

NTP Studies

The NTP routinely evaluates the potential toxic effect of environmental exposures to which humans may be exposed in rodent models (Fischer 344/N) and mice (B6C3F1 hybrid). The NTP performs appropriate toxicity studies (14-day and/or 90-day) in part to provide dose-setting information for its chronic toxicology/carcinogenicity studies and also to address specific deficiencies in the toxicology database for the environmental agent.

General toxicology screens are typically carried out as contracted studies at several commercial laboratories in the United States. Although designs are flexible, these studies usually involve exposures of rats and mice of both sexes to chemicals or occasionally to physical agents for periods of 14 to 90 days. Two-year studies in laboratory rodents remain the primary method by which chemicals or physical agents are identified as having the potential to be hazardous to humans. The NTP long-term toxicology and carcinogenesis studies (bioassays) in rodents generally employ both sexes of rats and mice with three exposure concentrations plus untreated controls in groups of 50 animals for two years.

The NTP is interested in identifying biomarkers for lipid/carbohydrate metabolism, lung function/injury, and cardiac function/injury to include in its 14-day and/or 90-day studies that can aid in characterization of endpoints of environmentally induced diseases or biological processes related to human disease etiology.

14-Day Toxicity Protocol

The goal is to identify potential target organs and toxicities and assist in setting doses for the 90-day exposure study.

Treatment:

After a 10- to 14-day quarantine period, animals are assigned at random to treatment groups. The study includes 5 treatment groups each administered a different concentration of test article per sex per species plus a control groups. Each group per sex per species contains 5 animals. The animals receive the test article through a designated route of exposure and the control animals receive vehicle alone. For dosed-feed and dosed-water studies animals are exposed for 14 consecutive days. For inhalation, gavage and dermal studies animals are exposed for 12 treatment days, not including weekends or holidays with at least two consecutive treatment days before the terminal sacrifice day. Male mice are housed individually and rats and female mice are housed in groups of five animals per cage except for inhalation and dermal exposure studies in which all rats and mice are housed individually.

	Animals	Sexes	Species	Test Groups	Total
Treatment	5	2	2	5	100
Control	5	2	2	1	20
					120

Observations:

Animals are weighed individually on day one on test, after seven days, and at sacrifice. The animals are observed twice daily, at least six hours apart (before 10:00 AM and after 2:00 PM) including holidays and weekends, for moribundity and death. Animals found moribund or showing clinical signs of pain or distress are humanely euthanized. Observations are made twice daily for clinical signs of pharmacologic and toxicologic effects of the chemical. For dosed-feed or dosed-water studies, food consumption/water consumption shall be measured and recorded weekly.

Necropsy and Histopathologic Evaluation:

Liver, thymus, right kidney, right testicle, heart, and lung weights are recorded for all animals surviving until the end of the study.

A complete necropsy is performed on all treated and control animals that either die or are sacrificed and all tissues are saved in formalin. (Necropsy List, see below)

Histopathologic evaluation is done only on those organs/tissues showing gross evidence of treatment-related lesions to a no-effect level plus corresponding tissues are evaluated in control animals. If specific targets are required they shall be read in the control and highest treatment group and the remaining groups to a no-effect level.

Necropsy List

Adrenal glands	Intestine, large (cecum, colon, rectum)	Oral cavity, larynx and pharynx	Spinal cord	Uterus
Brain	Intestine, small (duodenum, jejunum, ileum)	Ovaries	Spleen	Vagina
Clitoral glands	Kidneys	Pancreas	Stomach (forestomach and glandular)	Zymbal glands
Esophagus	Liver	Parathyroid glands	Testes, epididymides and vaginal tunics of testes	
Eyes	Lungs and mainstem bronchi	Pituitary gland	Thymus	
Femur	Lymph nodes - mandibular and mesenteric - bronchial and mediastinal (inhalation studies)	Preputial glands	Thyroid gland	
Gallbladder (mouse)	Mammary gland with adjacent skin	Prostate	Tissue masses	
Gross lesions	Muscle, thigh	Salivary glands	Tongue	
Harderian glands	Nerve, sciatic	Seminal vesicles	Trachea	
Heart and aorta	Nasal cavity and nasal turbinates	Skin, site of application (dermal studies)	Urinary bladder	

90-Day Toxicity Protocol

In addition to obtaining toxicological data, the purpose of this study is to determine the treatments for each strain and species to be used in the 2-year toxicology/carcinogenesis study.

Treatment:

After a 10- to 14-day quarantine period, animals are assigned at random to treatment groups. The study includes five treatment groups each administered a different concentration of the test article plus a control group. Each group contains 10 animals per sex per species. The animals receive the subject chemical by a designated route of exposure. Controls receive untreated water or feed or vehicle alone in gavage and dermal studies. For dosed-feed and dosed-water studies, animals are exposed for 90 days after which they are sacrificed with no recovery period. For inhalation, gavage and dermal studies animals are exposed five times per week, weekdays only until the day prior to necropsy. Male mice are housed individually and rats and female mice are housed in groups of five animals per cage except for inhalation and dermal exposure studies in which all rats and mice are housed individually.

	Animals	Sexes	Species	Test Groups	Total
Treatment	10	2	2	5	200
Control	10	2	2	1	40
Special "rats" for clinical lab studies	10	2	1	5	100
Special controls for clinical lab studies	10	2	1	1	20
					360

Observations:

Animals are weighed individually on day 1 on test, after 7 days, and at weekly periods thereafter. Animals are observed twice daily, at least 6 hours apart (before 10:00 AM and after 2:00 PM), including holidays and weekends, for moribundity and death. Animals found moribund or showing clinical signs of pain or distress are humanely euthanized. Formal clinical observations are performed and recorded weekly. For dosed-feed or dosed-water studies, food consumption/water consumption is measured and recorded weekly.

