



PRECLINICAL SAFETY TESTING PROGRAM FOR PHARMA/BIOTECH
Sample Study Designs

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Introduction

Toxikon is an ISO 17025 accredited contract research organization providing GLP and GMP laboratory support for toxicology, pharmacology, in-vitro, and analytical testing services. We serve various size research institutions, government agencies, biotech, device and combination products, and pharmaceutical organizations worldwide. Our preclinical and other early stage product development services assure product safety, regulatory compliance, and timely and professionally prepared reports to our customers for their Investigational New Drug (IND) and New Drug Application (NDA) submissions.

Our knowledgeable and experienced scientists and technical staff design and implement standard and customized studies. Toxikon has the ability to use a wide variety of small and large animal models including: mice, rats, guinea pigs, rabbits, dogs, cats, hamsters, goats, swine, and non-human primates. Toxikon also has GLP surgical suites that are fully equipped with technology to assess various indications. Toxikon specializes in performing drug safety studies in rodents and non-rodents with various administration routes available, such as oral, intravenous, ocular, subcutaneous, and intraperitoneal.

Study Design & Pricing Considerations

Study pricing is dependent on a number of variables for each study type, including overall design and evaluation parameters. The attached Sample Designs are included as an example of the types of studies performed at Toxikon. Study pricing is dependent on a number of study design factors and are quoted based on study outlines. The estimated ranges reflect the differences in price based on various options for:

- **Dose route:** Oral, IV bolus, IV continuous infusion, subcutaneous, intraperitoneal, intramuscular, dermal, topical ocular, intravitreal, etc.
- **Satellite Recovery Group:** In addition to main study animals it is recommended that Repeat Dose Studies include a satellite group for Recovery (High and Control doses). The Recovery group would be dosed for the same frequency as main study but would be scheduled for follow-up observations without dosing for a pre-determined period of time (i.e. 7, 14, or 28 days post-last dose). The intent of the observation period is to detect possible delayed occurrence, or persistence of, or recovery from toxic effects.
- **Clinical Pathology Evaluation:** Pricing is determined by Sponsor's specification of clinical pathology parameters. Standard clinical pathology options include:
 - Clinical Chemistry
 - Basic Chemistry Panel: Albumin, Alkaline Phosphatase, Bilirubin/Total, BUN, Calcium, Chloride, Cholesterol, Protein/Total, Creatinine, Sodium, ALT, AST, Phosphorus, Potassium, Glucose
 - Comprehensive Chemistry Panel: Basic Chemistry Panel, plus GGT, Uric Acid, Triglycerides
 - Hematology Parameters
 - Basic CBCD includes: Automated WBC, RBC, Hgb, Hct, Platelet Count, MPV, MCV, MCH, MCHC, and WBC Differential
 - Reticulocytes Count
 - Coagulation
 - Prothrombin Time (PT)
 - Activated Partial Thromboplastin Time (APTT)
 - Urinalysis Testing: Dipstick and/or Microscopic evaluation
- **Necropsy:**
 - Gross Necropsy: To examine the organs for potential abnormalities and lesions
 - Detailed: To examine all the organs and harvest for histopathological examination
- **Organ Weights:** As per Sponsor's specifications; standard organs are brain, heart, kidney, liver, lungs, ovaries/testes, spleen, thyroid, thymus, pituitary, adrenal.
- **Histology:** Prices will reflect standard processing of tissues by preservation in formalin, paraffin embedding, and slides with H&E or Trichrome stains. Other options are available at additional cost, such as immunohistochemistry and other specialized staining or preparation.
- **Pathology:** Histopathology is usually done on high dose and control groups. Organs from the mid and low dose groups are preserved for prospective tissue evaluation.

**Table 1:
Available Options for Tissue Collection, Organ Weights, and Pathology Evaluation**

Tissue	Standard List of Organ Weights per ICH	Example Partial Tissue List for Microscopic Examination—Main Tissues	Full Tissue List for Microscopic Examination
Adrenal gland	X		X
Aorta			X
Bone marrow smears			X
Bone with bone marrow, femur			X
Bone with bone marrow, sternum			X
Brain (cerebrum, cerebellum, medulla/pons)	X	X	X
Clitoral Glands (females)			X ^a
Epididymis (males)		X	X
Esophagus			X
Eyes (w/optic nerve)			X
Harderian gland			X ^a
Heart	X	X	X
Kidney	X	X	X
Lacrimal Gland (exorbital, L/R)			X
Large intestine, cecum			X
Large intestine, colon			X
Large intestine, rectum			X
Liver	X	X	X
Lung (with mainstem bronchi)	X	X	X
Lymph node, mesenteric			X
Lymph nodes, mandibular (L/R)			X
Mammary gland (females)			X
Nerve, sciatic			X
Ovaries (females)	X		X
Oviducts (females)			X
Pancreas			X
Pituitary gland	X		X
Prostate (males)			X
Salivary gland, mandibular (L/R)			X
Seminal vesicle (males)			X

