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90-Day Oral Toxicity Study on n-Nonane in Female Fisher 344 Rats and Male C57BL/6 Mice

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TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

//SIGNED//

MARK M. HOFFMAN
Deputy Chief, Biosciences and Protection Division
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Long chain petroleum hydrocarbons, ranging from C_9 to C_{15}, are predominant constituents in weathered jet-fuel spills. To evaluate potential toxic effects of a C_9 hydrocarbon following repeated oral gavage, *n*-nonane (neat) was administered to groups of 10 female Fischer 344 rats and 10 male C57BL/6 mice at daily doses of 5.0, 1.0, 0.1 and 0 (control) g/kg, 7 days/week for 90 days. Clinical observations included urogenital wetness, dark-colored urine, perianal alopecia and erythema, diarrhea, and hunched posture at 5.0 g/kg in both species. Mean body weights were similar between control and treated groups. Food consumption was decreased in rats in the 1.0 and 5.0 g/kg groups during the first two weeks. Measures of forelimb grip strength and overall locomotor activity indicated no consistent treatment-related effects. Statistically significant differences in hematology and serum chemistry values were observed; however, values were within normal species limits. Rats had increased liver, lung and adrenal weights and decreased spleen and ovary weights at 5.0 g/kg. Increased adrenal and decreased ovary weights also were observed at 1.0 g/kg. In mice, an increase in liver weight and a decrease in kidney weight were observed in the 1.0 and 5.0 g/kg groups. Individual rat blood levels of *n*-nonane, collected 2 hours post-dosing at the conclusion of the study, ranged from 0.44 (0.1 g/kg group) to 9.53 µg/g (5.0 g/kg group). Microscopic lesions consisted of varying degrees of hyperplasia and hyperkeratosis of squamous epithelium in the non-glandular stomach in all nonane-treated groups of both species. Mild inflammation of the proximal small intestinal mucosa was present in 20% of the high dose rats only. Mild perianal squamous hyperplasia was observed in high and mid dose rats and mice, but not in the 0.1 g/kg dose groups. The NOAEL for this study is 0.1g/kg in both species, for all biological endpoints except the lesions in the non-glandular forestomach. The lack of an analogous structure in the human stomach and the absence of lesions in the glandular stomach of the study animals suggest that the proliferative forestomach lesions represent a species-specific response, of no clinical significance to humans.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted by the Toxic Hazards Research staff managed by the Mantech/GEO-CENTERS Joint Venture contract. This document serves as a final report on the results of a 90-day oral toxicity study on *n*-nonane in female Fischer 344 rats and male C57BL/6 mice. The research described in this report began in June 1995 and was completed in October 1996 under the Department of the Air Force Contract Nos. F33615-90-C-0532 and F41624-96-C-9010. Lt Col Terry A. Childress served as Contract Officer Representative for the U.S. Air Force, Armstrong Laboratory, Toxicology Division (AL/OET). Dr. Darol E. Dodd served as Program Manager for the ManTech/GEO-CENTERS Joint Venture contract.

The authors gratefully acknowledge the scientific advice provided by E.R. Kinkead, Dr. J.W. Fisher, Dr. H.A. Barton, Dr. D.R. Mattie and Dr. D.A. Staats; technical assistance by M.L. Freedman, R.J. Godfrey, W.J. Malcomb, D.H. Ellis, SrA S.L. Southwell, J.W. Nicholson, M.A. Parish and G.A. Neely; quality assurance guidance by M.G. Schneider; and statistical analyses by C.D. Flemming and B. Most.

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90-Day Oral Toxicity Study on *n*-Nonane in Female Fischer 344 Rats and Male C57BL/6 Mice

INTRODUCTION

Contamination of soil and groundwater with petroleum products is a common environmental problem at Air Force (AF) bases and other Department of Defense (DOD) installations. At over 4000 groundwater contamination sites belonging to the AF, approximately 60 percent involve some type of petroleum product. Petroleum products include gasoline, diesel fuel and jet propulsion (JP) fuel. Millions of dollars are spent each year to assess and remediate petroleum contamination. However, much of this remediation may not be necessary. Site-specific evaluation of the risk to human health from petroleum contamination could save millions of dollars in unnecessary cleanup costs.

Though petroleum products are a complex mixture of hydrocarbons, cleanup of these contaminants are often regulated by a single numerical standard for total petroleum hydrocarbons (TPH). Soil cleanup standards for TPH vary between states from 10 to 1000 ppm. Most states have a TPH soil standard below 100 ppm; however, most state regulators cannot provide the scientific and/or technical basis for the TPH standards (Staats, 1997). Further, these standards may have originated as arbitrary values set for specific sites.

Long chain petroleum hydrocarbons (LCPH) are a category of petroleum hydrocarbons that comprise between 50 and 98 percent of most petroleum products (Hutcheson *et al.*, 1996). Petroleum hydrocarbons are commonly divided into four major groups: alkanes, alkenes, cycloalkanes and aromatics. A qualitative analysis of neat JP-4 fuel indicates that its hydrocarbon components lie in a carbon range of approximately C_5 to C_{15}.

Establishing health-based criteria for each of the components of JP-4 fuel is unreasonable. Similarly, assigning a single concentration value for a cleanup standard for the entire range of petroleum hydrocarbons as one mass is unfounded. One approach to determining risk based corrective action at petroleum contaminated sites is to identify a "reference compound" for each subgroup of petroleum hydrocarbons quantified at the site. Sufficient analytical data are not available to describe the subgroups of petroleum hydrocarbons that exist at a weathered JP-4 spill site, but the long chain alkanes with a carbon range of C_9 through C_{15} are suspected to represent a large portion of the TPH measured in soil at spill sites. Alkanes can be straight chain molecules (normal paraffins) or branched chain molecules (isoparaffins). Reference compounds for the LCPH subgroups C_5 through C_8, C_9 through C_{18} and C_{19} through C_{32} have been proposed (Hutcheson *et al.*, 1996; Massachusetts Department of Environmental Protection, 2001). They are *n*-hexane, *n*-nonane and eicosane, respectively. Due to limited information on the health effects of the proposed reference compounds, reference doses (RfDs) were derived with considerable conservatism and a large amount of uncertainty. Further, except for *n*-hexane, inhalation studies provided the only data available to derive oral RfDs. EPA considers a single, well-conducted, subchronic mammalian bioassay by the appropriate route as a minimum requirement for estimating a reference dose (RfD) (Dourson, 1994). Thus, this study was designed to provide mammalian toxicity information on one of the proposed reference compounds for the LCPH subgroups, *n*-nonane.

Oral toxicity studies with nonane are not reported in the open literature. Acute, 7-day and 90-day studies on rats with nonane vapor (Carpenter *et al.*, 1978; Nilsen *et al.*, 1988) indicated central nervous system or peripheral nervous system abnormalities (tremors, convulsions, coordination loss, limb paralysis) and irritation (lacrimation, salivation). Included were microscopic lesions in the liver (fatty changes), lungs (edema) and brain (loss of Purkinje cells) at high concentrations (approximately 2400 ppm); mild irritation, tremors, body weight depression with no microscopic lesions at intermediate concentrations (approximately 1600 ppm); and no observable effects at low concentrations (approximately 600 ppm).

Clearly, the database for nonane is minimal and inadequate for the purpose of establishing an oral RfD. The purpose of this study is to determine the no-observable-adverse-effect-level (NOAEL) and toxic effects associated with repeated exposure to nonane for a period of 90 days. In addition, blood and tissue samples were collected and analyzed for *n*-nonane to gain insight into the distribution and kinetics of this alkane following oral administration. This information can be of value for establishing safety criteria for human exposure.

MATERIALS AND METHODS

Test Substance - Source, Administration, Analysis, and Storage

n-Nonane (CAS No. 111-84-2, 99%, Lot No. 5921EL) was obtained from Aldrich Chemical Co., Milwaukee, WI. Gas chromatographic separation and mass spectrographic analysis of the test substance indicated there were no impurities. The test substance was stored in a chemical storage cabinet in Building 79. Test material stability was evaluated by performing analyses for composition prior to the initiation of the study and post study. No measurable impurities were detected on both occasions, indicating stability of the test substance throughout the study.

Neat *n*-nonane was administered orally (via gavage) on a daily basis throughout the study. Dosages were administered on the basis of weight of test substance (using a density correction of 0.72 g/mL for *n*-nonane) per animal body weight (not to exceed a volume of 1.0 mL/100 g body weight). Control animals received an equivalent volume (1 mL/100 g body weight) of distilled water. Animals were not fasted prior to dosing, but dosing was scheduled towards midday (approximately 11:00 A.M.) since rodents eat, in general, during the night. Using a glass syringe, the test substance or distilled water was administered by stomach intubation through a commercial 18-gauge ball-end stainless steel needle.

Animal Selection

Rodents are the preferred species for general toxicity testing. Members of the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) evaluated the "data gaps" on animal toxicity of LCPH and suggested that data from two species (rats and mice) would be of greater value than data from a single species for establishing a test substance reference dose. However, toxicity data from male rats may be difficult to interpret due to the development of α-2μ-globulin nephropathy, induced by many hydrocarbons (Alden, 1986). Therefore, to avoid the concern of male rat nephropathy and to keep animal numbers to a minimum, one sex of each species was considered appropriate for meeting the objectives of this study. Female rats and male mice were selected. Both the Fischer 344 rat and the C57BL/6 mouse were used extensively in the

Toxic Hazards Research Unit Laboratory (AMRL/TH) for the toxicity testing of jet fuels from 1973 to 1983. Except for the development of α-2μ-globulin nephropathy in male rats, differences between the sexes were not observed in the biological endpoints monitored. This study used the identical species and strains that the jet fuel studies used to strengthen the current database.

Animals and Animal Husbandry

Forty-four female Fischer 344 [CDF (F-344)/CrlBR, Lot T68] rats (six weeks of age) and forty-four male C57BL/6 [C57BL/6NCrlBR, Lot E42] mice (six weeks of age) were supplied by Charles River Breeding Laboratories, Raleigh, NC. Females were nulliparous and nonpregnant. Following identification procedures (white ink tail tattoos for mice), animals were assigned to different groups by a computer-generated randomization scheme (PATH/TOX system) that was stratified by body weight such that the body weights of all groups (per species) were homogeneous by statistical analysis at study initiation. Dosing began when the animals were approximately nine weeks old.

Animals were housed in the AL/OEVM vivarium (Building 838) upon receipt and subjected to a two-week quarantine. Quality control monitoring included body weight measurements and examinations for ecto- and endoparasites. Throughout the study, animals were housed individually in plastic cages with hardwood chip laboratory bedding (Sanichips). Cages were changed twice per week. Food (Purina Formulab #5002, powdered) and water (conditioned by AL/OEVM) were provided *ad libitum*. Animal room temperatures were targeted at 21 to 25°C, relative humidity ranged from 40 to 60%, and light/dark cycle was set at 12/12 hours.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee of Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHHS, National Institute of Health Publication 86-23, 1986; and the Animal Welfare Act of 1966, as amended.

Group Assignments and Dose Levels

A 7-day dose range-finding study was performed to assist in selecting target doses for the current 90-day study. In the 7-day study, female F-344 rats (5/group) and male C57BL/6 mice (5/group) were given oral (gavage) doses of *n*-nonane (neat) daily for 7 consecutive days. Doses were 0 (control), 0.7, 1.8, or 3.6 g/kg body weight. Clinical signs, neurobehavioral tests (locomotor, auditory startle response, and grip strength), body weights, gross necropsy, and organ weights were monitored for all animals. Three rats died after the first day due to dosing trauma. At the conclusion of the study, rats in the 3.6 g/kg group had decreased body weights compared to control rats and signs of irritation in the perianal area. Mice in the 3.6 g/kg group had increased liver and spleen weights compared to control mice. The only indication of toxicity in the lower dose groups was an increase in liver weights in mice of the 1.8 g/kg group. Neurobehavioral tests were inconclusive.

