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Effects of housing density on mouse physiology and behavior in NASA animal enclosure module simulators

Sujata Naidu
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**EFFECTS OF HOUSING DENSITY ON MOUSE PHYSIOLOGY AND
BEHAVIOR IN NASA ANIMAL ENCLOSURE MODULE SIMULATORS**

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Sujata Naidu

December, 1996

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ABSTRACT
**EFFECTS OF HOUSING DENSITY ON MOUSE PHYSIOLOGY AND
BEHAVIOR IN NASA ANIMAL ENCLOSURE MODULE SIMULATORS**
by Sujata Naidu

This study was part of a larger project to qualify the NASA Animal Enclosure Module (AEM) for use with female mice. Goals for this investigation were to determine the optimum number of adult mice that could be housed in the AEM, to verify that the exhaust filter would contain mouse odors, determine if water and food access were adequate, and to verify normal animal growth and behavior.

Three population densities of mice were evaluated: 8, 12 and 16. The results of the first experiment were confounded due to increased ammonia in some of the cages because fallen food bars accumulated urine and impeded ventilation. A second experiment was, therefore, performed taking precautions to prevent fallen food bars and ammonia build-up.

These findings indicate that mice housed in AEM simulators for 30 days at densities of 8, 12 or 16 are similar in terms of their growth, physiology and behavior.

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INTRODUCTION:

A. Background and Significance of the Study

This project was part of the effort to qualify the NASA Animal Enclosure Module (AEM) for use with female mice. Goals for the investigation were to determine the optimum number of adult mice that could be housed in the AEM, to verify that the exhaust filter would contain mouse odors, determine if water and food access were adequate, and to verify normal animal growth and behavior.

In order to determine if health and well-being of the mice were compromised by the AEM, the Stress Assessment Battery (S.A.B.) was developed. It was verified using immobilization as a stressor.

Three different population densities of mice were evaluated: 8, 12 and 16. The project consisted of two phases. The results of the first phase experiment were confounded due to increased ammonia in some of the cages because fallen food bars accumulated urine and impeded ventilation. A second phase experiment was, therefore, performed taking precautions to prevent fallen food bars and ammonia build-up.

B. Literature

Animal Stress: Historical Perspective

In the 1930's, Hans Selye (1936) developed a comprehensive theory of stress and described biological correlates associated with it. He suggested that a variety of stressors (e.g., pain, toxins, cold, heat, or starvation) when exposed to an animal chronically would result in physiological changes that may, in turn, develop into a pathological state. In his stress research, Selye repeatedly found an adrenal-cortical response. This led to the development of his General Adaptation Syndrome (GAS). GAS refers to a nonspecific response to stress and consists of three stages: (1) an immediate alarm reaction, (2) a resistance stage, and eventually (3) exhaustion (Selye, 1950).

The initial alarm reaction to an external stressor activates the sympathoadrenal system resulting in elevated levels of catecholamines. This reaction was described by Walter Canon (1929) as the "fight-flight" response whereby the animal prepares to escape or struggle against the noxious stimulus. The modern view, as summarized by Tepperman and Tepperman (1987) is that an immediate defense against volume depletion and hypotension is brought about by: a) an increase in autonomic sympathetic tone resulting in sympathoadrenal medullary activation thus increasing systemic catecholamines, b) activation of the hypothalamic-anterior pituitary-adrenocortical axis resulting in production of ACTH (adrenocorticotrophic hormone) and glucocorticosteroids, c) activation of the hypothalamic-posterior pituitary axis thus releasing ADH (anti-diuretic hormone), and d) autonomic activation of the renin-angiotensin pathway.

Selye's resistance stage was characterized by the release of adrenal glucocorticoids. The actions of glucocorticoids are slower than the rapid actions of catecholamines, but according to Selye, they provide the sustained secondary

response (resistance). The modern view is that the various physiological mechanisms respond to the insult (stress) to homeostatically maintain the dynamic conditions of the internal environment. From a metabolic stand point, this occurs in two phases. First, the insulin-glucagon ratio shifts such that the insulin secretion decreases and the glucagon secretion increases. This, along with the increased glucocorticoid levels, results in a metabolic profile that includes proteolysis, glycogenolysis, gluconeogenesis and lipolysis. In the next phase, the insulin glucagon ratio reverses, growth hormone and other mitogenic factors increase in concentration thus resulting in the reparative process such as wound healing, scar formation, antibody formation, hematopoiesis, etc.

When the animal is exposed to prolonged periods of stress, it eventually enters the third GAS stage, exhaustion. The modern view holds that if the physiological mechanisms can not maintain homeostasis then pathology may result. Prolonged high levels of glucocorticoids lead to a number of physiological changes including hypertrophy of the adrenal gland, suppression of the immune system, gastrointestinal ulceration and muscle atrophy.

Selye's early model failed to predict that hypersecretion of glucocorticosteroids would inhibit many systems including the immune system, mechanisms associated with inflammation and wound healing, mechanisms associated with extracellular fluid volume control and hormonal mechanisms associated with regulation of metabolism. Munck et al. (1984) suggest that glucocorticosteroids are permissive or "synergistic" with many hormones, prostaglandins, proteases, lymphokines and other "cellular mediators" in promoting full development and function of physiological systems and mechanisms involved in homeostasis and defence against disease and injury. During prolonged "chronic" stress situations, increased glucocorticosteroid secretion is not for protection from the source of the stress itself, but rather for protection against the effects of prolonged hyperactivity (overshoot) of the body's normal physiological

mechanisms that respond to stress. In other words, chronic overactivity of some mechanisms may be maladaptive and in themselves produce damage or be a threat to homeostasis. Immunosuppression would thus be an example of this adaptive mechanism, so that overactivity of the immune system might not damage endogenous tissues.

Effects of Chronic Stress

Elevated plasma corticosterone is still generally accepted as an indicator of stress. However, these levels fluctuate with the circadian rhythm of the animal (i.e., the plasma corticosterone concentration peaks just prior to activity onset). Another complication when evaluating plasma corticosterone is the time taken to withdraw a blood sample from the moment an animal's cage is disturbed. Any disturbance to the cage alarms the animal thus causing an acute adrenal activation. Plasma corticosterone concentrations in blood samples withdrawn within three minutes should not be greatly affected by this acute response (G. Moberg, personal communication). We therefore took precautions to account for our sample time. Most samples were withdrawn within three minutes from the time the cage was lifted from its chamber. Blood samples withdrawn after three minutes were considered questionable for determination of undisturbed plasma corticosterone concentrations. Because of the problems with sample time and circadian changes, adrenal gland weights may be a better indicator of chronic stress.

Chronic high plasma corticosterone is reflected by adrenal gland hypertrophy (Daniels-Severs, 1973) and leads to immunosuppression. We expected to see increased adrenal gland weights, decreased thymus and spleen weights, decreased total white blood cell counts (leukopenia) and a change in the leukocyte profile namely eosinopenia, and neutrophilia (which would be reflected in neutrophil to lymphocyte ratio) (Jain, 1986).

The immunosuppressive effect caused by chronic elevation of plasma corticosterone has recently been re-investigated. Garvy, et al. (1993), ascertained that elevated plasma corticosterone can cause a marked reduction in the number of cycling B cells in murine bone marrow and induce apoptosis. It has also been demonstrated that the stress-induced corticosterone response down-regulates mononuclear cell trafficking (Herman, et al., 1995)

There are now studies which indicate that another serum factor may be also responsible for the suppressed lymphocyte proliferation. Preliminary examination of this factor indicates that it is a large molecular weight protein that is stable to heat, temperature, organic solvents and proteases (Zha, et al., 1992). The serum factor is yet to be identified but has been shown by Zha, et al. to suppress lymphocyte proliferation when its production is induced by restraint stress in rodents.

Total plasma protein concentration is affected by a number of factors. For example, hormonal mechanisms, diet, hemoconcentration, pregnancy, lactation and age all play a role in influencing plasma protein concentrations. In our study, mice were of the same age and sex and were given the same diet. Hence, factors affecting the plasma protein concentration here are limited to hormonal control, stress related factors and hemoconcentration (e.g., dehydration). Insulin, growth hormone, estrogens and androgens are anabolic and act to facilitate protein synthesis and diminish nitrogen excretion. Thyroid hormones are anabolic at normal levels but catabolic at high doses. Glucocorticoids are catabolic and utilize amino acids for gluconeogenesis (via increased proteolysis).

It is difficult to conclude that a change in plasma protein is due to some hormonal change or to the level of hydration of the animal or to some combination of both factors. Nevertheless, it would be an indicator of a difference in physiological state of a test animal when compared to a control.

Lastly, under conditions of extreme prolonged stress, it is common to find stomach ulcerations due to chronic elevated glucocorticosteroid secretion. This mechanism is associated with a decrease in prostaglandins (PGE) and an increased hydrochloric acid secretion.

Crowding Stress in Mice

Historically, the concept of a nonspecific response to stress was generally accepted by physiologists. However, it should be noted that the actual hormonal response pattern and the various physiological changes differ among animal species, between strains within a species and from one type of stressor to another. It also affects male and female mice differently (Svec, et al., 1989).

"Crowding" has been defined as an increase in the intensity of competition for space (Christian, 1963). Social stress leading to increased aggressiveness occurs because mice form social dominance hierarchies when in groups. Fighting amongst mice, being a relatively natural form of physiological stress, has been used as a tool for studying normal mechanisms of neuro-endocrine adaptation and control. Fighting amongst male mice, in particular, has resulted in enlarged adrenals, spleens and hearts as well as marked increases in brain and adrenal catecholamines (Welch and Welch, 1969). Fighting also affects the immune system in dominant and submissive mice in a different manner than psychological or mild aggressive encounters (Hardy, et al., 1990). Hardy, et al. found that submissive mice had lower T-cell proliferation than dominant mice suggesting that a stronger stress response is elicited from submissive mice.

The physiological, socio-behavioral and morphological effects of overpopulation have been studied for many years. Increases in population size induce a number of endocrine responses. Besides increased pituitary-adrenocortical activity, there is decreased reproductive function (Christian, 1964, Edwards and Dean, 1977, and

Breazile, 1987). Factors implicated include rank of the females in the social hierarchy, fetal resorption, inhibition of estrus and sudden increases in infant mortality (Lloyd and Christian, 1969). Diverse stressors impede female receptivity and fertility. Epinephrine has been shown to disrupt lordosis behavior (thereby eliminating receptivity) but has not been shown to have an effect on implantation after insemination (deCatanzaro and Graham, 1992). Maternal stress has also been shown to induce a syndrome of fetal anomalies including encephalocele, supernumerary ribs, fused ribs and vertebral anomalies (Miller and Chernoff, 1995).

In summary, the effects of stress, particularly prolonged stress, have profound effects on mice which vary amongst strains, within a hierarchy in a strain and between the sexes (Griffiths and Anisman, 1994). The response to stress, while non-specific, has a profile that also varies between strains, within strains and between sexes and the type of stressor introduced to the mice. This study will focus on the crowding stressor using female Swiss Webster mice.

C. Hypothesis

Null Hypothesis: The health and well-being of mice housed in the AEM are not compromised given population densities of 8, 12 and 16.

Based on the premise that a noxious stressor elicits a non-specific response, an assessment battery of measures was developed. The assessment battery of tests (Stress Assessment Battery) includes:

- 1) General appearance of coat, nose, eyes and ears
- 2) Behavioral assessment
- 3) Food and water consumption
- 4) Body weight changes
- 5) Thymus, adrenal, spleen, heart and kidney weights
- 6) Plasma corticosterone concentration
- 7) Total plasma protein concentration
- 8) Total blood leukocyte count
- 9) Differential leukocyte count: neutrophil/lymphocyte ratio; eosinophil count
- 10) Gastric histology

Items for inclusion in the SAB were selected after consultation and review of the following:

- 1) report of NASA Workshop on Rodent Cage Sizing, March 30-31, 1990;
- 2) personal communication with Dr. G.P. Moberg, University of California, Davis, editor of Animal Stress, American Physiological Society, 1985.

MATERIALS AND METHODS:

A. Stress Assessment Battery Validation

1) Animals:

Twenty female Swiss-Webster mice, approximately 18 g (initial weight) were purchased from Simonsen Laboratories, Gilroy, CA. Ten became the experimental group that were exposed to daily immobilization stress (for fourteen days) and ten animals became the control group.

2) Food and Water:

Standard rodent laboratory chow (Teklad Sterilizable Rodent Diet, Harlan Teklad, Madison, WI) and distilled water were provided *ad libitum*.

3) Animal Housing:

Standard vivarium shoe-box cages (27.5 cm length X 17.2 cm width X 12.3 cm height) lined with "Beta chip" bedding (Northeastern Product Corp., Warrensburg, NY; alder hardwood chips) held 5 mice each. The experimental group was housed in isolation chamber DH 542 A and the control group was housed in isolation chamber DH 542 C. Filter bonnets were placed on all cages. All mice were exposed to a 12L:12D light cycle with lights on at 0700h. Lighting was standard fluorescent. Light intensity was approximately 330 - 470 lux

4) Restraint Method to Induce Stress Response:

Each animal from the experimental group was removed from its cage and placed in a plexiglass restraining device (catalogue identification number: 52-0460, Harvard Apparatus, South Natick, MA; 9.5cm X 2cm X 2cm) for three hours a day. The daily time chosen to restrain the animals was random between 1000 - 2100. The animals could not turn around within the restrainer and the tail gate of the restrainer

was adjusted to keep the animal contained without impairing its circulation. The mice were returned to their home cage immediately after the restraint period.

5) Daily measurements and health evaluation:

Water and food consumption were recorded daily from all rodent cages. A visual physical examination of the mice was done daily. Changes in the coat, eyes, nose and ears were taken as indicators of the physical well-being of the rodents.

<u>Observation</u>	<u>Acceptable</u>	<u>Questionable</u>
Hair coat	Smooth and glossy.	Dull, rough, loose or stained hair.
Eyes	Clear, open, "bright"	Puffy, partly closed, pink rings, discharge, ulceration.
Nose	No swelling	Discharge- clear yellowish pus, "bloody".
Ears	Clean, pink	Scaly, dirty, yellowish.
Tail	Clean, Smooth	Rough, scaly, stained
Behavioral	Exploring, grooming, playing	Fighting,
Postural	Head, back, tail	Arched back, head tilted

*Prepared by Dr. Sig Rich,
Campus Veterinarian

6) Behavior Assessment:

Mice were videotaped during the dark portion (mouse activity phase) of the light cycle using a Toshiba IK-M30M CCD camera system. Illumination came from one 15 watt Kodak darkroom lamp model A with a glass IR filter. A Magnavox VR9942 VHS videotape recorder recorded the camera video.

Videotapes were scored by observing 10 minutes per hour for the first two hours of each tape. The observer divided the cage volume into four quadrants. Each quadrant was scored independently by counting the number of times each mouse engaged in a particular type of activity over the 10 minute scoring period. Activity classes were defined as follows: 0 - sleeping, still, sniffing; 1 - eating, drinking; 2 - grooming, moving; 3 - social interaction; 4 - aggressive behavior; 5 - running. We defined social behavior as chasing or wrestling and aggressive behavior as biting. Scores for all mice and scoring periods were added together to obtain one number per activity class for each tape.

7) Euthanasia method:

The animals were euthanized one cage at a time. The cage was removed quietly from the isolation chamber and the cage was taken into an adjoining room where it was placed in a large container (approximately 5 gallon volume) pre-saturated with 100% carbon dioxide. A timer was started immediately upon initial handling of the cage. The animals were kept in the carbon dioxide until they were unconscious (about one minute). Subsequently, each mouse was given to a member of the dissection team for blood withdrawal via heart puncture.

The blood was collected using heparinized 3 cc syringes. An aliquot of the whole blood was immediately used to perform total white blood cell counts (Unopette microcollection system, Becton-Dickinson, Rutherford, New Jersey). Another aliquot was used to prepare slides for differential white blood cell counting. The remaining blood was centrifuged (at 4°C) and the plasma immediately frozen for later analysis.

All blood samples were labelled with time from initial handling of cage to completion of blood withdrawal. Samples that took longer than 3 minutes to obtain

were considered questionable for determination of undisturbed plasma corticosterone concentrations.

8) Dissection of mice:

The mice were weighed before they were dissected and their stomachs, hearts, spleens, kidneys, adrenals and thymus glands removed and weighed. The stomachs were gently washed with buffered 10% formalin and then stored in formalin-filled jars for later histopathological analysis by pathologists at CVD, Inc. (Sacramento, CA).

