**V1.1 Nonclinical Study Plan/Protocol Template for Repeat‑Dose Toxicology Studies**

**About This Template & Template Disclaimer**

This is a nonclinical study plan/protocol template provided for voluntary use by the public. It contains sections with proposed common text or text that may be used across study plans/protocols with little to no editing if the user chooses to do so. Company specific information may be included as appropriate. The use of this template is at the discretion of the user.

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**Components of the Study Plan/Protocol Template**

* **Individual Sections** contain study plan/protocol information common to most 28-day, FIH-enabling ‑repeat dose toxicology studies.
* **The Appendix** can be included at the discretion of the author to include specialty procedures such as punch biopsy diagrams, EEG information, etc.
*
* **Supporting Information** provides specific text that can be incorporated into the protocol when needed (such as clinical pathology parameters & tissue collection by species). Additional content may be added at a future date, such as lists of abbreviations and definitions for key terms. This section should be deleted before protocol finalization.

**Core Backbone Headings**

* Level 1 and 2 headings are intended to be consistent across study plan/protocols that use the template for reference and mapping purposes.
* Level 1 and 2 headings should not be deleted. If they are not relevant to the study, “Not applicable” should be inserted so that the numbering of subsequent sections is not changed.
* Level 2 headings may be added as needed. Level 3 and lower headings can be deleted/added/modified as needed.

**Terminology**

* *Various sections contain SEND terminology in brackets. This terminology was included to improve ease of compiling SEND datasets and to allow users to become familiar with SEND terminology. As SEND terminology is updated quarterly, users should refer to* *latest SEND controlled terminology document to ensure terminology accuracy by visiting* <https://www.cdisc.org/standards/terminology>

**Formatting and Text Conventions**

* Common Text: Black font is common or suggested language that may be harmonized across study plan/protocols. This text can be used as written to maintain consistency across documents but can be adapted if required.
* Variable Text: Blue bracketed text is variable text that should be addressed based on individual study needs.
* Instructional Text: Red italicized text is intended to aid in authoring of the study plan/protocol in this template. At times, example variables may be provided within instructional text for illustration purposes. All language should be reviewed to ensure it is appropriate for the specific study, and if not, be customized or altered as necessary.

Study Plan/Protocol

|  |  |
| --- | --- |
| Study Title [STITLE]: | A Repeat-Dose Toxicity **[and Toxicokinetic Study]** in **[SPECIES]** Administered **[TEST ITEM/ARTICLE]** **[DOSING FREQUENCY]** by **[ROUTE]** for **[DURATION]**‑weeks **[with a [DURATION]‑week Recovery Phase]**. |
| Test Facility Name [TSTFNAM]: | **[NAME]****[ADDRESS]** |
| Study Number [STUDYID]: | **[NUMBER]** |
| Sponsor [SSPONSOR]:Sponsor Reference ID [SPREFID]:*(optional)* | **[NAME][ADDRESS]****[UNIQUE STUDY ID]** |

GLP Status [GLPFL]: [**GLP/Non GLP]**

*Note: Title page may be updated to include company specific information and logo. Internal SOP reference or company statements may also be included as appropriate.*

Table of Contents

[Table of Contents 4](#_Toc94883253)

[Approval 6](#_Toc94883254)

[1. Objective 7](#_Toc94883255)

[2. Proposed Study Schedule 7](#_Toc94883256)

[3. Sponsor/Test Facility/Test Site Information 7](#_Toc94883257)

[4. Key Personnel 7](#_Toc94883258)

[5. Test Item/Article and Vehicle Control Item/Article 8](#_Toc94883259)

[5.1 Test Item/Article 8](#_Toc94883260)

[5.2 Vehicle Control Item/Article 9](#_Toc94883261)

[5.3 Bulk Test Item/Article Analysis 10](#_Toc94883262)

[6. Dose Formulation 10](#_Toc94883263)

[6.1 Analysis of Dose Formulations 10](#_Toc94883264)

[7. Test System and Husbandry 11](#_Toc94883265)

[7.1 Characterization of Test System 11](#_Toc94883266)

[7.2 Justification for Test System Selection 12](#_Toc94883267)

[7.3 Justification for Number of Animals 12](#_Toc94883268)

[7.4 Selection, Assignment and Replacement of Animals 12](#_Toc94883269)

[7.5 Pretest Handling/Acclimation 12](#_Toc94883270)

[7.6 Housing and Environmental Conditions 12](#_Toc94883271)

[7.7 Veterinary Care 13](#_Toc94883272)

[8. Experimental Design 13](#_Toc94883273)

[8.1 Dose Interval/Frequency 13](#_Toc94883274)

[8.2 Justification for Dose Selection 14](#_Toc94883275)

[8.3 Route of Dose Administration 14](#_Toc94883276)

[8.4 Justification for Route 14](#_Toc94883277)

[9. In-life Observations and Procedures 14](#_Toc94883278)

[9.1 Safety Pharmacology 15](#_Toc94883279)

[9.1.1 Cardiovascular 15](#_Toc94883280)

[9.1.2 Respiratory 16](#_Toc94883281)

[9.1.3 CNS 17](#_Toc94883282)

[10. Clinical Pathology 17](#_Toc94883283)

[10.1 Sample Collection 17](#_Toc94883284)

[10.2 Hematology 17](#_Toc94883285)

[10.3 Coagulation 18](#_Toc94883286)

[10.4 Clinical Chemistry 18](#_Toc94883287)

[10.5 Urinalysis 19](#_Toc94883288)

[11. Bioanalysis 19](#_Toc94883289)

[11.1 Bioanalytical Sample Collection 19](#_Toc94883290)

[12. Toxicokinetic Evaluation 22](#_Toc94883291)

[13. Other Testing 23](#_Toc94883292)

[14. Terminal Procedures 23](#_Toc94883293)

[14.1 Unscheduled Euthanasia and Decedents 24](#_Toc94883294)

[14.2 Scheduled Euthanasia 24](#_Toc94883295)

[14.3 Necropsy 25](#_Toc94883296)

[14.4 Bone Marrow Smears 25](#_Toc94883297)

[14.5 Organ Weights 25](#_Toc94883298)

[14.6 Tissue Collection and Preservation 25](#_Toc94883299)

[15. Histology and Histopathology 26](#_Toc94883300)

[15.1 Histology 26](#_Toc94883301)

[15.2 Histopathology 26](#_Toc94883302)

[16. Pathology Peer Review 27](#_Toc94883303)

[17. Data Evaluation and Statistical Analysis 27](#_Toc94883304)

[18. Regulatory Information 27](#_Toc94883305)

[18.1 Good Laboratory Practice 27](#_Toc94883306)

[18.2 Regulatory Testing Guidelines 27](#_Toc94883307)

[19. Quality Assurance 28](#_Toc94883308)

[20. Animal Welfare 29](#_Toc94883309)

[21. Major Computer Systems 29](#_Toc94883310)

[22. Amendments and Deviations 29](#_Toc94883311)

[23. Retention of Records, Samples and Specimens 30](#_Toc94883312)

[24. Study Classification 30](#_Toc94883313)

[25. Reporting 31](#_Toc94883314)

[26. References 33](#_Toc94883315)

[ATTACHMENT A 34](#_Toc94883316)

[ATTACHMENT B 37](#_Toc94883317)

[APPENDIX 38](#_Toc94883318)

[SUPPORTING INFORMATION 39](#_Toc94883319)

[SUMMARY OF CHANGES AND JUSTIFICATIONS 56](#_Toc94883320)

Approval

*Delete or add signature lines based on company specific requirements.*

The signature below <OR on the last page of the document> indicates that the Study Director approves the study plan/protocol.

 Date:

**[Insert Name, Credentials]**
Study Director

The signature below <OR on the last page of the document> acknowledges Test Facility Management’s responsibility to the study as defined by the relevant GLP regulations.

 Date:

**[Insert Name, Credentials]**
Test Facility Management

*Sponsor approvals should follow an organization’s preferred method.*

The signature below indicates that the Sponsor approves the study plan/protocol.

 Date:

**[Insert Name, Credentials]**
Sponsor Representative

 *OR*

The study plan/protocol was approved by the Sponsor by email on **[INSERT DATE]**.

# Objective

*The author may choose to add more or less detail* *based on expectations of Sponsor/CRO or other local requirements (class of compound, potential disease area, etc.).*

The purpose of this study is to evaluate the toxicity **[and determine toxicokinetics]** of the test item/article, **[TEST ITEM/ARTICLE NAME]**, when administered **[FREQUENCY, ROUTE, SPECIES, DURATION** (e.g. once daily by oral gavage to rats for at least 4 weeks)**]**, and to provide data to support the use of **[TEST ITEM/ARTICLE NAME]** in humans.

# Proposed Study Schedule

|  |
| --- |
| *Schedule details may vary and be edited based on study/sponsor/CRO needs. The black text in brackets may be included for studies requiring SEND.* |
| Experimental Start Date (date of first data collection) [EXPSTDTC]: | **[DATE]** |
| Dosing Start Date [DOSSTDTC]: | **[DATE]** |
| Dosing End Date [DOSENDTC]: | **[DATE]** |
| Experimental Completion Date (date of last data collected) [EXPENDTC]: | **[DATE]** |
| Audited Draft Report Date: | **[DATE]** |

# Sponsor/Test Facility/Test Site Information

|  |  |
| --- | --- |
| Sponsor [SSPONSOR]: | **[SPONSOR NAME & ADDRESS]** |
| Test Facility [TSTFNAM]: | **[TEST FACILITY NAME & ADDRESS]** |
| Test Site [TSNAM]: | **[TEST SITE NAME & ADDRESS]** |
| Repeat as needed for additional test sites. The black text in brackets may be included for studies requiring SEND. This section can be updated as needed. |

# Key Personnel

Listing meant to represent potential key personnel for inclusion. List can be added to or abbreviated based on the specific needs of the study; the author is recommended to include all key personnel formally referenced elsewhere in this document by role, where possible. Include relevant Quality Assurance and Management.

| **Role/Phase** | **Quality Assurance Unit** | **Name** | **Contact Information** |
| --- | --- | --- | --- |
| **Study Director [STDIR]** | *Insert laboratory name responsible for auditing study phase* |  | Address:Tel: E-mail:  |
| **Testing Facility Management** |  |  | Address:Tel: E-mail:  |
| **Analytical Chemistry****[CNTRBSC]** |  |  | Address:Tel: E-mail:  |
| **Ophthalmology [CNTRBSC]** |  |  | Address:Tel: E-mail:  |
| **Bioanalysis [CNTRBSC]** |  |  | Address:Tel: E-mail:  |
| **Toxicokinetics****[CNTRBSC]** |  |  | Address:Tel: E-mail:  |
| **Peer Review Pathologist****[CNTRBSC]** |  |  | Address:Tel: E-mail:  |

Repeat as needed for additional personnel. This section can be updated as needed.

# Test Item/Article and Vehicle Control Item/Article

## Test Item/Article

|  |  |
| --- | --- |
| Identification | **[ADD VALUE]** |
| Supplier | **[ADD VALUE]** |
| Lot Number(s) [EXLOT] | **[ADD VALUE]** |
| Purity | **[ADD VALUE]** |
| Correction Factor  | **[ADD VALUE]***(if needed) depending on potency* |
| Stability | **[ADD STATEMENT]***State how stability of test item/article will be demonstrated e.g. Certificate of Analysis (COA), testing, etc.* |
| Storage Conditions | **[ADD VALUE(S) / STATEMENT]** |
| Characteristics | **[ADD STATEMENT(s)] *or*** Information on synthesis methods, composition, or other characteristics defining the test item/article is on file with the Sponsor. |
| Safety | The Sponsor will provide relevant occupational safety information known about the test item/article (e.g., Safety Data Sheet, safety instructions, or test item/article identity). |
| Reserve Sample Collection | **[ADD STATEMENT(S)]** |
| Reserve Sample Storage Condition | **[ADD STATEMENT(S)]** |

This section can be updated as needed.

