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N-Methylneodecanamide/1993 | Neurotoxicity (§82-7) and Subchronic Toxicity (§82-1)

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DATA EVALUATION RECORD

STUDY TYPE: Neurobehavior/Subchronic Toxicity Study - Rat
OPPTS Number: 870.3800 OPP Guideline Number: §82-7/82-1

DP BARCODE: D228410 SUBMISSION CODE: S507937
P.C. CODE: 079052 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): N-Methylneodecanamide (96.04% a.i.) (Purity of the same lot of the test material was given as 95.9% a.i. in MRID's 44003202 and 43883914)

SYNONYMS: None

CITATION: Hoberman A.H. (1993) Reproductive and Neurobehavioral Effects of Sample No. 38674 Administered Orally via the Diet to Crl:CD BR VAF/Plus Rats for Two Generations. Argus Research Laboratories Inc., Horsham PA 19044. Laboratory Study number 91-02, December 17, 1993. MRID 43883913. Unpublished

SPONSOR: Colgate-Palmolive Company, Piscataway NJ

EXECUTIVE SUMMARY: In a 2-generation reproduction and neurobehavioral study (MRID 43883913), N-Methylneodecanamide (MNDA Sample No. 38674, 96.04% a.i.) was administered in the diet to 30 Crl:CD BR VAF/Plus (Sprague-Dawley®) rats/sex/dose. This DER only addresses the subchronic and neurotoxicity components of this study for the first (P_1) generation (MRID 43882913.DE1). Usually subchronic studies are terminated after 90 days, since the P_1 rats were not sacrificed until the F_1 rats were weaned, many of the subchronic data were collected at the end of the weaning period after 163-173 days of exposure. The test material was administered at dietary levels of 0, 94, 410 or 2500 ppm (equivalent to 0, 5.7, 25.1 or 152.4 mg/kg/day for males and 0, 6.7, 29.4 or 172.4 mg/kg/day for females; the dietary level was indicated as 0, 0.094, 0.41 or 2.5 mg/gm in the study report). Females were exposed to higher levels during gestation and lactation. Exposure to P_1 animals began at 51 days of age. Neurotoxicity and subchronic toxicity evaluations consisted of viability, general health, signs of pharmacotoxic or toxicologic effects, histopathology, and functional observational battery (FOB) and motor activity (MA) measurements. 12 rats/sex/dose were assessed for neurotoxicity parameters. The remaining 18 rats/sex/dose were subjected to hematological, clinical chemistry, urinalysis, and selected microscopic examinations for further subchronic toxicity evaluations.

12

Neurotoxicity was not demonstrated at dose levels of up to and including 2500 ppm. **The NOAEL for neurotoxicity is 2500 ppm (152.4 mg/kg/day for males and 172.4 mg/kg/day for females).**

At 410 ppm the liver was identified as a target organ as indicated by increased **liver to body** (10.3%, $p < 0.05$) and brain (6.5%, $p < 0.05$) weight rations in females and increased incidence of **hepatocellular hypertrophy** was increased in both males (14/30) and females (9/30). The magnitude of the liver weight increase and incidence of liver pathology increased at the 2500 ppm dietary level and **body weight** (5.5% - 8.2%) decreases were noted as were deceases in body weight gains (19.4-45.3%), and mean feed consumption values (10.5%). **Liver vacuolation** accompanied the hypertrophy especially in males. There were some apparent changes in the clinical chemistry and urinalysis values as follows: At 94 ppm females exhibited increased total serum protein levels (5%, $p \leq 0.05$). At 410 ppm females exhibited increased serum phosphorous (10%, $p \leq 0.05$) and total serum protein levels (8%, $p \leq 0.05$). Males had increased blood urea nitrogen (10%, $p \leq 0.05$) and protein in the urine. In addition, increases in blood urea nitrogen (11%, $p \leq 0.05$), and in total serum proteins (8%), and inorganic phosphorous (11%) were noted in the high dose females. These suggestions for a possible effect on the kidney could not be verified in a separate study (MRID No.: 43883910) assessed at a dose level of 200 mg/kg/day by gavage. **The systemic LOAEL is 410 ppm (25.1 mg/kg/day in males and 29.4 mg/kg/day in females) based on liver weight increases, hepatocellular hypertrophy and vacuolation. The systemic NOAEL is 94 ppm (5.7 mg/kg/day in males and 6.7 mg/kg/day in females).**

Classification: This FOB, motor activity and neuropathology aspects of this study are classified **ACCEPTABLE** and this study does satisfy the guideline requirement for neurotoxicity (OPPTS 870.3800, §82-7) study. However, this study does not satisfy the guideline requirement for a subchronic oral toxicity study (OPPTS 870.3800, §82-1) in the rat. The series 82-1 subchronic oral toxicity study requirement in the rat has been satisfied by a separate study (MRID No.: 43883910).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

124

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: N-Methylneodecanamide (Sample No. 38674)
Description: Light viscous liquid, stable at room temperature in plastic bottles protected from light for an unspecified interval
Lot #: CC #21709-17
Purity: 96.04% a.i.
CAS # and structure: Not provided.