9) Plasma corticosterone :

Plasma corticosterone concentration was determined using an ImmunochemTM ¹²⁵I Corticosterone RIA kit (catalogue number:07-120103, ICN Biomedicals, Inc., Irvine, CA). This double antibody radioimmunoassay is designed specifically for use with laboratory rats and mice.

10) Plasma protein:

Total plasma protein concentration was determined via the Lowry method using a diagnostic kit obtained from Sigma Chemical Co., St. Louis, MO, (Catalogue number 690A.)

11) Leukocyte analysis:

Total white blood cell count was determined using the Unopette method (Catalogue number 5853, Becton-Dickinson, Rutherford, New Jersey)). These counts were done within hours of the termination of the mice. Blood smears were stained with Diff-quick (Catalogue number: B4132-1, Scientific Products, McGaw Park, IL) and differential leukocyte counts were determined by Mr. Wayne Pinard, AHT, (Veterinary Lab Technician, Adobe Animal Hospital, Los Altos, CA).

12) Stomach histopathology:

The stomach tissue was trimmed, embedded, sectioned and examined by pathologists at CVD, Inc. (Sacramento, CA).

B. Experiment 1: Mouse Density Study

Animals

Eighty-eight female Swiss-Webster mice (Simonsen Laboratories, Gilroy, CA) were weighed (21.8 ± 1.0 grams, mean $\pm$ S.D.) and ear punched to allow individual identification. The mice were placed in eight cages on experiment day 0. Six NASA supplied animal enclosure module simulators (**Test Enclosures**), three with ALFCO #3A outlet filters, three with ALFCO #4 outlet filters, held two full food bar plates, a standard water box with four lixits, and 8, 12, or 16 mice. These six cages were housed in DH 639A. One large shoe box type cage (**Vivarium Shoe Box Control**, 42.5 cm length x 21 cm width x 12.3 cm height) in the Animal Care Facility and one AEM wire cage within a lexan housing (**Vivarium Control Enclosure**) in the Animal Care Facility each held eight of the remaining mice. The Vivarium Shoe Box Control was supplied with Techlad rodent diet (W) (Harlan Sprague-Dawley, Madison, WI) and distilled water from a standard vivarium cage water bottle. The Vivarium Control Enclosure held two food bar plates with NASA food bars and an AEM water box with four lixits. Distilled water from a graduated cylinder supplied the lixits. The study ran for 30 days. On Day 30, the animals were removed from the cages and the SAB conducted. Table 1 shows the combinations of cage type, number of mice, and filter type used.

Table 1 (Experiment 1). Mouse cage type and number, mouse ID numbers, housing location, filter type, food type, and water delivery method.

Cage Designator	Location	Number of mice	Mouse ID Numbers	Filter Type	Food Type	Water Delivery
Test enclosure #3	DH639-A	8	(#1-8)	ALFCO #3A	bars	lixit
Test enclosure #6	DH639-A	12	(#9-20)	ALFCO #3A	bars	lixit
Test enclosure #5	DH639-A	16	(#21-36)	ALFCO #3A	bars	lixit
Test enclosure #7	DH639-A	8	(#37-44)	ALFCO #4	bars	lixit
Test enclosure #1	DH639-A	12	(#45-56)	ALFCO #4	bars	lixit
Test enclosure #2	DH639-A	16	(#57-72)	ALFCO #4	bars	lixit
Test enclosure #4	DH639-A	0	N/A	ALFCO #4	N/A	N/A
Vivarium Shoe Box Control	ACF vivarium	8	(#73-80)	N/A	chow	bottle
Vivarium Control Enclosure	ACF vivarium	8	(#81-88)	N/A	bars	lixit

ACF = University Animal Care Facility (DH741A)

Lighting

All mice were exposed to a 12L:12D light cycle with lights on at 0700h. Light intensity was approximately 340 - 470 lux just outside the Lexan housing of the test enclosures. Lighting was standard fluorescent.

Test Enclosure Air Flow

Air speed in the 4" (internal diameter) PVC pipe attached to the test enclosures was set to 180 feet per minute on test day 0. This was equivalent to 14.8 cubic feet per minute of air flow. Neither the Vivarium Shoe Box Control nor the Vivarium Control Enclosure had forced air flow. Air speed was measured daily with a TSI, Inc. Velocicalc Model 8350 hot wire air flow meter.

Ammonia Measurement

Ammonia concentration within cages was measured using Sensidyne® Gastec No. 3L ammonia analyzer tubes (#32011-335, VWR Scientific, San Francisco, CA). A Sensidyne® Gastec pump (kit No. 800) drew either 100 ml or 200 ml of air through each analyzer tube. Test enclosure internal air was drawn from the upstream differential pressure gauge port located between the inner AEM cage and the outlet filter. Test enclosure outlet plenum air was drawn from the downstream differential pressure gauge port located in the wall of the outlet plenum (see Figure 1). Test enclosure internal ammonia concentration was measured on test days 15, 17, 21, 24, and 29. Test enclosure outlet plenum ammonia concentration was measured on test days 11, 14, 15, 21, 24, and 29.

C. Experiment 2: Mouse Density Study

Animals

Fifty-two female Swiss-Webster mice (Simonsen Laboratories, Gilroy, CA) were weighed (28.6 ± 0.3 grams, mean $\pm$ S.E.) and ear punched to allow individual identification. The mice were placed into five cages on experiment day 0. Three NASA supplied animal enclosure module simulators (**Test Enclosures**), all with ALFCO #3A outlet filters, held two full food bar plates, a standard water box with four lixits, and 8, 12, or 16 mice. These cages were housed in DH 639A. One large shoe box type cage (**Vivarium Shoe Box Control**, 42.5 cm length x 21 cm width x 12.3 cm height) and one AEM wire cage within a lexan housing (**Vivarium Control Enclosure**) each held eight of the remaining mice in the Animal Care Facility (DH 741A). The Vivarium Shoe Box Control was supplied with Techlad Rodent Diet (W) (Harlan Sprague-Dawley, Madison, WI) and distilled water from a standard vivarium cage water bottle. The Vivarium Control Enclosure held two food bar plates with NASA food bars and an AEM water box with four lixits. Distilled water from a graduated cylinder supplied the lixits. The study ran for 30 days. On Day 30, the animals were removed from the cages and the SAB conducted. Table 2 shows the combinations of cage type, number of mice, and filter type used.

Table 2 (Experiment 2). Mouse cage type and number, mouse ID numbers, housing location, filter type, food type, and water delivery method.

Cage Designator	Location	Number of mice	Mouse ID Numbers	Filter Type	Food Type	Water Delivery
Test Enclosure #2	DH639-A	8	(#1-8)	ALFCO #3A	bars	lixit
Test Enclosure #4	DH639-A	12	(#9-20)	ALFCO #3A	bars	lixit
Test Enclosure #1	DH639-A	16	(#21-36)	ALFCO #3A	bars	lixit
Test Enclosure #3	DH639-A	0	N/A	ALFCO #3A	N/A	N/A
Vivarium Control Enclosure	ACF vivarium	8	(#37-44)	N/A	bars	lixit
Vivarium Shoe Box Control	ACF vivarium	8	(#45-52)	N/A	chow	bottle

ACF = University Animal Care Facility (DH741)

Test Enclosures

Lighting

All mice were exposed to a 12L:12D light cycle with lights on at 0700h. Light intensity was approximately 330 - 470 lux just outside the Lexan housing of the Test Enclosures. Lighting was standard fluorescent.

Food Bar Plates

Food bar plates were prepared by NASA personnel using flight stock AEM food bars manufactured 6/21/93. Food bars were placed vertically rather than horizontally with respect to the launch orientation, as specified in the standard food bar plate preparation procedure. Two filled food bar plates were placed in each cage at the ends of the cage. The water box had no food bar plates attached. One full food bar plate was added to the Test Enclosure #1 (16 mice) water box, on test day 23.

Air Speed Measurement

Air speed in the 4" i.d. PVC pipe attached to each Test Enclosures was set to 180 feet per minute on test day 0. This was equivalent to 14.8 cubic feet per minute of air flow. Neither the Vivarium Shoe Box Control nor the Vivarium Control Enclosure had forced air flow.

Air speed was measured daily with a TSI, Inc. Velocicalc Model 8350 hot wire air flow meter. Air flow in cubic feet per minute, cfm, was calculated using the air speed and inside diameter of the pipe.

Ammonia Measurement

Ammonia concentration within the occupied cages and downstream from the outlet filter was measured using Sensidyne® Gastec No. 3L ammonia analyzer tubes (#32011-335, VWR Scientific, San Francisco, CA). A Sensidyne® Gastec pump (kit No. 800) drew either 100 ml or 200 ml of air through each analyzer tube. Test Enclosure internal air was drawn from the upstream differential pressure gauge port. Test Enclosure outlet plenum air was drawn from the downstream differential pressure gauge port (see Figure 1). Test Enclosure ammonia concentration was measured every day. Vivarium Control Enclosure ammonia concentration was measured every other day starting with test day 2.

Methylamine Measurement

Methylamine concentrations within the occupied cages and downstream from the outlet filter were measured using Dräger triethylamine analyzer tubes (number 5/A, Brenton Safety, Inc., South San Francisco, CA). An Accuro Delux pump drew either 100 ml or 200 ml of air through each analyzer tube. Test Enclosure internal air was drawn from the upstream differential pressure gauge port. Test Enclosure outlet plenum air was drawn from the downstream differential pressure gauge port (see Figure 1). Test Enclosure methylamine concentration was measured every day starting with test day 3. Vivarium Control Enclosure methylamine concentration was measured every other day starting with test day 4.

D. Data Analysis

Data Analysis Overview

Individual animal parameters comprise the bulk of the data collected from this study. Animal parameters included mouse body and organ weights and blood measurements. Data for each individual parameter were divided into two sets by cage number. One set of cages tested for effects due to the number of mice per cage. The other set tested for the effect of the type of cage used. Each cage set was checked for normality of distribution. Cage variances within a set were compared to test for homogeneity of variances. Sets which passed these checks were tested for differences using analysis of variance (ANOVA). Sets which did not pass the checks were tested for differences using the nonparametric Kruskal-Wallis test (Sokal and Rohlf, 1981).

Daily cage parameters, water and air flow, were also measured and recorded. Data for each daily cage parameter were divided into two sets of cages as for individual parameters. The sets of daily data were tested for differences among cages using a two way ANOVA without replication (Sokal and Rohlf, 1981).

Data Analysis Details

ANOVA requires the data in each cage to be normally distributed and the variances of cages within an ANOVA to be homogeneous. To test whether data conformed to the normal distribution, data for all mouse cages within each cage set were combined into one group after subtracting the cage mean from each cage value. We compared the frequency distribution of each combined group to the expected normal frequency distribution using the G test (Sokal and Rohlf, 1981), a test similar to but more accurate than the chi square test. Data were considered to be normally distributed for $P < .05$. Variances of cage means for cages within each cage set were then checked for homogeneity using the Burr-Foster Q-test. Variances were

homogeneous for $q < q_{\text{crit},0.01}$. A square root transform was applied to data which did not pass either the normality or the homogeneity test. These data were then reanalyzed. Data or transformed data which passed the normality and homogeneity of variances checks were tested using analysis of variance (ANOVA). Data which could not be transformed to correct normality or homogeneity problems were analyzed using the nonparametric Kruskal-Wallis test. Any parameter with a significant result in the ANOVA was also tested with the Student-Newman Keuls test (Glantz, 1992). This tested for significant differences between any two groups within the ANOVA for the characteristic which gave the significant ANOVA result.

Data for mice were divided, by cage, into two sets for analysis. Cage set one contained data from all three groups of mice in the Test Enclosures. Groups in this set differed in the number of mice per cage. One way ANOVAs were used to compare individual and daily parameters among these groups of mice. Any differences detected in measured parameters among these groups would be due to the number of mice per cage. Cage set two contained the Test Enclosure holding 8 mice, the Vivarium Control Enclosure (8 mice) and the Vivarium Shoe Box control (8 mice). This arrangement allowed mice in the two control cages to be compared to mice in the Test Enclosure. One way ANOVAs were used to compare individual and daily parameters among these groups. Differences detected by this test were due to cage type.

RESULTS:

A. Stress Assessment Battery Validation

Table 3 (S.A.B.). Video scoring data used for behavioral evaluation. The observer scored ten minutes of video per hour for six hours. The scores represent the sum frequency of each activity that occurred during the scoring epoch in all quadrants.

Treatment	Behavioral Score Category						Total
	0	1	2	3	4	5	
Stressed Score	412	120	826	195	0	118	1671
Stressed % Score	25%	7%	49%	12%	0%	7%	
Control Score	207	30	545	40	0	3	825
Control % Score	25%	4%	66%	5%	0%	0%	

Video Scoring Definitions

0 - sleeping, still, sniffing

1 - eating, drinking

2 - grooming, moving

3 - social interaction

4 - aggressive behavior

5 - running

Table 4 (S.A.B.): Daily food consumption (grams).

Day #	Stressed Group	Control Group
1	28.89	35.30
2	37.20	44.68
3	38.89	44.69
4	31.50	37.65
5	44.83	54.37
6	32.16	34.65
7	41.81	44.99
8	33.08	30.13
9	33.09	30.13
10	39.28	33.61
11	37.97	34.76
12	41.84	41.48
13	46.54	45.69
Mean	37.47	39.46
SEM	5.45	7.12

Table 5 (S.A.B.): Daily water consumption (ml).

Day #	Stressed Group	Control Group
1	49.0	62.0
2	52.0	65.0
3	53.0	65.0
4	50.0	52.0
5	70.0	74.0
6	52.0	49.0
7	69.0	83.0
8	47.0	45.0
9	47.0	46.0
10	51.0	55.0
12	54.0	49.0
13	73.0	69.0
Mean	55.7	58.9
SEM	9.0	11.8

Figure 1 (S.A.B.). Mean $\pm$ S.E.M. food consumption per mouse per day of the stressed and control mouse groups.

Figure 2 (S.A.B.). Mean $\pm$ S.E.M. water consumption per mouse per day of the stressed and control mouse groups.

Table 6 (S.A.B.). Mouse initial (day 0) and final (day 14) body weight, weight gain in grams and % weight gain.

Mouse #	Initial Wt(g)	Final Wt(g)	Gain (g)	Gain (%Initial)
Stress 1	18.11	20.30	2.19	12.09
Group 2	18.23	23.10	4.87	26.71
3	19.46	22.90	3.44	17.67
4	18.81	21.40	2.59	13.77
5	17.57	20.20	2.63	14.67
6	19.42	23.60	4.18	21.52
7	18.41	21.00	2.59	14.07
8	19.43	23.20	3.77	19.40
9	18.77	22.40	3.63	19.34
10	15.98	23.50	7.52	47.06
Mean	18.42	22.20	3.74	20.66
SEM	1.06	0.42	0.50	3.24

Mouse #	Initial Wt(g)	Final Wt(g)	Gain (g)	Gain (%Initial)
Control 11	17.76	24.20	6.44	36.26
Group 12	17.60	25.60	8.00	45.46
13	18.11	26.40	8.29	45.78
14	17.45	23.90	6.45	39.96
15	18.23	24.40	6.17	33.85
16	18.61	23.10	4.49	24.13
17	19.02	23.10	4.08	21.45
18	19.12	25.10	5.98	31.28
19	16.00	25.20	9.20	57.50
20	18.11	24.90	6.79	37.49
Mean	18.00	24.60	6.58	37.02
SEM	0.90	0.34	0.51	3.37

Figure 3 (S.A.B.). Mean $\pm$ S.E.M. body weight of the stressed and control mouse groups.

Figure 4 (S.A.B.). Mean $\pm$ S.E.M. weight gain and weight gain as a percentage of start weight.

Table 7 (S.A.B.). Organ weights in grams and as a percentage of final body weight (BW) of the stressed group.