## Vehicle Control Item/Article

|  |  |
| --- | --- |
| Identification [TRTV] | **[ADD VALUE(S)]***List excipients from highest concentration to lowest concentration with diluent as the last item. For excipient concentrations expressed as a percentage, include either w/v or v/v.* |
| Supplier | **[ADD VALUE]** |
| Batch/Lot Number(s) | **[ADD VALUE(S)] *or*** Batch/Lot numbers of the vehicle control article components (with the exception of water supplied by in‑house water systems) will be maintained in the raw data. |
| Purity | **[ADD VALUE(S)] *or*** Limited to the information listed on the label of these commercially available materials or on file with the manufacturer or supplier. *Consider specifying the grade of excipients used to make the vehicle.* |
| Storage Conditions | **[ADD STATEMENT(S)] *or*** Vehicle control article components will be stored according to the recommendations of the manufacturer/supplier. Water supplied by in-house water systems will be maintained under ambient conditions. The prepared vehicle control article will be stored in a refrigerator, set to maintain 2 to 8°C and used for test item/article formulation or for dosing within **[ADD VALUE]** days of completion of preparation. |
| Characteristics | **[ADD STATEMENT(S)] *or*** Information on synthesis methods, composition, or other characteristics defining the test item/article is on file with the Supplier. |
| Reserve Sample Collection | **[ADD STATEMENT(S)]** |
| Reserve Sample Storage Conditions | Store according to the recommendations of the manufacturer or supplier. |

This section can be updated as needed.

## Bulk Test Item/Article Analysis

*If samples are needed for analysis include instruction here. This section can be updated as needed.*

Each batch of **[TEST ITEM/ARTICLE]** will be used within the retest date stated on the Certificate of Analysis (COA). If it is necessary to use a batch of **[TEST ITEM/ARTICLE]** beyond the initial retest date, a sample of the batch of the test item/article will be submitted to the Sponsor or third party for reanalysis to extend the retest date.

# Dose Formulation

|  |  |
| --- | --- |
| Frequency  | *Describe the frequency of preparation for both Test Item/Article and Vehicle Control Item/Article.*The dosing formulations will be prepared **[daily/weekly/every 2 weeks etc.]**. |
| Procedures | *Include general instructions for preparing dose formulations.*Dosing formulations will be prepared based on Sponsor instructions at appropriate concentrations to meet dose level requirements. |
| Storage Conditions | Dosing formulations will be stored **[INSERT CONDITIONS,** ex freezer set to maintain -20°C**]**,and an aliquot will be dispensed **[DAILY/WEEKLY]**.*If applicable:*The dosing formulations will be removed from the refrigerator, stirred for at least [**INSERT TIME**, ex 30 minutes] before dosing and continuously during dosing. |

## Analysis of Dose Formulations

Add procedures to be used to demonstrate stability and homogeneity as appropriate. This section may be updated as needed based on SOPs or standard process.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample Type | Intervals (Week) | Concentration | Stratum | Number of Samplesper Concentration | Sample Volume (mL) | Sampling From |
| Collected | Analyzed | Backup |
| ConcentrationAnalyses |  | Group 1 | Middle |  |  |  |  | Preparation Vessel**OR**Dosing Jar**OR**Dosing jar following completion of dosing |
| Homogeneity |  |  |  |  |  |  |  |  |

All samples will be collected into **[VIAL DESCRIPTION]**. At minimum, the label for all containers will bear the following: **[INSERT LABEL REQUIREMENTS]. [Light exposure will be minimized by using an amber container or wrapping in foil].**

*If samples are being shipped for analysis:* Refer to Attachment A for shipping details.

Upon receipt at the analytical laboratory, the samples will be stored **[INSERT CONDITIONS].**

Analyses will be performed by **[INSERT METHOD, example HPLC]** using a substance-specific *validated analytical procedure or qualified method* **[INSERT ANALYTICAL TITLE & REFERENCE NUMBER, if available)**. A report of the dose formulation analysis results will be provided to the Study Director and included in the final report.

*Include and update if appropriate:* Any residual volumes and/or back-up samples will be discarded upon approval by the Sponsor and authorization by the Study Director.

# Test System and Husbandry

## Characterization of Test System

*This section can be updated as needed.*

|  |  |
| --- | --- |
| Species [SPECIES] | **[ADD VALUE]** |
| Strain [STRAIN] | **[ADD VALUE]** |
| Supplier [SPLRNAM] | **[ADD VALUE]** |
| Country of origin | **[ADD VALUE]** |
| Target weight or age at initiation of dosing | **[ADD VALUE]** |
| Number and sex | **[ADD VALUE]** |
| Method of Identification | **[ADD VALUE]** |
| Spare Animals | **[INSERT NUMBER]** spare animals (**#** males and **#** females) will be included in the transfer of study animals. These animals will be used to replace any individuals rejected during the pretest/study periods.Spare animals will be **[INSERT DISPOSITION DETAILS]** after completion of the replacement period. |

## Justification for Test System Selection

A number of factors should be taken into account when determining species relevancy. Factors typically required include pharmacological activity (for Biotechnology Derived‑ Pharmaceuticals this could include comparisons of target sequence homology between species, in vitro data on relative target binding affinities and receptor/ligand occupancy and kinetics and functional activity), acceptance as test species by regulatory agencies and experience from other toxicity studies. Availability of historical control data could also be of relevance. Example below can be modified to fit the needs of the study.

The **[TEST SYSTEM/SPECIES]** was chosen as the animal model for this study as it is an accepted test system for preclinical toxicity testing by regulatory agencies, is pharmacologically active and has been used in previous toxicity studies with **[INSERT STUDY CHARACTERISTICS OR JUSTIFICATION]**.

## Justification for Number of Animals

This section contains suggested wording; author is recommended to review for appropriate modifications.

The total number of animals used in this study, as well as the group size and number of groups, was considered to be the minimum required to properly characterize the effects of the test item/article and has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

**[INSERT NUMBER]** spare animals (**#** males and **#** females) will be included in the transfer of study animals. These animals will be used to replace any individuals rejected during the pretest phase.

## Selection, Assignment and Replacement of Animals

Provide any additional statements as deemed appropriate for this study on the selection, assignment, and replacement of animals, including randomization procedures. This section may also include a Level 3 Animal Screening sub-heading in which the organization provides any additional statements as deemed appropriate for this study on the screening of study animals.

## Pretest Handling/Acclimation

The length of the quarantine period is determined by the veterinary medical staff at each institution. Minimum acclimation time for rodents and large animals varies by region. Large animals require additional acclimation to housing and experimental and handling procedures.

## Housing and Environmental Conditions

|  |  |
| --- | --- |
| Housing  | *Caging, social vs single housing, bedding etc.* |
| Environmental Conditions | *Temperature, humidity, light cycle, etc.* |
| Food | *Food source, etc.**e.g. The feed is analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Test Facility.**e.g. It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.* |
| Water | *Origin of water supply, periodic analysis, etc.**e.g. It is considered that there are no known contaminants in the water that could interfere with the objectives of the study.* |
| Animal Enrichment | *e.g., For psychological/environmental enrichment, animals will be provided with items such as chewing object, except during study procedures/activities.* |

## Veterinary Care

This section contains suggested wording; author is recommended to review for appropriate modifications.

Palliative and prophylactic procedures may be based upon consensus agreement between the Study Director and attending laboratory animal Veterinarian. The Study Director and Sponsor/designee (if possible) will be included in discussions of palliative and prophylactic procedures (nonlife threatening conditions‑, including suspension of dosing and removal of animals from study) recommended by the attending Veterinarian. Final authority for decision making will be with the laboratory animal Veterinarian. All veterinary examinations and recommended therapeutic treatments will be documented in the study records.

# Experimental Design

Add indication of satellite group animals as appropriate. This section contains a suggested format; author is recommended to review and update for appropriate modifications.

| Group No. | Test Item/ Article or Vehicle | Dose Level (mg/kg/day) | Dose Volume (mL/kg) | Concentration (mg/mL) | No. of Animals |
| --- | --- | --- | --- | --- | --- |
| Dosing Phase | Recovery Phase | Toxicokinetics |
| Males | Females | Males | Females | Males | Females |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| - = not applicable. |  |

## Dose Interval/Frequency

This section contains suggested wording; author is recommended to review for appropriate modifications.

The test and vehicle control items/articles will be administered [once] [daily], [7] days per week, for at least [1] month.

## Justification for Dose Selection

The rationale for dose selection should be described. Two sample texts are provided below for illustration purposes.

*Example 1:* The chosen dose levels for this study were based on the findings from the maximum tolerated dose study **[STUDY IDENTITY]**.

*Example 2:* The low dose of **[INSERT VALUE (mg/kg/day)]** is margin of approximately 2 to the predicted pharmacological relevant exposure in humans. The high dose of **[INSERT VALUE (mg/kg/day)]** is more than ‑100-fold the maximum expected efficacy exposure in humans. The intermediate dose of **[INSERT VALUE (mg/kg/day)]** is chosen as it is the approximate geometric mean of the low and high dose.

## Route of Dose Administration

Typically the same route intended for human clinical trials. This section contains suggested wording; author is recommended to review for appropriate modifications.

The test and vehicle control items/articles will be administered by **[ROUTE]**, using **[a flexible tube fitted onto a syringe of appropriate size]**. Animals will be habituated to the dosing procedure prior to dose initiation. Doses will be calculated using the most recent body weights available.

Unused dose formulations will be discarded by the Testing Facility, unless otherwise directed by the Sponsor.

## Justification for Route

The route of administration should be the intended clinical route of administration. Deviation from this should be explained. This section contains suggested wording; author is recommended to review for appropriate modifications.

The intended route of administration in humans is **[ROUTE (e.g. oral, subcutaneous injection, etc.)]**, therefore **[ROUTE]** will be used for this study in **[SPECIES]**.

# In-life Observations and Procedures

Author may update table below accordingly.

| **Parameter** | **Population(s)** | **Frequency(minimum required)** | **Comments** |
| --- | --- | --- | --- |
| **Mortality** | All Dosing and Recovery Phase animals | Twice daily, (except on the day of arrival and on the day of scheduled necropsy), once in the morning and once in the afternoon, throughout the study | Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings. |
| **Cageside Observations** | All Dosing and Recovery Phase animals | Once daily, throughout the dosing and recovery phases; target time of **[X to Y]** hours postdose during the dosing phase | Animals will not be removed from the cage during observation, unless necessary for identification or confirmation of possible findings. |
| **Detailed Clinical Observations** | All Dosing and Recovery Phase animals | **[Daily or Weekly]** during the dosing phase, and at least every 2 weeks during the pretest phase | Animals will be removed from the cage for examination. |
| **Individual Body Weights** | All Dosing and Recovery Phase animals | Weekly during the dosing phase, and at least every 2 weeks during the pretest phase. | Animals will be individually weighed. A fasted weight will be recorded on the day of necropsy. |
| **Food Consumption**  | All Dosing and Recovery Phase animals | Weekly, starting Day -1, throughout the dosing **[and recovery phases]**. | *For rodents:* Food consumption will be quantitatively measured except for on the day of scheduled euthanasia. *For group housed animals include:* Food consumption will be measured on a per cage basis. Food consumption will be normalized to the number of rats/cages. *For dogs:* Food consumption will be quantitatively measured on each day, except for the day of scheduled euthanasia. *For monkeys:* Individual food evaluation will be assessed by visual inspection for overall appetite. |
| **Ophthalmic Examination** | All Dosing and Recovery Phase animals |  | Examinations will be performed by a board-certified veterinary ophthalmologist. All animals will be subjected to funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations. The mydriatic used will be **[INSERT]**. Animals **[will be/will not be]** anesthetized. Results will be provided for Study Director review & inclusion in final report. |
| **Veterinary Physical Examination** | All Dosing and Recovery Phase animals |  | *Animals* ***[will be/will not be]*** *removed from the cage for examination by veterinary staff. Animals* ***[will be/will not be]*** *anesthetized.* |

## Safety Pharmacology

The inclusion of safety pharmacology endpoints in repeat dose‑ studies is encouraged to reduce the number of animals involved in biomedical research. If safety pharmacology is not included on the study, say “Not Applicable” and delete subsections below. Sample text is provided for each parameter and may be updated at the author’s discretion.