2. Vehicle: Diet

3. Test animals: Species: rat
Strain: Crl:CD BR VAF/Plus (Sprague-Dawley®)
Age at start of dosing: Approximately 7 weeks
Weight at start of dosing: Males: 145-201 g, Females: 119-158 g
Source: Charles River Laboratories, Inc., Portage MI
Housing: Individually (except during mating) in wire bottom stainless steel cages suspended above absorbent paper liners except during the cohabitation and postpartum periods. During mating (2 rats/cage) in the male rat's wire bottom cage. Beginning no later than day 20 of presumed gestation, females were individually housed in nesting boxes and following delivery, each dam and litter were housed in a common nesting box during the 21-day postpartum period. Nesting boxes contained bed-o'-cobs® bedding.
Diet: PMI® Feeds Certified Rodent Diet (#5002), ad libitum
Water: Tap water processed through a reverse osmosis membrane, ad libitum
Environmental conditions:
Temperature: 74°F
Humidity: 40-70 %
Air changes: 10/hr (minimum), HEPA filtered
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period: 13 days

B. PROCEDURES AND STUDY DESIGN

1. Study schedule: Starting at approximately 51 days of age, P_1 parental animals were given test diets for 92 days before they were mated to produce the F_1 generation and until sacrifice (163-173 days on test diets). Neurotoxicity and subchronic toxicity evaluations (viability, general health, signs of pharmacotoxic or toxicologic effects, histopathology, functional observational battery (FOB) and Motor Activity (MA) measurements) were performed on 12 randomly selected P_1 generation rats/sex/dose. The remaining 18 P_1 generation rats/sex/dose were also subjected to hematological, clinical chemistry, urinalysis, and histopathological evaluations.

3. Animal assignment: Animals were randomly assigned to test groups as indicated in Table 1.

125

Table 1. Animal assignment for neurotoxicity and chronic toxicity evaluations[a]

Test Group	Dose in Diet[b] (mg/g)/ppm	Animals/Group	
		P1 Males	P1 Females
Control	0	30	30
Low (LDT)	0.094/94	30	30
Mid (MDT)	0.41/410	30	30
High(HDT)	2.5/2500	30	30

a 12 rats/sex/dose were evaluated for neurotoxicity and 18 rats/sex/dose were evaluated for subchronic toxicity.
b Diets were administered from the beginning of the study until sacrifice.

4. Dose selection rationale: The dietary concentrations tested were selected by the sponsor based on data obtained in a range-finding study (Argus Research Laboratories Study # 403-006P). In the range-finding study, dietary concentrations of 0, 0.5, 1.0, 2.5 and 5.0 mg/g were administered to male and female rats for 14 days before initiation of a 7-day cohabitation period. Males were administered the test diets for 47-days. Female rats were permitted to naturally deliver and rear their litters until day 4 postpartum. Results of this study included slight increases in chromorrhinorrhea in both sexes in the 200 and 400 mg/kg/day dose groups, depressed body weights (males in the 50-200 mg/kg/day treatment groups for the 47-day dosage period, females in the 200-400 mg/kg/day treatment groups during the 15-day premating period and females in the 400 mg/kg/day treatment group during gestation and lactation), reduced feed consumption (males in the 200-400 mg/kg/day treatment groups for the 47-day dosage period, females in the 50-400 mg/kg/day treatment groups during the 15-day premating period and females in the 100-400 mg/kg/day treatment groups during gestation and lactation). Pup body weights were reduced in the 200 and 400 mg/kg/day treated groups on days 1 and 4 postpartum. There were no effects on mating or fertility seen in this study. It was concluded that the appropriate dose levels for a developmental toxicity study were as high as 5.0 mg/g (400 mg/kg/day) and 1.0-2.5 mg/g (100-200 mg/kg/day) and less than 0.5 mg/g (50 mg/kg/day) as low- and mid-dose levels.

