



Title: Conventional Rodent Husbandry

SOP Number: 056

Purpose: To ensure proper rodent husbandry and to prevent the spread of disease between rodents housed in ventilated cages.

HOUSING AND CARE:

- A. Mice/rats are generally socially housed in ventilated cages.
 - 1. For any animal that is individually housed, justification must be given in the animal use protocol and approved by the IACUC or AV.
- B. All forms located in the room's notebook must be adequately filled out.
 - 1. Daily observation sheet
 - 2. Task sheets
 - 3. Temperature/humidity logs
 - a. Temperature range: 68-79 degrees F
 - b. Humidity range: 30-70%
- C. Rooms should be checked daily for any operational deficiencies. Any deficiencies must be reported immediately to the facility manager.
- D. Water bottles are filled as needed and changed out weekly.
- E. Lixits for cages on automatic watering are checked twice a week to ensure proper function. Lixits are changed out as needed due to malfunction or at minimum once a month.
- F. Fresh food is added to the cages as needed.
- G. Cage lids, wire bars, and enrichment devices are changed out at least once a month. Cages are changed out as follows:
 - a. Cages with 3 or more mice:
 - i. Total change outs occur every two weeks (this includes wire bars, lids, bottoms, and enrichment)
 - ii. Cage bottoms will be changed out every week (this includes new bedding and nesting material)
 - b. Cages with 2 mice:
 - i. Total change outs occur every two weeks (this includes wire bars, lids, bottoms, and enrichment)
 - c. Cages with 1 mouse:
 - i. Total change outs occur once a month (this includes wire bars, lids, bottoms, and enrichment)

FEED STORAGE:

- A. All rodent diets will be stored in accordance with the recommendations of *The Guide*
 - a. Feed bags will be stored off the floor on pallets, racks or carts, at least 4 inches off the wall, in a manner that facilitate sanitation and pest control.
 - b. Open bags of feed will be stored in vermin-proof containers to minimize contamination and avoid the potential spread of pathogens.
 - c. Natural ingredient, dry laboratory animal diets will be stored in a temperature-controlled room (less than 70°F and less than 50% humidity)

- d. All purified and specialized diets will be stored based on the recommendation of the manufacturer- often in a refrigerator (less than 39°F).
- B. All feed expiration dates will be determined based on the recommendations of the manufacturer.
 - a. This includes but is not limited to cereal grain diets, purified diets, specialized/custom diets, and food enrichment products.
 - i. Shelf life:
 - 1. Modified Cereal Diets- 9 months
 - 2. Purified Diets-6 months
 - 3. Irradiated Enrichment Products – 24 months
 - 4. Non-Irradiated Enrichment Products-12 months
 - b. Reference (see attachment at end of SOP)

ENRICHMENT:

- A. Rodents are housed in groups to provide social interaction, unless otherwise justified.
 - a. Strain Specific Mouse Housing:
 - i. CD-1: up to 3 mice per cage
 - ii. Swiss Webster: up to 3 mice per cage
 - iii. Balb/c: up to 5 mice per cage
 - iv. C57: up to 5 mice per cage
 - b. Rat Housing:
 - i. Up to three small juvenile rats per cage
 - ii. Up to 2 adult rats per cage
- **additional strains not mentioned above will be housed based on weight (*Guide* p.57)
- B. Nesting material is added to the cages to facilitate thermoregulation and provide opportunities for species-typical behavior (i.e. foraging, burrowing, nest building, etc.).
 - C. Enrichment tubes or huts are added to the cages to provide the rodents with shelter and also aid in thermoregulation.

ENRICHMENT IMPLEMENTATION:

- A. Enrichment is provided on a rotational schedule by the technicians at change-outs.
- B. Exceptions to enrichment require adequate justification as to why environmental enrichment would interfere with the study. These exceptions must be stated in the approved institutional animal care and use protocol.

CAGE SANITATION:

- A. Bedding from the cages is dumped in the trash and a scraper is used to remove bedding stuck to the bottom or corners of the cage. This is performed inside a bedding disposal station or in front of a biobubble. If neither are available, husbandry staff must wear an appropriately fitted n95 mask to dump bedding. The bedding is then removed from the facility and disposed in a designated dumpster outside the building.
- B. Water bottles, cage lids, wire bar lids, enrichment devices, and cages free of bedding, are cleaned in the facilities' cage or rack washer.