### 9.1.1 Cardiovascular

The details of the electro cardiology procedure should be described. Two sample texts are provided below for illustration purposes. Pretest and dosing phase ECGs should be collected. When using JET, individual regressions of QT interval on RR interval will be performed for each animal based on obtained baseline pretest data. Animals will be acclimated to jackets or sling procedures prior to each collection. If several endpoints (e.g., blood/urine sampling and ECG) should be evaluated in a given week, the ECG recordings will be conducted before the others are initiated. It is also important to state minimum number of spikes (restrained) or hours (JET).

Frequency and duration: **[INSERT]**

Procedure: All animals will have tracings recorded using the following limb leads:

 I, II, III, aVR, aVL and aVF.

 *If ECGs are collected via chest leads, use the following wording:* Tracings will also be recorded using the following chest leads: V1, V2, V3 and V4. Individual tracings will be recorded using a standard recording speed of 50 mm/sec and standard sensitivity may be set/adjusted as deemed necessary to capture a suitable waveform, e.g., 5 or 10 mm/mV. The tracing will be recorded while the animals are in a right lateral recumbency/on a platform/restrained on a sling.

 *If ECGs are collected via JET (Jacketed External Telemetry), use the following wording:*  Continuous ECG waveforms will be collected using a Modified Lead II on designated study days. All derived parameters will be logged in **[XX]** second averages and reported as **[X]** hour means. Typical quantitative parameters derived from the ECG waveforms include heart rate and the RR, PR, QRS, and QT intervals. QT corrected for heart rate (QTc) will be calculated using individual animal corrections. Qualitative analysis of abnormality in ECG rhythm or shape will be assessed using a **[XX]** minute sample at the following time points: [**XX**] hours following the start of dosing. The tracing will be recorded while the animals are [**XX]** housed in **[XX]** cages. Human activity in the animal room can have a significant effect on cardiovascular parameters and will be minimized to the greatest extent possible.

Evaluation: The recordings will be evaluated (qualitatively and quantitatively). A report will be included as an appendix to the Final Report.

### 9.1.2 Respiratory

Frequency and duration: **[INSERT]**

Procedure: Animals will be placed in a plethysmography chamber following dosing on Day X. Respiratory data will be collected from the time the animal is placed into the chamber to at least X hours post nominal dose time. Following the data collection period on Day X of the dosing phase, animals will be removed from chambers and returned to their home cage. Approximately X hours postdose, animals will be placed back into chambers, and respiratory data will be collected for at least X hours following exit of personnel from the room.

Evaluation: **[INSERT]**

### 9.1.3 CNS

*Refer to “Supporting Information” section for a list of CNS assessment observations in rodents. The author may update accordingly and should include this information in the Appendix of this study plan/protocol.*

Frequency and duration: **[INSERT]**

Procedure: Each animal will undergo a battery of behavioral tests and observations (see Appendix), approximately X, X, X, and X hourspostdose on Day X.

Evaluation: **[INSERT]**

# Clinical Pathology

Author should refer to Supporting Information for examples for this section. This section contains suggested wording; author is recommended to review for appropriate modifications.

## Sample Collection

*Author should complete the chart as applicable.*

| **Group Nos.** | **Time Point** | **Hematology** | **Coagulation** | **Clinical Chemistry** | **Urinalysis** |
| --- | --- | --- | --- | --- | --- |
| *All animals* | *Week –XXor Day -XX* | *X* | *X* | *X* | *X* |
| *1 to 4* | *Day -XX* |  | *X* |  |  |

X = samples to be collected.

Blood samples **[WILL BE/WILL NOT BE]** collected from study animals euthanized early.

Bone marrow smears will be collected from each animal at the scheduled euthanasia and prepared as described in the Tissue Collection and Preservation table in Section 14.6. Evaluation of stained smears may be added by amendment at the discretion of the Study Director in consultation with the clinical and/or anatomic pathologist and the Sponsor.

## Hematology

Animals **[WILL BE/WILL NOT BE]** fasted overnight before blood sampling.

*Author should complete the chart as applicable. Example is provided below.*

|  |  |
| --- | --- |
| Collection Site: | *Jugular vein* |
| Target Volume: | *0.5 ml* |
| Anticoagulant:  | *K2EDTA* |
| Container type: | *vacutainer tubes* |
| Processing: | *To plasma* |

Hematology Parameters

*Author should include list of required hematology parameters. Example is provided below. Refer to the Supporting Information for recommendation per species. Where applicable, follow the SEND controlled terminology.*

|  |  |
| --- | --- |
| *Erythrocytes [RBC]* | *Lymphocytes [LYM]* |
| *Hemoglobin [HGB]* | *Monocytes [MONO]* |
| *Hematocrit [HCT]* | *Eosinophils [EOS]* |
| *Ery. Mean Corpuscular Volume [MCV]* | *Basophils [BASO]* |
| *Erythrocytes Distribution Width [RDW]* | *Large Unstained Cells [LGUNSCE]* |
| *Ery. Mean Corpuscular Hemoglobin [MCH]* | *Platelets [PLAT]* |
| *Ery. Mean Corpuscular Hemoglobin Concentration [MCHC]* | *Reticulocytes [RETI]* |
| *Leukocytes (total [WBC] and absolute differential [WBCCE])* | *Cell morphology [CEMORPH]\** |
| *Neutrophils [NEUT]* |  |

*\* = blood smear to be prepared and stained for all animals at the time of phlebotomy or soon after.*

## Coagulation

Animals **[WILL BE/WILL NOT BE]** fasted overnight before blood sampling.

*Author should complete the chart as applicable. Example is provided below.*

|  |  |
| --- | --- |
| Collection Site: | *Jugular vein* |
| Target Volume: | *0.5 ml* |
| Anticoagulant:  | *Citrate* |
| Container type: | *vacutainer tubes* |
| Processing: | *To plasma* |

Coagulation Parameters

*Author should include list of required coagulation parameters. Example is provided below. Refer to Supporting Information for recommendation per species.*

|  |
| --- |
| *Activated Partial Thromboplastin Time [APTT]* |
| *Prothrombin Time [PT]* |
| *Fibrinogen [FIBRINO]* |

## Clinical Chemistry

Animals **[WILL BE/WILL NOT BE]** fasted overnight before blood sampling.

*Author should complete the chart as applicable. Example is provided below.*

|  |  |
| --- | --- |
| Collection Site: | *Jugular vein* |
| Target Volume: | *0.5 ml* |
| Anticoagulant:  | *None, collected in serum separator tubes* |
| Container type: | *vacutainer tubes* |
| Processing: | *To serum* |

Clinical Chemistry Parameters

*Author should include list of required clinical chemistry parameters. Example is provided below. Refer to Supporting Information for recommendation per species.*

|  |  |
| --- | --- |
| *Alanine Aminotransferase [ALT]* | *Creatinine [CREAT]* |
| *Aspartate Aminotransferase [AST]* | *Gamma Glutamyl Transferase [GGT]* |
| *Albumin [ALB]* | *Globulin [GLOBUL]* |
| *Albumin/Globulin [ALBGLOB]* | *Glucose [GLUC]* |
| *Alkaline phosphatase [ALP]* | *Ph Phosphate [PHOS]* |
| *Urea nitrogen [UREAN]* | *Potassium [K]* |
| *Calcium [CA]* | *Sodium [SODIUM]* |
| *Chloride [CL]* | *Bilirubin [BILI]* |
| *Cholesterol [CHOL]* | *Protein [PROT]* |
| *Creatine Kinase [CK]* | *Triglycerides [TRIG]* |

## Urinalysis

Urine will be collected overnight using **[METHOD,** e.g. metabolic cages**]**. Urine will be collected on wet ice until analysis or stored refrigerated (set to maintain 4° C). Animals **[WILL HAVE ACCESS TO FOOD AND WATER/WILL NOT HAVE ACCESS TO FOOD BUT WILL HAVE ACCESS TO WATER]** be deprived of food but not water during the urine collection procedure.

Urinalysis Parameters

*Author should include list of required urinalysis parameters. Example is provided below. Refer to Supporting Information for recommendation per species.*

|  |  |
| --- | --- |
| *pH [PH]* | *Occult Blood [OCCBLD]* |
| *Color [COLOR]* | *Glucose [GLUC]* |
| *Clarity [CLARITY]* | *Ketones [KETONES]* |
| *Volume [VOLUME]* | *Protein [PROT]* |
| *Specific Gravity [SPGRAV]* | *Sediment evaluation [SEDEXAM]* |
| *Bilirubin [BILI]* |  |

Urine Chemistry Parameters

*Author should include list of required urine chemistry parameters. Example is provided below. Refer to Supporting Information for recommendation per species.*

|  |  |
| --- | --- |
| *Creatinine (measured concentration) [CREAT]* | *Sodium (measured concentration) [SODIUM]**Sodium/Creatinine (calculated) [NACREAT]* |
| *Protein (concentration measured) [PROT]**Protein/Creatinine (calculated) [PROTCRT]* | *Chloride (measured concentration) [CL]**Chloride to creatinine ratio (calculated) [CLCREAT]* |
| *Glucose (concentration measured) [GLUC]**Glucose/Creatinine (calculated) [GLUCCRT]* |  |

# Bioanalysis

## Bioanalytical Sample Collection

This section contains suggested wording; author is recommended to review for appropriate modifications.

Blood will be collected by **[VENIPUNCTURE SITE]** venipuncture. Samples will be collected according to the following table (all times are nominal; actual collection times will be documented in the study data).

Select one of the tables below suitable for the species selected for this study. Tables assume time under 60 minutes is presented in minutes and time 60 minutes or more is presented in hours. Placeholder values are included in the table below for illustration purposes only; please refer to the key beneath the table.

Example TK Sample Collection Schedule with subgroups (Typical of Rodent)

|  |  |  |  |
| --- | --- | --- | --- |
| Group No. | Subgroup | No. of Males/Females | Sample Collection Time Points(Time Postdose) on Days [1] and [28] |
| [0 hra] | [XX min/hr] | [XXmin/hr] | [XXmin/hr] | [XXmin/hr] | [XXmin/hr] |
| 1 | A | 3/3 | X | - | - | X | - | - |
| B | 3/3 | - | X | - | - | X | - |
| C | 3/3 | - | - | X | - | - | X |
| 2 | A | 3/3 | X | - | - | X | - | - |
| B | 3/3 | - | X | - | - | X | - |
| C | 3/3 | - | - | X | - | - | X |
| 3 | A | 3/3 | X | - | - | X | - | - |
| B | 3/3 | - | X | - | - | X | - |
| C | 3/3 | - | - | X | - | - | X |
| 4 | A | 3/3 | X | - | - | X | - | - |
| B | 3/3 | - | X | - | - | X | - |
| C | 3/3 | - | - | X | - | - | X |
| X = Sample to be collected; - = Not applicable.a. = Sample will be collected before dosing. |