5. Dosage preparation and analysis: Test diets were prepared every 6 weeks or as needed by mixing appropriate amounts of the test substance with Purina Certified Rodent Diet (#5002) and samples of each batch of mixed feed were taken for test article analysis. Homogeneity (top, middle and bottom) was evaluated prior to the

126

start of the study for the test diets ranging in concentration from 0.094 to 2.5 mg/g. Prior to the start of the study, the stability of the test article in the diet was also evaluated for a period of up to 8 weeks at 4 C and up to 1 week (3 weeks for the 0.041 mg/g concentration) at ambient temperature.

Results - Homogeneity Analysis: The mean analytical values for the homogeneity determinations (20 determinations) ranged from 94-109% of the target levels.

Stability Analysis: At 4 C for 8 weeks, the 0.094, 0.41 and 2.5 mg/g diets were 110, 98 and 100% of nominal, respectively. At ambient temperature (23 C) for 1 week, the 0.094, 0.41 and 2.5 mg/g diets were 93, 91 and 94% of nominal.

Concentration analysis: The measured concentrations of the test article in the 0.094, 0.41 and 2.5 mg/g dose groups ranged from 0.089-0.113, 0.388-0.450 and 2.38-2.59 mg/g, respectively (95-120% of nominal).

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Parental animals: All animals were observed twice daily for viability and clinical signs of toxicity once daily during the periods test diets were provided and weekly during the acclimation and predosage periods. Body weights and feed consumption values were recorded weekly throughout the study and on the day of scheduled sacrifice. In addition, body weights of all rats selected for the neurotoxicity evaluations were recorded on the days those evaluations were made. Twelve P_1 rats/sex/dose were assigned to neurobehavioral evaluations and the remaining 18 P_1 rats/sex/dose were assigned to subchronic toxicity evaluations.

2. Neurotoxicity Observations: Neurotoxicity evaluations consisted of a FOB to assess autonomic function [lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, urination and defecation], reactivity and sensitivity [sensorimotor responses to visual, auditory, tactile and painful stimuli], excitability [reactions to handling and behavior in the open field], forelimb and hindlimb grip strength, gait, and sensimotor coordination [degree of motility, gait pattern in the open field, air righting reaction, visual placing response, and landing foot splay]. In addition, MA determinations were performed by observing individual rats in a stainless steel cage equipped with an automated apparatus (Coulbourn Instruments, Inc.) that recorded the number of movements and the time spent in movement. Each test session was 1.5 hours in duration with the number of and time spent in movements were tabulated at five minute intervals. Both the FOB and MA tests

12

were conducted before dosage began and during the fourth, eighth and thirteenth weeks of exposure.

3. Subchronic Toxicity Testing: The 18 rats/sex/treatment group not subjected to neurotoxicity testing were subjected to hematology, clinical chemistry and urine chemistry determinations and limited histopathology.

a. Blood

Blood was collected at sacrifice in appropriately labelled EDTA Vacutainer tubes for hematology parameters. Two blood smear slides were taken from each rat for leukocyte and reticulocyte counts. Tubes and slides were shipped on cold packs to Ani Lytics, Inc. for analysis of the hematology parameters. An additional blood sample was collected from each rat, allowed to clot for approximately 30 minutes at room temperature, centrifuged and the serum shipped (frozen) to Ani Lytics, Inc. for clinical chemistry parameter determinations. The CHECKED (X) hematology and clinical blood chemistry parameters were examined.

b. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		

* Required for subchronic toxicity studies.

c. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Cholesterol
X	Sodium*	X	HDL
		X	LDL
	ENZYMES	X	Globulins
		X	Glucose*
X	Alkaline phosphatase (ALK)	X	Total bilirubin
X	Creatine phosphokinase	X	Total serum protein (TP)*
X	Lactic acid dehydrogenase (LDH)		Triglycerides
X	Serum alanine aminotransferase (ALT, SGPT)*		
X	Serum aspartate aminotransferase (AST, SGOT)*		
X	Gamma glutamyl transferase (GGT)		

* Required for subchronic toxicity studies.

d. Urine

Urine was collected from all 18 rats/sex/treatment level over a 23-24 hour period beginning 24 hours prior to the scheduled sacrifice. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH	X	Occult blood
X	color	X	Nitrite
X	Protein	X	Ca
X	Specific Gravity	X	K
X	Sediment	X	Cl
X	Leukocytes	X	Phosphate
X	Urobilinogen		

* Urinalysis is not required for subchronic toxicity studies.

4. Sacrifice and Pathology

P_1 animals were sacrificed at approximately 1-3 weeks after the litter was weaned.