The results from the 7-day dose range-finding study were evaluated to assist in the selection of the doses for the 90-day study, presented in Table 1. Due to unexpected mortality during the first four days of dosing female rats of the 5.0 g/kg group, two additional rats were assigned to this group. The two rats were healthy and part of the original shipment of animals received for this investigation.

TABLE 1. GROUP IDENTIFICATION, GROUP SIZES, AND TARGET ORAL DOSE LEVELS

Group	Number of Animals		Dose Level of Test Substance (g/kg/day)
	Male mice	Female rats	
Control	10	10	0.0
Low	10	10	0.1
Middle	10	10	1.0
High	10	12*	5.0

* Due to unexpected mortality during the first four days of dosing, two additional rats were assigned to this group.

It was anticipated that at the highest dosage level, some toxicological or pharmacological effect(s) may be observed, and that at the lowest dosage level no treatment-related effects would be seen. The highest dose level is greater than EPA's "limit test" value for acute oral studies (EPA, 1998).

Experimental Evaluations

Clinical Observations

The animals were observed twice daily (a.m. and p.m.), including weekends and holidays. Symptoms or signs of toxicity were recorded.

Neurobehavioral Tests

Two neurobehavioral tests were used to measure integrity of the motor system in rodents treated with nonane. Specifically, animals were tested for forelimb grip strength and overall locomotor activity. All animals of each dose group were tested pre-exposure (week -1), 4 weeks into the exposure period, and near the conclusion of the exposure period (week 12).

Grip Strength. Animals were tested on a Columbus Instruments Automated Grip Strength Meter. The experimenter held individual animals such that the rear quarter was gently grasped and the forelimbs were unrestrained. The animal was placed over the dynamometer with the forelimbs in contact with the gripping screen. As the animal grasped the screen, the experimenter gently pulled the animal away from the meter until the animal released its grip. The force exerted by the grip was displayed on the grip meter and subsequently recorded by the experimenter. On each test day, individual animals completed five consecutive trials on the grip strength test. Scores from the five trials were averaged to produce one score per animal each test day.

Locomotor Activity. Animals were individually placed in Columbus Instruments Opto-Varimex Plexiglas open field measuring 30 cm^2. Each 20 minute test session was divided into 10 two-minute blocks for the purpose of statistical analyses. Along the perimeter of the field are infrared

photocells spaced one inch apart that detect quantity, size and direction of movement. In addition, there are infrared detectors at a second level used to detect vertical movements (rears). The apparatus automatically records the following types of motor movement: distance traveled, time spent resting, time spent in ambulatory movement, time spent in stereotypic movement, number of stereotypic movements and number of rears. The apparatus also analyzes rotational behavior by calculating the number of clockwise turns, number of counterclockwise turns and a ratio of clockwise to counterclockwise turns.

Body Weights and Food Consumption

Body weights were determined and recorded immediately prior to the initiation of the study and weekly thereafter. Body weight gains were computed. Food consumption was determined and recorded weekly on an individual animal basis.

Hematology

At the conclusion of the 90 days, animals were fasted for 12 hours and euthanized. Samples of blood were collected via the vena cava and the following determinations were performed: hematocrit (HCT), hemoglobin (HGB) concentration, erythrocyte (red blood cell, RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total and differential leukocyte (white blood cell, WBC) count, and a measure of clotting potential (platelet count).

Serum Chemistry

At the conclusion of the 90-day exposure period, the following determinations also were performed: calcium, phosphorous, chloride, sodium, potassium, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase, alkaline phosphatase, blood urea nitrogen (BUN), albumin, globulin, total protein, creatinine, and total bilirubin. A few additional measurements (e.g., cholesterol, triglycerides, magnesium) were performed on rat sera.

Blood and Tissue Sampling for Test Substance Analysis (rats only)

On two occasions during the study (weeks 5 and 10) and near the conclusion of the study (week 13), blood samples were drawn via the lateral tail vein and analyzed for nonane. During each collection period, two blood samples (approximately 0.1 g/sample) were taken per rat; one sample immediately prior to dosing and the second sample two hours after gavage dosing. In addition, tissue (fat, muscle, liver) samples (approximately 0.2 g) were taken for analysis at necropsy. Samples were immediately capped in 22 mL headspace vials to avoid loss of nonane. Tissues were digested in the vial by introducing 3 mL of 0.4 g NaOH/mL water and mixing on a Haake Buchler Vortex at 75°C until digestion was complete. Blood samples were not treated. Vial headspace samples of tissue or blood were injected onto a Varian 3700 Gas Chromatograph (GC) set up with the following conditions: 0.53 mm x 30 m SPB1 column, temperature ramp from 50°C to 90°C at 10°C/min, helium carrier of 5 mL/min, flame ionization detector temperature at 300°C, and 25 mL/min helium make-up gas flow. The GC output was automatically integrated

and stored using Nelson 2600 software. Standards were prepared in Tedlar bags and diluted into headspace vials. In general, headspace extraction recovery from blood, fat, muscle, and liver was 98, 27, 79, and 86%, respectively.

Necropsy

Euthanasia (via CO_2 asphyxiation) of all animals occurred following the completion of the 90-day study. Animals were fasted at least 12 hours prior to sacrifice and subjected to a complete gross necropsy. The gross necropsy included examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities, including their contents.

Organ Weights

Organs weighed included liver, kidneys (pair), adrenals (pair), gonads (pair), spleen, lungs, and brain.

Histopathology

Tissues and organs (listed below) were collected from all study animals. Tissues and organs from animals of the control and high-dose groups, "target" tissues from animals of the lower nonane dose groups, and gross lesions (identified at necropsy) were subjected to histopathologic examination. Additionally, a complete gross necropsy and histopathologic examination were conducted on any animal that died during the study.

- liver
- kidneys
- adrenals
- pancreas
- spleen
- pituitary
- thyroid/parathyroid
- thymus
- testes*
- ovaries
- heart
- trachea
- nasopharyngeal tissues
- esophagus
- stomach
- duodenum
- jejunum
- ileum
- cecum
- colon
- rectum
- urinary bladder
- uterus
- lungs
- sternum with bone marrow
- salivary glands
- accessory genital organs (epididymis, prostate, seminal vesicles)
- representative (ascending or descending) aorta
- brain, including sections of medulla/pons, cerebellar cortex and cerebral cortex
- spinal cord at three levels (cervical, midthoracic, and lumbar)
- peripheral nerve
- representative (submandibular or mesenteric) lymph nodes

*Note: testes fixed in Bouin's Fixative, sections stained with Periodic Acid and Schiff's (PAS), counterstained with hematoxylin

Statistical Analysis

Body weights and food consumption were intercompared using a repeated measures analysis of variance. Other continuous variables (e.g., organ weights, hematology and serum chemistry) were intercompared using an analysis of variance. Homogeneity of variance was tested using Levene's test. For significant F-values, multiple comparisons were conducted using a Bonferroni correction of t-tests.

Nonparametric data were transformed and, if normal in distribution, parametric tests were performed. If the transformed data were not normal, appropriate nonparametric tests were carried out. Frequency data were compared using chi-squared tests and multiple comparisons were made using Bonferroni-corrected Fisher's Exact Test. The fiducial limit of 0.05 (two-tailed) was used as the criterion for significance when assumptions for homoscedasticity and normality were not violated.

Statistical Analysis for Neurobehavioral Tests

Grip strength scores from the five trials were averaged to produce one score per animal each test day. The scores were subsequently analyzed in a repeated measures ANOVA. In the locomotor activity test, each 20 minute session was divided into 10 two-minute blocks for the purpose of statistical analyses. The different measures of motor activity were separately analyzed in repeated measures ANOVAs. Since the data were highly variable and not normally distributed, a Kruskal-Wallis (Hollander and Wolfe, 1973) analysis of variance was used for each test session, time block and dependent measure.

RESULTS

Clinical Observations

Results of pre-study quality control procedures were negative, indicating that the animals were healthy upon initiation of the study. Deaths, attributed to oral gavage trauma, were observed in both mice and rats (Table 2).

TABLE 2. MORTALITY IN THE 90-DAY ORAL STUDY WITH *n*-NONANE

Species and Sex	Dose (g/kg/day)	Number Dead/ Number on Study	Week of Death (Number dead)
Female Rats	0.0	0/10	-
	0.1	0/10	-
	1.0	1/10	13 (1)
	5.0	6/12	1 (2) 2 (3) 11 (1)
Male Mice	0.0	1/10	8 (1)
	0.1	0/10	-
	1.0	2/10	12 (1) 13 (1)
	5.0	2/10	3 (1) 12 (1)

Except for an occasional incidence of dry red material around the eyes of rats in the 0.0 (control), 0.1 and 1.0 g/kg groups, clinical signs of irritancy and/or toxicity were observed only in the high dose (5.0 g/kg) groups (6 of 9 rats, 7 of 10 mice). The clinical findings included wet urogenital/perianal areas, matted fur in the anal area, perianal alopecia (hair loss), perianal/hindlimb erythema, dark-colored urine, diarrhea, erythema/excreta at base of tail, hunched posture, dry red material around the eyes and nose, lower jaw alopecia, and matted "rough" body fur. In addition, mice of the 5.0 g/kg group had occasional redness and swelling of the penis and scrotal area. Mice of the 0, 0.1, and 1.0 g/kg groups were normal in appearance.

Neurobehavioral Tests

Grip Strength

There were no statistically significant differences related to nonane exposure in either the female rats or male mice (Tables 3 and 4, respectively). There were reliable differences across test sessions ($p < 0.001$), such that grip strength was greater during the week 12 test than during the pre-exposure or week 4 test. There was no interaction of treatment with test session indicating that increased grip strength was similar across all test groups and was likely an effect of age and experience with the test.

TABLE 3. MEAN[a] GRIP STRENGTH OF FEMALE RATS

	Nonane Dose (g/kg/day)			
Study Week	0.0[b]	0.1[b]	1.0[b]	5.0[c]
-1	505 ± 75	510 ± 56	479 ± 98	506 ± 50
4	507 ± 107	494 ± 73	501 ± 74	432 ± 49
12	824 ± 82	806 ± 127	780 ± 95	911 ± 64

[a]group mean (± SD); individual animal values are an average of five measurements; units are grams of isotonic force
[b]group size = 10 (weeks -1 through 12)
[c]group size = 10 (week -1); 7 (week 4); 6 (week 12)

TABLE 4. MEAN[a] GRIP STRENGTH OF MALE MICE

	Nonane Dose (g/kg/day)			
Study Week	0.0[b]	0.1[c]	1.0[c]	5.0[d]
-1	112 ± 10	113 ± 11	116 ± 12	114 ± 8
4	111 ± 11	111 ± 12	107 ± 16	112 ± 15
12	128 ± 7	120 ± 14	128 ± 17	116 ± 14

[a]group mean (± SD); individual animal values are an average of five measurements; units are grams of isotonic force
[b]group size = 10 (weeks -1 and 4); 9 (week 12)
[c]group size = 10
[d]group size = 10 (week -1); 9 (weeks 4 and 12)

Locomotor Activity

In female rats, there was a pattern of decreased motor activity during the first half of the week 12 locomotor activity test in the high dose group; however, the overall effect of *n*-nonane was not statistically significant (Figures 1 through 3). There were isolated cases of statistically significant treatment group differences at four weeks, but no consistent pattern emerged (Table 5).

Measures of clockwise rotations, counterclockwise rotations, and the ratio of the two were also analyzed. There was no indication of rotational behavior in any of the treatment groups during any of the three test sessions (data not shown).