- C. In facilities without access to a rack washer, racks should be cleaned and sanitized with Peroxigard every 6 months.
- D. In facilities with access to a rack washer, racks are to be changed out and cleaned in the rack washer every 6 months.

FEED CONTAINER SANITATION:

- A. Feed containers will not be transferred between areas of different risk of contamination without proper sanitation first.
 - a. Feed containers will be sprayed with approved disinfectant, allowed to sit the appropriate contact time and then wiped down before entering or leaving a room
- B. Feed storage containers are cleaned regularly
 - a. All feed containers are cleaned in the facilities cage or rack washer once every two weeks as needed but no less than once a month.

ANIMAL ROOM SANITATION:

- A. Floors are swept daily and mopped with disinfectant mixed according to manufacturer's recommendations at least twice a week. Designated brooms and mops are to be used in each room.
- B. All surfaces (including door frames, lights, and vents) are wiped down with disinfectant mixed according to manufacturer's recommendations, weekly.
- C. Floors, walls, and ceiling should be disinfected according to manufacturer's recommendations, monthly.

VENTILATED RACK MAINTENANCE:

- A. Check Daily:
 - 1. Power supply to rack
 - 2. Ensure all of the appropriate connections are capped
 - 3. Condensation in the cages- if present, report it to the facilities manager
- B. Check Every Two Weeks:
 - 1. Inspect pre-filter- clean if necessary
 - 2. Wipe down the unit
- C. Every 6 months:
 - 1. Change out the rack and hoses
- D. Yearly:
 - 1. Replace the EXHAUST HEPA filter
 - 2. Replace the pre-filters
- E. Every two years:
 - 1. Replace the SUPPLY HEPA filter

ANIMAL HEALTH MONITORING:

- A. Animals are observed daily by animal care staff for evidence of illness or change in behavior.
 - 1. Everyone with access to the animal facility is responsible for immediately informing the facility manager or a university veterinarian when an animal becomes ill or a change in behavior is observed.

2. Possible signs of illness are as follows:

Species	Behavior	Appearance
Rodents	Decreased activity; excessive licking/scratching; self-mutilation; avoidance or aggression; abnormal locomotion (stumbling); writhing; no nest building	Piloerection; rough or stained haircoat; abnormal stance or hunched back; porphyrin staining (rats); rapid, shallow respirations

B. In the event of suspected illness:

1. Record your observation on a sick animal card located in the ACS card system unit in the hallway of the animal facility- - fill out the information on the card completely. Be sure to place the white copy behind the cage card on the rack and bring the yellow copy to the animal health technician, veterinarian or facilities manager for further examination.
2. Contact the ACS facility manager or a university veterinarian:

ACS Attending Veterinarian
806-834-8588 Office
806-239-2120 Cell Phone

ACS Clinical Veterinarian
806-834-7373 Office
660-562-4425 Cell Phone

ACS Facilities Manager
806-834-2872 Office
602-758-0670 Cell Phone



How Storage Conditions, Product Stability, and the Manufacturer’s Warranty Work to Determine your TestDiet® Shelf Life

The purpose of this document is to provide clarification of nutrient stability and shelf life for products in three TestDiet® product segments: research specific complete nutrition diets (modified cereal grain or purified) and enrichment products.

TestDiet® laboratory animal feed products are formulated with precision and manufactured at our Food Safety System Certified 22000 (FSSC 22000) facility in Richmond, Indiana. The current issue of the Guide for Care and Use of Laboratory Animals and USDA facility inspectors suggest using products within a 6 month from the date of manufacture. The below excerpt from THE GUIDE references **manufacturer’s recommendations should be considered**:

Most natural-ingredient, dry laboratory animal diets stored properly can be used up to 6 months after manufacture. Refrigeration preserves nutritional quality and lengthens shelf life, but food storage time should be reduced to the lowest practical period and the manufacturers’ recommendations considered. 2011. Guide for the Care and Use of Laboratory Animals. p66.

The manufacturer’s warranty on nutrient stability as well as storage conditions for TestDiet® custom products vary depending on the exact formulation; many contain high levels of dietary fat, sensitive compounds, and purified, unstable forms of vitamins. Consequently, the guarantee for nutrient stability of custom diets is generally 6 months from the date of manufacture, and any extension of your diet usage by controlled storage varies depending on the type of diet. Custom diets should be stored under conditions of 39°F/4°C or lower and ≤ 50% relative humidity. If these storage conditions are not available at your facility, please consult with a LabDiet® nutritionist for further assistance. The recommended shelf life based on proven product stability for our TestDiet® items is provided below in a quick-reference table followed by a detailed description of each category of our products.