Tissue	Standard List of Organ Weights per ICH	Example Partial Tissue List for Microscopic Examination—Main Tissues	Full Tissue List for Microscopic Examination
Skeletal muscle, biceps femoris			X
Skin			X
Small intestine, duodenum ^b			X
Small intestine, ileum ^b			X
Small intestine, jejunum ^b			X
Spinal cord (cervical, mid-thoracic, lumbar)			X
Spleen	X	X	X
Stomach (glandular and non-glandular) ^b		X	X
Testes (males)	X*		X
Thymus	X		X
Thyroid (with parathyroid)	X		X
Tongue			X
Trachea			X
Ureter			X
Urinary bladder			X
Uterus with cervix (females)	X		X
Vagina (females)			X
Gross lesions			X

* Organ weights recorded for beagles only, does not apply to rats.

^a Tissues collected for rats only, does not apply to dogs.

^b Small intestine and stomach organ weights will include weights with and without contents.

PHARMACOKINETIC STUDIES—SAMPLE DESIGNS

Single Dose PK Study in Sprague Dawley Rats

Study Design

Species	Dose Route	No. of Animals per dose group	Total No. of Dose Groups	Collection Timepoints	Sample Collection
Sprague Dawley Rats	As specified	6	5	Approximately 8 timepoints up to 24 hours post-dose	As specified (i.e. whole blood, plasma, serum)

Animals: 30 Sprague Dawley rats

Dose Frequency: Single dose

Dose Route: To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)

Dose Formulation: Per Sponsor specifications

Collection Times: Standard collection time points are within normal working hours, 8:00am-5:00pm Monday through Friday. Sponsor-specified collections (i.e. 12 or 18 hour) outside of standard business hours may incur additional charges.

Blood Processing: Whole blood samples will be collected and immediately placed on wet ice until processed. A portion of each blood sample will be processed as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored at $-80 \pm 10^{\circ}\text{C}$. Samples will be shipped to Sponsor for analysis

Compliance: Non-GLP

Deliverables: Summary table with animal designation of dose group and collection time points

PHARMACOKINETIC STUDIES—SAMPLE DESIGNS (cont.)

Single Dose PK Study in Non-Naïve Beagle Dogs—Parallel Session

Study Design

Groups	Test Material	Animals per group	Dose Group	Dose Route	Approx. Time points
A	TBD	2M/2F	XX mg/kg	TBD	Approximately 8 timepoints up to 24 hours post-dose
B		2M/2F	XX mg/kg		

Total Animals: 8 Beagle Dogs (non-naïve)

No. of Dose Sessions: One session does in parallel

Dose Frequency: Single dose

Dose Route: To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)

Dose Formulation: Per Sponsor specifications

Collection Times: Standard collection time points are within normal working hours, 8:00am-5:00pm Monday through Friday. Sponsor-specified collections (i.e. 12 or 18 hour) outside of standard business hours will incur additional charges.

Blood Processing: Whole blood samples will be collected and immediately placed on wet ice until processed. A portion of each blood sample will be processed as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored at $-80 \pm 10^{\circ}\text{C}$. Samples will be shipped to Sponsor for analysis.

Compliance: Non-GLP

Deliverables: Summary table with animal designation of dose group and collection time points

PHARMACOKINETIC STUDIES—SAMPLE DESIGNS (cont.)

Single Dose PK Study in Non-Naïve Beagle Dogs —4 Crossover Sessions

Study Design

Species	Dosing Session	No. of Animals	Dose Route	Dose Frequency	Dose (mg/kg)	Blood Collection Time Points
Beagle Dogs (Naïve)	1	3M/3F	TBD	1x	TBD	Approximately 8 timepoints up to 24 hours post-dose
	2			1x	TBD	Approximately 8 timepoints up to 24 hours post-dose
	3			1x	TBD	Approximately 8 timepoints up to 24 hours post-dose
	4			1x	TBD	Approximately 8 timepoints up to 24 hours post-dose

Total Animals: Estimated 6 Beagle Dogs (non-naïve)

No. of Dose Sessions: 4 Sessions

Washout Period: As specified by Sponsor; minimum of 48 hours between doses

Dose Frequency: Single dose

Dose Route: To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)

Dose Formulation: Per Sponsor specifications

Collection Times: Standard collection time points are within normal working hours, 8:00am-5:00pm Monday through Friday. Sponsor-specified collections (i.e. 12 or 18 hour) outside of standard business hours will incur additional charges.

Blood Processing: Whole blood samples will be collected and immediately placed on wet ice until processed. A portion of each blood sample will be processed as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored at $-80 \pm 10^{\circ}\text{C}$. Samples will be shipped to Sponsor for analysis.

Compliance: Non-GLP

Deliverables: Summary table with animal designation of dose group and collection time points

PHARMACOKINETIC STUDIES—SAMPLE DESIGNS (cont.)