Example TK Sample Collection Schedule without subgroups (Typical of Dog, Monkey)

|  |  |
| --- | --- |
| Group No. | Sample Collection Time Points(Time Postdose) on Days [1] and [28] |
| [0 hr a]  | [XX min/hr] | [XX min/hr] | [XX min/hr] | [XX min/hr] | [XX min/hr] |
| 1 | X | X | X | X | X | X |
| 2 | X | X | X | X | X | X |
| 3 | X | X | X | X | X | X |
| 4 | X | X | X | X | X | X |
| X = Sample to be collected; - = Not applicable.a. = Sample will be collected before dosing. |

|  |
| --- |
| **Sample Collection and Storage Conditions** |
| Target Volume  | **[VALUE** (e.g. 0.5 mL)**]** |
| Anticoagulant | *To be confirmed by Sponsor; note that for biologics, serum is routinely collected.* **[VALUE** (e.g. K2EDTA or K3EDTA, Sodium Heparin or Lithium Heparin, None)**]** |
| Storage Conditions | **[INSERT VALUE** (e.g. temperature)**]** |
| **Bioanalytical Sample Processing and Analysis**  |
| Plasma Samples **OR** Serum Samples | *Plasma sample text, modify as needed:* Samples will be placed on crushed wet ice until centrifugation, which will be carried out as soon as is practical. The samples will be centrifuged **[as per standard procedures or insert details]**. The resultant plasma will be separated, transferred to clear polypropylene tubes bearing unique labels. At minimum, the label for all containers will bear the following: **[INSERT LABEL REQUIREMENTS].**  Plasma should be frozen immediately in the upright position over dry ice and transferred to a freezer set to maintain **[INSERT TEMPERATURE]**.*Serum sample text,modify as needed:*Samples will be mixed gently and allowed to clot under ambient conditions prior to centrifugation. The samples will be centrifuged **[as per standard procedures or insert details]**. The resultant serum will be transferred to clear polypropylene tubes bearing unique labels. At minimum, the label for all containers will bear the following: **[INSERT LABEL REQUIREMENTS].**  Serum should be frozen immediately over dry ice and transferred to a freezer set to maintain **[INSERT TEMPERATURE]**. |
| Control Sample Handling | *Review sample text below for applicability and update according to the needs of the study.***[ADD VALUE** (e.g. plasma/serum)**]** samples from control animals at the **[X]** hour sample will be packaged separately from samples from test item/article treated animals to minimize chances for artifactitious contamination. |
| Shipping Instructions | *For samples being shipped for analysis, review and update sample text below according to the needs of the study, also specifying shipping contact/address, shipping conditions (dry ice, ambient, etc.) and storage conditions at the lab receiving the samples.*All samples will be collected into **[VIAL DESCRIPTION]**. At minimum, the label for all containers will bear the following: **[INSERT LABEL REQUIREMENTS].** *If samples are being shipped for analysis:* Refer to Attachment A for shipping details. |
| Storage | Upon receipt at the bioanalytical laboratory, the samples will be stored **[INSERT CONDITIONS].** |
| Sample Analysis | **[ADD VALUE** (e.g. plasma/serum)**]** samples, including control samples collected at **[XX‑hours]** post dose**,** will be analyzed for concentration of test item/article using a validated bioanalytical procedure, and (if applicable) metabolite X by a qualified bioanalytical procedure. Analysis will be performed by **[METHOD TO BE USED, ex LC-MS/MS]** under the bioanalytical procedure, insert bioanalytical procedure title and reference number if available or indicate it will be included in the Bioanalytical Contributor report. Details of the bioanalytical procedure will be included in the bioanalytical contributor report. |
| Incurred Sample Reanalysis | Incurred sample reanalysis (ISR) will be performed for this study, if necessary, as per the appropriate SOP(s) of the bioanalytical laboratory. |
| Sample Disposition | Any residual/retained bioanalytical samples will be maintained for a minimum of **[INSERT TIMEFRAME]** following issuance of the **[AUDITED OR UNAUDITED]** Draft Report, after which samples will be discarded. |

# Toxicokinetic Evaluation

This section contains suggested wording; author is recommended to review for appropriate modifications.

Toxicokinetic parameters (AUC, Cmax and Tmax) will be calculated, as appropriate, from test item/article dosed animals only. Details of the toxicokinetic analysis will be included in the Toxicokinetics Report.

Parameters to be Estimated

*Update as necessary*

|  |  |
| --- | --- |
| Parameter | Description of parameter |
| Cmax | The maximum observed concentration |
| Tmax | The time after dosing at which the maximum observed concentration was observed |
| AUC | The area under the concentration versus time curve |

# Other Testing

*Use this section to describe other, non‑standard testing or optional evaluations (e.g. Micronucleus testing, Toxicogenomics, Biomarkers, Immunotoxicology, ADA). Due to the variety of approaches currently utilized across the industry, sample text is not provided within this template. Use Level 2 or Level 3 section headers as appropriate to maintain the Level 1 section header “Other Testing” so that the subsequent section headers remain as they are.*

# Terminal Procedures

Review the following sample text and tables to describe terminal procedures and update as necessary for this study. Placeholder values provided in the tables are for illustration purposes only. This section contains suggested wording; author is recommended to review for appropriate modifications.

***Rodents:*** *Euthanasia will be by carbon dioxide inhalation or anesthetized with sodium pentobarbital, ketamin/xylazine anesthesia or isoflurane inhalation followed by exsanguination. (Pick the appropriate method).*

***Nonrodents:*** *Animals will be administered a sedative [INSERT NAME] before being anesthetized with sodium pentobarbital and exsanguinated.*

Terminal procedures are summarized in the following table: *(Complete the Table as needed/appropriate). Author may update tables below accordingly.*

Terminal Procedures for Dosing Phase [and Recovery Phase] Animals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group No. | No. of Animals | Scheduled Euthanasia Day | Necropsy Procedures | Histology | Histopathology |
| M | F | Necropsy | Tissue Collection | Organ Weights |
| 1 |  |  |  | x | x | x | Full Tissuea | Full Tissuea |
| 2 |  |  |  | x | x | x | Gross Lesions Potential Target Tissuesb | Gross Lesions Potential Target Tissuesb |
| 3 |  |  |  | x | x | x | Gross Lesions Potential Target Tissuesb | Gross Lesions Potential Target Tissuesb |
| 4 |  |  |  | x | x | x | Full Tissuea | Full Tissuea |
| Unscheduled Deaths | x | x |  | Full Tissuea | Full Tissuea |
| Replaced animals (pretest) |  | Standard Diagnostic List per SOP |  | - | - |
| Replaced animals (dosing phase) | x | x |  | - | - |

a. See Tissue Collection and Preservation table in Section 14.5 for listing of tissues.

b. As requested by the Histopathology Pathologist.

**Toxicokinetic Animals**

|  | **Tissue Collection** | **Organ Weights** | **Histology Processing** | **Microscopic Evaluation** |
| --- | --- | --- | --- | --- |
| Toxicokinetic animals (found dead or unscheduled euthanasia) | X (limited)c | - | - | - |
| Toxicokinetic animals(scheduled euthanasia) | - | - | **-** | **-** |

c. Toxicokinetic animals that die on study or are euthanized for humane reasons will be subjected to a limited necropsy examination, consisting of an evaluation of the organs and tissues in the thoracic, abdominal, and pelvic cavities, with no tissues retained.

## Unscheduled Euthanasia and Decedents

This section contains suggested wording; author is recommended to review for appropriate modifications.

If a dosing phase **[or recovery phase]** animal dies on study, a necropsy will be conducted, and specified tissues will be saved. If necessary, the animal will be refrigerated (set to maintain 4°C) to minimize autolysis.

Animals that are euthanized for humane reasons, will undergo **[INSERT EUTHANASIA METHOD]** unless deemed inappropriate by the Study Director and /or the clinical veterinarian. These animals will undergo necropsy, **[blood collection will occur for the parameters listed below]** and specified tissues will be retained. If necessary, the animal will be refrigerated to minimize autolysis.

*If applicable, include chart below and update accordingly.*

| **Group Nos.** | **Hematology** | **Coagulation** | **Clinical Chemistry** | **BA, ADA, biomarkers etc.** |
| --- | --- | --- | --- | --- |
| *1 to 4* | *X* | *X* | *X* | *X* |
|  |  |  |  |  |

Animals found dead or euthanized before the initiation of dosing will be subject to necropsy and limited tissue retention (standard diagnostic tissue list). Any animal replaced after the start of dosing will be subject to necropsy and tissues will be retained (as per Tissue Collection and Preservation section).

## Scheduled Euthanasia

This section contains suggested wording; author is recommended to review for appropriate modifications, also adding euthanasia information for other satellite groups as appropriate.

Dosing phase **[and recovery phase]** animals surviving until scheduled euthanasia will have a terminal body weight recorded. Animals will be **[fasted /not fasted]** overnight but will retain access to water. The animals will undergo exsanguination from the **[INSERT LOCATION, ex abdominal aorta]** after **[INSERT ANESTHESIA METHOD, ex isoflurane]** and blood sample collection. *If a specific order of necropsy is required, please include.*

*Delete the following paragraph when there are no TK animals on study*

After completion of the blood collection schedule, the toxicokinetic animals will be euthanized by **[INSERT EUTHANASIA METHOD]**. There will be no examinations for the purposes of the study. Carcasses will be discarded.

## Necropsy

This section contains suggested wording; author is recommended to review for appropriate modifications, also adding necropsy information for other satellite groups as appropriate.

Dosing phase **[and recovery phase]** animalswill be subjected to a complete necropsy examination, which will include evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

At the discretion of the necropsy supervising pathologist, images may be generated for illustration of or consultation on gross observations. Generation of such images will be documented and communicated to the Study Director. Images and associated documentation will be retained and archived.

## Bone Marrow Smears

This section contains suggested wording; author is recommended to review for appropriate modifications.

Bone marrow smears will be collected from each animal at the scheduled euthanasia and prepared as described in the Tissue Collection and Preservation table in Section 14.6. Evaluation of stained smears may be added by amendment at the discretion of the Study Director in consultation with the clinical and/or anatomic pathologist and the Sponsor.

## Organ Weights

This section contains suggested wording; author is recommended to review for appropriate modifications.

The organs identified for weighing in the Tissue Collection and Preservation table in Section 14.6 will be weighed at necropsy for all scheduled dosing phase **[and recovery phase]** animals. Organ weights will not be recorded for animals found dead or euthanized in poor condition or in extremis. Paired organs will be weighed together. In the event of gross abnormalities, in addition to the combined weight, the weight of each organ of a pair may be taken and entered as a tissue comment. Organ to body weight ratio (using the terminal body weight) and organ to brain weight ratios will be calculated as percentages.

## Tissue Collection and Preservation

This section contains suggested wording; author is recommended to review for appropriate modifications.

Representative samples of the tissues identified in the Tissue Collection and Preservation table below will be collected from all animals and preserved in 10% neutral buffered formalin, unless otherwise indicated. Prior to its use in this study, the pH of each batch or lot of neutral buffered 10% formalin will be provided and maintained in file evidence that the neutral buffered 10% formalin used is of appropriate quality. Additional tissue samples may be collected at the discretion of the pathologist to elucidate abnormal findings. Missing tissue will be documented in histology data.

See Supporting Information for suggested tissue lists by species. Populate the table below with intended tissue collection and preservation procedures and additional context/comments as necessary. Include list of potential target tissue information as appropriate.

| Tissue | Collect | Weigh | Histology | Microscopic Evaluation | Comment |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| X = Procedure to be conducted; = Not applicable. |

# Histology and Histopathology

## Histology

This section contains suggested wording; author is recommended to review for appropriate modifications.

Tissues in the Tissue Collection and Preservation table from animals identified in the Terminal Procedures table will be embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

## Histopathology

This section contains suggested wording; author is recommended to review for appropriate modifications.

Histopathological evaluation will be performed for animals identified in the Terminal Procedures table.

Any additional stains or evaluations, if deemed necessary by the pathologist, will be added by study plan/protocol amendment following discussion with the Study Director and in consultation with the Sponsor.

At the discretion of the study pathologist and after acknowledgement by the Study Director, images may be captured for consultation purposes.

# Pathology Peer Review

This section contains suggested wording; author is recommended to review for appropriate modifications.

A pathology peer review will be conducted according to **[Sponsor’s SOP or the Organization for Economic Co-Operation and Development (OECD) Guidance No. 16]** by a pathologist designated by the Sponsor. *If samples are being shipped for analysis:* Refer to Attachment A for shipping details. The peer review statement will be included as an appendix to the final report.

