I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: June 1, 2009

SUBJECT: Flubendiamide Review of Toxicology Studies

PC Code: 027602
Decision No.: 366884
Petition No.: NA
Risk Assessment Type: New Registration
TXR No.: 0054319
MRID No.: see table below

DP Barcode: D331553
Registration No.: 6F7065
Regulatory Action: Tolerance Petition
Case No.: NA
CAS No.: 272451-65-7
40 CFR: NA

Ver.Apr.08

FROM: **Mary Manibusan, Toxicologist**
Toxicology and Epidemiology Branch
Health Effects Division (7509P)

THROUGH: **Brenda May, Acting Branch Chief** Marion Copley For BM 6/16/09
Science Information Management Branch
Health Effects Division (7509P)

TO: **Richard Gebken, Risk Manager 10**
Insecticide Branch
Registration Division (7505P)

I. CONCLUSIONS

The toxicology studies submitted to support the tolerance petition for the new chemical, flubendiamide, have been reviewed for completeness and general acceptability. These studies have been included in the hazard characterization and hazard identification for risk assessment.

II. ACTION REQUESTED

Review the submitted studies for the requested registration/risk assessment.

Revd in 7/7

EPA Reviewer: Jessica Kidwell
SIMB, Health Effects Division (7509P)
Work Assignment Manager: Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P)

Signature: Jessica Kidwell
Date: 6/26/07
Signature: MOttley
Date: 6/21/09
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity, dietary study in rats; OPPTS 870.4200a [§83-2a]; OECD 451.

PC CODE: 027602 **DP BARCODE:** D331553
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (96.7-97.8% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide; Flubendiamide

CITATION: Enomoto, A. (2004) NNI-0001: Carcinogenicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET 01-0080, April 30, 2004. MRID 46817219. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1- Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY - In this carcinogenicity study (MRID 46817219), NNI-0001 (Flubendiamide; 96.7-97.8% a.i.; Lot Nos.: 1FH0018P and 1FH0019M) was administered in the diet to Fischer (F344/DuCrj) rats (50/sex/dose) at nominal concentrations of 0, 50, 1000, or 20,000 ppm (equivalent to 0/0, 1.70/2.15, 33.9/43.7, and 705/912 mg/kg/day in males/females) for up to 104 weeks.

At 1000 and 20,000 ppm, hepatotoxicity was observed as follows: (i) increased ($p<=0.05$) absolute and/or relative to body liver weight in both sexes (incr 17-45%); (ii) increased ($p<=0.05$) incidences of darkly-colored liver (56-72% treated vs 4% controls), liver spot(s) (26-32% vs 6%), liver enlargement (14-62% vs 0%), and hair loss (44-56% vs 22%) were observed grossly in females; (iii) periportal hepatocyte fatty change in males (42-54% vs 14%; slight to severe severity) and females (34-36% vs 2%; slight to moderate); (iv) slight to severe diffuse hepatocyte fatty change in males (12% vs 2%; at 20,000 ppm only and not statistically significant [NS]) and females (20-32% vs 4%); and (v) slight diffuse hepatocyte hypertrophy in females (44-62% vs 0%). Increases ($p<=0.05$; except when noted) in the incidences (% treated vs % controls) of the following macroscopic lesions were noted in the 20,000 ppm males: (i) liver spot(s) (22% vs 6%); (ii) accentuated lobular pattern in the liver (48% vs 0%); (iii) coarse liver surface (40% vs 16%); (iv) liver enlargement (12% vs 2%; NS); and (v) liver mass(es) (14% vs 0%).

232

III. MRID Summary Table

Guideline #; Study Type	MRID	Comments
870.3100; 90-Day oral toxicity (rat)	46817210	New DER
870.3150; 90-Day oral toxicity (mouse)	46817211	New DER
870.3150; 90-Day oral toxicity (dog)	46817212, 46817242	New DER
870.3200; 28/29-Day dermal toxicity (rat)	46817213	New DER
870.3700a; Prenatal developmental in (rat)	46817215, 46817241	New DER
870.3700b; Prenatal developmental in (rabbit)	46817214, 46817240	New DER
870.3800, Two-generation Reproduction and fertility effects (rat)	46817216	New DER
Non-guideline; One-generation reproduction study in rat	46817239	New DER
Non-guideline; Histopathology of the Eyes of Weanlings in a One-generation Reproduction Study in Rats	46817238	New DER
Non-guideline; Perinatal Ocular Toxicity Study in CD-1 Mice Following exposure via diet	46817236	New DER
870.4100a, Chronic toxicity (rat)	46817217	New DER
870.4100b, Chronic toxicity (dog)	46817218	New DER
870.4200a, Carcinogenicity (rat)	46817219	New DER
870.4200b, Carcinogenicity (mouse)	46817220	New DER
870.5100, Gene mutation (in vitro bacteria)	46817221	New DER
870.5100, Gene mutation (in vitro bacteria)	46817222	New DER
870.5300, Gene Mutation (in vitro mammalian V79)	46817224	New DER
870.5375, Mammalian Cytogenetics (in vitro CHL)	46817223	New DER
870.5395, Mammalian Cytogenetics (micronucleus mouse)	46817225	New DER
870.5395, Mammalian Cytogenetics (micronucleus mouse)	46817226	New DER
870.6200, Acute neurotoxicity screening battery	46817227	New DER
870.6300, Developmental neurotoxicity	46817228	New DER
870.7485, Metabolism and pharmacokinetics (species)	46817229, 46817230, 46817131	New DER
870.7600, Dermal penetration (monkey)	46817234	New DER
870.7800, 4-week Immunotoxicity (plaque-forming assay in rat)	46817243	New DER
Non-guideline; Effects on Thyroid Hormones and Liver Enzymes in Female Rats	46817235	New DER
Non-guideline; In Vitro Metabolism in Rat	46817232	New DER
Non-guideline; Toxicokinetic Study in Rats and Mice	46817233	New DER

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.3100 [§82-1a], Subchronic Oral Toxicity Study in Rats

Work Assignment No. 4-1-124 A, formerly 3-1-124 A (MRID 46817210)

Prepared for
Health Effects Division
Office of Pesticide Programs
U S Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Rd, Bldg. 100, Ste. B
Durham, NC 27713

Primary Reviewer:
Ronnie J. Bever Jr., Ph.D.
Signature: Ronnie J. Bever Jr.
Date: 1/19/07

Secondary Reviewer:
Stephanie E. Foster, M.S.
Signature: Stephanie E. Foster
Date: 1/19/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 1/19/07

Quality Assurance:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 1/19/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica Kidwell Signature: Jessica Kidwell
SIMB, Health Effects Division (7509P) Date: 6/26/07
Work Assignment Manager: Myron Ottley Signature: 6/21/09
Registration Action Branch 3, Health Effects Division (7509P) Date: MOttley

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding]-[rat]; OPPTS 870.3100 [' 82-1a] (rodent); OECD 408.

PC CODE: 027602 **DP BARCODE:** D 331553
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 97.7% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Enomoto, A. (2003) NNI-0001: Repeated dose 90-day oral toxicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET 01-0013, February 18, 2003. MRID 46817210. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY - In a subchronic oral toxicity study (MRID 46817210), NNI-0001 (Flubendiamide; 97.7% a.i.; Lot No. 0FH0009P) was administered in the diet to SPF Fischer (F344/DuCrj) rats (10/sex/dose) at doses of 0, 20, 50, 200, 2000, or 20,000 ppm (equivalent to 0/0, 1.15/1.30, 2.85/3.29, 11.4/13.1, 116/128, and 1192/1320 mg/kg bw/day in males/females) for 13 weeks. Additionally, a group of controls and 20,000 ppm rats (10/sex/dose) were tested for 13 weeks followed by 4 weeks on the control diet.

No adverse, treatment-related effects were observed on mortality, clinical signs, functional observational battery parameters, motor activity, body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic examinations, hematology, or urinalysis.

Slight hepatotoxicity was indicated by increases in the following findings ($p<=0.05$) in the 2000 and 20,000 ppm females: (i) plasma gamma-glutamyl transpeptidase (incr 200% each dose group); (ii) absolute and relative to body liver weights (incr 26-48%); (iii) incidence of livers that were dark in color (2-7/10 treated vs 0/10 controls; not statistically significant [NS] at 2000 ppm); (iv) incidence of enlarged livers (5-10/10 treated vs 0/10 controls); (v) incidence of slight periportal hepatocyte fatty change (10/10 treated vs 0/10 controls; each dose group); and (vi) incidence of slight diffuse hepatocyte hypertrophy (6-10/10 treated vs 0/10 controls). Additionally, decreased serum triglycerides (decr 22-29%) and serum total cholesterol (at 20,000

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description: White crystals

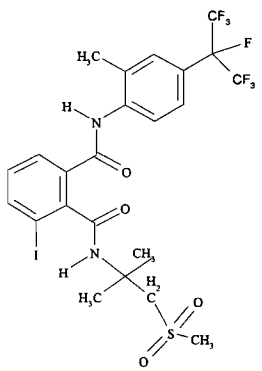
Lot No.: 0FH0009P

Purity (w/w): 97.7% a.i.

Stability of compound: Stability was confirmed in 20 and 20,000 ppm diets kept under a sealed, cold, and dark condition for 5 weeks, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient air for 8 days.

CAS #: 272451-65-7

Structure:



2. Vehicle: Diet

3. Test animals

Species: Rat

Strain: Fischer (F344/DuCrj)

Age and mean weight at initiation of treatment: 5 weeks old; 92-108 g males; 77-88 g females

Source: Atsugi Breeding Center, Charles River Japan, Inc. (Atsugi-shi, Japan)

Housing: Housed in groups of 2 by common sex and dose in wire mesh stainless steel cages in movable stainless steel racks

Diet: Certified diet MF Mash (Oriental Yeast Co., Ltd., Itabashi-ku, Tokyo), *ad libitum*

Water: Well water, filtered and sterilized with sodium hypochlorite and ultraviolet light, *ad libitum*

Environmental conditions

- **Temperature:** 24±2°C
- **Humidity:** 40-70%
- **Air changes:** ≥10 air changes/hour
- **Photoperiod:** 12 hours light/12 hours dark

Acclimation period: 9-10 days

B. STUDY DESIGN

1. In life dates Start: 02/22/01 End: Approximately 07/02/01

2. **Animal assignment/dose levels:** The animals were randomly assigned to the test groups shown in Table 1. After allocation, it was confirmed that there were no statistically significant differences in the mean body weights among groups, and all individual body weights were within ±20% of the mean value of each sex.

TABLE 1: Study design [a]

Test group	Dose to animal (ppm)	Dose to animal (mg/kg/day in M/F)	Main study No. rats/sex killed at Week 13	Satellite study No. rats/sex killed at Week 17 [b]
Control	0	0/0	10	10
Low	20	1.15/1.30	10	0
Mid-Low	50	2.85/3.29	10	0
Mid	200	11.4/13.1	10	0
Mid-High	2000	116/128	10	0
High	20,000	1192/1320 [c]	10	10

a Data were obtained from pages 22, 23 and 37 of MRID 46817210.
b All animals received control diet for the final 4 weeks.
c 1192/1320 mg/kg/day in M/F rats killed at Week 13 and 1199/1329 mg/kg/day in M/F rats killed at Week 17.

3. **Dose-selection rationale:** Based upon the results of a previously conducted 28-day range-finding study (IET 00-0156; not provided), the doses summarized in Table 1 were selected for the 90-day study. In study IET 00-0156, NNI-0001 was administered in the diet to Fischer rats (6/sex/dose) at dose levels of 0, 20, 50, 200, 2000, or 20,000 ppm for 28 days. No treatment-related changes were observed in the 20 and 50 ppm groups. At ≥200 ppm, increases in γ-glutamyl transpeptidase and liver weight were detected in females. At ≥2000 ppm, increased liver weight was noted in both sexes, and the following findings were observed in females: decreased mean corpuscular volume; alterations in several blood biochemical parameters including total cholesterol, triglyceride, and plasma cholinesterase; and liver enlargement. At 20,000 ppm, decreased mean corpuscular volume and alterations of several blood biochemical parameters in both sexes were observed.

4. **Dose preparation and analysis:** Dietary formulations were prepared by first making a premix of the appropriate amount of test substance with basal diet for each dose level. The premixes were then diluted with diet to the desired concentration. No adjustment for purity was made. Dietary formulations were sealed in plastic bags and stored in aluminum containers in the dark at 4°C until needed. Dietary formulations were prepared once prior to initiation of treatment and once every 4 weeks during the treatment period. Concentrations at each dietary level were measured monthly (including the first and last dietary preparations). Homogeneity (top, middle, bottom) of the test compound in each dietary formulation was tested in the first and last preparations. In a previous study (IET 00-0156), stability was confirmed in 20 and 20,000 ppm diets kept under a sealed, cold, and dark condition for 5 weeks, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient air for 8 days.

Results

Homogeneity analysis (% CV): 0.2-4.4%

Stability analysis (% of initial concentration): 97-100%

Concentration analysis (% of nominal concentration): 97-105%

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

5. **Statistics:** Significance was indicated at 5 and 1% probability. Main and satellite groups were evaluated independently.

PARAMETER	ANALYSIS CONDUCTED
Motor activity Grip strength Body weight Food consumption Urine specific gravity Urine volume Hematological parameters Clinical chemistry parameters Organ weights	Main study: Bartlett's test for equality of variance was conducted. One-way analysis of variance (ANOVA) and Dunnett's test were performed when group variances were homogeneous. The Kruskal-Wallis test and a Dunnett-type mean rank sum test were conducted when group variances were heterogeneous. Satellite study: F-test was conducted. Student's *t*-test was performed when group variances were homogeneous; otherwise, Aspen-Welch test was conducted.
Clinical observations (detailed) Sensorimotor responses Urinalysis (except for specific gravity and volume)	Main study: Kruskal-Wallis test and a Dunnett-type mean rank sum test were performed. Satellite study: Mann-Whitney's *U*-test was conducted.
Mortality Clinical observations (general) Ophthalmology Gross pathology Histopathology	Fisher's Exact test (one-tail analysis) was performed for both Main and Satellite studies.

These analyses were considered appropriate.

C. METHODS

1. Observations

1a. Cageside observations: Animals were observed at least twice daily for signs of toxicity and mortality, except once daily on weekends and holidays.

1b. Clinical examinations: Detailed clinical observations, including palpation of masses, were performed at least weekly throughout the study on all animals.

1c. Neurological evaluations: A limited functional observational battery (FOB) and locomotor activity tests were performed as detailed below.

(i) **Functional observational battery (FOB):** Detailed clinical observations (a limited functional observational battery) were performed once prior to treatment and once weekly during treatment on all animals. Environmental conditions, duration of observation in the open field, details about the observer, and positive control data were not reported. Animals were observed for the following signs, and findings were recorded using a scoring system (reported in Appendix 1 of this DER).

Home cage	Handling	Open field
Excitement	Handling difficulty	Jumping
Sedation	Changes in muscle tone	Circling
Abnormal posture	Tremors	Convulsions
Abnormal behavior	Palpebral closure	Abnormal gait
	Changes in pupil size	Spontaneous motor activity
	Salivation	Grooming
	Lacrimation	Rearing
	Discharges	Respiration
	Exophthalmos	Vocalization
	Changes in body temperature	Piloerection
	Abnormal respiratory sound	Urination
	Changes in fur	Defecation
	Changes in skin and mucous membranes	Abnormal posture
		Abnormal behavior

Additionally, all animals in the Main study during Week 11 and in the Satellite group at Week 17 were subjected to the following tests: grip strength (forelimb and hindlimb), approach response, auditory response, touch response, tail pinch response, and aerial righting reflex. Forelimb and hindlimb grip strengths were determined using a strain gauge, CPU gauge Model-9505 (Aiko Engineering Co., Ltd., Tokyo). Sensorimotor responses were assessed by scoring reactions to the approach of a pencil, sound of a clicker, touch on the rump, and tail pinch using forceps. The animals were then held supine and dropped from approximately 20 cm above the floor, and coordination scored. Order of animals for each test was counterbalanced across groups.

(ii) **Locomotor activity:** Locomotor activity was monitored in all animals in the Main study during Week 11 and in the Satellite group at Week 17 by an automated activity recording system (SUPER MEX®, Muromachi Kikai Co., Ltd., Chuo-ku, Tokyo) for one hour in 10 minute intervals. Order of animals for each test was counterbalanced across groups. Motor activity was assessed at approximately the same time as the sensorimotor tests.

2. **Body weight and body weight gain:** All animals were weighed prior to treatment, on Day 0, once weekly during the treatment and recovery periods until scheduled termination, and at termination. Body weight gains were not reported.

3. **Food consumption, food efficiency, and compound intake:** Food consumption was calculated as a mean value (g food/rat/day) for each cage. Mean food consumption was determined for a period of 2 consecutive days prior to treatment and 4 consecutive days weekly during the treatment and recovery periods. An average of the group mean food consumption during the treatment period was also calculated for each sex.

a

Food efficiency was calculated for each group weekly during treatment and recovery periods as a ratio (%) of group mean body weight gain to group mean food consumption at the week concerned. Averages of the group mean food efficiency during the 13 weeks of treatment and 4 weeks of recovery were also calculated for each sex. Compound intake (mg/kg bw/day) values were calculated as group mean food consumption x nominal concentration/ group mean body weight. A weighed average of the group mean test substance intake during the treatment period was also calculated for each sex.

4. **Ophthalmoscopic examination:** All animals were subjected to ophthalmoscopic examinations during the acclimatization period, and all animals in the 0 and 20,000 ppm groups were also examined at Week 13 (Main study) and Week 17 (Satellite study).

5. **Hematology and clinical chemistry:** Blood was collected from all animals at the scheduled termination. Animals were under ether anesthesia after overnight fasting, and blood samples were withdrawn from the posterior vena cava. Bone smears were prepared but not examined. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count (RET)
	Blood clotting measurements*		Blood cell morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin : globulin
X	Alanine aminotransferase (ALT/also SGPT)*	X	Plasma cholinesterase
X	Aspartate aminotransferase (AST/also SGOT)*	X	Total bile acid
	Sorbitol dehydrogenase*		
X	Gamma-glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis:** Samples were collected from all animals during the week of termination. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for 90-day oral rodent studies

7. **Sacrifice and pathology:** All animals were killed by exsanguination under deep ether anesthesia. Rats in the Main study were killed after 13 weeks of treatment; rats in the Satellite study were killed after 13 weeks of treatment followed by 4 weeks of control diet. All animals were subjected to gross pathological examination, and the following CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder (not rat)*
	Bile duct (rat)
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
XX	Ovaries*+
XX	Uterus*+ (horns and cervix)
X	Mammary gland*
	Oviducts
X	Vagina
X	Coagulating glands

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (retina, optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
	Lacrimal gland
X	Parathyroid*
XX	Thyroid*
	OTHER
X	Bone
X	Skeletal muscle
X	Skin* (and subcutis)
	Peyer's patches
X	Harderian gland
X	All gross lesions and masses*

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.

The collected tissues were fixed in 10% neutral-buffered formalin, except testes were fixed in a mixed solution of formalin, sucrose, and acetic acid (FSA solution). The lungs were instilled with formalin before fixation. Histopathological examinations were performed on all tissues collected from the 0 and 20,000 ppm groups from both the Main and Satellite studies; and the liver, thyroids, and all tissues/organs with gross lesions from all animals of the Main study. Microscopic lesions were graded as slight, moderate, or severe. In addition, the head and knee joint from all animals were collected and fixed, but were not examined. Tissues were prepared for microscopic examination using a routine method of paraffin-embedded, hematoxylin and eosin-stained sections.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** No mortality was observed.

2. **Clinical signs of toxicity:** No treatment-related clinical signs were observed during general cage-side observations or clinical examinations.

3. **Neurological evaluations:** In the open field, increased ($p \leq 0.05$) incidences of occasional rearing was observed in the 20,000 ppm females at Weeks 7, 8, and 11 (5-6/10 treated vs 0-1/10 controls). This finding was not considered adverse because the response was slight and transient without any further evidence to support a neurological effect. Increased (not statistically significant [NS]) incidences of occasional rearing in other dose groups occurred typically in 1-3/10 more animals in the treated group than controls. Forelimb grip strength was increased ($p \leq 0.05$) by 8% in the 20,000 ppm females following 4 weeks of control diet. This minor difference was considered incidental. No treatment-related effects were noted in any other FOB parameter, sensorimotor test, or during motor activity evaluations. Habituation was unaffected by treatment.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Increased ($p \leq 0.05$) body weights were observed in the males at 2000 ppm during Weeks 7-13 (↑7-8%), but these increases were unrelated to dose and were minor. All other mean bodyweights in treated groups were similar to controls.

TABLE 2. Mean (±SD) body weights and body weight gains (g) at selected intervals in rats treated with NNI-0001 in the diet for up to 3 months followed by a 4-week recovery period [a]

Week	Dose (ppm)					
	0	20	50	200	2000	20,000
Males						
0	100±4	101±4	101±3	101±3	101±3	100±3
7	258±15	265±13	263±14	270±9	275±11* (↑7)	267±9
13	306±17	316±16	312±16	321±12	328±14** (↑7)	312±12
14 [b]	321±13	NA	NA	NA	NA	320±18
17 [b]	332±13	NA	NA	NA	NA	333±16
BWG (0-13)	206	215	211	220	227	212
BWG (14-17) [b]	11	NA	NA	NA	NA	13
Females						
0	83±3	83±3	82±2	82±3	82±3	82±2
7	160±7	162±7	159±6	161±8	162±6	160±9
13	179±7	184±10	179±9	181±11	183±6	180±9
14 [b]	182±9	NA	NA	NA	NA	184±10
17 [b]	185±10	NA	NA	NA	NA	187±10
BWG (0-13)	96	101	97	99	101	98
BWG (14-17) [b]	3	NA	NA	NA	NA	3

a Data (n=10) were obtained from Tables 9-1 through 10-2 on pages 213-216 of MRID 46817210. Percent difference from controls, calculated by reviewers, is included in parentheses. Body weight gains were also calculated by the reviewers.

b Values in this row are for animals in the Satellite study (other values are for animals from the Main study). *Footnotes continue*

NA This dose was not tested

* Significantly different ($p \leq 0.05$) from the control group

** Significantly different ($p \leq 0.01$) from the control group

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** No treatment-related effect was observed on food consumption. Differences ($p \leq 0.05$) were minor and/or unrelated to dose. Slight increases were noted in average food consumption; however, no effects on body weight or food efficiency were observed.

2. **Compound consumption:** Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

3. **Food efficiency:** No effects were observed on food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects on the eyes were observed in the animals in the Main or Satellite groups.

E. BLOOD ANALYSES

1. **Hematology:** No adverse treatment-related effect was observed in hematology. All differences ($p \leq 0.05$) were minor, transient, and/or unrelated to dose.

   Evidence suggested a slight anemia in the ≥2000 ppm females, as indicated by slight decreases (↓3-7%; $p \leq 0.01$) in hematocrit, hemoglobin concentration, and mean corpuscular volume (Table 3a). Although treatment-related effects were observed, these changes were not considered adverse due to the small magnitude of change. Other minor differences ($p \leq 0.05$) included findings such as decreased mean corpuscular volume in the 20,000 ppm males (↓2%) and increases in platelets in the ≥2000 ppm males (↑7-9%) and females (↑10-16%). All differences were less following the 4 week recovery period.

TABLE 3a. Mean ±SD values (% from controls) for selected hematology findings female rats treated with NNI-0001 in the diet for 13 weeks[a]

Parameter	Dose (ppm)					
	0	20	50	200	2000	20,000
FEMALES						
Hematocrit (%)	39.9±1.7	39.4±1.6	40.0±1.3	38.5±1.3	37.6±1.6** (↓6%)[b]	37.1±1.1** (↓7%)
Hemoglobin (g/dl)	15.2±0.3	15.3±0.3	15.4±0.4	14.9±0.2	14.5±0.3** (↓5%)	14.4±0.3** (↓5%)
RBC (10^6/mm^3)	7.75±0.31	7.69±0.34	7.81±0.25	7.63±0.29	7.55±0.27	7.70±0.23
MCV (fl)	51.5±0.3	51.3±0.4	51.2±0.4	50.5±0.4** (↓2%)	49.7±0.6 ** (↓3%)	48.2±0.3** (↓6%)
Reticulocytes (/10^3 RBC)	14±4	16±3	14±3	14±3	15±2	13±3

a Data (n=10) were obtained from Table 22-1 on page 247 of MRID 46817210.
b % from control calculated by reviewer.
** Statistically different ($p \leq 0.01$) from the control.

2. **Clinical chemistry:** Plasma γ-glutamyl transferase was increased ($p \leq 0.01$) in the ≥2000 ppm females by 200% each (Table 3b), but were comparable to controls following 4 weeks on the control diet. Decreased ($p \leq 0.01$) total serum cholesterol in the 20,000 ppm females (↓24%) and triglycerides in the ≥2000 ppm females (↓22-29%) were observed. Serum total bile acid was decreased ($p \leq 0.05$) by 43% in the 20,000 ppm females, however, a decrease in total bile acid is usually considered to be an indicator of malabsorption from the intestine. However, food consumption and body weights did not support a diagnosis of malabsorption. Thus, the effect on bile acid was considered equivocal. Plasma cholinesterase was also decreased ($p \leq 0.01$) in the ≥2000 ppm females (↓22-43%); however, the toxicological significance of this finding is unclear. Other differences ($p \leq 0.05$) were observed, but were not considered adverse, treatment-related findings due to the small magnitude of change from controls, the effect was unrelated to dose, and/or no known toxicological significance was associated with the change. These differences ($p \leq 0.05$) included findings such as: (i) increased total cholesterol in all treated male groups; (ii) increased total protein and albumin in both sexes at 20,000 ppm; (iii) decreased alkaline phosphatase, glutamic pyruvic transaminase, and total bilirubin in the 2000 and/or 20,000 ppm females; and (iv) increased globulin and potassium in the 2000 and/or 20,000 ppm females. Only minor differences ($p \leq 0.05$) were observed after 4 weeks on the control diet, and reversibility was demonstrated for all treatment-related effects.

TABLE 3b. Mean (±SD) values for selected clinical chemistry findings in female rats treated with NNI-0001 in the diet for up to 3 months [a]

Parameter	Dose (ppm)					
	0	20	50	200	2000	20,000
γ-Glutamyl transpeptidase (U/L)	1±1	2±1	2±0	2±0	3±1** (↑200)	3±0** (↑200)
Total cholesterol (mg/dL)	58±5	65±8	63±7	65±8	53±5	44±5** (↓24)
Triglycerides (mg/dL)	51±5	52±6	51±6	51±8	40±7** (↓22)	36±9** (↓29)
Plasma cholinesterase (U/L)	630±47	658±59	670±52	582±40	493±76** (↓22)	359±50** (↓43)

a Data (n=10) were obtained from Tables 24-1 through 24-4 on pages 256-259 of MRID 46817210. Percent difference from controls, calculated by reviewers, is included in parentheses. No differences ($p \leq 0.05$) were noted in these parameters following 4 weeks on the control diet.

** Significantly different ($p \leq 0.01$) from the control group

F. URINALYSIS: No treatment-related effects were observed during urinalysis. A minor (3%) decrease ($p \leq 0.05$) in urinary specific gravity was noted in the 50 ppm females at Week 13. Urinary pH was increased ($p \leq 0.01$) in the 20,000 ppm males after 4 weeks on the control diet, but not during treatment. Without corroborating evidence of toxicity, this effect was not considered adverse.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** Increased ($p \leq 0.01$) terminal body weight was noted in the 2000 ppm males (↑8%; Table 4). Absolute and relative to body liver weights were increased ($p \leq 0.01$) in the 20,000 ppm males by 11-14% and in the ≥2000 ppm females by 26-48%. Increases ($p \leq 0.05$) in absolute and relative liver weights were also noted in the 200 ppm females (↑11-12%), however, this increase was minor and may be adaptive, and therefore, was not considered to be adverse. Absolute liver weight was increased ($p \leq 0.01$) by 13% in the 2000 ppm males; however, this was considered partially due to the increased mean weight of the group and was not considered adverse. These differences from controls were less following 4 weeks on the control diet. Other differences ($p \leq 0.05$) were not corroborated by gross or microscopic pathology, were unrelated to dose, and/or the weight differences from controls were minor. These differences included findings such as increased heart, kidney, adrenal, and ovary weights in the 20,000 ppm females.

TABLE 4. Mean (±SD) liver weights in rats treated with NNI-0001 in the diet for up to 3 months[a]

Parameter	Dose (ppm)					
	0	20	50	200	2000	20,000
Males						
Terminal BW (g)	292±17	300±16	297±15	306±11	314±14** (↑8)	298±11
Liver						
absolute (g)	6.47±0.45	6.50±0.39	6.48±0.32	6.69±0.38	7.33±0.41** (↑13)	7.36±0.36**(↑14)
relative to BW (%)	2.22±0.09	2.17±0.03	2.19±0.03	2.18±0.08	2.34±0.09	2.47±0.06**(↑11)
Females						
Terminal BW (g)	169±6	173±10	167±7	170±11	172±6	169±9
Liver						
absolute (g)	3.64±0.22	3.84±0.17	3.70±0.18	4.07±0.26**(↑12)	4.69±0.37** (↑29)	5.38±0.37**(↑48)
relative to BW (%)	2.16±0.08	2.23±0.05	2.21±0.08	2.40±0.07* (↑11)	2.72±0.15** (↑26)	3.18±0.10**(↑47)

a Data (n=10) were obtained from Tables 25-1 through 26-6 on pages 260-271 of MRID 46817210. Percent difference from controls, calculated by reviewers, is included in parentheses.
* Significantly different ($p \leq 0.05$) from the control group
** Significantly different ($p \leq 0.01$) from the control group

2. **Gross pathology:** Increased ($p \leq 0.05$) incidences of livers that were dark in color and/or enlarged were observed in the 20,000 ppm males (4-6/10 treated vs 0/10 controls) and females (7-10/10 treated vs 0/10 controls; Table 5). In the 2000 ppm females, livers were dark in color in 2/10 rats (not statistically significant [NS]) and enlarged in 5/10 rats ($p \leq 0.05$). These abnormalities were not observed after 4 weeks on control diet.

TABLE 5. Selected gross liver lesions (# affected/10) in rats treated with NNI-0001 in the diet for up to 3 months[a]

Abnormality	Dose (ppm)					
	0	20	50	200	2000	20,000
Males						
Dark in color	0	0	0	0	0	4*
Enlargement	0	0	0	0	0	6**
Females						
Dark in color	0	0	0	0	2	7**
Enlargement	0	0	0	0	5*	10**

a Data were obtained from Tables 27-1 through 28-2 on pages 272-277 of MRID 46817210. No differences ($p \leq 0.05$) were noted in these parameters following 4 weeks on the control diet.
* Significantly different ($p \leq 0.05$) from the control group
** Significantly different ($p \leq 0.01$) from the control group

3. **Microscopic pathology:** Selected histological lesions (slight severity) are detailed in Table 6. Increased incidences of periportal hepatocyte fatty change were noted in the 20,000 ppm males (3/10 treated vs 0/10 controls; NS) and ≥200 ppm females (5-10/10 treated vs 0/10 controls; $p \leq 0.05$). Increased ($p \leq 0.01$) incidences of diffuse hepatocyte hypertrophy were also noted in the ≥2000 ppm females (6-10/10 treated vs 0/10 controls). After 4 weeks on the control diet, the 20,000 ppm females still had an increased incidence of periportal hepatocyte fatty change (5/10 treated vs 0/10 controls), but this was an improvement over the incidence observed after 13 weeks treatment (10/10 treated). The other abnormalities were not observed after the recovery period.

The incidences of thyroid follicular cell hypertrophy were increased in the 20,000 ppm males (3/10 treated vs 0/10 controls; NS) and ≥200 ppm females (2-8/10 treated vs 0/10 controls; p≤0.05 in ≥2000 ppm females), however, this effect was not considered to be adverse in either sex since there was no other supporting evidence of thyroid toxicity in this study. The incidences of other lesions in treated groups were similar to controls.

TABLE 6. Selected microscopic liver lesions (# affected/10) in rats treated with NNI-0001 in the diet for up to 3 months [a]

Abnormality	Dose (ppm)					
	0	20	50	200	2000	20,000
Males						
Fatty change, hepatocyte, periportal	0	0	0	0	0	3
Females						
Fatty change, hepatocyte, periportal	0	0	0	5*	10**	10**
Hypertrophy, hepatocyte, diffuse	0	0	0	0	6**	10**

a Data were obtained from Tables 29-1 through 30-2 on pages 276-279 of MRID 46817210. All lesions were slight in severity. No differences (p≤0.05) were noted in these parameters following 4 weeks on the control diet, except that 5/10 females at 20,000 ppm had periportal hepatocyte fatty change.

* Significantly different (p≤0.05) from the control group

** Significantly different (p≤0.01) from the control group

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR=S CONCLUSIONS: The LOAEL was 200 ppm, based on differences detected in hematology, blood biochemistry, and pathology including organ weights in females at 200 ppm and both sexes at ≥2000 ppm (detailed in reviewers comments).

B. REVIEWER'S COMMENTS: No adverse, treatment-related effects were observed on mortality, clinical signs, functional observational battery parameters, motor activity, body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic examinations, hematology, or urinalysis.

Slight hepatotoxicity was noted in the 200 ppm females as indicated by increased (p≤0.05) absolute and relative to body liver weights (↑11-12%), and slight periportal hepatocyte fatty change (5/10 treated vs 0/10 controls). These changes were minimal and may have been adaptive. These changes were not considered adverse at this dose.

Slight hepatotoxicity was indicated by increases in the following findings (p≤0.05) in the 2000 and 20,000 ppm females: (i) plasma γ-glutamyl transpeptidase (↑200% each dose group); (ii) absolute and relative to body liver weights (↑26-48%); (iii) incidence of livers that were dark in color (2-7/10 treated vs 0/10 controls; NS at 2000 ppm); (iv) incidence of enlarged livers (5-10/10 treated vs 0/10 controls); (v) incidence of slight periportal hepatocyte fatty change (10/10 treated vs 0/10 controls; each dose group); and (vi) incidence of slight diffuse hepatocyte hypertrophy (6-10/10 treated vs 0/10 controls). Additionally, decreased serum triglycerides (↓22-29%) and serum total cholesterol (at 20,000 ppm only; ↓24%) were observed. These changes in lipid metabolism were considered slight and of unknown significance.

Slight hepatotoxicity was also observed in the 20,000 ppm males. Absolute and relative to body liver weights were increased ($p \leq 0.01$) by 11-14%. Increased ($p \leq 0.05$) incidences of livers that were dark in color and/or enlarged were observed (4-6/10 treated vs 0/10 controls). Increased incidences of periportal hepatocyte fatty change was noted (3/10 treated vs 0/10 controls; NS). Thus, toxicity was evident in males but females were the more sensitive species. Absolute liver weight was also increased ($p \leq 0.01$) by 13% in the 2000 ppm males; however, this was considered partially due to the increased mean weight of the group and was not considered adverse.

Serum total bile acid was decreased ($p \leq 0.05$) by 43% in the 20,000 ppm females; however, a decrease in total bile acid is usually considered to be an indicator of malabsorption from the intestine. However, food consumption and body weights did not support a diagnosis of malabsorption. Thus, the effect on bile acid was considered equivocal. Plasma cholinesterase was decreased ($p \leq 0.01$) in the $\geq$2000 ppm females ($\downarrow$22-43%); however, the toxicological significance of this finding is unclear.

The incidences of thyroid follicular cell hypertrophy were increased in the 20,000 ppm males (3/10 treated vs 0/10 controls; NS) and $\geq$200 ppm females (2-8/10 treated vs 0/10 controls; $p \leq 0.05$ in $\geq$2000 ppm females), however this effect was not considered to be adverse in either sex since there was no other supporting evidence of thyroid toxicity in this study.

The Sponsor suggested adverse treatment-related effects occurred on hematological parameters, including hematocrit, hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin. Although treatment-related effects were observed, these changes were not considered adverse due to the small magnitude of change. The Sponsor also considered the minor changes in platelets of importance. Likewise, any effect observed on serum alkaline phosphatase, total bilirubin, glutamic pyruvic transaminase, total protein, albumin, globulin, and potassium were only minor differences that were not adverse. Increased adrenal, ovary, kidney, and heart weights observed in females were minor and not corroborated by gross or histological evidence of toxicity.

Complete or partial recovery was noted for all treatment-related findings following 4 weeks of control diet.

The LOAEL is 2000 ppm (equivalent to 128 mg/kg bw/day in females), based on indications of slight hepatotoxicity in females. The NOAEL is 200 ppm (equivalent to 13.1 mg/kg bw/day in females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

C. **STUDY DEFICIENCIES:** No deficiency was noted.

APPENDIX 1

The following are pages 70-71 from MRID 46817210.

(IEI 01-00

Key to Tables 5 and 6
Standard key to scored clinical signs

Home cage:

Excitement
0. Negative
1. Slight
2. Moderate
3. Marked (may accompany vocalization)

Abnormal posture
0. Negative
1. Positive
(with description such as flattened, lying on side, etc.)

Handling:

Handling difficulty
0. No difficulties
1. Slight
2. Moderate
3. Marked (difficult to handle)

Tremors
0. Negative
1. Slight
2. Moderate
3. Marked

Changes in pupil size
-1. Contracted
0. Normal
1. Dilated

Lacrimation
0. Negative
1. Slightly excessive
2. Moderately excessive
3. Markedly excessive

Exophthalmos
0. Negative
1. Positive
(with description of side)

Abnormal respiratory sound
0. Negative
1. Positive

Changes in skin
(areas such as nose, limbs and tail)
0. Negative (normal)
1. Slightly congested
2. Moderately congested
3. Markedly congested

Sedation
0. Negative
1. Slight
2. Moderate
3. Marked

Abnormal behavior
0. Negative
1. Positive
(with description such as backward moving, stereotypies, self-destructive biting, etc.)

Changes in muscle tone
-3. Markedly decreased muscle tone
-2. Moderately decreased muscle tone
-1. Slightly decreased muscle tone
0. Negative (normal)
1. Slightly increased muscle tone
2. Moderately increased muscle tone
3. Markedly increased muscle tone

Palpebral closure
0. Negative (eyelids wide open)
1. Slight (eyelids closed less than half)
2. Moderate (eyelids closed half or more)
3. Marked (eyelids closed completely)
(with description of side)

Salivation
0. Negative
1. Slight (wetted fur on perioral region)
2. Moderate (wetted fur up to submandibular region)
3. Marked (wetted fur beyond submandibular region)

Discharges
0. Negative
1. Positive
(with description of type, i.e. nasal, auricular, and vaginal, color, side, etc.)

Changes in body temperature
-2. Moderately cold at handling
-1. Slightly cold at handling
0. Negative (normal)
1. Slightly warm at handling
2. Moderately warm at handling

Changes in fur
0. Negative (normal)
1. Slightly wetted fur in external genital region
2. Moderately wetted fur in external genital region
3. Markedly wetted fur in external genital region

Changes in mucous membrane
0. Negative (normal)
1. Slightly congested
2. Moderately congested
3. Markedly congested

(IET 01-0013)

Key to Tables 5 and 6 (continued)
Standard key to scored clinical signs

Open field:

Jumping
0. Negative
1. Positive

Circling
0. Negative
1. Positive

Convulsions
0. Negative
1. Positive

Abnormal gait
0. Negative
1. Positive
(with description such as staggering gait, dragging gait, paralysis of hind limbs, etc.)

Spontaneous motor activity
-3. Markedly decreased motor activity
-2. Moderately decreased motor activity
-1. Slightly decreased motor activity
0. Normal
1. Slightly increased motor activity
2. Moderately increased motor activity
3. Markedly increased motor activity

Grooming
0. No grooming
1. Occasional grooming
2. Frequent grooming
(about half of the observation period)
3. Continuous grooming
(almost all the observation period)

Rearing
0. No rearing
1. Occasional rearing
2. Frequent rearing
(about half of the observation period)
3. Continuous rearing
(almost all the observation period)

Respiration
-3. Markedly slow respiration
-2. Moderately slow respiration
-1. Slightly slow respiration
0. Normal
1. Slightly rapid respiration
2. Moderately rapid respiration
3. Markedly rapid respiration

Vocalization
0. Negative
1. Positive

Piloerection
0. Negative
1. Positive

Urination
0. No urination
1. 1-2 times
2. 3-4 times
3. 5 or more
(counts made during the observation period)

Defecation
0. No feces
1. 1-2 fecal boluses
2. 3-4 fecal boluses
3. 5 or more fecal boluses
(counts made during the observation period)

Abnormal posture
see Home cage observation

Abnormal behavior
see Home cage observation

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870 3100 [§82-1a], Subchronic Oral Toxicity Study in Mice

Work Assignment No 4-1-124 B (MRID 46817211)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Rd, Bldg. 100, Ste. B
Durham, NC 27713

Primary Reviewer:
Stephanie E. Foster, M.S.

Signature: Stephanie E. Foster
Date: 1/19/07

Secondary Reviewer:
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 1/19/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/19/07

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 1/19/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Marion Copley, DVM, DABT **Signature:** Marion Copley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/26/09
Work Assignment Manager: Myron Ottley **Signature:** Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity Feeding Study in Mice; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 027602 **DP BARCODE:** D 331553
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (98.5% a.i.)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Takeuchi, Y. (2002) NNI-0001: Repeated dose 90-day oral toxicity study in mice. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET 01-0049, April 17, 2002. MRID 46817211. Unpublished.

SPONSOR: Nihon Nohyaku Co. Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY - In a subchronic oral toxicity study (MRID 46817211), NNI-0001 (Flubendiamide; 98.5% a.i., Lot No. 0FH0010P) was administered to 10 SPF ICR (Crj:CD-1) mice/sex/dose in the diet at dose levels of 0, 50, 100, 1000, or 10,000 ppm (approximately equivalent to 0, 6.01/7.13, 11.9/14.7, 123/145, and 1214/1424 mg/kg/day in males/females) for at least 90 days.

No adverse, treatment-related effects were observed on mortality, clinical signs, body weight, food consumption, food efficiency, or hematology.

At 1000 and 10,000 ppm, slight hepatotoxicity was observed in both sexes. Increased ($p=<0.05$) absolute and relative to body liver weights (incr. 12-22%) were noted in females. Increased incidences ($p=<0.05$) of centrilobular hepatocyte fatty change was observed in the males (8-9/10 treated vs. 1/10 controls) and females (3-5/10 treated vs. 0/10 controls); and centrilobular hepatocyte hypertrophy was noted in the males (6-10/10 treated vs. 0/10 controls) and females (4-6/10 treated vs. 0/10 controls). For both lesions, the lesion severity was slight to moderate in males and slight in females.

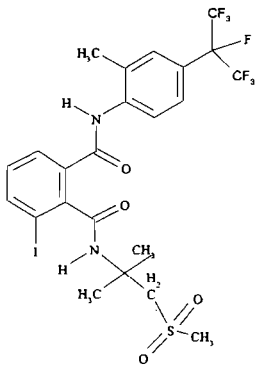
At 10,000 ppm in males, relative liver weights were increased ($p=<0.05$) by 14%, and an increased incidence of darkened liver was observed (4/10 treated vs. 0/10 controls). Increased

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: NNI-0001

Description: White crystal
Lot #: 0FH0010P
Purity: 98.5% a.i.
Compound stability: Stable in the test diets kept under a sealed, cold, and dark condition for 5 weeks, stored again under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient temperature for 8 days.
CAS # of TGAI: 272451-65-7
Structure:



2. Vehicle: Diet

3. Test animals

Species:	Mouse
Strain:	SPF ICR (Crj:CD-1)
Age/weight at study initiation:	5 weeks old; 28.2-36.0 g males; 22.0-27.7 g females
Source:	Charles River Japan, Inc. (Atsugi Breeding Center, Atsugi-shi, Kanagawa, Japan)
Housing:	2 animals of the same sex and group were housed in aluminum cages with wire-mesh floors.
Diet:	Certified diet MF Mash (Oriental Yeast Co., Ltd., Itabashi-ku, Tokyo, Japan), *ad libitum*
Water:	Filtered well water sterilized with sodium hypochlorite and UV light, *ad libitum*
Environmental conditions:	**Temperature:** 24±2EC **Humidity:** 55±15% **Air changes:** Minimum of 10/hr **Photoperiod:** 12 hours dark/ 12 hours
Acclimation period:	9 or 10 Days

B. STUDY DESIGN

1. In life dates: Start: 05/31/01 (males) End: 08/30/01
06/08/01 (females) 09/07/01

2. Animal assignment: Animals were randomly assigned to the test groups presented in Table 1. After allocation, it was confirmed that individual body weights were within 80-120% of the mean value of each sex.

TABLE 1: Study design [a]			
Test group	**Dose (ppm)**	**Mean Intake (mg/kg/day)**	**# Males/Females**
Control	0	0/0	10/10
Low	50	6.01/7.13	10/10
Mid-low	100	11.9/14.7	10/10
Mid-high	1000	123/145	10/10
High	10,000	1214/1424	10/10

a Data were obtained from page 19, and pages 47-48 of the study report.

3. **Dose-selection rationale:** The dose-selection was based on the results of a 28-day oral toxicity study in the same strain of mice (IET 00-0172; study not provided) in which doses of 0, 20, 200, 2000, or 20,000 ppm were administered in the diet. At 20,000 ppm, relative to body liver weights were significantly increased in males, and incidences of centrilobular hepatocellular hypertrophy and centrilobular hepatocellular fatty change were increased in both sexes. At 2000 ppm, an increased incidence of centrilobular hepatocellular hypertrophy was observed in both sexes, and centrilobular hepatocellular fatty change was observed in males.

4. **Treatment preparation, administration, and analysis:** Dietary formulations were prepared once prior to treatment and every four weeks during treatment by mixing the appropriate amount of test material with a small amount of diet to form a premix. The premix was further diluted with diet to achieve the desired concentration. The prepared diets were sealed and stored at 4°C in the dark until needed. The amount of test substance to be mixed was not corrected for purity. Top, middle, and bottom samples of all dietary formulations from the first and last preparations were analyzed for homogeneity and concentration verification. Also, samples of all dietary formulations were obtained twice after use in the study and analyzed for concentration. In a 28-day oral toxicity study in rats (IET 00-0156; study not provided), the stability of the test compound in the diet at 20 and 20,000 ppm was demonstrated for 5 weeks when kept in a sealed, cold, and dark condition, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient temperature for 8 days.

Results:

Stability (% of initial concentration): 97-100%

Homogeneity (% coefficient of variance): 0.8-5.7%

Concentration (% of nominal): 98-106%

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

5. **Statistics:** Group means were compared at the 5% and 1% significance levels. The following statistical procedures were used:

Parameter	Statistical Test
Body weight Food consumption Hematology Clinical chemistry Organ weights	Bartlett's test was performed to compare homogeneity of group variances. If Bartlett's was not significant, a one-way ANOVA was performed to compare the means. If the ANOVA was significant, means of the treated groups were compared with the mean of the control group using Dunnett's multiple comparison test. If Bartlett's was significant, group means were compared using the Kruskal-Wallis test for non-parametric comparison. If the Kruskal-Wallis test was significant, means of the treated groups were compared with the mean of the control group using a Dunnett-type mean rank sum test.
Mortality Clinical signs of toxicity Macroscopic and microscopic pathology.	Fisher's exact probability (one-tail)

Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

C. METHODS

1. Observations

1a. Cageside observations: Animals were observed twice daily on weekdays and once daily on weekends and holidays for mortality and moribundity. Daily cage-side examinations for clinical signs of toxicity were also performed.

1b. Clinical examinations: Detailed physical examinations (including palpation of masses) were performed at least once a week.

1c. Neurological evaluations: Neurological evaluations were not performed as a part of this study. A developmental neurotoxicity study (MRID 46817228) was concurrently submitted.

2. Body weight: All animals were weighed prior to treatment, weekly during treatment, and at necropsy.

3. Food consumption, food efficiency, and compound intake: Mean food consumption (g/animal/day) was determined weekly (for a "period of 3 or 4 consecutive days") and as an overall average for Weeks 1 to 13. Weekly food efficiency ratios (BWG x 100/FC) were also reported. Test substance intake (mg/kg bw/day) was calculated for each week and as an average for the overall study (Weeks 1-13) using the food consumption, body weight, and nominal dietary concentration data.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed.

5. **Hematology and clinical chemistry**: At Week 13, all animals were anesthetized with ether and blood samples for hematology and clinical chemistry were collected from the posterior vena cava. The CHECKED (X) parameters were examined.

   a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Activated Partial thromboplastin time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

   b. Clinical chemistry:

	ELECTROLYTES		OTHER
	Calcium	X	Albumin*
	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
	Phosphate	X	Total cholesterol*
	Potassium*	X	Globulins
	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis**: Urinalysis was not performed, but is not required by OPPTS Guideline 870.3100.

7. **Sacrifice and pathology**: At study termination, all animals were weighed, killed via exsanguination under ether anesthesia, and subjected to gross necropsy. The following CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue[a]	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina and optic nerve)*
X	Jejunum*	X	Thymus*+		**GLANDULAR**
X	Ileum*			X	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Thyroid (with parathyroid)*
X	Rectum*	X	Urinary bladder*	X	Harderian glands
XX	Liver*+[b]	X	Epididymides		**OTHER**
XX	Gall bladder (not rat)*[b]	XX	Testes*+	X	Skin*
	Bile duct (rat)	X	Prostate*	X	Bone
X	Pancreas*	X	Seminal vesicles*	X	Head[a]
	RESPIRATORY	XX	Ovaries*+	X	Knee joint[a]
X	Trachea*	X	Uterus (including cervix)*+	X	Muscle
X	Lung*	X	Mammary gland*	X	All gross lesions and masses*
X	Nasal cavity *	X	Vagina		
X	Pharynx*	X	Coagulating glands		
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
a The head and knee joint were fixed but not examined histologically.
b Liver was weighed with gallbladder

Samples to be examined microscopically were fixed in 10% neutral buffered formalin with the exception of the testes, which were fixed in FSA solution (Formalin-Sucrose-Acetic Acid). The lungs were instilled with 10% neutral-buffered formalin prior to fixation. Samples were prepared routinely and stained with hematoxylin and eosin. With the exception of tissues preserved with the head in situ (i.e., the tongue, paranasal sinuses, oral mucosa, and middle ears) and the knee joint, the above-listed tissues were examined microscopically in the control and high dose groups. Additionally in the intermediate dose groups, the liver, thyroids, and any gross lesions were examined; lesion severity was graded as slight, moderate, or severe.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All animals survived the treatment period.

2. **Clinical Signs of Toxicity:** No clinical signs of toxicity were observed.

B. BODY WEIGHT AND BODY WEIGHT GAIN: No treatment-related effects were observed on body weights or body weight gains (Table 2).

Table 2. Mean (±SD) body weights and body weight gains (g) in mice treated with Flubendiamide in the diet for up to 90 days [a]

Weeks on Study	Dose (ppm)				
	0	50	100	1000	10,000
Males					
0	30.7±1.8	31.2±2.2	30.3±1.7	30.6±1.6	31.0±1.8
6	43.2±4.0	42.5±5.9	41.7±2.6	42.2±2.8	41.3±2.6
13	49.8±6.3	48.0±7.3	47.0±3.5	48.5±3.8	46.9±2.9
BWG (0-13)	19.1	16.8	16.7	17.9	15.9
Females					
0	24.6±1.1	24.7±1.2	24.4±1.0	24.9±1.6	24.2±1.3
6	32.5±2.4	31.6±1.8	30.8±0.9	32.5±2.4	31.0±2.9
13	36.9±4.7	35.2±2.9	36.1±2.5	37.9±3.8	34.6±3.3
BWG (0-13)	12.3	10.5	11.7	13.0	10.4

a Data were obtained from Tables 5 and 6 on pages 43-44 of the study report, n=10.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

1. **Food consumption:** There were no treatment-related effects on food consumption or food efficiency ratios.

2. **Compound consumption:** Compound consumption data (mg/kg/day) are reported in Table 1 of this DER.

D. BLOOD ANALYSES

1. **Hematology:** No treatment-related effects were observed on hematology.

2. **Clinical chemistry:** In males and females, triglycerides were slightly increased at 10,000 ppm (↑26, 79%, respectively; not statistically significant [NS]). Total bilirubin was increased in the 10,000 ppm females (↑19%; $p \leq 0.05$); however, this slight magnitude in change did not indicate an adverse effect. All other differences in clinical chemistry were considered minor.

E. SACRIFICE AND PATHOLOGY

1. **Organ weight:** Increased ($p \leq 0.05$) absolute and relative to body liver weights were noted in the ≥1000 ppm females (↑12-22%). Additionally at 10,000 ppm, relative liver weights were increased ($p \leq 0.05$) by 14% in males. Histopathological findings were observed in the liver; therefore this effect on liver weight was considered related to treatment. Increased ($p \leq 0.05$) relative (to body) ovary weight (95, 131, 92, 137 g, controls to HDT, respectively) was observed at 10,000 ppm (↑37%); however, the effect was unrelated to dose and not corroborated by gross or microscopic examination. All other differences from controls were considered minor and related to treatment.

Table 3. Selected mean absolute (g) and relative to body (%) organ weights in mice treated with Flubendiamide in the diet for up to 90 days [a]

Organ	Dose (ppm)				
	0	50	100	1000	10,000
Males					
Terminal body weight	49.8±6.3	48.0±7.3	47.0±3.5	48.5±3.8	46.9±2.9
Liver absolute	2.52±0.52	2.48±0.74	2.29±0.19	2.44±0.22	2.69±0.36 (↑7)
relative to body	5.03±0.56	5.09±0.66	4.89±0.31	5.04±0.32	5.71±0.55* (↑14)
Females					
Terminal body weight	36.9±4.7	35.2±2.9	36.1±2.5	37.9±3.8	34.6±3.3
Liver absolute	1.71±0.2	1.67±0.19	1.76±0.09	1.99±0.25*(↑16)	1.99±0.29*(↑16)
relative to body	4.67±0.42	4.75±0.36	4.89±0.24	5.25±0.5**(↑12)	5.72±0.36**(↑22)

a Data were obtained from Tables 17-1, 17-2, 18-1, and 18-2 on pages 61-64 of the study report. Percent difference from controls (included in parentheses) were calculated by the reviewers, n=10.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

2. **Gross pathology:** At 10,000 ppm, darkened liver was observed in 4/10 males and 1/10 females compared with 0/10 respective controls (Table 4). All other changes were minor or unrelated to dose.

Table 4. Selected macroscopic findings (# affected/10 animals) in mice treated with Flubendiamide in the diet for up to 90 days [a]

Finding	Dose (ppm)				
	0	50	100	1000	10,000
Males					
Liver dark in color	0	0	0	0	4*
Females					
Liver dark in color	0	0	0	0	1

a Data were obtained from Tables 19 and 20 on pages 65-66 of the study report.
* Significantly different from controls; $p \leq 0.05$

3. **Microscopic pathology:** Selected microscopic findings are presented in Table 5. At 1000 ppm, increased incidence of slight to moderate centrilobular hepatocyte fatty change was observed in 8/10 males ($p \leq 0.01$; vs. 1/10 controls) and 3/10 females (NS; vs. 0/10 controls).

One male each from the 50 and 100 ppm dose groups were also noted with this effect. Increased incidence of slight centrilobular hepatocyte hypertrophy was observed in 6/10 males ($p \leq 0.01$) and 4/10 females ($p \leq 0.05$) vs. 0/10 of respective controls.