Necropsy and Histopathologic Evaluation:

Liver, thymus, right kidney, right testis, heart, and lung weights are recorded from all animals surviving until the end of the study.

A complete necropsy is performed on all treated and control animals that either die or are sacrificed. All tissues required for complete histopathology are trimmed, embedded, sectioned, and stained with hematoxylin and eosin for histopathologic evaluation.

Necropsy List

Adrenal glands	Intestine, large (cecum, colon, rectum)	Oral cavity, larynx and pharynx	Spinal cord	Uterus
Brain	Intestine, small (duodenum, jejunum, ileum)	Ovaries	Spleen	Vagina
Clitoral glands	Kidneys	Pancreas	Stomach (forestomach and glandular)	Zymbal glands
Esophagus	Liver	Parathyroid glands	Testes, epididymides and vaginal tunics of testes	
Eyes	Lungs and mainstem bronchi	Pituitary gland	Thymus	
Femur	Lymph nodes - mandibular and mesenteric - bronchial and mediastinal (inhalation studies)	Preputial glands	Thyroid gland	
Gallbladder (mouse)	Mammary gland with adjacent skin	Prostate	Tissue masses	
Gross lesions	Muscle, thigh	Salivary glands	Tongue	
Harderian glands	Nerve, sciatic	Seminal vesicles	Trachea	
Heart and aorta	Nasal cavity and nasal turbinates	Skin, site of application (dermal studies)	Urinary bladder	

A complete histopathologic evaluation inclusive of treatment-related gross lesions is done on all early death animals regardless of dose group, all control animals, all animals, and all animals in the highest treatment group with at least 60% survivors at the time of sacrifice plus all animals in higher treatment groups. Treatment-related lesions (target organs) are identified and these organs plus gross lesions are examined to a no-effect level.

Histopathology List

Adrenal glands	Larynx (inhalation studies)	Seminal vesicle
Brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons)	Liver (2 sections including left lateral lobe and median lobe)	Skin, site of application (dermal studies)
Clitoral glands	Lungs and mainstem bronchi	Spinal cord and sciatic nerve (if neurologic signs were present)
Esophagus	Lymph nodes - mandibular and mesenteric - bronchial & mediastinal (inhalation studies)	Spleen
Eyes	Mammary gland with adjacent skin	Stomach (forestomach and glandular)
Femur, including diaphysis with marrow cavity and epiphysis (femoral condyle with epiphyseal cartilage plate, articular cartilage and articular surface)	Muscle, thigh (only if neuromuscular signs were present)	Testes with epididymides
Gallbladder (mouse)	Nasal cavity and nasal turbinates (3 sections)	Thymus
Gross lesions	Ovaries	Thyroid gland
Harderian glands	Pancreas	Tissue masses
Heart and aorta	Parathyroid glands	Trachea
Intestine, large (cecum, colon, rectum)	Preputial glands	Urinary bladder

Intestine, small (duodenum, jejunum, ileum)	Prostate	Uterus
Kidneys	Salivary glands	

Specific Toxicologic Parameters Evaluated:

Clinical Laboratory Studies

Blood is collected from both sexes of "special study" rats, at days 4 ± 1 and 21 ± 2 and from the core study rats at the end of the study. These are processed for hematology and clinical chemistry determinations. Blood is collected from core study mice at the end of the study for hematology determinations.

Hematology	Clinical Chemistry
Erythrocyte count	Sorbitol dehydrogenase
Mean corpuscular volume	Alkaline Phosphatase
Hemoglobin	Creatine Kinase
Packed cell volume	Creatinine
Mean corpuscular hemoglobin	Total Protein
Mean corpuscular hemoglobin concentration	Albumin
Erythrocyte morphologic assessment	Urea Nitrogen
Leukocyte count	Total Bile Acids
Leukocyte differential	Alanine Aminotransferase
Reticulocyte count	Glucose
Platelet count and morphologic assessment	

Blood for Micronuclei

Blood samples are taken from mice and rats at study termination for micronuclei determinations.

Sperm Morphology and Vaginal Cytology Evaluations (SMVCE)

SMVCE are conducted on core study rats and mice. Mortality, body weight changes and clinical signs of toxicity are used to determine the 3 treatment groups used for the SMVC evaluations.

Additional Evaluations Added on a Selective Basis

Besides the routine evaluations for 14-day and 90-day studies, other evaluations may be added on a selective basis. The addition of these other evaluations is based upon findings in the literature or specific characteristics of the chemical or active agent suggesting a specific organ or metabolic pathway could be a target.

Examples of additional evaluations that NTP has included in its studies:

- Telemetry for detection of heart disease
- Lung lavage fluid analysis for detection of lung inflammation
- Special pathology stains (e.g, for detecting thrombosis, apoptosis, cell cycle, etc.)
- Serum cholesterol/triglyceride analysis for detection of altered lipid metabolism
- Serum hormone analyses (e.g., thyroid, reproductive, glucoregulatory)
- Gene analyses (cardiotoxicity, cancer mechanism characterization)
- Urine chemistry analyses for detection of kidney injury
- Special teratology and reproductive studies
- Immune-function studies
- Tissue p450 enzyme-induction studies