129

The 12 rats/sex/dose that were selected for neurobehavioral tests were anesthetized with a combination of sodium pentobarbital and sodium heparin and an initial examination was made of all external surfaces and orifices. The abdominal and pelvic cavities were opened and associated organs and tissues were examined. The thoracic cavity was perfused with a formaldehyde solution. Six of the 12 rats/sex in the control and high dose groups were selected for neuropathologic evaluations. The head, vertebral column and portions of the hindlimbs of these rats were preserved in formaldehyde for 24 hours and the Gasserian ganglia and portions of the cervical, thoracic and lumbar regions of the spinal cord, with dorsal root ganglia and associated nerve root fibers, were dissected. Samples of the sciatic, sural, tibial and fibular nerves in the hindlimbs were also dissected. These tissues were embedded, stained (hematoxylin and eosin, Bielschowsky's and either luxol fast blue/cresyl violet or toluidine blue) and examined microscopically.

The remaining 18 rats/sex/dose were sacrificed by complete blood collection following carbon dioxide anesthesia and a necropsy of the thoracic and abdominal cavities was performed. The tissues (X) listed below were prepared for microscopic examination and weighed (XX).

Histopathological evaluations were performed for all gross lesions and the liver and thymus of each rat (perfused and nonperfused) in each group. All reproductive organs, except the testes, were evaluated for the control and high dose group rats. All livers, thymuses and testes were examined (all dose groups).

	Neurotoxicity		Subchronic Toxicity
X	Gasserian ganglia	XX	Liver+
X	Spinal cord (cervical, thoracic and lumbar regions) with dorsal root ganglia and nerve root fibers	XX	Pituitary Gland
		XX	Thymus+
	Sciatic nerve	XX	Epididymis (both)
	Sural Nerve	XX	Prostate
	Tibular nerves	XX	Seminal Vesicles (with fluid)
X	Fibular nerves		Testes (both)
X		XX	Ovaries (both)
		XX	Uterus
		XX	Brain
		X	Coagulating gland
		X	Cervix
		X	Mammary Gland
		X	Vagina
		X	All gross lesions and masses*

* Target Organs

Note: From the rats in the subchronic study, representative samples of the adrenal glands, aorta, bone marrow, bone, brain, esophagus, eyes (with optic nerve) ,femur, heart, kidneys, lacrimal gland, large intestine, lungs, lymph nodes (mesenteric, mediastinal), male mammary gland, muscle, pancreas, parotid salivary gland, skin, small intestine, spleen, stomach, thyroid, trachea, urinary bladder, colon, cecum, rectum and anus were retained in neutral buffered 10% formalin for future histopathologic evaluation, if needed.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine statistical procedures.

2. Historical control data: Minimal historical control data included in this report were identified as "Reference Ranges" for hematological parameters (pages 1206-1219, 1255-1282), clinical chemistry parameters (pages 1221-1247) and urinalysis parameters (pages 1248-1254). These data did not include the numbers of studies nor the dates from which these data were extracted. For this reason, a more complete set of historical control data for CD rats 108-110 weeks of age from the CRC Handbook of Toxicology (1995 ed., page 523) were used for clinical chemistry parameter comparisons. Adequate historical positive control data for neurotoxicity tests are found in Volume 6, pages 1907-2117 of the study report.

A. **RESULTS**

1. Mortality and Clinical Signs of Toxicity: No treatment-related mortality or clinical findings were noted at any dose level. The causes of death for the three males (one each in the 0, 0.41 and 2.5 mg/g treatment groups) found dead and one control group female sacrificed moribund were all related to trauma. No other P_1 rats died or were found dead or sacrificed moribund during the study. Clinical observations included chromodacryorrhea, chromorhinorrhea, missing, broken and misaligned incisors, alopecia, red substance on penis, swollen, elongated and broken snout, emaciation, dehydration, gasping, red perioral substance, tip-toe walk, ungroomed coat, swollen ear, no feces in cage pan, red substance in cage pan, exophthalmos, moribund activity, decreased motor activity, impaired righting reflex, hindlimb paralysis, rales, urine-stained abdominal fur, dehydration, paleness, gasping, swollen limb and a lesion in one rat. These clinical signs were not considered to be treatment related because they were found in only a few rats and their incidences were not statistically significant.

2. Body Weight and Feed consumption: Body weight and selected feed consumption data are summarized in Tables 2. Females in the 2.5 mg/g dose group exhibited treatment-related decreases in mean body weights from study days 8-termination which were statistically significant from day 43 (5.5%, $p \leq 0.05$) to day 92 (8.2%, $p \leq 0.01$).

Body weight gains in the 2.5 mg/g dose group females throughout the premating period were decreased from day 1 and statistically significant on days 1-8 (19.8%, $p \leq 0.01$), days 50-57 (45.3%, $p \leq 0.05$) and overall from days 1-92 (19.4%, $p \leq 0.01$). These decreases were considered to be treatment-related.