The pattern of results seen in female rats was replicated in male mice. There was an overall decrease in the amount of motor activity in the high dose group during the first half of the week 12 locomotor activity test. These group differences, depicted in Figures 4 through 6, are found in the measures of distance traveled, time resting, and time ambulatory where the activity in the high dose group is reliably lower than in the control group. There are some spurious results in other measures; however, the group differences are transient and are not indicative of a dose-response effect (Table 6). As with female rats, no evidence of rotational behavior was found.

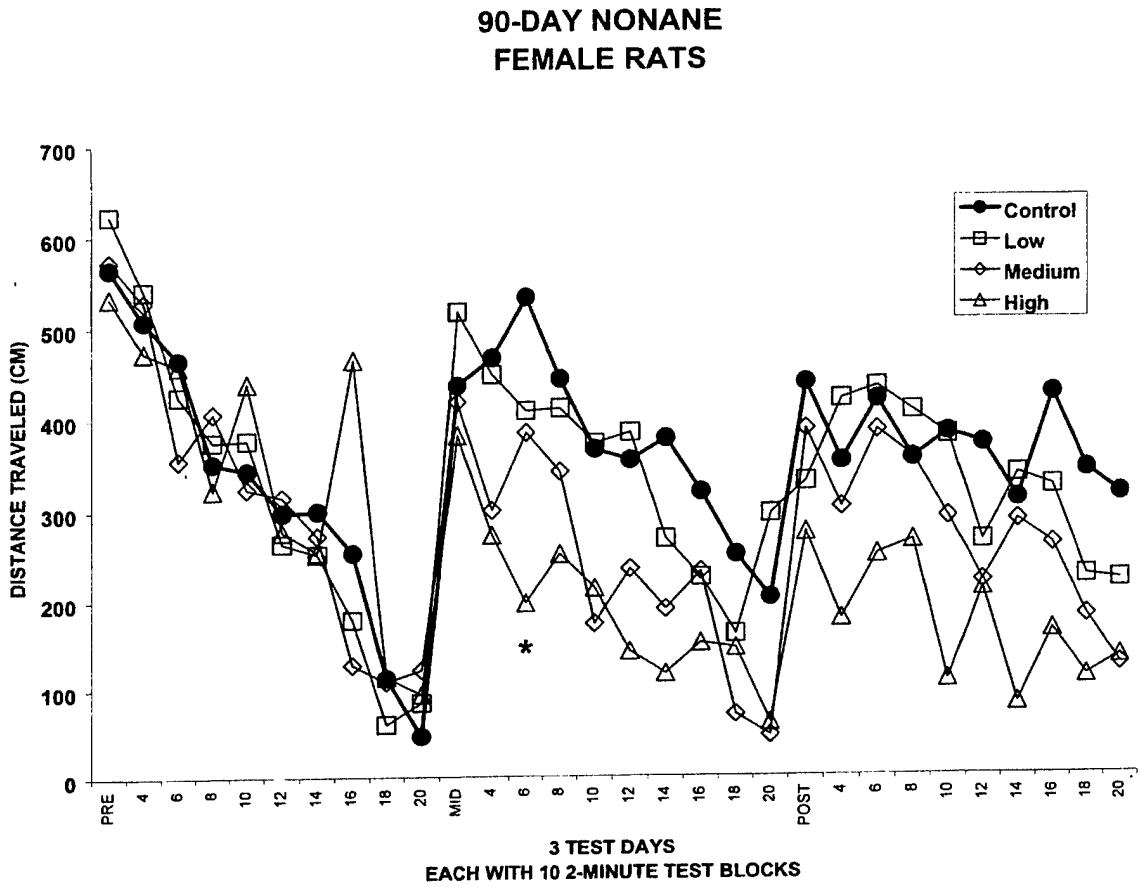

Figure 1. Distance traveled over three separate testing sessions (pre-exposure, week 4, week 12) for female rats. Each 20 minute session is divided into ten 2-minute blocks of time. A statistically significant difference ($p<0.05$) between the control and high dose groups is denoted with an asterisk (*).

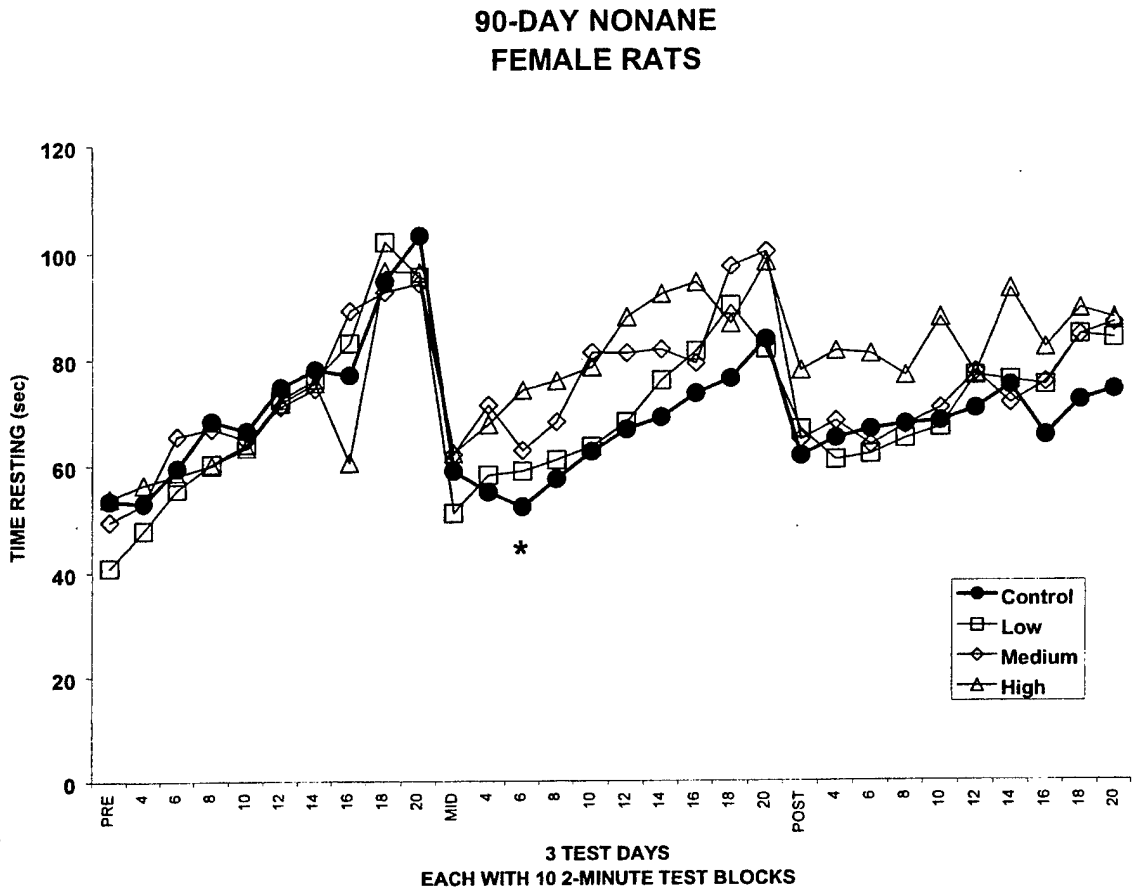

Figure 2. Time spent resting over three separate testing sessions (pre-exposure, week 4, week 12) for female rats. Each 20 minute session is divided into ten 2-minute blocks of time. A statistically significant difference ($p<0.05$) between the control and high dose groups is denoted with an asterisk (*).

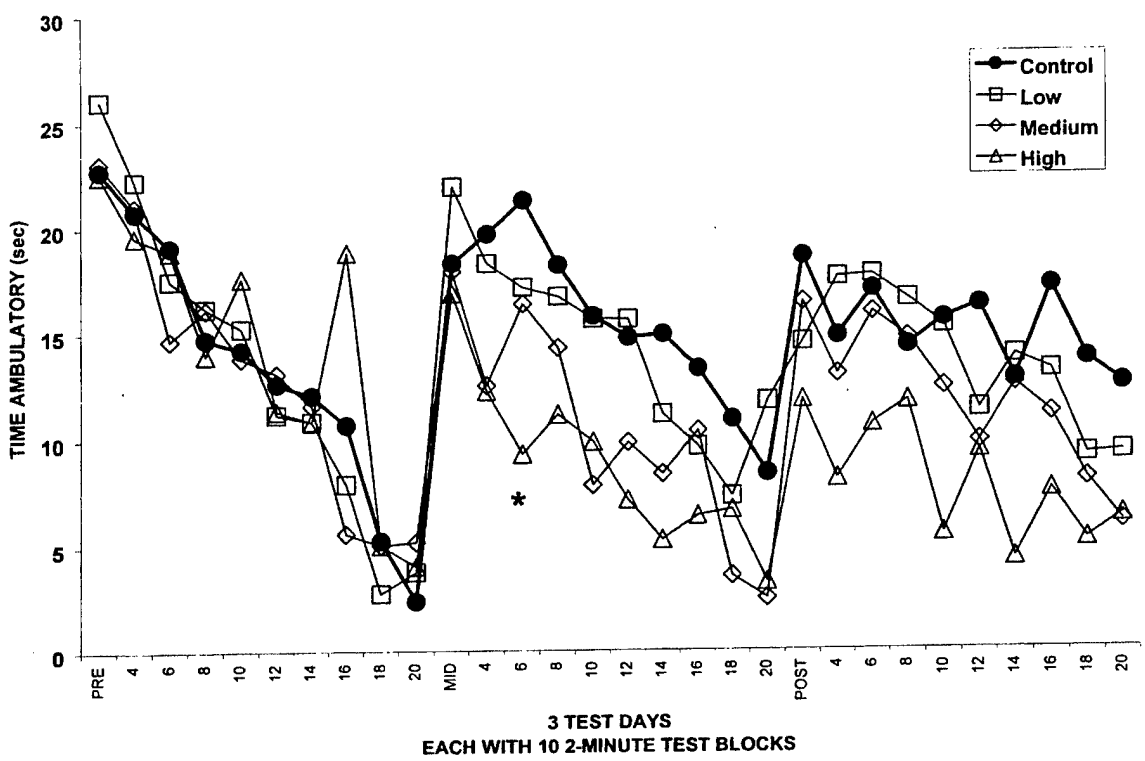

Figure 3. Time spent in non-stereotypic (ambulatory) movement over three separate testing sessions (pre-exposure, week 4, week 12) for female rats. Each 20 minute session is divided into ten 2-minute blocks of time. A statistically significant difference ($p<0.05$) between the control and high dose groups is denoted with an asterisk (*).