# Data Evaluation and Statistical Analysis

Due to the variety of approaches currently utilized across the industry, sample text for statistical analysis is not provided within this template. For efficiency, we suggest using the routine statistical analysis methods employed by the laboratory conducting the study.

# Regulatory Information

## Good Laboratory Practice

This section contains suggested wording; author is recommended to review for appropriate modifications. Author must highlight exceptions to compliance with GLPs.

This study will be performed in accordance with the OECD Principles of Good Laboratory Practice and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions to GLPs include the following study elements:

* *For example, include any non-validated systems as GLP exceptions.*

Each Principal Investigator will be responsible for compliance with OECD GLP and their national GLP regulations. *Specify here whether a PI acknowledgement form as documentation of this principle is required.*

## Regulatory Testing Guidelines

*This section contains suggested wording; author is recommended to review for appropriate modifications. Not all guidelines may be applicable to every study. Author may add additional guidelines as needed.*

The study will be performed in compliance with the following regulations or guidelines:

* European Parliament and Council Directive 2001/83/EC of 6 November 2001 of the Community Code Relating to Medicinal Products for Human Use, OJ L311/67‑128, 28 November 2001 as amended Commission Directive 2003/63/EC, OJ L159, 27 June 2003.
* Note for Guidance on Repeated Dose Toxicity (CPMP/SWP/1042/99) of the European Agency for Evaluation of Medicinal Products.
* OECD Guideline 407. Repeated Dose 28‑day Oral Toxicity Study in Rodents
* OECD Guideline 474. OECD Guideline for Testing of Chemicals - Mammalian Erythrocyte Micronucleus Test.
* OECD Guideline 13. The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies
* ICH Harmonised Tripartite Guideline M3 (R2). Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals and Marketing Authorization for Pharmaceuticals.
* ICH Harmonised Tripartite Guideline S3a. Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies.
* ICH Harmonised Tripartite Guideline S2 (R1). Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use.
* ICH Harmonised Tripartite Guideline S9. Nonclinical Evaluation for Anticancer Pharmaceuticals.
* ICH Harmonised Tripartite Guideline S6(R1). Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.
* ICH Harmonised Tripartite Guideline S8. Immunotoxicity Studies for Human Pharmaceuticals.
* ICH Harmonised Tripartite Guideline S2(R1). Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use.
* Japanese Guidelines for Nonclinical Studies of Drugs Manual (1995). Guidelines for Nonclinical Pharmacokinetic Studies, and Guidelines for Toxicity Studies of Drugs Chapter 3, “Repeated Dose Toxicity Studies”.
* Notification Number 24 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, dated 11 September 1989, as amended notification number 88 dated 10 August 1993 and notification number 655 dated 5 April 1999.

# Quality Assurance

This section contains suggested wording; author is recommended to review for appropriate modifications.

The Testing Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in accordance with Good Laboratory Practice regulations. The QAU will inspect the study at intervals adequate to assure integrity and review the Final Report to assure that it accurately describes the methods and that the results accurately reflect the raw data.

Portions of the study conducted at a test site will be monitored by the test site QAU. Copies of each test site inspection report will be made available to the Study Director, Testing Facility Management, and the Testing Facility QAU.

# Animal Welfare

*This section contains suggested wording; author is recommended to review for appropriate modifications, such as additions for regional animal regulations (for example, the European Directive on Animal Welfare).*

Institutional Animal Care and Use Statement (IACUC): All procedures in this study plan/protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the Sponsor, the study does not unnecessarily duplicate any previous work, and no other model can fulfil the study requirements.

In accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare, medical treatment necessary to prevent unacceptable pain and suffering, including euthanasia, is the sole responsibility of the attending laboratory animal Veterinarian.

# Major Computer Systems

This section contains suggested wording; author is recommended to review for appropriate modifications.

The following major computerized systems may be used in the study. The actual major computerized systems used will be specified in the Final Report.

Critical Computerized Systems

|  |  |
| --- | --- |
| **System Name** | **Description of Data Collected and/or Analyzed** |
|  |  |
|  |  |

# Amendments and Deviations

This section contains suggested wording; author is recommended to review for appropriate modifications.

Changes to the approved study plan/protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director **[add additional signatories if applicable]**. No study plan/protocol amendment will occur without the Sponsor’s consent.

All study plan/protocol and SOP deviations will be documented in the study records. Deviations from the study plan/protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/Contributing Scientist, and reported to the Study Director for authorization/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

# Retention of Records, Samples and Specimens

This section contains suggested wording; author is recommended to review for appropriate modifications.

All study-specific‑ raw data, documentation, study plan/protocol, samples, specimens, and interim (if applicable) and final reports from this study are the property of the Sponsor. Following dispatch of the Final Report, samples/specimens (if any, and except for those sent to the Sponsor test sites and resultant data which are the responsibility of the Sponsor test sites), all raw data and documents generated at **[NAME]** during this study, together with the original copy of the study plan/protocol (including amendments) and the Final Report (including the contributory reports generated by the Sponsor), will be transferred to the scientific archives of **[NAME]** for a period of approximately **[#]** years. Subsequently, storage details will be documented in the raw data.

Electronic data generated by the Test Facility will be archived, and the software and hardware required to produce it in a readable form will be maintained and available.

The Sponsor will be responsible for archival of all records, samples and specimens generated from phases or segments conducted by the Sponsor.

Third parties will be responsible for archival of all reports, records, samples and specimens generated from phases or segments conducted by third parties. **[DURATION OF ARCHIVING]**

Records to be maintained will include, but will not be limited to, documentation and data for the following:

|  |  |
| --- | --- |
| Study Plan/Protocol, study plan/protocol amendments, and deviations | Clinical pathology sample collection |
| Study schedule | Bioanalytical sample collection |
| Study-‑related correspondence | Gross observations and related data |
| Test system receipt, health, and husbandry | Organ weight measurements |
| Test item/article and reference item/control article receipt, identification and preparation | Statistical analysis results |
| In‑life measurements and observations | Test item/article reserve sample |
|  |  |

# Study Classification

*The author may choose to include the information below for studies requiring SEND. This section contains suggested wording; author is recommended to review for appropriate modifications.*

|  |  |
| --- | --- |
| Study Category [STCAT]: | Toxicology |
| Study Type [SSTYP]: | Repeat Dose Toxicity |
| Study Design [SDESIGN]: | PARALLEL*Or Choose:* *DOSE ESCALATION* |
| Primary Treatment CAS Registry Number [TRTCAS]: | *Insert Number Provided by Sponsor or Not Available* |
| Primary Treatment Unique Ingredient ID [TRTUNII]: | *Insert ID Provided by Sponsor* |
| Class of Compound [PCLASS]: | *Insert Class of Compound Provided by Sponsor or Not Available* |
| Planned Pharmacological Target [PPTEGSYM] | *"Insert the Entrez Gene Symbol provided by Sponsor or Not Available"**Refer to: https://www.ncbi.nlm.nih.gov/gene* |

# Reporting

This section contains suggested wording; author is recommended to review for appropriate modifications and/or to reflect standard operating procedures.

|  |  |
| --- | --- |
| Draft Report | A GLP compliant report will be prepared. Following experimental completion an audited draft report will be provided to the Sponsor for their review. The Draft report will be provided electronically in PDF (non‑hyperlinked) format. In addition, an MS Word copy of the text will be provided electronically.After receipt and review of the Sponsor’s comments, appropriate changes will be made, and revisions provided to the Sponsor. |
| Final Report | Once authorized by the Sponsor, the audited, signed final report will be issued.The Final report will be provided electronically in eCTD compliant fully hyperlinked PDF format. A Word file with the text of the report will also be provided.Any additions or corrections to an authorized final report will be documented as a formal addendum/amendment to the final report.A CTD Tabulated summary will be provided to the Sponsor as part of the Final report.*Optional statement on the provision of SEND data:* Study data will be prepared in SEND format following completion of the study and will be finalized following consultation with the Sponsor. The Sponsor will receive these data in an electronic version. |

Reports should be finalized within 6 months of issuance of the audited Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issuance, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

# References

Insert numbered references cited elsewhere within this document in a generally accepted format.

ATTACHMENT A

*Update the in-life activities schedule as needed based on specific study parameters and days of study. This section contains suggested wording/format; author is recommended to review for appropriate modifications.*

In Life Activities Schedule

|  |
| --- |
| **← DOSING PHASE →** |
| **Parameter** | **Protocol Section** | **Week:** | **-2** | **-1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **2** | **2** | **2** | **2** | **2** | **2** | **2** | **3** | **3** | **3** | **3** | **3** | **3** | **3** | **4** | **4** | **4** | **4** | **4** | **4** | **4** | **5** | **5** | **5** |
|   |  | **Day:** | **NA** | **NA** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** | **30** | **31** |
| **Dose Formulation Analysis (Sampling)** | 6 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Mortality Check** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Cageside Observations** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Veterinary Exam** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Body Weights**  | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Food Consumption** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Ophthalmoscopic Exam** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Cardiovascular/****Respiratory/CNS** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
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| **Hematology** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Coagulation** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Clinical Chemistry** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Urinalysis** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Bone Marrow Smears** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Bioanalysis** | 11 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Other Testing** | 13 | See Section 13 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Terminal Procedures** | 14 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Necropsy** | 14 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

**Attachment A *continued***

In Life Activities Schedule

|  |
| --- |
| **← RECOVERY PHASE →** |
| **Parameter** | **Protocol Section** | **Week:** |  |  |  | **5** | **5** | **5** | **5** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **7** | **7** | **7** | **7** | **7** | **7** | **7** | **8** | **8** | **8** | **8** | **8** | **8** | **8** | **9** | **9** | **9** |  |  |
|  |  | **Day:** |  |  |  | **32** | **33** | **34** | **35** | **36** | **37** | **38** | **39** | **40** | **41** | **42** | **43** | **44** | **45** | **46** | **47** | **48** | **49** | **50** | **51** | **52** | **53** | **54** | **55** | **56** | **57** | **58** | **59** |  |  |
| **Mortality Check** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Cageside Observations** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Veterinary Exam** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Body Weights**  | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Food Consumption** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Ophthalmoscopic Exam** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Cardiovascular/****Respiratory****/CNS** | 9 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
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| **Hematology** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Coagulation** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Clinical Chemistry** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Urinalysis** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Bone Marrow Smears** | 10 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Bioanalysis** | 11 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Other Testing** | 13 | See Section 13 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
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| **Terminal Procedures** | 14 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Necropsy** | 14 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

# ATTACHMENT B

*Author should update the chart below to indicate shipment information for samples being analyzed at a test site. This section contains suggested wording/format; author is recommended to review for appropriate modifications.*

Shipment of Samples

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Purpose** | **Day/****Week/****Aliquot** | **Proposed Shipment Date** | **Conditions**  | **Recipient/Address** |
| *Formulation* | *Dose Formulation Analysis* |  |  | For shipment: ex: Dry Ice, Ambient Temperature, etc.For storage:ex: Dry Ice, Ambient Temperature, etc. | NameAddressTel: E-mail:  |
| *Plasma* | *Bioanalysis* |  |  |  | NameAddressTel: E-mail:  |
| *Slides* | *Peer Review* |  |  |  | NameAddressTel: E-mail:  |
| *Plasma* | *Biomarker analysis* |  |  |  | NameAddressTel: E-mail:  |

# APPENDIX

*The Appendix can be included at the discretion of the author to include specialty procedures.*

# SUPPORTING INFORMATION

*This section contains further information about the parameters for clinical pathology, tissue collection per species and CNS Assessment Observations in Rodents. Text from this section can be pasted into the relevant sections of the protocol, or companies can use their own supporting information, as desired. Applicable systems or instruments used may also be included in relevant sections of the protocol (if appropriate). This supporting information should be removed from the protocol prior to finalization.*

**CLINICAL PATHOLOGY**

This section contains examples for commonly used designs for Clinical Pathology for rat, monkey & dog. Author should review for applicability to this study.