At 10,000 ppm, increased incidence ($p \leq 0.05$) of slight to moderate centrilobular hepatocyte fatty change was observed in 9/10 males (vs. 1/10 controls) and 5/10 females (vs. 0/10 controls). Increased incidence ($p \leq 0.05$) of slight to moderate centrilobular hepatocyte hypertrophy was observed in all (10/10) males and 6/10 females vs. 0/10 of respective controls.

These findings in the livers of males were increased in incidence and severity when compared with females.

Table 5. Selected microscopic findings (# affected/10 animals) in mice treated with Flubendiamide in the diet for up to 90 days [a]

Finding	Dose (ppm)				
	0	50	100	1000	10,000
Males					
Liver, centrilobular hepatocyte fatty change					
total	1	1	1	8**	9**
slight	0	0	1	4	3
moderate	1	1	0	4	6
hypertrophy					
total	0	0	0	6**	10**
slight	0	0	0	6	8
moderate	0	0	0	0	2
Females					
Liver, centrilobular hepatocyte fatty change					
total	0	0	0	3	5*
slight	0	0	0	3	5
hypertrophy					
total	0	0	0	4*	6**
slight	0	0	0	4	6

a Data were obtained from Tables 21 and 22 on pages 67-68, Appendices 16 and 17 on pages 166-235 of the study report.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: The Sponsor stated that the LOAEL was 1000 ppm based on increased absolute and relative liver weights in females, and increased incidences of centrilobular hepatocellular fatty change and centrilobular hepatocellular hypertrophy in both sexes.

B. REVIEWER'S COMMENTS: The reviewer agrees with the Investigator's conclusions.

No adverse, treatment-related effects were observed on mortality, clinical signs, body weight, food consumption, food efficiency, or hematology.

At 1000 and 10,000 ppm, slight hepatotoxicity was observed in both sexes. Increased ($p \leq 0.05$) absolute and relative to body liver weights (↑12-22%) were noted in females. Increased incidences ($p \leq 0.05$) of centrilobular hepatocyte fatty change was observed in the males (8-9/10 treated vs. 1/10 controls) and females (3-5/10 treated vs. 0/10 controls); and centrilobular hepatocyte hypertrophy was noted in the males (6-10/10 treated vs. 0/10 controls) and females (4-6/10 treated vs. 0/10 controls). For both lesions, the lesion severity was slight to moderate in males and slight in females.

At 10,000 ppm in males, relative liver weights were increased ($p \leq 0.05$) by 14%, and an increased incidence of darkened liver was observed (4/10 treated vs. 0/10 controls). Increased levels of serum triglycerides were noted in both sexes (↑26-79%); however, the difference was not statistically significant, and the toxicological relevance was considered equivocal.

The LOAEL is 1000 ppm (equivalent to approximately 123/145 mg/kg/day in males/females), based on slight hepatotoxicity in both sexes. The NOAEL is 100 ppm (equivalent to approximately 11.9/14.7 mg/kg/day in males/females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the mouse.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted but would not be expected to alter the conclusions of this study.

- $ Neurological evaluations were not performed.
- $ Ophthalmoscopic examinations were not performed.
- $ Blood clotting measurements, and sorbitol dehydrogenase, potassium and sodium levels were not obtained.
- $ The heart, thymus, uterus, and adrenal glands were not weighed.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.3150 [§82-1b], 90-Day Oral Toxicity Study in Dogs

Work Assignment No 4-1-124 C; formerly 3-1-124 C (MRIDs 46817212 and 46817242)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer
Stephanie E. Foster, M.S.

Signature: Stephanie E. Foster
Date: 1/08/07

Secondary Reviewer
John W. Allran, M.S.

Signature: John W. Allran
Date: 01-10-07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/8/07

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 1/20/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Ayaad Assaad, D.V.M., Ph.D. **Signature:** A Assaad
Toxicology Branch, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:** MJOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity in Dogs (feeding); OPPTS 870.3150 [82-1b]; OECD 409.

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 98.5% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Kuwahara, M. (2003) NNI-0001: Repeated dose 90-day oral toxicity study in dogs. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 01-0062, February 3, 2003. MRID 46817212. Unpublished.

Kuwahara, M. (2001) NNI-0001: Repeated dose 28-day oral toxicity study in dogs. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 01-0019, December 11, 2001. MRID 46817242. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRIDs 46817212 and 46817242), NNI-0001 (Flubendiamide; 98.5% a.i., Lot No. 0FH0010P) was administered to 4 beagle dogs/sex/dose group in the diet at concentrations of 0, 100, 2000, or 40,000 ppm (equivalent to 0/0, 2.58/2.82, 52.8/59.7, and 1076/1135 mg/kg/day in males/females) for 90 days.

No treatment-related adverse effects were observed on mortality, food consumption, ophthalmoscopic examination, or urinalysis.

At 2000 ppm females, loose stool was observed in 3/4 animals from each group compared with 1/4 control dogs. At 2000 ppm, APTT was shortened in males by 9-12% ($p \leq 0.05$) throughout the study and in females by 10-13% ($p \leq 0.05$), but only at Weeks 8 and 13. The significant decreases observed in APTT may indicate an increase in the pro-coagulant activity in the system. In the females, the following differences from controls were observed throughout the study: (i) ALP was increased by 395-892% ($p \leq 0.05$; NS at Week 4) at 2000 ppm; (ii) triglycerides were increased by 25-42% ($p \leq 0.05$; NS at Week 4) at 2000 ppm.

At 2000 ppm in males, absolute and relative to body adrenal weights were increased at ≥2000 ppm; coupled with microscopic findings of slight cortical cell hypertrophy of the adrenals in 2/4 dogs. Also in females, absolute and relative liver weights were increased (↑10-14%; NS). Dilated cerebral ventricle was observed in 1/4 dogs from each group compared with 0 control animals.

At 40,000 ppm, all males (4/4) were observed with loose stool compared with 0/4 control animals. This effect occurred in one dog for 11 weeks, 2 dogs for 4-5 weeks, and in the remaining dog for one week. Additionally in these animals, body weights gradually decreased by 5-10% (Not significant [NS]) beginning at Week 6 and lasting throughout the study, resulting in an overall (Week 0-13) decrease (decr. 41%; NS) in body weight gain.

In the 40,000 ppm males, hemoglobin was increased by 13-16% (p=<0.05) at Weeks 8 and 13, and red blood cell (RBC) counts were increased by 9-18% (only significant at Week 8) throughout the study. At 40,000 ppm, activated partial thromboplastin times (APTT) were shortened by 10-17% (p=<0.01) in both sexes throughout the study. At 2000 ppm, APTT was shortened in males by 9-12% (p=<0.05) throughout the study and in females by 10-13% (p=<0.05), but only at Weeks 8 and 13. The toxicological significance of these observed decreases in APTT is not clear, as this effect is not considered adverse.

At 40,000 ppm, the following effects on clinical chemistry were observed throughout the study: (i) alkaline phosphatase (ALP) was increased (p=<0.05) in males (incr. 263-613%) and females (incr. 641-1484%); and (ii) triglycerides (incr. 33-63%; p=<0.05) were increased in females. Additionally at 2000 ppm, ALP was also increased in males (incr. 71-192%; [NS]) and females (incr. 395-892%; p=<0.05; NS at Week 4), and triglycerides (incr. 25-42%; p=<0.05; NS at Week 4) were increased in females.

In the 40,000 ppm females, cholinesterase was increased by 19% (NS). Because there were no biologically adverse systemic clinical signs associated with increased cholinesterase levels in females, this effect was considered equivocal.

In the 40,000 ppm males, increased (NS) absolute and relative to body adrenal weights (incr. 27-39%) and liver weights (incr. 5-14%) were observed. Upon microscopic examination, slight to moderate cortical cell hypertrophy of the adrenal glands was observed in 2/4 males compared with 0/4 control dogs.

The LOAEL is 2000 ppm (equivalent to 52.7/59.7 mg/kg/day in M/F), based upon clinical signs of toxicity (loose stool), shortened APTT, increased ALP and triglicerides, increased adrenal weights, and microscopic effects on the adrenal glands in the males. The NOAEL is 100 ppm (equivalent to 2.58/2.82 mg/kg/day in M/F).

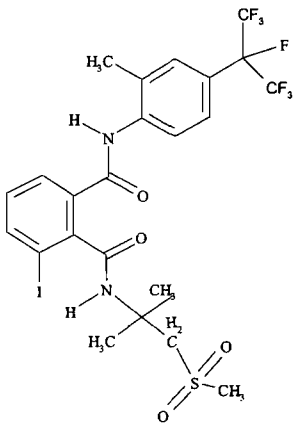
This study is classified as **acceptable/guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: NNI-0001 (Flubendiamide)

Description:	White crystal
Lot #:	0FH0010P
Purity:	98.5% a.i.
Compound stability:	Stable in the test diets kept under sealed, cold, and dark conditions for up to 4 weeks and then exposed to ambient temperature for 8 days.
CAS # if TGAI:	272451-65-7
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/body weight range at treatment initiation:	5-6 months old, 7.5-8.9 kg males; and 6 months old, 6.6-8.8 kg females
Source:	CSK Research Park, Inc. (Toyota, Suwa-shi, Nagano, Japan)
Housing:	Individually in stainless steel cages sustained in racks equipped with automatic sweepers. Exercise in a stainless steel cage was permitted twice a week during treatment for paired dogs of same sex and treatment group.
Diet:	Certified pellet diet DS (Oriental Yeast Co., Ltd., Azusawa, Itabashi, Tokyo, Japan); 250 g of pulverized diet moistened with 250 g of water/day
Water:	Filtered tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22±2° C **Humidity:** 55±15% **Air changes:** At least 10/hour **Photoperiod:** 12 hour light/dark
Acclimation period:	14 days (males) and 36 (females)

B. STUDY DESIGN

1. In life dates: Start: 07/04/01 End: 10/26/01

2. Animal assignment: The dogs were stratified by pre-exposure body weight and then randomly assigned to the test groups shown in Table 1. After allocation, there were no significant differences in the mean body weights among groups and all individual body weights were within 20% of the mean body weight value for each sex. Littermates were not

included in the same treatment group. One female (No. 19) that showed slight lens/vitreous opacity during the acclimatization period ophthalmological examination was allocated to the control group in order to minimize future interpretation of the effects of the test substance on the eye. Also, dogs that manifested outlying values in hematology and clinical chemistry during the acclimatization period were allocated to groups other than the high-dose.

Table 1. Study design [a]

Test Group	Diet concentration (ppm)	Achieved Intake (mg/kg/day, M/F)	# of Animals (M/F)
Control	0	0/0	4/4
Low	100	2.58/2.82	4/4
Mid	2000	52.7/59.7	4/4
High	40,000	1076/1135	4/4

a Data were obtained from page 22 and Tables 11 and 12 on pages 77-78 of MRID 46817212.

3. **Dose selection rationale:** The dose levels were selected based on the results of a 28-day oral toxicity study in dogs (MRID 46817242). A summary of this study is included as an appendix to this DER.

4. **Diet preparation and analysis:** Dietary formulations were prepared once prior to treatment and every four weeks during treatment by mixing the appropriate amount of test material with a small amount of diet to form a premix for each dose level. The premix was further diluted with diet to achieve the desired concentration. The prepared diets were sealed and stored in a cold, dark condition until needed. The amount of test substance to be mixed was not corrected for purity. Approximately 250 grams of diet were moistened with 250 g of water at the time of distribution and given daily to each animal. Top, middle, and bottom samples of all dietary formulations from the first and last preparations were analyzed for homogeneity and concentration verification. In a previously conducted 28-day study (MRID 46817242, reviewed concurrently), the stability of the test compound in the diet at 10 and 40,000 ppm was demonstrated for 4 weeks when kept sealed, cold, and dark and then exposed to ambient temperature for 8 days.

Results:

Stability (mean % of initial): 85-108%

Homogeneity (% coefficient of variation): 1.0-4.8%

Concentration (mean % of nominal): 96-101%

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

5. **Statistics:** Group means were compared at the 5% and 1% significance levels. The

following statistical procedures were used:

Parameter	Statistical Test
Body weight, hematology, clinical chemistry, urine specific gravity, urine volume, absolute and relative organ weights.	Bartlett's test was performed to compare homogeneity of group variances. If Bartlett's was not significant at $p \leq 0.05$, a one-way ANOVA was performed to compare the means. If the ANOVA was significant ($p \leq 0.05$), treated groups were compared with the control group using Dunnett's 2-sided test. If Bartlett's was significant ($p \leq 0.05$), group means were compared using the Kruskal-Wallis test for non-parametric comparison. If the Kruskal-Wallis test was significant ($p \leq 0.05$), treated groups were compared with the control group using a Dunnett-type mean rank sum test.
Food consumption	The non-parametric Kruskal-Wallis test was performed on group means. If $p \leq 0.05$, a Dunnett-type mean rank sum test was applied.

Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

C. METHODS

1. Observations

1a. Clinical Observations: Cage-side examinations for mortality and signs of morbidity were performed at least twice daily on weekdays and once/day on weekends and holidays during the treatment period. A detailed clinical examination was performed on all animals once prior to treatment and weekly throughout the study.

1b. Neurological evaluations: Neurological evaluations were conducted as part of the detailed clinical examinations and included the following checked (X) observations:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture/position		Tremor	X	Vocalization
X	Behavior (including stereotypies)		Lacrimation / chromodacryorrhea	X	Spontaneous motor activity
X	Convulsions (tonic, clonic)		Salivation		
X	Tremors		Piloerection	X	Convulsions
	Vocalization		Convulsion	X	Tremors
	Arousal upon opening cage		Palpebral closure		
X	Spontaneous motor activity		Respiratory rate	X	Urination / defecation
	Gait abnormalities	X	Appearance of fur/skin/nails	X	Changes in skin/fur/eye ball
	SENSORY OBSERVATIONS		Excessive vocalization	X	Gait abnormalities / posture
	Approach response		Exophthalmus	X	Respiration
X	Touch response	X	Muscle tone	X	Bizarre / stereotypic behaviour
X	Startle response	X	Social behavior during removal	X	Lacrimation/salivation
	Pain response		Ease of handling	X	Discharge (conjunctival, oral mucosa)
X	Pupil response/size			X	Palpebral closure
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension		Body weight		Hindlimb extensor strength
	Hindlimb extension		Body temperature		Forelimb grip strength
	Righting reflex				Hindlimb grip strength
	Olfactory orientation				Hindlimb foot splay
			OTHER OBSERVATIONS		Rotarod performance

2. **Body weight:** All animals were weighed prior to feeding one week prior to treatment (Week -1), on the first day of treatment (Week 0), weekly during the study, and at termination.

3. **Food consumption and compound intake:** Food consumption was measured daily during acclimatization and throughout the study. Mean weekly food consumption (g/animal/day) was then calculated from these data. Test substance intake (mg/kg bw/day) was calculated for each week and as an average for the overall study (Weeks 1-13) using the food consumption, body weight, and nominal dietary concentration data.

4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were conducted on all dogs pre-exposure and at Week 13.

5. **Hematology and clinical chemistry:** Blood samples for hematology and clinical chemistry analyses were collected from the cephalic vein of all animals (overnight food-fasted) prior to treatment and at Weeks 4, 8, and 13. The following CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg.,*)	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
X	Blood Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine amino-transferase (also SGPT)*		
X	Aspartate amino-transferase (also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

6. **Urinalysis:** Urine samples were collected from all animals prior to treatment and at Weeks 4, 8, and 13. Urine appearance, volume, and sediments were examined from samples collected for 24 hours, while the remaining parameters were examined from fresh samples. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

7. **Sacrifice and pathology:** At study termination, all animals were overnight fasted, weighed, killed via exsanguination under sodium pentobarbital anesthesia, and subjected to a gross necropsy. The following CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver (with gallbladder)*+
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta thoracic*
XX	Heart*+
X	Bone marrow (sternum, femur, rib)*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
XX	Prostate*
X	Penis
XX	Uterus (with cervix)*+
X	Mammary gland*
X	Vagina
XX	Ovaries*+
X	Oviducts

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve (sciatic)*
X	Spinal cord (3 levels)*
XX	Pituitary*
X	Eyes (with retina and optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal gland
XX	Thyroid (with parathyroid)*+
	OTHER
X	Bone (sternum, femur, rib)
X	Femoral muscle
X	Skin*
X	All gross lesions and masses*
X	Tonsils
X	Buccal mucosa of oral cavity
X	Peyer's Patches
X	Diaphragm

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
+ Organ weight required for non-rodent studies.

Tissue samples were fixed in 10% neutral-buffered formalin with the exception of the eye, and testes. The eyes were fixed for about 3 days in a phosphate-buffered solution of formalin and glutaraldehyde (pH 7.2) and then transferred into 10% neutral-buffered formalin. The testes were fixed for 5-6 days in a solution of formalin, sucrose, and acetic acid (FSA solution), trimmed, and then preserved in 10% neutral-buffered formalin. With the exception of the tonsils, tongue, buccal mucosa of the oral cavity, penis, oviducts, and diaphragm, all collected tissue samples were prepared routinely and stained with hematoxylin and eosin for microscopic examination. Bone marrow samples from ribs were prepared, but not examined. Findings were reported as present or assigned a severity grade.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All dogs survived the treatment period.

2. **Clinical signs of toxicity:** At 40,000 ppm, all males (4/4) were observed with loose stool compared with 0/4 control animals (Table 2). This effect occurred in one dog for 11 weeks, 2 dogs for 4-5 weeks, and in the remaining dog for one week. In the ≥2000 ppm females, loose stool was observed in 3/4 animals from each group compared with 1/4 control dogs. However, the duration (1-3 weeks) of loose stool in the ≥2000 ppm females was comparable to the duration (2 weeks) observed in the control group; therefore this finding was not considered to be adverse in the females. At 100 ppm, one male and one female were observed with loose stool for only one week; therefore, this was considered to be incidental.

   Increased incidence of urination was observed in the 40,000 ppm females. However, this effect was due to one female (# 32) that showed urination throughout the study (13/13 weeks); therefore, this effect was considered to be unrelated to treatment.

Table 2. Clinical signs of toxicity (animal number [duration in weeks]) in dogs treated with Flubendiamide in the diet for up to 90 days. [a]

Clinical Sign	Dose (ppm)			
	0	100	2000	40,000
Males				
Loose stool	0	6 [1]	0	13 [5] 14 [1] 15 [4] 16 [11]
Females				
Loose stool	19 [2]	21 [1]	26 [1] 27 [1] 28 [1]	29 [1] 31 [3] 32 [2]

a Data obtained from Text Table 1 on page 32.

B. **BODY WEIGHT:** Selected body weight and overall body weight gain data are presented in Table 3. In the 40,000 ppm males, body weights gradually decreased by 5-10% (Not significant [NS]) beginning at Week 6 and lasting throughout the study, resulting in an overall (Week 0-13) decrease (↓41%; NS) in body weight gain. Body weights and body weight gains in the remaining males and the treated females were comparable to respective controls.

Table 3. Mean (±SD) body weights and body weight gains (kg) at selected intervals in dogs treated with Flubendiamide in the diet for up to 90 days. [a]

Weeks on Study	Dose (ppm)			
	0	100	2000	40,000
Males				
0	8.3±0.5	8.3±0.5	8.3±0.5	8.3±0.3
6	9.7±0.6	9.6±0.5	9.4±0.7	9.2±0.3 (↓5)
13	11.0±0.7	10.7±0.8	10.4±1.1	9.9±0.4 (↓10)
BWG: 0-13 [b]	2.7	2.4	2.1	1.6 (↓41)
Females				
0	7.9±0.4	8.0±0.3	7.7±0.8	8.0±0.7
6	8.7±0.5	8.7±0.5	8.3±1.1	8.7±0.7
13	9.2±0.6	9.4±0.7	8.9±1.3	9.4±0.7
BWG: 0-13 [b]	1.3	1.4	1.2	1.4

a Data obtained from Tables 7 and 8 on pages 73-74 of MRID 46817212.
b Calculated by the reviewers as a difference in group mean body weight data presented in this table.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** All males ate all provided food throughout the study. Differences from controls in the females were minor and unrelated to dose.

2. **Compound intake:** Mean test material intake values for the overall study are reported in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were observed on ophthalmoscopy. One control female was noted with lens/vitreous body opacity prior to treatment and at Week 13. Otherwise, no abnormalities were detected.

E. BLOOD ANALYSES

1. **Hematology:** In the 40,000 ppm males, hemoglobin was increased by 13-16% ($p \leq 0.05$) at Weeks 8 and 13, and red blood cell (RBC) counts were increased by 9-18% (only significant at Week 8) throughout the study (Table 4a). At 40,000 ppm, activated partial thromboplastin times (APTT) were shortened by 10-17% ($p \leq 0.01$) in both sexes throughout the study.

   At 2000 ppm, APTT was shortened in males by 9-12% ($p \leq 0.05$) throughout the study and in females by 10-13% ($p \leq 0.05$), but only at Weeks 8 and 13. The significant decreases observed in APTT may indicate an increase in the pro-coagulant activity in the system.

   All other differences from controls were transient and/or minor.

Table 4a. Selected mean (±SD) hematology values in dogs treated with Flubendiamide in the diet for up to 90 days [a]

Parameter		Dose (ppm)			
		0	100	2000	40,000
Males					
Hemoglobin (g/dL)	0 weeks	13.9±0.8	13.5±0.7	14.2±0.7	14.2±0.9
	8 weeks	14.3±0.6	13.9±1.0	15.0±1.1	16.2±0.9*(↑13)
	13 weeks	13.7±1.0	14.5±0.9	15.2±0.8	15.9±0.9*(↑16)
RBC (10^6/mm^3)	0 weeks	5.87±0.37	5.82±0.12	5.88±0.40	6.03±0.35
	4 weeks	5.76±0.43	5.91±0.35	6.06±0.78	6.30±0.25 (↑9)
	8 weeks	6.11±0.26	6.07±0.45	6.37±0.54	7.09±0.33*(↑16)
	13 weeks	5.94±0.64	6.53±0.33	6.51±0.51	7.00±0.55 (↑18)
APTT (sec)	0 weeks	13.6±0.5	13.7±0.1	13.6±0.2	14.1±0.7
	4 weeks	13.4±0.4	13.0±0.2	12.2±0.5**(↓9)	12.1±0.3**(↓10)
	8 weeks	13.3±0.6	13.5±0.3	12.1±0.5*(↓9)	11.8±0.5**(↓11)
	13 weeks	13.5±0.7	13.2±0.3	11.9±0.2**(↓12)	11.7±0.2**(↓13)
Females					
APTT (sec)	0 weeks	13.5±0.6	13.4±0.3	13.9±0.5	13.6±0.9
	4 weeks	13.5±0.7	13.3±0.6	12.7±0.9	11.7±0.3**(↓13)
	8 weeks	14.0±0.4	13.5±0.8	12.6±0.7*(↓10)	11.6±0.2**(↓17)
	13 weeks	13.6±0.8	13.6±0.6	11.8±0.9**(↓13)	11.6±0.3**(↓15)

a Data obtained from Tables 17-1, 17-3, and 18-3 on pages 99, 101, and 107 of MRID 46817212.
* Statistically different from controls, $p \leq 0.05$.
** Statistically different from controls, $p \leq 0.01$.

2. **Clinical chemistry:** Selected clinical chemistry data are presented in Table 4b. In the males, alkaline phosphatase (ALP) was increased throughout the study at 40,000 (↑263-613%; $p \leq 0.05$) and 2000 ppm (↑71-192%; [NS]). Increases over controls increased in magnitude with time.

   In the females, the following differences from controls were observed throughout the study: (i) ALP was increased by 641-1484% ($p \leq 0.05$) at 40,000 ppm and by 395-892% ($p \leq 0.05$; NS at Week 4) at 2000 ppm; (ii) triglycerides were increased by 33-63% ($p \leq 0.05$) at 40,000 ppm and by 25-42% ($p \leq 0.05$; NS at Week 4) at 2000 ppm; and (iii) cholinesterase was increased by 19% (NS) at 40,000 ppm. Because there were no biologically adverse systemic clinical signs associated with increased cholinesterase levels in females, this effect was considered equivocal.

   In the 40,000 ppm males, total cholesterol was decreased (↓22-31%; $p \leq 0.05$) at Weeks 4 and 8; however, this finding was unrelated to dose and was not considered treatment-related. All other differences from controls were considered minor and/or transient.

Table 4b. Selected mean (±SD) clinical chemistry values in dogs treated with Flubendiamide in the diet for up to 90 days [a]

Parameter		Dose (ppm)			
		0	100	2000	40,000
Males					
ALP (U/L)	0 weeks	113±21	100±18	99±3	91±19
	4 weeks	104±19	90±13	178±26 (↑71)	377±287*(↑263)
	8 weeks	98±23	84±15	235±31 (↑140)	498±296*(↑408)
	13 weeks	84±17	77±15	245±48 (↑192)	599±362*(↑613)
Females					
ALP (U/L)	0 weeks	79±10	79±7	92±23	95±6
	4 weeks	78±13	81±14	386±192 (↑395)	578±203*(↑641)
	8 weeks	68±14	80±12	524±208*(↑671)	783±281**(↑1051)
	13 weeks	62±16	75±19	615±222*(↑892)	982±399**(↑1484)
Triglycerides (mg/dL)	0 weeks	43±3	54±11	49±6	49±8
	4 weeks	48±5	50±2	60±9 (↑25)	64±9*(↑33)
	8 weeks	43±7	49±3	61±6*(↑42)	70±15**(↑63)
	13 weeks	45±7	48±2	61±5*(↑36)	71±14**(↑58)
Cholinesterase (U/L)	0 weeks	335±58	334±30	316±63	349±62
	4 weeks	336±60	336±36	336±59	399±92 (↑19)
	8 weeks	332±67	324±46	339±50	396±82 (↑19)
	13 weeks	319±69	318±42	328±61	379±83 (↑19)

a Data obtained from Tables 19-1, 20-1, 20-5, and 20-7 on pages 112, 119, 123, and 125 of MRID 46817212.
* Statistically different from controls, $p \leq 0.05$.
** Statistically different from controls, $p \leq 0.01$.

F. URINALYSIS: No treatment-related effects were observed on urinalysis. At 2000 ppm, one female dog (# 27) had blood in the urine at Week 13. Upon microscopic examination, a luteal cyst in the ovary accompanied by an enlarged uterus was observed in this dog, and it was concluded that the urine sample was contaminated with bloody discharge from the vulva.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** In the males, absolute (↑15-27%) and relative to body (↑17-39%) adrenal weights were dose-dependently increased (NS) when compared with controls (Table 5). However, histopathological findings only corroborated this effect at 40,000 ppm. At 40,000 ppm, absolute (↑5%; NS) and relative to body (↑14%; NS) liver weights were also increased.

   There were no treatment-related effects in females. Absolute and relative to body adrenal weights were increased at ≥2000 ppm; however, this effect was not dose-dependent and was therefore not considered to be related to treatment. Microscopic findings revealed slight cortical cell hypertrophy of the adrenals in 2/4 dogs at 2000 ppm and 1/4 dogs at 40,000 ppm (compared with 0 controls); but this finding was slight and also not dose-dependent and was therefore not considered to be related to treatment. Also in females, absolute and relative liver weights were increased (↑10-14%; NS) at ≥2000 ppm, and absolute and relative thyroid weights were increased (↑10-21%; NS) at ≥100 ppm. However, there were no corroborating histopathological findings, so these effects were not considered treatment-related. At 2000 ppm, absolute and relative ovary and uterine weights were increased by 177-108%; however,

this was due to one animal (# 27; mentioned previously) with a luteal cyst in the ovary and hypertrophy of the uterus and was therefore not considered treatment-related.

Table 5. Selected mean (±SD) absolute (g) and relative to body (%) organ weights in dogs treated with Flubendiamide in the diet for up to 90 days [a]

Organ	Dose (ppm)			
	0	100	2000	40,000
Males				
Terminal body weight (kg)	11.0±0.7	10.8±0.8	10.5±1.0	10.0±0.4
Adrenals-absolute (mg)	937±116	1076±152 (↑15)	1090±81 (↑16)	1187±292 (↑27)
relative to body (%)	0.0085±0.001	0.01±0.0008 (↑17)	0.0105±0.0013 (↑24)	0.0118±0.0027 (↑39)
Liver-absolute (g)	286±20	275±35	296±28	299±5 (↑5)
relative to body (%)	2.61±0.31	2.56±0.31	2.83±0.38	2.98±0.12 (↑14)
Females				
Terminal body weight (kg)	9.2±0.5	9.4±0.7	8.9±1.3	9.4±0.7
Adrenals-absolute (mg)	1032±105	1005±191	1251±133 (↑21)	1233±142 (↑19)
relative to body (%)	0.0112±0.0015	0.0109±0.0027	0.0142±0.0016 (↑27)	0.0132±0.0024 (↑18)
Liver-absolute (g)	236±12	226±21	260±33 (↑10)	268±12 (↑14)
relative to body (%)	2.56±0.16	2.42±0.27	2.93±0.33 (↑14)	2.86±0.24 (↑12)

a Data obtained from Tables 21-1 thru 21-4 on pages 126-129 and 131-133 of MRID 46817212.

2. **Gross pathology:** In ≥2000 ppm males, dilated cerebral ventricle was observed in 1/4 dogs from each group compared with 0 control animals. However, this finding was observed in 2/4 females at 20,000 ppm, and in 1/4 males each at 0 (control) and 100 ppm in the corresponding chronic dog study (MRID 46817218, reviewed concurrently). Therefore, this effect was not considered treatment-related. In the 40,000 ppm females, abnormal lobulation of the liver was observed in 1/4 animals compared with 0 controls. This effect was considered minor and not adverse.

3. **Microscopic pathology:** Selected microscopic findings are presented in Table 6. In the males, the following were observed at 40,000 ppm: (i) slight to moderate cortical cell hypertrophy of the adrenal glands in 2/4 animals compared with 0/4 control dogs; and (ii) slight microgranuloma of the liver in 1/4 dogs compared with 0/4 controls. Hepatic microgranuloma in this one male dog was also observed in the 40,000 ppm females at the same incidence in a manner unrelated to dose; therefore this finding was considered unrelated to treatment.

   In the females, slight cortical cell hypertrophy of the adrenal glands was observed in 1/4 dogs at 40,000 ppm and 2/4 dogs at 2000 ppm compared with 0/4 control animals. Also, slight microgranuloma of the liver was noted in 1/4 dogs each in the control, 100, and 40,000 ppm treatment groups. Due to the lack of a dose-response effect, these findings in the females were not considered to be related to treatment.

   All other findings were minor and/or not dose-related.

Table 6. Selected microscopic findings (#affected) in dogs treated with Flubendiamide in the diet for up to 90 days [a]

Finding	Dose (ppm)			
	0	100	2000	40,000
Males				
Adrenal glands				
hypertrophy, cortical cell				
total	0	0	0	2
slight	0	0	0	1
moderate	0	0	0	1
Liver				
microgranuloma				
total (slight)	0	0	0	1
Females				
Adrenal glands				
hypertrophy, cortical cell				
total (slight)	0	0	2	1
Liver				
microgranuloma				
total (slight)	1	1	0	1

a Data (n=4) were obtained from Summary Tables 25 and 26 on pages 136 and 137, and Appendices 22-1 thru 23-16 on pages 250-281 of MRID 46817212.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: The LOAEL was 2000 ppm based on increased ALP and shortened APTT, increased triglycerides, and increased adrenal weights and adrenal cortical cell hypertrophy.

B. REVIEWER COMMENTS: No treatment-related adverse effects were observed on mortality, food consumption, ophthalmoscopic examination, or urinalysis.

At 2000 ppm females, loose stool was observed in 3/4 animals from each group compared with 1/4 control dogs. At 2000 ppm, APTT was shortened in males by 9-12% ($p \leq 0.05$) throughout the study and in females by 10-13% ($p \leq 0.05$), but only at Weeks 8 and 13. The significant decreases observed in APTT may indicate an increase in the pro-coagulant activity in the system. In the females, the following differences from controls were observed throughout the study: (i) ALP was increased by 395-892% ($p \leq 0.05$; NS at Week 4) at 2000 ppm; (ii) triglycerides were increased by 25-42% ($p \leq 0.05$; NS at Week 4) at 2000 ppm.

At 2000 ppm, one female dog had blood in the urine at Week 13. This finding was roled out as contamination with bloody discharge from the vulva.

At 2000 ppm in males, absolute and relative to body adrenal weights were increased at ≥2000 ppm; coupled with microscopic findings of slight cortical cell hypertrophy of the adrenals in 2/4 dogs. Also in females, absolute and relative liver weights were increased (↑10-14%; NS). Dilated cerebral ventricle was observed in 1/4 dogs from each group compared with 0 control animals.

At 40,000 ppm, all males (4/4) were observed with loose stool compared with 0/4 control animals. This effect occurred in one dog for 11 weeks, 2 dogs for 4-5 weeks, and in the remaining dog for one week. Additionally in these animals, body weights gradually decreased by 5-10% (NS) beginning at Week 6 and lasting throughout the study, resulting in an overall (Week 0-13) decrease (↓41%; NS) in body weight gain.

In the 40,000 ppm males, hemoglobin was increased by 13-16% ($p \leq 0.05$) at Weeks 8 and 13, and RBC counts were increased by 9-18% (only significant at Week 8) throughout the study. At 40,000 ppm, APTT were shortened by 10-17% ($p \leq 0.01$) in both sexes throughout the study. At 2000 ppm, APTT was shortened in males by 9-12% ($p \leq 0.05$) throughout the study and in females by 10-13% ($p \leq 0.05$), but only at Weeks 8 and 13. The toxicological significance of these observed decreases in APTT is not clear, as this effect is not considered adverse.

At 40,000 ppm, the following effects on clinical chemistry were observed throughout the study: (i) ALP activity was increased ($p \leq 0.05$) in males (↑263-613%) and females (↑641-1484%); and (ii) triglycerides (↑33-63%; $p \leq 0.05$) were increased in females. Additionally at 2000 ppm, ALP was also increased in males (↑71-192%; [NS]) and females (↑395-892%; $p \leq 0.05$; NS at Week 4), and triglycerides (↑25-42%; $p \leq 0.05$; NS at Week 4) were increased in females.

In the 40,000 ppm females, cholinesterase was increased by 19% (NS). Because there were no biologically adverse systemic clinical signs associated with increased cholinesterase levels in females, this effect was considered equivocal.

In the 40,000 ppm males, absolute and relative to body adrenal weights (↑27-39%) and liver weights (↑5-14%) were increased (NS) when compared with controls. Upon microscopic examination, slight to moderate cortical cell hypertrophy of the adrenal glands was observed in 2/4 males at 40,000 ppm compared with 0/4 control dogs.

The LOAEL is 2000 ppm (equivalent to 52.7/59.7 mg/kg/day in M/F), based upon clinical signs of toxicity (loose stool), shortened APTT, increased ALP and triglicerides, increased adrenal weights, and microscopic effects on the adrenal glands in the males. The NOAEL is 100 ppm (equivalent to 2.58/2.82 mg/kg/day in M/F).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in dogs.

C. **STUDY DEFICIENCIES:** Dose spacing was very large (0, 100, 2000, 40,000 ppm)

APPENDIX

Four Week Dietary Dose Range Finding Study in Dogs

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive subchronic oral toxicity study in dogs (MRID 46817212).

In a 28-day oral toxicity study (MRID 46817242), NNI-0001 (Flubendiamide; 98.5% a.i., Lot No. 0FH0010P) was administered to 1 beagle dog/sex/dose in the diet at doses of 0, 40, 400, 4000, or 40,000 ppm (equivalent to 0/0, 1.12/1.10, 10.7/12.0, 101.1/120, and 1111/1180 mg/kg/day in males/females) for 28 days.

No treatment-related effects were observed on mortality, clinical signs, food consumption, body weights, ophthalmoscopic examination, hematology, urinalysis, gross pathology, or histopathology.

In females, alkaline phosphatase (ALP) levels were increased throughout the study by 51-198% at 400 ppm, 125-415% at 4000 ppm, and 131-384% at 40,000 ppm.

In males at 400 and 4000 ppm, ALP was increased by 21-51% only at Week 4. At 40,000 ppm, ALP was increased over time and in a dose-dependent manner (45-251%).

In both sexes at 40,000 ppm, absolute and relative liver weights were increased by 9-35% over controls.

The LOAEL is 400 ppm (equivalent to 10.7/12.0 mg/kg/day in M/F), based upon increased ALP levels in both sexes. The NOAEL is 40 ppm (equivalent to 1.12/1.10 mg/kg/day in M/F).

This study is classified as **acceptable/non-guideline.**

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

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.3200 [§82-2]; 28-Day Dermal Toxicity in Rats

Work Assignment No. 4-1-124 D; formerly 3-1-124 D (MRID 46817213)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer: Ronnie J. Bever Jr., Ph.D.	Signature: Ronnie J. Bever Jr Date: 01/19/07
Secondary Reviewer Stephanie E. Foster, M.S.	Signature: Stephanie E Foster Date: 01/19/07
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 01/19/07
Quality Assurance: Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 01/19/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

57

EPA Reviewer: Mary Ko Manibusan **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/24/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity in Rats;
OPPTS 870.3200 [' 82-2] (rodent); OECD 410.

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (97.1% a.i.)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Krötlinger, F. (2004) 30-day toxicity study in the rat by dermal administration. Bayer HealthCare AG, Wuppertal, Germany. Laboratory Report No.: AT01704, December 10, 2004. MRID 46817213. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: In a dermal toxicity study (MRID 46817213), NNI-0001 (Flubendiamide; 97.1% a.i.; Batch no. 1FH0019M) was applied to the shaved skin of 10 Fisher rats/sex/dose at dose levels of 0, 10, 100, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week (7 days in Week 4) during a 28-day (males) or 29-day (females) period.

Based on minor increases in absolute and relative liver weights observed in both sexes at 1000 mg/kg/day, increased incidence and severity of minimal to slight elevated fat-positive reaction in the periportal zone in the 1000 mg/kg/day females and increased severity of the same lesion noted in the 1000 mg/kg/day males, along with increased incidence of minimal to slight follicular cell hypertrophy in the 1000 mg/kg/day females, the LOAEL is established at 1000 mg/kg/day and the NOAEL is 100 mg/kgday.

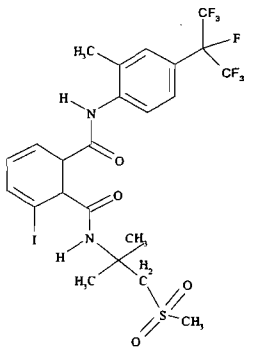
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3200; OECD 410) for a 28-day dermal toxicity study in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description:	White powder
Batch No.:	1FH0019M
Purity:	97.1% a.i.
Stability of compound:	Not tested in this study; test substance applied as undiluted solid
CAS #:	272451-65-7
Structure:	

2. Vehicle and/or positive control: None, applied as an undiluted solid onto a gauze pad moistened with tap water

3. Test animals

Species:	Rat
Strain:	Fisher (F344/NHsd, SPF)
Age and mean weight at start of study:	Approximately 12 weeks old; 230-257 g males; 158-196 g females
Source:	Harlan Netherlands Experimental Animal Breeders (Maastricht, Netherlands)
Housing:	Individually in cages of type IIIH
Diet:	Provimi Kliba® 3883.0.15 fixed-formula standard diet (Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	20-24EC
Humidity:	50-60%
Air changes:	≥10/hour
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	≥5 days

B. STUDY DESIGN

1. In life dates: Start: 11/04/03 End: 12/03/03

2. Animal assignment: Animals were randomly assigned, stratified by weight, to the test groups noted in Table 1.

TABLE 1: Study design [a]			
Test group	**Dose (mg/kg bw/day)**	**# Males**	**# Females**
Control	0	10	10
Low	10	10	10
Mid	100	10	10
High	1000	10	10

a Data were obtained from page 20 of MRID 46817213.

3. **Dose selection rationale:** A dose-selection rationale was not provided.

4. **Preparation and treatment of animal skin:** One day before the first application, the back and flanks of the rats were shorn over an area of at least 10% of the body surface (30.0 cm^2). Any regrowth of hair in areas marked for treatment was shaved off twice weekly. The test substance was transferred to a gauze patch (30.0 cm^2; moistened with tap water), and applied to the clipped skin. The patch was held in place with cohesive stretch tape. The mobility of the rats was impaired with a rat jacket from Lomir Biomedical Inc. The dressings were removed after 6 hours, and the application areas were cleaned with soap and water. The test substance was applied 5 days per week for each of the first 3 weeks and every day of the final week (applied on 8 consecutive days). The males were treated for 28 days and the females were treated for 29 days.

   Rats in the control group were exposed to tap water using the same procedure as described for the treated rats.

5. **Statistics:** Significance was reported at 1% and 5% probability. Outliers (data that appeared implausible) were excluded from analysis.

Parameter	**Statistical procedure**
Food consumption Water consumption Body weight	Statistical procedure was not reported.
Hematology	Analysis of variance (ANOVA) followed by Dunnett's test or the Kruskal-Wallis test followed by an adjusted U-test
Organ weight	ANOVA followed by Dunnett's test
Clinical chemistry	ANOVA followed by Dunnett's test, the Kruskal-Wallis test followed by an adjusted U-test, or the adjusted Welsh test
Urinalysis (specific gravity, volume, and pH)	Kruskal-Wallis test followed by an adjusted U-test

The statistical analyses were considered appropriate, provided that the assumptions of parametric testing were met before such testing was conducted. However, the statistical procedures used to analyze food consumption, water consumption, and body weight data were not reported. Also, it was not specified that an objective test for outliers was used to determine outliers.

55

C. METHODS:

1. Observations

1a. Cageside observations: Animals were observed daily for signs of mortality and toxicity. The shaved skin areas were examined before starting the study and each day of treatment during the study. Skin reddening was scored according to the Draize method. Skin swelling was evaluated on Days 1, 4, 8, 11, 15, 18, 22, 25, and 29 before treatment application. Skinfold thickness in the center of the treatment area was measured using a cutimeter or skinfold caliper by Kroeplin. Measurements were taken at two different locations within the treatment area, and a mean value was calculated.

1b. Clinical examinations: All animals were examined in the open field prior to treatment and weekly during the study. Any clinical findings were recorded. Body surfaces and orifices, posture, general behavior, breathing, excretion, and skin irritation at the dose site were evaluated.

1c. Neurological evaluations: In addition to the limited functional observational battery detailed above, a developmental neurotoxicity screening study (MRID 46817228) was concurrently submitted.

2. Body weight: Animals were weighed prior to initiation of the study and at the beginning of each study week.

3. Food and water consumption: Mean food and water consumption (g/animal/day and g/kg bw/day) were determined weekly.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were performed on all animals during acclimation and on all animals from the control and 1000 mg/kg/day groups on Day 24.

5. Hematology and clinical chemistry: Blood samples for the determination of glucose levels were collected from the caudal vein of all animals (non-fasted and non-anaesthetized) in the morning and deproteinized with perchloric acid (1:10). Blood for the assay of other parameters was withdrawn from the retroorbital venous plexus of all animals (non-fasted) under light carbon dioxide anesthesia prior to scheduled necropsy. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Thrombocyte count*	X	Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
X	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium* (K)		Globulins
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Gamma-glutamyltransferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

6. **Urinalysis:** Urine was collected overnight. All animals were given 5 ml of tap water/animal by gavage before transfer to metabolism cages. Urine was collected during a subsequent 14 hour period while food and water were withheld. A second transfer to metabolism cages was necessary on the next day for male animals, allowing no food during sampling, because the male rat urine was contaminated with food during the first collection. The CHECKED (X) parameters were examined.

	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for 28-day dermal toxicity studies

57

7. **Sacrifice and pathology:** All animals were sacrificed on schedule by exsanguination under deep ether anesthesia and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder* (not rat)
	Bile duct* (rat)
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta *
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
XX	Ovaries*+
XX	Uterus*+ (with cervix)
X	Mammary gland* (skin)
XX	Oviducts
X	Ureters
X	Urethra
X	Vagina

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal gland
X	Parathyroid*
X	Thyroid*
	OTHER
X	Bone (sternum and femur)
X	Skeletal muscle
X	Skin* (treated & untreated areas)
X	Knee joint
X	Head
X	Harderian glands
X	Eyelids
X	Peyer's patches
X	Zymbal's gland
X	All gross lesions and masses*

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
\+ Organ weights required.

All gross abnormalities, lung, liver, kidney, and thyroid gland samples from all animals were examined. Other tissues marked (X) above were examined in the control and 1000 mg/kg/day groups. Eyelids, lacrimal glands, Harderian glands, nasal cavity, larynx, optic nerves, pharynx, tongue, ureters, urethra, vagina, and Zymbal's gland were collected, but were not examined. The eyes, kidneys, eye parts of the optic nerve, and testes were fixed in Davidson's solution; all other tissue samples were fixed with neutral buffered 10% formalin. The lungs and urinary bladders were instilled with 10% neutral buffered formalin, and then postfixed with the other organs. All samples were processed routinely and stained with hematoxylin and eosin. Cryocuts obtained from the liver were stained with Oil Red O. Microscopic lesions were graded as minimal, slight, moderate, marked, or present. A second pathologist performed a cross-check of the diagnosis of all thyroid and liver samples, and all slides of 2 animals per sex of the high dose group.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed.

2. **Mortality:** One male control animal died during blood sampling at the end of the study. Histology revealed hemorrhage and an alveolar edema of the lungs. All other animals survived until scheduled termination.

3. **Neurological evaluations:** No evidence of neurological toxicity was noted.

4. **Dermal Irritation:** No dermal irritation and no effect on skinfold thickness were observed.

B. BODY WEIGHT AND WEIGHT GAIN: No treatment-related effect was observed on body weight or body weight gain (Table 2).

TABLE 2. Average body weights and body weight gains (±SD) in rats treated dermally with NNI-0001 for up to 29 days [a]

Days on Study	Dose (mg/kg/day)			
	0	10	100	1000
Males				
1	245±7.3	241±8.7	244±3.1	242±7.2
29	236±14.2	232±17.1	239±7.2	234±10.5
BWG (1-29)	9	9	5	8
Females				
1	176±8.3	175±5.9	183±10.2	174±8.7
29	163±6.2	160±7.2	166±10.9	161±6.5
BWG (1-29)	13	15	17	13

a Data (n=9-10) were obtained from pages 100-101 of MRID 46817213. Body weight gains (BWG) were calculated by reviewers.

C. FOOD AND WATER CONSUMPTION: No treatment-related effect was noted on food or water consumption. Increased ($p \leq 0.05$) food consumption (g/kg bw/day) was observed in the 100 and 1000 mg/kg/day females on Day 22 (↑8-9%). Increased ($p \leq 0.05$) water consumption (g/kg bw/day) was noted in each treated male group on Day 29 (↑10%, each group). However, these increases in food and water consumption were minor and/or unrelated to dose.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were observed during the ophthalmoscopic examinations.

E. BLOOD ANALYSES

1. **Hematology:** No treatment-related effects were noted on hematology parameters. All differences ($p \leq 0.05$) were minor and/or unrelated to dose, including minor decreases in mean corpuscular volume (↓3%) and mean corpuscular hemoglobin (↓1%) in the 1000 mg/kg/day females.

2. **Clinical chemistry:** No treatment-related effects were observed on clinical chemistry parameters. All differences ($p \leq 0.05$) were minor and/or unrelated to dose, including minor decreases in serum aspartate aminotransferase in the 1000 mg/kg/day females (↓15%).

3. **Urinalysis:** No treatment-related effects were observed on urinalysis parameters.

F. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No treatment-related, adverse effects were noted on organ weights. Minor increases ($p \leq 0.05$) in absolute and relative to body liver weights were observed in both sexes at 1000 mg/kg/day (↑10-16%). Absolute and relative to body uterine weights were decreased ($p \leq 0.05$) in the 1000 mg/kg/day females (↓20-21%); however, there was no corroborating pathological evidence of toxicity. All other differences ($p \leq 0.05$) were minor and/or unrelated to dose.

2. **Gross pathology:** No treatment-related effects were observed on gross pathology.

3. **Microscopic pathology:** No treatment-related effects were observed on microscopic pathology (Table 3). Increased incidence and severity of minimal to slight elevated fat-positive reaction in the periportal zone was observed in the 1000 mg/kg/day females (6/10 treated vs 3/10 controls), and increased severity of the same lesion was noted in the 1000 mg/kg/day males (4/10 treated vs 0/10 controls at slight severity). In the 1000 mg/kg/day females, decreased tinctorial density of the follicular colloid was noted; however, the severity was minimal. Increased incidence of minimal to slight follicular cell hypertrophy was also observed in the 1000 mg/kg/day females (4/10 treated vs 0/10 controls).

TABLE 3. Incidence (# affected/10) of microscopic lesions in selected organs of rats treated dermally with NNI-0001 for up to 29 days [a]

Parameter			Dose (mg/kg/day) 0	10	100	1000
Males						
Liver	Fat + in periportal zone	Total	3	2	6	5
		Minimal	3	2	5	1
		Slight	0	0	1	4
Females						
Liver	Fat + in periportal zone	Total	3	3	2	6
		Minimal	2	3	2	2
		Slight	1	0	0	4
Thyroid	Follicular cell hypertrophy	Total	0	0	0	4
		Minimal	0	0	0	2
		Slight	0	0	0	2
	Decreased colloid density	Total	0	0	0	3
		Minimal	0	0	0	3

a Data were obtained from pages 180, 184, 185 of MRID 46817213.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS: The LOAEL was 1000 mg/kg/day based on effects observed in the liver and thyroids, and decreased MCV, MCH, and aspartate aminotransferase. The NOAEL for dermal toxicity was 1000 mg/kg/day.

B. REVIEWER COMMENTS: Based on minor increases in absolute and relative liver weights observed in both sexes at 1000 mg/kg/day, increased incidence and severity of minimal to slight elevated fat-positive reaction in the periportal zone in the 1000 mg/kg/day females and increased severity of the same lesion noted in the 1000 mg/kg/day males, along with increased incidence of minimal to slight follicular cell hypertrophy in the 1000 mg/kg/day females, the LOAEL is established at 1000 mg/kg/day and the NOAEL is 100 mg/kgday.

C. STUDY DEFICIENCIES: The following deficiencies were noted, but do not alter the conclusions of this review:

- $ Reporting of the methods used for the statistical analyses was incomplete.
- $ Observations for mortality and morbidity should be made at least twice daily.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.3700a [§83-3a]; Developmental Toxicity Study in Rats

Work Assignment No. 4-1-124 F; formerly 3-1-124 F (MRIDs 46817215 and 46817241)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
Stephanie E. Foster, M.S.

Signature: Stephanie E. Foster
Date: 1/31/07

Secondary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 02/02/07

Project Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/31/07

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 2/2/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Myron S. Ottley **Signature:** [signature]
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/12/07
Work Assignment Manager: P.V. Shah **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/17/07
Template version 2/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (96.7% a.i.)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Aoyama, H. (2003) NNI-0001: Teratogenicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 02-0036, December 17, 2003. MRID 46817215. Unpublished.

Aoyama, H. (2002) NNI-0001: Preliminary teratogenicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 01-0113, December 9, 2002. MRID 46817241. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRIDs 46817215 and 46817241), NNI-0001 (Flubendiamide; 96.7% a.i., Lot No. 1FH0019M) in 1% sodium carboxymethylcellulose was administered daily via oral gavage to 24 time-mated specific pathogen-free Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats/group at a dose volume of 10 mL/kg at dose levels of 0, 10, 100, or 1000 mg/kg/day from gestation day (GD) 6 through 19. All surviving dams were killed on GD 20; their fetuses were removed by cesarean section and examined.

All females survived to scheduled sacrifice, and there were no clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body weight gains, food consumption, or gross pathology at any dose.

At >=100 mg/kg/day, both absolute (incr. 8-16%; p=<0.05) and relative to body (incr. 8-15%; p=<0.01) liver weights were increased when compared with controls. Liver weights at 10 mg/kg/day were comparable to controls.

Because clinical chemistry and histopathology were not performed as part of this study, it is difficult to determine the dose at which the increases in liver weights become adverse. In the absence of these data, the increased liver weights would ordinarily be considered equivocal.

However, in the concurrently reviewed subchronic (MRID 46817210) and chronic (MRID 46817217) oral toxicity studies in rats, the LOAEL was approximately 79.3-128 mg/kg/day based on slight hepatotoxicity in females as indicated by increased absolute and relative to body liver weights, hepatocyte hypertrophy and fatty change, increases in gamma-glutamyl transpeptidase, and changes in lipid metabolism. Additionally in the carcinogenicity study in rats (MRID 46817219), the LOAEL was approximately 33.9-43.7 mg/kg/day based on hepatotoxicity in both sexes. Finally, given the known mode of action of this compound (MRID 46817235; reviewed concurrently), the reviewers consider this increase in maternal liver weights a treatment-related adverse effect.

The maternal LOAEL is 100 mg/kg/day based on increased absolute and relative to body liver weights. The maternal NOAEL is 10 mg/kg/day.

There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, resorptions (early or late) or on fetal sex ratio, or post-implantation loss, fetal body weights, litter weights, placental weights, gravid uterine weights, or skeletal ossification in the fetuses. There were no treatment-related external, visceral, skeletal variations or malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

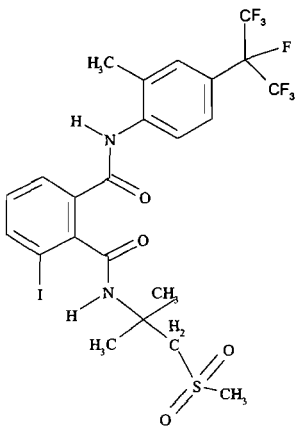
This study is classified **acceptable/guideline (OPPTS 870.3700a)** and satisfies the guideline requirements for a developmental study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide)

Description:	White crystal
Batch #s:	1FH0019M
Purity:	96.7% a.i.
Compound Stability:	Stable in the vehicle for at least two weeks at 5°C.
CAS #of TGAI:	272451-65-7
Structure:	

2. Vehicle - 1% sodium carboxymethylcellulose

3. Test animals

Species:	Rat
Strain:	Specific pathogen-free Wistar Hannover (BrlHan:WIST@Jcl[GALAS])
Age/weight at study initiation:	13 weeks old and 184-253 g on GD 0
Source:	Fuji Breeding Center (Clea, Japan)
Housing:	Individually in suspended, stainless steel, wire-mesh cages
Diet:	MF Mash, (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilized well water, *ad libitum*
Environmental conditions:	
Temperature:	22±2°C
Humidity:	55±15%
Air changes:	Minimum of 10/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	10 days

B. STUDY DESIGN

1. In life dates - Start: 07/22/02 End: 08/09/02

2. Mating - Sexually mature, adult nulliparous females in the proestrus or estrus cycle were placed in steel cages and naturally mated (1:1 basis) overnight with males of the same strain. Mating was confirmed by the presence of a vaginal plug or sperm in a vaginal smear, and the day on which mating was confirmed was designated as gestation day (GD) 0. This mating procedure was repeated for five consecutive days.

3. **Study design** - Female rats were stratified based on GD 0 body weights and then assigned to the test groups reported in Table 1.

Table 1. Study design [a]

Dose (mg/kg/day)	# Females
0	24
10	24
100	24
1000	24

a Data were obtained from page 17 of the study report.

4. **Dose selection rationale** - The dose levels were selected based on the results of a range finding developmental toxicity study in rats (MRID 46817241). A summary of this study is included as an appendix to this DER.