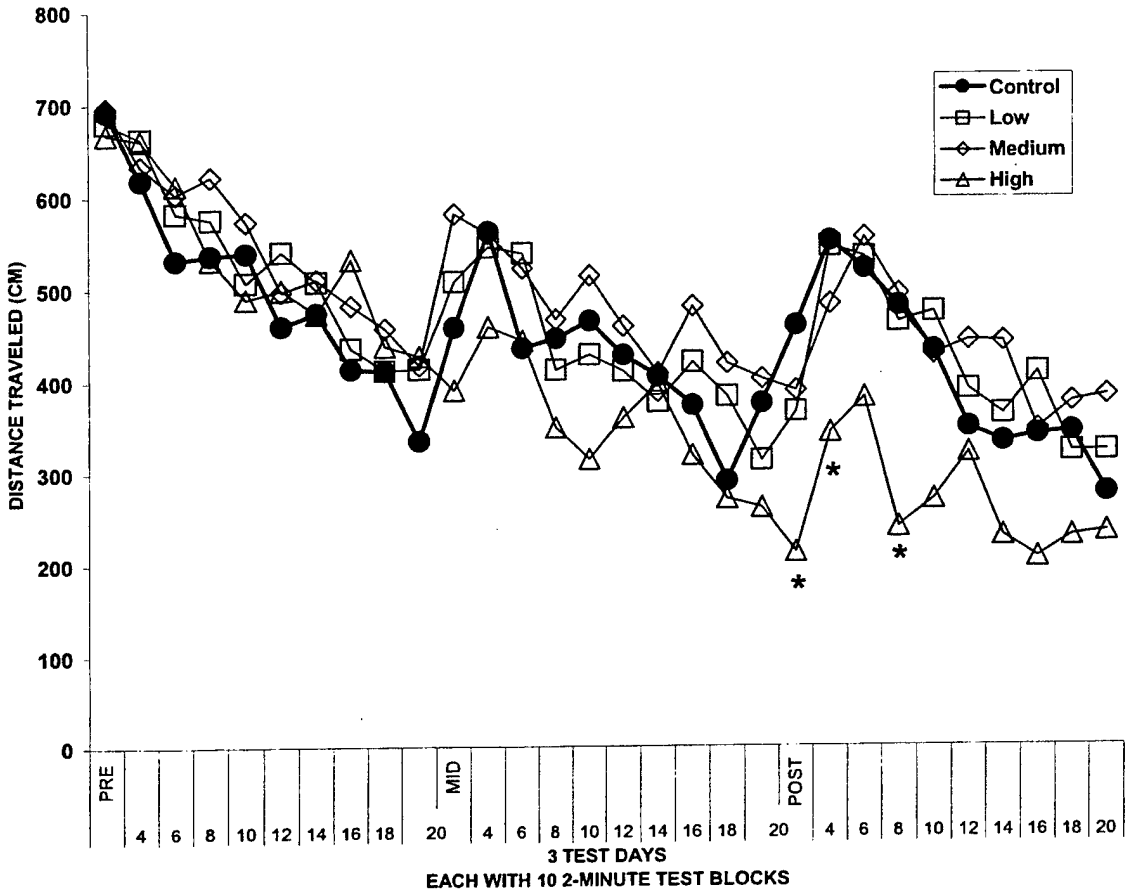

Figure 4. Distance traveled over three separate testing sessions (pre-exposure, week 4, week 12) for male mice. Each 20-minute session is divided into ten 2-minute blocks of time. Statistically significant differences ($p<0.05$) between the control and high dose groups are denoted with asterisks (*).

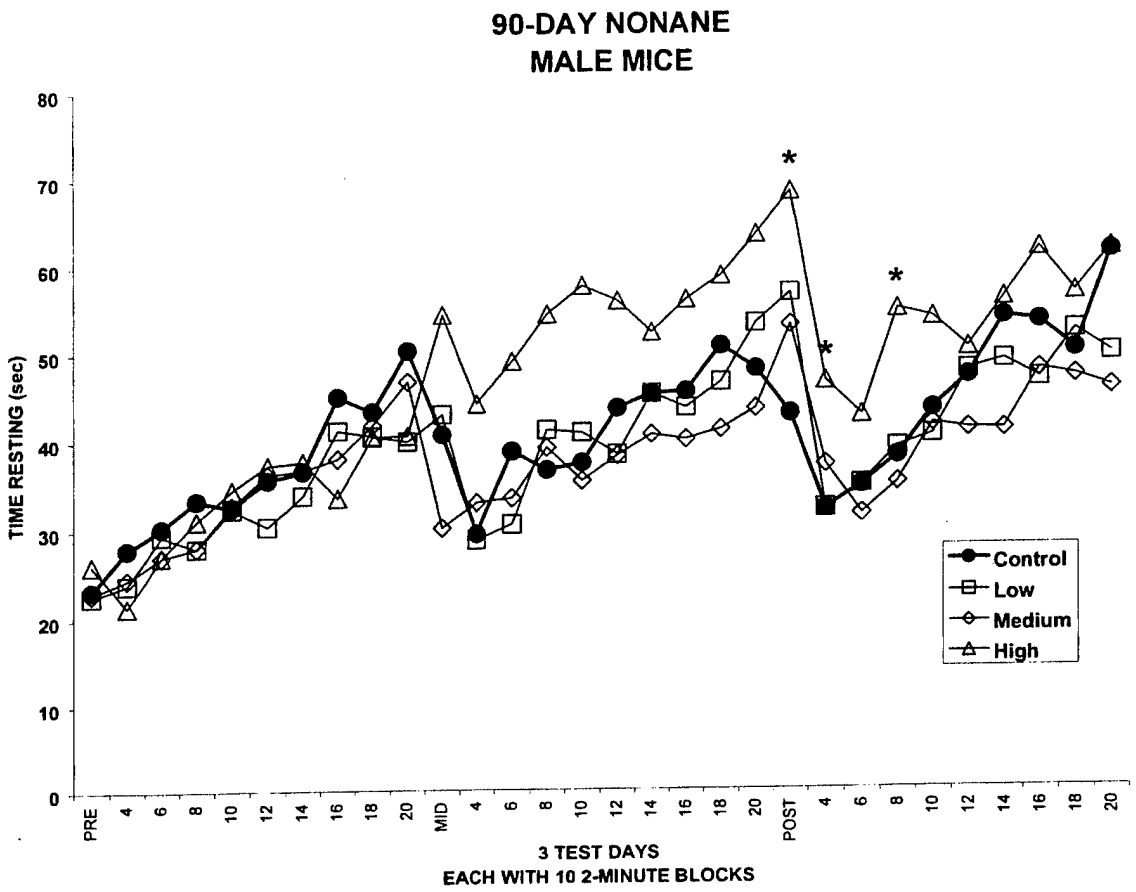

Figure 5. Time spent resting over three separate testing sessions (pre-exposure, week 4, week 12) for male mice. Each 20-minute session is divided into ten 2-minute blocks of time. Statistically significant differences ($p<0.05$) between the control and high dose groups are denoted with asterisks (*).

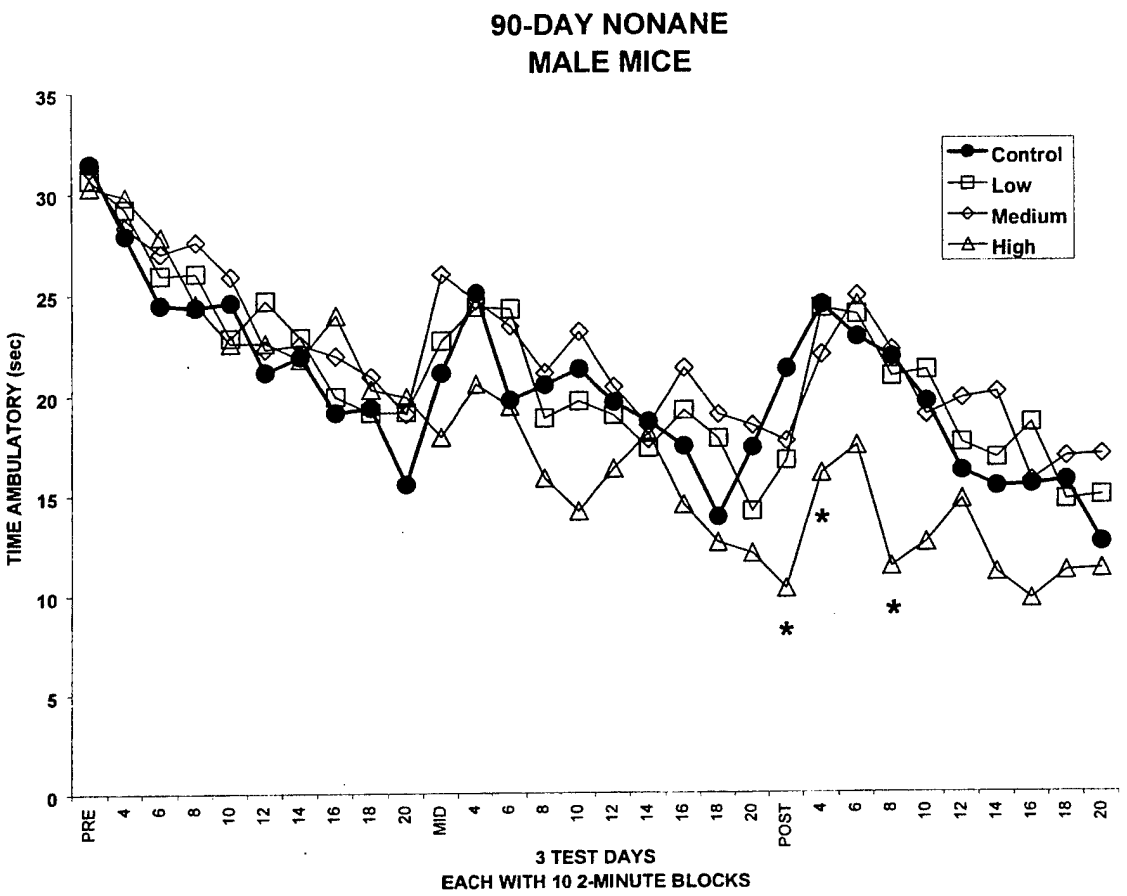

Figure 6. Time spent in non-stereotypic (ambulatory) movement over three separate (pre-exposure, week 4, week 12) testing sessions for male mice. Each 20 minute session is divided into ten 2-minute blocks of time. Statistically significant differences ($p<0.05$) between the control and high dose groups are denoted with asterisks (*).

TABLE 5. TRANSIENT GROUP DIFFERENCES IN FEMALE RAT LOCOMOTOR ACTIVITY

MEASURE	TEST SESSION	TIME BLOCK	DIFFERENCE
Distance Traveled	week 4	6 min block	0.0 g/kg > 5 g/kg
Time Resting	week 4	6 min block	1.0 g/kg > 5 g/kg
Time Ambulatory	week 4	6 min block	0.0 g/kg > 5 g/kg

TABLE 6. TRANSIENT GROUP DIFFERENCES IN MALE MOUSE LOCOMOTOR ACTIVITY

MEASURE	TEST SESSION	TIME BLOCK	DIFFERENCE
Time in Stereotypy	week 4	6 min block	0.1 g/kg > 5 g/kg
# of Stereotypical Movements	week 4	10 min block	1.0 g/kg > 5 g/kg
# of Stereotypical Movements	week 12	2 min block	0.0 g/kg > 5 g/kg
# of Stereotypical Movements	week 12	4 min block	0.0 g/kg > 5 g/kg
# of Vertical Movements	week 4	18 min block	1.0 g/kg > 5 g/kg
# of Vertical Movements	week 12	8 min block	0.0 g/kg > 5 g/kg

Body Weights and Food Consumption

Weekly mean body weight values are reported for rats (Table 7 and Figure 7) and mice (Table 8 and Figure 8). Weekly mean food consumption values are reported for rats and mice in Tables 9 and 10, respectively. For both rats and mice, there were no statistically significant differences in mean body weights between control and treated groups throughout the study. Food consumption values for female rats of the 5.0 and 1.0 g/kg groups were lower than the control values for the first two weeks on study. However, no further decreases from control means were observed, except for the 1.0 g/kg group on study Days 43 and 50. For male mice, there were no statistically significant differences between control and treated groups in mean food consumption throughout the 90-day study.