Repeat Dose PK Study in Non-Naïve Beagle Dogs

Study Design

Species	No. of Animals	Dose Route	Dose Frequency	Sample Collection	Collection Timepoints
Beagle Dog (non-naïve)	4 males/ 4 females	As requested	Once daily for 5-7 days	Clinical Pathology	Pre-dose and post-final dose
				TK	Post- final dose for approximately 6 timepoints (ex. 0.5, 1, 2, 4, 8, and 24 hours)

Total Animals: 8 Beagle Dogs (non-naïve)

Dose Frequency: Daily for 5-7 days

Dose Route: To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)

Dose Formulation: Per Sponsor specifications

Clinical Pathology: As specified by Sponsor. Options include clinical chemistry, hematology, coagulation, and/or reticulocytes.

TK Blood Processing: Whole blood samples will be collected and immediately placed on wet ice until processed. A portion of each blood sample will be processed as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored at $-80 \pm 10^{\circ}\text{C}$. Samples will be shipped to Sponsor for analysis.

Compliance: Non-GLP

Deliverables: Report with observations and dose administration table for in-life phase.

ACUTE TOXICITY—SAMPLE DESIGNS

Single-Dose Toxicity Study in Sprague Dawley Rats

Study Design

Dose Groups	No. of Animals	Dose Route	Dose Volume (mL/Kg)
1	3M/3F	TBD	TBD
2	3M/3F		TBD
3	3M/3F		TBD
4	3M/3F		TBD

Total Animals: 3/gender/group

No. of Dose Groups: 4 groups

Dose Frequency: Single dose

Dose Route: To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)

Study Duration: Up to 7 days

Clinical Observations: Daily

Body Weights: Pre-dose and prior to sacrifice

Clinical Pathology: Terminal collection. Options include clinical chemistry, hematology, and/or coagulation parameters.

Necropsy: Gross

Organ weights: As specified by Sponsor

Histopathology: As specified by Sponsor (i.e. Lesions only, target organs, or main organs); Standard histology includes paraffin embedding and slides prepared with H&E

Deliverables: Report with observations and dose administration table for in-life phase, as well as any clinical pathology, gross observations, and pathology results, as applicable.

Compliance: Non-GLP

ACUTE TOXICITY—SAMPLE DESIGNS (cont.)

Single-Dose Toxicity Study in Beagle Dogs

Study Design

Total Animals: 4 Beagle Dogs (2 M/2F)

Dose Frequency: Single dose

Dose Route: To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)

Study Duration: 48 hours

Clinical Observations: Daily

Body Weights: Pre-dose and study end

Clinical Pathology: Collection pre-dose, 24 and 48 hours post-dose. Options include clinical chemistry, hematology, and/or coagulation parameters.

Necropsy: Gross

Organ weights: As specified by Sponsor

Histopathology: As specified by Sponsor (i.e. Lesions only, target organs, or main organs); Standard histology includes paraffin embedding and slides prepared with H&E

Deliverables: Report with observations and dose administration table for in-life phase, as well as any clinical pathology, gross observations, and pathology results, as applicable.

Compliance: Non-GLP

ACUTE TOXICITY—SAMPLE DESIGNS (cont.)

MTD Determination with Repeat Dose Range-Finding in Sprague Dawley Rats

Study Design

Phase I: Single Dose MTD Determination

Animals: 24 Sprague Dawley Rats in four groups (3/sex/group)

Study Duration: Up to 7 days

Dosing: Single dose in an up/down procedure; 2 days between doses

Dose Route: As specified by Sponsor (i.e. Oral, iv, ip)

Clinical Observation: Twice daily

Body Weights: Pre-dose and Day 7

Blood Draws: Terminal for clinical chemistry and hematology

Gross Necropsy

Organ weights: Standard by ICH/OECD guidelines

Histopathology: Lesions as applicable; additional charges will be incurred for processing and evaluation

Phase II: 7-Day Repeat Dose Range-Finding Study in Sprague Dawley Rats

Animals: 24 Sprague Dawley Rats in four groups (3/sex/group)

Study Duration: 7 days

Dosing: Daily for 7 days

Dose Route: As specified by Sponsor (i.e. Oral, iv, ip)

Clinical Observation: Twice daily

Body Weights: Daily

Food Consumption: Daily

Clinical Pathology: Terminal draws for clinical chemistry and hematology

Gross Necropsy: Preserve any identified tissue lesions

Organ Weights: Standard by ICH/OECD guidelines

Histopathology: Lesions as applicable; additional charges will be incurred for preservation, processing, and evaluation

Deliverables: Report with observations and dose administration table for in-life phase, as well as clinical pathology, gross observations, and pathology results, as applicable.