5. **Dosage preparation and analysis** - Dose formulations were prepared by mixing the appropriate amount of the test material with the vehicle. The test formulations were prepared a total of two times at an interval of approximately once every 10 days during the study, and stored at 5° C in the dark until needed. In a previously conducted developmental dose range finding study in rabbits (MRID 46817240, reviewed concurrently), stability of the test substance in the vehicle at 20 and 2000 mg/kg was verified for up to 14 days at 5° C. Because the low dose (10 mg/kg) was not within the range of formulations previously tested for stability, it was analyzed as part of this study prior to initiation of dosing. Homogeneity (top, middle, and bottom) at 10 and 1000 mg/kg was verified prior to dosing only in the first preparation. Concentration analyses were performed on all dose formulations prior to use. Dose levels were not corrected for test substance purity.

Results:

Stability Analysis (range as % nominal): 99-100%

Homogeneity Analysis (range as % coefficient of variance): 0.4-1.1%

Concentration Analysis (range as % nominal): 93-103%

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

6. **Dosage administration** - Dosing suspensions were administered daily from GD 6-19 via oral gavage in a dose volume of 10 mL/kg. Dose volumes were adjusted daily based on the most recent body weight.

C. OBSERVATIONS

1. **Maternal observations and evaluations** - Cage-side observations were conducted at least once daily for clinical signs of toxicity and mortality. In addition, animals were subjected to a complete physical examination when being weighed. Body weights were recorded on GD 0, 6, 9, 12, 15, 18, and 20, with food consumption (g/animal/day) calculated for the corresponding intervals. Body weight gains were calculated by subtracting the body weight value on GD 6 from each value on GD 9, 12, 15, 18, and 20. On GD 20, all dams were euthanized (method not stated), and a gross necropsy was performed. The liver was removed, weighed, fixed, and preserved in 10% neutral-buffered formalin. The gravid uterus and ovaries of each dam were removed, and the following data were recorded: (1) gravid uterus weight; (2) the number of corpora lutea; (3) the number of implantations; (4) the number and position of live and dead fetuses *in utero*; and (5) the number, position, and classification (early, late) of resorption sites. It was not stated if the uteri of apparently non-pregnant females were examined for signs of implantation.

2. **Fetal evaluations** - Upon removal from the uterus, all fetuses were sexed and weighed (including placentas). All live fetuses were euthanized by intraperitoneal injection of sodium pentobarbital solution, and then fetuses were examined for external abnormalities (including those in the orifices). Even-numbered fetuses were examined for thoracic and abdominal visceral changes according to the method of Stuckhardt and Poppe[1]. After visceral examination, these fetuses were preserved in Bouin's solution along with the placentas for at least one week. After fixation, sections of the heads were made using Wilson's razor blade sectioning technique, and the eyes, brain, nasal passages, and tongue were examined. After examination, the head sections and the remaining body parts were preserved in Bouin's solution. Odd-numbered fetuses were fixed in 70% ethanol, stained with Alizarin Red S and Alcian Blue, cleared in 70% glycerin, and examined for skeletal alterations. After examination, skeletal specimens were preserved in 70% glycerin. All fetal alterations were classified as either a variation or a malformation. Examinations were performed blind to dose group designation.

[1] Stuckhardt, J.L., and Poppe, S.M. (1984) Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. Teratogenesis, Carcinogenesis, and Mutagenesis, 4:181-188.

D. DATA ANALYSIS

1. **Statistical analyses** - The following statistical tests were applied to the data:

Parameter	Statistical test
Body weights, body weight gains, adjusted body weights, food consumption, maternal liver weights, number of corpora lutea, implantations, and live fetuses, gravid uterine weights, and fetal and placental weights.	Bartlett's test for equality of variances. If data were homogeneous ($p>0.05$), a one-way ANOVA was used. If the ANOVA was significant, then Dunnett's t-test or Scheffe's test was used to detect any differences between the treated groups and the controls. If data were heterogeneous, then Kruskal-Wallis followed by a pairwise comparison with Dunnett-type mean rank test or Scheffe-type mean rank test were used.
Maternal clinical signs of toxicity, maternal gross findings, incidence of females having fetuses with malformations and variations, incidence of fetal malformations or variations, and fetal sex ratio.	Chi-square test (when all observed values were more than 5) or Fisher's exact probability test (when any one of the observed values was ≤ 5).
% pre-implantation loss, % incidence of resorptions and fetal deaths.	Mann-Whitney's *U*-test.

Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

2. **Indices** - The following index was provided in the study report:

Pre-implantation loss (%) = (# corpora lutea - # implantations)/# corpora lutea x 100

3. **Historical control data** - Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations** - All females survived the treatment period, and there were no clinical signs of toxicity.

2. **Body weight** - There were no treatment-related effects on maternal body weight or body weight gain (Table 2).

Table 2. Mean (±SD) maternal body weights and body weight gains (g) from female rats treated with Flubendiamide via oral gavage during gestational days 6-19 [a].

Interval	Dose in mg/kg/day			
	0	10	100	1000
Pre-treatment, body weight				
GD 0	215±13	218±16	217±13	217±12
Treatment, body weight gain				
GD 6-9	6±4	5±5	7±5	5±4
GD 6-15	31±6	32±7	34±5	32±6
Overall treatment, body weight gain				
GD 6-20	87±10	90±13	93±8	92±10
Gravid uterine weight	64±7	63±13	66±9	67±14
GD 6-20 (corrected)[b]	23	27	27	25

a Data were obtained from Tables 3 and 4 on pages 34 and 35 of MRID 46817215.
b Corrected body weight gains (GD 6-20) were calculated by the reviewers from data presented in this table, as body weight gain GD 6-20 - gravid uterine weight.

3. **Food consumption** - No treatment-related effect was observed on food consumption.

4. **Gross pathology** - No treatment-related effects were observed during necropsy. At ≥100 mg/kg/day, a hepatodiaphragmatic nodule was observed in one animal from each group. No other dose-related findings were observed.

5. **Liver weight** - At ≥100 mg/kg/day, both absolute (↑8-16%; p≤0.05) and relative to body (↑8-15%; p≤0.01) liver weights were increased when compared with controls (Table 3). Liver weights at 10 mg/kg/day were comparable to controls.

Table 3. Selected mean absolute (g) and relative to body (%) liver weights in female mice treated with Flubendiamide via oral gavage during gestational days 6-19 [a]

Organ	Dose (ppm)			
	0	10	100	1000
Adjusted body weight	264±18	270±19	266±16	268±12
Liver absolute	12.998±1.306	13.589±1.401	14.043±1.017*(↑8)	15.087±1.275***(↑16)
relative to body	4.92±0.34	5.04±0.31	5.29±0.25**(↑8)	5.64±0.38***(↑15)

a Data (n=23-24) were obtained from Table 7 on page 38 of MRID 46817215.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01
*** Significantly different from controls; p≤0.001

6. **Cesarean section data** - Cesarean section data are presented in Table 4. There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, resorptions (early or late) or on fetal body weights, litter weights, placental weights, sex ratio, or post-implantation loss.

Table 4. Cesarean section observations from female rats treated with Flubendiamide via oral gavage during gestational days 6-19 [a].

Observation	Dose (mg/kg/day)			
	0	10	100	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	23	24	24	24
Pregnancy Rate (%) [b]	95.83	100	100	100
# Nonpregnant	1	0	0	0
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea [c]	332	348	352	346
Corpora Lutea/Dam	14.4±1.6	14.5±1.7	14.7±1.6	14.4±1.3
Total # Implantations [c]	305	298	318	313
(Implantations/Dam)	13.3±1.7	12.4±2.6	13.3±2.1	13.0±1.9
Total # Litters	23	24	24	24
Total # Live Fetuses [c]	277	283	298	295
(Live Fetuses/Dam)	12.0±1.6	11.8±2.6	12.4±2.0	12.3±2.6
Total # Dead Fetuses [c]	0	2	1	0
(Dead Fetuses/Dam)	0	0.08	0.04	0
Total # Resorptions [c]	28	13	19	18
Early [c]	23	12	16	17
Late [c]	5	1	3	1
Total Resorptions/Dam	1.22	0.54	0.8	0.75
Early	1.0	0.5	0.67	0.71
Late	0.22	0.04	0.13	0.04
Complete Litter Resorption	0	0	0	0
Mean				
Male Fetal Weight (g)	3.544±2.43	3.455±3.03	3.559±1.43	3.528±2.75
Female Fetal Weights (g)	3.376±2.53	3.339±2.24	3.336±1.57	3.445±2.43
Litter Weight (g) [b]	6.92	6.79	6.90	6.97
Placental Weight (mg)	416±28	421±30	408±34	429±35
Sex Ratio (%Male)	48.0	49.1	48.0	51.2
Pre-implantation Loss (%)	8.0	14.5	9.6	9.7
Post-implantation Loss (%)	8.9	4.9	6.1	6.7

a Data were obtained from Table 1 on page 30, Table 8 on pages 39 and 40, and Appendices 25-28 on pages 70-73 of MRID 46817215.
b Calculated by the reviewers from data presented in this table.
c Tabulated by the reviewers from individual data.

B. DEVELOPMENTAL TOXICITY

1. **External examination** - No external variations or treatment-related fetal external malformations were observed. All of the external malformations that occurred at the high dose were found in the same litter (from Dam # 88). Each finding was either observed in only one or two fetuses or occurred with no apparent dose-response relationship.

Table 5. Selected External findings [% fetuses (% litters) affected] [a]				
Observations	**Dose (mg/kg/day)**			
	0	**10**	**100**	**1000** [b]
# Fetuses (# litters) examined	277 (23)	283 (24)	298 (24)	295 (24)
Malformations [c]				
Cleft palate	0 (0)	0 (0)	0 (0)	0.68 (4.17)
Micrognathia	0 (0)	0 (0)	0 (0)	0.34 (4.17)
Preaxial polydactyly	0 (0)	1.06 (4.17)	0 (0)	0 (0)
Acheiria	0 (0)	0 (0)	0 (0)	0.34 (4.17)
Oligodactyly	0 (0)	0 (0)	0 (0)	0.68 (4.17)
Omphalocele	0 (0)	0.35 (4.17)	0.34 (4.17)	0 (0)
Anal atresia	0 (0)	0 (0)	0 (0)	0.34 (4.17)
Short tail	0 (0)	0 (0)	0 (0)	0.68 (4.17)
Edema	0 (0)	0 (0)	0 (0)	0.34 (4.17)
Total number affected [# fetuses (# litters)]	0 (0)	4 (2)	1 (1)	2 (1)

a Data for fetal incidences were obtained from Table 11 on page 43, and Appendices 30-32 on pages 78, 80, 82, and 86 of MRID 46817215.
b All malformations observed at the high dose were found in 2 fetuses from a single litter (Dam # 88).
c Percent fetuses and litter incidence were calculated by the reviewers.

2. **Visceral examination** - No treatment-related visceral malformations or variations were observed. Each finding was either observed in only one fetus per group or occurred with no apparent dose-response relationship.

Table 6. Selected Visceral findings [% fetuses (% litters) affected] [a]				
Observations	**Dose (mg/kg/day)**			
	0	**10**	**100**	**1000** [b]
# Fetuses (# litters) examined	134 (23)	137 (24)	145 (24)	140 (24)
Malformations [c]				
Cleft palate	0 (0)	0 (0)	0 (0)	0.71 (4.17)
Ventricular septal defect	0 (0)	0 (0)	0 (0)	0.71 (4.17)
Undescended testis	0 (0)	0 (0)	0 (0)	0.71 (4.17)
Total number affected [# fetuses (# litters)]	0 (0)	0 (0)	0 (0)	1 (1)
Variations [c]				
Retroesophageal subclavian artery	0 (0)	0 (0)	0 (0)	0.71 (4.17)
Left umbilical artery	16.42 (60.87)	23.36 (62.50)	17.93 (66.67)	20.0 (70.83)
Total number affected [# fetuses (# litters)]	23 (14)	33 (15)	30 (17)	29 (17)

a Data for fetal incidences were obtained from Table 11 on pages 43 and 44, and Appendix 29 on pages 74 – 77 of MRID 46817215.
b All malformations observed at the high dose were found in a single fetus from Dam # 88.
c Percent fetuses and litter incidence were calculated by the reviewers.

3. **Skeletal examination** - All malformations were either observed in only one fetus or occurred with no apparent dose-response relationship. During examination for skeletal variations, incidence of cervical rib was significantly ($p \leq 0.05$) increased at 10 and 100 mg/kg/day, and incidence of supernumerary rib was significantly ($p \leq 0.05$) increased at 10 mg/kg/day when compared with controls. These findings were not considered treatment-related due to the

lack of a dose-response effect.

Table 7. Selected Skeletal findings [% fetuses (% litters) affected] [a]

Observations	Dose (mg/kg/day)			
	0	10	100	1000 [b]
# Fetuses (# litters) examined	143 (23)	146 (24)	153 (24)	155 (24)
Malformations [c]				
Extra ossification center of thoracic vertebral arch	0 (0)	0 (0)	0 (0)	0.65 (4.17)
Fused rib	0 (0)	0 (0)	0 (0)	0.65 (4.17)
Preaxial polydactyly	0 (0)	1.37 (4.17)	0 (0)	0 (0)
Absent ulna	0 (0)	0 (0)	0 (0)	0.65 (4.17)
Absent phalanx distal to carpal bone	0 (0)	0 (0)	0 (0)	0.65 (4.17)
Absent caudal vertebra	0 (0)	0 (0)	0 (0)	0.65 (4.17)
Total number affected [# fetuses (# litters)]	0 (0)	2 (1)	0 (0)	1 (1)
Variations [c]				
Cervical rib	6.99 (17.39)	1.37*(8.33)	1.96*(12.5)	3.23 (20.83)
Supernumerary rib	35.66 (86.96)	46.58*(91.67)	38.56 (79.17)	43.87 (87.5)
Total number affected [# fetuses (# litters)]	64 (20)	74 (24)	63 (20)	78 (23)

a Data for fetal incidences were obtained from Table 11 on pages 43 and 44, and Appendix 29 on pages 74 – 77 of MRID 46817215.
b All malformations observed at the high dose were found in a single fetus from Dam # 88.
c Percent fetuses and litter incidence were calculated by the reviewers.
* Statistically different from controls, $p \leq 0.05$.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - It was concluded that the maternal LOAEL was 100 mg/kg/day based on increased liver weights in dams. The developmental LOAEL was not observed.

B. REVIEWER COMMENTS

1. **Maternal toxicity** - All females survived to scheduled sacrifice, and there were no clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body weight gains, food consumption, or gross pathology at any dose.

   At ≥100 mg/kg/day, both absolute (↑8-16%; $p \leq 0.05$) and relative to body (↑8-15%; $p \leq 0.01$) liver weights were increased when compared with controls. Liver weights at 10 mg/kg/day were comparable to controls.

   Because clinical chemistry and histopathology were not performed as part of this study, it is difficult to determine the dose at which the increases in liver weights become adverse. In the absence of these data, the increased liver weights would ordinarily be considered equivocal. However, in the concurrently reviewed subchronic (MRID 46817210) and chronic (MRID 46817217) oral toxicity studies in rats, the LOAEL was approximately 79.3-128 mg/kg/day based on slight hepatotoxicity in females as indicated by increased absolute and relative to

body liver weights, hepatocyte hypertrophy and fatty change, increases in gamma-glutamyl transpeptidase, and changes in lipid metabolism. Additionally in the carcinogenicity study in rats (MRID 46817219), the LOAEL was approximately 33.9-43.7 mg/kg/day based on hepatotoxicity in both sexes. Finally, given the known mode of action of this compound (MRID 46817235; reviewed concurrently), the reviewers consider this increase in maternal liver weights a treatment-related adverse effect.

The maternal LOAEL is 100 mg/kg/day based on increased absolute and relative to body liver weights. The maternal NOAEL is 10 mg/kg/day.

2. Developmental toxicity

a. Deaths/Resorptions: There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, resorptions (early or late) or on sex ratio, or post-implantation loss.
b. Altered Growth: There were no treatment-related effects on fetal body weights, litter weights, placental weights, gravid uterine weights or skeletal ossification in the fetuses.
c. Developmental Variations: There were no treatment-related external, visceral, or skeletal variations.
d. Malformations: There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

This study is classified **acceptable/guideline (OPPTS 870.3700a)** and satisfies the guideline requirements for a developmental study in the rat.

C. STUDY DEFICIENCIES - The following deficiency was noted but does not affect the conclusions of this DER:

- Historical control data were not provided.

APPENDIX

Developmental Dose Range Finding Study in Rats

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive developmental toxicity study in rats (MRID 46817215).

In a range finding developmental toxicity study (MRID 46817241), NNI-0001 (Flubendiamide; 98.5% a.i., Lot No. OFH0010P) in 1% sodium carboxymethylcellulose was administered daily via oral gavage to 7 time-mated female specific pathogen-free Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats/group at a dose volume of 10 mL/kg at dose levels of 0, 20, 100, or 1000 mg/kg/day from gestation day (GD) 6 through 19. All surviving dams were killed on GD 20; their fetuses were removed by cesarean section and examined.

No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, or gross pathology at any dose.

The maternal LOAEL was not observed. The maternal NOAEL was 1000 mg/kg/day.

There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, resorptions (early or late) or on fetal body weights, sex ratio, or post-implantation loss, gravid uterine weights, or skeletal ossification in the fetuses. There were no treatment-related external, visceral, or skeletal variations or malformations. Increased ($p \leq 0.05$) incidence of supernumerary ribs was observed at 1000 mg/kg/day (22/37) when compared with controls (15/41); however, it was difficult to discern if this was a dose-related effect because only the control and high-dose groups were evaluated for skeletal variations and malformations. Also, it was stated that incidence of supernumerary ribs is commonly observed in this strain of rats.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day.

The study is classified as an **acceptable/non-guideline** developmental toxicity study in rats.

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

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.3700b [§83-3b]; Developmental Toxicity Study in Rabbits

Work Assignment No. 4-1-124 E; formerly 3-1-124 E (MRIDs 46817214 and 46817240)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
Stephanie E. Foster, M.S.
Signature: Stephanie E. Foster
Date: 1/16/07

Secondary Reviewer:
John W. Allran, M.S.
Signature: John W. Allran
Date: 01-16-07

Project Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 1/17/07

Quality Assurance:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 1/20/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Myron S. Ottley **Signature:** MSOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/12/07
Work Assignment Manager: P.V. Shah **Signature:** P.V.Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/17/07

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 96.7% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Takahashi, K. (2002) NNI-0001: Teratogenicity study in rabbits. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 01-0128, December 5, 2002. MRID 46817214. Unpublished.

Takahashi, K. (2001) NNI-0001: Preliminary teratogenicity study in rabbits. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 01-0030, September 21, 2001. MRID 46817240. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a developmental toxicity study (MRIDs 46817214 and 46817240), NNI-0001 (Flubendiamide; 96.7% a.i., Lot No. 1FH0019M) in 1% sodium carboxymethylcellulose was administered daily via oral gavage to 25 artificially inseminated female Japanese White (Kbl:JW) rabbits/group at a dose volume of 5 mL/kg at dose levels of 0, 20, 100, or 1000 mg/kg/day from gestation day (GD) 6 through 27. All surviving rabbits were killed on GD 28; their fetuses were removed by cesarean section and examined.

No effects of treatment were observed on mortality, body weight, body weight gain, or gross pathology at any dose level.

At 1000 mg/kg, incidence of loose stool was observed in 7/24 ($p=<0.01$) animals beginning on GD 20 compared with 0/22 control animals. Six of these animals only had one occurrence, while one animal (#89) was observed with loose stool three times during GD 26-28. Also at 1000 mg/kg/day, food consumption was decreased by 35% ($p=<0.05$) during the last day of treatment (GD 27-28).

The maternal LOAEL is 1000 mg/kg/day (the limit dose) based on incidence of loose stool, and decreased food consumption. The maternal NOAEL is 100 mg/kg/day.

There were no premature deliveries and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, or resorptions (early or late) per doe or on fetal sex ratio, post-implantation loss, fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses. There were no treatment-related external, visceral, or skeletal variations or malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day.

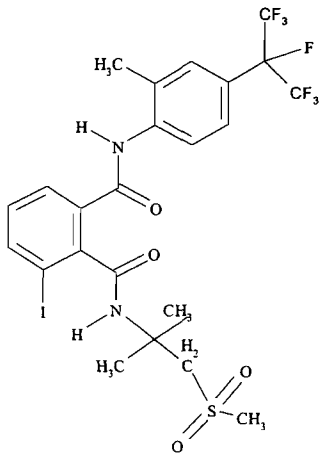
This study is classified **acceptable/guideline (OPPTS 870.3700b)** and satisfies the guideline requirements for a developmental study in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide)

Description:	White crystal
Lot #:	1FH0019M
Purity:	96.7% a.i.
Compound Stability:	Stable in the dosing suspension for at least two weeks at 5°C.
CAS #of TGAI:	272451-65-7
Structure:	

2. Vehicle: 1% sodium carboxymethylcellulose

3. Test animals

Species:	Rabbit
Strain:	Specific pathogen-free Japanese White (Kbl:JW)
Age/weight at study initiation:	18 weeks old and 3.231-4.196 kg
Source:	Minowa Breeding Center, Kitayama Labes Co., Ltd. (Kamiina-gun, Nagano, Japan)
Housing:	Individually in suspended aluminum cages with wire-mesh floors.
Diet:	Certified solid feed (LRC4, Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilized tap water, *ad libitum*
Environmental conditions:	
Temperature:	22±2°C
Humidity:	55±15%
Air changes:	Minimum of 10/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	Approximately 10 days

B. STUDY DESIGN

1. In life dates - Start: 01/21/02 End: 02/18/02

2. Mating - Sexually mature, adult nulliparous females were artificially inseminated with semen from males of the same strain from the same breeder. Semen was first taken from two or more males, then quality semen was pooled and diluted (10-fold) with physiological saline. Diluted semen (0.5 ml) was injected into the vagina of each female. After semen injection,

each female received 25 U of chorionic gonadotropin through the auricular vein to ensure ovulation. This procedure was repeated for five days at a rate of 20 females/day. The day of artificial insemination was designated as gestation day (GD) 0.

3. **Study design** - Female rabbits were stratified based on GD 0 body weights and then assigned to the test groups reported in Table 1.

Table 1. Study design [a]

Dose (mg/kg/day)	# Females
0	25
20	25
100	25
1000	25

a Data were obtained from page 16 of MRID 46817214.

4. **Dose selection rationale:** The dose levels were selected based on the results of a range finding developmental toxicity study in rabbits (MRID 46817240). A summary of this study is included as an appendix to this DER.

5. **Dosage preparation and analysis:** Dose formulations were prepared by mixing the appropriate amount of the test material with the vehicle. The test formulations were prepared approximately once every week during the study (four times in total). Stability of the test substance in the vehicle at 15 and 1000 mg/kg was verified for up to 14 days at 5° C. Homogeneity (top, middle, and bottom) at 20 and 1000 mg/kg was verified prior to dosing only in the first preparation. Concentration analyses were performed on all dose formulations weekly prior to use.

Results:

Stability (% of nominal): 94-106%

Homogeneity (% coefficient of variation): 1.3-1.4%

Concentration (mean % of nominal): 99-104%

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

6. **Dosage administration**: Dosing suspensions were administered daily from GD 6-27 via oral gavage in a dose volume of 5 mL/kg. Dose volumes were adjusted daily based on the most recent body weight.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** Cage-side observations were conducted twice daily during dosing to determine mortality or clinical signs of toxicity. In addition, animals were subjected to a complete physical examination when being weighed. Body weights were recorded on GD 0, 6, 9, 12, 15, 18, 21, 24, 27, and 28. Body weight gains were calculated by subtracting the body weight value on GD 6 from each value on GD 9, 12, 15, 18, 21, 24, 27, and 28. Individual food consumption was recorded on GD 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 28 and was reported as a daily mean (g/animal/day) between these intervals. On GD 28, all females were euthanized by intravenous injection of sodium pentobarbital, and a gross necropsy was performed. The gravid uterus and ovaries of each rabbit were removed, and the following data were recorded: (i) pregnancy status; (ii) gravid uterus weight; (iii) the number of corpora lutea; (iv) the number and position of live fetuses *in utero*; (v) the number and position of dead fetuses; and (vi) the number, position, and classification (early, late) of resorption sites. The uteri of apparently non-pregnant females were stained with a 10% ammonium sulfide solution and examined for signs of implantation.

2. **Fetal evaluations:** Upon removal from the uterus, all fetuses were sexed and weighed (including placentas). All live fetuses were euthanized by intraperitoneal administration of sodium pentobarbital solution, and then fetuses were examined for external abnormalities (including those in the orifices). The eyes were also examined after removing the palpebral skin. Then, all fetuses were examined for thoracic and abdominal visceral changes according to the method of Stuckhardt and Poppe[1]. After visceral examination, the thoracic and abdominal soft tissues were removed and preserved in Bouin's solution along with the placentas. All even-number fetuses were examined for eye abnormalities after removing the cranial skin. The eyes were then removed and preserved in Bouin's solution along with the thoracic and abdominal soft tissues. The brain of each rabbit was examined by making a transverse razor cut through the coronal suture of the skull. Then the odd-numbered fetuses were decapitated and the heads were fixed in Bouin's solution for at least four weeks. After fixation, sections of the heads were made using Wilson's razor blade sectioning technique, and the eyes, brain, nasal passages, and tongue were examined. Finally, sections of the head were preserved in Bouin's solution along with the thoracic and abdominal soft tissues. All fetal carcasses (except for the heads of the odd-numbered fetuses) were fixed in ethanol, eviscerated, cleared, stained by the Alizarin technique, and examined for skeletal alterations. All fetal alterations were classified as either a variation or a malformation. Examinations were performed blind to dose group designation.

D. DATA ANALYSIS

1. **Statistical analyses:** The following statistical tests were applied to the data:

[1] Stuckhardt, J.L., and Poppe, S.M. (1984) Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. Teratogenesis, Carcinogenesis, and Mutagenesis, 4:181-188.

Parameter	Statistical test
Maternal body weight, adjusted maternal body weights, maternal body weight gain, food consumption, number of corpora lutea, implantations, live fetuses, gravid uterine weights, and fetal and placental weights.	Bartlett's test for equality of variances. If variances were homogeneous ($p \geq 0.05$), a one-way ANOVA was used. If the ANOVA was significant, then Dunnett's t-test or Scheffe's test was used to detect any differences between the treated groups and the controls. If variances were heterogeneous, then Kruskal-Wallis followed by a pairwise comparison with Dunnett-type mean rank test or Scheffe-type mean rank test was used.
Maternal clinical signs of toxicity, maternal gross findings, incidence of females having fetuses with malformations and variations, incidence of fetal malformations or variations, and fetal sex ratio.	Chi-square test (when all observed values were >5) or Fisher's exact probability test (when any one of the observed values was ≤ 5).
% pre-implantation loss, % incidence of resorptions and fetal deaths.	Mann-Whitney's U-test.

Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

2. **Indices:** The following index was provided in the study report:

   Pre-implantation loss (%) = (# corpora lutea - # implantations)/# corpora lutea x 100

3. **Historical control data:** Historical control data, regarding fetal malformations and variations, were provided from 16 studies conducted from 1992-2001 at the performing laboratory. (See Appendices 40-1 thru 40-5 pages 118-122 of the study report).

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations:** All females survived the treatment period.

   At 1000 mg/kg, incidence of loose stool was observed in 7/24 ($p \leq 0.01$) animals beginning on GD 20 compared with 0/22 control animals. Six of these animals only had one occurrence, while one animal (#89) was observed with loose stool three times during GD 26-28. All other findings were minor and/or sporadic.

2. **Body weight:** No treatment-related effects were observed on maternal body weights or body weight gains. At 1000 mg/kg, body weight gain appeared to be slightly decreased when compared with controls. However there was no clear dose-response effect when compared among the treated groups and the decreases were not statistically significant, so an effect on body weight gain at 1000 mg/kg was difficult to discern.

Table 2. Mean (±SD) maternal body weight and body weight gain (g) in female rabbits treated with Flubendiamide via oral gavage during gestational days 6-27 [a].

Interval	Dose in mg/kg/day			
	0	20	100	1000
Pre-treatment				
GD 0	3745±229	3722±259	3721±246	3736±254
Treatment				
GD 6	3891±233	3847±263	3850±263	3878±287
GD 12	3906±250	3850±282	3883±274	3914±293
GD 21	4014±258	3942±291	3966±313	3999±297
GD 27	4108±219	4024±296	4057±312	4058±295
GD 27 (adjusted) [b]	3718	3631	3661	3631
Gravid uterine weight	390±121	393±125	396±137	427±111
BWG: GD 6-9 [c]	-9	-11	-5	9
BWG: GD 15-18 [c]	4	7	2	0
BWG: GD 18-21 [c]	45	39	32	29
BWG: GD 21-24 [c]	61	50	49	51
BWG: GD 24-27 [c]	33	32	42	8
BWG: GD 27-28 [c]	6	27	24	-7
BWG: GD 6-27	217±125	177±90	207±105	179±146

a Data were obtained from Tables 3 and 4 on pages 33 and 34 of MRID 46817214.
b Corrected body weight = body weight GD 27 – gravid uterine weight, calculated by the reviewers.
c Calculated by the reviewers from data presented in Table 3 on page 33 of MRID 46817214.

3. **Food consumption:** Food consumption data are reported in Table 3. At 1000 mg/kg/day, food consumption was decreased by 35% ($p \leq 0.05$) during the last day of treatment (GD 27-28). Food consumption in the remaining treatment groups was comparable to controls throughout the study.

Table 3. Mean selected food consumption values (g) in female rabbits treated with Flubendiamide via oral gavage during gestational days 6-27 [a].

Interval	Dose in mg/kg/day			
	0	20	100	1000
GD 0-3	166±19	171±24	160±21	173±28
GD 12-15	145±31	138±46	146±26	149±25
GD 24-27	118±42	114±44	115±33	89±36
GD 27-28	117±43	109±41	111±32	76±49* (↓35)

a Data were obtained from Table 5 on page 35 of MRID 46817214. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Statistically different from controls, $p \leq 0.05$.

4. **Gross pathology:** No treatment-related effects were observed during necropsy.

5. **Cesarean section data:** Cesarean section data are presented in Table 4. There were no premature deliveries and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, or resorptions (early or late) per doe or on fetal body weights, sex ratio, or post-implantation loss. There were complete litter resorptions in 3 control females and 1 female at 20 mg/kg/day.

Table 4. Cesarean section observations from female rabbits treated with Flubendiamide via oral gavage during gestational days 6-27 [a].

Observation	Dose (mg/kg/day)			
	0	20	100	1000
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	22	24	24	24
Pregnancy Rate (%) [c]	88	96	96	96
# Nonpregnant [b]	0	0	1	1
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea [b]	238	253	258	246
Corpora Lutea/Doe	10.8±2.4	10.5±2.2	10.8±2.5	10.3±1.5
Total # Implantations [b]	172	180	186	197
(Implantations/Doe)	7.8±2.9	7.5±2.7	7.8±3.0	8.2±2.3
Total # Litters	22	24	23	24
Total # Live Fetuses [b]	158	163	170	179
(Live Fetuses/Doe)	7.2±2.9	6.8±2.7	7.1±2.9	7.5±2.2
Total # Dead Fetuses [b]	2	4	5	5
(Dead Fetuses/Doe)	0.1	0.2	0.2	0.2
Total # Resorptions [b]	12	13	11	13
Early [b]	11	10	9	10
Late [b]	1	3	2	3
Total Resorptions/Doe [c]	0.5	0.5	0.5	0.5
Early	0.5	0.4	0.4	0.4
Late	0	0.1	0.1	0.1
Complete Litter Resorption	3	1	0	0
Mean				
Fetal Weight (g), Males	37.8±4.5	40.3±4.9	38.6±4.0	38.8±4.5
Fetal Weight (g), Females	38.1±5.1	39.3±5.3	38.0±4.4	38.4±4.6
Litter Weight (g) [b]	75.9	79.6	76.6	77.2
Placental weight (g)	5.333±1.0	5.577±0.7	5.196±0.9	5.583±0.9
Sex Ratio (% Male)	46.8	49.7	48.2	50.3
Pre-implantation Loss (%)	28.0	28.2	26.1	19.9
Post-implantation Loss (%)	8.8	10.0	11.5	8.6

a Data were obtained from Table 7 on pages 37-38 and Appendices 21-24 on pages 68-71 of MRID 46817214.
b Calculated by the reviewers from individual data in Appendices 21-24 on pages 68-71.
c Calculated by the reviewers from data presented in this table.

B. DEVELOPMENTAL TOXICITY

1. **External examination:** No treatment-related fetal external variations or malformations were observed. The only finding observed was within the historical control data range and is presented in Table 5a.

Table 5a. External findings [% fetuses (% litters) affected] [a]

Observations	Dose (mg/kg/day)				
	0	20	100	1000	Historical control [b]
# Fetuses (# litters) examined	158 (22)	163 (24)	170 (23)	179 (24)	117-182 (14-24)
Malformation [c]					
Local edema	0 (0)	0.61 (4.2)	0 (0)	0.56 (4.2)	0-0.56 (0-4.55)
Total number affected [# fetuses (# litters)]	0 (0)	1 (1)	0 (0)	1 (1)	NA

a Data for fetal incidences were obtained from Table 10 on page 41 and Appendices 26 and 28 on pages 76 and 84 of MRID 46817214.
b Data were obtained from Appendix 40-1 on page 118 of MRID 46817214.
c Percent fetuses and litter incidence were calculated by the reviewers.
NA Not available

2. **Visceral examination:** No treatment-related fetal visceral malformations or variations were observed (Table 5b). At 1000 mg/kg/day, incidence of enlarged thyroid gland was increased (0.56% of fetuses) when compared with concurrent (0 fetuses) and historical control (0 fetuses) values; however, this effect was only observed in one fetus and was considered to be minor. All other visceral findings were unrelated to dose.

Table 5b. Selected visceral findings [% fetuses (% litters) affected] [a]

Observations	Dose (mg/kg/day)				
	0	20	100	1000	Historical control [b]
# Fetuses (# litters) examined	85 (22)	88 (24)	91 (23)	95 (24)	117-182 (14-24)
Malformations [c]					
Small spleen	0 (0)	0 (0)	0 (0)	0.56 (4.2)	0-0.64 (0-5.56)
Enlarged thyroid gland	0 (0)	0 (0)	0 (0)	0.56 (4.2)	0 (0)
Total number affected [# fetuses (# litters)]	2 (2)	3 (3)	1 (1)	4 (3)	NA
Variations [c]					
Malpositioned carotid branch (left)	7.59 (31.82)	7.98 (37.5)	8.82 (30.43)	7.26 (25.0)	NA
Thymic remnant in the neck	1.90 (13.64)	1.84 (12.5)	2.94 (13.04)	1.68 (8.30)	NA
Extra artery arising from aortic arch	0 (0)	0.61 (4.2)	0 (0)	0 (0)	NA
Total number affected [# fetuses (# litters)]	15 (9)	17 (11)	20 (8)	16 (8)	NA

a Data for fetal incidences were obtained from Tables 10 and 11 on pages 41 and 42 of MRID 46817214.
b Data were obtained from Appendix 40-2 on page 119 of MRID 46817214.
c Percent fetuses and litter incidence were calculated by the reviewers.
NA Not available

3. **Skeletal examination:** All skeletal malformations and variations were either observed in a single fetus, not dose-related, or were within historical controls; therefore they were not considered treatment-related (Table 5c).

Table 5c. Selected skeletal findings [% fetuses (% litters) affected] [a]

Observations	Dose (mg/kg/day)				
	0	20	100	1000	Historical control [b]
# Fetuses (# litters) examined	158 (22)	163 (24)	170 (23)	179 (24)	117-182 (14-24)
Malformations [c]					
Fused sternebra	0 (0)	0.61 (4.2)	1.18 (8.7)	1.12 (8.3)	0-2.42 (0-18.75)
Absent rib	0 (0)	0 (0)	0 (0)	0.56 (4.2)	0-0.64 (0-5.56)
Short rib	0 (0)	0 (0)	0 (0)	0.56 (4.2)	0-0.68 (0-5.88)
Total number affected [# fetuses (# litters)]	0 (0)	1 (1)	5* (4)	3 (3)	NA
Variations [c]					
Supernumerary rib	34.81 (77.27)	44.79 (87.5)	35.29 (82.61)	28.49 (83.33)	NA
27 presacral vertebrae	8.86 (36.36)	14.11 (66.67)	7.06 (39.13)	8.94 (37.5)	NA
Total number affected [# fetuses (# litters)]	60 (18)	74 (21)	67 (20)	57 (21)	NA

a Data for fetal incidences were obtained from Tables 10 and 11 on pages 41 and 42 of MRID 46817214.
b Data were obtained from Appendices 40-2 and 40-3 on pages 119-120 of MRID 46817214.
c Percent fetuses and litter incidence were calculated by the reviewers.
NA Not available

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** It was concluded that the maternal LOAEL was 1000 mg/kg/day based on incidence of loose stool, decreased body weight, body weight gain, and food consumption. The developmental LOAEL was not observed, and the NOAEL was 1000 mg/kg/day (the limit dose).

B. REVIEWER COMMENTS

1. **Maternal toxicity:** At 1000 mg/kg, incidence of loose stool was observed in 7/24 ($p \leq 0.01$) animals beginning on GD 20 compared with 0/22 control animals. Six of these animals only had one occurrence, while one animal (#89) was observed with loose stool three times during GD 26-28. Also at 1000 mg/kg/day, food consumption was decreased by 35% ($p \leq 0.05$) during the last day of treatment (GD 27-28).

   At 1000 mg/kg body weight gain appeared to be slightly decreased when compared with controls, however there was no clear dose-response effect when compared among the treated groups, so an effect on body weight gain at 1000 mg/kg was difficult to discern.

The maternal LOAEL is 1000 mg/kg/day (the limit dose) based on incidence of loose stool, and decreased food consumption. The maternal NOAEL is 100 mg/kg/day.

2. Developmental toxicity

a. **Deaths/Resorptions:** There were no premature deliveries and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, or resorptions (early or late) per doe or on fetal body weights, sex ratio, or post-implantation loss. There were complete litter resorptions in 3 control females and 1 female at 20 mg/kg/day.
b. **Altered Growth:** There were no treatment-related effects on fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses.
c. **Developmental Variations:** There were no treatment-related external, visceral, or skeletal variations.
d. **Malformations:** There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day.

This study is classified **acceptable/guideline (OPPTS 870.3700b)** and satisfies the guideline requirements for a developmental study in the rabbit.

C. **STUDY DEFICIENCIES:** No deficiencies were noted.

APPENDIX

Developmental Dose Range Finding Study in Rabbits

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive developmental toxicity study in rabbits (MRID 46817214).

In this range finding developmental toxicity study (MRID 46817240), NNI-0001 (Flubendiamide; 98.5% a.i., Lot No. OFH0010P) in 1% sodium carboxymethylcellulose was administered daily via oral gavage to 6 artificially inseminated female Japanese White (Kbl:JW) rabbits/group at a dose volume of 5 mL/kg at dose levels of 0, 30, 100, 300, or 1000 mg/kg/day from gestation day (GD) 6 through 27. All surviving rabbits were killed on GD 28; their fetuses were removed by cesarean section and examined.

No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, or gross pathology at any dose.

One doe in the control group (No. 1002) aborted on GD 21 and was therefore euthanized on that day. Examination of this animal only revealed soiled fur in the perianal area, and the cause of the abortion was not found. One doe at 300 mg/kg/day was observed dragging the left hindlimb from GD 8 throughout the remainder of the study. However, upon examination, it was noted that this animal had a broken femur and this effect was not treatment-related.

The maternal LOAEL was not observed. The maternal NOAEL was 1000 mg/kg/day.

There were no effects of treatment on the numbers of litters, live fetuses, dead fetuses, or resorptions (early or late) per doe or on fetal sex ratio, post-implantation loss, fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses. There were complete litter resorptions in 2 females at 30 mg/kg/day, 1 female at 100 mg/kg/day, and in 1 female at 300 mg/kg/day. One control female aborted on GD 21. There were no treatment-related external, visceral, or skeletal variations or malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day.

The study is classified **acceptable/non-guideline.**

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

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.3800 [§83-4]; Multigeneration Reproduction Study in Rats

Work Assignment No. 4-1-124 G; formerly 3-1-124 G (MRID 46817216)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 01-29-07

Secondary Reviewer:
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 1/30/07

Project Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/30/07

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 1/30/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Myron S. Ottley **Signature:** MSOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/12/07
Work Assignment Manager: P.V. Shah **Signature:** P.V.Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/17/07
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - [rat]; OPPTS 870.3800 [§83-4]; OECD 416.

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (96.7% w/w)

SYNONYMS: Flubendiamide; N^2-(1,1-dimethyl-2-methylsulfonylethyl)-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Hojo, H. (2004) NNI-0001: Reproductive toxicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 01-0127, May 17, 2004. MRID 46817216. Unpublished.

SPONSOR: Nihon Nohyaku Co, Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 46817216), Flubendiamide (96.7%; Batch # 1FH0019M) was administered in the diet to 24 Wistar Hanover rats/sex/dose group at dietary levels of 0, 20, 50, 2000, or 20,000 ppm for two successive generations. The P generation animals were fed the test diets for 10 weeks prior to mating to produce the F1 litters. Upon weaning, F1 parents were fed the test diets for 10 weeks prior to mating to produce the F2 litters.

There were no treatment-related effects on body weights, body weight gains, or food consumption in either generation during pre-mating in the males or during pre-mating, gestation, or lactation in the females.

In the P generation, one 2000 ppm dam (#173) and one 20,000 ppm dam (#222) died during delivery on GD 22 or 23 without showing any clinical signs. In the F1 generation, two 20,000 ppm dams (#462 and 465) also died during delivery on GD 23 without showing any clinical signs. Clinical observations indicated enlargement of the eyeball throughout the study in one 20,000 ppm F1 dam.

Treatment-related effects on the **liver** were observed, including increased organ weights and gross and microscopic lesions. In both generations, absolute and relative liver weights were

increased ($p<0.001$) in the females at ≥2000 ppm (↑35-63%) and in the males at 20,000 ppm (↑11-25%). Relative liver weights were also increased ($p<0.05$) by 8% in the 2000 ppm P males. The following incidences of gross findings were increased ($p\leq0.05$) over controls (# affected/24 rats compared to 0-1/24 controls: (i) dark color and enlargement of the liver in the females of both generations at 2000 and 20,000 ppm (6-21); and (ii) enlargement (2/24) and heptodiaphragmatic nodule (3/24) in the 20,000 ppm F1 males. Incidences of the following microscopic findings (# affected/24 vs 0/24 controls) were significantly increased ($p<0.05$; unless otherwise stated) over controls: (i) periportal fatty change in the hepatocytes in both generations in the females at 2000 and 20,000 ppm (10-13) and in the males at 20,000 ppm (4-7, NS in P males); (ii) diffuse hepatocyte hypertrophy in the P and F1 females at 2000 and 20,000 ppm (5-16) and in the F1 males at 20,000 ppm (8); (iii) brown pigment deposition in the portal area in the liver in the F1 males and P and F1 females at 2000 and 20,000 ppm (6-24); (iv) bile duct proliferation in the 20,000 ppm dams of both generations (8-11); (v) focal hepatocyte necrosis in the P dams (3; NS) and massive hepatocyte necrosis in the F1 dams (1; NS); (vi) increased multinucleated hepatocytes in the 20,000 P dams (5); and (vii) hepatodiaphragmatic nodule in the 20,000 ppm F1 males (3; NS).

In both generations, absolute and relative **thyroid** weights were increased in the females at 2000 and 20,000 ppm and in the males at 20,000 ppm. Absolute thyroid weights were also increased by 19% in the 2000 ppm P males. Enlargement and brown color of the thyroid were significantly increased ($p<0.05$) in both sexes and both generations at 20,000 ppm (6-18 of 24 rats/group vs 0/24 controls), with brown color also observed in the females of both generations at 2000 ppm (5-6 of 24 rats/group vs 0 controls). Follicular cell hypertrophy in the thyroid was increased at 2000 and 20,000 ppm in both sexes and both generations (14-23 of 24 rats/groups vs 0 controls).

In the 2000 and 20,000 ppm P dams, relative **kidney** weights were increased (with absolute weights increased at 20,000 ppm). Tubular basophilic change and urinary cast in the kidneys were observed in these animals (9-11/24 treated vs 0/24 controls).

Additionally at 20,000 ppm: (i) absolute and relative **adrenal** weights were increased, and diffuse cortical cell hypertrophy was observed (16/24 treated vs 0/24 controls) in the P dams; and (ii) basophilic cell hydropic degeneration in the **pituitary** was observed in both sexes in the P generation and F1 females (3-4/24 treated vs 0 controls), and relative pituitary weights were decreased in both sexes in the F1 generation.

The LOAEL for parental toxicity is 2000 ppm (equivalent to 146.3/167.5 mg/kg/day in males/females) based on effects on the liver, thyroid, and kidneys as indicated by changes in organ weights corroborated by gross and microscopic lesions. The NOAEL is 50 ppm (equivalent to 3.68/4.27 mg/kg/day in males/females).

There were no treatment-related effects on the number of implantations, number of pups delivered, sex ratio, anogenital distance, or on the live birth, viability, or lactation indices.

At 20,000 ppm, pup body weights were decreased on PND 21 in both sexes in both generations. Sexual maturation was delayed in the males, as indicted by a dose-dependent increase ($p<=0.05$)

in the mean number of days until preputial separation at 50 ppm (42.5 days), 2000 ppm (43.0 days), and 20,000 ppm (43.7 days) compared to controls (41.3 days). Additionally at 2000 and 20,000 ppm, the body weight at which preputial separation occurred was increased ($p<=0.05$) by 5-7% over controls.

At >=2000 ppm, absolute and relative **liver** weights were increased in both sexes in both generations. The following lesions were observed in the liver in the >=2000 ppm pups of both generations (% incidence treated vs 0% controls): (i) dark-colored liver (26.6-49.3%); (ii) diffuse fatty change in the hepatocytes (43-72%); (iii) diffuse hepatocyte hypertrophy (39-100%); and (iv) brown pigment deposition in the portal area in the liver (70-100%); and (vi) bile duct proliferation (13-95%). Additionally, the following findings were observed in the liver: (i) hepatodiaphragmatic nodule at >=2000 ppm in the F2 pups (3.8-3.9%); (ii) enlargement of the liver at 20,000 ppm in the F2 pups (1.3%); and hepatocyte focal necrosis at 20,000 ppm in the F1 males (6%).

Relative **thyroid** weight was increased by 24% in the 20,000 ppm males. Enlargement of the thyroid was observed in the F1 pups at 20,000 ppm (5.3%). Incidences of follicular cell hypertrophy in the thyroid were increased at >=2000 ppm (35-100%) compared to 0 controls in both sexes of both generations.

Enlargement of the **eyeball** was observed in the F2 pups at >=2000 ppm (3.0-3.8%) compared to 0 controls. Incidences of opacity and enlargement of the eyeball in the F1 pups and opacity and discoloration of the eyeball in the F2 pups were noted, but were unrelated to dose. However, these effects were noted in the subsequent one-generation study (MRID 46817239).

The LOAEL for offspring toxicity is 2000 ppm (equivalent to 146.3/167.5 mg/kg/day in males/females) based on effects on the liver and thyroid as indicated by changes in organ weights corroborated by gross and microscopic lesions. The NOAEL is 50 ppm (equivalent to 3.68/4.27 mg/kg/day in males/females).

There were no effects of treatment on: the precoital interval; mating, fertility, or gestation indices; or gestation duration in either generation. Furthermore, the numbers of primordial ovarian follicles in the 20,000 ppm F1 dams were comparable to controls. No adverse effects were noted on estrous cycle duration or sperm parameters.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 20,000 ppm (equivalent to 1471.5/1692.5 mg/kg/day in males/females).

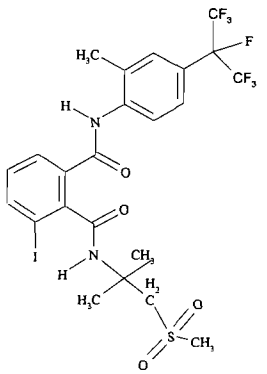
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide) technical

Description:	White crystals
Lot/batch #:	1FH0019M
Purity:	96.7% a.i.
Compound stability:	The test substance was stable in the diet for up to 4 weeks at 5°C followed by 3 weeks at room temperature.
CAS # of TGAI:	272451-65-7
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat	
Strain:	Wistar Hanover (BrlHan:WIST@Jcl[GALAS])	
Age at study initiation:	5 weeks	
Weight at study initiation:	156-185 g, males; 116-137 g, females	
Source:	Fuji Breeding Center, Clea Japan, Inc.	
Housing:	Acclimation (five/sex) and pre-mating (three/sex) - in suspended wire-mesh stainless steel cages. Mating - in pairs (1 male: 1 female) in TR-360 aluminum cages with wire-mesh floors and fronts. Gestation and lactation - Dams housed individually during gestation and with their litters during lactation in TR-358B cages. After weaning, dams were removed and pups continued to be housed by litter in TR-358B cages.	
Diet:	Certified pulverized feed, MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*	
Water:	Filtered (sand and charcoal) and sterilized (sodium hypochlorite) well water, *ad libitum*	
Environmental conditions:	**Temperature**	22 ± 2°C
	Humidity	55 ± 15%
	Air changes	At least 10/hour
	Light cycle	12 hours light/12 hours dark
Acclimation period:	11 days	

B. PROCEDURES AND STUDY DESIGN

1. **Mating procedure:** Each P male was placed in a TR-360 cage prior to the mating period. Daily vaginal smears were taken for up to two weeks prior to mating, and each female in proestrus was placed that evening in the cage with the male (1 male: 1 female) from the same dose group. Females that did not repeatedly show proestrus vaginal smears (i.e., did not have normal estrous cycling) were paired with males on the first day of the mating period (Week 11). Vaginal smears were taken daily and examined for positive evidence of mating as indicated by the presence of vaginal plugs or sperm in a vaginal smear. The day on which positive evidence of mating was detected was designated as gestation day (GD) 0. Females with positive evidence of mating were separated from the male and housed individually in breeding boxes with nesting materials, and the P males were returned to their cages. Females not showing evidence of mating during the two-week mating period were paired with another proven P male of the same dose group for an additional week. The same mating procedures were conducted on the F1 parental animals, with one exception. F1 parental animals not producing offspring (including four males and four females from the control group, one male and one female at 20 ppm, two males and one female at 50 ppm, four males and four females at 2000 ppm, and five males and four females at 20,000 ppm) were mated with proven control animals for a re-mating period of up to three weeks. No further matings were reported.

2. **Study schedule:** The P generation animals were fed the test diets for 10 weeks prior to mating to produce the F1 litters. On post-natal day (PND) 4, litters with nine or more pups were culled to yield 4 pups/sex/litter (as nearly as possible). On PND 21, the F1 offspring were weaned. During PND 21-25, one or two F1 pups/sex/litter (24/sex/dose group) were selected to be parents and were fed the same test diet concentration as their dam for 10 weeks prior to mating to produce the F2 litters. Parents were euthanized for terminal examination after weaning and selection of their offspring.

3. **Animal assignment:** The P animals were assigned to the test groups shown in Table 1 in such as way to equalize the group means and standard deviations of body weights as closely as possible.

TABLE 1. Animal assignment [a]

Test group	Dose (ppm) [b]	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	24	24	24	24
Low	20	24	24	24	24
Lower middle	50	24	24	24	24
Higher middle	2000	24	24	24	24
High	20,000	24	24	24	24

a Data were obtained from pages 27 and 29 of MRID 46817216.
b Exposure to the test substance was continuous throughout the study.

4. **Dose-selection rationale:** Doses for the current study were selected based on the results from a one-generation range-finding reproduction toxicity study in rats (IET 01-0029) in which 8 P

generation rats/sex/group were fed test diets at concentrations of 0, 20, 200, 2000, or 20,000 ppm beginning three weeks prior to mating and continuing until weaning of the F1 litters. This study was not provided; however, the following summary was obtained from pages 24-25 of the MRID 46817216. At 2000 ppm, approximately half of the P females had dark-colored, enlarged livers, and the liver weight was increased (although not significant) over controls. At 20,000 ppm, almost all of the females had dark-colored, enlarged livers, and the liver weight was significantly increased. Additionally at 20,000 ppm, pup body weights were significantly decreased on PND 21. There were no effects of treatment on reproductive performance.

5. **Test diet preparation and analysis:** For each dose level, the appropriate amount of the test substance was mixed with a small amount of basal feed. This pre-mix was stirred with additional feed to achieve the desired concentration. Test diets were prepared once every two to four weeks and were stored in plastic bags in the dark at approximately 5°C until use. From the first batch, samples were taken from the top, middle, and bottom of the mixer and analyzed for homogeneity and concentration. Stability of the test substance was determined in the 20 and 20,000 ppm diets after storage for 4 weeks at 5°C followed by up to 3 weeks at room temperature.

Results

Homogeneity: 0.2-4.4% C.V.

Stability: 97-105% of initial concentration

Concentration: 90-108% nominal

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

6. **Dosage administration:** The test material was administered in the diet continuously throughout the study (i.e., P generation adults were fed the test diets *ad libitum* beginning 10 weeks prior to mating, and the selected F1 adults were fed the same test diet concentrations as their parents beginning on PND 21-25).

C. OBSERVATIONS

1. **Parental animals:** All rats were examined for mortality and clinical signs of toxicity twice daily, except once daily on holidays. Detailed examinations were performed at the same time that body weights were recorded. Body weights of the males were recorded on the first day of treatment, weekly throughout the study, and on the day of necropsy. For the females, body weights were recorded: on the first day of treatment, weekly during pre-mating; on GD 0, 7, 14, and 20; on lactation day (LD) 0, 7, 14, and 21; and on the day of necropsy. Females that did not show any signs of parturition were weighed weekly from presumed GD 25 and on the day of necropsy. Weekly cumulative body weight gains and overall gains for pre-mating (Weeks 0-10), combined pre-mating and mating (Weeks 0-15), and overall study (Weeks 0-

16) periods were reported for the males. For the females, cumulative body weight gains from the beginning of each of the pre-mating, gestation, and lactation periods were reported, in addition to overall weight gains during each of these periods and for the overall (Weeks 0-18) study. Food consumption was recorded on the day of body weight measurement and was reported as a daily average (g food/rat/day) for each of the intervals for which body weight gains were determined. Estrous cycle periodicity and duration were determined from vaginal smears taken daily for at least 2 weeks prior to mating. From all P and F1 parental males, sperm enumeration, motility, and morphology were determined from the right caudal epididymis, and sperm head counts were taken from the right testis.

2. **Litter observations:** The following litter parameters (X) were recorded in all F1a, F2a, and F2b litters (Table 2):

TABLE 2. F_1 / F_2 Litter Observations [a]

Observation	Time of observation (lactation day)				
	Day 0	Day 4	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X
Number of dead pups	X	X	X	X	X
Litter weight	X				
Pup weight		X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X
External alterations	X	X	X	X	X
Anogenital distance [b]		X			

a Data obtained from pages 38-39 in MRID 46817216.
b Recorded for F2 pups, along with body weight, on PND 4 prior to culling.