TABLE 7. MEAN[a] BODY WEIGHTS OF FEMALE RATS

Study Day	Dose (g/kg/day)			
	0.0[b]	0.1[b]	1.0[b]	5.0[c]
1	131.3 ± 1.4	131.2 ± 1.6	130.9 ± 1.6	130.5 ± 2.2
6	133.9 ± 1.3	135.1 ± 1.2	132.6 ± 2.1	126.2 ± 2.1
13	140.3 ± 1.6	142.5 ± 1.3	137.5 ± 2.1	136.0 ± 2.4
20	149.6 ± 1.8	149.5 ± 1.1	146.2 ±1.8	144.4 ± 2.0
27	151.4 ± 2.1	150.5 ± 1.8	148.7 ± 2.3	150.0 ± 2.5
34	156.1 ± 2.1	154.3 ± 1.4	151.2 ± 2.5	155.0 ± 2.7
41	158.4 ± 2.3	157.5 ± 1.6	153.9 ± 3.1	159.5 ± 2.4
48	162.6 ± 2.4	160.7 ± 1.8	156.9 ± 2.8	164.0 ± 2.6
55	166.7 ± 2.4	164.7 ± 1.7	160.0 ± 2.6	166.7 ± 2.6
62	168.1 ± 2.4	166.6 ± 1.9	162.2 ± 2.8	169.3 ± 2.8
69	170.8 ± 2.6	166.9 ± 2.1	160.5 ± 3.7	167.9 ± 4.2
76	171.9 ± 2.6	168.7 ± 2.0	162.6 ± 2.5	166.7 ± 5.5
83	172.8 ± 2.7	168.8 ± 1.4	163.7 ± 2.7	170.6 ± 4.5

[a] mean ± SE; units are grams
[b] group size = 10 (Days 1-83)
[c] group size = 10 (Days 1-6), 7 (Days 13-69), 6 (Days 76-83)

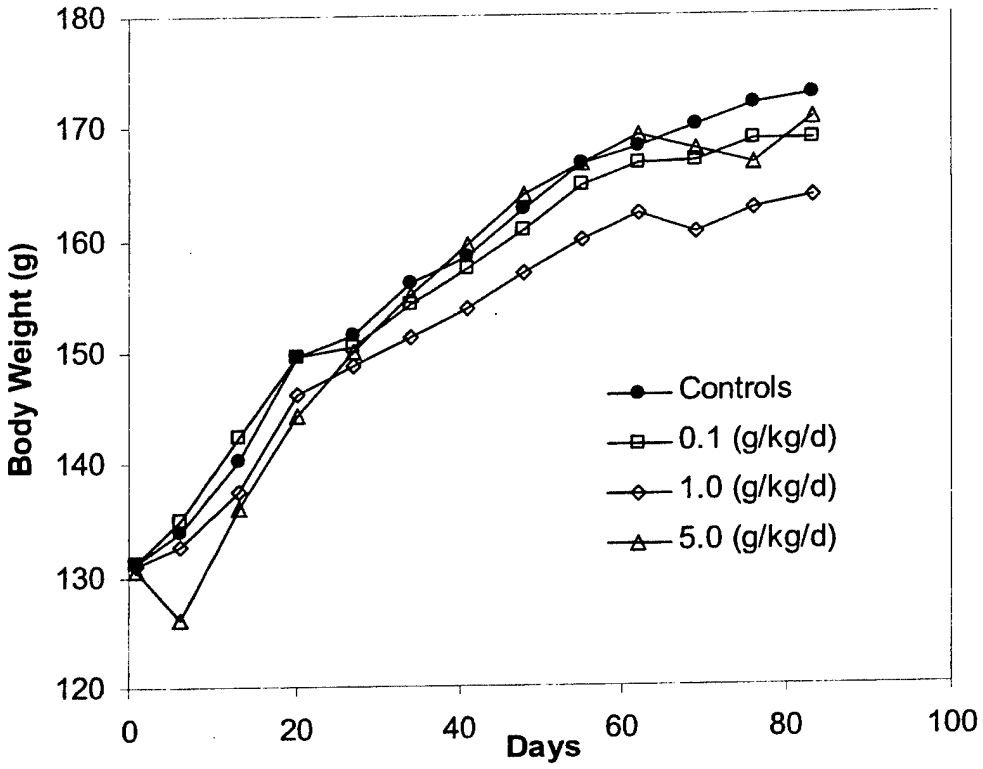

Figure 7. Body weights over time for female rats during 90-day oral dosing with *n*-nonane.

TABLE 8. MEAN[a] BODY WEIGHTS OF MALE MICE

	Dose (g/kg/day)			
Study Day	0.0[b]	0.1[c]	1.0[d]	5.0[e]
1	24.0 ± 0.3	24.1 ± 0.3	24.0 ± 0.3	24.0 ± 0.3
6	24.6 ± 0.4	24.2 ± 0.2	24.6 ± 0.4	23.4 ± 0.4
13	25.0 ± 0.4	24.7 ± 0.3	25.1 ± 0.4	24.5 ± 0.5
20	25.9 ± 0.3	25.6 ± 0.3	26.0 ± 0.5	26.1 ±0.5
27	26.2 ± 0.3	25.6 ± 0.4	25.7 ± 0.4	26.3 ± 0.4
34	26.9 ± 0.3	26.5 ± 0.4	26.6 ± 0.5	26.8 ± 0.4
41	27.4 ± 0.4	26.6 ± 0.4	27.0 ± 0.4	27.3 ± 0.5
48	28.1 ± 0.4	27.0 ± 0.3	27.4 ± 0.6	27.5 ± 0.5
55	28.4 ± 0.4	27.9 ± 0.5	27.9 ± 0.8	27.7 ± 0.5
62	29.4 ± 0.5	28.1 ± 0.4	27.8 ± 0.6	28.5 ± 0.6
69	29.9 ± 0.6	28.5 ± 0.6	28.5 ± 0.6	28.6 ± 0.5
76	30.6 ± 0.6	29.2 ± 0.6	28.9 ± 0.6	28.4 ± 0.5
83	30.5 ± 0.5	29.2 ± 0.5	28.7 ± 0.6	29.3 ± 0.5

[a] mean ± SE; units are grams
[b] group size = 10 (Days 1-48), 9 (Days 55-83)
[c] group size = 10 (Days 1-83)
[d] group size = 10 (Days 1-76), 9 (Day 83)
[e] group size = 10 (Days 1-13), 9 (Days 20-55, 69-76), 8 (Days 62 and 83)

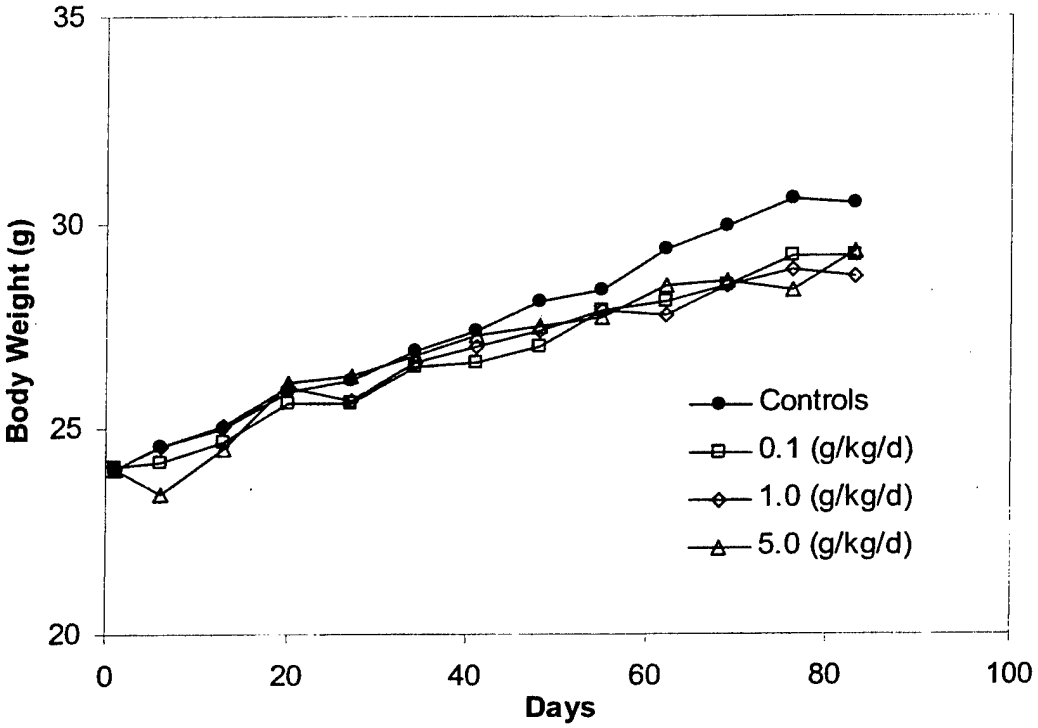

Figure 8. Body weights over time for male mice during 90-day oral dosing with *n*-nonane.

TABLE 9. MEAN[a] FOOD CONSUMPTION OF FEMALE RATS

	Dose (g/kg/day) and Group Size (N)							
Study Day	0.0	N	0.1	N	1.0	N	5.0	N
1	10.8 ± 0.2	10	10.1 ± 0.2	9	9.5[b] ± 0.3	10	7.9[b] ± 0.4	10
8	11.5 ± 0.2	10	11.4 ± 0.4	10	11.3 ± 0.3	10	9.4[b] ± 0.4	7
15	11.8 ± 0.2	10	11.3 ± 0.3	10	9.6[b] ± 0.4	10	9.8[c] ± 0.6	4
22	11.3 ± 0.3	10	10.7 ± 0.2	10	10.5 ± 0.3	10	10.8 ± 0.3	7
29	10.3 ± 0.3	10	11.2 ± 0.3	10	10.4 ± 0.5	10	9.9 ± 0.6	7
38[d]	10.6 ± 0.2	10	10.3 ± 0.3	8	9.6 ± 0.4	10	10.5 ± 0.7	7
43	10.6 ± 0.2	10	10.2 ± 0.3	10	8.3[b] ± 0.4	10	10.5 ± 0.4	7
50	10.7 ± 0.3	10	10.5 ± 0.2	10	9.2[b] ± 0.3	10	11.3 ± 0.6	7
57	10.9 ± 0.5	10	10.8 ± 0.3	10	9.7 ± 0.3	10	10.8 ± 1.0	7
64	10.2 ± 0.3	10	9.9 ± 0.4	10	9.7 ± 0.5	10	11.5 ± 0.4	7
71	10.6 ± 0.3	10	10.3 ± 0.3	10	9.3 ± 0.5	10	11.5 ± 0.5	7
78	10.4 ± 0.3	10	10.4 ± 0.6	10	9.3 ± 0.3	10	11.4 ± 0.4	6
85	10.6 ± 0.3	10	10.4 ± 0.3	10	10.0 ± 0.4	10	11.2 ± 0.4	6
90	10.6 ± 0.4	10	10.8 ± 0.9	10	8.7 ± 0.3	10	9.8 ± 0.8	6

[a] mean ± SE; units are grams
[b] $p<0.01$ compared to control
[c] $p<0.05$ compared to control
[d] = Day 38 was reported due to problems in data collection on Day 36