131

Mean feed consumption values (g/rat/day) in the 2.5 mg/g dose group females were statistically significantly decreased (9.2%, $p \leq 0.01$) for days 1-92 of premating period. For the high dose females, treatment-related reductions (7.4-10.6%, $p \leq 0.05$ or 0.01) in feed consumption were also noted at each interval recorded throughout the pre-mating period. No treatment-related effects on body weight, weight gain or feed consumption were noted for the P_1 males in this study.

Table 2. Premating body weight, weight gain and feed consumption data[a]

Observations/Study Week	Dietary Level (mg/g)			
	Cont. (0)	LDT (0.094)	MDT (0.41)	HDT (2.5)
Males				
Mean Body Weight (g/rat±SD) Day 92	504.6±38.7	498.8±62.2	499.2±50.9	496.8±42.0
Mean Body Weight (g/rat±SD) Day of Termination	565.5±51.4	555.8±71.6	548.7±62.5	556.9±50.9
Mean Weight Gain (g/rat±SD) Days 1-92	285.5±35.8	277.7±47.5	279.8±39.0	280.3±33.9
Mean Weight Gain (g/rat±SD) Days 1-termination	346.4±51.8	334.7±57.3	330.8±54.4	340.5±44.8
Mean Feed Consumption (g/rat/day±SD) Days 1-92	24.9±1.7	24.3±2.7	24.5±1.9	24.3±1.5
Mean Compound consumed per day[b]	-	5.4	25.1	152.4
Females				
Mean Body Weight (g/rat±SD) Day 92	283.6±26.9	286.5±30.6	281.2±27.9	260.4±22.5**
Mean Body Weight (g/rat±SD) Day of Termination	300.4±32.3	294.7±28.8	290.0±34.2	281.2±25.8
Mean Weight Gain (g/rat±SD) Days 1-92	120.4±19.7	123.6±25.0	117.9±19.6	97.0±15.2**
Mean Feed Consumption (g/rat/day±SD) Days 1-92	17.3±1.2	17.4±1.6	17.2±1.5	15.7±1.4**
Mean Compound consumed per day[b]	-	6.8	29.4	172.4

a Data extracted from study report Tables 8M, 9M, 10M, 8F, 9F, and 14F, pages 729-734, 908-911 and 916-917

b Taken from page 54 for males and page 58 for females.

* Statistically significant $p \leq 0.05$

** Statistically significant $p \leq 0.01$

132

3.Neurotoxicity Evaluations: N-methylneodecanamide administered continuously up to and including dietary concentrations of 2.5 mg/g for up to 13 weeks had a few statistically significant effects (decreased hindlimb grip strength [$p \leq 0.05$] in both sexes in the 0.41 mg/g treatment group, twitches and tremors in the limbs and/or whole body tremors or spasms in a few rats in all treatment groups during week 13 of the open field) which were not considered to be biologically significant because of the similarity of incidences in all treatment groups or the fact that they were not observed during the daily clinical observations. Adequate historical positive control data for neurotoxicity tests are found in Volume 6, pages 1907-2117 of the study report.

4.Hematological Evaluations: No biologically significant effects on hematological parameters in male or female rats were seen at any dose level in this study.

5.Clinical Chemistry Evaluations: Statistically significant clinical chemistry findings are summarized in Table 3. These data indicate that N-methylneodecanamide administered continuously for 13 weeks caused slight, but statistically significant elevations (10%-11%, $p \leq 0.05$) of serum blood urea nitrogen (BUN) in males in the 0.41 and 2.5 mg/g treatment groups and substantial statistically significant elevations ($p \leq 0.01$) in serum cholesterol (50%) and HDLs (high density lipoproteins) (59%) in males in the 2.5 mg/g treatment group. Females in all treatment groups exhibited slight (5%-8%), but statistically significant increases in total serum proteins ($p \leq 0.05$ at the 0.094 and 0.41 mg/g treatment levels and 0.01 at the 2.5 mg/g treatment level). Females in the 0.41 and 2.5 mg/g treatment groups exhibited statistically significant elevations in serum calcium (4%, $p \leq 0.01$), inorganic phosphorous (10-11%, $p \leq 0.01$) and globulins (9%, $p \leq 0.05$). Females in the 2.5 mg/g treatment group also exhibited a moderate (24%, $p \leq 0.01$) statistically significant elevation in serum cholesterol. All these values, except for phosphorous and total serum protein were within historical control values cited in the CRC Handbook of Toxicology (see footnote in Table 3), and were thus not considered to be of toxicological significance. The statistically significant elevations in BUN in the mid and high dose males, serum phosphorous in the high dose females and in total serum proteins at all doses in the females are considered to be both treatment-related and possibly, toxicologically significant in the absence of histopathological evaluations of the kidney tissues (see discussion).