Pups were examined daily for mortality and clinical signs of toxicity by cage-side observation throughout lactation. Sexual maturation was determined for all F1 parents. Daily checks were made for vaginal opening beginning on PND 26 and for preputial separation beginning on PND 35. Body weight was recorded for each rat on the day on which criterion was achieved.

3. **Postmortem observations**

a. **Parental animals:** At study termination, all surviving P males and females were anesthetized with ether and euthanized by exsanguination via the abdominal aorta and caudal vena cava and subjected to a gross necropsy. P males were necropsied after confirmation of parturition of the paired females and upon judgment by the study director that no further mating was needed. P dams were terminated after weaning of their F1 pups. F1 parents were anesthetized with ether and exsanguinated via decapitation, and a gross necropsy was performed following blood sampling. Vaginal smears were taken from P and F1 females at necropsy. Animals found dead during the study were subjected to a gross necropsy immediately after discovery.

The following checked (X) tissues from all animals, including decedents, were collected and fixed and preserved in neutral-buffered 10% formalin (except for the testes which were

preserved in formalin sucrose acetic acid [FSA] solution). Additionally from the animals surviving to scheduled termination, the (XX) organs were weighed:

XX	Brain	XX	Testes [a]	XX	Ovaries [a]
XX	Pituitary	XX	Epididymides [a]	XX	Uterus [c]
XX	Thyroids	XX	Seminal vesicles [b]	XX	Cervix [c]
XX	Liver	XX	Coagulating gland [b]	XX	Oviducts [c]
XX	Kidneys [a]	XX	Prostate gland (ventral lobe)	X	Vagina
XX	Adrenals [a]				Mammary gland (females)
XX	Spleen				
X	Gross lesions				

a Paired organs were weighed together and reported as an average of both sides.
b The seminal vesicles were weighed with the fluid, including coagulating glands
c The uterus was weighed with the oviducts and cervix.

The tissues listed above from the control and high dose animals were examined microscopically, with the exception of the brain from both sexes and the kidneys and spleen from the males. The kidneys and spleen from the control and high dose females were examined because of significant differences in organ weights compared to controls. Additionally, the following organs were examined from animals in the intermediate dose groups: (i) pituitary from all animals of both sexes and generations because of microscopic findings in the high dose groups; (ii) adrenals and kidneys in the P females because of microscopic findings in the high dose group; (iii) reproductive organs from paired parental males and females that did not show any evidence of copulation or pregnancy; and (iv) reproductive organs from the parental females that showed abnormal delivery or had a whole litter loss. The numbers of primordial follicles were counted from the F1 females from the control and high dose groups.

b. Offspring: F1 weanlings that were not selected to be parents and all F2 weanlings were euthanized by overdose of ether inhalation on PND 26. These animals, along with F1 and F2 pups culled on PND 4 and any decedents, were subjected to a gross necropsy. The brain, thyroids, thymus, spleen, liver, and uterus from one pup/sex/litter were weighed. With the exception of the brain, these organs were examined microscopically from animals in the control and high dose groups. Additionally in the intermediate dose groups, the thyroid and liver were examined microscopically because of findings in the high dose group. Because decreased thymus weights were observed in the F1 weanlings, samples of the thymus from the control and high dose groups in this generation were analyzed according to the TUNEL method in order to determine if this finding was associated with immunotoxicity. Lymphocytes in the cortex of the thymus stained using this method are considered "TUNEL positive" and indicate physiological cell death. The TUNEL index (%) = # TUNEL positive cell nuclei/total cell nuclei examined x 100

It was stated that 2 F2 weanlings/sex/litter were killed by decapitation under light ether anesthesia, and blood was collected. However, the Sponsor did not mention any hematology

parameters to be analyzed, and no data were reported.

D. DATA ANALYSIS

1. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure
Parental body weights Parental body weight gains Parental food consumption Number of implantation sites Sperm counts Number of pups delivered Parental and pup organ weights Anogenital distance of pups Pup body weights	Bartlett's test for homogeneity of variances. If variances are homogeneous, differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test or Scheffes' multiple comparison test to compare treated groups with controls if ANOVA is significant. If variances are not homogeneous, differences among groups were analyzed using Kruskal-Wallis followed by Dunnett type mean rank test or Scheffe-type mean rank test to compare treated groups with controls if Kruskal-Wallis is significant.
Follicular count	F test for homogeneity of variances. If variances are homogeneous, differences were analyzed using Student's t-test. If variances are not homogeneous, Aspin-Welch test was used.
Parental clinical signs Incidences of normal estrous cycle Mating, fertility, and gestation indices Parental gross pathology Parental histopathology Offspring histopathology Sex ratio	Fisher's exact probability test
Sexual development Estrous cycle length Pre-coital interval Gestation duration Sperm motility and morphology Viability indices Offspring clinical signs Offspring gross pathology TUNEL index	Mann-Whitney *U*-test

Statistical significance was denoted at $p \leq 0.05$, 0.01, and 0.001. Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

2. **Indices**

Reproductive indices: The following reproductive indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Male mating index (%) = # copulations/ # males used for mating x 100

Female mating index (%) = # copulations/ # females used for mating x 100

Pre-coital interval = # days from cohabitation of males and females to confirmation of copulation.
Fertility index (%) = # pregnancies/ # females copulated x 100

Gestation index (%) = # normal parturitions/ # pregnancies x 100

Gestation duration = # days from detection of copulation to completion of parturition.

Offspring viability indices: The following offspring indices were calculated by the performing laboratory from lactation records of litters in the study:

Sex ratio (% males) = # male pups/ # pups delivered

Live birth index (%) = # live pups on PND 0/ total # pups delivered x 100

Viability index (%) = # pups surviving to PND 4/ # pups alive on PND 0 x 100

Lactation indices (%) = # pups alive on PND 7, 14, or 21/ # pups selected on PND 4 x 100

Relative anogenital distance = anogenital distance (mm)/ [body weight (g) x 1000] $^{1/3}$

3. **Historical control data:** Not provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** In the P generation, one 2000 ppm dam (#173) and one 20,000 ppm dam (#222) died during delivery on GD 22 or 23 without showing any clinical signs. In the F1 generation, two 20,000 ppm dams (#462 and 465) also died during delivery on GD 23 without showing any clinical signs.

b. **Clinical signs of toxicity:** Enlargement of the eyeball was observed throughout the study in one F1 dam at 20,000 ppm. No other clinical signs could be attributed to treatment.

2. Body weight, body weight gain, and food consumption

a. **Pre-mating:** There were no treatment-related effects on body weights, body weight gains, or

food consumption in either sex in either generation during pre-mating (Table 3a). In the males from both generations, body weights and body weight gains of the treated groups were comparable to controls throughout the pre-mating and mating periods. In the P generation females, body weights and body weight gains of the treated groups were comparable to controls throughout pre-mating. In the F1 dams, increases ($p \leq 0.05$) were noted at 2000 ppm in body weights during Weeks 6-8 and in cumulative body weight gains during Weeks 4 through 9. At 20,000 ppm in this generation, body weights and cumulative body weight gains were increased at Week 7. However, these findings were neither dose-related nor adverse; and overall body weight gain for the pre-mating period was comparable to controls.

Food consumption was comparable to controls throughout pre-mating in the treated males of both generations and in the F1 dams. Food consumption was sporadically increased ($p \leq 0.05$) in the 2000 ppm P dams at Weeks 3 and 6. Again, these increases were neither dose-related nor adverse. Food consumption was also sporadically increased ($p \leq 0.05$) by 8-10% in the 2000 and 20,000 ppm males of both generations during the breeding period (data not depicted in table), but these findings were not considered adverse.

TABLE 3a. Selected mean (±SD) body weights (g) and body weight gains (g) during pre-mating [a]

Observation/study week		Dose Group (ppm)				
		0	20	50	2000	20,000
P Generation Males						
Body weight	Week 0	170±7	170±7	170±7	170±7	170±7
Body weight	Week 10	393±39	383±36	393±35	400±31	404±32
Body weight gain	Weeks 0-10	223±35	212±32	222±29	230±26	234±28
P Generation Females						
Body weight	Week 0	126±5	126±5	126±5	126±5	126±5
Body weight	Week 10	232±15	238±18	240±13	243±24	236±14
Body weight gain	Weeks 0-10	105±13	112±15	114±10	117±22	109±13
F1 Generation Males						
Body weight	Week 0	63±6	64±7	64±6	63±5	63±7
Body weight	Week 10	383±31	383±32	382±29	402±33	391±30
Body weight gain	Weeks 0-10	320±29	318±29	318±28	339±32	328±28
F1 Generation Females						
Body weight	Week 0	60±6	61±6	61±5	60±5	60±5
Body weight	Week 10	229±14	228±13	236±17	240±16	235±19
Body weight gain	Weeks 0-10	169±14	167±13	176±16	180±16	175±17

a Data (n = 24) were obtained from Tables 4 through 7 on pages 62-66 of MRID 46817216

b. Gestation: There were no effects of treatment on body weights, body weight gains, or food consumption during gestation in either generation (Table 3b). In the P dams, body weights, body weight gains, and food consumption of the treated groups were comparable to controls throughout gestation. In the F1 dams, increases ($p \leq 0.05$) were noted in body weights at 2000 ppm on GD 14 and 20 and in food consumption at 2000 and 20,000 ppm during GD 14-20; however, these increases were unrelated to dose, not adverse, and did not affect body weight

gains.

TABLE 3b. Selected mean (∀SD) body weights (g), body weight gains (g), and food consumption (g/animal/day) during gestation [a]

Observation/study week		Dose Group (ppm)				
		0	20	50	2000	20,000
P Generation						
Body weight	GD 0	231±16	237±18	241±14	246±24	238±14
Body weight	GD 7	258±17	261±17	263±14	273±22	263±15
Body weight	GD 14	285±19	287±20	289±16	301±25	289±17
Body weight	GD 20	348±27	348±25	345±23	364±30	352±26
Body weight gain	GD 0-20	117±16	111±14	105±21	118±14	114±18
Food consumption	GD 0-7	18.0±2.1	17.7±2.1	17.2±2.2	18.8±1.8	18.0±2.8
Food consumption	GD 7-14	20.2±2.5	19.6±1.9	19.6±2.1	20.5±2.2	20.4±3.0
Food consumption	GD 14-20	21.3±2.5	20.6±1.9	21.1±2.7	23.7±2.7	22.9±3.8
F1 Generation						
Body weight	GD 0	227±14	230±13	238±19	240±12	238±28
Body weight	GD 7	251±14	252±14	259±19	265±13	262±25
Body weight	GD 14	274±15	278±13	283±20	292±15*(↑22)	290±25
Body weight	GD 20	328±22	337±22	345±26	357±29*(↑49)	353±33
Body weight gain	GD 0-20	101±14	107±15	106±17	118±25	115±17
Food consumption	GD 0-7	16.6±2.4	17.0±2.1	17.4±1.9	17.2±1.8	17.2±2.3
Food consumption	GD 7-14	19.4±1.9	19.9±1.8	20.0±2.7	20.1±1.8	20.6±2.4
Food consumption	GD 14-20	20.2±2.2	21.2±1.8	22.1±2.5	23.0±2.3**(↑14)	22.9±2.9*(↑13)

a Data (n = 20-24) were obtained from Tables 5, 7, and 9 on pages 63, 65, and 67 of MRID 46817216. Percent differences from controls (calculated by reviewers) are included in parentheses.

* Significantly different from the control group at $p \leq 0.05$

** Significantly different from the control group at $p \leq 0.01$

c. **Lactation:** There were no effects of treatment on body weights, body weight gains, or food consumption during lactation in either generation (Table 3c). In the P dams, body weights, body weight gains, and food consumption of the treated groups were comparable to controls throughout lactation. In the F1 dams, food consumption and body weight gains were comparable to controls. Body weights were increased ($p \leq 0.05$) at 2000 ppm on LD 14 and 21; however, these increases were not considered adverse and were unrelated to dose.

TABLE 3c. Selected mean (∀SD) body weights (g), body weight gains (g), and food consumption (g/animal/day) during lactation [a]

Observation/study week		Dose Group (ppm)				
		0	20	50	2000	20,000
P Generation						
Body weight	LD 0	255±20	254±22	266±21	267±26	247±29
Body weight	LD 7	285±18	288±22	288±17	297±27	288±16
Body weight	LD 14	306±22	306±22	307±18	317±26	308±14
Body weight	LD 21	290±20	290±22	293±12	304±24	296±15
Body weight gain	LD 0-21	36±15	35±16	27±16	37±14	39±17
Food consumption	GD 0-7	35.9±6.1	36.0±5.3	34.5±5.1	36.9±4.8	35.2±5.0
Food consumption	GD 7-14	54.7±7.1	54.5±5.5	52.9±4.5	56.1±5.5	55.1±8.4
Food consumption	GD 14-21	64.5±7.7	65.8±6.3	64.0±5.2	67.8±6.1	62.8±7.4
F1 Generation						
Body weight	LD 0	261±16	264±17	268±23	269±20	267±30
Body weight	LD 7	282±17	282±15	291±21	301±17	293±25
Body weight	LD 14	297±19	298±16	304±19	321±18**(↑8)	305±30
Body weight	LD 21	283±13	280±13	287±18	306±15**(↑8)	299±22
Body weight gain	LD 0-21	22±13	17±12	19±13	36±12	32±17
Food consumption	GD 0-7	32.0±6.3	33.0±4.6	34.2±5.2	35.7±6.7	36.0±4.5
Food consumption	GD 7-14	49.3±8.3	50.6±7.4	52.1±5.1	52.9±10.3	54.6±4.2
Food consumption	GD 14-21	59.0±9.7	60.1±9.0	61.3±5.5	63.7±12.1	64.6±5.1

a Data (n = 18-25) were obtained from Tables 5, 7, and 9 on pages 63, 65, and 67 of MRID 46817216. Percent differences from controls (calculated by reviewers) are included in parentheses.

** Significantly different from the control group at p≤0.01

3. **Test substance intake:** Test substance intake (mg/kg/day) was calculated from the body weight and food consumption data, using the nominal concentration (ppm) of the diets. The mean test substance intake for both generations during pre-mating is considered to be representative of the achieved intake for the entire study (Table 4).

TABLE 4. Mean test substance intake (mg/kg/day in males/females) during pre-mating [a]

Generation	Dose (ppm)				
	0	20	50	2000	20,000
P generation	0/0	1.303/1.59	3.30/3.95	130.8/159	1307/1577
F1 generation	0/0	1.641/1.84	4.05/4.59	161.7/176	1636/1808
Mean [b]	**0/0**	**1.47/1.72**	**3.68/4.27**	**146.3/167.5**	**1471.5/1692.5**

a Data were obtained from Table 10 on page 68 of MRID 46817216.

b Calculated by the reviewers as the average of the P and F1 generations.

4. Reproductive function

a. **Estrous cycle length and periodicity:** The percentage of dams with normal estrous cycles in

the treated groups was comparable to controls in both generations. Estrous cycle duration in the treated groups was comparable to controls in the P generation, but was slightly shorter ($p \leq 0.05$) in the F1 dams at 20,000 ppm (4.0 days) compared to controls (4.2 days).

b. Sperm measures: The sperm count per testis and per gram testis in the treated P males was comparable to controls. Sperm count in the testis was 11% lower ($p \leq 0.05$) in the 20,000 ppm F1 males compared to controls. When considered per gram testis, this decrease was minor (↓6%) and not significant. The sperm counts per cauda epididymis and per gram cauda epididymis were unaffected by treatment. Sperm motility and morphology in the treated P and F1 males were comparable to controls.

5. Reproductive performance: There were no effects of treatment on: the precoital interval; mating, fertility, or gestation indices; or gestation duration in either generation (Table 5). Furthermore, the numbers of primordial ovarian follicles in the 20,000 ppm F1 dams were comparable to controls.

TABLE 5. Reproductive performance [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
P Generation (F1 litter)					
Precoital interval (days)	1.0 ± 0.0	1.2 ± 0.8	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.2
Mating index (%) male	24/24 (100)	24/24 (100)	24/24 (100)	23/24 (95.8)	24/24 (100)
female	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
Fertility index (%)	24/24 (100)	23/24 (95.8)	23/24 (95.8)	24/24 (100)	23/24 (95.8)
Gestation index (%)	24/24 (100)	23/23 (100)	22/23 (95.7)	23/24 (95.8)	22/23 (95.7)
Gestation duration (days)	22.3	22.1	22.1	22.3	22.5
F1 Generation (F2 litter)					
Precoital interval (days)	1.0 ± 0.2	1.2 ± 1.0	1.2 ± 1.1	1.0 ± 0.2	1.5 ± 1.9
Mating index (%) male	24/24 (100)	24/24 (100)	23/24 (95.8)	24/24 (100)	23/24 (95.8)
female	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
Fertility index (%)	20/24 (83.3)	23/24 (95.8)	23/24 (95.8)	20/24 (83.3)	20/24 (83.3)
Gestation index (%)	19/20 (95.0)	23/23 (100)	23/23 (100)	20/20 (100)	20/20 (100)
Gestation duration (days)	22.2	22.2	22.1	22.3	22.2
Number of ovarian follicles	384 ± 120	NE	NE	NE	440 ± 116

a Data were obtained Tables 14, 15, and 26 on pages 72, 73, and 91 of MRID 46817216.
NE Not examined

6. Parental postmortem results

a. Organ weights: Selected organ weights are presented in Tables 6a and 6b. In both generations, absolute and relative (to body weight) liver weights were increased ($p \leq 0.001$) in the females at ≥2000 ppm (↑35-63%) and in the males at 20,000 ppm (↑11-25%). Relative liver weights were also increased ($p \leq 0.05$) by 8% in the 2000 ppm P males. Similarly, in both generations, absolute and relative thyroid weights were increased in the females at ≥2000 ppm

(↑25-84%) and in the males at 20,000 ppm (↑48-74%); the thyroid weight increases attained significance ($p \leq 0.01$) except for the relative weight in the 2000 ppm F1 dams. Absolute thyroid weights were also increased ($p \leq 0.05$) by 19% in the 2000 ppm P males. Relative pituitary weights were decreased ($p \leq 0.01$) by 12-18% in the ≥2000 ppm F1 males and females. Additionally in the P dams, the following increases ($p \leq 0.05$) in organ weights were observed: (i) adrenal weights - absolute at ≥2000 ppm and relative at 20,000 ppm (↑11-14%); (ii) ovaries - absolute at ≥50 ppm and relative at 20,000 ppm (↑15-24%); and (iii) kidneys - relative at ≥2000 ppm and absolute at 20,000 ppm (↑9-19%).

The following organ weights differed significantly ($p \leq 0.05$) from controls but were considered unrelated to treatment because they were not corroborated by gross or microscopic findings indicating a treatment-related effect: (i) increased absolute adrenal weight in the 20,000 ppm P males; (ii) increased absolute and relative uterus weight in the ≥2000 ppm P dams and absolute uterus weight in the 20,000 ppm F1 dams; and (iii) decreased relative spleen weight in the P females and absolute and relative spleen weight in the F1 females at ≥2000 ppm. All other significant differences ($p \leq 0.05$) were unrelated to dose.

TABLE 6a. Selected absolute (mg) and relative to body weight (%) organ weights in males [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
P generation					
Terminal body weight (g)	434±43	418±34	432±37	437±36	449±39
Liver					
Absolute	13,726±1851	12,993±1313	13,963±1260	14,831±1791	17,136±1991***(↑25)
Relative	3.15±0.21	3.11±0.16	3.23±0.13	3.39±0.22*(↑8)	3.81±0.26***(↑21)
Thyroid					
Absolute	20.8±3.8	22.2±3.3	22.7±3.4	24.8±5.0*(↑19)	36.2±5.7***(↑74)
Relative	0.0047±0.00076	0.00529±0.00077	0.00522±0.00085	0.00570±0.00093	0.00816±0.00151***(↑71)
F1 generation					
Terminal body weight (g)	434±35	433±40	424±38	450±41	449±35
Liver					
Absolute	13910±1463	13596±1644	13391±1696	14427±1738	15990±1378**(↑15)
Relative	3.20±0.20	3.14±0.19	3.15±0.24	3.20±0.20	3.56±0.17***(↑11)
Thyroid					
Absolute	20.3±3.7	20.6±4.0	19.6±3.2	22.8±4.1	31.0±6.8***(↑53)
Relative	0.00465±0.00080	0.00476±0.00091	0.00463±0.00072	0.00506±0.00076	0.00690±0.00166***(↑48)
Pituitary					
Absolute	10.6±0.9	10.4±1.6	10.4±1.3	9.1±1.3* (↓14)	9.7±1.2
Relative	0.00244±0.00019	0.00241±0.00026	0.00245±0.0021	0.00201±0.00021***(↓18)	0.00215±0.00024**(↓12)

a Data were obtained from Tables 20 and 22 on pages 82 and 84 of MRID 46817216.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

TABLE 6b. Selected absolute (mg) and relative to body weight (%) organ weights in females [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
P generation					
Terminal body weight (g)	264±21	265±21	269±13	280±27	272±16
Liver					
Absolute	12092±1640	12222±1468	12004±1038	17354±2029***(↑44)	18505±2247***(↑53)
Relative	4.57±0.38	4.61±0.40	4.47±0.29	6.19±0.48***(↑35)	6.79±0.63***(↑49)
Thyroid					
Absolute	20.7±3.3	21.6±3.0	21.7±3.9	29.3±5.5***(↑42)	38.1±9.0***(↑84)
Relative	0.00781±0.00104	0.00814±0.00127	0.00807±0.00135	0.01046±0.00170***(↑34)	0.01408±0.00356***(↑80)
Adrenals					
Absolute	44.9±5.4	45.5±5.8	47.0±6.2	51.2±5.9**(↑14)	51.4±4.6**(↑14)
Relative	0.0170±0.0019	0.0172±0.0019	0.0175±0.0023	0.0183±0.0019	0.0189±0.0013*(↑11)
Ovaries					
Absolute	59.2±8.1	64.6±7.4	68.0±9.2*(↑15)	70.6±10.3**(↑19)	73.4±10.1***(↑24)
Relative	0.0224±0.0030	0.0244±0.0028	0.0253±0.0034	0.0254±0.0040	0.0271±0.0041**(↑21)
Kidneys					
Absolute	1010±74	1040±94	1038±90	1174±118	1197±124***(↑19)
Relative	0.384±0.032	0.393±0.031	0.387±0.030	0.419±0.025**(↑9)	0.441±0.040***(↑15)
F1 generation					
Terminal body weight (g)	262±15	266±14	273±19	285±14**(↑9)	274±19
Liver					
Absolute	10395±1100	11919±1896	11732±1902	15306±2295***(↑47)	16993±2058***(↑63)
Relative	3.97±0.40	4.48±0.59	4.29±0.49	5.37±0.71***(↑35)	6.19±0.58***(↑56)
Thyroid					
Absolute	16.4±2.0	18.6±3.2	17.1±3.0	22.3±4.5**(↑36)	28.3±7.1***(↑73)
Relative	0.00627±0.00089	0.00703±0.00127	0.00626±0.00095	0.00784±0.00149(↑25)	0.01029±0.00253***(↑64)
Pituitary					
Absolute	13.0±1.8	13.9±1.5	13.2±1.7	11.7±1.9	11.6±1.5
Relative	0.00497±0.00065	0.00524±0.00056	0.00483±0.00057	0.00412±0.00069**(↓17)	0.00424±0.00042**(↓15)

a Data were obtained from Tables 21 and 23 on pages 83 and 85 of MRID 46817216.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

b. Pathology

1) **Macroscopic examination:** At necropsy, treatment-related findings were observed in the liver and thyroid (Table 7). The following incidences of gross findings were increased ($p \leq 0.05$) over controls (# affected/24 rats compared to 0-1/24 controls: (i) enlargement and brown color of the thyroid in both sexes and both generations at 20,000 ppm (6-18), with brown color also observed in the females of both generations at 2000 ppm (5-6); (ii) dark color and enlargement of the liver in the females of both generations at ≥2000 ppm (6-21); and (iii) enlargement (2/24) and heptodiaphragmatic nodules (3/24) in the 20,000 ppm F1 males. No other macroscopic findings could be attributed to treatment.

TABLE 7. Selected gross pathological findings (# affected out of 24 rats) [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
P generation Males					
Thyroid					
Enlargement	1	2	3	2	15***
Brown in color	0	0	0	0	12***
F1 generation Males					
Thyroid					
Enlargement	1	1	0	2	13***
Brown in color	0	0	0	1	11***
Liver					
Enlargement	0	0	0	0	2
Hepatodiaphragmatic nodule	0	0	0	1	3
P generation Females					
Thyroid					
Enlargement	0	0	1	2	6*
Brown in color	0	0	0	6*	16***
Liver					
Enlargement	0	0	0	8**	16***
Dark in color	0	0	0	20***	19***
F1 generation Females					
Thyroid					
Enlargement	1	1	1	2	10**
Brown in color	0	0	0	5*	18***
Liver					
Enlargement	0	0	0	6*	13***
Dark in color	0	1	0	16***	21***

a Data were obtained from Tables 18 and 19 on pages 76-81 of MRID 46817216.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

2) **Microscopic examination:** Incidences of the following microscopic findings (# affected/24

vs 0/24 controls) were significantly increased ($p \leq 0.05$; unless otherwise stated) over controls (Tables 8a and 8b): (i) follicular cell hypertrophy in the thyroid at ≥2000 ppm in both sexes and both generations (14-23); (ii) periportal fatty change in the hepatocytes in both generations in the females at ≥2000 ppm (10-13) and in the males at 20,000 ppm (4-7, not significant [NS] in P males); (iii) diffuse hepatocyte hypertrophy in the P and F1 females at ≥2000 ppm (5-16) and in the F1 males at 20,000 ppm (8); (iv) brown pigment deposition in the portal area in the liver in the F1 males and P and F1 females at ≥2000 ppm (6-24); (v) hepatodiaphragmatic nodule in the 20,000 ppm F1 males (3; NS); (vi) basophilic cell hydropic degeneration in the pituitary in both sexes in both generations at 20,000 ppm (3-4; NS); (vii) interstitial vacuolation in the ovaries of the ≥2000 ppm P dams (4-6; NS at 2000 ppm) and 20,000 ppm F1 dams (3; NS); (viii) bile duct proliferation in the 20,000 ppm dams of both generations (8-11); (ix) increased multinucleated hepatocytes in the 20,000 P dams (5); (x) focal hepatocyte necrosis in the P dams (3; NS) and massive hepatocyte necrosis in the F1 dams (1; NS); (xi) diffuse adrenal cortical cell hypertrophy in the 20,000 ppm P dams (16); and (xii) tubular basophilic change and urinary cast in the kidneys in the ≥2000 ppm P dams (9-11). No other microscopic lesions could be attributed to treatment in the parents of either generation.

TABLE 8a. Selected microscopic findings (# affected out of 24 rats) in male rats [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
P generation Males					
Thyroid					
Hypertrophy, follicular cell	0	0	0	14***	21***
Liver					
Fatty change, hepatocyte, periportal	0	0	0	0	4
Pituitary					
Hydropic degeneration, basophilic cell	0	0	0	0	3
F1 generation Males					
Thyroid					
Hypertrophy, follicular cell	0	0	0	22***	22***
Liver					
Fatty change, hepatocyte, periportal	0	0	0	0	7**
Hypertrophy, hepatocyte, diffuse	0	0	0	0	8**
Deposition, brown pigment, portal area	0	0	0	16***	23***
Hepatodiaphragmatic nodule	0	0	0	1	3
Pituitary					
Hydropic degeneration, basophilic cell	0	0	0	0	3

a Data were obtained from Table 24 on pages 86-88 of MRID 46817216.

TABLE 8b. Selected microscopic findings (# affected out of 24 rats) in female rats [a]					
Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
P generation Females					
Thyroid					
Hypertrophy, follicular cell	0	0	0	22***	21***
Liver					
Fatty change, hepatocyte, periportal	0	0	0	10***	12***
Hypertrophy, hepatocyte, diffuse	0	0	0	8**	16***
Deposition, brown pigment, portal area	0	0	0	6*	18***
Proliferation, bile duct	0	0	0	0	8**
Increased multinucleated hepatocyte	0	0	0	0	5*
Necrosis, hepatocyte, focal	0	0	0	1	3
Pituitary					
Hydropic degeneration, basophilic cell	0	0	0	0	4
Ovary					
Vacuolation, interstitial cell	0	0	0	4	6*
Adrenal					
Hypertrophy, cortical cell, diffuse	0	0	0	0	16***
Kidney					
Tubular basophilic change	2	2	2	10**	11**
Urinary cast	1	1	2	9**	9**
F1 generation Females					
Thyroid					
Hypertrophy, follicular cell	0	0	0	23***	20***
Liver					
Fatty change, hepatocyte, periportal	0	0	0	12***	13***
Hypertrophy, hepatocyte, diffuse	0	0	0	5*	15***
Deposition, brown pigment, portal area	0	0	0	17***	24***
Proliferation, bile duct	0	0	0	1	11***
Necrosis, hepatocyte, massive	0	0	0	0	1
Pituitary					
Hydropic degeneration, basophilic cell	0	0	0	0	3
Ovary					
Vacuolation, interstitial cell	0	0	0	0	3

a Data were obtained from Table 25 on pages 89-90 of MRID 46817216.

B. OFFSPRING

1. **Viability and clinical signs:** There were no effects of treatment on the number of implantations, number of pups delivered, sex ratio, or on the live birth, viability, or lactation indices (Table 9). The viability index was slightly lower (not significant) at 20,000 ppm (84.3%) compared to controls (98.9%). However, the viability index at 20,000 ppm was comparable to concurrent controls in the F2 litter in the current study and in the subsequent one-generation reproduction study (MRID 46817239). Therefore this finding is considered unrelated to treatment.

TABLE 9. Litter parameters in the F1 and F2 generations [a]

Parameter		Dose Group (ppm)				
		0	20	50	2000	20,000
F1 litter						
Mean (±SD) implantations		13.8 ±1.6	13.9 ±2.0	12.3 ±2.9	13.5 ±2.2	14.0 ±2.1
Number of pups delivered		12.9 ±2.3	13.0 ±2.0	12.0 ±1.8	12.3 ±1.8	12.1 ±2.9
Sex ratio (% males)		0.484	0.482	0.445	0.486	0.461
Live birth index (%)	PND 0 [b]	99.7	99.0	100.0	99.7	90.0
Viability index (%)	PND 4 [c]	98.9	100.0	98.2	99.7	84.3
Lactation index (%)	PND 7 [d]	99.5	99.5	100.0	99.5	99.3
	PND 14 [d]	99.5	98.4	99.4	97.8	98.0
	PND 21 [d]	99.5	98.4	99.4	97.8	98.0
F2 litter						
Mean (±SD) implantations		11.3 ±3.9	12.0 ±3.0	12.3 ±2.6	12.6 ±3.2	12.6 ±2.3
Number of pups delivered		10.7 ±2.6	11.2 ±3.0	11.4 ±2.8	11.4 ±3.4	11.3 ±2.2
Sex ratio (% males)		0.443	0.416	0.510	0.480	0.473
Live birth index (%)	PND 0 [b]	96.3	99.7	99.7	98.2	98.8
Viability index (%)	PND 4 [c]	99.1	99.6	99.7	88.0	98.6
Lactation index (%)	PND 7 [d]	100.0	100.0	99.3	100.0	100.0
	PND 14 [d]	100.0	100.0	99.3	99.3	100.0
	PND 21 [d]	100.0	100.0	99.3	98.6	100.0

a Data were obtained from Table 15 on page 73 of MRID 46817216.

b Live birth index (denoted by the Sponsor as viability index on PND 0) = # alive on PND 0/ # delivered x 100

c Viability index (denoted by the Sponsor as viability index on PND 4) = # pups surviving to PND 4/ # pups alive on PND 0 x 100

d Lactation indices (denoted by the Sponsor as viability indices on PND 7,14, and 21) = # pups alive on PND 7, 14, or 21 (respectively)/ # pups selected on PND 4 x 100

2. **Body weight:** At 20,000 ppm, pup body weights were decreased ($p \leq 0.05$) by 8-9% on PND 21 in both sexes in both generations (Table 10). There were no other effects on body weights in the F1 or F2 offspring. Absolute and relative (to body weight) anogenital distances of the F2 pups were comparable to controls on PND 4.

TABLE 10. Mean (± SD) pup weights in the F1 and F2 generations [a]					
Post-natal day (PND)	Dose Group (ppm)				
	0	20	50	2000	20,000
F1 males					
0	5.8 ±0.4	5.7 ±0.2	5.9 ±0.5	6.3 ±0.6*(↑9)	6.1 ±0.8
4	10.3 ±1.0	10.4 ±0.7	10.7 ±1.1	11.2 ±1.4	11.3 ±1.6
7	17.0 ±1.6	17.3 ±1.1	17.4 ±1.6	17.8 ±2.2	17.7 ±2.4
14	36.1 ±3.0	36.4 ±1.8	35.7 ±2.4	36.0 ±3.1	34.7 ±3.7
21	56.6 ±4.3	57.2 ±2.8	56.1 ±3.2	55.5 ±4.7	51.4 ±5.0**(↓9)
F2 males					
0	6.2 ±0.6	6.3 ±0.6	6.3 ±0.6	6.3 ±0.6	6.4 ±0.4
4	11.1 ±1.1	11.7 ±1.5	11.3 ±1.5	11.8 ±1.5	12.1 ±1.1
7	18.1 ±1.2	18.8 ±1.7	18.3 ±2.0	18.5 ±3.1	18.9 ±1.4
14	36.8 ±2.7	36.9 ±2.2	36.9 ±2.6	36.9 ±5.3	36.9 ±2.4
21	57.5 ±4.2	57.9 ±3.1	57.1 ±4.3	56.7 ±7.1	52.9 ±2.4*(↓8)
F1 females					
0	5.4 ±0.4	5.4 ±0.3	5.6 ±0.4	6.0 ±0.5**(↑11)	5.7 ±0.7
4	9.9 ±0.8	10.1 ±0.7	10.5 ±1.1	10.9 ±1.2	10.8 ±1.5
7	16.4 ±1.2	16.9 ±1.1	17.0 ±1.6	17.4 ±2.0	17.1 ±2.4
14	34.9 ±2.7	35.5 ±1.6	34.9 ±2.5	35.2 ±2.7	34.1 ±3.7
21	54.3 ±3.9	55.1 ±2.8	54.2 ±3.1	53.5 ±4.0	50.2 ±4.9*(↓8)
F2 females					
0	6.0 ±0.6	6.0 ±0.6	5.9 ±0.5	6.1 ±0.5	6.0 ±0.5
4	11.0 ±1.5	11.3 ±1.5	11.0 ±1.3	11.5 ±1.3	11.6 ±1.1
7	17.9 ±1.8	18.2 ±1.7	17.6 ±1.7	17.8 ±2.8	18.2 ±1.4
14	36.4 ±3.4	35.9 ±2.1	36.0 ±2.4	35.6 ±4.9	35.9 ±2.3
21	55.8 ±4.6	55.6 ±2.7	55.0 ±3.6	54.8 ±6.3	51.4 ±2.1**(↓8)

a Data (n = 17-24) were obtained from Table 29 on page 97 of MRID 46817216.

3. **Sexual maturation:** Sexual maturation was delayed in the males, as indicted by a dose-dependent increase ($p \leq 0.05$) in the mean number of days until preputial separation at 50 ppm (42.5 days), 2000 ppm (43.0 days), and 20,000 ppm (43.7 days) compared to controls (41.3 days; Table 11). Additionally at 2000 and 20,000 ppm, the body weight at which preputial separation occurred was increased ($p \leq 0.05$) by 5-7% over controls. The number of days until vaginal opening and body weight at sexual maturation in the females were unaffected by treatment.

TABLE 11. Sexual maturation in the F1 generation pups [a]					
Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
F1 males					
Days until preputial separation	41.3 ± 2.0	41.5 ± 2.4	42.5 ± 1.8*	43.0 ± 2.1**	43.7 ± 2.5**
Body weight at criterion (g)	176.2 ± 16.0	175.0 ± 22.0	178.3 ± 13.6	188.9 ± 15.1**(↑7)	185.5 ± 18.2*(↑5)
F1 females					
Days until vaginal opening	31.8 ± 2.0	31.4 ± 2.0	31.5 ± 1.7	31.8 ± 2.5	32.2 ± 2.5
Body weight at criterion (g)	102.1 ± 10.0	97.2 ± 11.2	97.0 ± 10.8	100,2 ± 13.0	101.0 ± 13.0

a Data (n = 24) were obtained from Table 13 on page 71 of MRID 46817216.

4. Offspring postmortem results

a) Organ weights: Except for the liver and thyroid, there were no treatment-related effects on organ weights (Table 12). Absolute and relative liver weights were increased (↑14-38%; $p \leq 0.05$) at ≥2000 ppm in both sexes in both generations. With the exception of an increase of 24% ($p \leq 0.05$) in relative thyroid weight in the 20,000 ppm males, absolute and relative thyroid weights of the other groups were comparable to controls. Several other organ weights of the treated groups differed significantly ($p \leq 0.05$) from controls, but were uncorroborated by findings in gross or microscopic pathology and were likely related to the decreased terminal body weights in these animals. Furthermore, the TUNEL index showed no differences between the 20,000 ppm group and the control group in the F1 offspring, indicating a lack of evidence for immunotoxicity accounting for the decreased thymus weights.

TABLE 12a. Selected absolute (mg) and relative to body weight (%) organ weights in F1 and F2 weanling males at post-natal day 26 [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
F1 weanling males					
Terminal body weight (g)	80 ± 5	82 ± 3	81 ± 5	79 ± 7	72 ± 8**(↓10)
Liver					
Absolute	3441 ± 350	3640 ± 247	3527 ± 279	3938 ± 507**(↑14)	4087 ± 428***(↑19)
Relative	4.29 ± 0.27	4.45 ± 0.20	4.36 ± 0.18	5.00 ± 0.35***(↑17)	5.71 ± 0.22***(↑33)
Thyroid					
Absolute	8.3 ±1.5	8.3 ±1.7	8.7 ±1.7	8.4 ±1.5	9.1 ±2.2
Relative	0.01033 ± 0.00171	0.01011 ± 0.00210	0.01075 ± 0.00208	0.01068 ± 0.00184	0.01285 ± 0.00324*(↑24)
F2 weanling males					
Terminal body weight (g)	83 ± 5	83 ± 4	81 ± 6	83 ± 8	77 ± 4
Liver					
Absolute	3539 ± 376	3520 ± 193	3486 ± 366	4070 ± 502*(↑15)	4314 ± 311***(↑22)
Relative	4.28 ± 0.23	4.23 ± 0.16	4.28 ± 0.22	4.89 ± 0.21***(↑14)	5.60 ± 0.21***(↑31)
Thyroid					
Absolute	7.4 ± 1.2	8.0 ± 1.3	8.0 ± 1.5	7.9 ± 1.5	8.2 ± 1.4
Relative	0.00883 ± 0.00113	0.00957 ± 0.00165	0.00982 ± 0.00148	0.00960 ± 0.00211	0.1060 ± 0.00158

a Data (n = 17-24) were obtained from Tables 33 and 34 on pages 103-104 of MRID 46817216.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

TABLE 12b. Selected absolute (mg) and relative to body weight (%) organ weights in F1 and F2 weanling females at post-natal day 26 [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
F1 weanling females					
Terminal body weight (g)	74 ±5	75 ±5	74 ±5	73 ±4	66 ±5***(↓11)
Liver					
Absolute	3132 ±292	3275 ±238	3201 ±286	3803 ±383***(↑21)	3868 ±454***(↑23)
Relative	4.25 ±0.22	4.37 ±0.29	4.33 ±0.18	5.20 ±0.31***(↑22)	5.87 ±0.32***(↑38)
Thyroid					
Absolute	8.2 ±16	8.2 ±1.1	8.5 ±1.4	8.3 ±1.3	8.1 ±1.4
Relative	0.01113 ±0.00196	0.01097 ±0.00192	0.01145 ±0.00186	0.01142 ±0.00179	0.01234 ±0.00223
F2 weanling females					
Terminal body weight (g)	77 ±5	77 ±5	76 ±5	75 ±7	71 ±5*(↓8)
Liver					
Absolute	3199 ±301	3250 ±281	3198 ±309	3797 ±409***(↑19)	4082 ±357***(↑28)
Relative	4.16 ±0.19	4.20 ±0.19	4.21 ±0.24	5.05 ±0.22***(↑21)	5.73 ±0.26***(↑38)
Thyroid					
Absolute	7.5 ±1.4	7.5 ±1.0	7.5 ±1.6	7.8 ±1.4	7.4 ±1.5
Relative	0.00967 ±0.00178	0.00973 ±0.00125	0.00989 ±0.00210	0.01043 ±0.00222	0.1030 ±0.00216

a Data were obtained from Tables 33 and 34 on pages 103-104 of MRID 46817216.
* Significantly different from the control group at $p \leq 0.05$
*** Significantly different from the control group at $p \leq 0.001$

b) Pathology

1) **Macroscopic examination:** There were no treatment-related macroscopic findings in the F1 or F2 pups that were found dead during PND 0-4 or that were culled on PND 4. Among the pups found dead from PND 5-21 or surviving until scheduled termination, increased incidences of the following gross lesions were found compared to 0% controls (Table 13): (i) dark-colored liver in the F1 (26.6-35.9%; $p \leq 0.01$) and F2 (27.5-49.3%; $p \leq 0.01$) pups at ≥2000 ppm; (ii) thyroid enlargement in the F1 pups at 20,000 ppm (5.3%); (iii) liver enlargement in the F2 pups at 20,000 ppm (1.3%); (iv) hepatodiaphragmatic nodule in the F2 pups at ≥2000 ppm (3.8-3.9%); and (v) enlargement of the eyeball in the F2 pups at ≥2000 ppm (3.0-3.8%). Additionally, incidences of opacity and enlargement of the eyeball in the F1 pups and opacity and discoloration of the eyeball in the F2 pups were noted, but were unrelated to dose. No other gross lesions could be attributed to treatment.

TABLE 13. Selected gross findings (% litter incidence) in the F1 and F2 pups found dead during PND 5 and PND 21 or surviving until scheduled termination [a]

Parameter	Dose Group (ppm)				
	0	20	50	2000	20,000
F1 pups					
Liver - dark colored	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	26.6 ±16.6**	35.9 ±16.7**
Thyroid - enlargement	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	5.3 ±22.9
Eyeball - opacity	0.0 ±0.0	0.7 ±3.5	0.0 ±0.0	2.2 ±7.6	0.0 ±0.0
enlargement	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	12.3 ±25.2	3.7 ±12.1
F2 pups					
Liver - dark colored	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	27.5 ±28.9**	49.3 ±13.2**
enlargement	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.3 ±3.8
hepatodiaphragmatic nodule	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.9 ±8.7	3.8 ±7.1
Eyeball - opacity	0.0 ±0.0	0.0 ±0.0	1.1 ±5.2	0.7 ±2.9	0.6 ±2.8
enlargement	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.0 ±8.7	3.8 ±6.0
discoloration	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.8 ±3.4	0.0 ±0.0

a Data (n = 19-24) were obtained from Table 32 on pages 101-102 of MRID 46817216.
** Significantly different from the control group at $p \leq 0.01$

2) **Microscopic examination:** Incidences of the following microscopic findings (% incidence treated vs 0% controls) were significantly increased ($p \leq 0.05$; unless otherwise stated) at ≥2000 ppm over controls in both sexes of both generations (Table 14): (i) diffuse fatty change in the hepatocytes (43-72%); (ii) diffuse hepatocyte hypertrophy (39-100%); (iii) brown pigment deposition in the portal area in the liver (70-100%); (iv) bile duct proliferation (13-95%; NS in the F1 or F2 males at 2000 ppm); and (v) follicular cell hypertrophy in the thyroid (35-100%). Additionally at 20,000 ppm, hepatocyte focal necrosis was noted in the F1 males (6% treated vs 0 controls). No other microscopic lesions could be attributed to treatment in the offspring of either generation.

TABLE 14. Selected microscopic findings (# affected/ # examined) [a]					
	Dose Group (ppm)				
Parameter	0	20	50	2000	20,000
F1 male offspring					
Liver					
Fatty change, hepatocyte, diffuse	0	0	0	52***	72***
Hypertrophy, hepatocyte, diffuse	0	0	0	39***	100***
Deposition, brown pigment, portal area	0	0	0	70***	100***
Proliferation, bile duct	0	0	0	13	78***
Necrosis, hepatocyte, focal	0	0	0	0	6
Thyroid					
Hypertrophy, follicular cell	0	0	0	35**	100***
F1 female offspring					
Liver					
Fatty change, hepatocyte, diffuse	0	0	0	43***	67***
Hypertrophy, hepatocyte, diffuse	0	0	0	39***	94***
Deposition, brown pigment, portal area	0	0	0	78***	100***
Proliferation, bile duct	0	0	0	17*	94***
Thyroid					
Hypertrophy, follicular cell	0	0	0	48***	100***
F2 male offspring					
Liver					
Fatty change, hepatocyte, diffuse	0	0	0	65***	70***
Hypertrophy, hepatocyte, diffuse	0	0	0	53***	100***
Deposition, brown pigment, portal area	0	0	0	94***	100***
Proliferation, bile duct	0	0	0	24	75***
Thyroid					
Hypertrophy, follicular cell	0	0	0	47**	100***
F2 female offspring					
Liver					
Fatty change, hepatocyte, diffuse	0	0	0	44**	65***
Hypertrophy, hepatocyte, diffuse	0	0	0	56***	100***
Deposition, brown pigment, portal area	0	0	0	78***	100***
Proliferation, bile duct	0	0	0	39**	95***
Thyroid					
Hypertrophy, follicular cell	0	0	0	50***	90***

a Data were obtained from Table 35 on pages 105 and 107 of MRID 46817216.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS= CONCLUSIONS</u>: It was concluded that the LOAEL for the parents was 2000 ppm based on effects on the liver and thyroid. The LOAEL for offspring toxicity was 2000 ppm based on: increased incidences of clinical and gross pathological findings of enlargement of the eyeballs; dark-colored liver; increased weights of the thyroid, liver, and

uterus; and decreased thymus and spleen weights. Additionally at 20,000 ppm, maternal mortality occurred, and pup body weights were decreased. The LOAEL for reproductive toxicity was 20,000 ppm based on delayed sexual maturation in the F1 males which was of a high enough magnitude to be considered adverse (also observed at 2000 ppm). The investigators also noted that the sperm count per testis was decreased at 20,000 ppm, but that this effect was not evident when examined on a per gram basis.

B. REVIEWER COMMENTS

1. **PARENTAL ANIMALS:** There were no treatment-related effects on body weights, body weight gains, or food consumption in either generation during pre-mating in the males or during pre-mating, gestation, or lactation in the females.

   In the P generation, one 2000 ppm dam (#173) and one 20,000 ppm dam (#222) died during delivery on GD 22 or 23 without showing any clinical signs. In the F1 generation, two 20,000 ppm dams (#462 and 465) also died during delivery on GD 23 without showing any clinical signs. In the subsequent one-generation reproduction study (MRID 46817239), two maternal deaths occurred during delivery at 20,000 ppm. This finding adds further support that the deaths in the current study are treatment-related. However, because no deaths occurred in the one-generation study at 2000 ppm, it is possible that the death at this dose in the current study is unrelated to treatment.

   Clinical observations indicated enlargement of the **eyeball** throughout the study in one 20,000 ppm F1 dam.

   Treatment-related effects on the **liver** were observed, including increased organ weights and gross and microscopic lesions. In both generations, absolute and relative liver weights were increased ($p \leq 0.001$) in the females at ≥2000 ppm (↑35-63%) and in the males at 20,000 ppm (↑11-25%). Relative liver weights were also increased ($p \leq 0.05$) by 8% in the 2000 ppm P males. The following incidences of gross findings were increased ($p \leq 0.05$) over controls (# affected/24 rats compared to 0-1/24 controls: (i) dark color and enlargement of the liver in the females of both generations at ≥2000 ppm (6-21); and (ii) enlargement (2/24) and heptodiaphragmatic nodule (3/24) in the 20,000 ppm F1 males. Incidences of the following microscopic findings (# affected/24 vs 0/24 controls) were significantly increased ($p \leq 0.05$; unless otherwise stated) over controls: (i) periportal fatty change in the hepatocytes in both generations in the females at ≥2000 ppm (10-13) and in the males at 20,000 ppm (4-7, NS in P males); (ii) diffuse hepatocyte hypertrophy in the P and F1 females at ≥2000 ppm (5-16) and in the F1 males at 20,000 ppm (8); (iii) brown pigment deposition in the portal area in the liver in the F1 males and P and F1 females at ≥2000 ppm (6-24); (iv) bile duct proliferation in the 20,000 ppm dams of both generations (8-11); (v) focal hepatocyte necrosis in the P dams (3; NS) and massive hepatocyte necrosis in the F1 dams (1; NS); (vi) increased multinucleated hepatocytes in the 20,000 P dams (5); and (vii) hepatodiaphragmatic nodule in the 20,000 ppm F1 males (3; NS).

   In both generations, absolute and relative **thyroid** weights were increased in the females at

≥2000 ppm (↑25-84%) and in the males at 20,000 ppm (↑48-74%); the thyroid weight increases attained significance (p≤0.01) except in the relative weight in the 2000 ppm F1 dams. Absolute thyroid weights were also increased (p≤0.05) by 19% in the 2000 ppm P males. Enlargement and brown color of the thyroid were significantly increased (p≤0.05) in both sexes and both generations at 20,000 ppm (6-18/24 rats/group vs 0/24 controls), with brown color also observed in the females of both generations at 2000 ppm (5-6/24 rats/group vs 0 controls). Follicular cell hypertrophy in the thyroid was significantly increased (p≤0.001) at ≥2000 ppm in both sexes and both generations (14-23 of 24 rats/groups vs 0 controls).

Relative **pituitary** weights were decreased (p≤0.01) by 12-18% in the F1 males and females at ≥2000 ppm. Incidences of basophilic cell hydropic degeneration in the pituitary were increased (NS) in the P males and in the P and F1 females at 20,000 ppm (3-4 out of 24 treated vs 0 controls). Thus, the decreased pituitary weights at 2000 ppm were not considered adverse, because they were not corroborated by microscopic findings at that dose.

Absolute **adrenal** weights were increased (p≤0.05) at ≥2000 ppm and relative weights at 20,000 ppm (↑11-14%) in the P females; and incidences of diffuse cortical cell hypertrophy were increased (p≤0.001) in the 20,000 ppm P dams (16/24 treated vs 0/24 controls). Thus, the increased adrenal weights at 2000 ppm were not considered adverse, because they were not corroborated by microscopic findings at that dose. It should also be noted that the effects at 20,000 ppm were not observed in the concurrently-submitted chronic toxicity or carcinogenicity studies (MRIDs 46817217 and 46817219).

Additionally in the P dams, absolute **ovary** weights were increased (p≤0.05; ↑15-24%) at ≥50 ppm and relative weights at 20,000 ppm. Incidences of interstitial vacuolation in the ovaries were increased at ≥2000 ppm in the P dams (4-6/24/group vs 0 controls) and in the 20,000 ppm F1 dams (3/24 treated vs 0/24 controls), attaining significance (p≤0.05) only in the 20,000 ppm P dams. The increased ovary weights at 50 ppm were not considered adverse, because they were not corroborated by microscopic findings at that dose. There were no functional effects on fertility at any dose, and it is doubtful that the effects were treatment-related because there were no findings in the concurrently-submitted chronic toxicity or carcinogenicity studies.

Relative **kidney** weights were increased (p≤0.05) in the P dams at ≥2000 ppm and absolute weights at 20,000 ppm (↑9-19%); and incidences of tubular basophilic change and urinary cast in the kidneys were increased (p≤0.01) in the ≥2000 ppm P dams (9-11/24 vs 0/24 controls). In the concurrently-submitted carcinogenicity study, nephrotoxicity was indicated at 1000 and 20,000 ppm by increases (p≤0.05) in absolute and/or relative to body kidney weights in females (↑11-19%) and by slight to severe chronic nephropathy in males (84-92% treated vs 66% controls) and females (58-60% treated vs 18% controls). Dark-colored kidney (12% treated vs 2% controls; NS) was observed in the 20,000 ppm males in the carcinogenicity study.

The LOAEL for parental toxicity is 2000 ppm (equivalent to 146.3/167.5 mg/kg/day in males/females) based on effects on the liver, thyroid, ovaries, and kidneys as indicated by

changes in organ weights corroborated by gross and microscopic lesions. The NOAEL is 50 ppm (equivalent to 3.68/4.27 mg/kg/day in males/females).

2. **OFFSPRING:** There were no treatment-related effects on the number of implantations, number of pups delivered, sex ratio, or on the live birth, viability, or lactation indices.

   At 20,000 ppm, pup body weights were decreased ($p \leq 0.05$) by 8-9% on PND 21 in both sexes in both generations. There were no effects on body weights in the F1 or F2 offspring prior to PND 21; and absolute and relative (to body weight) anogenital distances of the F2 pups were comparable to controls on PND 4.

   Sexual maturation was delayed in the males, as indicted by a dose-dependent increase ($p \leq 0.05$) in the mean number of days until preputial separation at 50 ppm (42.5 days), 2000 ppm (43.0 days), and 20,000 ppm (43.7 days) compared to controls (41.3 days). Additionally at 2000 and 20,000 ppm, the body weight at which preputial separation occurred was increased ($p \leq 0.05$) by 5-7% over controls. The number of days until vaginal opening and body weight at sexual maturation in the females were unaffected by treatment.

   Absolute and relative **liver** weights were increased (↑14-38%; $p \leq 0.05$) at ≥2000 ppm in both sexes in both generations. Among the pups found dead from PND 5-21 or surviving until scheduled termination, increased incidences of the following gross lesions were found compared to 0% controls: (i) dark-colored liver in the F1 (26.6-35.9%; $p \leq 0.01$) and F2 (27.5-49.3%; $p \leq 0.01$) pups at ≥2000 ppm; (ii) hepatodiaphragmatic nodule in the F2 pups at ≥2000 ppm (3.8-3.9%); and (iii) enlargement of the liver in the F2 pups at 20,000 ppm (1.3%). Incidences of the following microscopic findings (% incidence treated vs 0% controls) were significantly increased ($p \leq 0.05$; unless otherwise stated) at ≥2000 ppm over controls in both sexes of both generations: (i) diffuse fatty change in the hepatocytes (43-72%); (ii) diffuse hepatocyte hypertrophy (39-100%); (iii) brown pigment deposition in the portal area in the liver (70-100%); and (iv) bile duct proliferation (13-95%; NS in 2000 ppm males of either generation). Additionally in the 20,000 ppm F1 males, hepatocyte focal necrosis (6% treated vs 0% controls) and hepatodiaphragmatic nodule (3/24 treated vs 0 controls) were observed.

   Absolute and relative **thyroid** weights were comparable to controls, with the exception of an increase of 24% ($p \leq 0.05$) in relative thyroid weight in the 20,000 ppm males. However, among the pups found dead from PND 5-21 or surviving until scheduled termination, enlargement of the thyroid was observed in the F1 pups at 20,000 ppm (5.3%; NS); and incidences of follicular cell hypertrophy in the thyroid were increased ($p \leq 0.01$) at ≥2000 ppm (35-100%) compared to 0% controls in both sexes of both generations.

   Enlargement of the **eyeball** was observed in the F2 pups at ≥2000 ppm (3.0-3.8%) compared to 0% controls. Incidences of opacity and enlargement of the eyeball in the F1 pups and opacity and discoloration of the eyeball in the F2 pups were noted, but were unrelated to dose. However, these effects were considered treatment-related in the subsequent one-generation study (MRID 46817239).