Mouse #	Body Wt (g)	Heart Wt(g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
1	20.3	0.094	0.463	0.281	1.384	0.0015	0.0074	0.056	0.276	0.066	0.325
2	23.1	0.124	0.537	0.346	1.498	0.0013	0.0056	0.088	0.381	0.062	0.268
3	22.9	0.097	0.424	0.301	1.314	0.0020	0.0087	0.042	0.183	0.042	0.183
4	21.4	0.110	0.514	0.308	1.439	0.0018	0.0084	0.067	0.313	0.068	0.318
5	20.2	0.099	0.490	0.265	1.312	0.0016	0.0079	0.045	0.223	0.062	0.307
6	23.6	0.115	0.487	0.366	1.551	0.0018	0.0038	0.048	0.203	0.047	0.199
7	21.0	0.096	0.457	0.303	1.443	0.0020	0.0095	0.042	0.200	0.060	0.286
8	23.2	0.106	0.457	0.288	1.241	0.0019	0.0082	0.048	0.207	0.063	0.272
9	22.4	0.090	0.402	0.289	1.290	0.0014	0.0063	0.064	0.286	0.086	0.384
10	23.5	0.100	0.426	0.285	1.213	0.0029	0.0012	0.047	0.200	0.068	0.289
Mean	22.2	0.103	0.466	0.303	1.369	0.0018	0.0082	0.055	0.247	0.062	0.283
SEM	0.42	0.003	0.013	0.0097	0.036	0.0001	0.0006	0.0046	0.020	0.0038	0.019

Table 8 (S.A.B.). Organ weights in grams and as a percentage of final body weight (BW) of the control group.

Mouse #	Body Wt (g)	Heart Wt(g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
11	24.2	0.108	0.446	0.300	1.240	0.0013	0.0054	0.060	0.248	0.048	0.198
12	25.6	0.119	0.465	0.253	0.988	0.0028	0.0109	0.085	0.332	0.099	0.387
13	26.4	0.100	0.379	0.376	1.424	0.0015	0.0057	0.069	0.261	0.123	0.466
14	23.9	0.109	0.456	0.342	1.431	0.0023	0.0096	0.077	0.322	0.106	0.444
15	24.4	0.113	0.463	0.242	0.992			0.080	0.328	0.089	0.365
16	23.1	0.088	0.381	0.258	1.117	0.0005	0.0022	0.062	0.268	0.089	0.385
17	23.1	0.100	0.433	0.320	1.385	0.0014	0.0061	0.070	0.303	0.086	0.372
18	25.1	0.116	0.462	0.324	1.291	0.0026	0.0104	0.052	0.207	0.096	0.382
19	25.2	0.126	0.500	0.309	1.226	0.0027	0.0107	0.096	0.381	0.106	0.421
20	24.9	0.106	0.426	0.347	1.394	0.0031	0.0124	0.098	0.394	0.100	0.402
Mean	24.6	0.109	0.441	0.307	1.249	0.0020	0.0082	0.075	0.304	0.094	0.382
SEM	0.34	0.003	0.012	0.0140	0.053	0.0003	0.0011	0.0048	0.019	0.0062	0.023

Figure 5 (S.A.B.). Mean $\pm$ S.E.M heart weight and heart weight as a percentage of final body weight (See also Tables 7 and 8).

Figure 6 (S.A.B.). Mean $\pm$ S.E.M. kidney weight and kidney weight as a percentage of final body weight (See also Tables 7 and 8).

Figure 7 (S.A.B.). Mean $\pm$ S.E.M. adrenal weight and adrenal weight as a percentage of final body weight (See also Tables 7 and 8).

Figure 8 (S.A.B.). Mean $\pm$ S.E.M. spleen weight and spleen weight as a percentage of final body weight (See also Tables 7 and 8).

Figure 9 (S.A.B.). Mean $\pm$ S.E.M. thymus weight and thymus weight as a percentage of final body weight (See also Tables 7 and 8).

Table 9 (S.A.B.). Plasma corticosterone ($\mu\text{g/dl}$), plasma protein, and total leukocyte count in the stressed and control groups.

Mouse #	Plasma Corticosterone $\mu\text{g/dl}$	Plasma Protein mg/dl	Total WBC cells/ml
Stress 1	2.08	5.13	7500
Group 2	6.10	6.36	6750
3	9.05	4.10	2000
4	3.86	3.67	8750
5	3.18	6.36	7500
6	13.71	6.41	2750
7	7.20	5.86	5500
8	41.52	4.24	4750
9	9.77	5.55	8750
10	4.50	4.41	5750
Mean	10.10	5.21	6000
SEM	3.66	0.33	735.0

Mouse #	Plasma Corticosterone $\mu\text{g/dl}$	Plasma Protein mg/dl	Total WBC cells/ml
Control 11	1.68	3.23	2500
Group 12	2.42	5.22	7000
13	6.70	5.17	7250
14	1.16	4.07	3500
15	3.07	4.15	ND
16	5.62	3.74	6250
17	22.20	3.46	9250
18	12.36	4.07	5750
19	19.11	5.10	6250
20	9.19	3.33	5250
Mean	8.35	4.15	5889
SEM	2.34	0.24	669.4

ND = not determined

Figure 10 (S.A.B.). Plasma corticosterone mean $\pm$ S.E.M. in the stressed and control groups (See also Table 9).

Figure 11 (S.A.B.). Plasma protein mean $\pm$ S.E.M. (See also Table 9).

Table 10 (S.A.B.). Differential WBC count, total eosinophil count and neutrophil to lymphocyte ratio of the stress group.

Mouse #	Eosinophil %WBC	Basophil %WBC	Neutrophil %WBC	Monocyte %WBC	Lymphocyte %WBC	Eosinophil cells/ μ l	N/L
1	0	0	5	3	92	0	0.05
2	3	0	6	2	89	203	0.07
3	0	0	8	5	87	0	0.09
4	1	0	2	9	88	88	0.02
5	2	0	8	5	85	150	0.09
6	2	0	15	7	76	55	0.20
7	0	0	14	9	77	0	0.18
8	0	0	6	4	90	0	0.07
9	1	0	12	5	82	88	0.15
10	4	0	7	6	83	230	0.08
Mean	1.3		8.3	5.5	84.9	81	0.10
SEM	0.448		1.309	0.734	1.703	27.7	0.018

Table 11 (S.A.B.). Differential WBC count, total eosinophil count and neutrophil to lymphocyte ratio of the control group.

Mouse #	Eosinophil %WBC	Basophil %WBC	Neutrophil %WBC	Monocyte %WBC	Lymphocyte %WBC	Eosinophil cells/ μ l	N/L
11	4	0	13	4	79	100	0.16
12	ND	ND	ND	ND	ND	ND	ND
13	4	0	1	4	91	290	0.01
14	0	0	8	2	90	0	0.09
15	ND	ND	ND	ND	ND	ND	ND
16	1	0	11	4	84	63	0.13
17	3	0	10	6	81	288	0.12
18	1	0	8	7	84	58	0.01
19	4	0	2	7	87	250	0.02
20	0	0	3	5	92	0	0.03
Mean	2.1		7	4.9	86	131	0.07
SEM	0.572		1.414	0.546	1.512	44.2	0.022

ND = not determined

B. Experiment 1: Mouse Density Study

Table 12 (Experiment 1). Daily total and per mouse water consumption for each cage (ml). Mean $\pm$ S.E.M. were calculated using days 4 - 30 only.

Day #	Test Enclosures														Viv shoe	Viv Cntrl
	#1		#2		#3		#5		#6		#7		box cntrl		Enclosure	
	(12 mice)		(16 mice)		(8 mice)		(16 mice)		(12 mice)		(8 mice)		(8 mice)		(8 mice)	
	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse
1	16	1.3	22	1.4	15	1.9	16	1.0	20	1.7	14	1.8	41	5.1	38	4.8
2	44	3.7	58	3.6	35	4.4	76	4.8	40	3.3	28	3.5			97	12.1
3	44	3.7	62	3.9	70	8.8	71	4.4	52	4.3	29	3.6	31	3.9	30	3.8
4	54	4.5	82	5.1	47	5.9	95	5.9	68	5.7	35	4.4	65	8.1	54	6.8
5	56	4.7	83	5.2	50	6.3	103	6.4	67	5.6	43	5.4			70	8.8
6	67	5.6	103	6.4	71	8.9	118	7.4	79	6.6	44	5.5	66	8.3	30	3.8
7	60	5.0	96	6.0	56	7.0	102	6.4	76	6.3	40	5.0	41	5.1	30	3.8
8	76	6.3	98	6.1	78	9.8	120	7.5	84	7.0	48	6.0	85	10.6	58	7.3
9	75	6.3	91	5.7	64	8.0	107	6.7	82	6.8	48	6.0	84	10.5	74	9.3
10	73	6.1	121	7.6	74	9.3	118	7.4	98	8.2	54	6.8	44	5.5	46	5.8
11	59	4.9	112	7.0	74	9.3	116	7.3	74	6.2	60	7.5	75	9.4	56	7.0
12	61	5.1	110	6.9	71	8.9	105	6.6	83	6.9	50	6.3	68	8.5	54	6.8
13	84	7.0	128	8.0	88	11.0	148	9.3	94	7.8	57	7.1	70	8.8	64	8.0
14	62	5.2	100	6.3	64	8.0	84	5.3	63	5.3	40	5.0	62	7.8	40	5.0
15	76	6.3	120	7.5	81	10.1	122	7.6	77	6.4	104	13.0	68	8.5	62	7.8
16	58	4.8	91	5.7	78	9.8	94	5.9	63	5.3	52	6.5	73	9.1	48	6.0
17	78	6.5	97	6.1	28	3.5	92	5.8	73	6.1	101	12.6	49	6.1	54	6.8
18	76	6.3	155	9.7	63	7.9	100	6.3	75	6.3	42	5.3	20	2.5	56	7.0
19	74	6.2	53	3.3	71	8.9	84	5.3	76	6.3	48	6.0	63	7.9	64	8.0
20	78	6.5	107	6.7	70	8.8	108	6.8	92	7.7	58	7.3	69	8.6	64	8.0
21	66	5.5	96	6.0	45	5.6	88	5.5	79	6.6	39	4.9	64	8.0	58	7.3
22	86	7.2	118	7.4	72	9.0	112	7.0	90	7.5	49	6.1	66	8.3	63	7.9
23	74	6.2	107	6.7	80	10.0	98	6.1	74	6.2	51	6.4	74	9.3	57	7.1
24	94	7.8	126	7.9	84	10.5	130	8.1	93	7.8	62	7.8	70	8.8	86	10.8
25	82	6.8	110	6.9	58	7.3	108	6.8	81	6.8	53	6.6	68	8.5	69	8.6
26	85	7.1	117	7.3	74	9.3	117	7.3	80	6.7	60	7.5	64	8.0	60	7.5
27	77	6.4	118	7.4	69	8.6	121	7.6	78	6.5	55	6.9	48	6.0	62	7.8
28	88	7.3	116	7.3	78	9.8	125	7.8	83	6.9	60	7.5	52	6.5	57	7.1
29	80	6.7	136	8.5	75	9.4	128	8.0	93	7.8	58	7.3	20	2.5	54	6.8
30	82	6.8	106	6.6	74	9.3	112	7.0	91	7.6	46	5.8	45	5.6	68	8.5
Mean	73.	6.1	107	6.7	68	8.5	109	6.8	80	6.7	54	6.7	60	7.6	58	7.2
S.E.M.	2	0.2	4	0.2	3	0.3	3	0.2	2	0.2	3	0.4	3	0.4	2	0.3

Figure 12 (Experiment 1). Mean $\pm$ S.E.M. daily water consumption per cage and per mouse (See Table 22).

Table 13 (Experiment 1). Total food use (grams) over 30 days.

	Test Encl #3 (8 mice)	Test Encl #6 (12 mice)	Test Encl #5 (16 mice)	Test Encl #7 (8 mice)	Test Encl #1 (12 mice)	Test Encl #2 (16 mice)
Total	690.83	665.17	666.11	724.75	1775.28	1218.38
Total/mouse/day	3.84	3.70	3.70	4.03	4.93	3.38

Table 14 (Experiment 1). Mouse initial (day 0) and final (day 30) body weight, weight gain in grams, and % weight gain.

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test enclosure #3	1	21.59	35.09	13.50	62.53
ALFCO #3A Filter	2	23.71	30.66	6.95	29.31
8 mice	3	21.61	28.17	6.56	30.36
	4	22.88	32.60	9.72	42.48
	5	21.60	30.32	8.72	40.37
	6	23.56	29.13	5.57	23.64
	7	21.54	27.53	5.99	27.81
	8	21.43	25.76	4.33	20.21
Mean		22.24	29.91	7.67	34.59
SEM		0.35	1.05	1.03	4.81

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test enclosure #6	9	23.31	29.07	5.76	24.71
ALFCO #3A Filter	10	21.81	30.31	8.50	38.97
12 mice	11	21.46	26.24	4.78	22.27
	12	21.07	30.13	9.06	43.00
	13	21.69	26.53	4.84	22.31
	14	21.50	23.87	2.37	11.02
	15	23.28	33.08	9.80	42.10
	16	23.17	31.38	8.21	35.43
	17	21.04	31.43	10.39	49.38
	18	20.22	27.70	7.48	36.99
	19	21.07	31.30	10.23	48.55
	20	22.24	29.60	7.36	33.09
Mean		21.82	29.22	7.40	33.99
SEM		0.29	0.77	0.72	3.39

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test enclosure #5 ALFCO #3A Filter 16 mice	21	21.21	25.42	4.21	19.85
	22	20.78	27.23	6.45	31.04
	23	21.79	32.28	10.49	48.14
	24	23.54	34.23	10.69	45.41
	25	23.66	31.37	7.71	32.59
	26	22.22	25.77	3.55	15.98
	27	22.91	30.50	7.59	33.13
	28	22.50	26.53	4.03	17.91
	29	22.12	28.00	5.88	26.58
	30	20.72	34.09	13.37	64.53
	31	21.15	24.84	3.69	17.45
	32	20.08	25.66	5.58	27.79
	33	21.35	24.52	3.17	14.85
	34	21.25	26.38	5.13	24.14
	35	21.14	29.67	8.53	40.35
	36	24.01	29.84	5.83	24.28
Mean		21.90	28.52	6.62	30.25
SEM		0.29	0.81	0.74	3.42

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test enclosure #7 ALFCO #4 Filter 8 mice	37	19.80	25.49	5.69	28.74
	38	22.66	30.10	7.44	32.83
	39	20.98	29.04	8.06	38.42
	40	21.21	27.59	6.38	30.08
	41	20.77	26.22	5.45	26.24
	42	22.22	31.63	9.41	42.35
	43	23.56	30.45	6.89	29.24
	44	20.47	26.99	6.52	31.85
Mean		21.46	28.44	6.98	32.47
SEM		0.44	0.78	0.46	1.90

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test enclosure #1 ALFCO #4 Filter 12 mice	45	20.19	27.19	7.00	34.67
	46	21.24	30.80	9.56	45.01
	47	21.06	27.24	6.18	29.34
	48	22.00	27.62	5.62	25.55
	49	22.46	24.74	2.28	10.15
	50	22.55	28.77	6.22	27.58
	51	22.42	27.22	4.80	21.41
	52	20.54	31.24	10.70	52.09
	53	22.54	31.31	8.77	38.91
	54	21.01	26.75	5.74	27.32
	55	23.91	30.55	6.64	27.77
	56	21.82	28.03	6.21	28.46
Mean		21.81	28.46	6.64	30.69
SEM		0.30	0.60	0.64	3.16

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test enclosure #2 ALFCO #4 Filter 16 mice	57	21.09	27.08	5.99	28.40
	58	21.95	27.27	5.32	24.24
	59	20.55	26.79	6.24	30.36
	60	21.42	29.29	7.87	36.74
	61	20.83	29.36	8.53	40.95
	62	21.44	27.26	5.82	27.15
	63	21.56	27.65	6.09	28.25
	64	23.28	28.66	5.38	23.11
	65	20.14	32.89	12.75	63.31
	66	22.75	28.47	5.72	25.14
	67	20.38	26.33	5.95	29.20
	68	22.01	26.22	4.21	19.13
	69	21.60	28.11	6.51	30.14
	70	20.63	26.42	5.79	28.07
	71	20.46	26.93	6.47	31.62
	72	22.10	31.26	9.16	41.45
Mean		21.39	28.12	6.74	31.70
SEM		0.22	0.46	0.51	2.58

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Vivarium Shoe Box Control 8 mice	73	22.91	26.48	3.57	15.58
	74	20.34	24.09	3.75	18.44
	75	23.86	29.05	5.19	21.75
	76	22.56	28.34	5.78	25.62
	77	21.13	25.53	4.40	20.82
	78	20.83	28.27	7.44	35.72
	79	22.26	28.18	5.92	26.59
	80	22.50	29.65	7.15	31.78
Mean		22.05	27.45	5.40	24.54
SEM		0.42	0.67	0.51	2.40

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Vivarium Control Enclosure 8 mice	81	20.88	26.14	5.26	25.19
	82	21.71	27.98	6.27	28.88
	83	23.17	38.81	15.64	67.50
	84	22.51	29.21	6.70	29.76
	85	21.77	27.90	6.13	28.16
	86	22.35	29.62	7.27	32.53
	87	23.24	30.32	7.08	30.46
	88	20.39	27.70	7.31	35.85
Mean		22.00	29.71	7.71	34.79
SEM		0.36	1.38	1.16	4.80

Table 15 (Experiment 1). Cage mean $\pm$ S.E.M for initial (day 0) and final (day 30) body weight, weight gain in grams, and % weight gain.