137

Table 3. Statistically significant clinical chemistry findings[a]

Parameter	Dietary Level (mg/g)				
	Controls (0)	LDT (0.094)	MDT (0.41)	HDT (2.5)	Hist. Controls
Males					
Calcium	11.66±0.57	11.73±0.37	11.72±0.54	11.96±0.81	9.8-12.2
Phosphorus	7.04±0.76	7.34±0.89	7.41±0.73	7.23±0.72	4.0-7.0
BUN	13.6±1.6	13.9±1.5	15.1±1.5*	15.0±1.4*	12-30
Globulin	2.62±0.21	2.57±0.32	2.62±0.26	2.84±0.33	2.0-4.5
TP	7.41±0.24	7.29±0.46	7.28±0.35	7.62±0.48	5.7-6.5
Cholesterol	69.1±18.9	79.4±25.3	74.4±23.7	103.6±23.3**	130-180
HDL	57.6±18.2	67.2±24.0	62.7±20.6	91.7±22.1**	-
Females					
Calcium	11.58±0.45	11.82±0.41	12.03±0.56*	12.06±0.36**	9.8-12.0
Phosphorus	7.66±0.76	8.28±0.82	8.41±0.93*	8.51±0.73**	4.0-7.0
BUN	16.2±2.2	16.0±2.5	16.1±3.2	17.6±2.7	12-30
Globulins	2.58±0.31	2.68±0.21	2.80±0.30*	2.82±0.26*	2.0-4.5
TP	7.49±0.51	7.90±0.45*	7.98±0.60*	8.07±0.38**	6.3-7.1
Cholesterol	88.9±11.9	95.8±26.1	97.4±24.0	110.2±17.4**	90-150
HDL	73.8±10.2	83.1±22.2	84.4±22.8	96.6±16.4	-

a Data (means±SD) extracted from study report Tables 14M and 22F, pages 750-754 and 937-941. Units are g/dl for total protein and globulins and mg/dl for calcium, phosphorous, BUN, cholesterol and HDL values. Data for historical controls from page 523 of the CRC Handbook of Toxicology 1995 ed., M.J. Derelanko and M.A. Hollinger

* Statistically significant $p \leq 0.05$

** Statistically significant $p \leq 0.01$

6.Urinalysis: Treatment-related substantial statistically significant ($p \leq 0.01$) increases were observed in the incidences of urine protein concentrations of 100 mg/dl in the 0.41 and 2.5 mg/g treatment group males (controls 4/17, 0.094 mg/g group 4/18, 0.41 mg/g group 9/17 [$p \leq 0.05$] and 2.5 mg/g group 12/17,[$p \leq 0.01$]). Females in the 2.5 mg/g treatment group exhibited a 4-fold statistically significant increase in the incidence of occasional phosphate crystals. However, the total number of females having any (few, occasional, moderate or many) phosphate crystals in the urine was comparable among groups.

7. Postmortem observations:

a. Organ weights: Absolute liver weights and relative (to both body and brain weights) liver weights were statistically significantly increased in both sexes in the 2.5 mg/g treatment group (males 13.6% [$p \leq 0.05$], 16.0% and 12.9% [$p \leq 0.01$]; females 18.8%, 26.4% [$p \leq 0.01$] and 16.7% [$p \leq 0.05$] for absolute, relative to body and relative to brain liver weights, respectively). Females in the 0.41 mg/kg treatment group also had statistically significantly ($p \leq 0.05$) increased (9.2%) relative to body weight liver weights. These changes in liver weights were considered toxic effects because of their magnitudes and that they were confirmed by histopathologic changes in the liver tissue. (See Pathology, below). Liver weight data are summarized in Table 4. Weights of other organs obtained from treated rats in this study were comparable to those of the control rats.