**1 Month Rat GLP Toxicology Studies**

**Blood Sample Intervals for Definitive GLP Studies**

In addition to near termination‑ samples, interim samples may be collected as suggested in the following table. Other sampling times may be taken as deemed necessary by the Pathologist in consultation with the Study Director. *For consideration: collecting same time points as previous study, earlier time points if need to define onset of a change, follow-through‑ recovery, etc.*

Rat Clinical Pathology Collection Schedule

|  |  |  |
| --- | --- | --- |
| Interval | 1‑Month Rat Study | Comments |
| Pretest | X | Research rats are a fairly homogenous population that are well characterized for hematology and clinical chemistry parameters. Given the small blood volume of a rat, pretest blood samples are collected only rarely in 1‑month studies. Requests for pretest phlebotomy are handled on a case‑by‑case basis. |
| Week 1 | X | In a 1‑month study an interim sample can be collected either on week 1 or week 2. These samples are often obtained from a peel-off group of animals. |
| Week 2 | OPT | OPT indicates optional collection times at discretion of Study Director and Study Pathologist. |
| Week 4 |  |  |
| Near Term | X | “Near Termination” refers to end of the dosing phase and, if applicable, end of the recovery phase. |

Routine interim samples consist of Hematology, Coagulation, and Clinical Chemistry on all animals and are usually determined on peel-off groups of rats.

Animals are routinely fasted for all scheduled clinical pathology blood sample analysis. Some nonscheduled (early termination of dose group, diagnostic samples for sick animals) blood collections may occur in nonfasted animals.

Although not routine, urinalysis may also be performed on a case‑by‑case basis when it is necessary to follow a urinalysis parameter during the course of the study. Consult with clinical pathologists to help you determine if interim urinalysis is necessary.

**Hematology (anti‑coagulant - potassium EDTA)**

Use 0.5- or 1-mL vacutainer tubes (lavender stopper) for blood collection. Some laboratories choose to use microtainer tubes (non‑vacuum tubes) of similar size (0.5 or 1 mL).

* Erythrocytes [RBC]
* Hemoglobin [HGB]
* Hematocrit [HCT]
* Ery. Mean Corpuscular Volume [MCV]
* Erythrocytes Distribution Width [RDW]
* Ery. Mean Corpuscular Hemoglobin [MCH]
* Ery. Mean Corpuscular Hemoglobin Concentration [MCHC]
* Leukocytes (total [WBC] and absolute differential [WBCCE])
* Neutrophils [NEUT]
* Lymphocytes [LYM]
* Monocytes [MONO]
* Eosinophils [EOS]
* Basophils [BASO]
* Large Unstained Cells [LGUNSCE]
* Platelets [PLAT]
* Reticulocytes [RETI]
* Cell morphology [CEMORPH] \*

\* = blood smear to be prepared and stained for all animals at the time of phlebotomy or soon after. Blood smear evaluation of all animals at all doses is ideal and preferred. However, an acceptable alternative in labs that do not do this routinely is to consider requesting evaluation of control and high dose groups. Evaluation of lower doses would occur only if potential compound-related findings occur in the high dose.

**Coagulation (anticoagulant - sodium citrate)**

Use 2 mL vacutainer tubes (blue stopper) for blood collection.

* Activated Partial Thromboplastin Time [APTT]

Prothrombin Time [PT]

Fibrinogen [FIBRINO]

**Clinical Chemistry (serum preferred) \***

Use 0.5- or 1-mL vacutainer tubes without anticoagulant (red stopper) for serum to provide adequate volume for tests listed below. Note: there are several sizes of vacutainer tubes for serum and they may be used for greater volume if need exists (serum biomarkers are desired.) At termination (necropsy), larger volumes of blood may be drawn for potential storage of extra serum for future studies if necessary. Some laboratories elect to use 0.5 or 1 mL microtainer tubes (non‑vacuum tubes) for rats.

Alanine Aminotransferase [ALT]

Aspartate Aminotransferase [AST]

Albumin [ALB]

Albumin/Globulin [ALBGLOB]

Alkaline phosphatase [ALP]

Urea nitrogen [UREAN]

Calcium [CA]

Chloride [CL]

Cholesterol [CHOL]

Creatine Kinase [CK]

Creatinine [CREAT]

Gamma Glutamyl Transferase [GGT]

Globulin [GLOBUL]

Glucose [GLUC]

Phosphate [PHOS]

Potassium [K]

Sodium [SODIUM]

Bilirubin [BILI]

Protein [PROT]

Triglycerides [TRIG]

Cardiac Troponin I (cTnI) - Used on a case-‑by-‑case basis.

\* = Amylase and lipase may be added at the discretion of the Clinical Pathologist for compounds known or suspected to cause pancreatic inflammation/injury.

**Urine Sample Intervals for Definitive GLP studies**

Urinalysis on all surviving rats are collected near termination. Cage pan collection is acceptable, although metabolic cage collection is preferred for all time points. As a standard, collect urine chilled on ice during an overnight collection. Cystocentesis at necropsy is also acceptable for nonstandard urine testing to include total protein and urine specific gravity only. Rat cystocentesis collections lack adequate urine volume for complete urinalysis. Please note that the preference is to *not* withhold water from animals prior to urine collection.

Unscheduled urine collection is recommended (by amendment) if an entire dose group is being terminated early and clinical chemistry samples are being collected. Collect urine from moribund animals by cystocentesis at necropsy when possible and only if clinical chemistry samples are collected. Do not collect and analyze urine from dead animals.

**Urinalysis**

(From scheduled termination animals. Cage pan collection is acceptable, although metabolic cage collection is preferred.)

pH [PH]

Color [COLOR]

Clarity [CLARITY]

Volume [VOLUME]

Specific Gravity [SPGRAV]

Bilirubin [BILI]

Occult Blood [OCCBLD]

Glucose [GLUC]

Ketones [KETONES]

Protein [PROT]

Sediment evaluation [SEDEXAM]

**Urine Chemistry**

* Creatinine (measured concentration) [CREAT]
* Protein (concentration measured) [PROT]
* Protein/Creatinine (calculated) [PROTCRT]
* Glucose (concentration measured) [GLUC]
* Glucose/Creatinine (calculated) [GLUCCRT]
* Sodium (measured concentration) [SODIUM]
* Sodium/Creatinine (calculated) [NACREAT]
* Chloride (measured concentration) [CL]
* Chloride to creatinine ratio (calculated) [CLCREAT]
* Albumin (concentration measured) [ALB]
* Albumin/Creatinine (calculated) [ALBCREAT]

**1 Month Monkey GLP Tox Studies**

**Blood Sample Intervals for Definitive GLP Studies**

In addition to near ‑termination samples, interim samples may be collected as suggested in the following table. Other sampling times may be taken as deemed necessary by the Pathologist in consultation with the Study Director. *For consideration: collecting the same time points as previous study, earlier time points if needed to define onset of a change, follow through recovery, etc.*

Monkey Clinical Pathology Collection Schedule

|  |  |  |
| --- | --- | --- |
| Interval | 1‑Month Monkey Study | Comments |
| Pretest | X | 1-2 pretest samples are recommended. Days ‑14 and ‑7 are preferred time for pretest blood collections. Some studies may opt for Days ‑7 and Day 1 (predose). |
| Week 1 | X | In a 1‑month study an interim sample can be collected either on week 1 or week 2. |
| Week 2 | OPT | OPT indicates optional collection times at discretion of Study Director and Study Pathologist. |
| Week 4 |  |  |
| Near Term | X | “Near Termination” refers to end of the dosing phase and, if applicable, end of the recovery phase. |

Routine interim samples consist of Hematology, Coagulation, and Clinical Chemistry on all animals.

Animals are routinely fasted for all scheduled clinical pathology blood sample analysis. Some nonscheduled (early termination of dose group, diagnostic samples for sick animals) blood collections may occur in nonfasted animals.

Although not routine, urinalysis may also be performed on a case-by‑case basis when it is necessary to follow a urinalysis parameter during the course of the study. Consult with clinical pathologists to help you determine if interim urinalysis is necessary.

**Hematology (anti‑coagulant - potassium EDTA)**

Use 1 mL vacutainer tubes (lavender stopper) for blood collection.

Erythrocytes [RBC]

Hemoglobin [HGB]

Hematocrit [HCT]

Ery. Mean Corpuscular Volume [MCV]

Erythrocytes Distribution Width [RDW]

Ery. Mean Corpuscular Hemoglobin [MCH]

Ery. Mean Corpuscular Hemoglobin Concentration [MCHC]

Leukocytes (total [WBC] and absolute differential [WBCCE])

Neutrophils [NEUT]

Lymphocytes [LYM]

Monocytes [MONO]

Eosinophils [EOS]

Basophils [BASO]

Large Unstained Cells [LGUNSCE]

Platelets [PLAT]

Reticulocytes [RETI]

Cell morphology [CEMORPH] \*

\* = blood smear to be prepared and stained for all animals at the time of necropsy or soon after. Blood smear evaluation of all animals at all doses is ideal and preferred. However, an acceptable alternative in labs that do not do this routinely is to consider requesting evaluation of control and high dose groups. Evaluation of lower doses would occur only if potential compound-related findings occur in the high dose.

**Coagulation (anticoagulant - sodium citrate)**

Use 2 mL vacutainer tubes (blue stopper) for blood collection.

Activated Partial Thromboplastin Time [APTT]

Prothrombin Time [PT]

Fibrinogen [FIBRINO]

**Clinical Chemistry (serum preferred) \***

Use 1 mL vacutainer tubes without anticoagulant (red stopper) for serum to provide adequate volume for tests listed below. Note: there are several sizes of vacutainer tubes for serum and they may be used for greater volume if need exists (Serum biomarkers are desired.) At termination (necropsy), larger volumes of blood may be drawn for potential storage of extra serum for future studies if necessary.

Alanine Aminotransferase [ALT]

Aspartate Aminotransferase [AST]

Albumin [ALB]

Albumin/Globulin [ALBGLOB]

Alkaline phosphatase [ALP]

Urea nitrogen [UREAN]

Calcium [CA]

Chloride [CL]

Cholesterol [CHOL]

Creatine Kinase [CK]

Creatinine [CREAT]

Gamma Glutamyl Transferase [GGT]

Globulin [GLOBUL]

Glucose [GLUC]

Phosphate [PHOS]

Potassium [K]

Sodium [SODIUM]

Bilirubin [BILI]

Protein [PROT]

Triglycerides [TRIG]

Cardiac Troponin I (cTnI) - Used on a case‑by‑case basis.

\* = Amylase and lipase may be added at the discretion of the Clinical Pathologist for compounds known or suspected to cause pancreatic inflammation/injury.

**Urine Sample Intervals for Definitive GLP studies**

Urinalysis on all surviving monkeys are taken pre‑test (2X) and near termination. Cage pan collection is acceptable for all time points. As a standard, collect urine chilled on ice during an overnight collection. Cystocentesis at necropsy is also acceptable. Please note that the preference is to ***not*** withhold water from animals prior to urine collection.

Urinalysis - Unscheduled urine collection is recommended (by amendment) if an entire dose group is being terminated early and clinical chemistry samples are being collected. Collect urine from moribund animals by cystocentesis at necropsy when possible and only if clinical chemistry samples are collected. Do not collect and analyze urine from dead animals.