	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Vivarium Shoe Box Control, 8 mice	22.05 $\pm$ 0.42	27.45 $\pm$ 0.67	5.40 $\pm$ 0.51	24.54 $\pm$ 2.40
Vivarium Control Enclosure, 8 mice	22.00 $\pm$ 0.36	29.71 $\pm$ 1.38	7.71 $\pm$ 1.16	34.79 $\pm$ 4.80
Test enclosure #3, ALFCO #3A Filter, 8 mice	22.24 $\pm$ 0.35	29.91 $\pm$ 1.05	7.67 $\pm$ 1.03	34.59 $\pm$ 4.81
Test enclosure #7, ALFCO #4 Filter, 8 mice	21.46 $\pm$ 0.44	28.44 $\pm$ 0.78	6.98 $\pm$ 0.46	32.47 $\pm$ 1.90
Test enclosure #6, ALFCO #3A Filter, 12 mice	21.82 $\pm$ 0.29	29.22 $\pm$ 0.77	7.40 $\pm$ 0.72	33.99 $\pm$ 3.39
Test enclosure #1, ALFCO #4 Filter, 12 mice	21.81 $\pm$ 0.30	28.46 $\pm$ 0.60	6.64 $\pm$ 0.64	30.69 $\pm$ 3.16
Test enclosure #5, ALFCO #3A Filter, 16 mice	21.90 $\pm$ 0.29	28.52 $\pm$ 0.81	6.62 $\pm$ 0.74	30.25 $\pm$ 3.42
Test enclosure #2, ALFCO #4 Filter, 16 mice	21.39 $\pm$ 0.22	28.12 $\pm$ 0.46	6.74 $\pm$ 0.51	31.70 $\pm$ 2.58

Figure 13 (Experiment 1). Mean $\pm$ S.E.M. starting body weight (day 1) and mean $\pm$ S.E.M. ending body weight (day 30). (See also table 15).

Figure 14 (Experiment 1). Mean $\pm$ S.E.M. body weight gain and mean $\pm$ S.E.M. per cent body weight gain. (See also table 15).

Table 16 (Experiment 1). Organ weights in grams and as a percentage of body weight (BW).

Test Enclosure #3, ALFCO #3A Filter, 8 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
1	0.121	0.345	0.358	1.020			0.062	0.177	0.052	0.148
2	0.122	0.398	0.378	1.233	0.0056	0.0183	0.067	0.219	0.087	0.284
3	0.116	0.412	0.384	1.363	0.0021	0.0075	0.126	0.447	0.054	0.192
4	0.116	0.356	0.384	1.178	0.0028	0.0086	0.082	0.252	0.039	0.120
5	0.134	0.442	0.462	1.524	0.0025	0.0082	0.112	0.369	0.125	0.412
6	0.128	0.439	0.388	1.332	0.0014	0.0048	0.109	0.374	0.076	0.261
7	0.120	0.436	0.417	1.515	0.0035	0.0127	0.086	0.312	0.108	0.392
8	0.109	0.423	0.340	1.320	0.0038	0.0148	0.082	0.318	0.067	0.260
Mean	0.121	0.406	0.389	1.311	0.0031	0.0107	0.091	0.309	0.076	0.259
SEM	0.003	0.013	0.013	0.060	0.0005	0.0018	0.008	0.032	0.010	0.037

Test Enclosure #6, ALFCO #3A Filter, 12 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
9	0.118	0.406	0.521	1.792	0.004	0.0138	0.121	0.416	0.090	0.310
10	0.135	0.445	0.466	1.537	0.0022	0.0073	0.122	0.403	0.061	0.201
11	0.108	0.412	0.323	1.231	0.002	0.0076	0.094	0.358	0.085	0.324
12	0.124	0.412	0.439	1.457	0.0014	0.0046	0.110	0.365	0.087	0.289
13	0.120	0.452	0.405	1.527	0.0053	0.0200	0.100	0.377	0.055	0.207
14	0.125	0.524	0.386	1.617	0.0039	0.0163	0.070	0.293	0.069	0.289
15	0.143	0.432	0.452	1.366	0.0023	0.0070	0.130	0.393	0.082	0.248
16	0.131	0.417	0.492	1.568	0.0050	0.0159	0.109	0.347	0.100	0.319
17	0.151	0.480	0.462	1.470	0.0069	0.0220	0.109	0.347	0.070	0.223
18	0.131	0.473	0.302	1.090	0.0012	0.0043	0.087	0.314	0.096	0.347
19	0.143	0.457	0.426	1.361	0.0047	0.0150	0.099	0.316	0.112	0.358
20	0.146	0.493	0.412	1.392	0.0014	0.0047	0.095	0.321	0.063	0.213
Mean	0.131	0.450	0.424	1.451	0.0034	0.0115	0.104	0.354	0.081	0.277
SEM	0.004	0.011	0.019	0.053	0.0005	0.0018	0.005	0.011	0.005	0.016

Test Enclosure #5, ALFCO #3A Filter, 16 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
21	0.116	0.456	0.362	1.424	0.0022	0.0087	0.082	0.323	0.086	0.338
22	0.130	0.477	0.421	1.546	0.0023	0.0084	0.100	0.367	0.046	0.169
23	0.147	0.455	0.551	1.707	0.0032	0.0099	0.089	0.276	0.060	0.186
24	0.147	0.429	0.587	1.715	0.0048	0.0140	0.147	0.429	0.087	0.254
25	0.126	0.402	0.393	1.253	0.0042	0.0134	0.105	0.335	0.090	0.287
26	0.124	0.481	0.389	1.510	0.0034	0.0132	0.080	0.310	0.065	0.252
27	0.132	0.433	0.441	1.446	0.0023	0.0075	0.125	0.410	0.062	0.203
28	0.153	0.577	0.495	1.866	0.0048	0.0181	0.104	0.392	0.143	0.539
29	0.113	0.404	0.406	1.450	0.0028	0.0100	0.098	0.350	0.069	0.246
30	0.134	0.393	0.561	1.646	0.0056	0.0164	0.161	0.472	0.096	0.282
31	0.108	0.435	0.333	1.341	0.0044	0.0177	0.086	0.346	0.027	0.109
32	0.121	0.472	0.369	1.438	0.0028	0.0109	0.086	0.335	0.059	0.230
33	0.109	0.445	0.329	1.342	0.0030	0.0122	0.091	0.371	0.040	0.163
34	0.123	0.466	0.424	1.607	0.0074	0.0281	0.099	0.375	0.039	0.148
35	0.120	0.404	0.392	1.321	0.0270	0.0910	0.074	0.249	0.046	0.155
36	0.128	0.429	0.410	1.374	0.0034	0.0114	0.095	0.318	0.056	0.188
Mean	0.127	0.447	0.429	1.499	0.0052	0.0182	0.101	0.354	0.067	0.234
SEM	0.003	0.011	0.020	0.042	0.0015	0.0050	0.006	0.014	0.007	0.025

Test Enclosure #7, ALFCO #4 Filter, 8 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
37	0.110	0.432	0.373	1.463	0.0038	0.0149	0.089	0.349	0.049	0.192
38	0.111	0.369	0.426	1.415	0.0044	0.0146	0.091	0.302	0.089	0.296
39	0.136	0.468	0.443	1.525	0.0016	0.0055	0.133	0.458	0.066	0.227
40	0.136	0.493	0.431	1.562	0.0029	0.0105	0.078	0.283	0.055	0.199
41	0.120	0.458	0.395	1.506	0.0020	0.0076	0.099	0.378	0.073	0.278
42	0.140	0.443	0.390	1.233	0.0035	0.0111	0.086	0.272	0.056	0.177
43	0.126	0.414	0.406	1.333	0.0036	0.0118	0.086	0.282	0.079	0.259
44	0.121	0.448	0.394	1.460	0.0042	0.0156	0.084	0.311	0.062	0.230
Mean	0.125	0.440	0.407	1.437	0.0033	0.0115	0.093	0.329	0.066	0.232
SEM	0.004	0.013	0.008	0.038	0.0004	0.0013	0.006	0.022	0.005	0.015

Test Enclosure #1, ALFCO #4 Filter, 12 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
45	0.104	0.382	0.370	1.361	0.0050	0.0184	0.094	0.346	0.080	0.294
46	0.133	0.432	0.416	1.351	0.0047	0.0153	0.114	0.370	0.069	0.224
47	0.141	0.518	0.436	1.601	0.0031	0.0114	0.095	0.349	0.077	0.283
48	0.113	0.409	0.430	1.557	0.0022	0.0080	0.142	0.514	0.059	0.214
49	0.121	0.489	0.435	1.758	0.0013	0.0053	0.091	0.368	0.038	0.154
50	0.120	0.417	0.420	1.460	0.0053	0.0184	0.080	0.278	0.040	0.139
51	0.130	0.478	0.386	1.418	0.0066	0.0242	0.073	0.268	0.046	0.169
52	0.128	0.410	0.482	1.543	0.0041	0.0131	0.146	0.467	0.098	0.314
53	0.139	0.444	0.469	1.498	0.0077	0.0246	0.111	0.355	0.076	0.243
54	0.120	0.449	0.420	1.570	0.0039	0.0146	0.110	0.411	0.070	0.262
55	0.148	0.484	0.465	1.522	0.0070	0.0229	0.095	0.311	0.050	0.164
56	0.100	0.357	0.400	1.427	0.0043	0.0153	0.080	0.285	0.060	0.214
Mean	0.125	0.439	0.427	1.505	0.0046	0.0160	0.103	0.360	0.064	0.223
SEM	0.004	0.014	0.0010	0.033	0.0005	0.0018	0.007	0.022	0.005	0.017

Test Enclosure #2, ALFCO #4 Filter, 16 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
57	0.128	0.473	0.435	1.606	0.0025	0.0092	0.094	0.347	0.095	0.351
58	0.126	0.462	0.388	1.423	0.0020	0.0073	0.090	0.330	0.046	0.169
59	0.124	0.463	0.429	1.601	0.0035	0.0131	0.099	0.370	0.070	0.261
60	0.136	0.464	0.432	1.475	0.0071	0.0242	0.093	0.318	0.064	0.219
61	0.138	0.470	0.482	1.642	0.0034	0.0116	0.121	0.412	0.086	0.293
62	0.113	0.415	0.389	1.427	0.0040	0.0147	0.095	0.348	0.087	0.319
63	0.118	0.427	0.447	1.617	0.0030	0.0108	0.091	0.329	0.057	0.206
64	0.112	0.391	0.415	1.448			0.149	0.520	0.115	0.401
65	0.121	0.368	0.498	1.514	0.0035	0.0106	0.094	0.286	0.072	0.219
66	0.114	0.400	0.385	1.352	0.0023	0.0081	0.085	0.299	0.098	0.344
67	0.114	0.433	0.474	1.800	0.0040	0.0152	0.087	0.330	0.052	0.197
68	0.112	0.427	0.425	1.621	0.0016	0.0061	0.084	0.320	0.083	0.317
69	0.128	0.455	0.455	1.619	0.0035	0.0125	0.106	0.377	0.145	0.516
70	0.125	0.473	0.329	1.245	0.0015	0.0057	0.099	0.375	0.086	0.326
71	0.115	0.427	0.381	1.415	0.0047	0.0175	0.112	0.416	0.091	0.338
72	0.119	0.381	0.439	1.404	0.0040	0.0128	0.108	0.345	0.120	0.384
Mean	0.121	0.433	0.425	1.513	0.0034	0.0120	0.100	0.358	0.085	0.304
SEM	0.002	0.009	0.011	0.035	0.0004	0.0012	0.004	0.014	0.007	0.023

Vivarium shoe box control, 8 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
73	0.128	0.483	0.418	1.579	0.0031	0.0117	0.100	0.378	0.090	0.340
74	0.100	0.415	0.320	1.328	0.0023	0.0095	0.110	0.457	0.080	0.332
75	0.120	0.413	0.410	1.411	0.0034	0.0117	0.090	0.310	0.120	0.413
76	0.130	0.459	0.360	1.270	0.0032	0.0113	0.090	0.318	0.100	0.353
77	0.120	0.470	0.380	1.488	0.0032	0.0125	0.070	0.274	0.060	0.235
78	0.130	0.460	0.360	1.273	0.0039	0.0138	0.080	0.283	0.138	0.488
79	0.130	0.461	0.410	1.455	0.0023	0.0082	0.090	0.319	0.080	0.284
80	0.150	0.506	0.440	1.484	0.0023	0.0078	0.160	0.540	0.060	0.202
Mean	0.126	0.458	0.387	1.411	0.0030	0.0108	0.099	0.360	0.091	0.331
SEM	0.005	0.011	0.014	0.039	0.0002	0.0008	0.0010	0.033	0.0010	0.033

Vivarium Control Enclosure, 8 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
81	0.122	0.467	0.388	1.484	0.0025	0.0096	0.105	0.402	0.034	0.130
82	0.140	0.500	0.420	1.501	0.0025	0.0089	0.097	0.347	0.050	0.179
83	0.163	0.420	0.543	1.399	0.0009	0.0023	0.152	0.392	0.086	0.222
84	0.153	0.524	0.426	1.458	0.0045	0.0154	0.075	0.257	0.073	0.250
85	0.125	0.448	0.428	1.534	0.0050	0.0179	0.092	0.330	0.068	0.244
86	0.148	0.500	0.375	1.266	0.0013	0.0044	0.090	0.304	0.068	0.230
87	0.148	0.488	0.405	1.336	0.0047	0.0155	0.089	0.294	0.092	0.303
88	0.125	0.451	0.399	1.440	0.0023	0.0083	0.125	0.451	0.070	0.253
Mean	0.141	0.475	0.423	1.427	0.0030	0.0103	0.103	0.347	0.068	0.226
SEM	0.005	0.012	0.018	0.032	0.0006	0.0020	0.009	0.023	0.007	0.018

Table 17 (Experiment 1). Cage mean $\pm$ S.E.M for organ weights in grams and as a percentage of body weight (BW).