ACUTE TOXICITY—SAMPLE DESIGNS (cont.)**MTD Determination with Repeat Dose Range-Finding in Beagle Dogs***Study Design***Phase I: Single Acute Dose****Animals:** 4 naïve Beagle dogs (2/sex)**Study Duration:** Approximately 9 days**Dosing:** Up to four dose administrations of a single dose with a sponsor-specified washout period between escalating doses**Dose Route:** As specified by Sponsor (i.e. Oral, iv, ip)**Clinical Observation:** Twice Daily**Body Weights:** Prior to dose administration**Blood Draws:** One sample per treatment for TK**Phase II: 7-Day Repeat Dose Range-Finding Study****Animals:** 24 naïve Beagle dogs (3/sex/group)**Study Duration:** 7 days**Dosing:** Daily for 7 days**Dose Route:** As specified by Sponsor (i.e. Oral, iv, ip)**Clinical Observation:** Twice Daily**Body Weights:** Daily**Blood Draws:** Pre-dose and study end for clinical chemistry and hematology**TK Blood Draws:** Days 1 and 7 for 6-9 timepoints per day**Gross Necropsy****Organ Weights:** Standard by ICH/OECD guidelines**Histopathology:** Lesions as applicable; additional charges will be incurred for preservation, processing, and evaluation**Deliverables:** Report with observations and dose administration table for in-life phase, as well as clinical pathology, gross observations, and pathology results, as applicable.

REPEAT DOSE TOXICITY STUDIES—GLP

14-Day Repeat Dose Toxicity Study in Rats

Study Design

	Group	Main Study		Optional: Recovery		Optional: Satellite TK	
		Males	Females	Males	Females	Males	Females
Sprague Dawley Rats	Control	10	10	5	5	0	0
	Low	10	10	0	0	6	6
	Mid	10	10	0	0	6	6
	High	10	10	5	5	6	6
Total # of Animals		N= 80		N= 20		N=48	
Dose Route	To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)						
Dose Frequency	Daily for 14 days						
In-Life Study Duration	14 days dosing, plus Recovery Period (i.e. 7 or 14 days)						
Dose Formulation	As specified by Sponsor—additional charges may apply						
Dose Sampling	Sampling on first and last dose days for dose verification (<i>Note: Bioanalytical sample analysis not included in quote</i>)						

Evaluation Parameters

Clinical Observation	Daily
Food Consumption	Weekly
Body Weights	Weekly
Ophthalmic Examination	As specified by Sponsor. Suggested prior to first dose and during week 2.
Clinical Pathology	Collection from main animals and recovery animals (if applicable) at sacrifice; standard panels include for clinical chemistry, hematology, coagulation, and/or urinalysis
Toxicokinetic Blood Draws for Satellite TK Group	Days 1 & 14; 3 rats/sex/timepoint for up to six timepoints per day. A refrigerated centrifuge will be used to process whole blood, as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored. Samples will be shipped to Sponsor on dry ice upon receipt of Sponsor request.
Necropsy	Main and recovery animals
Organ Weights	Standard tissues per ICH/OECD guidelines
Tissue Preservation	Mid and low level group tissues stored in formalin
Histology/Pathology	Standard tissues on high and control animals in main and recovery groups; Sponsor to specify request for histopathology on all tissues or preserved tissues
Reporting, Statistics, and Review	Standard GLP report

REPEAT DOSE TOXICITY STUDIES—GLP (cont.)

14-Day Repeat Dose Toxicity Study in Beagle Dogs

Study Design

	Group	Main Study		Optional: Recovery	
		Males	Females	Males	Females
Beagle Dogs	Control	4	4	3	3
	Low	4	4	0	0
	Mid	4	4	0	0
	High	4	4	3	3
Total # of Animals		N=32		N= 12	
Dose Route	To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)				
Dose Frequency	Daily for 14 days				
In-Life Study Duration	14 days dosing, plus Recovery Period (i.e. 7 or 14 days)				
Dose Formulation	As specified by Sponsor—additional charges may apply				
Dose Sampling	Sampling on first and last dose days for dose verification (<i>Note: Bioanalytical sample analysis not included in quote</i>)				

Evaluation Parameters

Clinical Observation	Daily
Food Consumption	Weekly
Body Weights	Weekly
Ophthalmic Examination	As specified by Sponsor. Suggested prior to first dose and during week 2.
Clinical Pathology	All animals pre-dose and at sacrifice; standard panels for clinical chemistry, hematology, and coagulation; terminal collection for urinalysis
Toxicokinetic Blood Draws	Approximately six timepoints on Day 1 and Day 14
TK Blood Processing	A refrigerated centrifuge will be used to process whole blood to plasma. Plasma samples will be directly transferred to appropriate tubes and stored at -80 ± 10°C. Samples will be shipped to Sponsor on dry ice upon receipt of shipping request.
Necropsy	Main and recovery animals
Organ Weights	Standard tissues per ICH/OECD guidelines
Tissue Preservation	Mid and low level group tissues stored in formalin
Histology/Pathology	Standard tissues on high and control animals in main and recovery groups; Sponsor to specify request for histopathology on all tissues or preserved tissues
Reporting, Statistics, and Review	Standard GLP report