**Urinalysis**

(Scheduled euthanasia animals. Cystocentesis at necropsy or overnight cage pan collection are acceptable). For interim samples, catheterization may also be considered.

pH [PH]

Color [COLOR]

Clarity [CLARITY]

Volume [VOLUME]

Specific Gravity [SPGRAV]

Bilirubin [BILI]

Occult Blood [OCCBLD]

Glucose [GLUC]

Ketones [KETONES]

Protein [PROT]

Sediment evaluation (microscopic) [SEDEXAM]

**Urine Chemistry**

* Creatinine (measured concentration) [CREAT]
* Protein (concentration measured) [PROT]
* Protein/Creatinine (calculated) [PROTCRT]
* Glucose (concentration measured) [GLUC]
* Glucose/Creatinine (calculated) [GLUCCRT]
* Sodium (measured concentration) [SODIUM]
* Sodium/Creatinine (calculated) [NACREAT]
* Chloride (measured concentration) [CL]
* Chloride to creatinine ratio (calculated) [CLCREAT]

**1‑Month Dog GLP Toxicology Studies**

**Blood Sample Intervals for Definitive GLP studies**

In addition to near ‑termination samples, interim samples may be collected as suggested in the following table. Other sampling times may be taken as deemed necessary by the Study Director in consultation with the Clinical Pathologist. *For consideration: collecting same time points as previous study, earlier time points if need to define onset of a change, follow through‑ recovery, etc.*

Dog Clinical Pathology Collection Schedule

|  |  |  |
| --- | --- | --- |
| Interval | 1‑Month Dog Study | Comments |
| Pretest | X | 1-2 pretest samples are recommended (excluding urinalysis). Days ‑14 and ‑7 are preferred time for prestudy blood collections. Some studies may opt for Days ‑7 and Day 1 (predose). |
| Week 1 | X | In a 1‑month study an interim sample can be collected either on week 1 or week 2. |
| Week 2 | OPT | OPT indicates optional collection times at discretion of Study Director and study pathologist. |
| Week 4 |  |  |
| Near Term | X | “Near Termination” refers to end of the dosing phase and, if applicable, end of the recovery phase (preferably within 3 days of necropsy). |

Routine interim samples consist of Hematology, Coagulation, and Clinical Chemistry on all animals.

Animals are routinely fasted for all scheduled clinical pathology blood sample analysis. Some non‑scheduled (early termination of dose group, diagnostic samples for sick animals) blood collections may occur in nonfasted animals.

Although not routine, urinalysis may also be performed on a case ‑by ‑case basis when it is necessary to follow a urinalysis parameter during the course of the study. Consult with clinical pathologists to help you determine if interim urinalysis is necessary.

**Hematology (anti‑coagulant - potassium EDTA)**

[K2EDTA or K3EDTA]. Use 1 mL vacutainer tubes (lavender stopper) for blood collection (or other appropriate tube).

Erythrocytes [RBC]

Hemoglobin [HGB]

Hematocrit [HCT]

Ery. Mean Corpuscular Volume [MCV]

Erythrocytes Distribution Width [RDW]

Ery. Mean Corpuscular Hemoglobin [MCH]

Ery. Mean Corpuscular Hemoglobin Concentration [MCHC]

Leukocytes (total [WBC] and absolute differential [WBCCE])

Neutrophils [NEUT]

Lymphocytes [LYM]

Monocytes [MONO]

Eosinophils [EOS]

Basophils [BASO]

Large Unstained Cells [LGUNSCE]

Platelets [PLAT]

Reticulocytes [RETI]

Cell morphology [CEMORPH] \*

\* = blood smear to be prepared and stained for all animals at the time of necropsy or soon after. Blood smear evaluation of all animals at all doses or of control and high dose only may be performed. Evaluation of lower doses would occur only if potential compound-related findings occur in the high dose. Alternatively, blood smears may only be evaluated to confirm findings of an automated analyzer or based on reflex criteria (generally SOP‑based).

**Coagulation (anti‑coagulant - sodium citrate)**

Use 2 mL vacutainer tubes (blue stopper) for blood collection. (When using vacutainer tubes, citrate tubes should be collected prior to EDTA and serum tubes.)

Activated Partial Thromboplastin Time [APTT]

Prothrombin Time [PT]

Fibrinogen [FIBRINO]

**Clinical Chemistry (serum preferred) \***

Use 1 mL vacutainer tubes without anticoagulant (red stopper) for serum to provide adequate volume for tests listed below. Note: there are several sizes of vacutainer tubes for serum and they may be used for greater volume if need exists (e.g. Serum biomarker are desired.) At termination (necropsy), larger volumes of blood may be drawn for potential storage of extra serum for future studies if necessary.

Alanine Aminotransferase [ALT]

Aspartate Aminotransferase [AST]

Albumin [ALB]

Albumin/Globulin [ALBGLOB]

Alkaline phosphatase [ALP]

Urea nitrogen [UREAN]

Calcium [CA]

Chloride [CL]

Cholesterol [CHOL]

Creatine Kinase [CK]

Creatinine [CREAT]

Gamma Glutamyl Transferase [GGT]

Globulin [GLOBUL]

Glucose [GLUC]

Phosphate [PHOS]

Potassium [K]

Sodium [SODIUM]

Bilirubin [BILI]

Protein [PROT]

Triglycerides [TRIG]

**Urine Sample Intervals for Definitive GLP studies**

Urinalysis on all surviving dogs taken pre‑test (1X) and near termination. Cage pan collection is acceptable for all time points. As a standard, collect urine chilled on ice during a timed overnight collection. Cystocentesis at necropsy is also acceptable. Please note that the preference is to ***not*** withhold water from animals prior to urine collection.

Urinalysis - Unscheduled urine collection is recommended (by amendment) if an entire dose group is being terminated early and clinical chemistry samples are being collected. Collect urine from moribund animals by cystocentesis at necropsy when possible and only if clinical chemistry samples are collected. Do not collect and analyze urine from dead animals.

**Urinalysis**

(Scheduled euthanasia animals. Cystocentesis at necropsy or overnight (timed) cage pan collection are acceptable). For interim samples, catheterization may also be considered.

pH [PH]

Color [COLOR]

Clarity [CLARITY]

Volume [VOLUME]

Specific Gravity [SPGRAV]

Bilirubin [BILI]

Occult Blood [OCCBLD]

Glucose [GLUC]

Ketones [KETONES]

Protein [PROT]

**Urine Chemistry**

* Creatinine (measured concentration) [CREAT]
* Protein (concentration measured) [PROT]
* Protein/Creatinine (calculated) [PROTCRT]
* Glucose (concentration measured) [GLUC]
* Glucose/Creatinine (calculated) [GLUCCRT]
* Sodium (measured concentration) [SODIUM]
* Sodium/Creatinine (calculated) [NACREAT]
* Chloride (measured concentration) [CL]
* Chloride to creatinine ratio (calculated) [CLCREAT]

**TISSUE COLLECTION**

*This section contains examples for tissue collection for rat, dog & monkey. Author should review f**or applicability to this study.*

**Tissue Collection for Rats**

Tissue Collection and Preservation

| Tissue | Weigh | Collect | Histology | Microscopic Evaluationa | Comment |
| --- | --- | --- | --- | --- | --- |
| Animal identification | ‑ | X | ‑ | ‑ | ‑ |
| Artery, aorta | ‑ | X | X | X | ‑ |
| Bone Marrow | ‑ | X | ‑ | ‑ | Three (3) bone marrow smears will be collected from the femur at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin. |
| Bone marrow, femur/joint, femorotibial | ‑ | X | X | X | Femur and Tibia bone marrow (joint femorotibial) |
| Bone marrow, sternum | ‑ | X | X | X |  |
| Bone, femur/joint, femorotibial | ‑ | X | X | X | Evaluation includes bones of femur and tibia and other joint components |
| Bone, sternum | ‑ | X | X | X | ‑ |
| Brain | X | X | X | X | Seven brain levels to be examined to include olfactory bulb (Examine in nasal cavity, nasal section level 4) |
| Cervix | ‑ | X | X | X | Collected and weigh with uterus |
| Epididymis | X | X | X | X | Paired examination |
| Esophagus | ‑ | X | X | X | ‑ |
| Eye | ‑ | X | X | X | Paired examination; Fixed in Davison’s fixative (all animals). |
| Gland, adrenal | X | X | X | X | Paired weight and examination. |
| Gland, harderian (Ocular accessory gland) | ‑ | X | X | X | Collect both, 1 required for microscopic examination |
| Gland, mammary | ‑ | X | X | X | Inguinal |
| Gland, parathyroid | - | X | X | X | Collected with thyroid; At least one examined microscopically. One recut/recheck of wet tissue will be conducted to find missing tissue. |
| Gland, pituitary | X | X | X | X | ‑ |
| Gland, prostate/Gland, seminal vesicle | X | X |   |   | Separated for Microscopic Evaluation |
| Gland, prostate |  |  | X | X | Weighed with Seminal vesicle |
| Seminal vesicle |  |  | X | X | Weighed with prostate |
| Gland, salivary, submandibular | ‑ | X | X | X | Submandibular; collected bilateral; 1 required for microscopic examination |
| Gland, thyroid | X | X | X | X | Paired weight and examination; weight includes parathyroid. |
| Gross lesions/masses | ‑ | X | X | X | ‑ |
| Heart | X | X | X | X | Including section of aorta |
| Injection site (s) | ‑ | X | X | X | ‑ |
| Kidney | X | X | X | X | Paired weight and examination. Cross and longitudinal sections collected and examined. |
| Large intestine, cecum | ‑ | X | X | X | ‑ |
| Large intestine, colon | ‑ | X | X | X | ‑ |
| Large intestine, rectum | ‑ | X | ‑ | ‑ | ‑ |
| Larynx | ‑ | X | ‑ | ‑ |  |
| Liver | X | X | X | X | Sample of two lobes |
| Lung | ‑ | X | X | X | All lobes retained. Sample of left lateral lobe, longitudinal section for microscopic examination examined |
| Lymph node, mandibular | ‑ | X | X | X | Only one required for microscopic examination; collect more than one |
| Lymph node, mesenteric | ‑ | X | X | X | ‑ |
| Muscle, Quadriceps femoris  | ‑ | X | X | X | cross and longitudinal sections processed. |
| Muscle, Diaphragm |  |  |  |  | cross and longitudinal sections processed. |
| Nerve, optic | ‑ | X | X | X | Both fixed in Davidson’s fixative (all animals) |
| Nerve, sciatic | ‑ | X | X | X | Collect bilateral; 1 required for microscopic examination. Cross and longitudinal sections processed. |
| Ovary | X | X | X | X | Paired weight and examination |
| Pancreas | ‑ | X | X | X | ‑ |
| Skin | ‑ | X | X | X | Dorsal thoracic |
| Small intestine, duodenum | ‑ | X | X | X | ‑ |
| Small intestine, ileum | ‑ | X | X | X | ‑ |
| Small intestine, jejunum | ‑ | X | X | X | ‑ |
| Spinal cord | ‑ | X | X | X | Cervical, thoracic, lumbar. Cross and longitudinal sections processed and examined. |
| Spleen | X | X | X | X | ‑ |
| Stomach | ‑ | X | X | X | Cardiac, Fundic, pyloric and non-glandular regions |
| Testis | X | X | X | X | Paired weight and examination; Fixed in Modified Davidson’s fixative (all animals). |
| Thymus | X | X | X | X | Collect tissue from region if not identified grossly. |
| Tongue | ‑ | X | X | X | ‑ |
| Trachea | ‑ | X | X | X | ‑ |
| Urinary bladder | ‑ | X | X | X | ‑ |
| Uterus | ‑ | X | X | X | Horns and body |
| Vagina | ‑ | X | X | X | ‑ |
| - = Not applicable; X = Procedure to be conducted.a. Efforts will be made to evaluate all study plan/protocol-required‑ tissues microscopically; however, it is not always feasible for every study plan/protocol-required‑ tissue to be present on every slide. Study plan/protocol required‑ tissues that are not examined will be documented in the histopathology data and the impact of these missing tissues on the study will be documented in the pathology report. |