	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW
Vivarium Shoe Box Control, 8 mice	0.126 $\pm$ 0.005	0.458 $\pm$ 0.011	0.387 $\pm$ 0.014	1.411 $\pm$ 0.039
Vivarium Control Enclosure, 8 mice	0.141 $\pm$ 0.005	0.475 $\pm$ 0.012	0.423 $\pm$ 0.018	1.427 $\pm$ 0.032
Test enclosure #3, ALFCO #3A Filter, 8 mice	0.121 $\pm$ 0.003	0.406 $\pm$ 0.013	0.389 $\pm$ 0.013	1.311 $\pm$ 0.060
Test enclosure #7, ALFCO #4 Filter, 8 mice	0.125 $\pm$ 0.004	0.440 $\pm$ 0.013	0.407 $\pm$ 0.008	1.437 $\pm$ 0.038
Test enclosure #6, ALFCO #3A Filter, 12 mice	0.131 $\pm$ 0.004	0.450 $\pm$ 0.011	0.424 $\pm$ 0.019	1.451 $\pm$ 0.053
Test enclosure #1, ALFCO #4 Filter, 12 mice	0.125 $\pm$ 0.004	0.439 $\pm$ 0.014	0.427 $\pm$ 0.001	1.505 $\pm$ 0.033
Test enclosure #5, ALFCO #3A Filter, 16 mice	0.127 $\pm$ 0.003	0.447 $\pm$ 0.011	0.429 $\pm$ 0.020	1.499 $\pm$ 0.042
Test enclosure #2, ALFCO #4 Filter, 16 mice	0.121 $\pm$ 0.002	0.433 $\pm$ 0.009	0.425 $\pm$ 0.011	1.513 $\pm$ 0.035

	Adrenal Wt (mg)	Adrenal % BW $\times 10^{-3}$	Spleen Wt (g)% BW	Spleen
Vivarium Shoe Box Control, 8 mice	3.0 $\pm$ 0.2	10.8 $\pm$ 0.8	0.099 $\pm$ 0.001	0.360 $\pm$ 0.033
Vivarium Control Enclosure, 8 mice	3.0 $\pm$ 0.6	10.3 $\pm$ 2.0	0.103 $\pm$ 0.009	0.347 $\pm$ 0.023
Test enclosure #3, ALFCO #3A Filter, 8 mice	3.1 $\pm$ 0.5	10.7 $\pm$ 1.8	0.091 $\pm$ 0.008	0.309 $\pm$ 0.032
Test enclosure #7, ALFCO #4 Filter, 8 mice	3.3 $\pm$ 0.4	11.5 $\pm$ 1.3	0.093 $\pm$ 0.006	0.329 $\pm$ 0.022
Test enclosure #6, ALFCO #3A Filter, 12 mice	3.4 $\pm$ 0.5	11.5 $\pm$ 1.8	0.104 $\pm$ 0.005	0.354 $\pm$ 0.011
Test enclosure #1, ALFCO #4 Filter, 12 mice	4.6 $\pm$ 0.5	16.0 $\pm$ 1.8	0.103 $\pm$ 0.007	0.360 $\pm$ 0.022
Test enclosure #5, ALFCO #3A Filter, 16 mice	5.2 $\pm$ 1.5	18.2 $\pm$ 5.0	0.101 $\pm$ 0.006	0.354 $\pm$ 0.014
Test enclosure #2, ALFCO #4 Filter, 16 mice	3.4 $\pm$ 0.4	12.0 $\pm$ 1.2	0.100 $\pm$ 0.004	0.358 $\pm$ 0.014

	Thymus Wt (g)	Thymus % BW
Vivarium Shoe Box Control, 8 mice	0.091 $\pm$ 0.001	0.331 $\pm$ 0.033
Vivarium Control Enclosure, 8 mice	0.068 $\pm$ 0.007	0.226 $\pm$ 0.018
Test enclosure #3, ALFCO #3A Filter, 8 mice	0.076 $\pm$ 0.010	0.259 $\pm$ 0.037
Test enclosure #7, ALFCO #4 Filter, 8 mice	0.066 $\pm$ 0.005	0.232 $\pm$ 0.015
Test enclosure #6, ALFCO #3A Filter, 12 mice	0.081 $\pm$ 0.005	0.277 $\pm$ 0.016
Test enclosure #1, ALFCO #4 Filter, 12 mice	0.064 $\pm$ 0.005	0.223 $\pm$ 0.017
Test enclosure #5, ALFCO #3A Filter, 16 mice	0.067 $\pm$ 0.007	0.234 $\pm$ 0.025
Test enclosure #2, ALFCO #4 Filter, 16 mice	0.085 $\pm$ 0.007	0.304 $\pm$ 0.023

Figure 15 (Experiment 1). Mean $\pm$ S.E.M. heart weight. and mean $\pm$ S.E.M. heart weight as a percent of final body weight. (See also Table 17).

Figure 16 (Experiment 1). Mean $\pm$ S.E.M. kidney weight. and mean $\pm$ S.E.M. kidney weight as a percent of final body weight. (See also Table 17).

Figure 17 (Experiment 1). Mean $\pm$ S.E.M. bilateral adrenal weight. and mean $\pm$ S.E.M. bilateral adrenal weight as a percent of final body weight. (See also Table 17).

Figure 18 (Experiment 1). Mean $\pm$ S.E.M. spleen weight, and mean $\pm$ S.E.M. spleen weight as a percent of final body weight. (See also Table 17).

Figure 19 (Experiment 1). Mean $\pm$ S.E.M. thymus weight, and mean $\pm$ S.E.M. thymus weight as a percent of final body weight. (See also Table 17).

Table 18 (Experiment 1). Total white blood cell count (WBC), plasma corticosterone, plasma protein, eosinophil (EOS) count, basophil (BAS) count, neutrophil (NEU) count, monocyte (MON) count, lymphocyte (LYM) count, and neutrophil to lymphocyte ratio (N/L).

Test Enclosure #3, ALFCO #3A Filter, 8 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L ratio
1	4.36	4.07	10.38	0	0	2	3	95	0.021
2	2.52	4.50							
3	1.90	6.24	7.88	5	0	7	9	79	0.089
4	10.48	5.25	5.00	4	0	9	3	84	0.107
5	0.19	3.85	15.38	0	0	0	1	99	0.000
6	1.88	3.50	10.63	0	0	3	3	94	0.032
7	1.52	3.98	9.38	2	0	2	0	96	0.021
8	2.52	3.85	14.25	0	0	9	9	82	0.110
Mean	3.17	4.41	10.41	1.6	0.0	4.6	4.0	90	0.054
S.E.M	1.12	0.32	1.35	0.8	0.0	1.4	1.4	3	0.017

Test Enclosure #6, ALFCO #3A Filter, 12 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L ratio
9	12.22	2.99	11.38	1	0	0	2	97	0.000
10	21.85	2.74	7.50	3	0	3	4	90	0.033
11	19.55	3.16	10.25	0	0	4	0	96	0.042
12	3.95	2.96	4.50	2	2	3	4	91	0.033
13			2.00	6	0	9	2	83	0.108
14	9.17	2.65	3.63	3	0	7	4	86	0.081
15	15.74	3.98	9.13	2	0	9	3	86	0.105
16	22.69	3.98	13.63	0	0	1	3	96	0.010
17	12.12	2.54	4.38	1	0	3	1	95	0.032
18	31.20	3.27	11.75	1	0	8	4	87	0.092
19	3.41	0.04	15.75	2	0	4	2	92	0.043
20	7.48	1.02	4.00	10	0	3	5	82	0.037
Mean	14.49	2.67	8.16	2.6	0.2	4.5	2.8	90.1	0.051
S.E.M	2.61	0.36	1.29	0.8	0.2	0.9	0.4	1.5	0.010

Test Enclosure #5, ALFCO #3A Filter, 16 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L ratio
21	26.65	4.34	4.88	2	0	5	4	89	0.056
22	24.76		5.00	1	0	5	2	92	0.054
23	25.45		5.13						
24	22.11		16.38	2	0	1	2	95	0.011
25	23.22		11.63	3	0	0	1	96	0.000
26	15.50	2.10	3.13	3	0	3	1	93	0.032
27	5.49		4.63	2	0	8	3	87	0.092
28	30.85	4.08	5.75	5	0	5	1	89	0.056
29	17.30								
30	38.06	2.99	24.38	1	0	3	1	95	0.032
31	37.17	3.73	4.50	2	0	4	1	93	0.043
32	8.79	3.78	9.63	2	0	0	1	97	0.000
33	18.29	1.49	31.63	2	0	2	2	94	0.021
34	31.04	4.69	5.13	4	0	12	3	81	0.148
35	13.75	3.34	7.25	1	0	2	3	94	0.021
36	11.94	4.30	5.25	2	0	12	4	82	0.146
Mean	21.90	3.48	9.62	2.3	0.0	4.4	2.1	91.2	0.051
S.E.M	2.40	0.33	2.15	0.3	0.0	1.0	0.3	1.3	0.013

Test Enclosure #7, ALFCO #4 Filter, 8 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L ratio
37		4.17	13.38	1	0	4	2	93	0.043
38	1.93		17.75	0	0	7	2	91	0.077
39	6.35	4.69	3.75	1	0	5	1	93	0.054
40	4.30	4.21	8.50	2	0	4	2	92	0.043
41	23.31	4.35	7.25						
42	4.92	4.69	7.63	2	0	6	5	87	0.069
43	6.53	4.28	5.88	4	0	6	2	88	0.068
44	8.64	5.46	6.88	3	0	8	2	87	0.092
Mean	8.00	4.55	8.88	1.9	0.0	5.7	2.3	90.1	0.064
S.E.M	2.67	0.17	1.60	0.5	0.0	0.6	0.5	1.0	0.007

Test Enclosure #1, ALFCO #4 Filter, 12 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L ratio
45	14.82		22.50	1	0	1	2	96	0.010
46	3.52	4.99	8.88	2	0	10	3	85	0.118
47	10.24	4.82	11.00	2	0	4	2	92	0.043
48		4.04	10.88	2	0	7	5	84	0.083
49	8.34	7.42	9.75	3	0	0	3	94	0.000
50	4.46	5.42	13.13	2	0	9	3	86	0.105
51			7.38	4	0	9	3	84	0.107
52	4.76	5.77	2.75	0	0	0	5	95	0.000
53	4.33	2.99	6.63	2	0	5	7	86	0.058
54	2.99	3.72	10.50	4	0	5	4	87	0.057
55	11.75	4.50	9.13	0	0	7	6	87	0.080
56	26.80	5.64	2.88	1	0	5	5	89	0.056
Mean	9.20	4.93	9.61	1.9	0.0	5.2	4.0	88.8	0.060
S.E.M	2.32	0.39	1.48	0.4	0.0	1.0	0.5	1.3	0.012

Test Enclosure #2, ALFCO #4 Filter, 16 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L ratio
57	24.44	5.94	10.88	2	0	7	3	88	0.080
58	9.32	5.65	17.50	0	0	2	2	96	0.021
59	10.21	3.17	3.25	3	0	2	0	95	0.021
60	15.82	2.24	7.00	0	0	3	1	96	0.031
61	20.56	5.68	5.13	1	0	7	3	89	0.079
62	31.41	4.34	7.63	0	0	4	2	94	0.043
63	13.88	4.69	5.63	1	0	2	3	94	0.021
64	8.74	5.51							
65			7.63	0	0	2	5	93	0.022
66	8.24	3.56	8.50	1	0	3	1	95	0.032
67	14.14	3.86	10.00	2	0	5	0	93	0.054
68	21.33	1.31	13.38	1	0	0	0	99	0.000
69			31.75	3	0	2	4	91	0.022
70	35.45	5.69	4.13	4	0	13	2	81	0.160
71	17.12	4.91	8.25	5	0	10	2	83	0.120
72	13.00	5.00	12.38	2	0	8	2	88	0.091
Mean	17.40	4.40	10.20	1.7	0.0	4.7	2.0	91.7	0.053
S.E.M	2.25	0.38	1.82	0.4	0.0	0.9	0.4	1.3	0.012

Vivarium shoe box control, 8 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
73	4.82	3.71	6.50	0	0	1	4	95	0.011
74	3.33	3.62	4.88	1	0	1	1	97	0.010
75	7.16	4.52	12.13	3	0	10	3	84	0.119
76	3.35	4.73	11.00	2	0	7	0	91	0.077
77	5.28	0.90	6.88	2	0	8	10	80	0.100
78	25.31	2.11	5.25	0	0	1	1	98	0.010
79	3.71	2.86	2.13	0	0	5	3	92	0.054
80	14.02	3.49	2.13	1	0	1	1	97	0.010
Mean	8.37	3.24	6.36	1.1	0.0	4.3	2.9	91.8	0.049
S.E.M	2.72	0.45	1.30	0.4	0.0	1.3	1.1	2.3	0.016

Vivarium Control Enclosure, 8 mice

Mouse id #	Plasma Corticosterone µg/dl	Plasma Protein g/dl	Total WBC cells x 10 ³ /ul	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
81	17.86	1.82	4.75	3	0	4	0	93	0.043
82	17.55	3.78	2.00	1	0	4	2	93	0.043
83	12.12	3.65	2.63	0	0	3	3	94	0.032
84	12.66	4.34	4.88	3	0	5	5	87	0.057
85	11.16	3.69	3.88	2	0	3	5	90	0.033
86	10.22	1.14	5.50	2	0	4	3	91	0.044
87	10.43	1.82							
88		3.78	5.00						
Mean	13.14	3.00	4.09	1.8	0.0	3.8	3.0	91.3	0.042
S.E.M	1.22	0.43	0.50	0.5	0.0	0.3	0.8	1.1	0.004

Table 19 (Experiment 1). Mean $\pm$ S.E.M. white blood cell count (WBC), plasma corticosterone, plasma protein, eosinophil (EOS) count, basophil (BAS) count, neutrophil (NEU) count, monocyte (MON) count, lymphocyte (LYM) count, and neutrophil to lymphocyte ratio (N/L).

	Plasma Corticosterone $\mu\text{g/dl}$	Plasma Protein g/dl	Total WBC cells $\times 10^3/\text{ul}$	EOS count % WBC
Vivarium Shoe Box Control, 8 mice	8.37 $\pm$ 2.72	3.24 $\pm$ 0.45	6.36 $\pm$ 1.30	1.1 $\pm$ 0.4
Vivarium Control Enclosure, 8 mice	13.14 $\pm$ 1.22	3.00 $\pm$ 0.43	4.09 $\pm$ 0.50	1.8 $\pm$ 0.5
Test enclosure #3, ALFCO #3A Filter, 8 mice	3.17 $\pm$ 1.12	4.41 $\pm$ 0.32	10.41 $\pm$ 1.35	1.6 $\pm$ 0.8
Test enclosure #7, ALFCO #4 Filter, 8 mice	8.00 $\pm$ 2.67	4.55 $\pm$ 0.17	8.88 $\pm$ 1.60	1.9 $\pm$ 0.5
Test enclosure #6, ALFCO #3A Filter, 12 mice	14.49 $\pm$ 2.61	2.67 $\pm$ 0.36	8.16 $\pm$ 1.29	2.6 $\pm$ 0.8
Test enclosure #1, ALFCO #4 Filter, 12 mice	9.20 $\pm$ 2.32	4.93 $\pm$ 0.39	9.61 $\pm$ 1.48	1.9 $\pm$ 0.4
Test enclosure #5, ALFCO #3A Filter, 16 mice	21.90 $\pm$ 2.40	3.48 $\pm$ 0.33	9.62 $\pm$ 2.15	2.3 $\pm$ 0.3
Test enclosure #2, ALFCO #4 Filter, 16 mice	17.40 $\pm$ 2.25	4.40 $\pm$ 0.38	10.20 $\pm$ 1.82	1.7 $\pm$ 0.4
	basophil count % WBC	neutrophil count % WBC	monocyte count % WBC	lymphocyte count % WBC
Vivarium Shoe Box Control, 8 mice	0.0 $\pm$ 0.0	4.3 $\pm$ 1.3	2.9 $\pm$ 1.1	91.8 $\pm$ 2.3
Vivarium Control Enclosure, 8 mice	0.0 $\pm$ 0.0	3.8 $\pm$ 0.3	3.0 $\pm$ 0.8	91.3 $\pm$ 1.1
Test enclosure #3, ALFCO #3A Filter, 8 mice	0.0 $\pm$ 0.0	4.6 $\pm$ 1.4	4.0 $\pm$ 1.4	90.0 $\pm$ 3.0
Test enclosure #7, ALFCO #4 Filter, 8 mice	0.0 $\pm$ 0.0	5.7 $\pm$ 0.6	2.3 $\pm$ 0.5	90.1 $\pm$ 1.0
Test enclosure #6, ALFCO #3A Filter, 12 mice	0.2 $\pm$ 0.2	4.5 $\pm$ 0.9	2.8 $\pm$ 0.4	90.1 $\pm$ 1.5
Test enclosure #1, ALFCO #4 Filter, 12 mice	0.0 $\pm$ 0.0	5.2 $\pm$ 1.0	4.0 $\pm$ 0.5	88.8 $\pm$ 1.3
Test enclosure #5, ALFCO #3A Filter, 16 mice	0.0 $\pm$ 0.0	4.4 $\pm$ 1.0	2.1 $\pm$ 0.3	91.2 $\pm$ 1.3
Test enclosure #2, ALFCO #4 Filter, 16 mice	0.0 $\pm$ 0.0	4.7 $\pm$ 0.9	2.0 $\pm$ 0.4	91.7 $\pm$ 1.3
	N/L Ratio			
Vivarium Shoe Box Control, 8 mice	0.049 $\pm$ 0.016			
Vivarium Control Enclosure, 8 mice	0.042 $\pm$ 0.004			
Test enclosure #3, ALFCO #3A Filter, 8 mice	0.054 $\pm$ 0.017			
Test enclosure #7, ALFCO #4 Filter, 8 mice	0.064 $\pm$ 0.007			
Test enclosure #6, ALFCO #3A Filter, 12 mice	0.051 $\pm$ 0.010			
Test enclosure #1, ALFCO #4 Filter, 12 mice	0.060 $\pm$ 0.012			
Test enclosure #5, ALFCO #3A Filter, 16 mice	0.051 $\pm$ 0.013			
Test enclosure #2, ALFCO #4 Filter, 16 mice	0.053 $\pm$ 0.012			

Figure 20 (Experiment 1). Mean $\pm$ S.E.M. plasma corticosterone. and mean $\pm$ S.E.M. plasma protein. (See also Table 19).