The LOAEL for offspring toxicity is 2000 ppm (equivalent to 146.3/167.5 mg/kg/day in males/females) based on effects on the liver and thyroid as indicated by changes in organ weights corroborated by gross and microscopic lesions. The NOAEL is 50 ppm (equivalent to 3.68/4.27 mg/kg/day in males/females).

3. **REPRODUCTIVE TOXICITY:** There were no effects of treatment on: the precoital interval; mating, fertility, or gestation indices; or gestation duration in either generation. Furthermore, the numbers of primordial ovarian follicles in the 20,000 ppm F1 dams were comparable to controls.

   Estrous cycle duration in the treated groups was comparable to controls in the P generation, but was slightly shorter ($p \leq 0.05$) in the F1 dams at 20,000 ppm (4.0 days) compared to controls (4.2 days). However, the percentage of dams with normal estrous cycles in the treated groups was comparable to controls in both generations. Thus, the minor change in duration was not considered adverse.

   The sperm count per testis and per gram testis in the treated P males was comparable to controls. Sperm count in the testis was 11% lower ($p \leq 0.05$) in the 20,000 ppm F1 males compared to controls. However, it is unlikely that this decrease is treatment-related because, when considered per gram testis, this decrease was minor (↓6%) and not significant. Furthermore, the sperm count per cauda epididymis and per gram cauda epididymis were unaffected by treatment. Sperm motility and morphology in the treated P and F1 males were comparable to controls.

   The LOAEL for reproductive toxicity was not observed. The NOAEL is 20,000 ppm (equivalent to 1471.5/1692.5 mg/kg/day in males/females).

   This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

C. **STUDY DEFICIENCIES:** No study deficiencies were noted.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; One-generation Reproduction Study in Rats

Work Assignment No. 4-1-124 V; formerly 3-1-124 V (MRID 46817239)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
John W. Allran, M.S.

Signature: John W Allran
Date: 01-30-07

Secondary Reviewer:
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J Bever Jr.
Date: 1/30/07

Project Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/30/07

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature:
Date: 1/30/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Myron S. Ottley **Signature:** MSOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/12/07
Work Assignment Manager: P.V. Shah **Signature:** P.V.Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/17/07
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - [rat]; Non-guideline

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (96.7% w/w)

SYNONYMS: Flubendiamide; N^2-(1,1-dimethyl-2-methylsulfonylethyl)-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]1,2-benzenedicarboxamide

CITATION: Hojo, H. (2004) NNI-0001: One-generation reproductive toxicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 03-0013, May 17, 2004. MRID 46817239. Unpublished.

SPONSOR: Nihon Nohyaku Co, Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In the previously conducted two-generation reproductive toxicity study (MRID 46817216), statistically significant differences were noted in various parameters, including sexual maturation in the F1 males at dose levels of 50 ppm and above. In order to determine if these effects were reproducible and therefore due to treatment, the present one-generation reproduction toxicity study (MRID 46817239) was conducted, in which Flubendiamide (96.7%; Batch # 1FH0019M) was administered in the diet to 24 Wistar Hanover rats/sex/dose group at dietary levels of 0, 50, 200, 2000, or 20,000 ppm. The P generation animals were fed the test diets for 10 weeks prior to mating to produce the F1 litters. On PND 21, the F1 offspring were weaned, and one or two F1 pups/sex/litter (24/sex/dose group) were selected to continue on study until sexual maturation and were fed the same test diet concentration as their dam. The remaining F1 offspring were killed and subjected to a gross necropsy; and selected organs were weighed from one pup/sex/litter (as nearly as possible).

There were no adverse treatment-related effects on clinical signs, body weights, body weight gains, or food consumption during pre-mating, gestation, or lactation in the P or F1 adults.

At 20,000 ppm, two P females died during delivery without showing any clinical signs.

Absolute and relative **liver** weights were increased ($p \leq 0.001$) in both generations in the ≥2000 ppm female (↑24-53%) and 20,000 ppm male (↑11-16%) adults. Incidences of the following

gross findings in the liver were significantly increased ($p \leq 0.05$) over controls (# affected/24 treated vs 0/24 controls): (i) dark in color in the ≥200 ppm P dams (8-22), ≥2000 ppm F1 dams (22-24), and 20,000 ppm F1 males (17); and (ii) enlarged liver in the ≥2000 ppm P dams (11-20) and 20,000 ppm F1 dams (24).

Absolute and relative **thyroid** weights were increased at ≥2000 ppm in the females of both generations (↑17-24%); these increases were significant ($p \leq 0.05$), except for the absolute weights in the F1 dams at 20,000 ppm. Incidences of the following gross findings in the thyroid were significantly increased ($p \leq 0.05$) over controls (# affected/24 treated vs 0/24 controls, unless otherwise noted): (i) enlarged in the 20,000 ppm P males (8 treated vs 2 controls); and (ii) brown in color in the ≥2000 ppm P dams (20-22) and in the 20,000 ppm P and F1 males (18-21).

At ≥2000 ppm in the F1 generation, absolute and relative **pituitary** weights were decreased ($p \leq 0.05$) in the males (↓9-13%) and females (↓19-24%). However, decreased pituitary weights were corroborated by hydropic degeneration only at 20,000 ppm in the 2-generation reproduction study.

Additionally in the females, absolute and relative **kidney** weights were increased (↑6-16%) at ≥2000 ppm in the P generation and at ≥200 ppm in the F1 generation; these increases were significant ($p \leq 0.05$), except for the relative weights in the P generation at 20,000 ppm. In the concurrently-submitted carcinogenicity study, nephrotoxicity was indicated at 1000 and 20,000 ppm by increases ($p \leq 0.05$) in absolute and/or relative to body kidney weights in females (↑11-19%) and by slight to severe chronic nephropathy in males (84-92% treated vs 66% controls) and females (58-60% vs 18%). Dark-colored kidney was observed in the 20,000 ppm males in the carcinogenicity study (12% treated vs 2% controls; not significant [NS]).

It is difficult to interpret the few organ weight and macroscopic findings that occurred at 200 ppm in the current study because this dose group fell between the LOAEL and the NOAEL in the two-generation study, and because microscopic examinations were not conducted on the organs in the current one-generation study. Thus, the finding of dark-colored liver in the P dams at this dose was considered equivocal.

The LOAEL for parental toxicity is 2000 ppm (equivalent to 127.2/148.9 mg/kg/day in males/females) based on effects on the liver, thyroid, and kidneys. The NOAEL is 200 ppm (equivalent to 12.91/14.97 mg/kg/day in males/females).

There were no effects of treatment on the number of implantations, number of pups delivered, sex ratio, or on the live birth, viability, or lactation indices.

Clinical signs of toxicity were limited to effects on the eyes and the following increased (NS, unless otherwise noted) incidences were observed (% treated vs 0% controls): (i) eyeball discoloration at >=2000 ppm during PND 8-14 and at 20,000 ppm during PND 15-21 (2.4-2.6%); (ii) opacity at >=2000 ppm during PND 15-21 (1.0-3.0%); and (iii) enlargement at 2000 ppm (3.6%) and 20,000 ppm (14.4%; p<=0.01) during PND 15-21. Among the pups found dead from PND 5-21 or surviving until scheduled termination, increased incidences of opacity (2.1-6.8%)

and enlargement (5.6-21.4%; p≤0.01 at 20,000 ppm) were found in the eyeballs at ≥2000 ppm compared to 0 controls. Additionally at 20,000 ppm, the incidence of discoloration of the eyeball was increased (1.6%; NS) compared to 0% controls.

At 20,000 ppm, F1 pup body weights were decreased by 9% in each sex compared to controls on PND 21. Absolute and relative (to body weight) anogenital distances were increased by in the >=2000 ppm male pups on PND 4. These parameters were comparable to controls in the treated females.

Sexual maturation was delayed in both sexes. Increases (p<=0.05) in the mean number of days until preputial separation were observed in the males at 2000 ppm (42.2 days) and 20,000 ppm (43.1 days) compared to controls (41.0 days) and in the number of days until vaginal opening in the 20,000 ppm females (32.3 days) compared to controls (30.4 days). Additionally at 20,000 ppm, the body weight at which sexual maturation occurred was increased (p<=0.05) over controls. This finding confirmed that the delayed preputial separation observed in the 2000 ppm F1 males in the 2-generation study was due to treatment.

Except for the **liver**, there was no evidence of any treatment-related effects on organ weights in the F1 offspring in the current study. At >=2000 ppm, absolute and relative liver weights were increased (p<=0.001) in both sexes. At necropsy, incidences of dark-colored liver were increased (p<=0.01) at >=2000 ppm (25.7-42.8%) compared to 0 controls.

The LOAEL for offspring toxicity 2000 ppm (equivalent to 127.2/148.9 mg/kg/day in males/females) based on effects on the eyes and liver and on increased anogenital distance and delayed sexual maturation in the males. The NOAEL is 200 ppm (equivalent to 12.91/14.97 mg/kg/day in males/females).

There were no effects of treatment on: the precoital interval; mating, fertility, or gestation indices; or gestation duration. The percentage of P dams with normal estrous cycles and the estrous cycle duration in the treated groups were comparable to controls, although in two-generation study, estrous cycle duration was slightly shorter (p<=0.05) in the F1 dams at 20,000 ppm (4.0 days) compared to controls (4.2 days). No measurements were performed on sperm counts, motility, or morphology in the current study.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 20,000 ppm (equivalent to mg/kg/day in 1287/1490 in males/females).

This study is classified as an **acceptable/non-guideline** one-generation reproduction study in the rat.

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

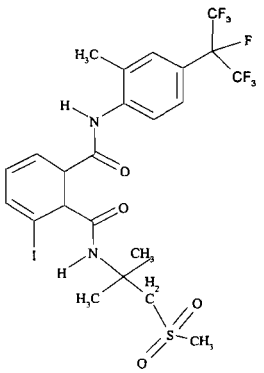
127

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide) technical

Description: White crystals
Lot/batch #: 1FH0019M
Purity: 96.7% a.i.
Compound stability: The test substance was stable in the diet for up to 4 weeks at 5°C followed by 3 weeks at room temperature.
CAS # of TGAI: 272451-65-7
Structure:



2. Vehicle: Diet

3. Test animals

Species:	Rat	
Strain:	Wistar Hanover (BrlHan:WIST@Jcl[GALAS])	
Age at study initiation:	5 weeks	
Weight at study initiation:	146-168 g, males; 117-136 g, females	
Source:	Fuji Breeding Center, Clea Japan, Inc.	
Housing:	Acclimation (five/sex) and pre-mating (three/sex) - in suspended wire-mesh stainless steel cages. Mating - in pairs (1 male: 1 female) in TR-360 aluminum cages with wire-mesh floors and fronts. Gestation and lactation - Dams housed individually during gestation and with their litters during lactation in TR-358B cages. After weaning, dams were removed and pups continued to be housed by litter in TR-358B cages.	
Diet:	Certified pulverized feed, MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*	
Water:	Filtered (sand and charcoal) and sterilized (sodium hypochlorite) well water, *ad libitum*	
Environmental conditions:	**Temperature**	22 ± 2°C
	Humidity	55 ± 15%
	Air changes	At least 10/hour
	Light cycle	12 hours light/12 hours dark
Acclimation period:	11 days	

B. PROCEDURES AND STUDY DESIGN

1. **Purpose:** The objective of the current study was to determine if the statistically significant differences from controls observed in various parameters in the concurrently-submitted 2-generation study were reproducible (i.e., whether or not they could be considered treatment-related). The delayed sexual maturation in the F1 males was of particular interest.

2. **Mating procedure:** Each P male was placed in a TR-360 cage prior to the mating period. Daily vaginal smears were taken for up to two weeks prior to mating. Each female in proestrus was placed that evening in the cage with the male (1 male: 1 female) from the same dose group. Females that did not repeatedly show proestrus vaginal smears (i.e., did not have normal estrous cycling) were paired with males on the first day of the mating period (Week 11). Vaginal smears were taken daily and examined for positive evidence of mating as indicated by the presence of vaginal plugs or sperm in a vaginal smear. The day on which positive evidence of mating was detected was designated as gestation day (GD) 0. Females with positive evidence of mating were separated from the male and housed individually in breeding boxes with nesting materials, and the P males were returned to their cages. No further matings were reported.

3. **Study schedule:** The P generation animals were fed the test diets for 10 weeks prior to mating to produce the F1 litters. On post-natal day (PND) 4, litters with nine or more pups were culled to yield 4 pups/sex/litter (as nearly as possible). On PND 21, the F1 offspring were weaned. During PND 21-25, one or two F1 pups/sex/litter (24/sex/dose group) were selected to continue on study until sexual maturation and were fed the same test diet concentration as their dam. P animals were euthanized for terminal examination after selection of the F1 offspring. The remaining F1 offspring were killed on PND 26 and subjected to a gross necropsy; and selected organs were weighed from one pup/sex/litter (as nearly as possible).

4. **Animal assignment:** The P animals were assigned to the test groups shown in Table 1 in such as way to equalize the group means and standard deviations of body weights as closely as possible.

TABLE 1. Animal assignment [a]

Test group	Dose (ppm) [b]	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	24	24	24	24
Low	50	24	24	24	24
Lower middle	200	24	24	24	24
Higher middle	2000	24	24	24	24
High	20,000	24	24	24	24

a Data were obtained from pages 22 and 24 of MRID 46817239.
b Exposure to the test substance was continuous throughout the study.

5. **Dose-selection rationale:** Doses for the current study were selected based on the results from a concurrently submitted two-generation reproduction toxicity study in rats (MRID 46817216) in which 24 P generation rats/sex/group were fed test diets at concentrations of 0, 20, 50, 2000, or 20,000 ppm. Parental toxicity at ≥2000 ppm entailed effects on the liver, thyroid, and kidneys as indicated by changes in organ weights corroborated by gross and microscopic lesions. Similarly, offspring toxicity occurred at ≥2000 ppm, characterized by effects on the liver and thyroid as indicated by changes in organ weights corroborated by gross and microscopic lesions. Although it was determined that no reproductive toxicity was evident in the 2-generation study; the purpose of this study was to determine if the delayed sexual maturation observed in the F1 males in the 2-generation study occurred again in the current study. Thus, the doses selected in the current study (0, 50, 200, 2000, or 20,000 ppm) were similar, but not identical, to the doses employed in the 2 generations study (0, 20, 50, 2000, or 20,000 ppm).

6. **Test diet preparation and analysis:** For each dose level, the appropriate amount of the test substance was mixed with a small amount of basal feed. This pre-mix was stirred with additional feed to achieve the desired concentration. Test diets were prepared once every two to four weeks and were stored in plastic bags in the dark at approximately 5°C until use. Concentration analyses were performed on samples taken from the middle of the mixer at the time of each preparation. The homogeneity of the test substance in the diet was verified in the previously conducted two-generation reproduction toxicity study (MRID 46817216) by taking samples from the top, middle, and bottom of the mixer from the first batch. Stability of the test substance in the diet was verified in the two generation study in 20 and 20,000 ppm diets after storage for 4 weeks at 5°C followed by up to 3 weeks at room temperature.

   Results

   Homogeneity (% C.V.): 0.2-4.4%

   Stability (% initial): 97-105%

   Concentration (mean % nominal): 92-107%

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

7. **Dosage administration:** The test material was administered in the diet continuously throughout the study (i.e., P generation adults were fed the test diets *ad libitum* beginning 10 weeks prior to mating, and the selected F1 adults were fed the same test diet concentrations as their parents beginning on PND 21-25 until study termination upon sexual maturation of the F1 generation).

C. OBSERVATIONS

1. **Parental animals:** All rats were examined for mortality and clinical signs of toxicity twice

daily (once daily on holidays). Detailed examinations were performed at the same time that body weights were recorded. Body weights of the males were recorded on the first day of treatment, weekly throughout the study, and on the day of necropsy. For the females, body weights were recorded: on the first day of treatment, weekly during pre-mating; on GD 0, 7, 14, and 20; on lactation day (LD) 0, 7,14, and 21; and on the day of necropsy. Females that did not show any signs of parturition were weighed weekly from presumed GD 25 and on the day of necropsy. Weekly cumulative body weight gains and overall gains for pre-mating (Weeks 0-10), combined pre-mating and mating (Weeks 0-15), and overall study (Weeks 0-16) periods were reported for the males. For the females, weekly cumulative body weight gains were reported for the pre-mating, gestation, and lactation periods, in addition to overall weight gains during each of these periods. Food consumption was recorded on the day of body weight measurement and was reported as a daily average (g food/rat/day) for each of the intervals for which body weight gains were determined. Estrous cycle periodicity and duration were determined from vaginal smears taken daily beginning at least 2 weeks prior to mating. Sperm enumeration, motility, and morphology were not determined in this study.

2. **Litter observations:** The following litter parameters (X) were recorded in all F1 offspring (Table 2):

TABLE 2. F_1 Litter Observations [a]

Observation	Time of observation (lactation day)				
	Day 0	Day 4	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X
Number of dead pups	X	X	X	X	X
Litter weight	X				
Pup weight		X	X	X	X
Sex of each pup (M/F)	X				
External alterations	X	X	X	X	X
Anogenital distance [b]		X			

a Data obtained from pages 28-29 in MRID 46817239.
b Recorded for F1 pups, along with body weight, on PND 4 prior to culling.

Pups were examined daily for mortality and clinical signs of toxicity by cage-side observation throughout lactation. In addition, F1 pups were given a complete external examination when they were weighed. Sexual maturation was determined for all F1 weanlings. Daily checks were made for vaginal opening beginning on PND 26 and for preputial separation beginning on PND 35. Body weight was recorded for each rat on the day on which criterion was achieved.

3. **Postmortem observations**

a. **Parental animals:** At study termination, all surviving P and F1 males and females were anesthetized with ether, euthanized by exsanguination via the abdominal aorta and caudal vena cava, and subjected to a gross necropsy according to the following schedule: P males after confirmation of parturition of the paired females and upon judgment by the study director

that no further mating was needed; P dams after weaning of their F1 pups; and F1 animals (selected to continue on study after weaning) upon sexual maturation. Vaginal smears were taken from P and F1 females at necropsy. Animals found dead during the study were subjected to a gross necropsy immediately after discovery.

The following checked (X) tissues were collected from all animals, including decedents, and fixed and preserved in neutral-buffered 10% formalin (with the exception of the testes which were fixed in Formalin-Sucrose-Acetic acid [FSA]). Additionally from the animals surviving to scheduled termination, the (XX) organs were weighed:

XX	Brain	XX	Testes [a]	XX	Ovaries [a]
XX	Pituitary	XX	Epididymides [a]	XX	Uterus [c]
XX	Thyroids	XX	Seminal vesicles [b]	XX	Cervix [c]
XX	Liver	XX	Coagulating gland [b]	XX	Oviducts [c]
XX	Kidneys [a]	XX	Prostate gland (ventral lobe)	X	Vagina
XX	Adrenals [a]				Mammary gland (females)
XX	Spleen				
X	Gross lesions				

a Paired organs were weighed together and reported as an average of both sides.
b The seminal vesicles were weighed with the fluid, including coagulating glands
c The uterus was weighed with the oviducts and cervix.

All of the above-mentioned tissues and organs from all groups were examined macroscopically, and any gross lesions were recorded. Microscopic examinations were not conducted.

b. Offspring: F1 weanlings that were not selected to continue on study to examine sexual maturation were euthanized by overdose of ether inhalation on PND 26. These animals, along with F1 pups culled on PND 4 and any decedents, were subjected to a gross necropsy. Additionally, from among the F1 offspring terminated on PND 26, one pup/sex/litter was selected for organ weight measurement and tissue preservation; the brain, thyroids, thymus, spleen, liver, and uterus from these animals were weighed following ether anesthesia and exsanguination. In addition to these organs, the head (including eyes and pituitary), kidneys, adrenals, reproductive organs, and any gross lesions were fixed and preserved in neutral-buffered 10% formalin. Microscopic examinations were not performed.

D. DATA ANALYSIS

1. Statistics: The following statistical procedures were used:

Parameter	Statistical procedure
Parental body weights Parental body weight gains Parental food consumption Number of implantation sites Number of pups delivered Parental and pup organ weights Anogenital distance of pups Pup body weights	Bartlett's test for homogeneity of variances. If variances are homogeneous, differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test or Scheffes' multiple comparison test to compare treated groups with controls if ANOVA is significant. If variances are not homogeneous, differences among groups were analyzed using Kruskal-Wallis followed by Dunnett type mean rank test or Scheffe-type mean rank test to compare treated groups with controls if Kruskal-Wallis is significant.
Parental clinical signs Incidences of normal estrous cycle Mating, fertility, and gestation indices Parental gross pathology Sex ratio	Fisher's exact probability test
Sexual development Estrous cycle length Pre-coital interval Gestation duration Viability indices Offspring clinical signs Offspring gross pathology	Mann-Whitney *U*-test

Statistical significance was denoted at $p \leq 0.05$, 0.01, and 0.001. Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

2. Indices

Reproductive indices: The following reproductive/viability indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Male mating index (%) = # copulations/ # males used for mating x 100

Female mating index (%) = # copulations/ # females used for mating x 100

Pre-coital interval = # days from cohabitation of males and females to confirmation of copulation.

Fertility index (%) = # pregnancies/ # females copulated x 100

Gestation index (%) = # normal parturitions/ # pregnancies x 100

Gestation duration = # days from detection of copulation to completion of parturition

Offspring indices: The following viability indices were calculated by the performing laboratory from lactation records of litters in the study:

Sex ratio (% males) = # male pups/ # pups delivered x 100

Live birth index (%) = # live pups on PND 0/ total # pups delivered x 100

Viability index (%) = # pups surviving to PND 4/ # pups alive on PND 0 x 100

Lactation indices (%) = # pups alive on PND 7, 14, or 21/ # pups selected on PND 4 x 100

Relative anogenital distance = anogenital distance (mm)/ [body weight (g) x 1000] $^{1/3}$

3. **Historical control data:** Not provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** At 20,000 ppm, two P females (#224 and 231) died during delivery without showing any clinical signs.

b. **Clinical signs of toxicity:** Enlargement of the eyeball was observed in 1/24 F1 dams, each, at 2000 and 20,000 ppm compared to 0/24 controls. However, this finding was noted in the F1 males in one control animal and in one 20,000 ppm rat. Furthermore, no effects on the eyes were noted in the concurrently-submitted chronic or carcinogenicity studies (MRIDs 46817217 and 46817219). Thus, although effects are seen on the eyes in the offspring, the findings of enlarged eyeball in individual parents are considered unrelated to treatment. No other clinical observations could be attributed to treatment.

2. Body weight, body weight gain, and food consumption

a. **Pre-mating:** There were no treatment-related effects on body weights, body weight gains, or food consumption in the P generation during the pre-mating period. However, in the F1 females, cumulative body weight gains were increased (↑9-13%; $p \leq 0.05$) at 2000 ppm at Week 1 and at 20,000 ppm for Weeks 1-4 (Table 3). Additionally in the F1 dams, food consumption was increased by 6-10% during Weeks 1 and 2 at 50, 200, and 20,000 ppm. However, these increases were considered minor, not adverse, and (in the case of food consumption) unrelated to dose.

b. **Gestation:** There were no effects of treatment on body weights, body weight gains, or food consumption in the P dams during gestation.

c. **Lactation:** There were no effects of treatment on body weights, body weight gains, or food consumption in the P dams during lactation.

TABLE 3. Selected mean (∀SD) body weight gains (g) and food consumption (g/animal/day) in the F1 dams during pre-mating [a]

Observation/ study week	Dose Group (ppm)				
	0	50	200	2000	20,000
F1 Generation					
Body weight gains					
Week 0-1	30 ± 3	32 ± 3	32 ± 4	33 ± 4** (↑10)	34 ± 3*** (↑13)
Week 0-2	60 ± 6	62 ± 7	61 ± 8	64 ± 6	66 ± 6* (↑10)
Week 0-3	79 ± 9	83 ± 9	81 ± 13	85 ± 9	87 ± 9* (↑10)
Week 0-4	99 ± 11	102 ± 11	99 ± 15	104 ± 11	108 ± 11* (↑9)
Food consumption					
Week 1	9.7 ± 0.4	10.5 ± 0.5** (↑8)	10.7 ± 0.4*** (↑10)	10.1 ± 0.4	10.6 ± 0.3*** (↑10)
Week 2	13.1 ± 0.6	13.9 ± 0.3* (↑6)	14.0 ± 0.4* (↑7)	13.8 ± 0.8	14.0 ± 0.6** (↑7)

a Data (n = 24) were obtained from Tables 7 and 9 on pages 53 and 55 of MRID 46817239. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from the control group at p≤0.05
** Significantly different from the control group at p≤0.01
*** Significantly different from the control group at p≤0.01

3. **Test substance intake:** Test substance intake (mg/kg/day) was calculated from the body weight and food consumption data, using the nominal concentration (ppm) of the diets. The mean test substance intake for the P generation during pre-mating is considered to be representative of the achieved intake for the entire study (Table 4). The F1 generation data are not included because they were terminated after 5 weeks.

TABLE 4. Mean test substance intake (mg/kg/day in males/females) during pre-mating [a]

Generation	Dose (ppm)				
	0	50	200	2000	20,000
P generation	0/0	3.25/3.84	12.91/14.97	127.2/148.9	1287/1490

a Data were obtained from Table 10 on page 56 of MRID 46817239.

4. **Reproductive function**

a. **Estrous cycle length and periodicity:** The percentage of P dams with normal estrous cycles and the estrous cycle duration in the treated groups were comparable to controls.

b. **Sperm measures:** Not conducted.

5. **Reproductive performance:** There were no effects of treatment on: the precoital interval;

mating, fertility, or gestation indices; or gestation duration (Table 5).

TABLE 5. Reproductive performance [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
P Generation (F1 litter)					
Precoital interval (days)	1.0 ± 0.0	1.1 ± 0.4	1.2 ± 0.9	1.0 ± 0.2	1.0 ± 0.0
Mating index (%) male	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
female	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)	24/24 (100)
Fertility index (%)	23/24 (95.8)	24/24 (100)	22/24 (91.7)	24/24 (100)	23/24 (95.8)
Gestation index (%)	23/23 (100)	24/24 (100)	22/22 (100)	24/24 (100)	21/23 (91.3)
Gestation duration (days)	22.3	22.4	22.3	22.3	22.5

a Data were obtained Tables 14 and 15 on pages 60-61 of MRID 46817239.

6. Parental postmortem results

a. Organ weights: Selected organ weights are presented in Tables 6a and 6b. Absolute and relative (to body weight) liver weights were increased ($p \leq 0.001$) in both generations in the 20,000 ppm male (↑11-16%) and ≥2000 ppm female (↑24-53%) adults. At ≥2000 ppm in the F1 generation, absolute and relative pituitary weights were decreased ($p \leq 0.05$) in the males (↓9-13%) and females (↓19-24%).

Additionally in the females, absolute and relative kidney weights were increased (↑6-16%) at ≥2000 ppm in the P generation and at ≥200 ppm in the F1 generation; these increases were significant ($p \leq 0.05$), except for the relative weights in the P generation at 20,000 ppm. Increases ($p \leq 0.05$) were observed in absolute ovary weights at ≥200 ppm in the P dams and in absolute and relative weights in the 50, 2000, and 20,000 ppm F1 dams. Absolute and relative thyroid weights were increased at 20,000 ppm in both generations (↑17-24%); these increases were significant ($p \leq 0.05$), except for the absolute weights in the F1 generation at 20,000 ppm.

Several other organ weights differed significantly ($p \leq 0.05$) from controls but were considered unrelated to treatment because these differences, if present at all in the two-generation reproduction study (MRID), were not corroborated by gross or microscopic findings.

TABLE 6a. Selected absolute (mg) and relative to body weight (%) organ weights in males [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
P generation					
Terminal BW (g)	424 ± 29	421 ± 29	417 ± 37	421 ± 29	431 ± 25
Liver					
Absolute (mg)	13080 ± 1768	12972 ± 1446	13225 ± 1314	13499 ± 1604	14775 ± 1537*** (↑13)
Relative (%)	3.07 ± 0.26	3.08 ± 0.25	3.17 ± 0.16	3.20 ± 0.26	3.42 ± 0.25*** (↑11)
F1 generation					
Terminal BW (g)	366 ± 29	370 ± 30	364 ± 36	376 ± 30	378 ± 35
Liver					
Absolute (mg)	13127 ± 1662	13236 ± 1450	13230 ± 1710	14020 ± 1710	15275 ± 2472*** (↑16)
Relative (%)	3.58 ± 0.26	3.57 ± 0.20	3.62 ± 0.23	3.73 ± 0.32	4.03 ± 0.37*** (↑13)
Pituitary					
Absolute (mg)	9.3 ± 0.9	9.1 ± 0.7	8.7 ± 1.4	8.5 ± 0.8* (↓9)	8.3 ± 1.3** (↓11)
Relative (%)	0.00255 ± 0.00024	0.00247 ± 0.00024	0.00237 ± 0.00027	0.00227 ± 0.00029**(↓11)	0.00221 ± 0.00025***(↓13)

a Data (n = 24) were obtained from Tables 18 and 20 on pages 66 and 68 of MRID 46817239.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

TABLE 6b. Selected absolute (mg) and relative to body weight (%) organ weights in females [a]					
	Dose Group (ppm)				
Parameter	0	50	200	2000	20,000
P generation					
Terminal BW (g)	265±16	265±20	269±17	273±15	275±19
Liver					
Absolute (mg)	11657±1308	11682±1678	12489±1256	16326±1619***(↑40)	17836±2135*** (↑53)
Relative (%)	4.40±0.37	4.41±0.44	4.64±0.40	5.99±0.41***(↑36)	6.49±0.59*** (↑48)
Kidneys					
Absolute (mg)	1021±96	1010±108	1079±119	1146±86**(↑12)	1122±113* (↑10)
Relative (%)	0.385±0.026	0.382±0.028	0.401±0.037	0.421±0.022**(↑9)	0.408±0.024 (↑6)
Ovaries					
Absolute (mg)	60.7±6.9	64.0±8.6	69.4±11.4* (↑14)	69.6±6.8* (↑15)	70.2±11.8* (↑16)
Relative (%)	0.0230±0.0028	0.0242±0.0027	0.0259±0.0044	0.0256±0.0028	0.0257±0.0049
Thyroid					
Absolute (mg)	26.2±7.8	27.8±9.7	23.4±3.1	33.6±21.3	31.6±4.9* (↑21)
Relative (%)	0.00989±0.00293	0.01050±0.00356	0.00869±0.00112	0.01232±0.00782	0.01156±0.00201* (↑17)
F1 generation					
Terminal BW (g)	166±13	168±11	167±16	168±12	172±16
Liver					
Absolute (mg)	7250±742	7347±659	7550±1071	9109±880***(↑26)	10782±1253*** (↑49)
Relative (%)	4.37±0.40	4.37±0.24	4.52±0.33	5.42±0.32***(↑24)	6.26±0.38*** (↑43)
Pituitary					
Absolute (mg)	9.0±1.0	8.5±1.3	8.3±1.0	6.9±0.8*** (↓23)	7.3±1.0*** (↓19)
Relative (%)	0.00544±0.00061	0.00506±0.00070	0.00500±0.00051*(↓8)	0.00412±0.00045***(↓24)	0.00424±0.00046***(↓22)
Kidneys					
Absolute (mg)	718±62	756±58	786±90** (↑9)	789±60** (↑10)	830±100*** (↑16)
Relative (%)	0.432±0.023	0.450±0.022	0.471±0.024***(↑9)	0.470±0.027***(↑9)	0.483±0.032*** (↑12)
Ovaries					
Absolute (mg)	38.4±4.8	43.7±7.0* (↑14)	41.6±8.2	46.2±8.1***(↑20)	46.4±7.1***(↑21)
Relative (%)	0.0232±0.0027	0.0260±0.0036*(↑12)	0.0250±0.0043	0.0275±0.0043***(↑19)	0.0270±0.0032**(↑16)
Thyroid					
Absolute (mg)	14.7±4.3	17.2±5.7	16.5±5.1	16.5±3.6	18.2±3.5 (↑24)
Relative (%)	0.00887±0.00243	0.01031±0.00386	0.00993±0.00278	0.00982±0.00185	0.01056±0.00157***(↑19)

a Data (n = 21-24) were obtained from Tables 19 and 21 on pages 67 and 69 of MRID 46817239.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

b. Gross pathology: Treatment-related findings at necropsy were limited to the liver and thyroid (Table 7). Incidences of the following gross findings were significantly increased ($p \leq 0.05$) over controls (# affected/24 treated vs 0/24 controls, unless otherwise noted): (i) thyroids that were enlarged (8 treated vs 2 controls) and brown in color (21) in the 20,000 ppm P males; (ii) liver that was dark in color (17) and thyroids that were brown in color (18) in the 20,000 ppm F1 males; (iii) enlarged liver in the ≥2000 ppm P dams (11-20); (iv) liver that was dark in color in the ≥200 ppm P dams (8-22); (v) thyroids that were brown in color in the ≥2000 ppm P dams (20-22); (vi) liver that was enlarged at 20,000 ppm (24) and dark in color at ≥2000 ppm in the F1 dams (22-24). No other macroscopic findings in the adults could be attributed to treatment.

TABLE 7. Selected gross pathological findings (# affected out of 24 rats) [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
P generation Males					
Thyroid					
Enlargement	2	2	5	1	8*
Brown in color	0	0	0	0	21***
F1 generation Males					
Liver					
Dark in color	0	0	0	0	17***
Thyroid					
Brown in color	0	0	0	1	18***
P generation Females					
Liver					
Enlargement	0	0	0	11***	20***
Dark in color	0	0	8**	22***	22***
Thyroid					
Brown in color	0	0	0	20***	22***
F1 generation Females					
Liver					
Enlargement	0	0	0	0	24***
Dark in color	0	0	0	22***	24***

a Data were obtained from Tables 16 and 17 on pages 62-65 of MRID 46817239.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

B. OFFSPRING

1. **Viability and clinical signs:** Clinical signs of toxicity were limited to effects on the eyes and the following increased (not significant, unless otherwise noted) incidences were observed (% treated vs 0% controls; Table 8): (i) eyeball discoloration at ≥2000 ppm during PND 8-14 and at 20,000 ppm during PND 15-21 (2.4-2.6%); (ii) opacity at ≥2000 ppm during PND 15-21 (1.0-3.0%); and (iii) enlargement at 2000 ppm (3.6%) and 20,000 ppm (14.4%; $p \leq 0.01$) during PND 15-21. No other clinical observations could be attributed to treatment. There were no effects of treatment on the number of implantations, number of pups delivered, sex ratio, or on the live birth, viability, or lactation indices.

TABLE 8. Litter parameters in the F1 generation [a]						
Parameter		Dose Group (ppm)				
		0	50	200	2000	20,000
F1 litter						
Mean (±SD) implantations		12.9 ± 2.1	12.5 ± 3.7	13.5 ± 2.2	14.3 ± 2.0	14.0 ± 2.3
Number of pups delivered		12.3 ± 2.2	11.6 ± 3.5	12.5 ± 2.5	13.2 ± 1.9	12.2 ± 2.4
Sex ratio (% males)		47.7	45.5	47.3	49.1	45.5
Live birth index (%)	PND 0 [b]	100.0	97.2	98.3	98.2	95.0
Viability index (%)	PND 4 [c]	99.3	95.8	98.0	98.8	95.8
Lactation index (%)	PND 7 [d]	100.0	99.5	100.0	99.5	99.4
	PND 14 [d]	100.0	98.9	99.4	99.5	96.4
	PND 21 [d]	100.0	98.9	99.4	99.5	96.4
Eyeball (%) discoloration	PND 8-14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 7.4	2.5 ± 6.5
	PND 15-21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 6.4
opacity	PND 15-21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 5.1	3.0 ± 6.7
enlargement	PND 15-21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.6 ± 9.4	14.4 ± 17.4**

a Data (n = 21-24)were obtained from Tables 15 and 23 on pages 61 and 73 of MRID 46817239.
b Live birth index (denoted by the Sponsor as viability index on PND 0) = # alive on PND 0/ # delivered x 100
c Viability index (denoted by the Sponsor as viability index on PND 4) = # pups surviving to PND 4/ # pups alive on PND 0 x 100
d Lactation indices (denoted by the Sponsor as viability indices on PND 7,14, and 21) = # pups alive on PND 7, 14, or 21 (respectively)/ # pups selected on PND 4 x 100
** Significantly different from the control group at $p \leq 0.01$

2. **Body weight:** At 20,000 ppm, F1 pup body weights were decreased by 9% ($p \leq 0.01$) in each sex compared to controls on PND 21 (Table 9). There were no other differences in body weights during the post-natal period.

TABLE 9. Mean (± SD) pup weights in the F1 generation [a]						
Post-natal day (PND)	Dose Group (ppm)					
	0	50	200	2000	20,000	
F1 males						
0	6.0 ± 0.4	6.0 ± 0.4	6.0 ± 0.6	6.2 ± 0.7	6.3 ± 0.6	
4	11.0 ± 1.1	10.8 ± 1.1	11.2 ± 1.5	11.0 ± 1.4	11.5 ± 1.4	
7	18.1 ± 1.3	17.9 ± 1.7	18.2 ± 2.6	17.5 ± 2.2	18.0 ± 2.4	
14	36.8 ± 2.2	37.2 ± 2.2	37.1 ± 3.9	35.2 ± 3.3	35.9 ± 3.3	
21	57.8 ± 3.2	58.7 ± 3.9	58.4 ± 4.9	54.3 ± 4.5	52.7 ± 4.2** (↓9)	
F1 females						
0	5.7 ± 0.5	5.8 ± 0.4	5.8 ± 0.6	5.9 ± 0.6	6.0 ± 0.6	
4	10.5 ± 1.2	10.5 ± 1.1	10.9 ± 1.4	10.7 ± 1.4	11.3 ± 1.3	
7	17.4 ± 1.4	17.1 ± 1.8	17.8 ± 2.1	17.1 ± 2.1	17.6 ± 2.3	
14	35.8 ± 2.2	35.3 ± 3.8	36.4 ± 2.8	34.3 ± 3.2	35.1 ± 3.1	
21	55.4 ± 3.1	55.3 ± 4.9	55.9 ± 3.6	51.9 ± 4.3	50.5 ± 4.0** (↓9)	

a Data (n = 21-24)were obtained from Table 24 on page 75 of MRID 46817239.
** Significantly different from the control group at $p \leq 0.01$

3. **Anogenital distance:** Absolute and relative (to body weight) anogenital distances were increased by 4-6% ($p \leq 0.05$) compared to controls in the ≥2000 ppm F1 males on PND 4 (Table 10). These parameters were comparable to controls in the treated females.

TABLE 10. Mean (± SD) anogenital distance in F1 generation pups on PND 4 [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
F1 males					
Body weight (g) [b]	10.8 ± 1.1	10.6 ± 1.1	11.0 ± 1.5	10.8 ± 1.5	11.3 ± 1.4
Absolute anogenital distance (mm)	5.02 ± 0.21	5.14 ± 0.23	5.20 ± 0.35	5.23 ± 0.20* (↑4)	5.31 ± 0.25**(↑6)
Relative anogenital distance [c]	0.228 ± 0.009	0.234 ± 0.006	0.234 ± 0.009	0.238 ± 0.009**(↑4)	0.237 ± 0.007**(↑4)
F1 females					
Body weight (g) [b]	10.4 ± 1.2	10.3 ± 1.1	10.7 ± 1.5	10.3 ± 1.4	11.1 ± 1.4
Absolute anogenital distance (mm)	2.47 ± 0.09	2.50 ± 0.13	2.55 ± 0.17	2.55 ± 0.13	2.57 ± 0.11
Relative anogenital distance [c]	0.114 ± 0.004	0.115 ± 0.004	0.116 ± 0.006	0.118 ± 0.006	0.116 ± 0.005

a Data were obtained from Table 25 on page 76 of MRID 46817239.
b Body weight prior to culling on PND 4
c Relative anogenital distance = absolute anogenital distance in mm/(body weight in grams x 1000)^3
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$

4. **Sexual maturation:** Sexual maturation was delayed in both sexes (Table 11). Increases ($p \leq 0.05$) in the mean number of days until preputial separation were observed in the males at 2000 ppm (42.2 days) and 20,000 ppm (43.1 days) compared to controls (41.0 days) and in the number of days until vaginal opening in the 20,000 ppm females (32.3 days) compared to controls (30.4 days). Additionally at 20,000 ppm, the body weight at which sexual maturation occurred was increased ($p \leq 0.05$) by 8-12% over controls.

TABLE 11. Sexual maturation in the F1 generation pups [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
F1 males					
Days until preputial separation	41.0 ± 1.7	40.9 ± 1.4	41.1 ± 1.5	42.2 ± 1.9*	43.1 ± 2.1**
Body weight at criterion (g)	175.8 ± 11.9	175.8 ± 12.3	173.5 ± 14.5	183.0 ± 17.3	189.4 ± 21.9* (↑8)
F1 females					
Days until vaginal opening	30.4 ± 1.8	30.4 ± 2.1	30.2 ± 2.1	31.3 ± 1.8	32.3 ± 1.9**
Body weight at criterion (g)	92.8 ± 11.7	94.0 ± 10.4	93.2 ± 11.0	98.2 ± 11.3	103.7 ± 8.9** (↑12)

a Data (n = 24) were obtained from Table 13 on page 59 of MRID 46817239.
* Significantly different from the control group at $p \leq 0.05$
** Significantly different from the control group at $p \leq 0.01$

5. Offspring postmortem results

a) **Organ weights:** Except for the liver, there was no evidence of any treatment-related effects on organ weights in the F1 offspring in the current study. At ≥2000 ppm, absolute and relative liver weights were increased (p≤0.001) by 15-37% in both sexes (Table 12). Although decreased (p≤0.05) absolute thyroid weights were observed at ≥2000 ppm in the males and at 20,000 ppm in the females, these findings were considered incidental because increased (and not decreased) thyroid weights were noted in the two-generation study (MRID 46817216). In the two-generation study, the increased thyroid weights were corroborated by follicular cell hypertrophy. Several other organ weights differed significantly (p≤0.05) from controls but were considered unrelated to treatment because these differences, if present at all in the two-generation reproduction study, were not corroborated by gross or microscopic findings, and in the current study were likely related to the decreased terminal body weights in these animals.

TABLE 12. Selected absolute (mg) and relative to body weight (%) organ weights in selected F1 weanlings at post-natal day 26 [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
F1 males					
Terminal BW (g)	82±4	84±6	84±6	78±7	75±6**(↓9)
Liver					
Absolute	3307±291	3438±431	3447±293	3827±403***(↑16)	4140±370***(↑25)
Relative	4.03±0.24	4.09±0.29	4.10±0.20	4.90±0.36***(↑22)	5.51±0.36***(↑37)
Thyroid					
Absolute	9.8±2.3	8.4±1.6	8.7±2.0	7.8±1.2*(↓20)	7.9±1.8*(↓19)
Relative	0.01191±0.00273	0.01008±0.00195	0.01047±0.00284	0.00997±0.00140	0.01049±0.00199
F1 females					
Terminal BW (g)	76±5	76±7	77±4	71±7	68±4***(↓11)
Liver					
Absolute	3125±336	3100±376	3273±191	3605±343***(↑15)	3820±547***(↑22)
Relative	4.08±0.26	4.05±0.24	4.26±0.22	5.05±0.28***(↑24)	5.59±0.59***(↑37)
Thyroid					
Absolute	9.5±1.8	8.6±1.2	8.8±2.2	8.3±1.7	7.8±1.5*(↓18)
Relative	0.01247±0.00222	0.01136±0.00232	0.01152±0.00310	0.01170±0.00242	0.01148±0.00215

a Data were obtained from Tables 28 and 29 on pages 79-80 of MRID 46817239.
* Significantly different from the control group at p≤0.05
** Significantly different from the control group at p≤0.01
*** Significantly different from the control group at p≤0.001

b) **Gross pathology:** There were no treatment-related macroscopic findings in the F1 pups that were found dead during PND 0-4 or that were culled on PND 4. Among the pups found dead from PND 5-21 or surviving until scheduled termination, increased incidences of the following gross lesions were found at ≥2000 ppm (% treated vs 0% controls; Table 13): (i) dark-colored liver (25.7-42.8%; p≤0.01); (ii) opacity of the eyeball at ≥2000 ppm (2.1-6.8%);

and (iii) enlargement of the eyeball (5.6-21.4%; p≤0.01 at 20,000 ppm). Additionally at 20,000 ppm, the incidence of discoloration of the eyeball was increased (1.6%; NS) compared to 0% controls. No other gross lesions could be attributed to treatment.

TABLE 13. Selected gross findings (% incidence) in the pups found dead during PND 5 and PND 21 or surviving until scheduled termination [a]

Parameter	Dose Group (ppm)				
	0	50	200	2000	20,000
F1 pups					
Liver - dark colored	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	25.7 ± 13.9**	42.8 ± 19.9**
Eyeball					
opacity	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 7.5	6.8 ± 13.3
enlargement	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.6 ± 13.6	21.4 ± 26.6**
discoloration	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 7.3

a Data (n = 24) were obtained from Table 27 on pages 78 of MRID 46817239.
** Significantly different from the control group at p≤0.01

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS= CONCLUSIONS: It was concluded that the LOAEL for the parents was 200 ppm based on dark colored liver in the P dams. Additionally at 2000 and 20,000 ppm in the adults, macroscopic findings of enlarged and dark colored liver and brown colored thyroid were observed. Differences in weights of the pituitary, liver, kidneys, uterus, ovaries, and thyroid were found in this study and were considered treatment-related at 2000 and 20,000 ppm due to similar observations in the two-generation study. Similar to the two-generation study, two P dams died during delivery without showing any clinical signs. The apparently delayed sexual maturation in the F1 males at 50 ppm in the previous 2-generation study was considered incidental because no delay in preputial separation was observed in the F1 males in the 50 and 200 ppm groups in the current study. However, significantly delayed preputial separation observed at 2000 and 20,000 ppm were observed in both studies and were thus considered treatment-related. The LOAEL for offspring toxicity was 2000 ppm. There were no effects on any reproductive parameter; thus the LOAEL for reproductive toxicity was not observed, and the NOAEL was 20,000 ppm.

B. REVIEWER COMMENTS

1. PARENTAL ANIMALS: There were no adverse treatment-related effects on clinical signs, body weights, body weight gains, or food consumption during pre-mating, gestation, or lactation in the P or F1 adults.

At 20,000 ppm, two P females (#224 and 231) died during delivery without showing any clinical signs. Maternal mortality also occurred in several P and F1 dams during delivery in the two-generation reproduction study, indicating that these deaths were treatment-related.

Absolute and relative **liver** weights were increased ($p \leq 0.001$) in both generations in the ≥2000 ppm female (↑24-53%) and 20,000 ppm male (↑11-16%) adults. Incidences of the following gross findings in the liver were significantly increased ($p \leq 0.05$) over controls (# affected/24 treated vs 0/24 controls): (i) dark in color in the ≥200 ppm P dams (8-22), ≥2000 ppm F1 dams (22-24), and 20,000 ppm F1 males (17); and (ii) enlarged liver in the ≥2000 ppm P dams (11-20) and 20,000 ppm F1 dams (24).

Absolute and relative **thyroid** weights were increased at ≥2000 ppm in the females of both generations (↑17-24%); these increases were significant ($p \leq 0.05$), except for the absolute weights in the F1 dams at 20,000 ppm. Incidences of the following gross findings in the thyroid were significantly increased ($p \leq 0.05$) over controls (# affected/24 treated vs 0/24 controls, unless otherwise noted): (i) enlarged in the 20,000 ppm P males (8 treated vs 2 controls); and (ii) brown in color in the ≥2000 ppm P dams (20-22) and in the 20,000 ppm P and F1 males (18-21).

At ≥2000 ppm in the F1 generation, absolute and relative **pituitary** weights were decreased ($p \leq 0.05$) in the males (↓9-13%) and females (↓19-24%). However, decreased pituitary weights were corroborated by hydropic degeneration only at 20,000 ppm in the 2-generation reproduction study.

Additionally in the females, absolute and relative **kidney** weights were increased (↑6-16%) at ≥2000 ppm in the P generation and at ≥200 ppm in the F1 generation; these increases were significant ($p \leq 0.05$), except for the relative weights in the P generation at 20,000 ppm. In the concurrently-submitted carcinogenicity study, nephrotoxicity was indicated at 1000 and 20,000 ppm by increases ($p \leq 0.05$) in absolute and/or relative to body kidney weights in females (↑11-19%) and by slight to severe chronic nephropathy in males (84-92% treated vs 66% controls) and females (58-60% vs 18%). Dark-colored kidney was observed in the 20,000 ppm males in the carcinogenicity study (12% treated vs 2% controls; NS).

Increases (↑12-21%; $p \leq 0.05$) were observed in absolute **ovary** weights at ≥200 ppm in the P dams and in absolute and relative weights in the 50, 2000, and 20,000 ppm F1 dams. It should be noted that the increased ovary weights were also observed in the 2-generation reproduction study and were accompanied by corroborating microscopic findings of interstitial vacuolation. However, the vacuolation was only noted at ≥2000 ppm in the P dams and at 20,000 ppm in the F1 dams, and only attained significance ($p \leq 0.05$) in the 20,000 ppm P dams. Furthermore, there were no functional effects on fertility at any dose, and it is doubtful that the effects were treatment-related because there were no findings in the ovaries in the concurrently-submitted chronic toxicity or carcinogenicity studies at dose levels up to 20,000 ppm.

It is difficult to interpret the few organ weight and macroscopic findings that occurred at 200 ppm in the current study because this dose group fell between the LOAEL and the NOAEL in the two-generation study, and because microscopic examinations were not conducted on the organs in the current one-generation study. Thus, the finding of dark-colored liver in the P dams at this dose was considered equivocal.

The LOAEL for parental toxicity is 2000 ppm (equivalent to 127.2/148.9 mg/kg/day in males/females) based on effects on the liver, thyroid, and kidneys. The NOAEL is 200 ppm (equivalent to 12.91/14.97 mg/kg/day in males/females).

2. **OFFSPRING:** There were no effects of treatment on the number of implantations, number of pups delivered, sex ratio, or on the live birth, viability, or lactation indices.

Clinical signs of toxicity were limited to effects on the eyes and the following increased (not significant, unless otherwise noted) incidences were observed (% treated vs 0% controls): (i) eyeball discoloration at ≥2000 ppm during PND 8-14 and at 20,000 ppm during PND 15-21 (2.4-2.6%); (ii) opacity at ≥2000 ppm during PND 15-21 (1.0-3.0%); and (iii) enlargement at 2000 ppm (3.6%) and 20,000 ppm (14.4%; $p \leq 0.01$) during PND 15-21. Among the pups found dead from PND 5-21 or surviving until scheduled termination, increased incidences of opacity (2.1-6.8%) and enlargement (5.6-21.4%; $p \leq 0.01$ at 20,000 ppm) were found in the eyeballs at ≥2000 ppm compared to 0 controls. Additionally at 20,000 ppm, the incidence of discoloration of the eyeball was increased (1.6%; NS) compared to 0% controls. Although no obvious ocular toxicity was noted in the offspring in the 2-generation study, treatment-related effects on the eyes were observed in the offspring in the concurrently-submitted developmental neurotoxicity study (MRID 46817228). These findings included corneal opacity, dark red eyes, enlarged eyes, exophthalmia, retinal degeneration, hemorrhage, cataract, and/or atrophy of the optic nerve in both sexes at 12,000 ppm.

At 20,000 ppm, F1 pup body weights were decreased by 9% compared to controls on PND 21. Absolute and relative (to body weight) anogenital distances were increased by 4-6% in the ≥2000 ppm male pups on PND 4. These parameters were comparable to controls in the treated females.

Sexual maturation was delayed in both sexes. Increases ($p \leq 0.05$) in the mean number of days until preputial separation were observed in the males at 2000 ppm (42.2 days) and 20,000 ppm (43.1 days) compared to controls (41.0 days) and in the number of days until vaginal opening in the 20,000 ppm females (32.3 days) compared to controls (30.4 days). Additionally at 20,000 ppm, the body weight at which sexual maturation occurred was increased ($p \leq 0.05$) by 8-12% over controls. Sexual maturation was also delayed in the developmental neurotoxicity study at ≥1200 ppm in the males and at 12,000 ppm in the females.

Except for the **liver**, there was no evidence of any treatment-related effects on organ weights in the F1 offspring in the current study. At ≥2000 ppm, absolute and relative liver weights were increased ($p \leq 0.001$) by 15-37% in both sexes. At necropsy, incidences of dark-colored liver were increased ($p \leq 0.01$) at ≥2000 ppm (25.7-42.8%) compared to 0 controls, from among the pups found dead from PND 5-21 or surviving until scheduled termination.

Although decreased ($p \leq 0.05$) absolute **thyroid** weights were observed at ≥2000 ppm in the males and at 20,000 ppm in the females, these findings were considered incidental because increased (and not decreased) thyroid weights were noted in the two-generation study (MRID 46817216). In the two-generation study, the increased thyroid weights were corroborated by follicular cell hypertrophy.

The LOAEL for offspring toxicity 2000 ppm (equivalent to 127.2/148.9 mg/kg/day in males/females) based on effects on the eyes and liver and on increased anogenital distance and delayed sexual maturation in the males. The NOAEL is 200 ppm (equivalent to 12.91/14.97 mg/kg/day in males/females).

REPRODUCTIVE TOXICITY: There were no effects of treatment on: the precoital interval; mating, fertility, or gestation indices; or gestation duration. The percentage of P dams with normal estrous cycles and the estrous cycle duration in the treated groups were comparable to controls, although in two-generation study, estrous cycle duration was slightly shorter ($p \leq 0.05$) in the F1 dams at 20,000 ppm (4.0 days) compared to controls (4.2 days). No measurements were performed on sperm counts, motility, or morphology in the current study.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 20,000 ppm (equivalent to mg/kg/day in 1287/1490 in males/females).

This study is classified as an **acceptable/non-guideline** one-generation reproduction study in the rat.

C. **STUDY DEFICIENCIES:** No study deficiencies were noted.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; Histopathology of the Eyes of Weanlings in a One-generation Reproduction Study in Rats

Work Assignment No. 4-1-124 U; formerly 3-1-124 U (MRID 46817238)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 01-31-07

Secondary Reviewer:
Ronnie J. Bever, Jr., Ph.D.

Signature: Ronnie J. Bever Jr
Date: 1/31/07

Project Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/31/07

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 1/31/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Myron S. Ottley **Signature:** MSOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/12/07
Work Assignment Manager: P.V. Shah **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/17/07
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Histopathology of the Eyes of Weanlings in a One-generation Reproduction Study in Rats; Non-guideline

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (96.7% w/w)

SYNONYMS: Flubendiamide; N^2-(1,1-dimethyl-2-methylsulfonylethyl)-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Takeuchi, Y. (2005) NNI-0001: One-generation reproductive toxicity study in rats histopathological examination of the eyes of weanlings. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 04-0075, June 8, 2005. MRID 46817238. Unpublished.

SPONSOR: Nihon Nohyaku Co, Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: A one-generation reproduction toxicity study (MRID 46817239) was conducted in which Flubendiamide (96.7%; Batch # 1FH0019M) was administered in the diet to 24 Wistar Hanover rats/sex/dose group at dietary levels of 0, 50, 200, 2000, or 20,000 ppm. Treatment-related ocular lesions were observed at necropsy in the F1 weanlings of both sexes at 2000 and 20,000 ppm. The current non-guideline study (MRID 46817238) was conducted in order to confirm microscopic changes in eyes in which gross ocular lesions were found and to determine whether or not any microscopic findings were present in eyes that appeared normal upon gross examination.