REPEAT DOSE TOXICITY STUDIES—GLP

28-Day Repeat Dose Toxicity Study in Rats

Study Design

	Group	Main Study		Optional: Recovery		Optional: Satellite TK	
		Males	Females	Males	Females	Males	Females
Sprague Dawley Rats	Control	10	10	5	5	0	0
	Low	10	10	0	0	6	6
	Mid	10	10	0	0	6	6
	High	10	10	5	5	6	6
Total # of Animals		N= 80		N= 20		N=48	
Dose Route	To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)						
Dose Frequency	Daily for 28 days						
In-Life Study Duration	28 days dosing, plus Recovery Period (i.e. 14 days)						
Dose Formulation	As specified by Sponsor—additional charges may apply						
Dose Sampling	Sampling on first and last dose days for dose verification (<i>Note: Bioanalytical sample analysis not included in quote</i>)						

Evaluation Parameters

Clinical Observation	Daily
Food Consumption	Weekly
Body Weights	Weekly
Ophthalmic Examination	As specified by Sponsor. Suggested prior to first dose and during week 4.
Clinical Pathology	Collection from main animals and recovery animals (if applicable) at sacrifice; standard panels include for clinical chemistry, hematology, coagulation, and/or urinalysis
Toxicokinetic Blood Draws for Satellite TK Group	Days 1 & 28; 3 rats/sex/timepoint for up to six timepoints per day. A refrigerated centrifuge will be used to process whole blood, as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored. Samples will be shipped to Sponsor on dry ice upon receipt of Sponsor request.
Necropsy	Main and recovery animals, as applicable
Organ Weights	Standard tissues per ICH/OECD guidelines
Tissue Preservation	Mid and low level group tissues stored in formalin
Histology/Pathology	Standard tissues on high and control animals in main and recovery groups; Sponsor to specify request for histopathology on all tissues or preserved tissues
Reporting, Statistics, and Review	Standard GLP report

REPEAT DOSE TOXICITY STUDIES—GLP (cont.)

28-Day Repeat Dose Toxicity Study in Beagle Dogs

Study Design

	Group	Main Study		Optional: Recovery	
		Males	Females	Males	Females
Beagle Dogs	Control	4	4	3	3
	Low	4	4	0	0
	Mid	4	4	0	0
	High	4	4	3	3
Total # of Animals		N=32		N= 12	
Dose Route	To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)				
Dose Frequency	Daily for 28 days				
In-Life Study Duration	28 days dosing, plus Recovery Period (i.e. 14 days)				
Dose Formulation	As specified by Sponsor—additional charges may apply				
Dose Sampling	Sampling on first and last dose days for dose verification (<i>Note: Bioanalytical sample analysis not included in quote</i>)				

Evaluation Parameters

Clinical Observation	Daily
Food Consumption	Weekly
Body Weights	Weekly
Ophthalmic Examination	As specified by Sponsor. Suggested prior to first dose and during week 4.
Clinical Pathology	All animals pre-dose and at sacrifice; standard panels for clinical chemistry, hematology, and coagulation; terminal collection for urinalysis
Toxicokinetic Blood Draws	Approximately six timepoints on Day 1 and Day 28
TK Blood Processing	A refrigerated centrifuge will be used to process whole blood to plasma. Plasma samples will be directly transferred to appropriate tubes and stored at $-80 \pm 10^{\circ}\text{C}$. Samples will be shipped to Sponsor on dry ice upon receipt of shipping request.
Necropsy	Main and recovery animals
Organ Weights	Standard tissues per ICH/OECD guidelines
Tissue Preservation	Mid and low level group tissues stored in formalin
Histology/Pathology	Standard tissues on high and control animals in main and recovery groups; Sponsor to specify request for histopathology on all tissues or preserved tissues
Reporting, Statistics, and Review	Standard GLP report

REPEAT DOSE TOXICITY STUDIES—GLP

90-Day Repeat Dose Toxicity Study in Rats

Study Design

	Group	Main Study		Optional: Recovery		Optional: Satellite TK	
		Males	Females	Males	Females	Males	Females
Sprague Dawley Rats	Control	10	10	10	10	0	0
	Low	10	10	0	0	6	6
	Mid	10	10	0	0	6	6
	High	10	10	10	10	6	6
Total # of Animals		N= 80		N= 40		N=48	
Dose Route	To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)						
Dose Frequency	Daily for 90 days						
In-Life Study Duration	90 days dosing, plus Recovery Period (i.e. 14 or 28 days)						
Dose Formulation	As specified by Sponsor—additional charges may apply						
Dose Sampling	Sampling on first and last dose days for dose verification (<i>Note: Bioanalytical sample analysis not included in quote</i>)						

Evaluation Parameters

Clinical Observation	Daily
Food Consumption	Weekly
Body Weights	Weekly
Ophthalmic Examination	As specified by Sponsor. Suggested prior to first dose and prior to sacrifice.
Clinical Pathology	Collection from main animals and recovery animals (if applicable) at sacrifice; standard panels include for clinical chemistry, hematology, coagulation, and/or urinalysis
Toxicokinetic Blood Draws for Satellite TK Group	Days 1, 45, & 90; 3 rats/sex/timepoint for up to six timepoints per day. A refrigerated centrifuge will be used to process whole blood, as specified by Sponsor. Samples will be directly transferred to appropriate tubes and stored. Samples will be shipped to Sponsor on dry ice upon receipt of Sponsor request.
Necropsy	Main and recovery animals, as applicable
Organ Weights	Standard tissues per ICH/OECD guidelines
Tissue Preservation	Mid and low level group tissues stored in formalin
Histology/Pathology	Standard tissues on high and control animals in main and recovery groups; Sponsor to specify request for histopathology on all tissues or preserved tissues
Reporting, Statistics, and Review	Standard GLP report

REPEAT DOSE TOXICITY STUDIES—GLP (cont.)