Figure 21 (Experiment 1). Mean $\pm$ S.E.M. total leukocyte count and mean $\pm$ S.E.M. eosinophil count. (See also Table 19).

Figure 22 (Experiment 1). Mean $\pm$ S.E.M. basophil count and mean $\pm$ S.E.M. neutrophil count. (See also Table 19).

Figure 23 (Experiment 1). Mean $\pm$ S.E.M. monocyte count and mean $\pm$ S.E.M. lymphocyte count. (See also Table 19).

Figure 24 (Experiment 1). Mean $\pm$ S.E.M. neutrophil to lymphocyte ratio. (See also Table 19).

Table 20 (Experiment 1). Video scoring data used for behavioral evaluation. The observer scored ten minutes of video per hour for four hours. Scores represent the total number of times any mouse engaged in the particular activity summed over all quadrants.

Number of mice	Behavioral Score Category					
	0	1	2	3	4	5
Test Enclosure #6, ALFCO #3A, 12 mice	198	7	544	0	0	9
Test Enclosure #5, ALFCO #3A, 16 mice	229	39	929	0	0	20
Test Enclosure #1, ALFCO #4, 12 mice	200	37	567	16	0	30
Test Enclosure #2, ALFCO #4, 16 mice	157	49	701	15	0	79

Behavioral Score Category Definitions

0 - sleeping, still, sniffing

1 - eating, drinking

2 - grooming, moving

3 - social interaction

4 - aggressive behavior

5 - running

Table 21 (Experiment 1). Cage temperatures (°C) for each test enclosure and the daily vivarium room temperature at the time of animal maintenance.

Day Number	Test Encl #3 (8 mice)	Test Encl #6 (12 mice)	Test Encl #5 (16 mice)	Test Encl #7 (8 mice)	Test Encl #1 (12 mice)	Test Encl #2 (16 mice)	Vivarium (8 mice)
1	22.5	23.0	23.0	23.0	23.0	23.5	
2	22.0	23.5	23.0	24.0	23.0	23.0	
3	24.0	24.5	24.5	24.5	24.5	24.5	21.0
4	24.5	24.0	25.0	25.0	26.0	25.5	19.0
5							
6	26.0	26.0	26.0	26.0	26.0	26.0	
7	24.0	23.5	23.5	24.0	23.5	24.0	
8	24.0	24.0	24.0	24.0	24.0	25.0	
9	24.5	24.0	25.0	24.5	24.5	25.0	24.5
10	25.0	24.5	25.0	25.0	24.5	25.0	24.5
11	24.5	25.0	25.0	25.0	24.5	25.0	24.5
12	25.0	25.0	24.5	25.0	24.5	25.0	24.5
13	23.0	23.0	23.0	23.0	23.0	24.0	24.5
14	23.0	23.0	23.0	23.0	23.0	23.5	25.0
15	24.0	24.0	24.0	24.0	24.0	24.0	24.0
16	25.0	25.0	25.0	25.0	24.0	25.0	24.0
17		24.0	24.0	24.0	23.5	24.0	24.0
18	24.0	24.0	24.0	24.0	24.0	24.0	24.5
19	21.5	22.0	22.0	22.0	22.0	22.0	24.5
20	22.0	22.0	22.0	22.0	22.0	22.0	24.5
21		21.5	21.5	21.5	21.5	21.5	23.5
22		23.0	23.0	23.0	23.0	23.0	23.5
23	24.5	24.0	24.0	24.0	24.0	24.0	25.5
24	24.0	24.0	24.0	24.0	23.5	24.0	24.0
25	23.5	23.5	23.5	24.0	23.5	24.0	24.5
26	25.0	25.0	25.0	25.0	25.0	25.0	22.0
27	23.0	23.0	23.0	23.0	23.0	23.0	20.0
28	22.0	22.0	22.0	22.0	22.0	22.0	16.0
29	25.0	25.0	25.0	25.0	25.0	25.0	30.0
30		24.0	26.0	26.0	25.0	25.0	

Table 22 (Experiment 1). Results of testing for the effects of the number of mice per cage and the filter type used. Column two shows adjusted G from the test for normally distributed data. A single asterisk indicates data that is not normally distributed ($G_{adj} > \chi^2_{.05[v]}$). Column five shows Burr-Foster q-test results for homogeneity of variances. Two asterisks indicates data where the variances are not homogeneous ($q > .q_{crit .05[v=10, P=6]}$, 0.250). Columns six and seven show the test used and significance results of the test. Three asterisks indicate a significant difference found.

	G_{adj}	v	$\chi^2_{0.05[v]}$	q	Test Used	Significance
Initial Wt (grams)	15.795	10	18.307	0.175	ANOVA	
Final Wt (grams)	5.421	11	19.675	0.196	ANOVA	
Gain (grams)	17.513	12	21.026	0.192	ANOVA	
arcsin(sqrt(Gain (%body)))	8.712	11	19.675	0.188	ANOVA	
Heart Wt (grams)	5.814	10	18.307	0.194	ANOVA	
arcsin(sqrt(Heart Wt (%body)))	11.210	10	18.307	0.175	ANOVA	
Kidney Wt (grams) **	11.165	9	16.919	0.253	N/A	
Sqrt (Kidney Wt)	12.376	11	19.675	0.180	ANOVA	
arcsin(sqrt(Kidney Wt (%body)))	3.416	12	21.026	0.189	ANOVA	*** #
Adrenal Wt (grams)	10.250	11	19.675	0.194	ANOVA	
arcsin(sqrt(Adrenal Wt (%body)))	14.867	12	21.026	0.192	ANOVA	
Spleen Wt (grams) *	21.792	11	19.675	0.185	Kruskal-Wallis	
Sqrt (Spleen Wt) *	23.901	12	21.026	0.139	N/A	
arcsin(sqrt(Spleen Wt (%body)))	10.508	10	18.307	0.221	ANOVA	
Thymus Wt (grams)	10.537	15	24.996	0.201	ANOVA	*** F
arcsin(sqrt(Thymus Wt (%body)))	10.871	12	21.026	0.208	ANOVA	*** F
Plasma Corticosterone *	32.661	11	19.675	0.209	N/A	
sqrt(Plasma Corticosterone)	7.100	12	21.026	0.191	ANOVA	*** # F
Plasma Protein	8.714	11	19.675	0.239	ANOVA	*** #
Total Leukocyte *	30.974	13	22.362	0.226	N/A	
sqrt(Total Leukocyte)	10.958	10	18.307	0.199	ANOVA	

- Significant result associated with number of mice per cage.

F - Significant result associated with filter type.

Table 23 (Experiment 1). Results of testing for the effects of the cage type used. Column two shows adjusted G from the test for normally distributed data. A single asterisk indicates data that is not normally distributed ($G_{adj} > \chi^2_{.05[v]}$). Column five shows Burr-Foster q-test results for homogeneity of variances. Two asterisks indicates data where the variances are not homogeneous ($q > q_{crit, .05[v=10, P=6]}$, 0.413). Columns six and seven show the test used and significance results of the test. Three asterisks indicate a significant difference found.

	G_{adj}	v	$\chi^2_{0.05[v]}$	q	Test Used	Results
Initial Wt (grams)	3.211	7	14.067	0.260	ANOVA	
Final Wt (grams)	7.382	10	18.307	0.328	ANOVA	
Gain (grams)	9.844	6	12.592	0.367	ANOVA	
arcsin(sqrt(Gain (%body))) *	20.025	9	16.919	0.347	Kruskal Wallis	
Heart Wt (grams)	7.603	10	18.307	0.294	ANOVA	***
arcsin(sqrt(Heart Wt (%body)))	5.031	7	14.067	0.260	ANOVA	***
Kidney Wt (grams)	8.680	9	16.919	0.310	ANOVA	
arcsin(sqrt(Kidney Wt (%body)))	6.742	9	16.919	0.330	ANOVA	
Adrenal Wt (grams)	3.315	7	14.067	0.346	ANOVA	
arcsin(sqrt(Adrenal Wt (%body)))	8.968	10	18.307	0.366	ANOVA	
Spleen Wt (grams)	18.055	12	21.026	0.274	ANOVA	
arcsin(sqrt(Spleen Wt (%body)))	5.989	10	18.307	0.285	ANOVA	
Thymus Wt (grams)	7.204	11	19.675	0.319	ANOVA	
arcsin(sqrt(Thymus Wt (%body)))	9.548	10	18.307	0.346	ANOVA	***
Plasma Corticosterone *	21.349	10	18.307	0.398	N/A	
sqrt(Plasma Corticosterone)	11.692	9	16.919	0.342	ANOVA	***
Plasma Protein	6.742	9	16.919	0.327	ANOVA	***
Total Leukocyte	12.535	12	21.026	0.345	ANOVA	***

C. Experiment 2: Mouse Density Study

Table 24 (Experiment 2). Daily total and per mouse water consumption for each cage (ml). Mean $\pm$ S.E.M. were calculated using test days 4 through 30 only (See also Figure 25).

Test Day Number	<u>Test Encl #2</u> (8 mice)		<u>Test Encl #4</u> (12 mice)		<u>Test Encl #1</u> (16 mice)		<u>VivShoeBox</u> (8 mice)		<u>VivCntrlEncl</u> (8 mice)	
	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse	ml	ml per mouse
1	28	3.5	40	3.3	48	3.0	50	6.3	48	6.0
2	40	5.0	52	4.3	67	4.2	52	6.5	64	8.0
3	60	7.5	74	6.2	90	5.6	45	5.6	40	5.0
4	52	6.5	70	5.8	85	5.3	41	5.1	50	6.3
5	45	5.6	80	6.7	78	4.9	21	2.6	19	2.4
6	50	6.3	88	7.3	90	5.6	47	5.9	56	7.0
7	50	6.3	56	4.7	93	5.8	60	7.5	94	11.8
8	56	7.0	72	6.0	102	6.4	58	7.3	52	6.5
9	76	9.5	81	6.8	108	6.8	70	8.8	35	4.4
10	63	7.9	84	7.0	109	6.8	42	5.3	30	3.8
11	62	7.8	80	6.7	102	6.4	54	6.8	52	6.5
12	62	7.8	76	6.3	108	6.8	n.d.	n.d.	n.d.	n.d.
13	78	9.8	100	8.3	150	9.4	52	6.5	54	6.8
14	65	8.1	73	6.1	148	9.3	50	6.3	58	7.3
15	69	8.6	95	7.9	100	6.3	45	5.6	n.d.	n.d.
16	65	8.1	116	9.7	125	7.8	58	7.3	55	6.9
17	75	9.4	119	9.9	123	7.7	51	6.4	60	7.5
18	57	7.1	92	7.7	140	8.8	52	6.5	61	7.6
19	85	10.6	112	9.3	158	9.9	56	7.0	72	9.0
20	82	10.3	115	9.6	156	9.8	63	7.9	70	8.8
21	80	10.0	98	8.2	132	8.3	72	9.0	76	9.5
22	56	7.0	90	7.5	141	8.8	54	6.8	60	7.5
23	56	7.0	70	5.8	97	6.1	67	8.4	64	8.0
24	79	9.9	83	6.9	90	5.6	61	7.6	72	9.0
25	72	9.0	78	6.5	95	5.9	60	7.5	68	8.5
26	87	10.9	100	8.3	108	6.8	50	6.3	56	7.0
27	86	10.8	94	7.8	126	7.9	58	7.3	63	7.9
28	80	10.0	92	7.7	125	7.8	70	8.8	78	9.8
29	79	9.9	85	7.1	115	7.2	64	8.0	44	5.5
30	41	5.1	82	6.8	82	5.1	n.d.	n.d.		n.d.
Mean		8.4		7.4		7.1		6.9		7.3
SEM		0.32		0.25		0.28		0.28		0.41

Figure 25 (Experiment 2): Daily water consumption per mouse for each cage.

Table 25 (Experiment 2). Food consumption (grams) over 30 days.

	Test Enclosure #2, 8 mice	Test Enclosure #4, 12 mice	Test Enclosure #1, 16 mice	Vivarium Shoe Box Control, 8	Vivarium Cntrl Enclosure, 8
Total	1234.3	1660.6	1461.5	977.0	1212.9
Total/mouse/day	154.3	138.4	91.3	122.1	151.6

Table 26 (Experiment 2). Mouse initial (test day 0) and final (test day 30) body weight, weight gain in grams, and % weight gain.

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test Enclosure #2 ALFCO #3A Filter 8 mice	1	14.91	18.19	3.28	22.00
	2	18.72	26.19	7.47	39.90
	3	17.35	29.53	12.18	70.20
	4	16.80	26.94	10.14	60.36
	5	18.98	26.83	7.85	41.36
	6	16.11	27.60	11.49	71.32
	7	16.50	33.15	16.65	100.91
	8	18.65	30.38	11.73	62.90
Mean		17.25	27.35	10.10	58.62
S.E.M.		0.51	1.54	1.40	8.55

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test Enclosure #4 ALFCO #3A Filter 12 mice	9	17.20	27.73	10.53	61.22
	10	16.33	26.84	10.51	64.36
	11	18.10	27.97	9.87	54.53
	12	17.58	30.49	12.91	73.44
	13	18.56	31.32	12.76	68.75
	14	18.12	26.98	8.86	48.90
	15	17.35	30.16	12.81	73.83
	16	17.13	31.99	14.86	86.75
	17	17.97	26.83	8.86	49.30
	18	17.91	30.81	12.90	72.03
	19	15.82	26.93	11.11	70.23
	20	17.62	27.66	10.04	56.98
Mean		17.47	28.81	11.34	65.03
S.E.M.		0.23	0.57	0.54	3.27

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test Enclosure #1 ALFCO #3A Filter 16 mice	21	16.68	25.67	8.99	53.90
	22	16.61	29.00	12.39	74.59
	23	17.82	28.37	10.55	59.20
	24	16.99	28.85	11.86	69.81
	25	17.58	27.52	9.94	56.54
	26	17.08	26.78	9.70	56.79
	27	16.79	31.00	14.21	84.63
	28	18.92	25.50	6.58	34.78
	29	17.19	28.36	11.17	64.98
	30	17.49	26.11	8.62	49.29
	31	16.83	28.52	11.69	69.46
	32	16.70	29.48	12.78	76.53
	33	18.35	30.99	12.64	68.88
	34	15.22	30.49	15.27	100.33
	35	16.68	28.93	12.25	73.44
	36	17.72	28.18	10.46	59.03
Mean		17.17	28.36	11.19	65.76
S.E.M.		0.21	0.43	0.54	3.80

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Vivarium Shoe Box Control 8 mice	37	17.70	29.93	12.23	69.10
	38	16.94	30.19	13.25	78.22
	39	17.02	29.82	12.80	75.21
	40	16.89	25.92	9.03	53.46
	41	17.73	30.05	12.32	69.49
	42	18.19	28.42	10.23	56.24
	43	16.62	29.35	12.73	76.59
	44	17.71	28.52	10.81	61.04
Mean		17.35	29.03	11.67	67.42
S.E.M.		0.19	0.50	0.52	3.35

	Mouse #	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Vivarium Control Enclosure 8 mice	45	17.92	30.20	12.28	68.53
	46	18.32	28.96	10.64	58.08
	47	17.02	31.10	14.08	82.73
	48	19.63	32.52	12.89	65.66
	49	16.62	30.16	13.54	81.47
	50	17.15	26.50	9.35	54.52
	51	16.41	23.90	7.49	45.64
	52	17.40	30.62	13.22	75.98
Mean		17.56	29.24	11.69	66.58
S.E.M.		0.37	0.98	0.82	4.69

Table 27 (Experiment 2). Cage mean $\pm$ S.E.M. for initial (test day 0) and final (test day 30) body weight, weight gain in grams, and % weight gain.

	Initial Wt (g)	Final Wt (g)	Gain (g)	Gain (% Initial Weight)
Test Enclosure #2, 8 mice	17.25 $\pm$ 0.51	27.35 $\pm$ 1.54	10.10 $\pm$ 1.40	58.62 $\pm$ 8.55
Test Enclosure #4, 12 mice	17.47 $\pm$ 0.23	28.81 $\pm$ 0.57	11.34 $\pm$ 0.54	65.03 $\pm$ 3.27
Test Enclosure #1, 16 mice	17.17 $\pm$ 0.21	28.36 $\pm$ 0.43	11.19 $\pm$ 0.54	65.76 $\pm$ 3.80
Vivarium Shoe Box Cntrl, 8 mice	17.35 $\pm$ 0.19	29.03 $\pm$ 0.50	11.67 $\pm$ 0.52	67.42 $\pm$ 3.35
Vivarium Control Encl, 8 mice	17.56 $\pm$ 0.37	29.24 $\pm$ 0.98	11.69 $\pm$ 0.82	66.58 $\pm$ 4.69

Figure 26 (Experiment 2): Mean $\pm$ S.E.M. starting body weight (day 1) and mean $\pm$ S.E.M. ending body weight (day 30) (See also Table 27).