Table 4. Liver weight data[a]

Observation	Dietary Level (mg/g)			
	Control (0)	LDT (0.094)	MDT (0.41)	HDT (2.5)
Males				
Absolute Liver Weight g/liver±SD	15.95±2.00	15.98±2.96	16.39±2.42	18.12±2.01*
Liver Weight/Body Weight, mean±SD	2.88±0.23	2.92±0.25	3.05±0.39	3.34±0.29**
Liver Weight/Brain Weight, mean±SD	698.3±89.2	702.8±98.9	715.7±106.6	788.1±91.4*
Females				
Absolute Liver Weight mean±SD	8.67±0.92	9.03±1.11	9.38±0.92	10.30±2.54**
Liver Weight/Body Weight, mean±SD	3.03±0.29	3.15±0.24	3.31±0.24*	3.83±0.74**
Liver Weight/Brain Weight, mean±SD	419.1±44.5	432.9±58.2	446.2±44.4	489.0±113.4*

a Data extracted from study report Tables 5M-7M and 5F-7F, pages 723-193 and 905-907; weights of perfused organs were excluded.
* Statistically significant $p \leq 0.05$
** Statistically significant $p \leq 0.01$

b. Pathology

1) Macroscopic examination: There were no biologically or statistically significant necropsy findings for the rats in this study.

135

2) Microscopic examination: Treatment-related, minimal, usually reversible histopathologic lesions of the liver (hepatocellular hypertrophy and hepatocellular vacuolization) were seen in both sexes in the 0.41 and 2.5 mg/g dose groups. These findings are summarized in Tables 5. No histopathologic findings were seen in the other tissues examined, including thymuses, reproductive, central and peripheral nervous system tissues, or in the livers of the 0.094 mg/kg treatment group rats that were considered to be treatment related or not commonly seen in laboratory rats of this age and strain. Although 11-12 P_1 generation livers examined were from rats in which the livers were perfused for neurotoxicity evaluations, the reviewer has included these data in Table 10 for completeness and accuracy of the histopathologic database.

Table 10. Microscopic liver changes[a]

Observation	Dietary Level (mg/g)			
	(0)	(0.094)	(0.41)	(2.5)
Males				
Hepatocellular Hypertrophy				
Perfused	0/12[b] 1/17	2/12	6/11	12/12
Non-Perfused		0/18	8/19	6/17
Hepatocellular Vacuolization				
Perfused	0/12	0/12	0/11	7/12
Non-Perfused	0/17	0/18	2/19	15/17
Females				
Hepatocellular Hypertrophy				
Perfused	0/12	0/12	3/12	12/12
Non-Perfused	0/18	0/18	6/18	17/18
Hepatocellular Vacuolization				
Perfused	0/12	0/12	0/12	0/12
Non-Perfused	0/18	0/18	0/18	1/18

a Data extracted from study report page 2321
b Data expressed as number of findings/number of livers examined and apparently, not subjected to statistical analyses.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that the LOAEL is 0.41 mg/g based on reversible histopathologic changes (hepatocellular hypertrophy and vacuolization of the type and intensity seen in these rats is usually reversible upon discontinuation of treatment) in the liver of both sexes and an increase in urine protein in males. The NOAEL for neurotoxicity is 2.5 mg/g (the highest level tested).

136

B. REVIEWER'S DISCUSSION:

Important Special Note: The following discussion was prepared by the contractor. TB-II does not concur with these opinions and has provided a justification as below. The following is being retained in this review for possible future reference.

N-methylneodecanamide, Sample No. 38674 (96.04% pure) was administered to 30 Crl:CD BR VAF/Plus (Sprague-Dawley®) rats/sex/dose in the diet at dose levels of 0, 0.094, 0.41 and 2.5 mg/g.

Exposure to P_1 animals began at 51 days of age and lasted for 92 days before they were mated (1:1 ratio) to produce the F_1 generation. Access to the test diets for the P_1 animals continued until they were sacrificed 1-3 weeks after weaning the F_1 rats (163-173 days of exposure). Neurotoxicity and subchronic toxicity studies were conducted as part of the evaluation of the P_1 generation rats. These evaluations consisted of viability, general health, signs of pharmacotoxic or toxicologic effects, histopathology, FOB and MA measurements of 12 randomly selected P_1 generation rats/sex/dose. The remaining 18 P_1 generation rats/sex/dose were also subjected to hematological, clinical chemistry, urinalysis, and histopathological evaluations.

The average achieved test substance intakes were 5.7, 25.1 and 152.4 mg/kg/day for the males and 4.2, 18.4, and 112.3 mg/kg/day for the females. [Note added by TB-II: This depends on the method of calculation and the time intervals for feeding, TB-II calculations differ from this set of doses.

Treatment-related systemic toxicity was characterized in the 2.5 mg/g dietary treatment group females as statistically significant ($p \leq 0.01$ or 0.05) reductions in body weights (5.5-8.2% days 43-92), body weight gains (19.4-45.3%) and mean feed consumption values (10.5%) at various weeks throughout the 92-day premating period. In addition, increases in blood urea nitrogen (11%, $p \leq 0.05$),in males, and in total serum proteins (8%), inorganic phosphorous (11%) were seen in the high dose females. Males in this group had a increased treatment-related incidence of urine protein levels. Both sexes in this treatment group had moderate increases in absolute (males 13.6%,$p \leq 0.05$) and females 18.8%, $p \leq 0.01$]), relative liver to body weights (males 16.0%,$p \leq 0.01$ and females 26.4%,$p \leq 0.01$) and relative liver to brain weights (males 12.9%, $p \leq 0.05$ and females 16.7%, $p \leq 0.05$) and reversible histopathologic changes (hepatocellular hypertrophy and vacuolization) in the liver.

