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Policies

General Policies and Terms:

- We are unable to guarantee the performance of NASH mice for any specific applications. NASH mice are provided under our normal <u>Terms and Conditions for the</u> <u>Sale of Products.</u>
- In the event you must cancel a booked order, be sure to cancel prior to the cut-off time or cancellation fees up to the entire price of the order will be applied. <u>Click here for more details on Taconic cancellation policies.</u>

Recommended Care and Housing for NASH mice

Production at Taconic

Taconic Biosciences maintains an inventory of C57BL/6NTac male* mice conditioned on a modified Amylin liver NASH (AMLN) diet. <u>Diet # D09100310i</u> (source Research Diets) contains 40 kcal% fat, 20 kcal% fructose and 2% cholesterol and is an irradiated diet. C57BL/6NTac males are put on this diet at 6 weeks of age and group housed at reduced density relative to normal C57BL/6 holding. Exact caging and density vary by production site. Control males are housed in the same location, also at reduced density, and are fed <u>NIH-31M</u> chow diet. Control males can also be generated using a low-fat purified diet upon request. Water source is filtered and either hyperchlorinated or autoclaved. Light cycle is 12:12. Production locations are maintained within an allowable temperature range of 20-26° C.

Phenotype Information

NASH B6 mice become obese, get fatty enlarged livers and develop liver inflammation and fibrosis after 26+ weeks on diet, with some inter-animal variability observed for development onset and severity of the inflammation and fibrosis phenotype. NASH and control mice through 59 weeks of age should be bright, alert and responsive. Fur coat in NASH mice may be visibly oily, whereas controls should be well-groomed to mildly oily. Hairshaft tips may have intermittent lighter color.



- Relevant characterization and efficacy data is posted on the <u>NASH product</u> webpage.
- Phenotypic penetrance of certain NASH aspects is incomplete, so animals conditioned on diet for the same length of time will vary in disease phenotype.
- Taconic recommends a pilot study to determine the appropriate number of mice to order for a specific study type.
- Incidence of liver tumors: Development of liver tumors in the Taconic NASH model appears to be rare through 53 weeks on diet. Mice were examined at 27, 35 and 53 weeks on diet (n=10/timepoint). No lesions were observed via gross necropsy or histopathological evaluation of a section of the medial lobe in mice on diet for 27 and 35 weeks. Among 10 mice on diet for 53 weeks, one mouse displayed a large liver adenoma, but no other tumors were observed in the cohort (histopathological assessment is pending on 7/10 mice, but no gross lesions were observed in those samples).

* Note that female C57BL/6 mice are resistant to diet-induced obesity and NASH and thus Taconic only offers male NASH mice.

Recommendations for maintenance by users

Key factors to consider for NASH and control mice are recovery of body weight loss in shipment prior to start on study and minimizing aggression.

Diet: Be sure to have NASH diet on hand prior to receipt of mice. Diet # D09100310i may be ordered from <u>Research Diets</u>. Lead time to receive diet may be several weeks. If ordering a custom diet compounded with a test article, lead time may be longer. Research Diets has <u>certified distributors</u> in many countries. Lead time for diet receipt may vary by country. Taconic cannot provide the NASH diet beyond the amount the mice are shipped with in the Taconic Transit Cage (TTC) for use in transit.

High fat diet pellets may clump together and not flow freely in the hopper. Ensure that animals have clear access to diet during cage observations. If this occurs, fluff pellets so they come into contact with bottom of hopper. Taconic recommends that diet be completely changed once per week, with old feed discarded.

Water: NASH and control mice are maintained on water bottles at Taconic. If your facility uses an automatic watering system, be sure that animals acclimate properly to the new water source so that dehydration does not occur. Detailed suggestions may be found in the Taconic Insight <u>Acclimating Rodents to Automated Watering</u>.



Taconic recommends use of hyperchlorinated or autoclaved water. Use of acidified water is likely to shift the animal's microbiome, which may have an unknown effect on the NASH phenotype.

Unpacking and housing: NASH and control mice are packed as full groups of cagemates, one cage per TTC. This is designed to minimize aggression. If an order quantity is not evenly divisible, there may be one TTC with a partial group of cagemates. Note that there is significant inter-animal variation in weight gain on the NASH diet, so each group of cagemates will contain mice with varying body weights. Body weight does not appear to be strongly correlated with liver phenotypes at later ages. Taconic cannot accept orders for NASH or control mice by weight.

TTC density will vary based on production location:

- Germantown, NY: 8-11 mice per TTC
- San Diego, CA: 4 mice per TTC
- Hallingore, Denmark: 4-9 mice per TTC

<u>To reduce potential aggression, do not ever recombine NASH or control mice from</u> <u>different housing groups</u>. As obese mice may require more floor space per animal based on body weight, appropriate housing densities should be reviewed by your institution's attending veterinarian and IACUC or oversight body. Unpack and house the mice in their final study configurations. Housing options are:

- house exactly as received, with contents of one TTC placed in one cage
- break down into smaller groups, unpacking a single TTC into several cages, but never recombining mice from different TTCs
- singly house one mouse per cage (with prior approval by IACUC or oversight body).

Weight loss: NASH B6 mice in transit for extended periods (more than 48 hours by ground or any air transit) can lose 10-20% of their body weight and **may require extended acclimation (~2-4 weeks).** Weight loss induced by transit may delay disease phenotype relative to animals which have remained in the same facility for the entire conditioning period.

Weight loss is a stress response. Minimize stress in mice by minimizing handling during the acclimation period. Unpack mice and leave them undisturbed as much as possible for the first week. After the first week, begin taking body weight measurements (same time/day frequency, once per week) to determine when any weight loss due to transit is rectified. Researchers may request to receive animal weights at time of pack when



placing a NASH B6 order with Customer Service. Note that individual animals will not have identifying marks, though weights for each animal within a Taconic Transit Cage will be provided.

Read the related Taconic Insight:

<u>Acclimating Research Animals Through Effective Nurturing</u>

Aggression: Aged C57BL/6 models are prone to aggression. Carefully observe mice for signs of aggression and fight wounds. Mice with fight wounds may require veterinary care or euthanasia. Taconic recommends enrichment with the use of chew sticks and a form of nesting material in all NASH and control cages. Transfer of used nesting material (but not dirty bedding) from the old cage to the new cage during cage changes may reduce aggression. The use of hides (huts, shacks) is not recommended as it may increase aggression. If aggression continues, separation of mice may be considered to avoid injury and welfare concerns. Handling may contribute to aggression. Gentle handling and consistent care staff is recommended.

Alopecia: Alopecia may occur in NASH mice and is thought to be a consequence of the metabolic disease phenotype. As long as the skin is normal and intact and no pruritus is observed, no treatment is required and the mice may be used in experiments.

Light Cycle: Maintain a consistent light cycle without interruption of the dark period. Disruption of the light cycle may have adverse phenotypic effects such as increased tumor formation.

<u>Contact Taconic</u> for any questions regarding the above recommendations. Requirements for care will vary by facility. Please consult your veterinarian or facility manager for more information on working with NASH and other metabolic and diet-induced models.

Additional Resources

- 1. Phenotypic Data (located near the bottom of this page)
- 2. Diet Induced NASH B6 Mouse Flyer
- 3. NASH Diet Formulation



- 4. <u>The More Things Change, The More They Stay the Same: The Amylin Liver Nash</u> (AMLN) Diet
- 5. Webinar Q&A The Diet Induced Nash B6: A Translational Nash Model For Drug Discovery