90-Day Repeat Dose Toxicity Study in Beagle Dogs

Study Design

	Group	Main Study		Optional: Recovery	
		Males	Females	Males	Females
Beagle Dogs	Control	4	4	3	3
	Low	4	4	0	0
	Mid	4	4	0	0
	High	4	4	3	3
Total # of Animals		N=32		N= 12	
Dose Route	To be specified (ex. oral, bolus iv, infusion, subcutaneous injection, intraperitoneal injection, topical ocular, etc.)				
Dose Frequency	Daily for 90 days				
In-Life Study Duration	90 days dosing, plus Recovery Period (i.e. 14-28 days)				
Dose Formulation	As specified by Sponsor—additional charges may apply				
Dose Sampling	Sampling on first and last dose days for dose verification (<i>Note: Bioanalytical sample analysis not included in quote</i>)				

Evaluation Parameters

Clinical Observation	Daily
Food Consumption	Weekly
Body Weights	Weekly
Ophthalmic Examination	As specified by Sponsor. Suggested prior to first dose and prior to sacrifice.
Clinical Pathology	All animals pre-dose and at sacrifice; standard panels for clinical chemistry, hematology, and coagulation; terminal collection for urinalysis
Toxicokinetic Blood Draws	Approximately six timepoints on Day 1, 45, and 90
TK Blood Processing	A refrigerated centrifuge will be used to process whole blood to plasma. Plasma samples will be directly transferred to appropriate tubes and stored at $-80 \pm 10^{\circ}\text{C}$. Samples will be shipped to Sponsor on dry ice upon receipt of shipping request.
Necropsy	Main and recovery animals
Organ Weights	Standard tissues per ICH/OECD guidelines
Tissue Preservation	Mid and low level group tissues stored in formalin
Histology/Pathology	Standard tissues on high and control animals in main and recovery groups; Sponsor to specify request for histopathology on all tissues or preserved tissues
Reporting, Statistics, and Review	Standard GLP report

GENOTOXICITY STUDIES

Ames Reverse mutation assay in *S. typhimurium* and *E.coli*

The test is conducted per OECD 471 using four strains of *S.typhimurium* containing a mutation in the histidine gene and one strain of *E.coli* containing a mutation in the tryptophan gene. To determine toxicity and select test concentrations, an initial range finding study is conducted Conducted with Salmonella typhimurium TA98 and/or TA100 using 6 concentrations of the test substance in the absence of metabolic activation. The definitive assay is conducted with 4 strains of *S.typhimurium* and one strain of *E.coli* in the presence and absence of metabolic activation by plate incorporation method using concentrations based on the range finding study. A confirmatory assay is performed in the event of negative results using a modified method of exposure, such as pre-incubation. *Potential solvents include: USP Water for Injection (WFI), USP 0.9% Sodium Chloride for Injection (NaCl), Dimethylsulfoxide (DMSO), Acetone, 95% Ethanol (EtOH), and Cottonseed Oil.*

Chromosomal Aberration Assay with CHO cells

The test is conducted per OECD 473 using the Chinese hamster ovary (CHO) cell line. The cells are exposed to the test substance at three concentrations in the presence and absence of metabolic activation. An initial range finding assay is performed to determine the toxicity of the test substance and to select the test concentrations. Metaphase cells (N200) in the negative and test groups are examined for structural chromosomal aberrations. Specific positive control substances are used in the activated and non-activated conditions. A confirmatory assay is performed in the event of negative results and uses an extended period of exposure.

Chromosomal Aberration Assay with Human Lymphocytes

The test is conducted per OECD 473 using Human Lymphocytes. An initial range finding assay is performed to determine the toxicity of the test substance and to select the test concentrations. The cells are exposed to the test substance at three concentrations in the presence and absence of metabolic activation. Metaphase cells (N200) in the negative and test groups are examined for structural chromosomal aberrations. Specific positive control substances are used in the activated and non-activated conditions. A confirmatory assay is performed in the event of negative results and uses an extended period of exposure. *Potential solvents include: Ham's F12, Dimethylsulfoxide (DMSO), and 95% Ethanol (EtOH).*