Figure 27 (Experiment 2): Mean $\pm$ S.E.M. body weight gain and mean $\pm$ S.E.M. per cent body weight gain (See also Table 27).

Table 28 (Experiment 2). Organ weights in grams and as a percentage of final body weight (BW)

Test Enclosure #2 (ALFCO #3A Filter, 8 mice)

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
1	0.069	0.379	0.236	1.297	0.0050	0.0275	0.060	0.330	0.083	0.456
2	0.133	0.508	0.405	1.546	0.0061	0.0233	0.108	0.412	0.124	0.473
3	0.129	0.437	0.410	1.388	0.0033	0.0112	0.106	0.359	0.104	0.352
4	0.116	0.431	0.392	1.455	0.0032	0.0119	0.106	0.393	0.085	0.316
5	0.113	0.421	0.393	1.465	0.0046	0.0171	0.110	0.410	0.075	0.280
6	0.126	0.457	0.406	1.471	0.0070	0.0254	0.125	0.453	0.122	0.442
7	0.125	0.377	0.426	1.285	0.0044	0.0133	0.094	0.284	0.129	0.389
8	0.126	0.415	0.436	1.435	0.0028	0.0092	0.089	0.293	0.090	0.296
Mean	0.117	0.428	0.388	1.418	0.0046	0.0174	0.100	0.367	0.101	0.376
SEM	0.007	0.015	0.022	0.032	0.0005	0.0025	0.007	0.021	0.007	0.027

Test Enclosure #4 (ALFCO #3A Filter, 12 mice)

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
9	0.117	0.422	0.386	1.392	0.0054	0.0195	0.088	0.317	0.079	0.285
10	0.132	0.492	0.496	1.848	0.0005	0.0019	0.110	0.410	0.131	0.488
11	0.143	0.511	0.470	1.680	0.0047	0.0168	0.101	0.361	0.108	0.386
12	0.136	0.446	0.488	1.601	0.0068	0.0223	0.148	0.485	0.099	0.325
13	0.151	0.482	0.393	1.255	0.0067	0.0214	0.161	0.514	0.086	0.275
14	0.108	0.400	0.406	1.505	0.0065	0.0241	0.082	0.304	0.080	0.297
15	0.142	0.471	0.480	1.592	0.0051	0.0169	0.118	0.391	0.088	0.292
16	0.148	0.463	0.502	1.569	0.0052	0.0163	0.106	0.331	0.104	0.325
17	0.120	0.447	0.374	1.394	0.0071	0.0265	0.083	0.309	0.089	0.332
18	0.135	0.438	0.468	1.519	0.0042	0.0136	0.130	0.422	0.101	0.328
19	0.114	0.423	0.403	1.496	0.0034	0.0126	0.105	0.390	0.088	0.327
20	0.128	0.463	0.346	1.251	0.0048	0.0174	0.108	0.390	0.059	0.213
Mean	0.131	0.455	0.434	1.508	0.0050	0.0174	0.112	0.385	0.093	0.323
SEM	0.004	0.009	0.016	0.049	0.0005	0.0018	0.007	0.019	0.005	0.019

Test Enclosure #1 (ALFCO #3A Filter, 16 mice)

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
21	0.134	0.522	0.406	1.582	0.0028	0.0109	0.110	0.429	0.094	0.366
22	0.134	0.462	0.432	1.490	0.0031	0.0107	0.149	0.514	0.098	0.338
23	0.141	0.497	0.446	1.572	0.0039	0.0137	0.106	0.374	0.102	0.360
24	0.142	0.492	0.465	1.612	0.0050	0.0173	0.077	0.267	0.114	0.395
25	0.148	0.538	0.468	1.701	0.0044	0.0160	0.101	0.367	0.085	0.309
26	0.123	0.459	0.450	1.680	0.0048	0.0179	0.095	0.355	0.090	0.336
27	0.116	0.374	0.420	1.355	0.0032	0.0103	0.109	0.352	0.094	0.303
28	0.120	0.471	0.408	1.600	0.0019	0.0075	0.091	0.357	0.101	0.396
29	0.143	0.504	0.489	1.724	0.0006	0.0021	0.096	0.339	0.109	0.384
30	0.111	0.425	0.372	1.425	0.0024	0.0092	0.110	0.421	0.099	0.379
31	0.142	0.498	0.379	1.329	0.0048	0.0168	0.133	0.466	0.126	0.442
32	0.120	0.407	0.405	1.374	0.0133	0.0451	0.096	0.326	0.080	0.271
33	0.139	0.449	0.488	1.575	0.0021	0.0068	0.100	0.323	0.099	0.319
34	0.149	0.489	0.395	1.296	0.0018	0.0059	0.130	0.426	0.106	0.348
35	0.146	0.505	0.483	1.670	0.0026	0.0090	0.150	0.518	0.107	0.370
36	0.135	0.479	0.431	1.529	0.0028	0.0099	0.109	0.387	0.103	0.366
Mean	0.134	0.473	0.434	1.532	0.0037	0.0131	0.110	0.389	0.100	0.355
SEM	0.003	0.011	0.001	0.035	0.0007	0.0024	0.005	0.017	0.003	0.011

Vivarium Control Enclosure (8 mice)

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
37	0.146	0.488	0.424	1.417	0.0041	0.0137	0.106	0.354	0.089	0.297
38	0.140	0.464	0.496	1.643	0.0024	0.0079	0.111	0.368	0.090	0.298
39	0.143	0.480	0.485	1.626	0.0036	0.0121	0.120	0.402	0.162	0.543
40	0.146	0.563	0.386	1.489	0.0069	0.0266	0.108	0.417	0.124	0.478
41	0.152	0.506	0.467	1.554	0.0047	0.0156	0.142	0.473	0.060	0.200
42	0.120	0.422	0.382	1.344	0.0054	0.0190	0.096	0.338	0.151	0.531
43	0.160	0.545	0.441	1.503	0.0030	0.0102	0.112	0.382	0.110	0.375
44	0.130	0.456	0.464	1.627	0.0035	0.0123	0.109	0.382	0.101	0.354
Mean	0.142	0.490	0.443	1.525	0.0042	0.0147	0.113	0.389	0.111	0.385
SEM	0.004	0.016	0.015	0.038	0.0005	0.0021	0.005	0.015	0.012	0.043

Vivarium Shoe Box Control, 8 mice

Mouse id #	Heart Wt (g)	Heart % BW	Kidney Wt (g)	Kidney % BW	Adrenal Wt (g)	Adrenal % BW	Spleen Wt (g)	Spleen % BW	Thymus Wt (g)	Thymus % BW
45	0.156	0.517	0.404	1.338	0.0052	0.0172	0.086	0.285	0.109	0.361
46	0.132	0.456	0.483	1.668	0.0035	0.0121	0.116	0.401	0.166	0.573
47	0.148	0.476	0.446	1.434	0.0047	0.0151	0.085	0.273	0.134	0.431
48	0.142	0.437	0.381	1.172	0.0032	0.0098	0.099	0.304	0.135	0.415
49	0.180	0.597	0.464	1.538	0.0033	0.0109	0.080	0.265	0.127	0.421
50	0.155	0.585	0.474	1.789	0.0045	0.0170	0.079	0.298	0.088	0.332
51	0.126	0.527	0.333	1.393	0.0039	0.0163	0.069	0.289	0.122	0.510
52	0.139	0.454	0.439	1.434	0.0030	0.0098	0.113	0.369	0.126	0.411
Mean	0.147	0.506	0.428	1.471	0.0039	0.0135	0.091	0.311	0.126	0.432
SEM	0.006	0.022	0.018	0.068	0.0003	0.0011	0.006	0.017	0.008	0.027

Table 29 (Experiment 2). Mean $\pm$ S.E.M organ weights by cage.

	Heart Wt (g)	Heart Wt (% Final Wt)	Kidney Wt (g)	Kidney Wt (% Final Wt)
Test Enclosure #2, 8 mice	0.117 $\pm$ 0.0073	0.428 $\pm$ 0.0149	0.388 $\pm$ 0.0224	1.418 $\pm$ 0.0317
Test Enclosure #4, 12 mice	0.131 $\pm$ 0.0040	0.455 $\pm$ 0.0092	0.434 $\pm$ 0.0158	1.508 $\pm$ 0.0494
Test Enclosure #1, 16 mice	0.134 $\pm$ 0.0030	0.473 $\pm$ 0.0108	0.434 $\pm$ 0.0095	1.532 $\pm$ 0.0348
Vivarium Shoe Box Cntrl, 8 mice	0.147 $\pm$ 0.0060	0.506 $\pm$ 0.0215	0.428 $\pm$ 0.0183	1.471 $\pm$ 0.0682
Vivarium Control Encl, 8 mice	0.142 $\pm$ 0.0044	0.490 $\pm$ 0.0165	0.443 $\pm$ 0.0152	1.525 $\pm$ 0.0382

	Adrenal Wt (g)	Adrenal Wt (% Final Wt)	Spleen Wt (g)	Spleen Wt (% Final Wt)
Test Enclosure #2, 8 mice	0.0046 $\pm$ 0.00052	0.017 $\pm$ 0.0025	0.100 $\pm$ 0.0068	0.367 $\pm$ 0.0215
Test Enclosure #4, 12 mice	0.0050 $\pm$ 0.00053	0.017 $\pm$ 0.0018	0.112 $\pm$ 0.0071	0.386 $\pm$ 0.0193
Test Enclosure #1, 16 mice	0.0037 $\pm$ 0.00071	0.013 $\pm$ 0.0024	0.110 $\pm$ 0.0051	0.389 $\pm$ 0.0174
Vivarium Shoe Box Cntrl, 8 mice	0.0039 $\pm$ 0.00028	0.014 $\pm$ 0.0011	0.091 $\pm$ 0.0060	0.310 $\pm$ 0.0171
Vivarium Control Encl, 8 mice	0.0042 $\pm$ 0.00051	0.015 $\pm$ 0.0021	0.113 $\pm$ 0.0048	0.389 $\pm$ 0.0148

	Thymus Wt (g)	Thymus Wt (% Final Wt)
Test Enclosure #2, 8 mice	0.102 $\pm$ 0.0075	0.376 $\pm$ 0.0268
Test Enclosure #4, 12 mice	0.093 $\pm$ 0.0052	0.323 $\pm$ 0.0192
Test Enclosure #1, 16 mice	0.100 $\pm$ 0.0028	0.355 $\pm$ 0.0105
Vivarium Shoe Box Cntrl, 8 mice	0.126 $\pm$ 0.0079	0.432 $\pm$ 0.0274
Vivarium Control Encl, 8 mice	0.111 $\pm$ 0.0119	0.385 $\pm$ 0.0435

Figure 28 (Experiment 2). Mean $\pm$ S.E.M. heart weight. and mean $\pm$ S.E.M. heart weight as a percent of final body weight. (See also Table 29)

Figure 29 (Experiment 2). Mean $\pm$ S.E.M. kidney weight, and mean $\pm$ S.E.M. kidney weight as a percent of final body weight (See also Table 29).

Figure 30 (Experiment 2). Mean $\pm$ S.E.M. bilateral adrenal weight. and mean $\pm$ S.E.M. bilateral adrenal weight as a percent of final body weight (See also Table 29).

Figure 31 (Experiment 2). Mean $\pm$ S.E.M. spleen weight. and mean $\pm$ S.E.M. spleen weight as a percent of final body weight (See also 29).

Figure 32 (Experiment 2). Mean $\pm$ S.E.M. thymus weight, and mean $\pm$ S.E.M. thymus weight as a percent of final body weight (See also 29).

Table 30 (Experiment 2). Total white blood cell count (WBC), plasma corticosterone, plasma protein, eosinophil (EOS) count, basophil (BAS) count, neutrophil (NEU) count, monocyte (MON) count, lymphocyte (LYM) count, and neutrophil to lymphocyte ratio (N/L)

Test Enclosure #2 (ALFCO #3A Filter, 8 mice)

Mouse id #	Plasma Corticosterone $\mu\text{g}/100\text{ml}$	Plasma Protein g/dl	Total WBC cells x $10^3/\text{ul}$	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
1	2.49	1.20	8.63	0	0	1	2	97	0.010
2	12.56	1.10	11.00	3	0	3	3	84	0.036
3	16.55	1.22	8.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	15.73	1.29	15.75	5	0	1	1	87	0.011
5	3.49	0.24	6.25	0	0	3	1	96	0.031
6	26.52	1.14	9.13	1	0	6	1	92	0.065
7	7.10	1.22	11.25	2	0	10	4	84	0.119
8	16.68	1.23	8.00	4	0	15	5	76	0.197
Mean	12.64	1.08	9.77	2	0	6	2	88	0.067
S.E.M	2.84	0.12	1.03	1	0	2	1	3	0.026

Test Enclosure #4 (ALFCO #3A Filter, 12 mice)

Mouse id #	Plasma Corticosterone $\mu\text{g}/100\text{ml}$	Plasma Protein g/dl	Total WBC cells x $10^3/\text{ul}$	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
9	5.24	1.15	14.25	3	0	11	2	84	0.131
10	8.34	0.34	11.00	0	0	7	4	89	0.079
11	2.46	0.35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12	10.26	1.01	2.63	0	0	8	3	89	0.090
13	8.69	0.72	5.00	0	0	1	1	98	0.010
14	16.57	1.10	9.63	3	0	3	6	88	0.034
15	4.08	0.25	32.75	0	0	0	1	99	0.000
16	7.58	0.53	10.25	2	0	3	3	92	0.033
17	14.09	0.85	10.63	2	0	5	2	91	0.055
18	26.03	0.87	7.75	1	0	4	3	92	0.043
19	5.25	0.50	8.38	2	0	2	5	91	0.022
20	7.34	0.75	6.50	3	0	8	3	86	0.093
Mean	9.66	0.70	10.80	1	0	5	3	91	0.054
S.E.M	1.89	0.09	2.39	0.4	0	1	0.5	1	0.012

Test Enclosure #1 (ALFCO #3A Filter, 16 mice)

Mouse id #	Plasma Corticosterone $\mu\text{g}/100\text{ml}$	Plasma Protein g/dl	Total WBC cells $\times 10^3/\text{ul}$	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
21	5.67	0.46	14.50	2	0	2	4	92	0.022
22	28.47	1.38	13.38	5	0	3	2	90	0.033
23	6.42	0.40	27.63	0	0	2	4	94	0.021
24	15.67	1.23	7.50	2	0	7	1	90	0.078
25	16.63	1.12	9.88	1	0	8	2	89	0.090
26	8.51	1.21	9.88	2	0	11	3	84	0.131
27	15.85	0.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28	21.17	1.08	13.75	3	0	5	2	90	0.056
29	9.96	1.26	20.38	2	0	11	0	87	0.126
30	14.13	0.66	9.50	2	0	4	4	90	0.044
31	13.39	1.17	19.38	1	0	6	5	88	0.068
32	9.76	0.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
33	13.19	1.38	10.38	5	0	8	3	84	0.095
34	16.00	1.08	13.00	1	0	6	9	84	0.071
35	5.77	0.81	14.50	1	0	0	1	98	0.000
36	10.30	1.09	6.75	2	0	17	3	78	0.218
Mean	13.18	0.97	13.60	2	0	6	3	88	0.075
S.E.M	1.50	0.08	1.51	0.4	0	1	0.6	1	0.015

Vivarium Control Enclosure (8 mice)

Mouse id #	Plasma Corticosterone $\mu\text{g}/100\text{ml}$	Plasma Protein g/dl	Total WBC cells $\times 10^3/\text{ul}$	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
37	18.49	1.11	10.50	1	0	2	2	95	0.021
38	13.85	1.37	7.38	2	0	10	3	85	0.118
39	9.41	1.14	15.88	2	0	2	2	94	0.021
40	27.23	1.30	9.25	0	0	5	5	90	0.056
41	23.94	1.50	18.00	1	0	6	2	91	0.066
42	14.09	1.09	9.50	0	0	11	2	87	0.126
43	29.26	1.46	11.25	3	0	5	3	89	0.056
44	16.25	1.03	9.50	0	0	3	2	95	0.032
Mean	19.06	1.25	11.41	1	0	6	3	91	0.062
S.E.M	2.49	0.06	1.28	0.4	0	1	0.4	1	0.014

Vivarium Shoe Box Control (8 mice)

Mouse id #	Plasma Corticosterone $\mu\text{g}/100\text{ml}$	Plasma Protein g/dl	Total WBC cells $\times 10^3/\text{ul}$	EOS count % WBC	BAS count % WBC	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count
45	40.93	1.48	9.25	2	0	6	2	90	0.067
46	20.71	0.99	10.38	1	0	7	5	85	0.082
47	5.93	1.10	9.63	0	0	1	2	97	0.010
48	10.85	1.19	6.00	0	0	14	5	81	0.173
49	42.10	1.27	9.00	0	0	7	2	91	0.077
50	27.44	1.06	12.25	3	0	5	1	91	0.055
51	22.50	1.28	7.75	2	0	2	5	91	0.022
52	12.94	1.40	8.75	2	0	5	3	90	0.056
Mean	22.93	1.22	9.13	1	0	6	3	90	0.068
S.E.M	4.73	0.06	0.65	0.4	0	1	1	2	0.017

Table 31 (Experiment 2). Mean $\pm$ S.E.M blood test values by cage.

