxicologic Pathology, 34:806–809, 2006"

**III. QUALITY ASSURANCE****A. Type of Study**

This is a nonclinical laboratory study and will require compliance with the FDA Good Laboratory Practice Regulations. Data from this study will be included as part of a final report to be submitted to the FDA.

**B. Standard Operating Procedures**

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the standard operating procedures of the laboratory and any deviations will be documented.

**C. Protocol Amendments**

All changes in or revisions of an approved protocol and the reasons

therefore will be documented, signed, and dated by the Principal Investigator, Study Director and the NCI Project Officer. Amendments will be maintained with the protocol. Verbal approval for changes in the protocol may be granted by the NCI Project Officer, but a written amendment will follow.

**D. Records**

Data will be verified by the laboratory's Quality Assurance Unit and stored in accordance with the Good Laboratory Practice Regulations.

**IV. REPORTING AND DISCUSSION OF DATA**

**A. Progress Reports**

Status reports summarizing the progress of the study will be provided at monthly intervals. These reports will detail the status of the study on the reporting date, any problems encountered and proposed means of resolution.

**B. Final Report**

The data and results of this study will be submitted as a separate draft report, due 30 working days after the last rat sacrifice in this study. The final report will be due 30 working days after return of the draft report for revision.

Reporting of Pathology data should follow as closely as possible the basic guidelines presented in "Toxicologic Pathology, 34:806–809, 2006"

The final report will accurately and completely describe the study design, procedures and findings, present an analysis and summary of the data followed by the conclusions derived from the analyses. The report will also include: (a) a cover page which will include the title, contract number, authors, laboratory address, dates of initiation and completion, and sponsor; (b) the NTIS Report Documentation Page, to be placed at the beginning of the final report; (c) a comprehensive summary to be placed after the NTIS page; and (d) the signature of the Study Director and any others deemed necessary.

**Protocol Approvals:**

Study Director: \_\_\_\_\_ (Date)  
Principal Investigator: \_\_\_\_\_

Project Officer:

\_\_\_\_\_

(Date)

(Date)