TABLE 10. MEAN* FOOD CONSUMPTION OF MALE MICE

	Dose (g/kg/day) and Group Size (N)							
Study Day	0.0	N	0.1	N	1.0	N	5.0	N
1	7.2 ± 0.5	8	7.1 ± 0.7	7	7.0 ± 0.7	7	7.9 ± 0.6	7
8	4.9 ± 0.2	10	5.0 ± 0.4	10	5.8 ± 0.4	10	5.2 ± 0.5	10
15	4.4 ± 0.4	10	4.1 ± 0.3	10	4.5 ± 0.4	10	5.4 ± 0.3	10
22	5.2 ± 0.4	10	4.3 ± 0.5	10	4.8 ± 0.6	9	5.7 ± 0.5	9
29	4.5 ± 0.6	10	4.8 ± 0.3	10	4.3 ± 0.6	10	4.4 ± 0.5	9
36	4.2 ± 0.2	10	4.3 ± 0.3	10	3.7 ± 0.3	10	3.7 ± 0.5	9
43	4.7 ± 0.2	10	4.8 ± 0.5	10	4.9 ± 0.4	10	5.0 ± 0.3	9
50	5.0 ± 0.4	10	5.3 ± 0.3	10	4.4 ± 0.3	9	4.4 ± 0.4	8
57	2.6 ± 0.3	8	3.4 ± 0.5	10	4.0 ± 0.7	10	4.4 ± 0.3	9
64	5.3 ± 0.3	9	5.5 ± 0.7	10	5.0 ± 0.5	9	5.1 ± 0.2	9
71	4.7 ± 0.4	9	4.7 ± 0.2	10	5.2 ± 0.4	10	5.5 ± 0.3	9
78	4.2 ± 0.2	9	4.3 ± 0.2	10	4.9 ± 0.5	10	4.9 ± 0.3	9
85	4.6 ± 0.2	9	4.8 ± 0.4	10	4.9 ± 0.3	9	5.6 ± 0.3	8
88	5.4 ± 0.5	8	4.8 ± 0.3	10	5.9 ± 0.5	8	6.4 ± 0.4	8

* mean ± SE; units are grams

Hematology and Serum Chemistry

Mean hematologic values of rats and mice at the conclusion of the 90-day study are given in Tables 11 and 12, respectively. In rats of the 0.1 and 5.0 g/kg groups, the white blood cell count was increased compared to the control value. The mean percentages of neutrophils and basophils were also increased in the 5.0 g/kg group, but the lymphocyte percentage was decreased. In mice, decreases in red blood cell count, hemoglobin concentration, hematocrit percentage and percent lymphocytes were observed in the 5.0 g/kg group. Neutrophil percentage was increased compared to control in the 0.1, 1.0, and 5.0 g/kg groups.

TABLE 11. MEAN[a] HEMATOLOGIC VALUES OF FEMALE RATS

Parameters	Dose (g/kg/day)			
	0.0	0.1	1.0	5.0
N	10	10	9	5
WBC (10^3)	6.8 ± 0.8	8.7[b] ± 1.1	7.9 ± 1.5	9.6[b] ± 1.2
RBC (10^6)	8.9 ± 0.4	8.8 ± 0.5	8.7 ± 0.6	8.9 ± 0.8
HGB (g/dL)	15.4 ± 0.6	15.5 ± 0.8	15.2 ± 1.0	15.5 ± 1.2
HCT (%)	47.2 ± 1.7	46.9 ± 2.5	46.6 ± 2.9	47.2 ± 3.8
MCV (fL)	53.2 ± 0.6	53.3 ± 0.2	53.5 ± 0.6	52.8 ± 0.6
MCH (pg)	17.4 ± 0.3	17.6 ± 0.2	17.4 ± 0.3	17.3 ± 0.2
MCHC (g/dL)	32.7 ± 0.6	33.0 ± 0.2	32.6 ± 0.3	32.8 ± 0.3
Platelets (10^3)	886 ± 69	955 ± 86	908 ± 77	965 ± 97
Neutrophils (%)	21.1 ± 3.2	22.1 ± 3.9	24.4 ± 3.5	29.5[b] ± 7.3
Lymphocytes (%)	73.8 ± 3.2	73.2 ± 4.0	70.9 ± 3.6	65.3[b] ± 6.9
Monocytes (%)	4.2 ± 0.9	3.6 ± 1.2	3.9 ± 1.2	3.7 ± 1.1
Eosinophils (%)	0.9 ± 0.3	0.8 ± 0.2	0.7 ± 0.4	0.7 ± 0.4
Basophils (%)	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.8[b] ± 0.7

[a] mean ± SD
[b] $p<0.01$ compared to control

TABLE 12. MEAN[a] HEMATOLOGIC VALUES OF MALE MICE

Parameter	Dose (g/kg/day)			
	0.0	0.1	1.0	5.0
N	4	9	6	8
WBC (10^3)	5.6 ± 1.3	7.1 ± 2.6	5.9 ± 1.4	7.6 ± 2.0
RBC (10^6)	11.5 ± 1.2	11.4 ± 0.6	10.9 ± 0.9	9.7[b] ± 1.5
HGB (g/dL)	16.7 ± 0.7	16.0 ± 0.3	15.5 ± 0.5	14.5[c] ± 2.0
HCT (%)	53.7 ± 5.1	52.4 ± 4.1	49.4 ± 2.9	44.1[c] ± 6.9
MCV (fL)	46.8 ± 1.3	46.1 ± 2.8	45.5 ± 1.8	45.4 ± 3.2
MCH (pg)	14.6 ± 1.6	14.1 ± 0.7	14.3 ± 1.0	14.9 ± 1.2
MCHC (g/dL)	31.2 ± 2.9	30.7 ± 2.6	31.5 ± 1.5	33.1 ± 4.8
Platelets (10^3)	1326 ± 240	1596 ± 295	1612 ± 344	1322 ± 468
Neutrophils (%)	9.6 ± 5.5	10.4[c] ± 8.1	10.7[c] ± 5.5	25.8[c] ± 10.9
Lymphocytes (%)	85.6 ± 4.4	86.2 ± 6.8	85.5 ± 4.2	70.1[c] ± 14.5
Monocytes (%)	3.5 ± 2.0	2.7 ± 3.2	2.7 ± 0.7	3.5 ± 4.1
Eosinophils (%)	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.1
Basophils (%)	1.2 ± 2.0	0.6 ± 1.0	0.9 ± 1.4	0.5 ± 0.6

[a] mean ± SD
[b] $p<0.05$ compared to control
[c] $p<0.01$ compared to control

Mean serum chemistry values of rats and mice are given in Tables 13 and 14, respectively. In rats of the 5.0 g/kg group, there were decreases in mean values of cholesterol, triglycerides, and albumin and an increase in alanine aminotransferase (ALT). Rats of the 1.0 g/kg group had lower albumin and total protein concentrations compared to the control group. No additional statistically significant difference was observed in serum chemistry values in rats. Decreases in the mean values of chloride, aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, and albumin were observed in mice of the 5.0 g/kg group. Mice of the 1.0 g/kg group had lower chloride, alkaline phosphatase and albumin values compared to control mice. Alkaline phosphatase was also lower in the 0.1 g/kg group. No other statistically significant differences between treated and control groups were observed (Table 14).

TABLE 13. MEAN[a] SERUM CHEMISTRY VALUES OF FEMALE RATS

Parameter	Dose (g/kg/day)			
	0.0	0.1	1.0	5.0
N	10	10	9	6
BUN (mg/kg)	18.7 ± 1.7	19.2 ± 2.0	17.0 ± 1.7	17.2 ± 1.5
Creatinine (mg/dL)	0.5 ± <0.1	0.5 ± <0.1	0.5 ± 0.1	0.5 ± <0.1
Chloride (mmol/L)	98.7 ± 1.3	98.8 ± 1.9	99.7 ± 0.9	98.3 ± 2.1
Calcium (mg/dL)	11.3 ± 0.2	11.3 ± 0.4	11.0 ± 0.3	10.9 ± 0.4
Phosphorus (mg/dL)	10.1 ± 1.0	9.6 ± 0.8	9.4 ± 0.5	10.0 ± 0.8
Total Protein (g/dL)	6.3 ± 0.2	6.3 ± 0.2	5.9[b] ± 0.2	6.1 ± 0.3
AST (IU/L)	85.6 ± 23.0	78.5 ± 8.2	83.1 ± 15.0	97.3 ± 15.3
ALT (IU/L)	48.8 ± 4.1	47.9 ± 7.2	44.7 ± 5.8	61.8[b] ± 5.0
Alkaline phosphatase (IU/L)	127 ± 13	124 ± 21	113 ± 11	157 ± 53
Glucose (mg/dL)	131 ± 22	141 ± 20	142 ± 23	151 ± 19
Sodium (mmol/L)	147 ± 2	147 ± 2	146 ± 2	146 ± 1
Triglycerides (mg/dL)	62.2 ± 14.8	69.4 ± 18.3	49.8 ± 6.7	37.2[c] ± 11.4
Magnesium (mg/dL)	2.8 ± 0.2	2.6 ± 0.2	2.7 ± 0.2	2.7 ± 0.2
Potassium (mmol/L)	5.4 ± 0.5	5.2 ± 0.5	5.5 ± 0.3	5.3 ± 0.2
Cholesterol (mg/dL)	77.9 ± 7.5	79.1 ± 5.1	72.7 ± 3.2	64.5[b] ± 6.6
Total Bilirubin (mg/dL)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Albumin (g/dL)	3.6 ± 0.1	3.6 ± 0.1	3.2[b] ± 0.2	3.2[b] ± 0.2
Globulin (g/dL)	2.8 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	3.0 ± 0.3

[a] mean ± SD
[b] $p<0.05$ compared to control
[c] $p<0.01$ compared to control

TABLE 14. MEAN[a] SERUM CHEMISTRY VALUES OF MALE MICE

	Dose (g/kg/day)			
Parameter	**0**	**0.1**	**1**	**5**
N	4	9	6	8
BUN (mg/kg)	19.6 ± 1.7	19.6 ± 3.3	21.7 ± 4.6	18.6 ± 5.4
Creatinine (mg/dL)	0.2 ± 0.1	0.2 ± <0.1	0.2 ± 0.1	0.2 ± 0.1
Chloride (mmol/L)	115.0 ± 4.0	115 ± 2.0	113.0[b] ± 1.0	111.0[c] ± 2.0
Calcium (mg/dL)	9.9 ± 0.8	9.6 ± 0.3	9.5 ± 0.3	9.8 ± 0.2
Phosphorus (mg/dL)	8.7 ± 0.4	8.5 ± 1.0	8.5 ± 0.9	8.6 ± 0.8
Total Protein (g/dL)	5.0 ± 0.3	4.8 ± 0.3	4.8 ± 0.1	4.6 ± 0.3
AST (IU/L)	64.1 ± 4.4	52.3 ± 5.4	54.5 ± 13.5	50.6[b] ± 5.6
ALT (IU/L)	16.8 ± 6.0	15.8 ± 7.3	24.5 ± 14.5	19.3 ± 9.1
Alkaline Phosphatase (IU/L)	98.3 ± 15.0	96.0[b] ± 17.4	89.5[c] ± 7.7	65.5[c] ± 18.0
Glucose (mg/dL)	223 ± 57	193.0 ± 36.0	193.0 ± 28.0	202.0 ± 33.0
Sodium (mmol/L)	154 ± 3.0	155 ± 2.0	155 ± 3.0	153 ± 2.0
Potassium (mmol/L)	7.3 ± 0.6	7.0 ± 0.7	7.0 ± 1.1	7.6 ± 0.8
Total Bilirubin (mg/dL)	0.4 ± 0.1	0.3 ± 0.1	0.3 ± <0.1	0.2[b] ± 0.1
Albumin (g/dL)	2.6 ± 0.2	2.5 ± 0.1	2.4[b] ± 0.1	2.2[c] ± 0.2
Globulin (g/dL)	2.3 ± 0.2	2.3 ± 0.2	2.4 ± 0.1	2.4 ± 0.2

[a] mean ± SD
[b] $p<0.05$ compared to control
[c] $p<0.01$ compared to control

n-Nonane Analysis in Blood and Tissues (rats only)

Blood and tissue concentrations of *n*-nonane in female rats during the 90-day study are given in Tables 15 and 16, respectively. Minimum and maximum concentrations were reported due to inter-animal variability. Blood concentrations increased with dose and were considerably lower in value prior to dosing compared to post-dosing (Table 15). Further, blood concentrations were consistent between study weeks at each dose level (Figure 9). At the conclusion of the study, concentrations of *n*-nonane were the highest in fat tissue compared to muscle or liver (Table 16). Though inter-animal variability was large, tissue concentrations consistently increased with dose.