Rodent Bone Marrow Micronucleus Test

The test is conducted as per OECD 474. An initial range finding study is performed using a maximum dose of 2,000-mg/kg body weight followed by four lower concentrations of the test substance to determine toxicity. Animals are observed for signs of toxicity such as, loss of weight, clinical symptoms of toxicity, and alteration of ratio of immature to mature red blood cells in the bone marrow. In the event of toxicity, the definitive test will be performed using three doses of the test substance including a dose with partial toxicity as the highest dose. In the definitive assay male and female mice are administered the test substance by an appropriate route of administration. Bone marrow smears are prepared after 24 and 48 hours of treatment. The treatment groups consist of negative, test, and positive control groups. The bone marrow smears are examined to count approximately 2,000 immature and a corresponding number of mature erythrocytes per animal in each group. Clastogenicity is measured as the proportion of micronucleated erythrocytes. *Potential solvents include: USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil.*

Dose-Verification Study

High Performance Liquid Chromatography (HPLC) will be used to confirm doses for the Ames Assay, Chromosomal Aberration, and Mouse Micronucleus studies.

DEVELOPMENTAL & REPRODUCTIVE TOXICOLOGY

Embryo Fetal Development Study in Rats—Dose Range Finding

Study Design

	Group	Females
New Zealand White Rabbits	Control	6
	1	6
	2	6
	3	6
	4	6
Total # of Animals	<i>N</i> = 30	
Dose Administration	TBD	
Dose Frequency	Daily on Gestation Days (GD) 8-21	
Dose Formulation	As specified by Sponsor—additional charges may apply	
Dose Sampling	Sampling on first and last dose days for verification by Sponsor	

Evaluation Parameters

Observations	Twice daily for Clinical observations & Mortality/Moribundity check
Body Weights	Prior to randomization, and twice weekly from start of dose period to sacrifice
Food Consumption	Along same schedule as body weight measurement
Blood Draws	None
C-Section	GD 22
Fetal Observations	Body weight and external exam
Reporting, Statistics, and Review	Standard GLP Report
Compliance	GLP

DEVELOPMENTAL & REPRODUCTIVE TOXICOLOGY (cont.)

Embryo Fetal Development Study in Rats—Definitive

Study Design

	Group	Females
Sprague Dawley Rats	Control	25
	Low	25
	Mid	25
	High	25
Total # of Animals	<i>N= 100</i>	
Dose Administration	TBD	
Dose Frequency	Daily on Gestation Days (GD) 7-20	
Dose Formulation	As specified by Sponsor—additional charges may apply	
Dose Sampling	Sampling on first and last dose days for verification by Sponsor	

Evaluation Parameters

Observations	Twice daily for Clinical observations & Mortality/Moribundity check
Body Weights	Prior to randomization, and GD 0, 4, 7, 11, 14, 17, 20, 21, and prior to sacrifice
Food Consumption	Along same schedule as body weight measurement
Blood Draws	None
C-Section	GD 22
Fetal Observations	Gender, body weight, and gross external exam for all fetuses; Visceral exam, skeletal exam, and head evaluations for 1/2 of each litter
Reporting, Statistics, and Review	Standard GLP Report
Compliance	GLP

DEVELOPMENTAL & REPRODUCTIVE TOXICOLOGY (cont.)

Embryo Fetal Development Study in Rabbits—Dose Range Finding

Study Design

	Group	Females
New Zealand White Rabbits	Control	6
	1	6
	2	6
	3	6
	4	6
Total # of Animals	<i>N</i> = 30	
Dose Administration	TBD	
Dose Frequency	Daily on Gestation Days (GD) 8-21	
Dose Formulation	As specified by Sponsor—additional charges may apply	
Dose Verification	Sampling on first and last dose days for verification by Sponsor	

Evaluation Parameters

Observations	Twice daily for Clinical observations & Mortality/Moribundity check
Body Weights	Prior to randomization, and twice weekly from start of dose period to sacrifice
Food Consumption	Along same schedule as body weight measurement
Blood Draws	None
C-Section	GD 30
Fetal Observations	Body weight and external exam
Reporting, Statistics, and Review	Standard GLP Report
Compliance	GLP

DEVELOPMENTAL & REPRODUCTIVE TOXICOLOGY (cont.)

Embryo Fetal Development Study in Rabbits—Definitive

Study Design

	Group	Females
New Zealand White Rabbits	Control	20
	Low	20
	Mid	20
	High	20
Total # of Animals	<i>N</i> = 80	
Dose Administration	TBD	
Dose Frequency	Daily on Gestation Days (GD) 8-21	
Dose Formulation	As specified by Sponsor—additional charges may apply	
Dose Sampling	Sampling on first and last dose days for verification by Sponsor	

Evaluation Parameters

Observations	Twice daily for Clinical observations & Mortality/Moribundity check
Body Weights	Prior to randomization, and GD 0, 4, 8, 11, 14, 17, 20, 23, 26, 29, and prior to sacrifice
Food Consumption	Along same schedule as body weight measurement
Blood Draws	None
C-Section	GD 30
Fetal Observations	Gender, body weight, and gross external exam for all fetuses; Visceral exam and skeletal for 1/2 of each litter
Reporting, Statistics, and Review	Standard GLP Report
Compliance	GLP

DEVELOPMENTAL & REPRODUCTIVE TOXICOLOGY (cont.)