<i>Product</i>	<i>Formula Provides the Stability to Assure a Recommended Shelf Life for</i>
TestDiet® Modified Cereal Grain Diets	9 months
TestDiet® Purified Diets	6 months
LabDiet® & TestDiet® Irradiated Enrichment Products	24 months
Non-Irradiated Enrichment Products	12 months

TestDiet® Modified Cereal Grain Diets:

Often research-specific products are created from the cereal grain LabDiet® product already in-use at your facility, or a very similar variation. Slight nutrient modification, or addition of a compound of interest, to one of our proven cereal grain diets allows seamless transition of your research animals onto a study and can also eliminate the need for a specialized control diet.

The major constituents of cereal grain diets are commodity crops including corn, wheat, and soybean meal. These complex, nutrient-dense ingredients make up the majority of these formulas with micro-nutrients being

added, when needed, to meet requirements specific to the intended species. Exhaustive data supports nutrient stability of diets made using cereal grains for a minimum of 9 months from the date of manufacture. Supporting data illustrating long-term nutrient stability of LabDiet® products and similar research-specific diets, can be found on our website (<https://www.labdiet.com/ProductStability/index.html>).

While the standard ingredients in cereal grain diets are nutrient stable at ($\leq 70^{\circ}\text{F}/21^{\circ}\text{C}$), special consideration may be needed to account for unique aspects of individual diets based on research-specific needs (stability of added compound, for example). When possible, storage at $\leq 39^{\circ}\text{F}/4^{\circ}\text{C}$ or even $\leq 0^{\circ}\text{F}/-20^{\circ}\text{C}$ is strongly recommended.

TestDiet® Purified Diets:

Purified diets are created from purified ingredients, such as casein, corn starch, cellulose, and individual vitamins and minerals. The American Institute of Nutrition (AIN) originally created the three formulas on which most purified diets used today are based, AIN-76A, AIN-93M, and AIN-93G. The AIN first developed a universally available formula in 1972, updated that formula in 1982 (what is known today as AIN-76A). Further improvements were made in 1993 resulting in today's common purified formulas, AIN-93M (maintenance) and AIN-93G (growth). In general, the nutrient levels were chosen to very closely meet rodent requirements. Little effort was made to exceed animal requirements to account for possible degradation over time.

With consideration of sensitivity of micro-nutrients, the formulation level relative to animal requirement, and the intended short-term use of these diets, the developing team recommended storage for 3 months under refrigeration ($\leq 39^{\circ}\text{F}/4^{\circ}\text{C}$) and for 6 months under freezer conditions ($\leq 0^{\circ}\text{F}/-20^{\circ}\text{C}$; Reeves et al., 1993). Additional data is needed to evaluate nutrient-stability of purified diets under varying conditions; at this time, we recommend storage of purified diets no longer than 6 months from date of manufacture at ($\leq 39^{\circ}\text{F}/4^{\circ}\text{C}$, or preferably ($\leq 0^{\circ}\text{F}/-20^{\circ}\text{C}$), when possible.

LabDiet® & TestDiet® Enrichment Products:

Dietary enrichment products provide supplemental nutrients but are typically not designed to be nutritionally complete diets. Dietary enrichment products should be stored under conditions of $\leq 70^{\circ}\text{F}/21^{\circ}\text{C}$ and $\leq 50\%$ relative humidity. Because they are typically not the sole source of the animals' daily ration, the assured product stability or shelf-life for our enrichment products is 12 months from the date of manufacture; all irradiated enrichment products are assured for stability up to 24 months.

We are confident that TestDiet® products maintain their nutritional integrity and remain palatable and safe for the timeframes outlined above. However, the contents of this publication do not supersede or override the decisions, protocols, and/or procedures of the inspection agencies, laboratory animal governing bodies, or directors or managers of lab animal facilities at research institutions. Our intention is to provide information about how our feeds are formulated and how product stability can be extended based on factual data we have accumulated over time. To review data supporting the stability of our products, please visit the Product Stability section at www.testdiet.com.

If questions or concerns arise, please do not hesitate to contact one of our LabDiet® team members by emailing info@testdiet.com.

TestDiet®

Reeves, P.G., F.H. Nielsen, and G.C. Fahey, Jr. 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J. Nutrition. Committee Report 1939-1951.