TABLE 15. FEMALE RAT BLOOD CONCENTRATION[a] OF *n*-NONANE

Study Week	Dose (g/kg/day) 0.1[b] Prior[d]	0.1[b] Post[d]	1.0[b] Prior	1.0[b] Post	5.0[c] Prior	5.0[c] Post
5	0.05 - 0.14	0.27 - 1.58	0.45 – 0.81	1.98 - 6.42	1.33 - 1.88	5.04 - 9.43
10	0.07 - 0.28	0.70 - 2.06	0.40 – 1.11	1.74 - 4.34	1.00 - 1.80	4.09 - 11.7
13	0.06 - 0.17	0.44 - 1.59	0.46 – 1.07	1.34 - 2.93	1.43 - 1.95	3.72 - 9.53

[a] values represent minimum to maximum concentration (μg/g) of individual animals
[b] group size = 10
[c] group size = 7 (weeks 5 and 10); 6 (week 13)
[d] samples were collected immediately prior to dosing and two hours post-dosing

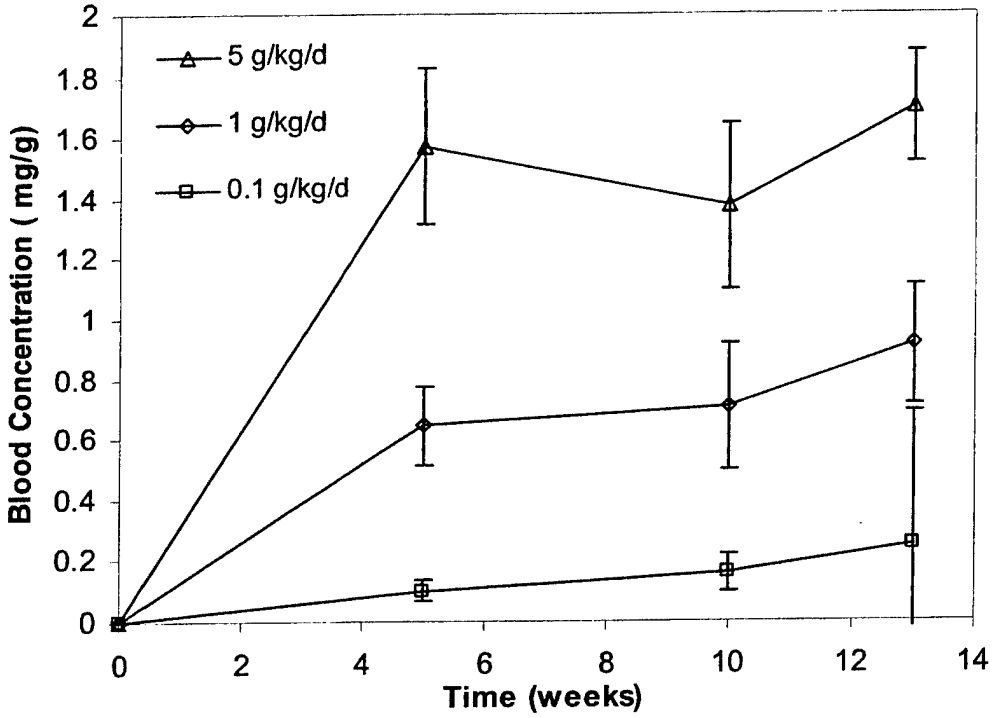

Figure 9. Blood concentration in female rats immediately prior to daily dosing of *n*-nonane. Values are mean ± SD.

TABLE 16. FEMALE RAT TISSUE CONCENTRATION[a] OF *n*-NONANE

	Dose (g/kg/day)		
Tissue	0.1[b]	1.0[c]	5.0[d]
Fat	6.4 – 41	109 – 402	0 - 847
Liver	0.0 - 2.2	1.1 - 7.0	0.0 - 7.6
Muscle	0.0 - 9.7	0.6 – 51	0 - 70

[a]values represent minimum to maximum concentration (μg/g) of individual animals
[b]group size = 10
[c]group size = 8 or 9
[d]group size = 6

Necropsy and Organ Weights

Mild to moderate perianal alopecia and inflammation were observed in a majority of the rats and mice of the 5.0 g/kg groups. Five high dose group rats were found dead between days 1 and 10, with gross and histologic lesions suggestive of dosing accidents (pulmonary hemorrhage; severe transmural hemorrhagic gastritis). Another high dose rat died during week 11, and an intermediate dose rat died during week 13. These deaths also were attributed to dosing accidents. Two high dose mice died on days 19 and 74; lesions again were suggestive of dosing accidents. Two intermediate dose mice and one control mouse died as well, presumably to dosing accidents. No other treatment-related lesions were observed in the remaining groups.

Mean organ weight values for female rats and male mice are presented in Tables 17 through 19 and 20 through 22, respectively. The mean values between control and treated groups were similar for final body weights and absolute brain weights in both rats (Table 17) and mice (Table 20). Hence, statistically significant differences in absolute organ weight values agreed, in the majority of cases, with statistically significant differences in relative organ weight values. Female rats of the 5.0 g/kg group had increased liver, lung, and adrenal weights, but decreased spleen and ovary weights. The increase in adrenal weights and decrease in ovary weights were also observed in the 1.0 g/kg female rats, but there were no differences in organ weights between the control and 0.1 g/kg groups.

In male mice, liver weights were increased and kidney weights were decreased in the 5.0 and 1.0 g/kg groups. There were no statistically significant differences in mean organ weights between the control and 0.1 g/kg groups.

TABLE 17. MEAN[a] ORGAN WEIGHTS AND FINAL BODY WEIGHT OF FEMALE RATS

Organ	Dose (g/kg/day)			
	0.0[b]	0.1[c]	1.0[c]	5.0[d]
Liver	4.19 ± 0.10	4.10 ± 0.11	4.03 ± 0.09	4.82[e] ± 0.18
Kidneys	1.17 ± 0.02	1.14 ± 0.02	1.13 ± 0.02	1.21 ± 0.04
Lungs	1.32 ± 0.12	1.25 ± 0.07	1.21 ± 0.09	2.28[e] ± 0.44
Spleen	0.39 ± 0.01	0.38 ± 0.01	0.35 ± 0.01	0.34[e] ± 0.02
Adrenals	0.054 ± 0.003	0.053 ± 0.002	0.060 ± 0.002	0.066 ± 0.003
Ovaries	0.091 ± 0.002	0.088 ± 0.002	0.076[e] ± 0.003	0.067[e] ± 0.008
Brain	1.69 ± 0.02	1.67 ± 0.01	1.64 ± 0.03	1.67 ± 0.02
Body weight	163.3 ± 2.5	161.9 ± 1.5	154.0 ± 2.5	160.8 ± 4.4

[a] mean ± SE, units are grams
[b] group size = 10
[c] group size = 9
[d] group size = 6
[e] $p<0.05$ compared to control

TABLE 18. MEAN[a] RELATIVE (TO BODY WEIGHT) ORGAN WEIGHTS OF FEMALE RATS

Organ	Dose (g/kg/day)			
	0.0[b]	0.1[b]	1.0[c]	5.0[d]
Liver	2.56 ± 0.04	2.53 ± 0.06	2.62 ± 0.04	3.00[e] ± 0.06
Kidneys	0.72 ± 0.01	0.71 ± 0.01	0.74 ± 0.01	0.75 ± 0.01
Lungs	0.80 ± 0.07	0.77 ± 0.04	0.79 ± 0.05	1.43[e] ± 0.30
Spleen	0.24 ± <0.01	0.23 ± 0.01	0.23 ± <0.01	0.21[e] ± 0.01
Adrenals	0.033 ± 0.002	0.033 ± 0.001	0.039[e] ± 0.001	0.041[e] ± 0.001
Ovaries	0.056 ± 0.001	0.055 ± 0.001	0.050 ± 0.002	0.041[e] ± 0.004
Brain	1.04 ± 0.01	1.03 ± 0.01	1.07 ± 0.01	1.04 ± 0.03

[a] mean ± SE, units are percent
[b] group size = 10
[c] group size = 9
[d] group size = 6
[e] $p<0.05$ compared to control

TABLE 19. MEAN[a] RELATIVE (TO BRAIN WEIGHT) ORGAN WEIGHTS OF FEMALE RATS

	Dose (g/kg/day)			
Organ	**0.0[b]**	**0.1[b]**	**1.0[c]**	**5.0[d]**
Liver	248 ± 5	245 ± 6	246 ± 4	289[e] ± 10
Kidneys	69.3 ± 1.0	68.3 ± 0.6	69.2 ± 0.9	72.7 ± 2.2
Lungs	77.5 ± 6.4	75.1 ± 4.8	73.8 ± 5.1	138[e] ± 28
Spleen	22.9 ± 0.5	22.5 ± 0.5	21.2 ± 0.4	20.4 ± 1.0
Adrenals	3.21 ± 0.19	3.16 ± 0.08	3.66[e] ± 0.13	3.97[e] ± 0.18
Ovaries	5.41 ± 0.09	5.28 ± 0.13	4.65 ± 0.18	4.00[e] ± 0.45

[a] mean ± SE, units are percent
[b] group size = 10
[c] group size = 9
[d] group size = 6
[e] $p<0.05$ compared to control

TABLE 20. MEAN[a] ORGAN WEIGHTS AND FINAL BODY WEIGHT OF MALE MICE

	Dose (g/kg/day)			
Organ	**0.0[b]**	**0.1[c]**	**1.0[d]**	**5.0[d]**
Liver	1.14 ± 0.04	1.20 ± 0.03	1.27 ± 0.06	1.40[e] ± 0.08
Kidneys	0.45 ± 0.01	0.41 ± 0.01	0.40[e] ± 0.01	0.40[e] ± 0.02
Lungs	0.34 ± 0.01	0.31 ± 0.02	0.29 ± 0.02	0.28 ± 0.02
Spleen	0.05 ± <0.01	0.06 ± <0.01	0.07 ± < 0.01	0.12 ± 0.04
Adrenals	0.006 ± 0.001	0.007 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Testes	0.22 ± <0.01	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Brain	0.43 ± <0.01	0.42 ± <0.01	0.42 ± <0.01	0.41 ± 0.01
Body weight	28.5 ± 0.5	27.6 ± 0.5	27.6 ± 0.9	26.9 ± 0.7

[a] mean ± SE, units are grams
[b] group size = 9
[c] group size = 10
[d] group size = 8
[e] $p<0.05$ compared to control

TABLE 21. MEAN[a] RELATIVE (TO BODY WEIGHT) ORGAN WEIGHTS OF MALE MICE

	Dose (g/kg/day)			
Organ	0.0[b]	0.1[c]	1.0[d]	5.0[d]
Liver	4.01 ± 0.13	4.35 ± 0.08	4.60[e] ± 0.11	5.18[e] ± 0.20
Kidneys	1.58 ± 0.02	1.50 ± 0.03	1.46[e] ± 0.02	1.47 ± 0.05
Lungs	1.21 ± 0.03	1.13 ± 0.07	1.04 ± 0.06	1.04 ± 0.04
Spleen	0.19 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.46 ± 0.14
Adrenals	0.023 ± 0.004	0.025 ± 0.004	0.018 ± 0.003	0.020 ± 0.003
Testes	0.78 ± 0.02	0.74 ± 0.03	0.77 ± 0.03	0.76 ± 0.03
Brain	1.51 ± 0.03	1.54 ± 0.02	1.53 ± 0.06	1.54 ± 0.04