The eyes with gross lesions at 2000 and 20,000 ppm were confirmed to have the following microscopic changes (% affected): (i) synechia in both sexes (88-100%); (ii) hemorrhage in both sexes (88-100%); (iii) keratitis in males (73-75%); (iv) iritis in both sexes (47-100%); (v) cataracts in both sexes (38-100%); (vi) hydropic degeneration of the basal layer of the corneal epithelium in males (75-87%); and (vii) corneal epithelial vacuolation in males (7-13%). Additionally in the 20,000 ppm females, keratitis (62%), hydropic degeneration of the basal layer of the corneal epithelium (46%), and corneal epithelial vacuolation (8%) were observed.

Microscopic examination of the eyes without grossly observable ocular lesions revealed various histopathological findings, including synechia, hemorrhage, keratitis, and/or hydropic degeneration of the basal layer of the corneal epithelium. However, the incidences of these lesions were not statistically significant compared to controls, and they were not dose-related.

In conclusion, microscopic analyses of the eyes of the offspring from the one-generation reproductive toxicity study confirmed ocular lesions at 2000 and 20,000 ppm, but did not lower the LOAEL, as no microscopic lesions were observed at doses lower than were observed grossly (i.e., there were no microscopic lesions at 200 ppm).

The LOAEL for offspring toxicity is 2000 ppm (equivalent to 127.2/148.9 mg/kg/day in males/females) based on confirmed microscopic effects on the eyes in both sexes. The NOAEL is 200 ppm (equivalent to 12.91/14.97 mg/kg/day in males/females).

This study is classified as **acceptable/non-guideline**.

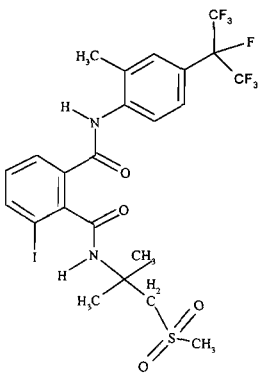
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide) technical

Description: White crystals
Lot/batch #: 1FH0019M
Purity: 96.7% a.i.
Compound stability: The test substance was stable in the diet for up to 4 weeks at 5°C followed by 3 weeks at room temperature.
CAS # of TGAI: 272451-65-7
Structure:



2. Vehicle: Diet

3. Test animals

Species:	Rat	
Strain:	Wistar Hanover (BrlHan:WIST@Jcl[GALAS])	
Age at study initiation:	5 weeks	
Weight at study initiation:	146-168 g, males; 117-136 g, females	
Source:	Fuji Breeding Center, Clea Japan, Inc.	
Housing:	Acclimation (five/sex) and pre-mating (three/sex) - in suspended wire-mesh stainless steel cages. Mating - in pairs (1 male: 1 female) in TR-360 aluminum cages with wire-mesh floors and fronts. Gestation and lactation - Dams housed individually during gestation and with their litters during lactation in TR-358B cages. After weaning, dams were removed and pups continued to be housed by litter in TR-358B cages.	
Diet:	Certified pulverized feed, MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*	
Water:	Filtered (sand and charcoal) and sterilized (sodium hypochlorite) well water, *ad libitum*	
Environmental conditions:	**Temperature**	22 ± 2°C
	Humidity	55 ± 15%
	Air changes	At least 10/hour
	Light cycle	12 hours light/12 hours dark
Acclimation period:	11 days	

B. PROCEDURES AND STUDY DESIGN

1. **Study objective:** A one-generation reproduction toxicity study (MRID 46817239) was conducted in which Flubendiamide was administered at dietary levels of 0, 50, 200, 2000, or 20,000 ppm; and treatment-related ocular lesions were observed at necropsy in the F1 weanlings of both sexes at 2000 and 20,000 ppm. However, the eyes were not examined microscopically. Thus, the current non-guideline study was conducted in order to confirm microscopic changes in eyes in which gross ocular lesions were found and to determine whether or not any microscopic findings were present in eyes that appeared normal upon gross examination.

2. **Animal assignment:** The P animals had been assigned to the test groups shown in Table 1 in such as way to equalize the group means and standard deviations of body weights as closely as possible.

TABLE 1. Animal assignment [a]

Test group	Dose (ppm) [b]	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	24	24	24	24
Low	50	24	24	24	24
Lower middle	200	24	24	24	24
Higher middle	2000	24	24	24	24
High	20,000	24	24	24	24

a Data were obtained from page 10 of MRID 46817238.
b Exposure to the test substance was continuous throughout the study.

3. **Study rationale (clinical and gross findings in the F1 generation):** In the one-generation study, enlargement of the eyeball was observed in 1/24 F1 dams, each, at 2000 and 20,000 ppm compared to 0/24 controls. In the F1 weanlings, clinical signs of toxicity were limited to effects on the eyes, and the following increased (not significant, unless otherwise noted) incidences were observed (% treated vs 0% controls): (i) eyeball discoloration at ≥2000 ppm during PND 8-14 and at 20,000 ppm during PND 15-21 (2.4-2.6%); (ii) opacity at ≥2000 ppm during PND 15-21 (1.0-3.0%); and (iii) enlargement at 2000 ppm (3.6%) and 20,000 ppm (14.4%; $p \leq 0.01$) during PND 15-21. Among the pups found dead from PND 5-21 or surviving until scheduled termination, increased incidences of the following gross lesions were found in the eyeballs at ≥2000 ppm compared to 0 controls: (i) discoloration (2.5-2.6%; NS); (ii) opacity (2.1-6.8%); and (iii) enlargement (5.6-21.4%; $p \leq 0.01$ at 20,000 ppm).

4. **Study schedule/design:** During the one-generation reproduction study (MRID 46817239), F1 weanlings that had not been selected to continue through sexual maturation were euthanized on PND 26. These animals, along with F1 pups culled on PND 4 and any decedents, were subjected to a gross necropsy. However, from among the F1 offspring terminated on PND 26, one pup/sex/litter was selected for organ weight measurement and tissue preservation. The heads (including eyes) from these selected animals (along with any

animals showing grossly visible ocular lesions) were removed, fixed in neutral-buffered 10% formalin and decalcified. Cross sections of the eyes were prepared, embedded in paraffin, sectioned, and stained with hematoxylin and eosin by a routine method. Because grossly observable lesions were observed at 2000 and 20,000 ppm, microscopic analyses were first performed on the eyes from the rats in these groups and the controls. After microscopic lesions confirmed the gross lesions at 2000 ppm, the 200 ppm group was examined microscopically. Because no microscopic lesions were found at 200 ppm, no examinations were performed on the eyes of the rats from the 50 ppm group.

D. DATA ANALYSIS: Fisher's exact probability test (one-tailed) was performed on the incidences of histopathological findings in the eyes without gross abnormalities. Statistical significance was denoted at $p \leq 0.05$ and 0.01. The statistical methods were considered appropriate.

II. RESULTS

A. Microscopic analyses of eyes WITH gross abnormalities: The eyes with gross lesions at 2000 and 20,000 ppm were confirmed to have the following microscopic changes (% incidence): (i) synechia in males (88-100%) and females (92-100%); (ii) hemorrhage in males (88-100%) and females (100%); (iii) keratitis in males (73-75%); (iv) iritis in males (47-50%) and females (54-100%); (v) cataracts in males (38-60%) and females (77-100); (vi) hydropic degeneration of the basal layer of the corneal epithelium in males (75-87%); and (vii) corneal epithelial vacuolation in males (7-13%; Table 2). Additionally in the 20,000 ppm females, keratitis (62%), hydropic degeneration of the basal layer of the corneal epithelium (46%), and corneal epithelial vacuolation (8%) were observed.

B. Microscopic analyses of eyes WITHOUT gross abnormalities: Microscopic examination of the eyes without grossly observable ocular lesions revealed various histopathological findings, including synechia, hemorrhage, keratitis, and/or hydropic degeneration of the basal layer of the corneal epithelium (Table 3). However, the incidences of these lesions were not statistically significant compared to controls and they were not dose-related.

TABLE 2. Incidences of microscopic findings in the eyes with gross abnormalities [# (%) rats affected] [a]				
	Dose Group (ppm) [b]			
Ocular microscopic lesion	0	200	2000	20,000
Males				
Number of rats examined	0	0	8	15
Synechia	--	--	7 (88)	15 (100)
Hemorrhage	--	--	7 (88)	15 (100)
Keratitis	--	--	6 (75)	11 (73)
Iritis	--	--	4 (50)	7 (47)
Cataract	--	--	3 (38)	9 (60)
Hydropic degeneration, basal layer of corneal epithelium	--	--	6 (75)	13 (87)
Vacuolation, basal layer of corneal epithelium	--	--	1 (13)	1 (7)
Females				
Number of rats examined	0	0	1	13
Synechia	--	--	1 (100)	12 (92)
Hemorrhage	--	--	1 (100)	13 (100)
Keratitis	--	--	0 (0)	8 (62)
Iritis	--	--	1 (100)	7 (54)
Cataract	--	--	1 (100)	10 (77)
Hydropic degeneration, basal layer of corneal epithelium	--	--	0 (0)	6 (46)
Vacuolation, basal layer of corneal epithelium	--	--	0 (0)	1 (8)

a Data were obtained from Table 1 on page 19 of MRID 46817238. Incidences include all findings from both eyes, even though one eye may have been considered grossly normal. The percent of rats with a given eye lesion out of rats with grossly observable eye lesions were calculated by the reviewers and are included in parentheses.
b The eyes from the 50 ppm group were not examined because no microscopic lesions were found at 200 ppm.
-- Not examined because there were no gross findings in these groups

TABLE 3. Incidences of microscopic findings in the eyes without gross abnormalities (# rats affected) [a]				
	Dose Group (ppm) [b]			
Ocular microscopic lesion	0	200	2000	20,000
Males				
Number of rats examined	23	21	22	14
Synechia	2	1	3	--
Hydropic degeneration, basal layer of corneal epithelium	5	5	7	2
Females				
Number of rats examined	23	21	23	16
Synechia	2	2	3	3
Hemorrhage	2	--	1	1
Keratitis	--	--	--	1
Hydropic degeneration, basal layer of corneal epithelium	3	2	7	5

a Data were obtained from Table 2 on page 20 of MRID 46817238. Incidences include all findings from both eyes, even though one eye may have been considered grossly normal.
b The eyes from the 50 ppm group were not examined because no microscopic lesions were found at 200 ppm.
-- No microscopic findings

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS:** Although various histological changes were confirmed in the eyes with gross abnormalities, no effects of the test substance were suggested histopathologically in the grossly normal eyes at any dose levels examined. In the 200 ppm group in which no ocular lesions were detected macroscopically, there were no treatment-related microscopic lesions at any dose in either sex. Therefore, the LOAEL was determined to be 2000 ppm for both sexes.

B. **REVIEWER COMMENTS:** The eyes with gross lesions at 2000 and 20,000 ppm were confirmed to have the following microscopic changes (% affected): (i) synechia in both sexes (88-100%); (ii) hemorrhage in both sexes (88-100%); (iii) keratitis in males (73-75%); (iv) iritis in both sexes (47-100%); (v) cataracts in both sexes (38-100%); (vi) hydropic degeneration of the basal layer of the corneal epithelium in males (75-87%); and (vii) corneal epithelial vacuolation in males (7-13%). Additionally in the 20,000 ppm females, keratitis (62%), hydropic degeneration of the basal layer of the corneal epithelium (46%), and corneal epithelial vacuolation (8%) were observed.

Microscopic examination of the eyes without grossly observable lesions revealed that the weanlings of either sex in all groups (including the controls) had various ocular lesions at incidences ranging up to 32%, including synechia, hemorrhage, keratitis, and/or hydropic degeneration of the basal layer of the corneal epithelium. However, the incidences of these lesions were neither significantly different from the controls nor dose-related.

In conclusion, microscopic analyses of the eyes of the offspring from the one-generation reproductive toxicity study confirmed ocular lesions at 2000 and 20,000 ppm, but did not lower the LOAEL, as no microscopic lesions were observed at doses lower than were observed grossly (i.e., there were no microscopic lesions at 200 ppm).

Although no obvious ocular toxicity was noted in the offspring the in 2-generation study (MRID 46817216), treatment-related ocular toxicity was observed in the offspring in the concurrently-submitted developmental neurotoxicity study (MRID 46817228). Findings included corneal opacity, dark red eyes, enlarged eyes, exophthalmia, retinal degeneration, hemorrhage, cataract, and/or atrophy of the optic nerve in both sexes at 12,000 ppm.

The LOAEL for offspring toxicity is 2000 ppm (equivalent to 127.2/148.9 mg/kg/day in males/females) based on confirmed microscopic effects on the eyes in both sexes. The NOAEL is 200 ppm (equivalent to 12.91/14.97 mg/kg/day in males/females).

Note that the equivalent achieved intake in mg/kg/day is based on food consumption data reported in the one-generation study (MRID 46817239) not reiterated in this study report.

This study is classified as an **acceptable/non-guideline**.

C. **STUDY DEFICIENCIES:** No study deficiencies were noted.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; Perinatal Ocular Toxicity Study in Mice

Work Assignment No. 4-1-124 S; formerly 3-1-124 S (MRID 46817236)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 01-16-07

Secondary Reviewer:
Ronnie J. Bever, Jr., Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 01-16-07

Project Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/17/07

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 1/17/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Myron S. Ottley **Signature:** MSOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/23/07
Work Assignment Manager: PV Shah **Signature:** PVShah
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/23/07

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Perinatal ocular toxicity study in mice following exposure via diet; Non-guideline

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 technical; (97.1% a.i.)

SYNONYMS: Flubendiamide; 1,2-benzenedicarboxamide, N^2-(1,1-dimethyl-2-methylsulfonylethyl)-3-iodo-N^1-{2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl] – (9CI)

CITATION: Langewische, F.W. (2006) NNI-0001: Perinatal ocular toxicity study in CD-1 mice following exposure via diet. Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany. Laboratory Study No.: T2073354, January 20, 2006. MRID 46817236. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim Germany

EXECUTIVE SUMMARY: In a non-guideline perinatal ocular toxicity study, NNI-0001 (Flubendiamide; Lot No. 1FH0019M; 97.1% a.i.) was administered in the diet to naturally mated Crl:CD1(ICR) mice in a control group and a treated group. After positive evidence of mating was confirmed, 24 females each were alternately allocated to each group. Thereafter, one additional female was placed in the control group, and an additional four females were added to the treated group to substitute for females that were either not pregnant or had no surviving pups. Animals in the treated group were fed a test diet concentration of 4500 ppm from gestation day (GD) 6 through lactation day (LD 3). Due to increased food consumption during lactation, the dietary concentrations were then reduced to a concentration of 2000 ppm for the remainder of the lactation period (LD 3-21) in order to approximate the limit dose of 1000 mg/kg/day throughout the study. The overall study intake of the test compound was 1052 mg/kg/day. Control mice were fed the basal diet throughout the study. After weaning, the pups were raised to post-natal day (PND) 42 for the stated purpose of being evaluated for any potential effects of the test substance on perinatal ocular development. Clinical observations, body weights, body weight gains, food consumption, and reproductive data were evaluated in the dams. Litter data, clinical signs, body weights, and body weight gains were evaluated in the pups.

There were no effects of treatment on maternal survival, clinical signs, body weights, body

weight gains, food consumption, or water consumption.

The LOAEL for parental toxicity was not observed. The NOAEL is 4500/2000 ppm (equivalent to 1052.3 mg/kg/day).

There were no effects of treatment on the duration of gestation or on fertility, gestation, or rearing indices

The LOAEL for reproductive toxicity was not observed. The NOAEL is 4500/2000 ppm (equivalent to 1052.3 mg/kg/day).

There were no treatment-related effects on pup viability or clinical signs.

Pup body weights in the treated group were decreased by 4-6% compared to the control group: in the males on PND 7, 14, and 21; in the females on PND 21; and in the combined sexes on PND 21. Pup body weight gains were decreased by 9-10% in the males and combined sexes for PND 4-7 and by 7% in the females and combined sexes for PND 14-21.

The LOAEL for offspring toxicity is 4500/2000 ppm (equivalent to 1052.3 mg/kg/day) based on decreased pup body weights and body weight gains. The NOAEL was not established.

This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirements for a developmental or reproductive study in rodents.

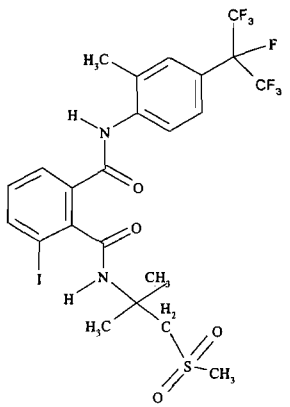
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description: White powder
Lot/batch #: 1FH0019M
Purity: 97.1% a.i.
Compound stability: The test substance was stable in the diet for up to 15 days at room temperature.
CAS # of TGAI: 272451-65-7
Structure:



2. Vehicle: Diet

3. Test animals

Species:	Mouse
Strain:	Crl:CD1(ICR)
Age at study initiation:	6-7 weeks
Weight at study initiation:	31-34 g, males; 23-33 g, females
Source:	Charles River Wiga (Deutschland) GmbH, Sulzfeld, Germany
Housing:	Females were housed: in groups during acclimation in Type III Makrolon cages; and individually during gestation and with litters during lactation in Makrolon Type II cages. Each litter was housed in Makrolon Type III cages following weaning. Males were housed individually in Makrolon Type II cages, with the exception of mating.
Diet:	Standard Diet: Provimi Kliba SA 25 G4, Art.-No. 3883.9.25, females Provimi Kliba Maus/Ratte-Haltung-GLP, Art.-No. 3883.0.15, males (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature** 22 ± 2°C **Humidity** Approximately 50 ± 20% **Air changes** At least 10/hour **Light cycle** 12 hours light/12 hours dark
Acclimation period:	At least 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: August 10, 2005 End: November 8, 2005

2. **Objective:** It was stated that the purpose of this study was to evaluate the offspring for any potential effects on perinatal ocular development via dams fed the test substance in the diet from implantation through weaning.

3. **Mating procedure:** The animals were mated during the morning for approximately 3-4 hours by placing up to two females together in a cage with one male. Positive evidence of mating was defined by the existence of a vaginal plug after mating or the presence of sperm in a vaginal smear (taken in cases of no vaginal plug). The day on which positive evidence of mating was detected was designated as gestation day (GD) 0.

4. **Study design/schedule:** The male animals were used for mating only and were not treated. After positive evidence of mating was confirmed, 24 females each were alternately allocated to a control and a treated group. Thereafter, one additional female was placed in the control group, and an additional four females were added to the treated group to substitute for females that were either not pregnant or had no surviving pups. The test substance was administered in the diet to presumed pregnant mice in the control (25 mice) and treated (28 mice) groups. Animals in the treated group were fed a test diet concentration of 4500 ppm from GD 6 through lactation day (LD 3). Due to increased food consumption during lactation, the dietary concentration was then reduced to 2000 ppm for the remainder of the lactation period (LD 3-21) in order to approximate the limit dose of 1000 mg/kg/day throughout the study. Control mice were fed the basal diet throughout the study. After weaning, the pups were raised to post-natal day (PND) 42 for the stated purpose of being evaluated for any potential effects of the test substance on perinatal ocular development.

5. **Animal assignment:** To the test groups shown in Table 1.

TABLE 1. Animal assignment [a]

Test group	Diet concentration (ppm)	Achieved intake (mg/kg/day)			# Females
		Gestation	Lactation	Overall	
Control	0	0	0	0	24 [c]
High (HDT)	4500/2000 [b]	930.9	1133.2	1052.3	24 [c]

a Data were obtained from pages 12 and 22 of the study report.
b Animals in the treated group were fed a test diet concentration of 4500 ppm from GD 6 through LD 3. Due to increased food consumption during lactation, the dietary concentration was then reduced to 2000 ppm for LD 3-21 in order to approximate the limit dose of 1000 mg/kg/day throughout the study.
c 24 females each were alternately allocated to a control and a treated group. Thereafter, one additional female was placed in the control group, and an additional four females were added to the treated group to substitute for females that were either not pregnant or had no surviving pups.

6. **Dose-selection rationale:** Doses for the current study were selected based on the results from pilot study (T3073355), in which the test substance was administered in the diet to 7 pregnant mice/group in a control group and a treated group. Animals in the treated group were fed a test diet concentration of 4500 ppm from GD 6 until sometime between LD 0-8. Due to increased food consumption during lactation, the dietary concentrations were then reduced to a concentration of 2250 ppm for the remainder of the lactation period in order to approximate

the limit dose of 1000 mg/kg/day throughout the study. Control mice were fed the basal diet throughout the study. The dams were allowed to deliver and rear their pups through weaning for the stated purpose of being evaluated for any potential effects of the test substance on perinatal ocular development. There were no treatment-related effects on survival, clinical signs (including lactation behavior), food consumption, or water consumption in the dams during gestation or lactation. One control female delivered on GD 12, which must have been due to an earlier unnoticed insemination, because the pups were normally developed. There were no treatment-related effects on pup viability on post-natal day (PND) 1 or 2, between the treated group (five dead pups in two litters) and the control group (4 pups). The percent of males was low in the treated group (37.6%); however, this finding was considered incidental because fetal sex is already determined prior to the initiation of treatment on GD 6; and it was considered that final evaluation was not possible due to the low numbers of litters in the pilot study. It was stated that the surviving pups showed no treatment-related effects on survival, clinical observations, body weight development, or eye lesions during the lactation period. However, no data were provided.

7. **Dosage preparation and analysis:** The appropriate amount of test substance was mixed with the diet to achieve the desired concentration of test diet. Stability for 15 days frozen or for up to 15 days at room temperature, in addition to homogeneity, were tested at dietary concentrations of 25 and 5000 ppm in a prior study (F9011188; March 29, 2004). In the current study, homogeneity and concentration were verified at 2000 and 4500 ppm. These data are reported in the analytical report (Study No. T2073354) in the Annex on pages 188-191 of the study report (MRID 46817236).

Results

Homogeneity: 2.87-8.51% relative standard deviation

Stability: 104-111% initial concentration for up to 15 days at room temperature
102-107% initial concentration after 15 days frozen

Concentration: 107-110% nominal

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

C. OBSERVATIONS

1. **Parental observations:** Dams were checked for mortality and clinical signs of toxicity twice daily, except on weekends, public holidays, and on the day of necropsy, when they were examined once daily. Excretory products were examined by visual assessment once daily. Body weights were measured on GD 0 and 3 and daily from GD 6 through LD 21. Food consumption was measured for GD 0-3, 3-6, and daily from GD 6 through LD 21. Water consumption was determined once weekly by visual estimation. The course of birth was observed when parturition occurred during the day, and lactation behavior was noted during the twice-daily observations.

2. **Litter observations:** Pups were checked for mortality and clinical signs of toxicity twice daily (once daily on weekends, public holidays, and on the day of necropsy), with special focus on potential eye lesions. The number of pups in each litter was assessed at birth (PND 0) and on PND 4, 7, 14, 21, and 42. Litters were standardized on PND 4 to a maximum of 8 pups/litter (4/sex/litter, as near as possible). Sex ratio was determined at birth. Individual pup body weights were recorded on the days that the pups were counted.

3. **Indices**

Reproductive indices: The following reproductive/viability indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Fertility index (%) = # females which became pregnant / # inseminated x 100

Gestation index (%) = # females delivering pups / # females which became pregnant x 100

Rearing index (%) = # females which reared pups / # females which littered x 100

Offspring viability indices: The following viability indices were calculated by the performing laboratory from lactation records of litters in the study:

Live birth index (%) = # live pups on PND 0/total # pups born x 100

Viability index (%) = # pups surviving to PND 4 (pre-cull)# pups live pups on PND 0 x 100

Lactation index (%) = # pups surviving to PND 21/# pups on PND 4 (post-cull) x 100

4. **Postmortem examinations**

a. **Parents:** The dams were killed by cervical dislocation under deep carbon dioxide anesthesia after weaning their litters (PND 21). The uterus of any female who did not litter was examined for implantation sites after staining with 10% ammonium sulfide. The carcasses were discarded.

b. **Offspring:** The pups were killed by cervical dislocation under deep carbon dioxide anesthesia at scheduled termination (PND 42-44) or when litters were culled (PND 4). The head of each animal was removed and preserved in Davidson's solution, and the remaining carcass was discarded. However, it was stated that microscopic examination of the heads was not performed due to the results of this study.

D. DATA ANALYSIS

1. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure
Maternal and pup body weights and body weight gains Maternal food consumption Duration of gestation Live birth index Number of pups delivered Number of live pups Sex ratio of pups Survival rate of the pups (viability and lactation indices)	Analysis of variance (ANOVA) followed by Dunnett's test for pair-wise comparisons of treated groups with controls if ANOVA was significant ($p \leq 0.05$)
Fertility index Gestation index Numbers of dams with live pups, stillborn pups, and all pups stillborn Numbers of dams with live born pups but no pups alive on PND 4 and PND 21 Numbers of stillborn pups and pups that were dead, missing, killed, and/or cannibalized. Rearing index	2 by N Chi-square test followed by Fisher's exact test with Bonferroni correction for pair-wise comparisons of treated groups with controls if Chi-square test was significant ($p \leq 0.05$)

Statistical significance was denoted at $p \leq 0.05$ and 0.01. The statistical methods were considered appropriate.

2. **Historical control data:** Historical control data for fertility index were reported on page 34 of the study report. No other historical control data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. Mortality: All dams survived until scheduled termination.

b. Clinical signs of toxicity: There were no remarkable clinical signs during gestation or lactation.

2. Body weight and body weight gain: There were no effects of treatment on parental body weights or body weight gains. Body weights of the treated group and control group were comparable throughout gestation and lactation. Body weight gains of the treated and control groups were comparable during gestation and lactation, except for an incidental increase of 66% ($p \leq 0.05$) during LD 17-18.

3. Food and water consumption: There were no effects of treatment on parental food consumption. Food consumption of the treated and control groups were comparable during gestation and lactation, except for an incidental increase in absolute (↑47%) and relative to body weight (↑42%) food consumption during GD 3-6 and a minor decrease of 9% in relative

food consumption during GD 17-18. Water intake (by visual estimation) was unaffected by treatment during gestation and lactation.

4. **Test substance intake:** Test substance intake (mg/kg/day) was calculated from the body weight and food consumption data, using the nominal concentration (ppm) of the diets. The mean test substance intakes for the gestation and lactation periods and for the overall study are presented in Table 1 of this DER.

5. **Reproductive performance:** There were no effects of treatment on the duration of gestation or on fertility, gestation, or rearing indices (Table 2).

TABLE 2. Reproductive performance [a]

Parameter	Dose Group (mg/kg/day)	
	0	1052
Fertility index (%)	92.0	85.7
Gestation index (%)	100	100
Rearing index (%)	95.7	95.8
Duration of gestation (days)	19.13 ± 0.344	19.13 ± 0.338

a Data were obtained from page 68 of the study report.

B. OFFSPRING

1. **Viability and clinical signs:** No treatment-related clinical signs were observed in the pups. Similarly, there were no effects of treatment on sex ratio or on live birth, viability, or lactation indices (Table 3).

TABLE 3. Litter parameters [a]

Parameter	Dose Group (mg/kg/day)	
	0	1052
Live birth index (%)	98.86 ± 3.073	99.36 ± 2.172
Viability index (%)	92.52 ± 20.835	92.99 ± 20.221
Lactation index (%)	98.30 ± 4.391	98.37 ± 4.304
Sex ratio (%), PND 0	44.51 ± 19.140	51.17 ± 13.310
PND 21	47.86 ± 12.534	49.88 ± 8.265

a Data were obtained from pages 69 and 70 of the study report.

2. **Body weight:** Pup body weights in the treated group were decreased by 4-6% compared to the control group: in the males on PND 7, 14, and 21; in the females on PND 21; and in the combined sexes on PND 21. Pup body weight gains were decreased by 9-10% in the males and combined sexes for PND 4-7 and by 7% in the females and combined sexes for PND 14-21 (Table 4).

TABLE 4. Mean (± SD) pup body weights and body weight gains (g) [a]			
Post-natal day (PND), gender		Dose Group (ppm)	
		0	4500/2000
Body weights			
PND 7	males	5.15 ± 0.368	4.83 ± 0.525* (↓6)
PND 14	males	8.75 ± 0.705	8.26 ± 0.713* (↓6)
PND 21	males	14.16 ± 1.050	13.28 ± 1.074** (↓6)
	females	13.19 ± 0.810	12.61 ± 0.923* (↓4)
	males + females	13.66 ± 0.881	12.95 ± 0.948* (↓5)
Body weight gains			
PND 4-7	males	2.10 ± 0.270	1.88 ± 0.207** (↓10)
	males + females	2.07 ± 0.266	1.89 ± 0.206* (↓9)
PND 14-21	females	4.69 ± 0.500	4.34 ± 0.417* (↓7)
	males + females	5.04 ± 0.503	4.68 ± 0.529* (↓7)

a Data were obtained from pages 72-75 of the study report.

3. **Postmortem investigation:** Although the head of each animal was removed and preserved in Davidson's solution, it was stated that microscopic examination of the heads was not performed due to the results of this study.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS:** It was concluded that no effects on perinatal ocular development occurred following dietary administration at the limit dose. Maternal food consumption was decreased at several time points beginning on LD 17. Pup body weights were slightly decreased in the treated group compared to the controls between PND 7-21. There were no other effects of treatment on any of the maternal, reproductive, or offspring parameters examined.

B. REVIEWER COMMENTS

1. **PARENTAL ANIMALS:** There were no effects of treatment on maternal survival, clinical signs, body weights, body weight gains, food consumption, or water consumption.

   The LOAEL for parental toxicity was not observed. The NOAEL is 4500/2000 ppm (equivalent to 1052.3 mg/kg/day).

2. **REPRODUCTIVE TOXICITY:** There were no effects of treatment on the duration of gestation or on fertility, gestation, or rearing indices

   The LOAEL for reproductive toxicity was not observed. The NOAEL is 4500/2000 ppm (equivalent to 1052.3 mg/kg/day).

3. **OFFSPRING:** There were no treatment-related effects on pup viability or clinical signs.

Pup body weights in the treated group were decreased by 4-6% compared to the control group: in the males on PND 7, 14, and 21; in the females on PND 21; and in the combined sexes on PND 21. Pup body weight gains were decreased by 9-10% in the males and combined sexes for PND 4-7 and by 7% in the females and combined sexes for PND 14-21.

The LOAEL for offspring toxicity is 4500/2000 ppm (equivalent to 1052.3 mg/kg/day) based on decreased pup body weights and body weight gains. The NOAEL was not established.

This study is classified as **unacceptable/non-guideline** but is **upgradeable** pending submission of ophthalmology and/or gross or microscopic ocular data.

C. STUDY DEFICIENCIES: None. The reported results of the study would have been more complete if microscopic examinations were conducted on the eyes. No summary or individual data were reported for ophthalmology, gross pathology, or microscopic pathology. Since the head of each animal was removed and preserved in Davidson's solution, it is possible to obtain these data if deemed necessary. However, the data from the reproductive and developmental neurotoxicity studies in rats [MRIDs 46817228, 46817216, 46817238, 46817239] show that ocular effects at the microscopic level were observed only in the presence of gross ocular observations. Since no gross observations were noted in this mouse study at dose levels at or near the limit dose, the need for microscopic evaluations appears to be obviated at this time.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870 7800; 28-Day Immunotoxicity Study in Rats

Work Assignment No. 4-01-124 Q; formerly 3-01-124 Q (MRID 46817243)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer
John W. Allran, M.S.

Signature: John W. Allran
Date: 01-17-07

Secondary Reviewer
Ronnie J. Bever, Jr., Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 01-17-07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/17/07

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 1/17/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Toxicology Branch, Health Effects Division (7509P) **Date:** 6/26/2007
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Immunotoxicity Study in Rats (Diet); OPPTS 870.7800

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 technical; (97.1% a.i.)

SYNONYMS: Flubendiamide; 1,2-benzenedicarboxamide, N^2-(1,1-dimethyl-2-methylsulfonylethyl)-3-iodo-N^1-{2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl] – (9CI)

CITATION: Krötlinger, F. and H.W. Vohr (2005) Immunotoxicity study in rats – plaque assay (4 weeks administration by diet). Bayer HealthCare AG, Wuppertal, Germany. Laboratory Study No.: T1073902, June 13, 2005. MRID 46817243. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 46817243), NNI-0001 (Flubendiamide; 97.1% a.i.; Batch # 1FH0019M) was administered in diet to Wistar rats (10/sex/group) at dietary concentrations of 0, 40, 400, or 4000 ppm (equivalent to 0, 3.3, 33.6, or 336.3 mg/kg/day for males and 0, 4.0, 38.4, or 358.8 mg/kg/day for females) for 28 days. Five days prior to necropsy, the animals were immunized via intravenous (i.v.) injection with 100 µL sheep red blood cells (SRBC) at a concentration of 1 x 10^9 per mL. Immunotoxicological investigations included spleen cell counts, FACScan analyses, antibody titers, and plaque forming cell assays.

There were no mortalities and no treatment-related adverse effects on clinical observations, body weights, body weight gains, food consumption, water consumption, hematology, clinical chemistry, or gross pathology.

At 400 and 4000 ppm, treatment-related effects on the liver and thyroid were observed, as indicated by increased organ weights. Absolute and relative liver weights were increased by 18-50% ($p<0.05$) over controls in 400 and 4000 ppm females. Additionally at 4000 ppm, an increase of 8% ($p<0.05$) in relative liver weight was observed in the males. Absolute thyroid weight was increased by 80% ($p<0.05$) in the 4000 ppm females but the relative thyroid weight was not significantly different from the control. However, there were no macroscopic findings in

these organs and no histopathology was performed. It should be noted that in the concurrently submitted subchronic (MRID 46817210) and chronic (MRID 46817219) studies on rats with this chemical, hepatotoxicity was observed at 2000 ppm (equivalent to approximately 100 mg/kg/day) in the females. Additionally in a study on effects of the test substance on thyroid and liver function (MRID 46817235), similar increases in liver and thyroid weights were observed at 1000 and 10,000 ppm.

There were no significant treatment-related effects on immunological findings. Total spleen cell counts, enumeration of total B cells, T cells, and T cell subpopulations, antibody (IgM) titers, and anti-SRBC plaque forming cells response in the treated groups were comparable to controls for both sexes. No effects were observed in antibody titers of IgA and IgG; however, results were not meaningful since the optimum time of collection for IgA or IgG (usually is different from IgM response) was not provided.

Under conditions of this study, the NOAEL for immunotoxicity is 4000 ppm (equivalent to 336.3/358.8 mg/kg/day in males/females) based on no effects were observed on anti-SRBC (IgM) plaque forming cells response and other immunological parameters. The LOAEL has not been achieved.

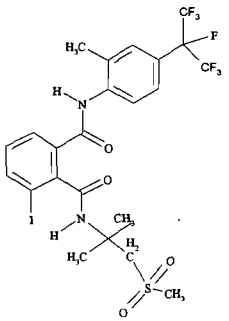
This study is classified as **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study **(OPPTS 870.7800)** in rats.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description:	White powder
Batch #:	1FH0019M
Purity:	97.1% a.i.
Compound stability:	The test substance was stable in the diet for up to 15 days at room temperature followed by 15 days frozen
CAS # of TGAI:	272451-65-7
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Rat	
Strain:	SPF-bred Wistar (Hsd:Cpb:WU)	
Age/weight at study initiation:	7-8 weeks old; 154-191 g, males; 131-156 g, females	
Source:	Harlan Winkelmann GmbH, Borchen Germany	
Housing:	Individually in Type IIIh cages on racks; low-dust wood granulate bedding.	
Diet:	Fixed formula standard powdered Diet No. 3883.9.25 (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 2EC
	Humidity:	55 ± 5%
	Air changes:	≥10/hour
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	8 days	

B. STUDY DESIGN:

1. In life dates: Start: 05/25/04 End: 06/23/04

2. Animal assignment: Animals were randomly assigned (stratified by body weight) to the test groups shown in Table 1. The weight of each animal was within 20% of the mean weight for each sex.

Table 1. Study design [a]				
Test Group	Diet concentration (ppm)	Achieved dose (M/F; mg/kg/day)	# Animals	
			Males	Females
Control	0	0/0	10	10
Low	40	3.3/4.0	10	10
Mid	400	33.6/38.4	10	10
High	4000	336.3/358.8	10	10

a Data were obtained from page 9 of MRID 46817243.

3. **Dose selection rationale:** It was stated that dose levels were selected by the Sponsor based on results obtained from previous studies. However, no further information was provided.

4. **Dose preparation, administration, and analysis:** The test diets were prepared once per week (except twice during Week 1, due to a public holiday) by mixing the required amount of the test material with diet to achieve the desired concentrations. Storage temperature and other conditions were not provided. Homogeneity was verified prior to the study by analyzing samples from the top, middle, and bottom of the mixing granulator containing 2 kg batches at dietary concentrations of 25 and 5000 ppm (Study No. F9011188). Stability of the test substance in the diet at these concentrations was analyzed after 4, 8, and 15 days at room temperature followed by 15 days deep frozen. Concentration analyses were performed on each dietary level once during the current study.

Results

Homogeneity (% relative standard deviation): 3.30-4.33%

Stability analysis (% of initial concentration): 102-111%

Concentration (%nominal): 86-105%

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

5. **Statistics:** The following statistical analyses were applied to the noted parameters:

PARAMETER	ANALYSIS CONDUCTED
Body weight Organ weights Erythrocytes Hemoglobin Hematocrit MCH MCHC MCV Thrombocytes (Platelets) Triglycerides	One-way analysis of variance (ANOVA) followed by Dunnett's test.
Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Cholesterol	Adjusted Welch test
Food consumption Water consumption Atypical leukocytes Basophils Eosinophils Hepato-quick Leukocytes Lymphocytes Monocytes Neutrophils Reticulocytes	Kruskal-Wallis test followed by Steel-Test
Immunotoxicity (spleen cell counts, FACScan analyses, antibody titers, and PFC assay)	Homogeneity of variance using Cochran test followed by Mann-Whitney or Wilcoxon significance test (Rank Sum Test or One Way ANOVA or Kruskal-Wallis ANOVA)

Immunotoxicity parameters were first analyzed for homogeneity of variances. The remaining parameters in this study were analyzed according to the following decision tree: (i) Dunnett's test if assumptions of normal distribution and homogeneous variances are met; (ii) p-value adjusted Welch test if heteroscedasticity appeared more likely; or (iii) Kruskal-Wallis test followed by adjusted Mann-Whitney-Wilcoxon test, where appropriate, if historical control data indicate that the assumptions of parametric ANOVA are not met. Significance was indicated at 5 and 1% probability.

C. METHODS

1. **Observations:** Cage-side observations for mortality and moribundity were conducted at least once a day. Detailed clinical examinations, including observations outside the home cage, were conducted once prior to treatment and weekly throughout the study.

2. **Body weight:** Each rat was weighed prior to treatment, on Day 1, and weekly throughout the study. Additionally, terminal body weights were determined immediately prior to necropsy for the determination of relative (to body) organ weights.

3. **Food/water consumption and compound intake:** Individual food and water intakes were determined weekly, based on the difference in the weight of the food (or water) supplied and the weight not consumed. These data were presented for each week as mean daily absolute (g/rat/day) and relative to body weight (g/kg bw/day) food (or water) consumption.

Individual achieved intake (mg/kg/day), reported in Table 1 of this DER, and was calculated from the food consumption and body weight data and the nominal dietary concentration.

4. **Hematology and clinical chemistry:** On Day 21/22, blood samples were collected from the retro-orbital venous plexus of each animal under carbon dioxide anesthesia. The following checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Blood cell morphology
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		
X	Hepato Quick		

a

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium		Albumin
	Chloride		Creatinine
	Magnesium		Urea nitrogen
	Phosphorus	X	Total cholesterol
	Potassium		Globulins
	Sodium		Glucose
	ENZYMES		Total bilirubin
	Alkaline phosphatase (ALP)		Total protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)		
X	Aspartate aminotransferase (AST/also SGOT)		
	Sorbitol dehydrogenase		
	Gamma-glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

5. Sacrifice and pathology

a. Gross necropsy: At study termination, all rats were killed by exsanguination under deep ether anesthesia and were subjected to a gross necropsy.

b. Organ weights: The adrenals, liver, spleen, thyroid, and thymus of each animal were weighed following exsanguination.

c. Tissue preparation/histopathology: The adrenals, liver, half of the spleen, thymus, and

thyroids (left half, with parathyroids) from all animals as well as any gross lesions or masses were preserved in 10% formalin for possible future microscopic analyses.

6. Immunotoxicity

a. Spleen cell counts: The spleen of each rat was crushed through a metal sieve to produce a single cell suspension. Spleen cells were counted manually for viability by Trypan blue exclusion.

b. Enumeration of total B cells, total T cells, and T cell subpopulations: Flow cytometry (FACScan) analyses were conducted to determine subpopulations of the spleen cells on a per animal basis. The cells were stained with the surface marker for B cells (PanB; OX-33), antigen presenting cells (I-a; OX3), T helper cells (CD4; OX-35), lymphocytes (CD45RC; OX-22), T cells (CD5; OX-19), and cytotoxic T cells (CD8; OX-8). Double labeling was done with CD4/CD45RC, CD4/CD8, CD5/CD8, and PanB/I-a. It was stated that previous studies using cyclophosphamide as a positive control showed dose-dependent immunosuppression using FACScan[1, 2]

c. Antibody titers: Whole blood samples were centrifuged to obtain sera preparations which were then stored at -80°C until evaluation. Antibody (IgG, IgM, and IgA) titers in the sera were measured on a per animal basis using the Sandwich enzyme-linked immunosorbent assay (ELISA) method.

d. Antibody plaque-forming cell (PFC) assay: Five days prior to necropsy, the animals were immunized with sheep red blood cells (SRBC) via intravenous (i.v.) injection. SRBC suspensions were adjusted to 1 x 10^9 per mL BSS. Humoral immune reactions were induced by i.v. injection of 100 µL of this suspension per animal. Five days after this *in vivo* stimulation (Day 28/29), the animals were killed, and the spleens were removed for further *in vitro* analysis. The spleens were crushed through a metal sieve resulting in single cell suspensions. The suspensions were adjusted to 1 x 10^7 cells per mL for further analysis. For each animal, four glass slides were prepared for evaluation using a duplicate for each of two different volume suspensions (100µL and 10 µL) after incubation with the appropriate amount of guinea pig complement. Evaluation was done by calculating the amount of PFC per 10^6 spleen cells. It was stated that previous studies using cyclophosphamide as a positive control showed dose-dependent immunosuppression using ELISA.[1, 2] Additionally, the Sponsor cited previous studies in which the optimal time course of SRBC immunization and the optimal concentration of antigen and complement were determined [3, 4, 5].

---

1 Vohr, H.W. Suppression of the Immune Response to SRBC in Rats by Cyclophosphamide (Validation of the function of FACScan analyses and ELISA) Report No. PH-33585, October 2004.

2 Vohr, H.W. Confirmation of the Function of a Plaque Forming Cell Assay in Rats with Cyclophosphamide Report No. PH-33481, September 2004.

3 Vohr, H.W. Titration of complement for the validation of a Plaque Forming Cell Assay in Rats. Report No. PH-30640, January 2001.

4 Vohr, H.W. Application of SRBC for the validation of a Plaque Forming Cell Assay in Rats. Report No. PH-28711, May 1999.

5 Vohr, H.W. Adjustment of the optimal time point for the application of SRBC for the validation of a Plaque Forming Cell Assay in Rats. Report No. PH-27046, August 1998.

168

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All rats survived until scheduled termination.

2. **Clinical signs of toxicity:** There were no treatment-related clinical signs of toxicity. The only findings were unrelated to dose and minimal in incidence and included: injury and loss of hair on the neck in one 4000 ppm male; thin coat on one control female; and increased motility in one 40 ppm female.

B. BODY WEIGHTS AND BODY WEIGHT GAINS: There were no significant treatment-related effects on body weights or body weight gains. The mean body weight gain was increased by 23% ($p \leq 0.05$) in the 40 ppm males for the first week (Days 1-8). Body weights and body weight gains in all other treated groups were comparable to controls throughout the study.

C. FOOD CONSUMPTION: There were no treatment-related effects on food consumption. In the males, absolute and relative (to body weight) food consumption was increased ($p \leq 0.05$) by 6-11% in all treated groups compared to controls on Day 8. In the 400 and 4000 ppm females, absolute and relative food consumption was decreased by 17-20% on Day 15. These differences were transient and/or unrelated to dose. The fact that the changes in food consumption were not consistent between the sexes (i.e., increased in the males but decreased in the females) further indicates that these differences were not treatment-related.

D. WATER CONSUMPTION: Absolute and relative (to body weight) water consumption in the treated males and females were comparable to controls throughout the study.

E. HEMATOLOGY: There were no significant treatment-related effects on hematology. In the males, clotting time was decreased ($p \leq 0.05$) by 7-10% in all treated groups compared to controls. In the 400 and 4000 ppm females, hemoglobin and hematocrit were decreased ($p \leq 0.05$) by 5-7%. Additionally in the 4000 ppm females, erythrocytes were decreased ($p \leq 0.05$) by 6%. However, these differences were minor and not considered adverse. There were no other dose-related differences in any hematology parameter.

F. CLINICAL CHEMISTRY: There were no significant treatment-related effects on clinical chemistry parameters. At 4000 ppm, minor decreases ($\downarrow$12-18%; $p \leq 0.05$) were noted in alanine aminotransferase (ALT) in both sexes and in aspartate aminotransferase (AST) in the males (Table 2). Although *increases* in these enzymes may indicate liver toxicity, the toxicological significance of *decreases* in ALT and AST are unknown. Cholesterol was increased ($p \leq 0.05$) by 14-20% in all treated groups in the males compared to controls; however, these increases were not dose-dependent.

Table 2. Clinical chemistry parameters in rats fed NNI-0001 in the diet for 21/22 days [a]				
Parameter	Dose (ppm)			
	0	40	400	4000
Males				
Alanine aminotransferase (U/L)	76.0 ± 9.28	70.0 ± 3.53	68.0 ± 5.26	62.1 ± 6.16** (↓18%)
Aspartate aminotransferase (U/L)	66.1 ± 5.94	64.5 ± 5.76	61.9 ± 12.70	58.3 ± 4.24* (↓12%)
Cholesterol (mmol/L)	2.05 ± 0.182	2.46 ± 0.240** (↑20%)	2.44 ± 0.249** (↑19%)	2.33 ± 0.326* (↑14%)
Females				
Alanine aminotransferase (U/L)	61.0 ± 7.62	59.5 ± 6.97	58.9 ± 7.04	51.8 ± 7.48* (↓15%)

a Data were obtained from pages 110-111 of the study report; n=10. Percent differences from controls, calculated by the reviewers, are included in parentheses.
* Statistically different from controls, p≤0.05.
** Statistically different from controls, p≤0.01.

G. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** An increased incidence of pale kidneys was noted in the 4000 ppm males (3/10) compared to controls (0/10; Table 3). This finding was also noted in 1/10 animals/group in the 40 ppm males and females, in the 400 ppm males, and in the 4000 ppm females. Limited clinical chemistry was performed and microscopic evaluations of the tissues were not conducted, making interpretation of this finding problematic. However, in the concurrently submitted subchronic (MRID 46817210) and chronic (MRID 46817219) studies on rats with this chemical, no adverse effects on the kidney were noted at doses up to 20,000 ppm.

Table 3. Incidences of pale kidney (# affected/10) in rats fed NNI-0001 in the diet for 28 days [a]			
Dose (ppm)			
0	40	400	4000
Males			
0	1	1	3
Females			
0	1	0	1

a Data were obtained from pages 115-116 of the study report; n=10

2. **Organ weight:** Increases (p≤0.05) over controls were observed in absolute and relative (to body) liver weights in the females at 400 ppm (↑18-23%) and 4000 ppm (↑46-50%; Table 4). Additionally at 4000 ppm, increases (p≤0.05) were observed in relative liver weight in the males (↑8%) and absolute thyroid weight in the females (↑80%); however, the relative thyroid weight was not significantly different from the control.

Table 4. Mean (±SD) terminal body weight and selected absolute (mg) and relative to body weight (mg/100 g BW) organ weights in rats fed NNI-0001 in the diet for 28 days [a]

Organ Parameter		Dose (ppm)			
		0	40	400	4000
Males					
Terminal body weight		308 ± 20.7	310 ± 14.3	299 ± 27.7	300 ± 27.9
Liver	Absolute	13,080 ± 1190.8	12,920 ± 933.3	12,898 ± 1614.7	13,771 ± 1202.8
	Relative	4239 ± 217.6	4169 ± 136.1	4307 ± 228.7	4599 ± 199.2* (↑8%)
Thyroid	Absolute	7 ± 2.5	8 ± 2.7	7 ± 2.2	9 ± 4.0
	Relative	2 ± 0.8	3 ± 0.8	2 ± 0.7	3 ± 1.2
Females					
Terminal body weight		204 ± 18.4	204 ± 16.7	196 ± 14.7	209 ± 15.8
Liver	Absolute	8305 ± 968.9	8184 ± 862.0	9829 ± 1139.1* (↑18%)	12,487 ± 1351.4** (↑50%)
	Relative	4076 ± 206.5	4016 ± 282.3	5017 ± 372.2** (↑23%)	5956 ± 347.5** (↑46%)
Thyroid	Absolute	5 ± 2.4	8 ± 4.3	7 ± 1.8	9 ± 2.0* (↑80%)
	Relative	3 ± 1.1	4 ± 2.4	3 ± 0.8	4 ± 1.1

a Data were obtained from pages 119, 120, 124, and 125 of the study report; n=10. Percent differences from controls, calculated by the reviewers, are included in parentheses.
* Statistically different from controls, $p \leq 0.05$.
** Statistically different from controls, $p \leq 0.01$.

H. IMMUNOTOXICITY

1. **Spleen cell counts:** There were no significant treatment-related effects on spleen cell counts (Table 5). The only difference noted was a decrease ($p \leq 0.05$) of 31% in females at 400 ppm, no dose response relationship was evidenced.

Table 5. Mean (±SD) spleen cell counts (10^6 cells/organ) in rats fed NNI-0001 in the diet for 28 days [a]

Dose (ppm)			
0	40	400	4000
Males			
403.6 ± 93.4	308.8 ± 75.0	367.2 ± 78.9	331.7 ± 102.6
Females			
294.9 ± 75.2	291.2 ± 105.6	203.4 ± 63.0* (↓31%)	246.2 ± 56.8

a Data were obtained from page 143 of the study report; n=10. Percent differences from controls, calculated by the reviewers, are included in parentheses.
* Statistically different from controls, $p \leq 0.05$.

2. **Enumeration of total B cells, total T cells, and T cell subpopulations:** Flow cytometry (FACScan) analyses results are presented in Table 6.

   At 400 and 4000 ppm in both sexes: the CD45 ^{total} positive cells were dose-dependently decreased by 5-13%; CD45 ^{high} positive cells (mature/activated lymphocytes) were dose-dependently decreased by 13-29%; and CD45 ^{low} positive cells (more immature cells) were dose-dependently increased by 12-33%. These differences were significant ($p \leq 0.05$), with the exception of the CD45 ^{low} positive cells in the 400 ppm females. Because these values are expressed as a percentage of cells out of 15,000 counted in a sample, the relative increase observed in CD45 ^{low} positive cells would correspond with the relative decrease noted in CD45 ^{high} positive cells. However, the lower percentage of CD45 ^{total} positive cells at these doses indicates that there is also an absolute decrease in these cells in general, irrespective of

the shift from mature/activated lymphocytes to more immature cells. A minor decrease of 5% ($p \leq 0.05$) in CD45 ^{total} positive cells was also noted in the 40 ppm males. There were no other dose-related differences in any cell populations in either sex. In the 4000 ppm females, CD5 ^{total} positive cells were decreased ($p \leq 0.05$) by 8%. The study report stated that most of the statistically significant changes were within the normal range of variance for the relevant parameters except the changes detected at 4000 ppm just exceed the borderline of normal variance and were considered as treatment-related. No historical normal ranges of these parameters were provided.

Table 6. B and T cell subpopulation enumeration via FACScan flow cytometry (% of positive cells in a 15,000 count sample) in spleen cell suspensions from rats fed NNI-0001 in the diet for 28 days [a]

Cell type/surface marker	Dose (ppm)			
	0	40	400	4000
Males				
CD4 total	39.34 ± 1.94	39.71 ± 2.64	39.70 ± 2.95	39.39 ± 3.87
CD45 total	73.59 ± 1.61	70.27 ± 2.80* (↓5)	68.27 ± 3.13* (↓7)	64.31 ± 3.56* (↓13)
CD45 low	17.45 ± 1.18	19.12 ± 2.12	20.71 ± 2.74* (↑19)	23.20 ± 2.80* (↑33)
CD45 high	21.90 ± 1.93	20.59 ± 1.53	18.99 ± 1.89* (↓13)	16.20 ± 2.23* (↓26)
CD4 total [b]	39.87 ± 2.18	39.37 ± 7.45	40.87 ± 3.01	39.82 ± 3.88
CD8 total	26.91 ± 1.51	25.75 ± 2.71	26.22 ± 1.52	24.45 ± 2.10
CD4/CD8 ratio	1.48	1.53	1.56	1.63
CD5 total	54.92 ± 3.51	54.38 ± 6.36	56.34 ± 3.58	55.19 ± 5.84
CD5 neg/CD8 pos (NK)	6.95 ± 1.59	7.44 ± 2.13	6.99 ± 1.14	6.24 ± 1.50
Pan B total	31.32 ± 1.70	30.59 ± 3.46	29.83 ± 2.64	30.80 ± 4.36
I-a total	36.23 ± 2.20	35.61 ± 4.90	33.96 ± 3.26	34.05 ± 4.21
Females				
CD4 total	41.80 ± 2.60	39.84 ± 3.18	39.26 ± 3.38	38.91 ± 3.01
CD45 total	73.24 ± 3.13	71.72 ± 1.73	69.25 ± 3.51* (↓5)	66.60 ± 3.78 (↓9)
CD45 low	17.45 ± 1.43	17.88 ± 1.55	19.49 ± 2.52 (↑12)	21.63 ± 3.02* (↑24)
CD45 high	24.35 ± 2.34	21.96 ± 2.20	19.77 ± 2.30* (↓19)	17.28 ± 3.00* (↓29)
CD4 total [b]	43.76 ± 2.38	41.27 ± 3.49	40.60 ± 3.48	40.25 ± 3.29
CD8 total	28.75 ± 3.11	28.27 ± 3.57	29.46 ± 3.32	27.55 ± 2.26
CD4/CD8 ratio	1.52	1.46	1.38	1.46
CD5 total	59.98 ± 4.86	58.37 ± 4.78	56.76 ± 2.92	55.47 ± 3.33* (↓8)
CD5 neg/CD8 pos (NK)	5.80 ± 1.39	5.61 ± 0.94	7.30 ± 1.66* (↑26)	6.32 ± 1.25
Pan B total	27.02 ± 4.31	28.09 ± 3.39	28.64 ± 3.45	30.85 ± 3.38
I-a total	31.79 ± 5.04	32.39 ± 4.22	34.03 ± 2.61	35.82 ± 3.34

a Data were obtained from pages 144-146 of the study report; n=10. Percent differences from controls, calculated by the reviewers, are included in parentheses.

b From CD4/CD8 double labeling

* Statistically different from controls, $p \leq 0.05$.