Fertility Study in Rats

Study Design

	Group	Males	Females
Sprague Dawley Rats	Control	25	25
	Low	25	25
	Mid	25	25
	High	25	25
Total # of Animals	N= 200		
Dose Administration	TBD		
Dose Frequency	<u>Females:</u> Daily for a minimum of two weeks prior to cohabitation, during cohabitation (up to 21 days), and from GD 1-GD 7 <u>Males:</u> Daily for four weeks prior to cohabitation and until necropsy (scheduled on confirmation of mating)		
Dose Formulation	As specified by Sponsor—additional charges may apply		
Dose Sampling	Sampling on first and last dose days for verification by Sponsor		

Evaluation Parameters

Observations	Twice daily for Clinical observations & Mortality/Moribundity check
Body Weights	<u>Females:</u> Prior to randomization, twice weekly while not pregnant, and GD0, 4, 7, 10, 13, 16 and prior to sacrifice <u>Males:</u> Prior to randomization, and twice weekly
Food Consumption	Along same schedule as body weight measurement
Blood Draws	None
Vaginal Cytology	Daily for a minimum of two weeks prior to cohabitation and during cohabitation until mating confirmed
Sperm Analysis	Sperm motility on all groups; Sperm density, morphology, and spermatid head count for control and high group animals
Necropsy	<u>Females:</u> Gross necropsy at GD 14-16, including # of corpora lutea, # of implantations with type and placement, and uterine weight <u>Males:</u> Gross necropsy after completion of female necropsies
Histopathology	<u>Females:</u> Ovaries, uterus, and all gross lesions preserved <u>Males:</u> Gross lesions preserved; Pathological examination of testes and epididymis
Reporting, Statistics, and Review	Standard GLP Report
Compliance	GLP

DEVELOPMENTAL & REPRODUCTIVE TOXICOLOGY (cont.)

Pre and Post Natal Development Study in Rats

Study Design

	Group	Females
Sprague Dawley Rats	Control	25
	Low	25
	Mid	25
	High	25
Total # of Animals	N= 100	
Dose Administration	Dosing of Parental generation (P1); Fetal Generation 1 (F1) exposed <i>in utero</i> and through lactation	
Dose Frequency	P1 animals daily from GD7 until necropsy	
Dose Formulation	As specified by Sponsor—additional charges may apply	
Dose Sampling	Sampling on first and last dose days for verification by Sponsor	

Evaluation Parameters

Observations	Daily for Clinical observations & Mortality/Moribundity check
Body Weights	<u>P1</u> : Prior to randomization, GD0, 4, 7, 10, 14, 17, 22, and Postnatal Day (PND) 1, 4, 7, 10, 14, 17, 22
	<u>F1</u> : PND 24, 31, 38, 45, 52, 59, 66, 73, 80, 87, 90
	<u>F2</u> : Prior to sacrifice
Food Consumption	<u>P1 animals</u> : along same schedule as body weight measurement
Breeding	<u>F1</u> : A set of offspring (1 pup/sex/litter) will be assigned for breeding with vaginal cytology and mating evaluation
Vaginal Cytology	<u>F1 Females</u> : Daily vaginal smears beginning two weeks prior to cohabitation, or within 14 days of cohabitation, whichever first
Offspring Evaluations	<u>F1 Pre-Weaning</u> : PND 1, 2, 4, 8, 15, 22 for # of live/dead pups, # of males/females, pup weight, and external exams
	<u>F1 Post-Weaning</u> : Litters will be culled to 2/sex/litter; Females examined daily for vaginal opening PND 27 until successful; Males examined daily for prepuital skinfold separation PND 35 until successful
	<u>F2</u> : All pups PND 1 & 4 for # of live/dead pups, # of males/females, pup weights and external exams
Developmental & Neurotoxicity Assessment	<u>F1</u> : 1 pup/sex/litter will be used to assess function and behavior for sensory response at PND 24, motor activity at PND 30, passive avoidance at PND 45, 46, and 52
Scheduled Sacrifice	<u>P1</u> : At completion of weaning (PND 22)

	<p><u>F1</u>: PND 4 - litters culled to 4/sex/litter; PND 22 - culled to 2/sex/litter; PND 98 - Sacrifice remaining F1 males and nonmated F1 females; F2 PND 4 - Sacrifice mated F1 females</p> <p><u>Nonparturient mated females</u>: P1 and F1 mated females not completely delivering a litter by GD 24</p> <p><u>Nonviable litters</u>: P1 and F1 females with no live pups</p> <p><u>F2</u>: PND 4</p>
Necropsy Observations	<p>Gross observation for moribund animals</p> <p><u>Nonparturient mated females</u>: Uterine contents examined for implantations, resorptions, live/dead fetuses</p> <p><u>Nonviable litters</u>: Uterine implantation sites counted</p>
Reporting, Statistics, and Review	Standard GLP Report
Compliance	GLP