[a] mean ± SE, units are percent
[b] group size = 9
[c] group size = 10
[d] group size = 8
[e] $p<0.05$ compared to control

TABLE 22. MEAN[a] RELATIVE (TO BRAIN WEIGHT) ORGAN WEIGHTS OF MALE MICE

	Dose (g/kg/day)			
Organ	0.0[b]	0.1[c]	1.0[d]	5.0[d]
Liver	266 ± 10	283 ± 7	304[e] ± 16	339[e] ± 21
Kidneys	105 ± 2	97.4 ± 1.4	95.8 ± 3.4	96.1 ± 4.9
Lungs	79.9 ± 1.9	73.4 ± 4.6	68.6 ± 5.5	67.7 ± 3.6
Spleen	12.7 ± 0.5	14.7 ± 0.5	15.9 ± 1.1	30.4 ± 9.6
Adrenals	1.50 ± 0.25	1.61 ± 0.26	1.22 ± 0.25	1.31 ± 0.19
Testes	51.9 ± 1.1	48.2 ± 1.7	50.4 ± 1.7	49.1 ± 1.6

[a] mean ± SE, units are percent
[b] group size = 9
[c] group size = 10
[d] group size = 8
[e] $p<0.05$ compared to control

Histopathology

Tissue lesion incidence is presented in Table 23. Lesions in the alimentary tract were present in both rats and mice of all *n*-nonane treated groups, but not in the controls. Most lesions were in the non-glandular stomach (forestomach). These lesions consisted of varying degrees of hyperplasia and hyperkeratosis of the squamous epithelium, often accompanied by infiltrates of neutrophils, eosinophils, lymphocytes, and lesser macrophages in the lamina propria and submucosa. Occasionally, erosion and ulceration of the mucosa were present. In the most severe manifestations, the squamous epithelium was thickened up to 6-fold, often producing pronounced invaginating folds. The keratinized layer was similarly thickened, occasionally with

dense aggregates of degenerating neutrophils (intracornual abscesses). The glandular stomach was histologically normal in all animals.

TABLE 23. INCIDENCE* OF TISSUE LESIONS

	Dose (g/kg/day)							
	0.0		0.1		1.0		5.0	
Finding	**Rats**	**Mice**	**Rats**	**Mice**	**Rats**	**Mice**	**Rats**	**Mice**
Stomach (non-glandular):								
Squamous epithelial hyperplasia or hyperkeratosis with or without inflammation – marked/moderate	0/10	0/9	0/10	5/10	4/10	6/8	10/11	8/8
Squamous epithelial hyperplasia or hyperkeratosis with or without inflammation - mild	0/10	0/9	8/10	1/10	6/10	1/8	0/11	0/8
Duodenum (proximal):								
Mucosal inflammation - mild	0/10	0/7	0/10	0/10	0/10	0/10	2/10	0/10
Rectum:								
Perianal hyperplasia, hyperkeratosis and inflammation	0/10	0/9	0/10	0/10	5/10	2/10	9/11	8/10
Nasal Turbinates:								
Rhinitis	0/10	0/9	1/9	0/10	7/10	0/10	9/10	4/10

* number with finding / number examined

Eleven high dose (5.0 g/kg) rats were examined (including five who died during the study). Ten had marked forestomach hyperplasia and hyperkeratosis. The remaining one had a normal forestomach, but died on day 7 due to a dosing injury. Seven of these animals also had gastric inflammation, and two (18%) had mild inflammation of the proximal duodenal mucosa. Additionally, in nine of the high dose rats (82%), perianal epidermal hyperplasia and hyperkeratosis, often with mild inflammation, was noted. Of ten medium dose (1.0 g/kg) rats (including one who died during the study), six had mild and four had marked forestomach squamous hyperplasia and hyperkeratosis. Eight of the animals had gastric inflammatory changes; in four, the changes were marked. Five (50%) medium dose rats exhibited mild perianal squamous hyperplasia; however, inflammation was noted in only one animal (10%). In low dose (0.1 g/kg) rats, mild forestomach hyperplasia and hyperkeratosis were present in eight of ten animals. Seven had accompanying mild gastric inflammation; perianal lesions were not noted. Control rats were normal.

In the high dose mice, seven of eight had marked forestomach squamous hyperplasia and hyperkeratosis, four with inflammation; one had moderate hyperplasia and hyperkeratosis with no inflammation. Eight of ten high dose mice had hyperplasia, hyperkeratosis, and inflammation in the perianal epithelium similar to that noted in high dose rats. In medium dose mice, six of eight had moderate to marked forestomach hyperplasia and hyperkeratosis, four with inflammation, and one had mild hyperplasia (no inflammation); perianal lesions were not noted in these animals. In low dose mice, five of ten had moderate to marked forestomach squamous hyperplasia and hyperkeratosis, four accompanied by inflammation, and one animal

had mild hyperplasia (no inflammation); perianal lesions were not noted. Control mice were normal.

The other significant lesion noted in this study was multifocal, minimal to mild necrosis and suppurative inflammation of the nasal turbinates, primarily at level 4, affecting the ethmoid turbinates (olfactory epithelium). In rats, these changes were present in most medium dose and high dose animals, while in mice they were only occasionally present, primarily in high dose animals. In rats but not mice, most animals with nasal turbinate lesions also had multifocal pulmonary lesions consistent with aspiration of foreign material, ranging from peribronchial histiocytic infiltrates to necrohemorrhagic bronchopneumonia.

DISCUSSION

Neurobehavioral effects of petroleum products (Knave *et al.*, 1979) and more importantly, alkanes (Kristiansen and Nielsen, 1988; Nilsen *et al.*, 1988) are well documented, so the neurotoxic potential of surrogate LCPH should be included in health hazard assessments. The only indication that chronic nonane exposure may effect overall motor system integrity was in the measure of locomotor activity of male mice. In this case, the mice with the highest exposure dose exhibited a reduction in their activity following 12 weeks of exposure. This difference was most apparent during the first half of the testing session. During the second half of the session, a similar trend was evidenced; however, activity levels in all animals were gradually decreasing and reducing the between-group differences. Overall, the evidence suggests *n*-nonane exposure over 12 weeks resulted in a decrease of gross locomotor activity but had no effect on forelimb grip strength in male mice. Comparable effects of nonane exposure were not observed in the female rats. Indeed, the data fail to indicate an effect of chronic exposure on the integrity of the motor system as there were not treatment group differences in any measures of general locomotion or in forelimb grip strength.

Food consumption was significantly lower for rats of the 1.0 and 5.0 g/kg groups for the first two weeks. This observation was not noted in the mice. No statistical differences in mean body weights between control and treated groups of both species were noted. Female rats had increased liver, lung, and adrenal weights and decreased spleen and ovary weights at 5.0 g/kg. Increased adrenal and decreased ovary weights also were observed at 1.0 g/kg. In male mice, increased liver weight was noted with increasing dose while kidney weights decreased at 1.0 and 5.0 g/kg. There were no significant differences in mean organ weights between the control and 0.1 g/kg group of both species. Mild to moderate perianal alopecia (hair loss) and erythema, hunched posture, and dark-colored urine were noted at 5.0 g/kg for both species.

Though statistically significant hematologic alterations were seen in high dose groups of both rats and mice, values were still well within normal limits for the species. Increases in neutrophil percentage and corresponding decreases in lymphocyte percentage in high dose animals are consistent with normal physiologic response to stress and minor inflammation, and correlate well with the histopathologic findings in the alimentary tract and nasal passages. Similarly, alterations in serum chemistries noted in both rats and mice, primarily in high dose groups, while statistically significant, were well within normal range and represent mild, clinically insignificant changes. While the blood and tissue levels of nonane in the rat both pre- and post-dosing show wide variations, the blood values (Table 15) are quite consistent with four hour nose-only inhalation studies conducted previously in this laboratory (Robinson, 2000) over the range of 100 to 1000 ppm nonane. In these inhalation studies (in which a physiologically-based

pharmacokinetic (PBPK) model was fitted to the data), blood levels during the exposure reached about 0.7, 5 and 18 μg/g, and fell to about 0.05, 0.3 and 1 μg/g after cessation of exposure to 100, 500 and 1000 ppm nonane, respectively. Values during inhalation exposure can roughly be compared with "post-exposure" values in the present paper, and the lower values with those measured prior to daily gavage dosing. This suggests roughly similar systemic loads at low, medium, and high exposures in the two series of experiments, and the likely applicability of the nonane PBPK model to oral as well as inhalation exposures.

In this study, orally administered nonane was clearly irritating to the nonglandular stomach in both rats and mice, at all dose levels, with no apparent dose response. A NOAEL was not seen in either species for this target tissue; however, whether this is of physiological significance in attempting to extrapolate the data to humans for risk assessment purposes is questionable, as there is no morphologically consistent structure in the human stomach. The non-glandular stomach, or forestomach, serves as a storage organ in rodents. Due to the delayed gastric emptying at this site, it is thought to act as a primary site of absorption and interaction with ingested xenobiotics. Hyperplasia and hyperkeratosis of the nonglandular stomach mucosa, often accompanied by inflammatory infiltrates, is a well-recognized entity in rodent stomachs, most often seen in gavage studies. In the glandular stomach (analogous to the human stomach), there were no lesions observed in either rats or mice.

Mild inflammation of the proximal small intestinal mucosa was present in two (20%) of high dose rats, but not in any other animals. Mild perianal squamous hyperplasia was present in 82% and 80% of high dose rats and mice, respectively, and in 50% and 20% of mid dose rats and mice, respectively. Low dose (0.1g/kg) rats and mice were free of perianal lesions, indicating a NOAEL at this dose level.

The multifocal nature of the lesions in the nasoturbinate epithelium suggests that the lesions resulted from direct contact with the gavage material, rather than from specific xenobiotic targeting of olfactory epithelium. This interpretation is supported by the pulmonary foreign body response often accompanying nasal lesions. It suggests that small amounts of the nonane gavage material was either eructed, or more likely, inadvertently deposited in the distal esophagus during gavage administration, permitting reflux into the nasopharynx, and subsequent passage into the nasal cavity and lungs. In any event, a NOAEL for nasal turbinate and lung lesions was found at the low dose level (0.1g/kg) in both rats and mice.

The Total Petroleum Hydrocarbon Criteria Working Group evaluated the toxicity of LCPH to use in their Risk Based Corrective Action (RBCA) approach to determining cleanup levels for weathered petroleum contaminated soil. The TPHCWG assigned RfD values for aliphatic and aromatic fractions as determined by their analytical method. This study was conducted because *n*-nonane is a potential surrogate for the C_9-C_{16} aliphatic fraction RfD. Should the TPHCWG reassess this fraction, this study has potential for use as one of the key studies used to assign the RfD. Using a NOAEL of 0.1 g/kg/day and a minimum uncertainty factor of 1000 (10 for animal to human extrapolation, 10 for sensitive subpopulations and 10 for subchronic to chronic duration of study), the RfD for *n*-nonane would be 0.1 mg/kg/day. This RfD is equal to the current TPHCWG RfD for the C_9-C_{16} aliphatic fraction based on an unpublished oral 90-day rat study using a dearomatized aliphatic mixture of C_9-C_{12} alkanes, with application of similar uncertainty adjustments (TPHCWG, 1997).

CONCLUSION

In conclusion, a No Adverse Effect Level was found at the low dose level (0.1 g/kg) in both rats and mice, for all lesions except the proliferative and inflammatory lesions in the non-glandular forestomach. While *n*-nonane is clearly irritating to this tissue, the lack of an analogous structure in the human stomach and the absence of lesions in the glandular stomach of the study animals suggest that the proliferative forestomach lesions represent a species-specific response of no clinical significance to humans.

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