3. **Antibody titers:** Serum antibody titers for IgG, IgM, and IgA in the treated males were comparable to controls (Table 7). For females, decreases ($p \leq 0.05$) of 32-33% were observed in IgA at 4000 ppm and in IgM at 400 and 4000 ppm. It was stated that the mean IgM concentrations at 400 and 4000 ppm were within the normal range of variation; however, no historical control data were presented for antibody titer data. Additionally, the Sponsor noted that the values in the control and 40 ppm groups in the females were inexplicably high. The optimum time for collection of IgA and IgG was not determined and was not submitted. For example, the optimum time for antibody IgG production was latter than IgM for a few weeks; therefore, the result of the IgA and IgG was not meaningful since it was not measured at the

optimum time of production.

Table 7. Mean (±SD) antibody titer (µg/mL) in the serum of rats fed NNI-0001 in the diet for 28 days [a]				
Immunoglobulin	Dose (ppm)			
	0	40	400	4000
Males				
IgA	110.0 ± 31.9	80.5 ± 33.9	145.3 ± 25.5	114.1 ± 28.9
IgM	1784.2 ± 618.3	1350.5 ± 508.2	1748.4 ± 446.7	1894.6 ± 517.8
IgG	521.0 ± 95.9	391.1 ± 150.9	388.7 ± 100.4	498.7 ± 150.9
Females				
IgA	166.6 ± 50.1	173.7 ± 46.2	124.4 ± 51.5	114.1 ± 28.9* (↓32%)
IgM	5771.8 ± 1390.1	6344.1 ± 1515.2	3848.5* ± 1129.8 (↓33%)	3903.3 ± 1221.4* (↓32%)
IgG	1068.5 ± 298.9	877.3 ± 291.1	769.2 ± 274.9	1183.6 ± 317.1

a Data were obtained from pages 147 of the study report; n=10. Percent differences from controls, calculated by the reviewers, are included in parentheses.

* Statistically different from controls, $p \leq 0.05$.

4. **Antibody plaque-forming cell (PFC) assay:** There were no significant treatment-related effects on anti-SRBC (IgM) plaque-forming cell response. The numbers of anti-SRBC plaque forming cells per million spleen cells examined in the treated groups were comparable to controls for both sexes (Table 8).

Table 8. Individual and mean (±SD) PFC per 10^6 spleen cells from rats fed NNI-0001 in the diet for 28 days [a]				
	Dose (ppm)			
	0	40	400	4000
Males				
1st animal of the group	1395	1835	2075	1780
2nd animal of the group	1235	705	1240	2300
3rd animal of the group	1125	3860	1695	3360
4th animal of the group	1085	2435	1065	2095
5th animal of the group	5820	1435	2295	2045
6th animal of the group	995	6*	1265	1980
7th animal of the group	1080	960	815	770
8th animal of the group	525	1170	2615	1105
9th animal of the group	605	2490	2275	1965
10th animal of the group	2240	725	1900	1560
Mean	1611 ± 1552	1735 ± 1041	1724 ± 604	1896 ± 699
Females				
1st animal of the group	2850	2510	3220	1695
2nd animal of the group	1225	2165	348	2030
3rd animal of the group	2310	2355	985	2440
4th animal of the group	1005	2200	1545	1895
5th animal of the group	2755	2430	875	1480
6th animal of the group	4190	1060	510	875
7th animal of the group	1790	1325	1500	3465
8th animal of the group	1320	1930	1870	1990
9th animal of the group	1735	1990	3360	2850
10th animal of the group	2770	2490	3435	2830
Mean	2195 ± 976	2046 ± 495	1765 ± 1181	2155 ± 757

a Data were obtained from pages 148 of the study report; n=10. Standard deviations were calculated by the

reviewer.
* value was not used for evaluation.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: The study author concluded that treatment with the test substance revealed no *primary* immunotoxicity because the PFC assay showed no functional changes up to the highest dose tested. The authors also suggested that the changes in isolated immune parameters were considered either as due to chance variation or are interpreted as secondary changes due to the live toxicity observed at 400 ppm and above.

B. REVIEWER COMMENTS: There were no mortalities and no treatment-related adverse effects on clinical observations, body weights, body weight gains, food consumption, water consumption, hematology, clinical chemistry, or gross pathology.

At 400 and 4000 ppm, treatment-related effects on the liver and thyroid were observed, as indicated by increased organ weights. Increased absolute and relative liver weights were observed in females by 18-50% ($p \leq 0.05$) over controls at doses ≥400 ppm while increased relative liver weight ($p \leq 0.05$) was observed in males (↑8%) at 4000 ppm. There were no macroscopic findings in these organs and no histopathology was performed. Increased absolute thyroid weight ($p \leq 0.05$) was observed in the 4000 ppm females only; however, the relative thyroid weight was not significantly different compared to the control.

It should be noted that in the concurrently submitted subchronic (MRID 46817210) and chronic (MRID 46817219) studies on rats with this chemical, hepatotoxicity was observed at 2000 ppm (equivalent to approximately 100 mg/kg/day) in the females. In a study on effects of the test substance on thyroid and liver function (MRID 46817235) which was also concurrently reviewed, similar increases in liver and thyroid weights were observed at 1000 and 10,000 ppm. Cytochrome (CYP) P-450 protein and EROD activity were increased, which suggested a selective induction of CYP 1A2. Also, UDP-GT was induced, which would lead to increased elimination of T4 from the blood stream and initiate the feedback loop that would eventually induce thyroid follicular cell hyperplasia and increase the thyroid weight.

For immunological parameters, no treatment-related effects were seen in total spleen cell counts, antibody titers (IgA, IgM, IgG), and anti-SRBC plaque forming cells (PFC) response for both sexes. The optimum time for collection of IgA and IgG was not determined and was not submitted. For example, the optimum time for antibody IgG production was latter than IgM for a few weeks; therefore, the results of the IgA and IgG at days 5 was not meaningful.

FACScan analyses for enumeration of total B cells, total T cells, and T cell subpopulations at 400 and 4000 ppm in both sexes indicated a minor decrease in lymphocytes in general ($CD45^{total}$ positive cells) and a shift from mature/activated lymphocytes ($CD45^{high}$ positive cells) to more immature cells ($CD45^{low}$ positive cells). Additionally in the 4000 ppm females, $CD5^{total}$ positive cells were decreased by 8%. It was stated that most of the statistically significant changes at 40 and 400 ppm were within the normal range of variation, and the findings at 4000 ppm just exceeded this range. However, the historical control data provided on p. 58 of the study report (see attachment to this DER) only comprised mean, standard deviation, and

relative standard deviation, but did not include a range for each parameter, nor were the number of studies reported. The only finding at 40 ppm was a minor decrease of 5% ($p \leq 0.05$) in CD45 ^{total} positive cells in the males.

Under the condition of this study, the NOAEL for immunotoxicity is 4000 ppm (equivalent to 336.3/358.8 mg/kg/day for males/females), the highest dose tested, based on anti-SRBC (IgM) plaque forming cells response and other immunological parameters. The LOAEL has not been achieved.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study **(OPPTS 870.7800)** in rats.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted but do not affect the conclusions of this DER:

- A detailed dose-selection rationale was not provided.
- The optimum time of collection for antibody IgA or IgG was not submitted.
- Historical control data of flow cytometry did not include ranges and the number of studies was not provided.

ATTACHMENT

The following is page 58 of the study report

Historical Data of Flow Cytometry

	Percentage of positive cells of vehicle treated animals					
Marker	MALE MEAN	SD	SD%	FEMALE MEAN	SD	SD%
$CD4^{TOTAL}$	37.69	3.98	10.56	39.70	3.89	9.80
$CD45^{TOTAL}$	74.69	4.60	6.15	73.84	4.40	5.96
$CD45^{LOW}$	15.80	2.82	17.84	15.60	2.75	17.61
$CD45^{HIGH}$	21.85	4.77	21.83	24.09	3.36	13.94
$CD4^{TOTAL}$ (from CD4/CD8)	39.68	4.39	11.05	40.75	3.54	8.68
$CD8^{TOTAL}$	25.57	2.41	9.44	25.42	3.06	12.04
$CD5^{NEG.}$ $CD8^{POS.}$ (NK)	5.69	1.41	24.79	5.60	1.45	25.88
$CD5^{TOTAL}$	54.08	7.45	13.77	55.56	8.30	14.94
PAN B^{TOTAL}	29.77	4.15	13.92	25.76	3.83	14.86
$I\text{-}a^{TOTAL}$	31.12	4.27	13.71	28.15	4.08	14.49

12

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; Effects on Thyroid Hormones and Liver Enzymes in Female Rats

Work Assignment No. 4-01-124 R; formerly 3-01-124 R (MRID 46817235)

Prepared for
Health Effects Division
Office of Pesticide Programs
U S Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer
John W. Allran, M.S.

Signature: John W. Allran
Date: 01-22-07

Secondary Reviewer
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/22/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 1/22/07

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 1/22/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

178

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Toxicology Branch, Health Effects Division (7509P) **Date:** 6/26/2007
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Effects on Thyroid Hormones and Liver Enzymes in Female Rats; Non-guideline.

PC CODE: 027602 **DP BARCODE:** D331553
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 technical; (96.7% a.i.)

SYNONYMS: N^2-(1,1-dimethyl-2-methylsulfonylethyl)-3-iodo-N^1-{2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]1,2-benzenedicarboxamide

CITATION: Amanuma T. (2005) Effect of NNI-0001 administration on the thyroid-related hormones and liver drug-metabolizing enzymes in female F-344 rats. Product Safety & Pharmaceutical Research Unit, Research Center, Research & Development Division, Nohon Nohyaku Co., Ltd. Osaka, Japan. Laboratory Study Protocol No.: GA-11, 02-0162, Final Report No.: LSRC-T05-041A, June 29, 2005, amended October 26, 2005. MRID 46817235. Unpublished.

SPONSOR: Nihon Nohyaku Co, Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a non-guideline study (MRID 46817235), NNI-0001 (Flubendiamide, lot# 1FH0019M, 96.7% a.i.) was administered in the diet to female Fischer 344 rats (10/dose group) at dietary concentrations of 0, 1000, or 10,000 ppm (equivalent to 0, 83, or 812 mg/kg/day, respectively) for 7 or 28 days. Blood samples were periodically drawn for analysis of T4, T3, and thyroid stimulating hormone (TSH) levels. At necropsy, the liver, thyroids, and pituitary were weighed and examined microscopically. A portion of the liver from each rat was processed for determinations of cytochrome P-450 content and enzyme activities of uridine diphosphate-glucuronyl transferase (UDP-GT), ethoxyresorufin O-dealkylase (EROD), and pentoxyresorufin O-dealkylase (PROD).

There were no mortalities and no treatment-related adverse effects on clinical observations, body weights, food consumption, and gross pathology.

Liver microsomal protein (mg/g liver) was decreased at 10,000 ppm on Days 7 and 28 while cytochrome P-450 concentrations (ng/mmol protein) were increased at 1000 and 10,000 ppm on Days 7 and 28. For liver enzymes, UDP-GT and EROD activities were increased at 1000 and 10000 ppm on Days 7 and 28. PROD activity was unaffected by the treatment.

Serum concentrations of T3 and TSH were increased at 1000 and 10000 ppm beginning on Day 7, with TSH concentrations being increased in both a time- and dose-dependent manner. T4 was increased at ≥1000 ppm beginning on Day 1. Because of the concern that individual hormonal level has a large variability, actual concentrations of T3, T4 and TSH were evaluated together with the calculated changes of concentrations from pre-treatment values. The differences from pre-treatment values showed a similar pattern with T3 and TSH. These increases were dose-dependent but not increased with time. Compared with pre-treatment values, the differences in T4 at 10000 ppm were greater than controls on Days 1 and 7; however, they were comparable to controls thereafter. Although T4 concentrations relative to levels prior to treatment were not increased over controls after Day 7, it is difficult to explain the fact that absolute concentrations of T4 did not decrease with induction of UDP-GT. It is possible that the increased TSH stimulated production of T4 to a concentration that saturated the available UDP-GT.

Absolute and relative liver weights were increased at 1000 and 10000 ppm on Days 7 and 28. Microscopic pathology showed increased incidences and severity of diffuse hepatocyte hypertrophy and vacuolation of hepatocytes that were dose-dependently increased at these doses on Days 7 and 28. In the thyroid, increased absolute thyroid weights were observed at 1000 and 10000 ppm on Day 28 only. Microscopic pathology showed increased incidences of follicular cell hypertrophy in all rats in the 1000 and 10,000 ppm groups (10/10 each treated) compared to controls (0/10) on Days 7 and 28. The severity of this finding increased with both time and dose dependent manner. For pituitary, there were no treatment-related effects seen.

In summary, the findings in this study generally support the mechanism of effect of NNI-0001 on the thyroid via induction of enzymes in the liver. Direct effects on the liver included increases in liver weights, cytochrome P450, UDP-GT and EROD activities; and incidences of hepatocyte hypertrophy and vacuolation. The increased enzymatic activity of the liver resulted in indirect effects on the thyroid, including increases in thyroid weights; T3, T4, and TSH serum concentrations, and incidences of follicular cell hypertrophy. The mechanism of induction of UDP-GT activity without reduction in serum T4 concentration was not clearly explained. It is possible that the TSH-mediated increased production of T4 saturated the available UDP-GT.

This study is classified as **acceptable/non-guideline**. No particular test guideline was directly applicable to the study design.

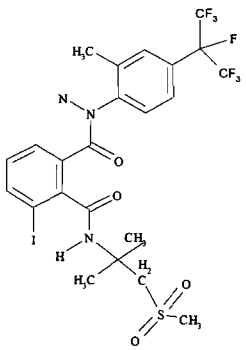
COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. INTRODUCTION AND OBJECTIVES: This study was conducted to clarify the mechanism of the effect of NNI-0001 on thyroid function in female Fischer 344 rats by examining: (i) the levels of thyroid-related hormones in serum; (ii) changes in histology in the thyroid and pituitary; and (iii) the induction of liver enzymes which contribute to thyroid hormone metabolism.

II. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide) technical

Description:	White crystal
Batch #:	1FH0019M
Purity:	96.7% a.i.
Compound stability:	The test substance was stable in the diet for up to 5 weeks under cold conditions, followed by 5 days at room temperature, followed by 8 days exposed to ambient air (from Study No. IET 00-0156; cited in MRID 46817210).
CAS # of TGAI:	272451-65-7
Structure:	

2. Vehicle: Diet

3. Test animals:

Species:	Rat (females)	
Strain:	Fisher-344 (F344/DuCrj)	
Age/weight at study initiation:	6 weeks old; 92.89-111.95 g	
Source:	Charles River Japan (Atsugi, Kanagawa, Japan)	
Housing:	5/cage during acclimation and 2/cage during the experimental period in FRP resin cages with wire mesh floors sustained on a movable stainless steel rack.	
Diet:	Labo-MR stock (pelleted during quarantine period and subsequently powdered (Nohsan Corporation, Yokohama, Kanagawa, Japan). On the day prior to animal assignment, the diet was finally switched to powdered MF diet stock (Oriental Yeast Co., Ltd, Chiba, Japan), *ad libitum*	
Water:	Filtered (5μm) tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25EC
	Humidity:	30-74%
	Air changes:	12-15/hour
	Photoperiod:	12 hours light/12 hours dark
Acclimation period:	7 days	

B. STUDY DESIGN

1. **In life dates:** Start: 10/08/02 End: 11/05/02

2. **Animal assignment:** All animals were ranked in descending order of their body weight, and five animals (the heaviest one and the lowest four) were excluded from the study to minimize the variation of body weight. The rats were then assigned randomly, stratified by body weights, to the test groups shown in Table 1. Animals in each group were randomly divided into two subgroups to allow for interim blood sampling and to obtain organ samples at different time points.

Table 1. Study design [a]

Test Group	Diet concentration (ppm)	Achieved dose (mg/kg/day)	# Animals	
			Subgroup B Interim sacrifice [b]	Subgroup A Terminal sacrifice [c]
Control	0	0	10	10
Low	1000	83	10	10
High	10,000	812	10	10

a Data were obtained from pages 18 and 34 of MRID 46817235.
b Interim sacrifice (Subgroup B) occurred after 7 days of treatment.
c Terminal sacrifice (Subgroup A) occurred after 28 days of treatment.

3. **Dose selection rationale:** The high dose group in this study was selected based on the results of a 28-day repeated dose oral toxicity study of NNI-0001 in the rat conducted by the Institute of Environmental Toxicology (Study No: IET00-0156)[1] in which a dietary concentration of 20,000 ppm was equivalent to an achieved concentration of 1605 mg/kg/day. Therefore, 10,000 ppm was chosen for the current study to approximate 1000 mg/kg/day, the limit dose in 28- and 90-day repeated dose oral toxicity studies in rodents. The low dose group (1000 ppm) was selected to show a clear dose-response.

4. **Dose preparation, administration, and analysis:** The test diets were prepared once prior to treatment. The required amount of the test material was weighed, ground, and made into a pre-mix by adding a small portion of pre-weighed powdered diet. This pre-mix was mixed with the appropriate amount of additional diet to achieve the 10,000 ppm dietary concentration. The 1000 ppm diet was prepared by diluting a portion of the 10,000 ppm test diet with additional basal diet. Test diets were stored at ≤4°C until use. Concentration and homogeneity of the test diets were determined by analyzing samples from the top, middle, and bottom of the mixing container. Stability was confirmed in 20 and 20,000 ppm diets kept under a sealed, cold, and dark condition for 5 weeks, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient air for 8 days (Study No. IET00-0156). Although this study not provided, the data were presented on p. 24 in a concurrently submitted 90-day dietary study in rats (MRID 46817210).

1 Institute of Environmental Toxicology, 28 days repeated oral toxicity of NNI-0001 in the rat (Study No.: IET00-0156)

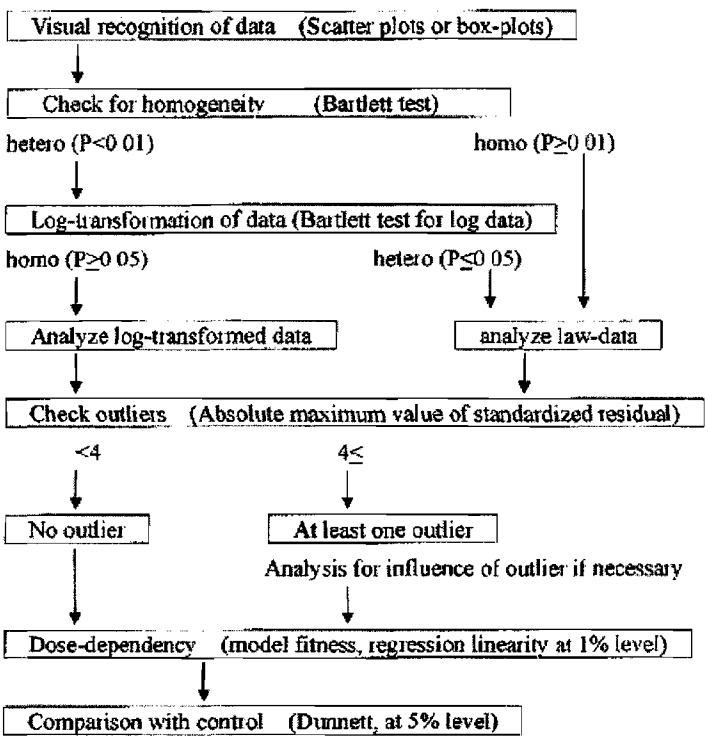
Results

Homogeneity (% relative standard deviation): 0.8-2.1%

Concentration (95% confidence interval of %nominal): 94.1-101.0%

Stability analysis (% of initial concentration): 97-100%

5. **Statistics:** Continuous data were analyzed using the following tree-type algorithm statistical procedures described by Hamada[2]:



Schematic illustration of the tree type algorithm by Hamada et al[7].

Statistical significance for Dunnett's test was denoted at $p \leq 0.05$ and 0.01, and dose-dependency was defined at $p \leq 0.01$. For statistical analyses of microscopic findings in histopathology, each severity grade (-, ±,+, ++, +++) was transformed to 1, 2, 3, 4, and 5, respectively, and subjected to Mann-Whitney's U-test (two-tailed). The statistical methods were considered appropriate.

C. METHODS

2 C. Hamada et. al. "Tree-type algorithm for statistical analysis in chronic toxicity studies" J. Toxicol. Sciences, 23, p. 173.

1. **Observations:** All rats were observed daily.

2. **Body weight:** All animals were weighed just prior to assignment and on Days 7, 14, 21, and 28.

3. **Food consumption:** During the treatment period, the food container was weighed on the first and third day of each week, and daily food consumption (g/rat/day) was calculated.

4. **Test material intake:** Individual achieved intake (mg/kg/day), reported in Table 1 of this DER, was calculated from the food consumption and body weight data, using the nominal concentrations.

5. **Serum thyroid hormone analyses:** Blood samples were drawn from the cervical vein of all animals under diethyl ether anesthesia prior to treatment. Cervical vein blood samples were drawn again from the Subgroup B animals on Day 1 and from the Subgroup A animals on Days 3 and 14. At termination, blood was taken from the vena cava on Day 7 (interim sacrifice; Subgroup B) or Day 28 (terminal sacrifice; Subgroup A). Serum samples were stored under -80 °C and analyzed for T3, T4, and thyroid stimulating hormone (TSH) concentrations using commercially available enzyme-immunoassay (EIA) kits.

6. **Sacrifice and pathology**

a. **Necropsy, organ weights, and sampling of organs:** At study termination, all rats were killed by diethyl ether anesthesia and were subjected to a gross necropsy. The liver, thyroid, and pituitary were weighed. A portion of the left lateral lobe of the liver was removed and immersed in phosphate-buffered 10% formalin. The remaining portion of the liver was weighed and processed for biochemical analysis. The thyroid (right and left lobe) and pituitary were collected, preserved in phosphate-buffered 10% formalin, and then weighed.

b. **Histopathology:** Samples of the liver, thyroid, and pituitary were processed, sectioned at 5 µm, stained with hematoxylin and eosin, and examined microscopically. Severity was graded as "no abnormal finding, minimum (very slight), slight, moderate, or severe".

7. **Biochemical analysis of the liver:** The liver tissue for biochemical analysis was homogenized in 1.15% KCl buffered with 10 mM Na-K phosphate and centrifuged to obtain a pellet, referred to as the microsomal fraction. This pellet was re-suspended in the same buffer and centrifuged again to obtain a final pellet (washed microsomal fraction), which was then suspended in 100 mM Na-K phosphate containing 10% (v/v) glycerol to make a final homogenate (referred to as a microsomal suspension). Microsomal suspensions, stored at -80°C until analysis, were analyzed for protein content, cytochrome P-450 content, and activities of ethoxyresorufin O-dealkylase (EROD), pentoxyresorufin O-dealkylase (PROD), and uridine diphosphate-glucuronyl transferase (UDP-GT) using spectrophotometry and fluorescent spectrophotometry. Sample size for this analysis at each sampling time was limited to 8 per treatment group due to the available capacity of ultracentrifuge equipment.

II. RESULTS

A. OBSERVATIONS:

1. **Mortality:** All rats survived until scheduled termination.

2. **Clinical signs of toxicity:** There were no treatment-related clinical signs of toxicity. The only finding was stained fur in one rat in the 1000 ppm group, which was considered incidental and not related to the treatment.

B. BODY WEIGHTS: There were no treatment-related effects on body weights. The mean body weight in the 10,000 ppm group was increased by 5% ($p \leq 0.05$) over controls on Day 28. Because this was an increase in body weight instead of a decrease and because it was minor, it was not considered adverse. Body weights in all other treated groups were comparable to controls throughout the study.

C. FOOD CONSUMPTION: There were no treatment-related effects on food consumption. A statistically significant increase of food consumption over controls was seen in the 1000 ppm group during Week 3 but the data of 10000 ppm was comparable to the control. Furthermore, a second measurement of food consumption was made during Week 3, and all treated groups were comparable to controls. Thus, this increase was considered incidental.

D. LIVER MICROSOMAL PROTEIN AND CYTOCHROME P-450: Decreased liver microsomal protein concentrations (mg/g liver) were observed on Day 7 (11%, $p \leq 0.01$) and Day 28 (14%, not significant [NS]; Table 2) at 10,000 ppm. Increased cytochrome P-450 concentration (ng/mmol protein) was observed at 1000 and 10,000 ppm (36-57%) compared to controls; these increases grew in magnitude with time from Day 7 (↑36-47%) to Day 28 (↑51-57%).

Table 2. Microsomal protein and cytochrome P-450 content in the liver of female rats fed NNI-0001 in the diet for up to 28 days [a]

Day	Dose (ppm)		
	0	1000	10,000
Microsomal protein (mg/g liver)			
7	20.1±1.7	20.2±1.7	17.8±0.8**(↓11%)
28	15.3±1.2	14.5±1.8	13.1±2.3 (↓14%)
Cytochrome P-450 (ng/mmol protein)			
7	0.74±0.06	1.09±0.06**(↑47%)	1.01±0.08**(↑36%)
28	0.47±0.09	0.74±0.08**(↑57%)	0.71±0.03**(↑51%)

a Data were obtained from Table 7 on page 38 of MRID 46817235; n=8, except for the 1000 and 10,000 ppm groups on Day 28. Percent differences from controls, calculated by the reviewers, are included in parentheses.

** Statistically different from controls, $p \leq 0.01$.

E. LIVER ENZYMES: UDP-GT activity was statistically increased at 10000 ppm on Day 7 and at 1000 and 10000 ppm on Day 28 compared to controls (Table 3). These increases were both time- and dose-dependent and became more pronounced when standardized according to the mass of whole liver (mmol/min/mg whole liver; ↑31-97%) compared to the mass of

protein (mmol/min/mg protein; ↑10-44%). EROD activity was increased by 24-53% ($p \leq 0.05$) at these doses on Day 7 and Day 28; but did not increase in magnitude with time. PROD activity was unaffected by treatment. A statistically significant increase of PROD activity was seen at 1000 ppm on Day 7; however, the increase was not dose- and time-dependent and was considered not treatment-related.

Table 3. Microsomal enzyme activities in the liver of female rats fed NNI-0001 in the diet for up to 28 days [a]

Day	Dose (ppm)		
	0	1000	10,000
UDP-GT (mmol/min/mg protein)			
7	49±3	54±7 (↑10%)	62±4**(↑27%)
28	39±4	52±4**(↑33%)	56±6**(↑44%)
UDP-GT (mmol/min/mg whole liver)			
7	4393±669	5765±964**(↑31%)	6650±582**(↑51%)
28	3150±696	5461±975**(↑73%)	6214±1469**(↑97%)
Ethoxyresorufin O-dealkylase (EROD) activity (nmol/min/mg protein)			
7	18.83±3.24	28.72±7.19**(↑53%)	24.52±1.86*(↑30%)
28	68.72±14.13	85.24±12.41*(↑24%)	88.86±10.34*(↑29%)
Pentoxyresorufin O-dealkylase (PROD) activity (pmol/min/mg protein)			
7	7.31±0.90	8.52±1.24*(↑17%)	8.36±0.47
28	11.32±2.10	9.96±1.14	10.46±0.94

a Data were obtained from Table 8 on page 39 of MRID 46817235; n=8, except for the 1000 and 10,000 ppm groups on Day 28. Percent differences from controls, calculated by the reviewers, are included in parentheses.

** Statistically different from controls, $p \leq 0.01$.

* Statistically different from controls, $p \leq 0.05$.

F. THYROID HORMONES: The serum concentration of T3 was increased by 14-28% ($p<0.01$) at doses of 1000 and 10,000 ppm over controls on Days 7, 14, and 28 (Table 4a). Serum T4 level was increased by 7-34% at these doses beginning on Days 1, 3, 14 and 28; and these increases were significant ($p<0.05$), with the exception of the 1000 ppm group on Day 1 and the 10,000 ppm group on Day 28. TSH concentration was increased ($p<0.05$) at 1000 ppm on Days 14 (↑19%) and 28 (↑36%) and at 10,000 ppm on Days 14 (↑30%) and 28 (↑64%); thus TSH concentrations were increased in a time- and dose-dependent manner.

The study author stated that "it is well known that the individual hormonal level has a large variability"; therefore, actual concentrations of T3, T4 and TSH were evaluated together with the calculated changes of concentrations from pre-treatment values. The changes from pre-treatment values in T3 were greater ($p<0.05$) at 1000 ppm (↑126-230%) and 10,000 ppm (↑205-318%) than the change in the controls on Days 7, 14, and 28 (Table 4b). The changes from pre-treatment values in T4 showed the decrease in treatment groups were smaller than the control. Changes in TSH were greater ($p<0.05$) at 1000 ppm on Day 28 (↑114%) and at 10,000 ppm on Days 14 and 28 (↑207-233%). These increases were dose-dependent but not increased with time. At 10,000 ppm, the differences from pre-treatment values in T4 were greater than controls on Day 1 (↑49%) and Day 7 (↑95%); however, they were comparable to controls thereafter.

Table 4a. Selected mean (±SD) concentrations of thyroid hormones in serum of rats fed NNI-0001 in the diet for up to 28 days [a]

Day	Subgroup	Dose (ppm)		
		0	1000	10,000
T3 (ng/dL)				
-1	A	111±12	118±12	109±9
	B	128±12	127±8	126±14
1	A	ND	ND	ND
	B	118±7	117±10	119±7
3	A	133±13	142±21	138±13
	B	ND	ND	ND
7	A	ND	ND	ND
	B	140±12	160±11**(↑14%)	160±13**(↑14%)
14	A	130±7	162±21**(↑25%)	166±16**(↑28%)
	B	-	-	-
28	A	122±12	145±15**(↑19%)	155±13**(↑27%)
	B	-	-	-
T4 (µg/dL)				
-1	A	9.9±15	10.8±1.7	10.8±2.2
	B	7.8±1.4	7.7±1.0	7.3±1.0
1	A	ND	ND	ND
	B	4.1±06	4.9±0.9	5.5±0.8**(↑34%)
3	A	7.4±0.9	9.0±1.1**(↑22%)	9.4±0.8**(↑27%)
	B	ND	ND	ND
7	A	ND	ND	ND
	B	5.9±1.0	7.1±1.1*(↑20%)	7.2±0.9**(↑22%)
14	A	7.6±0.9	9.7±1.2**(↑28%)	9.1±0.4**(↑20%)
	B	-	-	-
28	A	7.6±1.2	8.8±0.6**(↑16%)	8.1±0.5
	B	-	-	-
TSH (ng/mL)				
-1	A	30±4	31±4	31±5
	B	21±3	24±3*(↓20%)	23±2
1	A	ND	ND	ND
	B	23±7	23±6	28±7
3	A	28±2	30±3	30±4
	B	ND	ND	ND
7	A	ND	ND	ND
	B	28±6	31±6	33±6
14	A	27±4	32±2*(↑19)	35±6**(↑30)
	B	-	-	-
28	A	45±5	61±8**(↑36)	74±13**(↑64)
	B	-	-	-

a Data were obtained from Tables 9-1 through 9-3 on pages 40-42 of MRID 46817235. Percent differences from controls, calculated by the reviewers, are included in parentheses.

* Statistically different from controls, $p \leq 0.05$.

** Statistically different from controls, $p \leq 0.01$.

ND Not determined

\- Not applicable (animals had already been sacrificed by this time)

Table 4b. Selected mean (±SD) changes in thyroid hormone concentrations from pre-treatment values in serum of rats fed NNI-0001 in the diet for up to 28 days [a]

Day	Subgroup	Dose (ppm)		
		0	1000	10,000
T3 (ng/dL)				
1	A	ND	ND	ND
	B	-9±16	-10±14	7±16
3	A	22±14	23±12	29±17
	B	ND	ND	ND
7	A	ND	ND	ND
	B	10±17	33±7**(↑230%)	34±19**(↑240%)
14	A	19±14	43±16**(↑126%)	58±19**(↑205%)
	B	-	-	-
28	A	11±15	26±8*(↑136%)	46±14(↑318%)
	B	-	-	-
T4 (µg/dL)				
1	A	ND	ND	ND
	B	-3.7±1.9	-2.8±1.4	-1.9±1.3*(↑49%)
3	A	-2.5±1.5	-1.8±1.5	-1.4±2.4
	B	ND	ND	ND
7	A	ND	ND	ND
	B	-2.0±1.7	-0.6±1.1	-0.1±1.4*(↑95%)
14	A	-2.4±1.8	-1.1±1.8	-1.7±2.3
	B	-	-	-
28	A	-2.3±1.7	-2.0±1.6	-2.7±2.1
	B	-	-	-
TSH (ng/mL)				
1	A	ND	ND	ND
	B	2±9	-1±6	4±8
3	A	-2±4	-1±3	-1±4
	B	ND	ND	ND
7	A	ND	ND	ND
	B	7±7	7±5	10±8
14	A	-3±5	1±5	4±8*(↑233%)
	B	ND	ND	ND
28	A	14±7	30±8**(↑114%)	43±14**(↑207%)
	B	ND	ND	ND

a Data were obtained from Tables 10-1 through 10-3 on pages 43-45 of MRID 46817235. Percent differences from controls, calculated by the reviewers, are included in parentheses.
* Statistically different from controls, $p \leq 0.05$.
** Statistically different from controls, $p \leq 0.01$.
ND Not determined
\- Not applicable (animals had already been sacrificed by this time)

G. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** There were no macroscopic findings that could be attributed to treatment. The only macroscopic finding observed was hepatodiaphragmatic nodule in the liver. This lesion was noted in 1-2 rats in all groups, including the controls.

2. **Organ weight:** Increased absolute and relative (to body) liver weights ($p \leq 0.01$) were

observed in the 1000 ppm (↑18-38%) and 10,000 ppm (↑33-62%) groups compared to controls on Days 7 and 28 (Table 5). Absolute and relative thyroid weights were dose-dependently increased at 1000 ppm (↑8-9%) and 10,000 ppm (↑27-33%) on Day 28; these increases were significant ($p \leq 0.05$), except for the relative weight at 1000 ppm. Pituitary weights of the treated groups were comparable to controls at both time points.

Table 5. Selected mean (±SD) absolute (g) and relative to bodyweight (g /100 g BW) organ weights in rats fed NNI-0001 in the diet for up to 28 days [a]

Day	Organ Parameter	Dose (ppm)		
		0	1000	10,000
Liver				
7	Absolute	4.3 ± 0.3	5.2 ± 0.5** (↑21%)	5.9 ± 0.3** (↑37%)
	Relative to body weight	3.9 ± 0.2	4.6 ± 0.2** (↑18%)	5.2 ± 0.1** (↑33%)
28	Absolute	5.2 ± 0.3	7.2 ± 0.5** (↑38%)	8.4 ± 0.6** (↑62%)
	Relative to body weight	3.5 ± 0.1	4.7 ± 0.1** (↑34%)	5.4 ± 0.2** (↑54%)
Thyroid				
7	Absolute	10.2 ± 2.1	9.9 ± 1.3	10.8 ± 1.3
	Relative to body weight	9.1 ± 1.8	8.8 ± 1.4	9.5 ± 1.1
28	Absolute	11.0 ± 0.9	12.0 ± 1.1* (↑9%)	14.6 ± 1.0** (↑33%)
	Relative to body weight	7.3 ± 0.5	7.9 ± 0.6 (↑8%)	9.3 ± 0.6** (↑27%)
Pituitary				
7	Absolute	6.4 ± 0.7	6.1 ± 0.8	7.1 ± 0.9
	Relative to body weight	5.7 ± 0.6	5.5 ± 0.9	6.2 ± 0.8
28	Absolute	9.3 ± 0.8	9.8 ± 0.9	10.1 ± 1.6
	Relative to body weight	6.2 ± 0.5	6.4 ± 0.7	6.4 ± 1.1

a Data were obtained from Tables 6-1 and 6-2 on pages 36-37 of MRID 46817235; n=10, except for the thyroid weight in the 1000 ppm group on Day 28 (n=9). Percent differences from controls, calculated by the reviewers, are included in parentheses.

* Statistically different from controls, $p \leq 0.05$.

** Statistically different from controls, $p \leq 0.01$.

3. **Microscopic pathology:** In the liver (Table 6a), increased incidences of diffuse hepatocyte hypertrophy and periportal vacuolation of hepatocytes were observed at 1000 ppm and 10,000 ppm on Days 7 and 28. The incidence and severity were increased in a time- and dose-dependent manner. In the thyroid (Table 6b), increased incidences of follicular cell hypertrophy were observed at 1000 ppm and 10000 ppm compared to controls on Days 7 and 28. The lesion also increased in severity with both time and dose. There were no significant treatment related effects seen on the pituitary.

Table 6a Selected microscopic findings (#affected/10 animals) in the liver in female rats fed NNI-0001 in the diet for up to 28 days [a]

Finding		Dose (mg/kg/day)		
		0	1000	10,000
Day 7				
Hepatocyte hypertrophy – diffuse,	Total	0	2	10**
	Minimal	0	2	0
	Slight	0	0	1
	Moderate	0	0	9
Vacuolation of hepatocytes – periportal,	Total	0	10**	10**
	Minimal	0	10	6
	Slight	0	0	4
Day 28				
Hepatocyte hypertrophy – diffuse,	Total	0	10**	10**
	Slight	0	2	0
	Moderate	0	8	7
	Severe	0	0	3
Vacuolation of hepatocytes – periportal,	Total	0	10**	10**
	Minimal	0	8	6
	Slight	0	2	4

a Data obtained from Table 11-1 pages 46 of MRID 46817235; n=10.

Table 6b Incidences of follicular cell hypertrophy (#affected/10 animals) in the thyroid in female rats fed NNI-0001 in the diet for up to 28 days [a]

Finding		Dose (mg/kg/day)		
		0	1000	10,000
Day 7				
Follicular cell hypertrophy	Total	0	10**	10**
	Minimal	0	3	0
	Slight	0	7	4
	Moderate	0	0	6
Day 28				
Follicular cell hypertrophy	Total	0	10**	10**
	Slight	0	3	0
	Moderate	0	7	0
	Severe	0	0	10

a Data obtained from Table 11-2 pages 47 of MRID 46817235; n=10.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The study author concluded that the thyroid hormones are rapidly eliminated from blood stream after consecutive administration of NNI-0001. After NNI-0001 treatment, both absolute and relative weight of liver increased significantly by dose and time dependent manner, and the relative thyroid weight increased significantly on Day 28, but pituitary weight remained unchanged. Increased induction of UDP-GT which would lead to increased elimination of T4 from the blood stream and initiate the feedback loop that would increase serum TSH concentration. The TSH increase was sufficient enough to induce thyroid follicular cell hypertrophy and to increase the relative thyroid weight. The induction of UDP-GT activity without concomitant reduction in serum T4 concentration may be explained by the feedback mechanism restoring T4 at a faster rate than it is being conjugated and excreted by UDP-GT. Increased cytochrome (CYP) P-450 protein and EROD activity suggested a selective induction of CYP 1A2. There were no effects on the pituitary gland suggested that such activation remain within mild, physiologically adaptable level under these treatment conditions.

B. **REVIEWER COMMENTS:** The purpose of this non-guideline study was to investigate the mechanism of the effects of oral administration of NNI-0001 on thyroid function in female rats by examining: (i) the levels of thyroid-related hormones in serum; (ii) the histological changes of the thyroid glands and pituitary; and (iii) the induction of liver enzymes which contribute to thyroid hormone metabolism.

The test substance (NNI-0001) was administered in the diet to 10 female Fischer 344 rats/dose group at dietary concentrations of 0, 1000, or 10,000 ppm for 7 or 28 days. There were no mortalities and no treatment-related adverse effects on clinical observations, body weights, food consumption, or gross pathology.

Decreased liver microsomal protein (mg/g liver) was observed at 10,000 ppm on Days 7 and 28. Cytochrome P-450 concentration (ng/mmol protein) was increased at 1000 and 10,000 ppm; these increases grew in magnitude with time from Day 7 to Day 28. UDP-GT activity was increased at 1000 ppm and 10,000 ppm groups. These increases were both time- and dose-dependent and became more pronounced when standardized according to the mass of whole liver (mmol/min/mg whole liver) compared to the mass of protein (mmol/min/mg protein). EROD activity was increased at these doses; but did not increase in magnitude with time. PROD activity was unaffected by treatment.

Increased absolute and relative liver weights were observed at 1000 and 10000 ppm in a time- and dose-dependent relationship. Increased incidences of diffuse hepatocyte hypertrophy were observed at these doses on Days 7 and 28. These findings showed increases in incidence (2-10/10 treated rats vs 0/10 controls) and in severity (from minimal to severe) with both time and dose. Increased incidences of minimal to slight vacuolation of the hepatocytes were seen at 1000 and 10000 ppm (10/10 each treated) compared to controls (0/10).

Increased serum concentrations of T3 and TSH were observed at 1000 and 10000 ppm over controls beginning on Day 7, with TSH concentrations being increased in both a time- and dose-dependent manner. The differences from pre-treatment values showed a similar pattern with T3 and TSH. Beginning on Day 7, the changes from pre-treatment values in T3 were greater at ≥1000 ppm compared to the change in the controls. Changes in TSH were greater at 1000 ppm on Day 28 and at 10,000 ppm on Days 14 and 28. These increases were dose-dependent but not increased with time. T4 was increased at ≥1000 ppm beginning on Day 1. Compared with pre-treatment values, the differences in T4 at 10000 ppm were greater than controls on Days 1 and 7; however, they were comparable to controls thereafter. Although T4 concentrations relative to levels prior to treatment were not increased over controls after Day 7, it is difficult to explain the fact that absolute concentrations of T4 did not decrease with induction of UDP-GT. It is possible that the increased TSH stimulated production of T4 to a concentration that saturated the available UDP-GT.

In the thyroid, absolute and relative thyroid weights were dose-dependently increased at ≥1000 ppm on Day 28. Follicular cell hypertrophy was observed in all rats in the 1000 and 10,000 ppm groups (10/10 each treated) compared to controls (0/10). This finding increased in severity (minimal to severe) with both time and dose. There were no effects of treatment on the pituitary.

Thyroid hormones are degraded primarily by conjugation in the liver. T4 is conjugated on the outer phenolic ring with glucuronic acid in a reaction catalyzed by thyroxine-UDP glucuronyl transferase, and excreted in the bile. Induction of UDP-GT results in increased elimination of T4. The increased elimination of T4 activates the positive feedback loop to stimulate the pituitary to release TSH. TSH acts on the thyroid follicular cells to produce T3/T4; and when this process is prolonged, it results in hypertrophy, hyperplasia, and eventually, tumor formation (See Attachment to this DER). The findings in this study generally support this indirect effect on the thyroid via induction of enzymes in the liver.

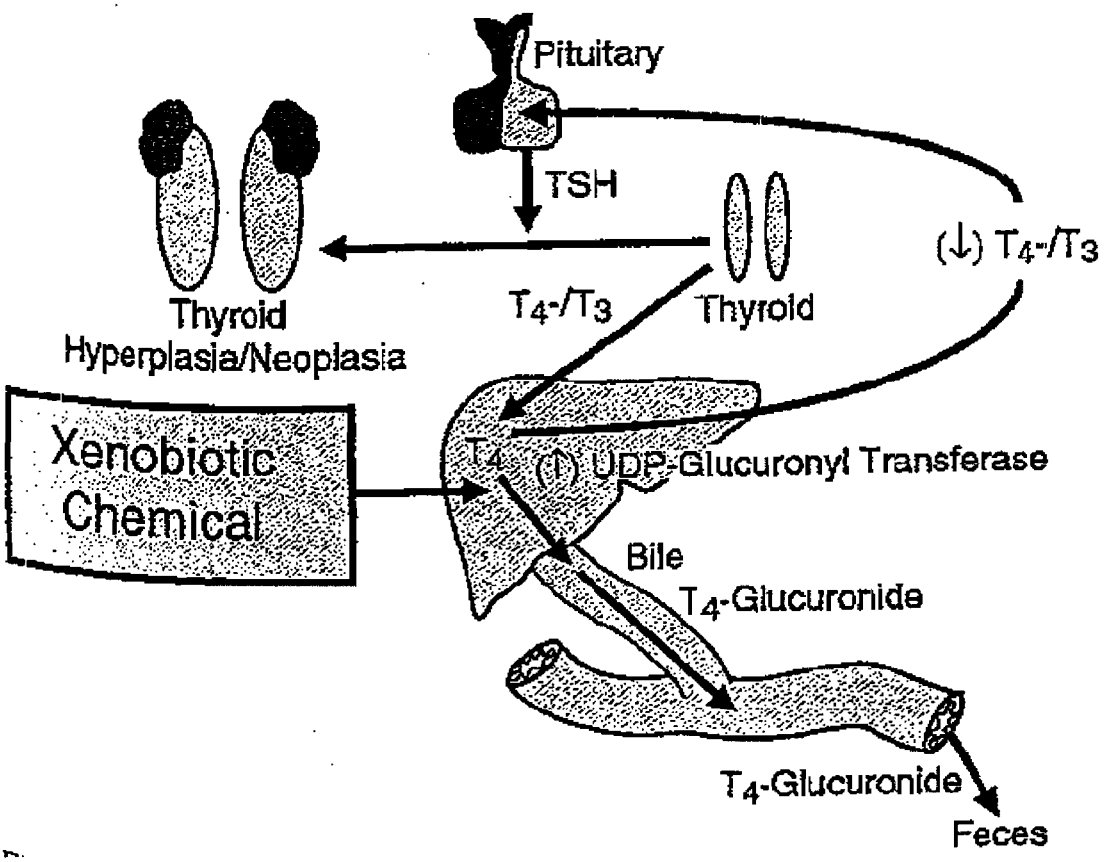
In summary, the findings in this study generally support this indirect effect on the thyroid via induction of enzymes in the liver. Direct effects on the liver included increases in: organ weights; cytochrome P450, UDP-GT and EROD activities; and incidences of hepatocyte hypertrophy and vacuolation. The increased enzymatic activity of the liver resulted in indirect effects on the thyroid, including increases in organ weight; T3, T4, and TSH serum concentrations; and incidences of follicular cell hypertrophy. Although T4 would be expected to decrease with induction of UDP-GT, it is possible that the TSH-mediated increased production of T4 saturated the available UDP-GT.

This study is classified as an **acceptable/non-guideline.** No particular test guideline was directly applicable to the study design.

C. **STUDY DEFICIENCIES:** There were no deficiencies.

ATTACHMENT

Figure 21-22. Hepatic Microsomal enzyme induction by the chronic administration of xenobiotic chemical, leading to thyroid follicular cell hyperplasia and neoplasia



Casarett & Doull's Toxicology: The Basic Science of Poisons, 6th Edition. Curtis D. Klaassen

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

OPPTS 870.4100 [§83-1a]; Chronic Toxicity Study in Rats

Work Assignment No. 4-1-124 H, formerly 3-1-124 H (MRID 46817217)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Rd, Bldg. 100, Ste. B
Durham, NC 27713

Primary Reviewer:
Ronnie J. Bever Jr., Ph.D.
Signature: Ronnie J. Bever Jr.
Date: 1/29/07

Secondary Reviewer:
John W. Allran, M.S.
Signature: John W. Allran
Date: 01-29-07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 1/30/07

Quality Assurance:
Steve Brecher, Ph.D., D.A.B.T.
Signature: Steven Brecher
Date: 1/29/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Marion Copley, DVM, DABT **Signature**: Marion Copley
Science Information Branch, Health Effects Division (7509P) **Date**: 6/26/07
Work Assignment Manager: Myron Ottley **Signature**: MOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date**: 6/2/07

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity in rats; feeding study; OPPTS 870.4100a [§83-1a]; OECD 452.

PC CODE: 027602 **DP BARCODE**: D331553
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (97.8% a.i.)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Enomoto, A. (2004) NNI-0001: Repeated dose 1-year oral toxicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET 01-0079, April 1, 2004. MRID 46817217. Unpublished.

SPONSOR: Nihon Nohyaku Co, Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY - In a chronic toxicity study (MRID 46817217), NNI-0001 (Flubendiamide; 97.8% a.i.; Lot No. 1FH0018P) was administered in the diet to SPF Fischer (F344/DuCrj) rats (25/sex/dose) at doses of 0, 20, 50, 2000, or 20,000 ppm (equivalent to 0, 0.8/1.0, 2.0/2.4, 79.3/97.5, and 822/998 mg/kg bw/day in males/females) for up to 1 year.

No adverse, treatment-related effects were observed on mortality, clinical signs, functional observational battery parameters, motor activity, body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic examinations, urinalysis, or neoplasia.

There was evidence of slight anemia in the females at 2000 and 20,000 ppm and in males at 20,000 ppm. This was only considered adverse in the high dose females. There was a statistical decrease (up to 5 %) throughout the study in hematologic parameters including hematocrit, RBC, hemoglobin, and MCV and a 6 – 38 % increase in reticulocytes (NS) in the 2000 ppm females and 20,000 ppm males. The minimal nature of the response and the increase (although not statistical) in reticulocytes indicates that the rats can compensate for the anemia at these doses. In the high dose females however, these parameters were often decreased between 5 and 10 %. The reticulocytes were decreased (not statistical) at 52 weeks from control values indicating that the rats are no longer able to adequately compensate for the anemia.

19V

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description: White crystals

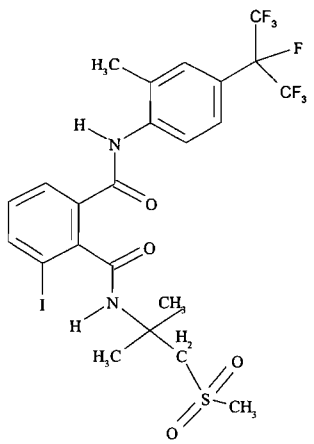
Lot No.: 1FH0018P

Purity (w/w): 97.8% a.i.

Stability of compound: Stability was confirmed in 20 and 20,000 ppm diets stored in a sealed container in the dark at 4°C for 4 weeks and then in ambient air in the animal room for 3 weeks.

CAS #: 272451-65-7

Structure:



2. Vehicle: Diet

3. Test animals

Species: Rat

Strain: SPF Fischer (F344/DuCrj)

Age and mean weight at initiation of treatment: 5 weeks old; 92-104 g males; 79-92 g females

Source: Atsugi Breeding Center, Charles River Japan, Inc. (Atsugi-shi, Kanagawa)

Housing: Housed in groups of 5 by common sex and dose in wire mesh stainless steel cages in movable stainless steel racks

Diet: Certified diet MF Mash (Oriental Yeast Co., Ltd., Itabashi-ku, Tokyo), *ad libitum*

Water: Well water, filtered and sterilized with sodium hypochlorite and ultraviolet light, *ad libitum*

Environmental conditions

- **Temperature:** 24±2°C
- **Humidity:** 38-70%
- **Air changes:** ≥10 air changes/hour
- **Photoperiod:** 12 hours light/12 hours dark

Acclimation period: 9-10 days

B. STUDY DESIGN

1. **In life dates** Start: 08/02/01 End: Approximately 08/10/02

2. **Animal assignment/dose levels:** The animals were randomly assigned to the test groups shown in Table 1. After assignment, it was confirmed that there were no statistically significant differences in the mean body weights among groups, and all individual body weights were within ±20% of the mean value of each sex.

TABLE 1. Study design [a]

Nominal concentration in diet (ppm)	Dose to animal (mg/kg/day; M/F)	Terminal Sacrifice (52 Weeks; # rats/sex)
0	0/0	25
20	0.781/0.960	25
50	1.953/2.403	25
2000	79.3/97.5	25
20,000	822/998	25

a Data were obtained from pages 25, 153, and 154 of MRID 46817217.

3. **Dose-selection rationale:** Dose-selection was based on the results of a concurrently submitted subchronic oral toxicity study (MRID 46817210; IET 01-0013), where NNI-0001 was administered in the diet to same strain and the main study rats (10/sex/dose) at doses of 0, 20, 50, 200, 2000, or 20,000 ppm for 13 weeks. Additionally, a group of controls and 20,000 ppm rats (10/sex/dose) were tested for 13 weeks followed by 4 weeks on the control diet. Slight hepatotoxicity was indicated by increases in the following findings in the 2000 (LOAEL) and 20,000 ppm females: (i) plasma γ-glutamyl transpeptidase; (ii) absolute and relative to body liver weights; (iii) incidence of livers that were dark in color; (iv) incidence of enlarged livers; (v) incidence of slight periportal hepatocyte fatty change; and (vi) incidence of slight diffuse hepatocyte hypertrophy. Additionally, decreased serum triglycerides and serum total cholesterol were noted. These changes in lipid metabolism were considered slight and may not be adverse. Slight hepatotoxicity was also observed in the 20,000 ppm males. Serum total bile acid was decreased in the 20,000 ppm females, and plasma cholinesterase was decreased in the ≥2000 ppm females; however, these differences were considered equivocally adverse. Complete or partial recovery was noted for all treatment-related findings following 4 weeks of control diet.

4. **Dose preparation and analysis:** Dietary formulations were prepared by first making a premix of the appropriate amount of test substance with basal diet for each dose level. The premixes were then diluted with diet to the desired concentration. No adjustment for purity was made. Dietary formulations were sealed in plastic bags and stored in aluminum containers in the dark at 4°C. Dietary formulations were prepared once prior to initiation of treatment and once every 4 weeks during the treatment period. Concentrations at each dietary level were measured in the first dietary formulations, in preparations at approximately 3, 6, and 9 months, and in the final preparations. Homogeneity (top, middle, bottom) of the test compound in each dietary formulation was tested in the first and last preparations and at

approximately 6 months. In a previously conducted 28-day dietary study (data not available), stability of the compound in the diet in a sealed container was tested in 20 and 20,000 ppm diets following storage in the dark at 4°C for 5 weeks, followed by storage in the dark at room temperature for 5 days, and then ambient air in the animal room for 8 days. Stability was further tested in a concurrently submitted 2-generation reproductive toxicity study (MRID 46817216) using 20 and 20,000 ppm diets stored in a sealed container in the dark at 4°C for 4 weeks and then the ambient air in the animal room for 3 weeks.

Results

Homogeneity analysis (% CV): 0.5-7.8%

Stability analysis (% of initial concentration): 96-105%

Concentration analysis (% of nominal concentration after preparation): 94-103%

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

5. **Statistics:** Significance was indicated at 5 and 1% probability.

PARAMETER	ANALYSIS CONDUCTED
Motor activity Grip strengths Body weight Food consumption Urine specific gravity Urine volume Hematological parameters (except for bone marrow cytology) Clinical chemistry Brain cholinesterase activity Organ weights	Bartlett's test was conducted. One-way analysis of variance (ANOVA) and Dunnett's test were performed when group variances were homogeneous. The Kruskal-Wallis test and a Dunnett-type mean rank sum test were conducted when group variances were heterogeneous.
Clinical observations (detailed) Sensorimotor responses Urinalysis (except for specific gravity and volume)	Kruskal-Wallis test and a Dunnett-type mean rank sum test were performed.
Bone marrow cytology	Data from the 0 and 20,000 ppm groups were analyzed using Mann-Whitney's U-test. Data from the 0, 2000, and 20,000 groups were evaluated by the Kruskal-Wallis test and a Dunnett-type mean rank sum test.
Mortality Clinical observations (general) Ophthalmology Gross pathology Histopathology	Fisher's Exact test (one-tail analysis)

These analyses were considered appropriate.