##

**Tissue Collection for Dogs**

Tissue Collection and Preservation

| Tissue | Weigh | Collect | Histology | Microscopic Evaluationa | Comment |
| --- | --- | --- | --- | --- | --- |
| Animal identification | ‑ | X | ‑ | ‑ | ‑ |
| Artery, aorta | ‑ | X | X | X | ‑ |
| Bone marrow | ‑ | X | ‑ | ‑ | Bone marrow smears will be collected from the 5th to 7th rib at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin. |
| Bone marrow, rib | ‑ | X | X | X | ‑ |
| Bone marrow, sternum | - | X | X | X |  |
| Bone marrow, femur | - | X | X | X |  |
| Bone, femur | ‑ | X | X | X | Collect distal femur‑ |
| Bone, rib | - | X | X | X |  |
| Bone, sternum | ‑ | X | X | X | ‑ |
| Brain | X | X | X | X | Five brain levels to be examined*\*\*\*Note that a 3-level scheme is also available if requested by the sponsor\*\*\** |
| Cervix | ‑ | X | X | X | ‑ |
| Epididymis | X | X | X | X | ‑ |
| Esophagus | ‑ | X | X | X | ‑ |
| Eye | ‑ | X | X | X | ‑ |
| Gallbladder | ‑ | X | X | X | ‑ |
| Gland, adrenal | X | X | X | X | ‑ |
| Gland, mammary | ‑ | X | X | X | ‑ |
| Gland, parathyroid | ‑ | X | X | X | ‑ |
| Gland, pituitary | X | X | X | X | ‑ |
| Gland, prostate | X | X | X | X | ‑ |
| Gland, salivary | ‑ | X | X | X | ‑ |
| Gland of the third eyelid (ocular accessory gland) | ‑ | X | X | X | - |
| Gland, thyroid | X | X | X | X | weigh with parathyroid |
| Gross lesions/masses | ‑ | X | X | X | ‑ |
| Heart | X | X | X | X | ‑ |
| Injection sites |  | X | X | X | Only if applicable |
| Kidney | X | X | X | X | ‑ |
| Large intestine, cecum | ‑ | X | X | X | ‑ |
| Large intestine, colon | ‑ | X | X | X | ‑ |
| Large intestine, rectum | ‑ | X | ‑ | ‑ | Collect but do not process |
| Liver | X | X | X | X | weigh with gallbladder (drained) |
| Lung | ‑ | X | X | X | ‑ |
| Lymph node, mandibular | ‑ | X | X | X | ‑ |
| Lymph node, mesenteric | ‑ | X | X | X | ‑ |
| Lymph node, other | ‑ | X | X | X | *\*\*\*Delete if not required for study\*\*\** Specify other lymph node collected (i.e. renal, popliteal, etc.). Collect lymph nodes that would be expected to receive primary exposure to the test item/article (i.e. lymph node draining the administration site. |
| Muscle, Quadriceps femoris  | ‑ | X | X | X | cross and longitudinal sections processed. |
| Muscle, Diaphragm | - | X | X | X | cross and longitudinal sections processed. |
| Nerve, optic | ‑ | X | X | X | ‑ |
| Nerve, sciatic | ‑ | X | X | X | ‑ |
| Ovary | X | X | X | X | ‑ |
| Pancreas | ‑ | X | X | X | ‑ |
| Skin | ‑ | X | X | X | ‑ |
| Small intestine, duodenum | ‑ | X | X | X | ‑ |
| Small intestine, ileum | ‑ | X | X | X | Include GALT in section if possible |
| Small intestine, jejunum | ‑ | X | X | X | ‑ |
| Spinal cord | ‑ | X | X | X | ‑ |
| Spleen | X | X | X | X | ‑ |
| Stomach | ‑ | X | X | X | ‑ |
| Testis | X | X | X | X | ‑ |
| Thymus | ‑ | X | X | X | ‑ |
| Tongue | ‑ | X | X | X | ‑ |
| Trachea | ‑ | X | X | X | ‑ |
| Urinary bladder | ‑ | X | X | X | ‑ |
| Uterus | ‑ | X | X | X | ‑ |
| Vagina | ‑ | X | X | X | ‑ |
| ‑ = Not applicable; X = Procedure to be conducted.a. Efforts will be made to evaluate all study plan/protocol-required‑ tissues microscopically; however, it is not always feasible for every study plan/protocol-required‑ tissue to be present on every slide. Study plan/protocol-required‑ tissues that are not examined will be documented in the histopathology data and the impact of these missing tissues on the study will be documented in the pathology report. |

**Tissue Collection for Monkeys**

Tissue Collection and Preservation

| Tissue | Weigh | Collect | Histology | Microscopic Evaluationa | Comment |
| --- | --- | --- | --- | --- | --- |
| Animal identification | ‑ | X | ‑ | ‑ | ‑ |
| Artery, aorta | ‑ | X | X | X | ‑ |
| Bone marrow | ‑ | X | ‑ | ‑ | Bone marrow smears will be collected from the 5th to 7th rib at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears are allowed to air dry and are not fixed in formalin. |
| Bone marrow, rib | ‑ | X | X | X | ‑ |
| Bone marrow, sternum |  | X | X | X |  |
| Bone marrow, femur |  | X | X | X |  |
| Bone, femur | ‑ | X | X | X | Collect distal femur |
| Bone, sternum | ‑ | X | X | X | ‑ |
| Bone, rib |  | X | X | X |  |
| Brain | X | X | X | X | Five brain levels to be examined. |
| Cervix | ‑ | X | X | X | Collect with uterus |
| Epididymis | X | X | X | X | Paired examination |
| Esophagus | ‑ | X | X | X | ‑ |
| Eye | ‑ | X | X | X | Paired examination; Fixed in Davidson’s fixative (all animals). |
| Gallbladder | ‑ | X | X | X | With adjacent liver |
| Gland, adrenal | X | X | X | X | Paired weight and examination |
| Gland, lacrimal (ocular accessory gland) | - | X | X | X |  |
| Gland, mammary | ‑ | X | X | X | ‑ |
| Gland, parathyroid | ‑ | X | X | X | Collect with thyroid; at least one examined. One recut/recheck of wet tissue will be conducted to find missing tissue |
| Gland, pituitary | X | X | X | X | ‑ |
| Gland, prostate/ Gland, seminal vesicle | X | X | X | X | - |
| Gland, salivary | ‑ | X | X | X | ‑ |
| Gland, thyroid | X | X | X | X | Paired weight and examination; weight willinclude parathyroid |
| Gross lesions/masses | ‑ | X | X | X | ‑ |
| Heart | X | X | X | X | Including sections of aorta |
| Injection site(s) | ‑ | X | X | X | ‑ |
| Kidney | X | X | X | X | Paired weight and examination. Cross and longitudinal sections collected and examined. |
| Large intestine, cecum | ‑ | X | X | X | ‑ |
| Large intestine, colon | ‑ | X | X | X | ‑ |
| Large intestine, rectum | ‑ | X | ‑ | ‑ | Collect but do not process |
| Liver | X | X | X | X | Weight with gallbladder (drained); sample of left and right lateral lobes |
| Lung | ‑ | X | X | X | Infuse with 10% neutral buffered formalin. Sample of right middle and left caudal lobes examined. All lobes retained |
| Lymph node, mandibular | ‑ | X | X | X | Only 1 required for examination |
| Lymph node, mesenteric | ‑ | X | X | X | ‑ |
| Lymph node, other | ‑ | X | X | X | Specify other lymph node collected (i.e. renal, popliteal, etc.). Collect lymph nodes that would be expected to receive primary exposure to the test item/article (i.e. lymph node draining the administration site. |
| Muscle, Quadriceps femoris  | ‑ | X | X | X | cross and longitudinal sections processed. |
| Muscle, Diaphragm | - | X | X | X | cross and longitudinal sections processed. |
| Nerve, optic | ‑ | X | X | X | Fixed in Davidson’s fixative (all animals) |
| Nerve, sciatic | ‑ | X | X | X | Only 1 required for examination. Cross and longitudinal sections processed and examined |
| Ovary | X | X | X | X | ‑ |
| Pancreas | ‑ | X | X | X | ‑ |
| Skin | ‑ | X | X | X | Dorsal thoracic |
| Small intestine, duodenum | ‑ | X | X | X | ‑ |
| Small intestine, ileum | ‑ | X | X | X | Include GALT in section, if possible. |
| Small intestine, jejunum | ‑ | X | X | X | ‑ |
| Spinal cord | ‑ | X | X | X | Cervical, thoracic, lumbar. Cross and longitudinal sections processed and examined. |
| Spleen | X | X | X | X | Spleen will be dissected free of fat prior to weighing. *To be included with spleen immunophenotyping:* The portion to be used for immunophenotyping will also be weighed |
| Stomach | ‑ | X | X | X | Cardiac, fundic, pyloric and non-glandular regions. |
| Testis | X | X | X | X | Paired weight and examination; fixed in Modified Davidson’s fixative (all animals) |
| Thymus | ‑ | X | X | X | Examined only if present in thymic area |
| Tongue | ‑ | X | X | X | ‑ |
| Trachea | ‑ | X | X | X | ‑ |
| Urinary bladder | ‑ | X | X | X | ‑ |
| Uterus | ‑ | X | X | X | Body |
| Vagina | ‑ | X | X | X | ‑ |
| ‑ = Not applicable; X = Procedure to be conducted.a. Efforts will be made to evaluate all study plan/protocol-required‑ tissues microscopically; however, it is not always feasible for every study plan/protocol-required‑ tissue to be present on every slide. Study plan/protocol-‑required tissues that are not examined will be documented in the histopathology data and the impact of these missing tissues on the study will be documented in the pathology report. |

**CNS ASSESSMENT OBSERVATIONS IN RODENTS**

Variations in the test conditions will be minimized to avoid systematic bias relating to dose group. Observational measurements will be made in the following order; however, due to system limitations, observations may not be documented in the exact order listed. Documentation of observations in the computer system or manually generated form indicates the observations were conducted in the correct order unless otherwise noted. Related Irwin observations will be grouped into functional domains (CNS activity [A], CNS excitability [E], sensorimotor [S], neuromuscular [N], physiological P], and autonomic [U]), as applicable, in the Final Report.

|  |  |
| --- | --- |
| **1. Home Cage Observations**Restlessness (E)Aggression to cagemate (E) Respiration (A)Excretion (U)**2. Body Temperature** (via Transponder; U)**3. Arena Assessments**Latency (A)Number of rears (A)Number of urine pools (U)Numbers of Fecal Boli (U)Activity (A)Alertness (E)Posture (P)Gait (N)Tail (N)Behavior-Stereotype (A)Behavior-Other (A)Involuntary movements (N)Auditory startle response (S)Approach response (S) | **4. Handheld Observations**Reactivity to Handling (E)Vocalization (E)Body Tone (P)Extensor Thrust (N)Lacrimation (U)Eyes (P)Eye closure (N)Salivation (U)Discharge (U)Skin color (P)Pelage (P)**5. Elicited Behaviors**Visual Response (S)Bar Test (N)Waxy Rigidity (N)Grip Strength (N)Pinna Response (S)Palpebral Reflex (U)Pupil Status (U)Pupil Response (A)Grasping Loss (N)Righting Reflex (N)Proprioception (N)Pain Response (S)**6. Body Temperature** (via Transponder or an Alternative Method; U) |

# SUMMARY OF CHANGES AND JUSTIFICATIONS

*When a protocol amendment is needed this template may be used and included in the beginning of the protocol, after the title page. Be sure to include the Amendment #, amendment and section(s), reason for change, and date of Study Director signature. For a subsequent amendment be sure to copy and paste “Amendment #” and “Date of Study Director Signature:<Date>” fields to the next empty row and add rows as needed.*

This Protocol Amendment includes changes to the following portions of the Protocol.

Note: When applicable, additions are indicated by **bold and underlined** text and

deletions are indicated by double strikethrough text directly to the affected sections of the

document. Section and/or page numbering may be renumbered in the Protocol or Table of Contents to account for additions or removal of sections, as applicable; these changes will not be detailed in this summary of changes.

The Protocol was issued on <Date>.

| **Amendment and Section(s)** | **Reason for Change** |
| --- | --- |
| **Amendment <#>** | **Date of Study Director Signature: <Date>** |
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