	Plasma Corticosterone $\mu\text{g}/100\text{ml}$	Plasma Protein g/dl	Total WBC cells $\times 10^3/\text{ul}$	EOS count % WBC	BAS count % WBC
Test Enclosure #2, 8 mice	12.64 $\pm$ 2.84	1.08 $\pm$ 0.12	9.77 $\pm$ 1.03	2 $\pm$ 1.0	0 $\pm$ 0
Test Enclosure #4, 12 mice	9.66 $\pm$ 1.89	0.70 $\pm$ 0.09	10.80 $\pm$ 2.39	1 $\pm$ 0.4	0 $\pm$ 0
Test Enclosure #1, 16 mice	13.18 $\pm$ 1.50	0.97 $\pm$ 0.08	13.60 $\pm$ 1.51	2 $\pm$ 0.4	0 $\pm$ 0
Vivarium Shoe Box Cntrl, 8 mice	19.06 $\pm$ 2.49	1.25 $\pm$ 0.06	11.41 $\pm$ 1.28	1 $\pm$ 0.4	0 $\pm$ 0
Vivarium Control Encl, 8 mice	22.93 $\pm$ 4.73	1.22 $\pm$ 0.06	9.13 $\pm$ 0.65	1 $\pm$ 0.4	0 $\pm$ 0

	NEU count % WBC	MON count % WBC	LYM count % WBC	N/L count % WBC
Test Enclosure #2, 8 mice	6 $\pm$ 2	2 $\pm$ 1.0	88 $\pm$ 3	0.067 $\pm$ 0.026
Test Enclosure #4, 12 mice	5 $\pm$ 1	3 $\pm$ 0.5	91 $\pm$ 1	0.054 $\pm$ 0.012
Test Enclosure #1, 16 mice	6 $\pm$ 1	3 $\pm$ 0.6	88 $\pm$ 1	0.075 $\pm$ 0.015
Vivarium Shoe Box Cntrl, 8 mice	6 $\pm$ 1	3 $\pm$ 0.4	91 $\pm$ 1	0.062 $\pm$ 0.014
Vivarium Control Encl, 8 mice	6 $\pm$ 1	3 $\pm$ 1.0	90 $\pm$ 2	0.068 $\pm$ 0.017

Figure 33 (Experiment 2). Mean $\pm$ S.E.M. plasma corticosterone and mean $\pm$ S.E.M. plasma protein (See also Table 31).

Figure 34 (Experiment 2): Mean $\pm$ S.E.M. total leukocyte count and mean $\pm$ S.E.M. eosinophil count. (See also Table 31).

Figure 35 (Experiment 2): Mean $\pm$ S.E.M. neutrophil count and mean $\pm$ S.E.M. monocyte count(See also Table 31).

Figure 36 (Experiment 2): Mean $\pm$ S.E.M. lymphocyte count and mean $\pm$ S.E.M. neutrophil to lymphocyte ratio(See also Table 31).

Table 32 (Experiment 2). Video scoring data used for behavioral evaluation. The observer scored twenty minutes of video per hour for two hours. Scores represent the total number of times any mouse engaged in the particular activity summed over all quadrants.

Total Count for Each Behavior Category						
Number of mice	0	1	2	3	4	5
Test Enclosure #2, 8 mice	84	10	218	4	0	21
Test Enclosure #4, 12 mice	154	10	578	5	0	22
Test Enclosure #1, 16 mice	147	14	447	5	0	41

Count Per mouse for Each Behavior Category						
Number of mice	0	1	2	3	4	5
Test Enclosure #2, 8 mice	10.5	1.2	27.2	0.5	0	2.6
Test Enclosure #4, 12 mice	12.8	0.8	48.2	0.4	0	1.8
Test Enclosure #1, 16 mice	9.2	0.9	27.9	0.3	0	2.6

Behavioral Score Category Definitions

- 0 - sleeping, still, sniffing
- 1 - eating, drinking
- 2 - grooming, moving
- 3 - social interaction (chasing or wrestling)
- 4 - aggressive behavior (biting)
- 5 - running

Table 33 (Experiment 2). Adjusted G for ANOVA 1 from the test for normally distributed data and Burr-Foster q-test results for homogeneity of variances. A single asterisk indicated data that is not normally distributed ($G_{adj} > \chi^2_{.05[v]}$). Two asterisks indicates data where the variances are not homogeneous ($q > .q_{crit .05[v=10, P=6]}$, 0.512). Three asterisks indicate a significant difference found.

	G_{adj}	v	$\chi^2_{0.05[v]}$	q	Test Used	Results
Initial Wt (grams)	4.887	9	16.919	0.456	ANOVA	
Final Wt (grams) **	7.165	8	15.507	0.641		
sqrt(Final Wt (grams)) **	7.656	9	15.507	0.697		
Gain (grams)	18.704	11	19.675	0.501	ANOVA	
arcsin(sqrt(Gain (%body)))	9.333	9	16.919	0.410	ANOVA	
Heart Wt (grams) *	22.440	10	18.307	0.409	N/A	
sqrt(Heart Wt (grams))	11.598	9	18.307	0.473	ANOVA	***
arcsin(sqrt(Heart Wt (%body)))	3.601	9	16.919	0.354	ANOVA	***
Kidney Wt (grams)	14.258	9	16.919	0.390	ANOVA	
arcsin(sqrt(Kidney Wt (%body)))	13.464	9	16.919	0.378	ANOVA	
Adrenal Wt (grams)	10.363	8	15.507	0.417	ANOVA	
arcsin(sqrt(Adrenal Wt (%body)))	9.006	8	15.507	0.349	ANOVA	
Spleen Wt (grams)	6.040	9	16.919	0.346	ANOVA	
arcsin(sqrt(Spleen Wt (%body)))	8.455	10	18.307	0.335	ANOVA	
Thymus Wt (grams)	2.886	9	16.919	0.419	ANOVA	
arcsin(sqrt(Thymus Wt (%body)))	5.671	9	16.919	0.403	ANOVA	
Plasma Corticosterone	13.055	8	15.507	0.353	ANOVA	
Plasma Protein	10.034	9	16.919	0.336	ANOVA	***
Total White Blood Count	13.820	8	15.507	0.481	ANOVA	
arcsin(sqrt(Eosinophil (% total))) *	25.063	9	16.919	0.437		
arcsin(sqrt(Neutrophil (% total)))	6.911	9	16.919	0.384	ANOVA	
arcsin(sqrt(Monophil (% total)))	3.957	9	16.919	0.432	ANOVA	
arcsin(sqrt(Lymphocyte (% total)))	12.295	9	16.919	0.420	ANOVA	
Neutrophil/Lymphocyte	10.819	9	16.919	0.435	ANOVA	

Table 34 (Experiment 2). Adjusted G for ANOVA 1 from the test for normally distributed data and Burr-Foster q-test results for homogeneity of variances. A single asterisk indicated data that is not normally distributed ($G_{adj} > \chi^2_{.05[v]}$). Two asterisks indicates data where the variances are not homogeneous ($q > .q_{crit .05[V=7, P=3]}$, 0.539). Three asterisks indicate a significant difference found.

	G_{adj}	v	$\chi^2_{0.05[v]}$	q	Test Used	Results
Initial Wt (grams)	9.819	9	16.919	0.465	ANOVA	
Final Wt (grams)	12.578	10	18.307	0.514	ANOVA	
Gain (grams)	14.894	10	18.307	0.518	ANOVA	
arcsin(sqrt(Gain (%body)))	10.093	8	15.507	0.435	ANOVA	
Heart Wt (grams)	6.669	9	16.919	0.381	ANOVA	***
arcsin(sqrt(Heart Wt (%body)))	7.089	8	15.507	0.357	ANOVA	***
Kidney Wt (grams)	3.175	8	15.507	0.390	ANOVA	
arcsin(sqrt(Kidney Wt (%body)))	5.572	10	18.307	0.486	ANOVA	
Adrenal Wt (grams)	4.219	9	16.919	0.394	ANOVA	
arcsin(sqrt(Adrenal Wt (%body)))	2.957	9	16.919	0.402	ANOVA	
Spleen Wt (grams)	14.187	9	16.919	0.360	ANOVA	***
arcsin(sqrt(Spleen Wt (%body)))	6.446	8	15.507	0.371	ANOVA	***
Thymus Wt (grams)	3.691	10	18.307	0.402	ANOVA	
arcsin(sqrt(Thymus Wt (%body)))	7.407	9	16.919	0.431	ANOVA	
Plasma Corticosterone	6.153	9	16.919	0.450	ANOVA	
Plasma Protein	13.481	8	15.507	0.493	ANOVA	
Total White Blood Count	8.594	10	18.307	0.411	ANOVA	
arcsin(sqrt(Eosinophil (% total))) *	19.261	9	16.919	0.366	Kruskal-Wallis	
arcsin(sqrt(Neutrophil (% total)))	7.707	9	16.919	0.393	ANOVA	
arcsin(sqrt(Monocyte (% total)))	9.494	8	15.507	0.407	ANOVA	
arcsin(sqrt(Lymphocyte (% total)))	3.598	8	15.507	0.455	ANOVA	
Neutrophil/Lymphocyte	8.930	9	16.919	0.419	ANOVA	

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DISCUSSION OF SAB, EXPERIMENT 1 AND EXPERIMENT 2

Stress Assessment Battery

Two weeks exposure to 3 hours/day restraint stress resulted in significant changes in body weight, thymus weight, spleen weight, and plasma protein concentration.

There was significantly less growth (weight gain) in the stress group than in the control group, even though there was very little difference in the food and water consumption between the two groups. This supports the assumption that the difference in weight gain can be attributed to the restraint treatment imposed on the stress group and the altered physiology resulting from this treatment.

The lower thymus weights and spleen weights in the stress group may be indicative of immunosuppression. This would be consistent with chronic hypercortisosterone secretion. While the differences in plasma corticosterone concentrations were variable and not statistically significant, the mean plasma corticosterone concentration was higher in the stress group. This trend is also consistent with chronic hypercortisosterone secretion.

The higher plasma protein concentration in the stress group is difficult to interpret without a more complete analysis of the differential blood protein profile. There were no significant changes in total white blood cell counts, total eosinophil counts and the neutrophil to lymphocyte ratio. In addition, the pathology report indicated that there was no evidence of any stomach ulcerations in any of the mice. These results, along with the fact that there was little difference in mean adrenal weights between the stress and control groups, leads us to believe that this stress response was not a strong one. Perhaps the type of stressor used was not severe enough or the period of stress exposure was not long enough. Also, the number of animals in each experimental group may not have been great enough to resolve small changes given the variability that was noted. Finally, it is also possible that some adaptation to the 3 hour restraint occurred over the 14 day exposure period.

Experiment 1

There were no trends seen with increasing population density in most of the parameters of the SAB in Experiment 1. Statistical analysis indicated that there were significant differences seen in plasma corticosterone and kidney weights. The mean plasma corticosterone levels were highest in the cages housing 16 mice and lowest in the cages housing 8 mice. Normalized kidney weights were lowest in the cages housing eight mice.

The results from the SAB performed on the animals in Experiment 1 were, unfortunately, inconclusive. Food bars in the test enclosures came loose in some of the cages. This could have happened because the bars were old and beginning to dry out or because they were not prepared and glued to the plates properly. In any case, these fallen food bars impeded the filtering system and became contaminated with urine thus providing a constant source of odor contamination. The ammonia levels in the affected cages were consistently high and, therefore, became a confounding variable in this study. High ammonia levels have been shown to be a stressor that can play a significant role in compromising the health of rodents (Broderson, Lindsey and Crawford, 1976 and Schaerdel et al., 1983) with the most frequently reported toxic effect of ammonia involving the respiratory tract.

Ammonia is produced by the action of urea-splitting bacteria on urine. Specifically, bacterial ureases catalyze the hydrolysis of urea to ammonia and carbamate (Collins and D'Orazio, 1993) while a second series of enzymes, D- and L-amino acid oxidases can deaminate amino acids to their corresponding keto acid and ammonia. Adverse effects of ammonia include aggravation of respiratory infections (Broderson, Lindsey and Crawford, 1976) subclinical histologic changes in tracheal epithelium (Gamble and Clough, 1976), decreased immune function (Targowski, Klucinski and Babiker, 1984) and impairment of hepatic drug metabolism (Veseli, Lang and White, 1973). In addition, it has been shown to affect some of the parameters of the SAB such as adrenal weights, plasma corticosterone concentration, peripheral

lymphocyte populations and overall growth in mice (Peters and Festing, 1990 and Peng, et al., 1989) .

Population density has been shown to be positively correlated with intracage ammonia levels (Serrano, 1971). While there is no definitive rule as to the duration and concentration of ammonia that will induce pathological changes mice, Broderson et al. (1976) showed that rats exposed to 200 ppm of ammonia for four and eight days developed sub-clinical pathological changes ranging from mild thickening through gross stratification of the tracheal epithelium, to cilia loss and extensive mucous membrane folding.

The effect of environmental ammonia challenging the well-being of the mice can be seen in this study if we compare the animals housed in the test enclosures without the filtering system. The mean plasma corticosterone level of the mice in this cage was greater than that of the mice in the vivarium shoe-box cage control and the test-enclosure with the filtering system, all housing similar population densities of 8. Also, these mice had the lowest mean total leukocyte counts and the greatest heart weights when compared to the other two cages housing 8 mice.

As such, the few significant differences seen in the cages housing 16 mice can not be interpreted as being due to the effect of the increased population density. The experiment was, therefore, repeated (Experiment 2) with precautions made so that the confounding variable introduced by the fallen food bars was minimized.

Experiment 2

In this experiment the number of mice per Test Enclosure affected the amount of water used per mouse per day, heart weight, and plasma protein concentration. Mice in the Test Enclosure housing 16 mice used less water than mice in the Test Enclosure housing 8 mice. Heart weights of mice in the Test Enclosures housing 12 and 16 mice were greater than those of mice in the Test Enclosure housing 8 mice. The mean normalized heart weights of the mice Test Enclosure housing 16 mice was greater than those of mice in the Test Enclosures housing 8 animals. Mice in the Test Enclosure housing 12 mice had greater heart weights and lower plasma protein concentrations than mice in the Test Enclosure housing 8 mice.

Interpretation of the effects of the number of mice per cage in this study has its complications. Reduced water consumption by the 16 mouse Test Enclosure mice corresponded to and could be due to the reduced amount of food consumed (Dr. Sig Rich, campus veterinarian, personal communication). The 16 mouse Test Enclosure mice probably ate less because of the improved thermal efficiency of a 16 mouse huddle. Greater heart weights of 16 and 12 mouse Test Enclosure mice (15% and 12%) can be partially explained by the greater body weights of those mice (4% and 5%). The experimental results are further complicated by the presence of ammonia and methylamine only in the 12 mouse Test Enclosure (which were not caused by fallen bars in this study).

In any case, the differences in plasma corticosterone and kidney weights seen in the cage housing 16 mice in Experiment 1 is not seen in Experiment 2. This suggests odor contamination from fallen food bars in the previous study was not a confounding variable in this study.

In conclusion, these findings indicate that mice housed in AEM simulators at densities of 8, 12 or 16 per simulator are similar in terms of their growth, physiology and behavior. After 30 days, there was no evidence of compromised health or well-being in any of the 3 housing densities.

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