Females in the 0.41 mg/g dietary treatment group exhibited increased serum phosphorous (10%, $p \leq 0.05$), total serum protein levels (8%, $p \leq 0.05$) and increased relative liver/body weight(9.2%, $p \leq 0.05$). Males had increased blood urea nitrogen (10%, $p \leq 0.05$) levels, protein in the urine and both sexes exhibited hepatocellular hypertrophy and vacuolization in the liver.

Females in the 0.094 mg/g dietary treatment group exhibited increased total serum protein levels (5%, $p \leq 0.05$).

137

The primary issue of concern for these reviewers is if and to what extent, N-methylneodecanamide administered to females at a dietary level of 0.094 mg/g may have caused renal injury in these rats. Data obtained in the 0.41 mg/g and 2.5 mg/g treatment group rats (elevated blood urea nitrogen [males only], serum phosphorous, and total protein levels and protein in the urine [males only]) suggest nephrotoxicity. The kidneys were not weighed at necropsy and, according to information on page 1297 of the study report, most of the kidneys were not subjected to histopathologic examinations. However, samples of kidney tissue were retained for such evaluations, should they be required. Although the levels of total serum proteins in the 0.094 mg/g treatment group rats were only slightly elevated (5%), they were statistically significant, were not due to the presence of outliers in any of the treatment groups (pages 1254-1259 of the study report) and outside the range of the historical control data found on page 523 of the 1995 edition of the CRC Handbook of Toxicology. In light of these findings, it would seem appropriate to subject all the kidney tissues from this study to microscopic evaluation. Data from this exercise may be useful in ascertaining whether or not the slight increases in serum total protein seen in this study were indicative of nephrotoxicity.

The subchronic LOAEL for females is 0.094 mg/g (4.2 mg/kg/day), based on slight but statistically significant increases in total serum proteins. A subchronic NOAEL for females is not established.

The subchronic LOAEL for males is 0.41 mg/g (25.1 mg/kg/day), based on elevated blood urea nitrogen levels, increased protein in the urine and reversible histopathologic changes in the liver. The systemic NOAEL for males is 0.094 mg/g.

Neurotoxicity was not demonstrated at dietary treatment levels of up to 2.5 mg/g in this study. **The NOAEL for neurotoxicity is 2.5 mg/g (152.4 mg/kg/day for males and 112.3 mg/kg/day for females).**

C. STUDY DEFICIENCIES No blood clotting determinations were made and most of the tissues required to be evaluated microscopically for subchronic studies were not evaluated; however, samples were retained. Except for the microscopic evaluation of the kidney tissue, these deficiencies are not expected to affect the outcome of the study. Without renal histopathology, the submitted study is unacceptable. However, if the renal histopathology data indicate no lesions in the 0.094 mg/g group rats, the study may be upgraded.

C. **Toxicology Branch II Discussion.** TB-II ***does not concur*** with the above discussion as prepared by the contractor. The suggestion of possible kidney effects as indicated by the blood chemistry and urinary parameters were not also noted in second subchronic dosing study (MRID No.: 43883910). This study in which the same strain of rat was dosed at 0, 3, 30, 100 or 200 mg/kg by gavage for 90 days included histopathological assessment of the kidneys and there were no remarkable treatment related findings. The parameters mentioned by the contractor above for clinical chemistry and urinalysis are not considered by TB-II to be sufficient to warrant a conclusion that the kidney is a target organ for MNDA toxicity. There were no similar changes in these parameters in the second subchronic study (MRID No.: 43883910). TB-II, however,

recommends that the kidney be reconsidered as a possible target organ for toxicity in the review of the chronic feeding studies with MNDA.

Since the liver was established as the target organ for toxicity of MNDA TB-II has determined that the NOAEL and LOAEL statement for the systemic toxicity in this study should be as follows. **The systemic LOAEL is 0.41 mg/kg (410 ppm or 25.1 mg/kg/day in males and 29.4 mg/kg/day in females) based on liver effects. The systemic NOAEL is 0.094 mg/gm (94 ppm or 5.7 mg/kg/day in males and 6.7 mg/kg/day in females).**

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139