C. METHODS

1. Observations

1a. Cageside observations: Animals were inspected at least twice daily for moribundity and mortality, except on weekends and holidays when animals were observed at least once daily. Cage-side observations for general condition were performed at least once daily.

1b. Clinical examinations: Clinical examinations, including palpation of masses, were conducted at least weekly.

1c. Neurological evaluations: A limited functional observational battery (FOB) and locomotor activity tests were performed as detailed below.

(i) Functional observational battery (FOB): Detailed clinical observations (a limited functional observational battery) were performed once prior to treatment and once weekly during treatment on all animals. Environmental conditions, duration of observation in the open field, details about the observer, and positive control data were not reported. Animals were observed for the following signs, and findings were recorded using a standard scoring system reported on pages 71-72 of the study report (see attachment):

Home cage	Handling	Open field
Excitement	Handling difficulty	Jumping
Sedation	Changes in muscle tone	Circling
Abnormal posture	Tremors	Convulsions
Abnormal behavior	Palpebral closure	Abnormal gait
	Changes in pupil size	Spontaneous motor activity
	Salivation	Grooming
	Lacrimation	Rearing
	Discharges	Respiration
	Exophthalmos	Vocalization
	Changes in body temperature	Piloerection
	Abnormal respiratory sound	Urination
	Changes in fur	Defecation
	Changes in skin and mucous membranes	Abnormal posture
		Abnormal behavior

Additionally, 10 rats/sex/dose group were evaluated during Week 49 with the following tests: grip strength (forelimb and hindlimb), approach response, auditory response, touch response, tail pinch response, and aerial righting reflex. Forelimb grip strength was also determined in 10 females/dose group at 17 weeks of treatment to confirm findings in a previous 90 day study. Forelimb and hindlimb grip strengths were determined using a strain gauge, CPU gauge Model-9505 (Aiko Engineering Co., Ltd. Nagoya-shi, Aichi). Sensorimotor responses were assessed by scoring reactions to the approach of a rod, sound of a clicker, touch on the rump, and tail pinch using forceps. The animals were then held supine and dropped from approximately 20 cm above the floor, and coordination scored. Order of animals for each test was counterbalanced across groups.

(ii) **Locomotor activity:** Locomotor activity was monitored in 10 rats/sex/dose group during Week 49 by an automated activity recording system (SUPER MEX®, Muromachi Kikai Co., Ltd., Chuo-ku, Tokyo) for one hour in 10 minute intervals. Order of animals for each test was counterbalanced across groups. Motor activity was assessed at approximately the same time as the sensorimotor tests.

2. **Body weight and body weight gain:** All animals were weighed prior to treatment, on Day 1, once weekly for the first 13 weeks, once every 4 weeks from Week 16, and at termination. Body weight gains were not reported.

3. **Food consumption, food efficiency, and compound intake:** Food consumption was calculated as a mean value (g food/rat/day) for each cage. Mean food consumption was determined for a period of 4 consecutive days weekly during the first 13 weeks, and every 4 weeks from Week 16 until termination. A weighed average of the group mean food consumption during the treatment period was also calculated for each sex. Food efficiency was calculated for each group during the first 13 weeks of treatment as a ratio (%) of group mean body weight gain to group mean food consumption at the week concerned. An average of the group mean food efficiency during the 13 weeks of treatment was also calculated for each sex. Compound intake (mg/kg bw/day) values were calculated as group mean food consumption x nominal concentration/ group mean body weight. A weighed average of the group mean test substance intake during the treatment period was also calculated for each sex.

4. **Ophthalmoscopic examination:** All animals during the acclimatization period and all animals in the 0 and 20,000 ppm groups at 52 weeks were subjected to ophthalmoscopic examinations.

5. **Hematology and clinical chemistry:** Blood was collected from 10 rats/sex/dose group after 13, 26, and 52 weeks of treatment. Animals were fasted overnight. Blood samples were withdrawn (under ether anesthesia) from the cephalic vein after 13 and 26 weeks of treatment and from the posterior vena cava after 52 weeks. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Femur bone marrow smears [a]
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.

a Bone marrow cytology was determined after 52 weeks of treatment in the control and 20,000 ppm groups (both sexes) and in the 2000 ppm females.

b. Clinical chemistry

	ELECTROLYTES
X	Calcium
X	Chloride
	Magnesium
X	Phosphorus
X	Potassium*
X	Sodium*
	ENZYMES (more than 2 hepatic enzymes eg., *)
X	Alkaline phosphatase (ALK)*
	Cholinesterase (ChE) (see OTHER)
	Creatine phosphokinase
	Lactic acid dehydrogenase (LDH)
X	Alanine amino-transferase (ALT/ SGPT)*
X	Aspartate amino-transferase (AST/ SGOT)*
X	Gamma-glutamyl transferase (GGT)*
	Sorbitol dehydrogenase*
	Glutamate dehydrogenase

	OTHER
X	Albumin*
X	Creatinine*
X	Urea nitrogen*
X	Total cholesterol*
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total protein (TP)*
X	Triglycerides
	Serum protein electrophoresis
X	Total bile acid
X	Albumin:globulin
X	Plasma cholinesterase (only after 52 weeks)
X	Brain cholinesterase (only after 52 weeks)
X	Erythrocyte cholinesterase (only after 52 weeks)

* Recommended for chronic studies based on Guideline 870.4100.

For acetylcholinesterase analyses, erythrocytes were separated from heparinized blood samples, and 20% (v/v) suspensions were prepared. Erythrocyte cholinesterase activity was determined using an AutoAnalyzer II (Bayer Corp.) under the conditions with or without eserine (cholinesterase inhibitor). The DTNB method with acetylthiocholine iodide as a substrate was used for the analysis. Animals (10 rats/sex/dose group) not subjected to hematological and blood biochemical examinations were assayed for brain cholinesterase activity using an automated biochemical analyzer (JCA-BM1250) on 20% (w/v) brain homogenate by DTNB method with acetylthiocholine iodide as a substrate.

6. **Urinalysis:** Samples were collected from 10 rats/sex/dose group after 13, 26, and 51 weeks of treatment. The CHECKED (X) parameters were examined.

X	Appearance* [b]	X	Glucose* [a]
X	Volume* [b]	X	Ketones [a]
X	Specific gravity / osmolality* [a]	X	Bilirubin [a]
X	pH* [a]	X	Blood/blood cells* [a]
X	Sediment (microscopic) [b]		Nitrate
X	Protein* [a]	X	Urobilinogen [a]

* Recommended for chronic studies based on Guideline 870.4100.

a Analysis was performed on fresh urine samples.

b Analysis was performed on urine samples collected overnight in individual metabolic cages.

7. **Sacrifice and pathology**: After 52 weeks of treatment, animals were fasted overnight, and killed by exsanguination under deep ether anesthesia. Where possible, all animals were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder* (not rat)
	Bile duct (rat)
X	Pancreas*

	RESPIRATORY
X	Trachea*
X	Lung*++
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta, thoracic*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
X	Thymus

	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicle*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland*
X	Coagulating glands
X	Vagina

	NEUROLOGIC
XX	Brain (multiple sections)*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (retina, optic nerve)*

	GLANDULAR
XX	Adrenal gland*+
	Lacrimal gland
X	Parathyroids*
XX	Thyroids*

	OTHER
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin*
X	Harderian gland
X	Knee joint
X	All gross lesions and masses*

* Required for chronic studies based on Guideline 870.4100.
+Organ weight required in chronic studies.
++Organ weight required if inhalation route.

The collected tissues were fixed in 10% neutral-buffered formalin. The lungs were instilled with formalin before fixation. The fixed tissues were embedded in paraffin, stained with hematoxylin and eosin, routinely prepared, and examined microscopically as follows. Histopathological examinations were performed on all tissues collected from the 0 and 20,000 ppm groups; all tissues from all dose groups in animals found dead; and the liver, kidneys, thyroids, and all gross lesions from all animals. Microscopic lesions were graded as slight, moderate, or severe.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** No treatment-related effect was observed on mortality. Animals found dead included one control male (week 28), one 50 ppm male (week 44), and one 2000 ppm female (week 43). No animals were killed *in extremis*.

2. **Clinical signs of toxicity:** No treatment-related clinical signs were observed during general cage-side observations or clinical examinations.

3. **Neurological evaluations:** In the open field, increased ($p \leq 0.05$) incidences of occasional rearing were observed in the 20,000 ppm females at Weeks 6, 8-10, 15, and 52 (6-9/25 treated vs 0-1/25 controls). This finding was not considered adverse because the response was slight and transient without any further evidence to support a neurological effect. Increased ($p \leq 0.05$) incidences of occasional rearing in other dose groups occurred in only 1-2 weeks and were unrelated to dose. No treatment-related effects were noted in any other FOB parameter, sensorimotor test, or during motor activity evaluations. Habituation was unaffected by treatment.

B. BODY WEIGHT AND WEIGHT GAIN:

No treatment-related effect was observed on body weight or body weight gain (Table 2). Minor increases ($p \leq 0.05$) of 3% were noted in the body weights of ≥2000 ppm females during Week 2.

TABLE 2. Mean (±SD) body weights and body weight gains (g) at selected intervals in rats treated with NNI-0001 in the diet for up to 1 year [a]

Week	Dose (ppm)				
	0	20	50	2000	20,000
Males (n=24-25)					
0	97±3	97±3	97±3	97±3	97±3
13	319±21	325±19	317±16	320±13	322±13
52	422±26	434±33	428±29	434±19	439±21
BWG (0-13)	222	228	220	223	225
BWG (13-52)	103	109	111	114	117
BWG (0-52)	325	337	331	337	342
Females (n=24-25)					
0	86±3	86±3	86±3	86±3	86±3
13	178±7	180±7	182±9	181±7	182±9
52	220±15	223±11	225±14	220±11	219±10
BWG (0-13)	92	94	96	95	96
BWG (13-52)	42	43	43	39	37
BWG (0-52)	134	137	139	134	133

a Data were obtained from Tables 9-1 through 10-2 on pages 145-148 of MRID 46817217. No statistical difference in body weight was found between the treated groups and controls at Weeks 0, 13, or 52. Body weight gains were calculated by the reviewers.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** No adverse, treatment-related effect was observed on food consumption. At 20,000 ppm, minor increases ($p \leq 0.05$) in food consumption were noted in males (↑5-11%) at Weeks 3, 6-7, 9, 12, 20-24, and 32-52; and in females (↑5-9%) at Weeks 2-6, 8-9, 11-13, 24, and 40. These increases contributed to an increase in average overall (Weeks 0-52) food consumption of only 7% in males and 4% in females. Incidental minor increases ($p \leq 0.05$) in food consumption were also noted in the 2000 ppm males at Weeks 3 and 52 (↑5-7%). The minor effects observed on food consumption did not lead to differences in body weights or food efficiency and were not considered adverse.

2. **Compound consumption (time-weighted average):** Compound intake is reported in Table 1.
3. **Food efficiency:** No treatment-related effect was noted on food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related finding was observed during ophthalmoscopic examination.

E. BLOOD ANALYSES

1. **Hematology:** As can be seen in Table 3, no adverse treatment-related hematological effect was observed in the males at any dose or in females at 2000 ppm and below. However, in females at 20,000 ppm there was evidence that the anemia was adverse. Evidence suggested a slight condition of microcytic anemia and reactive hematopoiesis in the 20,000 ppm males and 2000 ppm females, as indicated by slight decreases (↓2-6.9%, generally throughout the study; $p \leq 0.05$) in erythrocytes, hematocrit, hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin and slight increases ($p \leq 0.05$) in reticulocytes (↑15% in males at Week 52 and transient ↑64% in females [both groups] at Week 13). However, females at 20,000 ppm had decreases ranging mostly between 5 and 10 % throughout the study. Reticulocytes, although increased non-statistically between 6 and 13 % at 13 and 26 week were decreased non-statistically at 52 weeks by about 12 %. This may indicate that the rats can no longer adequately compensate for the anemia by regenerating new RBC.

   Minor increases ($p \leq 0.01$) in platelet count were noted at Weeks 13, 26, and 52 in the 20,000 ppm males (↑8-10%). There was a decrease ($p \leq 0.05$) in segmented neutrophils at Weeks 13 and 26 in the 20,000 ppm females (↓33-50%), but it was similar to controls at Week 52. Decreased ($p \leq 0.05$) eosinophils (↓25%) were observed at Week 52 in the 20,000 ppm males, but this effect was minor. Minor decreases ($p \leq 0.01$) in basophils were noted in the 2000 and 20,000 ppm females at Week 52 (0.00 ± 0.01 x10^3/µL treated vs 0.01 ± 0.01 x10^3/µL controls). Due to the magnitude of these changes, the findings were considered not adverse. All other differences ($p \leq 0.05$) were minor, transient, and/or unrelated to dose.

TABLE 3. Mean ±SD values (% from controls) for selected hematology findings in male and female rats treated with NNI-0001 in the diet for up to 1 year[a]

Parameter	Dose (ppm)				
	0	20	50	2000	20,000
MALES					
Hematocrit (%)					
Week 13	42.3±1.2	41.5±0.7	41.7±1.2	41.2±1.0	39.8**±1.3 (↓5.9)[b]
Week 26	42.7±1.3	42.2±1.3	42.3±1.5	41.2±1.5	39.7**±1.0 (↓7.0)
Week 52	45.1±1.1	44.9±0.8	45.2±1.2	44.7±0.5	44.0±0.5 (↓2.4)
Hemoglobin (g/dl)					
Week 13	15.4±0.4	15.1±0.2	15.2±0.4	15.0*±0.4 (↓2.6)	14.6**±0.3 (↓5.1)
Week 26	15.0±0.4	14.8±0.3	15.0±0.4	14.7±0.3	14.3**±0.3 (↓4.7)
Week 52	15.4±0.3	15.3±0.3	15.5±0.4	15.2±0.2	14.9*±0.2 (↓3.3)
RBC (10^6/mm^3)					
Week 13	8.76±0.22	8.57±0.14	8.55±0.25	8.53±0.23	8.39**±0.24 (↓4.2)
Week 26	8.74±0.28	8.63±0.25	8.61±0.26	8.56±0.24	8.38**±0.19 (↓4.1)
Week 52	9.42±0.24	9.36±0.15	9.47±0.26	9.38±0.18	9.37±0.14 (↓5.3)
MCV (fl)					
Week 13	48.3±0.7	48.4±0.2	48.7±0.3	48.3±0.5	47.4*±0.3 (↓1.9)
Week 26	48.8±0.8	48.9±0.4	49.1±0.8	48.1±0.4	47.3±0.6 (↓3.1)
Week 52	47.9±0.3	48.0±0.7	47.7±0.9	47.7±0.6	46.9**±0.5 (↓2.1)
Reticulocytes					
Week 13 (/10^3 RBC)	13±5	16±5	15±5	17±5	18±5 (↑38)
Week 26 (/10^3 RBC)	14±3	14±3	14±5	18±6	18±4 ↑ (29)
Week 52 (/10^9 l)	153.6±10.6	159.2±9.5	158.1±13.3	166.5*±8.2 (↓8.4)	176.7**±13.1(↑15)
FEMALES					
Hematocrit (%)					
Week 13	40.1±0.9	40.0±1.9	38.7±0.7	37.5**±1.3 (↓6.5)	36.4**±0.9 (↓9.0)
Week 26	41.2±1.0	40.2±1.0	40.5±1.5	39.0**±1.3 (↓5.3)	37.6**±1.3 (↓8.7)
Week 52	44.0±0.8	43.3±0.9	42.6**±1.3 (3.2)	41.0**±0.6 (↓6.8)	40.2**±0.5 (↓8.6)
Hemoglobin (g/dl)					
Week 13	14.9±0.3	14.8±0.5	14.5±0.3	14.4*±0.5 (↓3.3)	13.8**±0.4 (↓7.4)
Week 26	14.9±0.2	14.4±0.3	14.6±0.6	14.3*±0.4 (↓4.0)	13.9**±0.2 (↓6.7)
Week 52	15.9±0.3	15.6±0.5	15.3±0.5	14.8**±0.2 (↓6.9)	14.4**±0.1 (↓9.4)
RBC (10^6/mm^3)					
Week 13	7.79±0.18	7.84±0.35	7.61±0.16	7.60±0.25	7.53±0.20 (↓3.3)
Week 26	7.92±0.22	7.69±0.16	7.85±0.27	7.73±0.23	7.68±0.23 (↓3.0)
Week 52	8.56±0.15	8.44±0.16	8.4±0.22	8.29**±0.08(↓3.2)	8.32**±0.13 (↓2.8)
MCV (fl)					
Week 13	51.5±0.5	51.2±0.4	50.9**±0.3 (↓1.2)	49.3**±0.4 (↓4.3)	48.4**±0.2 (↓6.0)
Week 26	52.1±0.4	52.3±0.6	51.6±0.5	50.4**±0.6 (↓3.3)	49.0**±0.4 ↓ (6.3)
Week 52	51.4±0.5	51.4±0.4	50.6**±0.3 (↓1.6)	49.4**±0.5 (↓3.9)	48.3**±0.5 (↓6.0)
Reticulocytes					
Week 13 (/10^3 RBC)	11±5	11±3	15±4	18±5 (↑6.3)	18**±4 (↑6.3)
Week 26 (/10^3 RBC)	10±3	9±3	11±4	12±2	13±3 (↑30)
Week 52 (/10^9 l)	143.0±9.6	133.3±25.4(↓7.0)	145.6±19.7(↑1.8)	132.7±28.2(↓7.0)	124.9±18.2(↓12.6)

a Data (n=10) were obtained from Tables 21-1, 21-3, 21-5, 22-1, 22-3, 22-5, on pages 173, 175, 177, 181, 183, 185 of MRID 46817217.

b % from control calculated by reviewer.

* Statistically different ($p \leq 0.05$) from the control.

** Statistically different ($p \leq 0.01$) from the control.

204

2. **Clinical Chemistry:** In the females, increased ($p \leq 0.05$) plasma γ-glutamyl transpeptidase was noted at 2000 and 20,000 ppm during Weeks 13 (2 U/L each treated group vs 0 U/L controls), 26 (2-3 U/L vs 1 U/L), and 52 (4 U/L vs 2 U/L; Table 4). At 20,000 ppm, decreased ($p \leq 0.01$) total serum cholesterol (↓20-23%) and triglycerides (↓51-63%) were observed throughout the study. At 2000 ppm, decreased ($p \leq 0.05$) triglycerides were noted during Weeks 13 and 52 (↓40-46%), and decreased ($p \leq 0.01$) total cholesterol was observed at Week 26 (↓14%). Plasma cholinesterase was decreased ($p \leq 0.01$) at ≥2000 ppm throughout the study (↓18-34%); however, the toxicological significance of this finding is unclear. Erythrocyte cholinesterase was slightly increased ($p \leq 0.01$) by 14-16% at Week 52 at ≥2000 ppm; however, brain cholinesterase levels were unchanged. The Sponsor postulated that this discrepancy may be due to the increased surface area per volume of the smaller erythrocytes in these animals. Serum total bile acid was decreased ($p \leq 0.05$) by 40-53% at ≥2000 ppm. A decrease in total bile acid is usually considered to be an indicator of malabsorption from the intestine. However, food consumption and body weights did not support a diagnosis of malabsorption. Thus, the effect on bile acid was considered equivocal.

   Other differences ($p \leq 0.05$) were observed, but were not considered adverse, treatment-related findings due to the small magnitude of change from controls, the effect was unrelated to dose, transient, and/or no known toxicological significance associated with the change. These differences ($p \leq 0.05$) included increases in parameters such as: (i) plasma γ-glutamyl transpeptidase in the ≥50 ppm males; (ii) albumin in the ≥50 ppm males and ≥2000 ppm females; (iii) total protein in the 20,000 ppm males and the ≥2000 ppm females; (iv) inorganic phosphorus in the ≥2000 ppm females; (v) potassium in the 20,000 ppm females; and (vi) total cholesterol in the ≥50 ppm males.

TABLE 4. Mean ±SD (% from controls) values for selected clinical chemistry findings in female rats treated with NNI-0001 in the diet for up to 1 year[a]

Parameter	Dose (ppm)				
	0	20	50	2000	20,000
γ-Glutamyl transpeptidase (U/L)					
Week 13	0±0	1±0	1±0	2±1**	2±0**
Week 26	1±0	1±1	2±1	2±1** (↑100)[b]	3±1** (↑200)
Week 52	2±1	3±1	3±1	4±1* (↑100)	4±1** (↑100)
Total cholesterol (mg/dL)					
Week 13	67±5	70±6	67±5	61±6	52±6** (↓22)
Week 26	83±7	81±7	85±6	71±7** (↓14)	64±7** (↓23)
Week 52	92±8	100±9	107±10** (↑16)	88±7	74±7** (↓20)
Triglycerides (mg/dL)					
Week 13	30±8	26±13	18±5* (↓40)	18±8* (↓40)	14±7** (↓53)
Week 26	39±14	30±13	28±11	25±10	19±12** (↓51)
Week 52	41±13	39±14	38±11	22±4* (↓46)	15±4** (↓63)
Plasma cholinesterase (U/L)					
Week 13	752±43	815±52	754±68	599±62** (↓20)	506±52** (↓33)
Week 26	841±39	867±39	861±54	693±56** (↓18)	594±44** (↓29)
Week 52	847±70	848±66	827±38	645±54** (↓24)	561±51** (↓34)
Erythrocyte cholinesterase U/L)					
Week 52	0.58±0.04	0.57±0.04	0.58±0.07	0.67±0.03** (↑16)	0.66±0.03** (↑14)
Brain cholinesterase (U/L)					
Week 52	39±1	37±2	37±2	39±2	38±2

a Data (n=10) were obtained from Text-Table 4 on pages 45-46 and Tables 24-1 through 24-6 and Table 26 on pages 196-201 and 203 of MRID 46817217.
b % from control calculated by reviewer.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

F. URINALYSIS: No treatment-related effect was observed during urinalysis.

G. SACRIFICE AND PATHOLOGY

1. **Organ weights:** Absolute and relative to body liver weights were increased ($p \leq 0.01$) in the 20,000 ppm males by 16-18% and in the ≥2000 ppm females by 23-41% (Table 5). Absolute and relative thyroid weights were increased ($p \leq 0.05$) in the 20,000 ppm males by 23-26%. Other differences ($p \leq 0.05$) noted in females (generally at ≥2000 ppm) were not considered adverse because an effect was not corroborated by gross or microscopic pathology, or the weight differences from controls were minor. These differences included increased heart, kidney, adrenal, and ovary weights, and decreased spleen weights.

TABLE 5. Mean ±SD (% from controls) liver and thyroid weights in rats treated with NNI-0001 in the diet for up to 1 year [a]

Parameter	Dose (ppm)				
	0	20	50	2000	20,000
Males					
Liver: Absolute (g)	8.53±0.72	8.82±0.79	8.48±0.95	9.03±0.68	10.05±0.87** ↑18)[b]
Relative to body (%)	2.04±0.06	2.05±0.09	2.04±0.09	2.17±0.10** (↑6)	2.36±0.11** (↑16)
Thyroid: Absolute (mg)	18.2±3.1	19.1±3.4	19.8±3.0	20.2±2.7	22.9±2.2** (↑26)
Relative to body (%)	0.0044 ±0.0008	0.0045 ±0.0008	0.0048 ±0.0009	0.0049 ±0.0006	0.0054* (↑23) ±0.0006
Females					
Liver: Absolute (g)	4.25±0.37	4.27±0.27	4.53±0.40	5.23±0.43** (↑23)	5.93±0.36** (↑40)
Relative to body (%)	1.98±0.14	1.99±0.12	2.11±0.10	2.50±0.13** (↑26)	2.80±0.15** (↑41)

a Data (n=10) were obtained from Text-Table 6 on page 49 and Tables 27-1 through Table 28-2 on pages 204-207 of MRID 46817217.
b % from control calculated by reviewer.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

2. **Gross pathology:** In ≥2000 ppm females, the liver was dark in color and/or enlarged in 13-24/25 rats (vs 0/25 controls; $p \leq 0.01$; Table 6). Male livers were not similarly affected. The incidences of other lesions were not increased in the treated groups.

TABLE 6. Selected gross liver lesions (# affected/25 [%]) in female rats treated with NNI-0001 in the diet for up to 1 year [a]

Parameter	Dose (ppm)				
	0	20	50	2000	20,000
Dark in color	0 (0)	0 (0)	0 (0)	19** (76)	24** (96)
Enlargement	0 (0)	0 (0)	0 (0)	13** (52)	24** (96)

a Data were obtained from Tables 30-3 on page 213 of MRID 46817217.
** Statistically different ($p \leq 0.01$) from the control.

3. **Microscopic pathology:** Selected non-neoplastic lesions are detailed in Table 7; no treatment-related effect on neoplasia was observed. Increased ($p \leq 0.01$) incidences of slight to severe periportal hepatocyte fatty change and slight to moderate diffuse hepatocyte hypertrophy were noted in the ≥2000 ppm females (92-100% treated vs 0% controls). In the 20,000 ppm males, a slight increase (NS) was observed in slight to severe bile duct hyperplasia in the liver (44% treated vs 32% controls), but severity was unrelated to dose and this finding was considered incidental. A minor increase in incidence of slight to moderate chronic nephropathy (32% treated vs 12% controls) was noted in the 20,000 ppm males. There was no further evidence to support nephrotoxicity, and the slight increase in the common finding of chronic nephropathy was considered not adverse. The incidences of thyroid follicular cell hypertrophy were increased ($p \leq 0.01$) in the ≥2000 ppm males (64-100% treated vs 0% controls) and females (96-100% treated vs 0% controls), although this may be considered a secondary effect resulting from increased liver metabolism, it is still considered adverse. The incidences of other lesions in treated groups were similar to controls.

TABLE 7. Incidence (# affected/25 [%]) of microscopic lesions in selected organs of rats treated NNI-0001 in the diet for up to 1 year[a]

Parameter	Dose (ppm)				
	0	20	50	2000	20,000
Males					
Liver					
Hyperplasia, bile duct	8 (32)	8 (32)	8 (32)	8 (32)	11 (44)
Slight	6	3	6	8	8
Moderate	2	3	1	0	2
Severe	0	2	1	0	1
Kidney					
Chronic nephropathy	3 (12)	2 (8)	4 (16)	3 (12)	8 (32)
Slight	3	2	3	3	6
Moderate	0	0	1	0	2
Thyroid follicular cell hypertrophy (slight)	0	0	0	16** (64)	25** (100)
Females					
Liver					
Fatty change, hepatocyte, periportal	0 (0)	0 (0)	0 (0)	23** (92)	23** (92)
Slight	0	0	0	16	15
Moderate	0	0	0	7	7
Severe	0	0	0		1
Hypertrophy, hepatocyte, diffuse	0 (0)	0 (0)	0 (0)	23** (92)	25** (100)
Slight	0	0	0	15	4
Moderate	0	0	0	8	21
Thyroid follicular cell hypertrophy (slight)	0	0	0	24 (96)	25 (100)

a Data were obtained from Tables 31-6 through Tables 31-8 on pages 219-220 and Tables 32-6 through 32-8 on pages 227-229 of MRID 46817217. Severity data were tabulated by reviewers from individual data.

** Statistically different ($p \leq 0.01$) from the control.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: The LOAEL was 2000 ppm, based on differences detected in hematology, blood biochemistry, and pathology including organ weights in both sexes (detailed in reviewers' comments). The test substance was not carcinogenic in the rat.

B. REVIEWER COMMENTS: No adverse, treatment-related effects were observed on mortality, clinical signs, functional observational battery parameters, motor activity, body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic examinations, hematology, urinalysis, or neoplasia.

In the females, slight hepatotoxicity was indicated by the following findings at 2000 and 20,000 ppm (except as noted). In the females, increased ($p \leq 0.05$) plasma γ-glutamyl transpeptidase was noted at 2000 and 20,000 ppm during Weeks 13 (2 U/L each treated group vs 0 U/L controls), 26 (2-3 U/L vs 1 U/L), and 52 (4 U/L vs 2 U/L). Decreased ($p \leq 0.01$) total serum cholesterol (↓20-23%) and triglycerides (↓51-63%) were observed throughout the study (at 20,000 ppm only). At 2000 ppm, decreased ($p \leq 0.05$) triglycerides were noted during Weeks 13 and 52 (↓40-46%), and decreased ($p \leq 0.01$) total cholesterol was observed at Week 26 (↓14%). These changes in lipid metabolism were considered slight and may not be adverse. Absolute and relative liver weights were increased by 23-41%. These differences may be due to hepatic enzyme induction (demonstrated in the concurrently submitted special study, MRID 46817235). Increased incidences of liver that was dark in

color and/or enlarged were noted grossly (52-96% treated vs 0% controls). Increased ($p \leq 0.01$) incidences of slight to severe periportal hepatocyte fatty change and slight to moderate diffuse hepatocyte hypertrophy were also observed (92-100% treated vs 0% controls).

Indications of hepatotoxicity in the 20,000 ppm males were not considered adverse. Absolute and relative liver weights were increased ($p \leq 0.01$) in males by 16-18%, but this effect was considered to be due to enzyme induction. A slight increase (NS) was observed in slight to severe bile duct hyperplasia in the liver (44% treated vs 32% controls). Severity was unrelated to dose. No further evidence was suggestive of hepatotoxicity in the males.

Plasma cholinesterase was decreased ($p \leq 0.01$) in the ≥2000 ppm females throughout the study (↓18-34%); however, the toxicological significance of this finding is unclear. Erythrocyte cholinesterase was slightly increased ($p \leq 0.01$) by 14-16% at Week 52 in the ≥2000 ppm females; however, brain cholinesterase levels were unchanged. The Sponsor postulated that this discrepancy may be due to the increased surface area per volume of the smaller erythrocytes in the ≥2000 ppm females. The reviewers conclude that an adverse effect on cholinesterase was not observed in this study.

Absolute and relative thyroid weights were increased ($p \leq 0.05$) in the 20,000 ppm males by 23-26%. The incidences of slight thyroid follicular cell hypertrophy were increased ($p \leq 0.01$) in the ≥2000 ppm males (64-100% treated vs 0% controls) and females (96-100% treated vs 0% controls). Although these changes may be due to increased liver metabolism, they are still considered adverse.

The reviewer agrees with the Sponsor that the effects on hematological parameters are adverse in the female high dose animals (treatment-related effects occurred on hematological parameters, including hematocrit, hemoglobin concentration, erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin, reticulocyte count, segmented neutrophils, and erythroblast count, as well as spleen weight). The changes in the 2000 ppm females and 20,000 ppm males are considered to be adaptive, indicating that at these doses the rats can handle the insult to their hematologic system. However, the changes in spleen weight are not considered to be treatment related. The Sponsor also indicated that they considered serum albumin, total protein, inorganic phosphorus, and potassium to be adversely affected, but the reviewers also considered these to be minor changes.

The LOAEL is 2000 ppm (equivalent to 97.5 mg/kg bw/day in females), based on indications of slight hepatotoxicity in females. The NOAEL is 50 ppm (equivalent to 2.4 mg/kg bw/day in females).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements for a chronic oral study [OPPTS 870.4100, OECD 452] in rats.

C. **STUDY DEFICIENCIES:** The starting weight for each dose group of a common sex was reported as exactly the same, however, this does not affect the conclusions of this review.

ATTACHMENT

The following are pages 71 and 72 of the study report

(IE I 01-0079)

Key to Tables 5 and 6
Standard key to scored clinical signs

Home cage:

Excitement
0. Negative
1. Slight
2. Moderate
3. Marked (may accompany vocalization)

Sedation
0. Negative
1. Slight
2. Moderate
3. Marked

Abnormal posture
0. Negative
1 Positive
(with description such as flattened, lying on side, etc.)

Abnormal behavior
0. Negative
1. Positive
(with description such as backward moving, stereotypies, self-destructive biting, etc.)

Handling:

Handling difficulty
0. No difficulties
1. Slight
2. Moderate
3. Marked (difficult to handle)

Changes in muscle tone
-3 Markedly decreased muscle tone
-2. Moderately decreased muscle tone
-1 Slightly decreased muscle tone
0. Negative (normal)
1. Slightly increased muscle tone
2. Moderately increased muscle tone
3. Markedly increased muscle tone

Tremors
0 Negative
1. Slight
2. Moderate
3 Marked

Palpebral closure
0. Negative (eyelids wide open)
1. Slight (eyelids closed less than half)
2. Moderate (eyelids closed half or more)
3. Marked (eyelids closed completely)
(with description of side)

Changes in pupil size
-1 Contracted
0. Normal
1 Dilated

Salivation
0. Negative
1. Slight (wetted fur on perioral region)
2. Moderate (wetted fur up to submandibular region)
3. Marked (wetted fur beyond submandibular region)

Lacrimation
0 Negative
1. Slightly excessive
2. Moderately excessive
3. Markedly excessive

Discharges
0 Negative
1. Positive
(with description of type, i.e. nasal, auricular, and vaginal, color, side, etc.)

Exophthalmos
0 Negative
1 Positive
(with description of side)

Changes in body temperature
-2 Moderately cold at handling
-1. Slightly cold at handling
0. Negative (normal)
1 Slightly warm at handling
2. Moderately warm at handling

Abnormal respiratory sound
0. Negative
1. Positive

Changes in fur
0. Negative (normal)
1. Slightly wetted fur in external genital region
2. Moderately wetted fur in external genital region
3. Markedly wetted fur in external genital region

Changes in skin
(areas such as nose, limbs and tail)
0 Negative (normal)
1. Slightly congested
2. Moderately congested
3. Markedly congested

Changes in mucous membrane
0 Negative (normal)
1. Slightly congested
2. Moderately congested
3. Markedly congested

(IET 01-0079)

Key to Tables 5 and 6 (continued)
Standard key to scored clinical signs

Open field:

Jumping
0. Negative
1. Positive

Circling
0. Negative
1 Positive

Convulsions
0. Negative
1 Positive

Abnormal gait
0. Negative
1. Positive
(with description such as staggering gait, dragging gait, paralysis of hind limbs, etc.)

Spontaneous motor activity
-3. Markedly decreased motor activity
-2. Moderately decreased motor activity
-1. Slightly decreased motor activity
0. Normal
1. Slightly increased motor activity
2. Moderately increased motor activity
3. Markedly increased motor activity

Grooming
0. No grooming
1. Occasional grooming
2. Frequent grooming
(about half of the observation period)
3. Continuous grooming
(almost all the observation period)

Rearing
0. No rearing
1 Occasional rearing
2 Frequent rearing
(about half of the observation period)
3. Continuous rearing
(almost all the observation period)

Respiration
-3. Markedly slow respiration
-2 Moderately slow respiration
-1. Slightly slow respiration
0. Normal
1. Slightly rapid respiration
2. Moderately rapid respiration
3. Markedly rapid respiration

Vocalization
0 Negative
1. Positive

Piloerection
0 Negative
1. Positive

Urination
0. No urination
1. 1-2 times
2 3-4 times
3 5 times or more
(counts made during the observation period)

Defecation
0. No feces
1. 1-2 fecal boluses
2 3-4 fecal boluses
3. 5 or more fecal boluses
(counts made during the observation period)

Abnormal posture
see Home cage observation

Abnormal behavior
see Home cage observation

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.4100b [§83-1b], Chronic Toxicity Study in Dogs

Work Assignment No. 4-1-124 I; formerly 3-1-124 I (MRID 46817218)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer
Stephanie E. Foster, M.S.
Signature: Stephanie E Foster
Date: 01/19/07

Secondary Reviewer:
John W. Allran, M.S.
Signature: John W Allran
Date: 01/19/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 01/19/07

Quality Assurance:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 01/19/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

215

EPA Reviewer: Ayaad Assaad, D.V.M., Ph.D. **Signature:**
Toxicology Branch, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity in Dogs (feeding); OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 96.7% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Kuwahara, M. (2004) NNI-0001: 52-week chronic toxicity study in dogs. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID.: IET 02-0035, April 16, 2004. MRID 46817218. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46817218), NNI-0001 (Flubendiamide, 96.7% a.i., Lot #: 1FH0019M) was administered to 4 beagle dogs/sex/dose group in the diet for 52 weeks at concentrations of 0, 100, 1500, or 20,000 ppm (equivalent to 0/0, 2.21/2.51, 35.2/37.9, and 484/533 mg/kg/day in males/females).

No treatment-related adverse effects were observed on mortality, clinical signs of toxicity, food consumption, ophthalmoscopic examination, or urinalysis.

A gradual decrease of 5-6% (Not significant [NS]) in bodyweights was observed in the 1500 ppm males during Weeks 24-52, resulting in a decrease of 21% (NS) in overall (Weeks 0-52) bodyweight gain. At 20,000 ppm, bodyweights were decreased in males by 5-11% (NS) beginning at Week 11 and in females by 6-10% (NS) beginning at Week 16, resulting in a decrease of 42-46% (NS) in overall bodyweight gains in both sexes.

The liver appeared to be the target organ. At >=1500 ppm, alkaline phosphatase activity (ALP) was increased in both males (incr. 485-1514%) and females (incr. 547-2648%) throughout the study. Additionally at 20,000 ppm, ALT was increased throughout the study in both males (incr. 39-211%) and females (incr. 118-240%); albumin and A/G ratio were decreased by 7-19% in males during Weeks 26-52; and triglycerides were increased (incr. 100-117%) throughout the study in females. The increases in liver enzymes were an obvious indication of hepatocellular

suggest an effect on hepatic lipid and protein metabolism.

At 1500 ppm, absolute (incr. 7-10%) and relative to body (incr. 14%) liver weights were slightly increased in both sexes. At 20,000 ppm, absolute (incr. 12% [M], incr. 23% [F]) and relative to body (incr. 26% [M], incr. 37% [F]) liver weights were more noticeably increased in both males and females. Upon necropsy, enlarged liver was observed in 1/4 females at 20,000 ppm compared with 0/4 controls. The liver in this animal was also dark in color, which was correlated microscopically with brown pigment deposition in the Kupffer cells. This pigment was determined to be lipofuscin, and not hemosiderin or bile pigment. Additionally at 20,000 ppm, incidence of slight deposition of brown pigment in liver Kupffer cells was also observed in 2/4 males.

At >=1500 ppm, activated partial thromboplastin times (APTT) were shortened by 6-21% in both sexes throughout the study. The decreases observed in APTT may indicate an increase in the pro-coagulant activity in the system.

Serum cholinesterase levels were increased in the >=1500 ppm males (incr. 25-35%) and the 20,000 ppm females (incr. 38-46%). Because there were no biologically adverse systemic clinical signs associated with increased cholinesterase levels, this effect was considered equivocal.

The LOAEL is 1500 ppm (equivalent to 35.2/37.9 mg/kg/day in M/F), based on: decreased bodyweight and bodyweight gain in males; increased ALP in both sexes; and increased absolute and relative liver weights in both sexes. The NOAEL is 100 ppm (equivalent to 2.21/2.51 mg/kg/day in M/F).

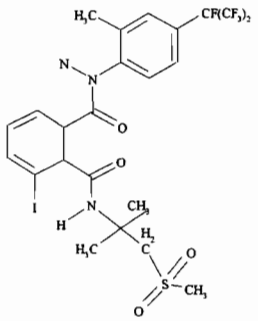
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: NNI-0001 (Flubendiamide)

Description:	White crystal
Lot #:	1FH0019M
Purity:	96.7% a.i.
Compound stability:	Stable in the test diets kept under sealed, cold, and dark conditions for up to 4 weeks and then exposed to ambient temperature for 8 days.
CAS # of TGAI:	272451-65-7
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	5-6 months; 7.8-9.6 kg males, 7.1-9.8 kg females
Source:	CSK Research Park, Inc. (Toyota, Suwa-shi, Nagano, Japan)
Housing:	Individually in stainless steel cages sustained in racks equipped with automatic sweepers. Exercise in a stainless steel cage was permitted for ten minutes, twice a week during treatment for paired dogs of same sex and treatment group.
Diet:	Certified pellet diet DS-A (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, Japan); 250 g of pulverized diet moistened with 250 g of water/day.
Water:	Filtered well water sterilized with hypochlorite and UV light, *ad libitum*
Environmental conditions:	**Temperature:** 23±2° C **Humidity:** 55±15% **Air changes:** ≥10/hour **Photoperiod:** 12 hour light/dark
Acclimation period:	49 days (males) and 57 (females)

B. STUDY DESIGN:

1. In life dates: Start: 05/22/02 End: 05/30/03

2. Animal assignment: The dogs were randomly assigned to the test groups shown in Table 1. After allocation, there were no significant differences in the mean body weights among groups and all individual body weights were within 20% of the mean body weight value for each sex. Littermates were not included in the same treatment group. One male (# 4) that showed an abnormality during the acclimatization period ophthalmological examination was

allocated to the control group in order to minimize future interpretation of the effects of the test substance on the eye. Also, two female dogs (#s 22 and 27) that had outlying alkaline phosphatase values during the acclimatization period were allocated to groups other than the high-dose.

Table 1. Study design [a]

Test Group	Diet concentration (ppm)	Achieved Intake (mg/kg/day, M/F)	# of Animals (M/F)
Control	0	0/0	4/4
Low	100	2.21/2.51	4/4
Mid	1500	35.2/37.9	4/4
High	20,000	484/533	4/4

a Data were obtained from page 23 and Tables 11 and 12 on pages 94-95 of the study report.

3. **Dose selection rationale:** The dose-selection was based on the results of a subchronic toxicity study (MRID 46817212, reviewed concurrently with this study) in which the dogs were fed dietary concentrations of 0, 100, 2000 or 40,000 ppm for at least 90 days.

   At ≥2000 ppm, the following were observed: (i) an increase in alkaline phosphatase (ALP) and shortened activated partial thromboplastin time (APTT) were observed in both sexes; (ii) triglycerides were increased in females; (iii) increased adrenal weights were observed in females at 2000 ppm and in males at 40,000 ppm; and (iv) cortical hypertrophy of the adrenals was observed in 2/4 females at 2000 ppm and in 2/4 males and 1/4 females at 40,000 ppm. Additionally at 40,000 ppm, decreased body weight gains and decreased cholesterol were observed in males.

4. **Diet preparation and analysis:** Dietary formulations were prepared once prior to treatment and every four weeks during treatment by mixing the appropriate amount of test material with a small amount of diet to form a premix for each dose level. The premix was further diluted with diet to achieve the desired concentration. The prepared diets were sealed and stored in cold, dark conditions until needed. The amount of test substance to be mixed was not corrected for purity. Approximately 250 grams of diet was moistened with 250 g of water and given daily to each animal. Homogeneity (top, middle, and bottom) and concentration analyses were performed on all dietary formulations prior to treatment and at Weeks 22 and 51. In a previously conducted 28-day study (MRID 46817242, reviewed concurrently), the stability of the test compound in the diet at 10 and 40,000 ppm was demonstrated for 4 weeks when kept sealed, cold, and dark and then exposed to ambient temperature for 8 days.

Results:

Stability (mean % of initial): 85-108%

Homogeneity (% coefficient of variation): 0.3-5.0%

Concentration (mean % of nominal): 97-101%

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

5. **Statistics:** Group means were compared at the 5% and 1% significance levels. The following statistical procedures were used:

Parameter	Statistical Test
Body weight, hematology, clinical chemistry, urine specific gravity, urine volume, absolute and relative organ weights.	Bartlett's test was performed to compare homogeneity of group variances. If Bartlett's was not significant at $p \leq 0.05$, a one-way ANOVA was performed to compare the means. If the ANOVA was significant ($p \leq 0.05$), treated groups were compared with the control group using Dunnett's 2-sided test. If Bartlett's was significant ($p \leq 0.05$), group means were compared using the Kruskal-Wallis test for non-parametric comparison. If the Kruskal-Wallis test was significant ($p \leq 0.05$), treated groups were compared with the control group using a Dunnett-type mean rank sum test.
Food consumption	The non-parametric Kruskal-Wallis test was performed on group means. If $p \leq 0.05$, a Dunnett-type mean rank sum test was applied.

Before proceeding with parametric analyses, the assumption of normal distribution of the data should have been verified. Otherwise, the statistical methods were considered appropriate.

C. METHODS

1a. **Clinical Observations:** Cage-side examinations for mortality and signs of morbidity were performed at least twice daily on weekdays and once/day on weekends and holidays during the treatment period. Animals were examined once daily for clinical signs of toxicity. A detailed clinical examination was performed on all animals once prior to treatment and weekly throughout the study.

1b. **Neurological evaluations:** Neurological evaluations were conducted as part of the detailed clinical examinations and included the following checked (X) observations:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture/position		Tremor	X	Vocalization
X	Behavior (including stereotypies)		Lacrimation / chromodacryorrhea	X	Spontaneous motor activity
X	Convulsions (tonic, clonic)		Salivation	X	Convulsions
X	Tremors		Piloerection	X	Tremors
	Vocalization		Convulsion	X	Urination / defecation
	Arousal upon opening cage		Palpebral closure	X	Changes in skin/fur/eye ball
X	Spontaneous motor activity		Respiratory rate	X	Gait abnormalities / posture
	Gait abnormalities	X	Appearance of fur/skin/nails	X	Respiration
	SENSORY OBSERVATIONS		Excessive vocalization	X	Bizarre / stereotypic behavior
	Approach response		Exophthalmus	X	Lacrimation/salivation
X	Touch response	X	Muscle tone	X	Discharge (conjunctival, oral mucosa, ocular, nasal, auricular, and vaginal)
X	Startle response	X	Social behavior during removal	X	Palpebral closure
	Pain response		Ease of handling		
X	Pupil response/size				
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension		Body weight		Hindlimb extensor strength
	Hindlimb extension		Body temperature		Forelimb grip strength
	Righting reflex				Hindlimb grip strength
	Olfactory orientation				Hindlimb foot splay
			OTHER OBSERVATIONS		Rotarod performance

2. **Body weight:** All animals were weighed prior to feeding one week prior to treatment (Week -1), on the first day of treatment (Week 0), weekly during Weeks 1-13, and once every four weeks during Weeks 16-52.

3. **Food consumption and compound intake:** Food consumption was measured weekly during acclimatization and during Weeks 1-13, and every four weeks during Weeks 16-52. Mean daily food consumption (g/animal/day) was then calculated from these data and reported for these intervals. Test substance intake (mg/kg bw/day) was calculated for each week and as an average for the overall study (Weeks 1-52) using the food consumption, body weight, and nominal dietary concentration data.

4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were conducted on all dogs pre-exposure and at Week 52.

5. **Hematology and clinical chemistry:** Blood samples for hematology and clinical chemistry analyses were collected from the cephalic vein of all animals (overnight food-fasted) prior to treatment and at Weeks 13, 26, 39, and 52, except for total bile acid which was examined only at Weeks 26, 39, and 52. Blood smears were prepared, but not examined. Also, blood samples for possible mechanistic studies were collected from the cephalic vein of all animals (non-fasted) prior to termination. The following CHECKED (X) parameters were examined.

a. 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*		
	(Thromboplastin time)		
X	(Activated partial thromboplastin time)		
X	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg. *)	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
X	Serum cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Alanine aminotransferase (ALT/ SGPT)*	X	Total bile acid
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for chronic studies based on Guideline 870.4100.

6. **Urinalysis:** Urine samples were collected from all animals prior to treatment and at Weeks 13, 26, 39, and 52. Urine appearance, volume, and sediments were examined from samples collected for 24 hours, while the remaining parameters were examined from fresh samples. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)		Nitrite
X	Protein*	X	Urobilinogen

* Recommended for chronic studies based on Guideline 870.4100.

7. **Sacrifice and pathology:** At study termination, all animals were overnight fasted, weighed, killed via exsanguination under sodium pentobarbital anesthesia, and subjected to a gross necropsy. The following CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver (with gallbladder)*+
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*++
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta thoracic*
XX	Heart*+
X	Bone marrow (sternum, femur, rib)*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
XX	Prostate*
X	Penis
XX	Uterus (with cervix)*+
X	Mammary gland*
X	Vagina
XX	Ovaries *+
X	Oviducts

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve (sciatic)*
X	Spinal cord (3 levels)*
XX	Pituitary*
X	Eyes (with retina and optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal gland
XX	Thyroid (with parathyroid)*+
	OTHER
X	Bone (sternum, femur, rib)
X	Femoral muscle
X	Skin*
X	All gross lesions and masses*
X	Tonsils
X	Buccal mucosa of oral cavity
X	Peyer's Patches
X	Diaphragm

* Required for chronic studies based on Guideline 870.4100.
\+ Organ weight required in chronic studies.
++ Organ weight required if inhalation route.

Tissue samples were fixed in 10% neutral-buffered formalin with the exception of the eye, and testes. The eyes were fixed for about 3 days in a phosphate-buffered solution of formalin and glutaraldehyde (pH 7.2) and then transferred into 10% neutral-buffered formalin. The testes were fixed for 5-6 days in a solution of formalin, sucrose, and acetic acid (FSA solution), trimmed, and then preserved in 10% neutral-buffered formalin. With the exception of the tonsils, tongue, buccal mucosa of the oral cavity, penis, oviducts, and diaphragm, all collected tissue samples were prepared routinely and stained with hematoxylin and eosin for microscopic examination. Bone marrow samples from ribs were prepared, but not examined. Findings were reported as present or assigned a severity grade.

II. RESULTS:

A. OBSERVATIONS:

1. **Mortality:** All dogs survived the treatment period.

2. **Clinical/Neurological signs of toxicity:** There were no treatment-related clinical and/or neurological signs of toxicity. At 20,000 ppm, loose stool was observed in 3/4 males (vs. 1/4 controls) and 2/4 females (vs. 0/4 controls); however, this finding was considered incidental because it was observed on only one occasion/dog. However, in the 90-day dog study of the same compound, loose stool was observed in several animals.

B. BODY WEIGHT AND WEIGHT GAIN: A gradual decrease (↓5-6%; Not significant [NS]) in bodyweights was observed in the 1500 ppm males during Weeks 24-52, resulting in a decrease (↓21%; NS) in overall (Weeks 0-52) bodyweight gain (Table 2). At 20,000 ppm, bodyweights were decreased in males (↓5-11%; NS) beginning at Week 11 and in females (↓6-10%; NS) beginning at Week 16, resulting in a decrease (↓42-46%; NS) in overall bodyweight gains in both sexes. Bodyweights and bodyweight gains in the remaining treatment groups were comparable to controls throughout the study.

Table 2. Mean (±SD) body weights and body weight gains (kg) at selected intervals in dogs treated with Flubendiamide in the diet for up to 1 year [a]

Weeks on Study	Dose (ppm)			
	0	100	1500	20,000
Males				
0	8.9±0.7	8.9±0.4	8.9±0.3	8.9±0.8
11	10.5±1.0	10.7±0.3	10.3±0.3	10.0±0.8 (↓5)
13	10.7±1.1	10.8±0.4	10.3±0.5	10.1±0.8 (↓6)
24	11.3±1.3	11.4±0.6	10.7±0.8 (↓5)	10.5±0.8 (↓7)
36	11.8±1.5	12.0±0.7	11.1±0.9 (↓6)	10.7±0.8 (↓9)
52	12.2±1.8	12.4±0.8	11.5±1.0 (↓6)	10.8±1.0 (↓11)
BWG: 0-52 [b]	3.3	3.5	2.6 (↓21)	1.9 (↓42)
Females				
0	8.3±0.4	8.3±1.1	8.2±0.5	8.4±0.9
16	9.9±0.6	9.7±1.3	9.7±0.8	9.3±1.1 (↓6)
24	10.1±0.8	9.9±1.6	9.9±1.0	9.3±1.2 (↓8)
36	10.7±0.9	10.4±1.6	10.3±1.0	9.7±1.3 (↓9)
52	10.9±1.0	10.7±1.9	10.5±1.4	9.8±1.4 (↓10)
BWG: 0-52 [b]	2.6	2.4	2.3	1.4 (↓46)

a Data obtained from tables 7-1, 7-2, 8-1, and 8-2 on pages 86-89 of the study report; n=4. Percent differences from controls (calculated by reviewers) are included in parentheses.

b Calculated by the reviewers from data in this table as a difference in group mean body weight data.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** No treatment-related effect was observed on food consumption. All dogs ate all of the food provided throughout the study.

2. **Compound intake:** Mean test material intake values for the overall study are reported in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were observed on ophthalmoscopy. One control male was noted with congestion of the conjunctiva prior to treatment.

E. BLOOD ANALYSES

1. **Hematology:** Selected hematology data are presented in Table 3a. At ≥1500 ppm, activated partial thromboplastin times (APTT) were shortened by 6-21% compared to controls in both sexes throughout the study. The decreases observed in APTT may indicate an increase in the pro-coagulant activity in the system. In the ≥1500 ppm females, platelets were increased by 38-51% ($p \leq 0.01$) during Weeks 39 and 52. However, platelets were also increased by 27% prior to treatment at 1500 ppm, therefore this effect was considered incidental and not related to treatment in this group.

   Eosinophils were increased in all treated males throughout the study (↑32-109%); however, eosinophils were increased (↑52-74%) in these animals prior to treatment. Therefore, this finding was not considered treatment-related.

   All other differences from controls were either not dose-dependent and/or transient and were not considered treatment-related.

Table 3a. Selected mean (±SD) hematology values in dogs treated with Flubendiamide in the diet for up to 1 year [a]

Parameter		Dose (ppm)			
		0	100	1500	20,000
Males					
APTT (sec)	0 weeks	13.1±1.0	14.0±1.0	13.6±1.3	13.1±0.6
	13 weeks	13.1±0.4	13.7±0.9	12.3±1.0 (↓6)	11.4±0.3* (↓13)
	26 weeks	13.0±0.7	13.3±1.0	11.8±1.1 (↓9)	10.6±0.5**(↓18)
	39 weeks	13.0±0.6	13.5±0.9	12.0±1.3 (↓8)	10.9±0.4* (↓16)
	52 weeks	13.0±0.4	13.5±0.8	11.7±0.9* (↓10)	10.7±0.4** (↓18)
Females					
APTT (sec)	0 weeks	14.0±0.7	13.5±0.5	13.7±1.1	13.5±0.9
	13 weeks	15.0±0.4	13.5±0.6**	12.5±0.9**(↓17)	11.8±0.2**(↓21)
	26 weeks	14.0±0.6	12.7±1.1	12.2±0.7**(↓13)	11.5±0.2**(↓18)
	39 weeks	14.0±0.4	13.0±0.5	12.0±0.9**(↓14)	11.3±0.6**(↓19)
	52 weeks	14.3±0.5	13.0±0.7	12.2±0.9**(↓15)	11.5±0.5**(↓20)
Platelets ($x10^3/\mu L$)	0 weeks	364±24	361±37	463±32** (↑27)	397±54
	39 weeks	315±16	402±34	442±36** (↑40)	475±79** (↑51)
	52 weeks	328±19	391±15	452±36** (↑38)	452±69** (↑38)

a Data obtained from Tables 17-5, 18-4, and 18-5 on pages 124, 132, and 133 of the study report.
* Statistically different from controls, $p \leq 0.05$.
** Statistically different from controls, $p \leq 0.01$.

2. **Clinical chemistry:** At ≥1500 ppm, alkaline phosphatase activity (ALP) was increased both with dose and time in both males (↑485-1514%) and females (↑547-2648%) throughout the

study (Table 3b). Serum cholinesterase levels were increased in the ≥1500 ppm males (↑25-35%) and the 20,000 ppm females (↑38-46%). Because there were no biologically adverse systemic clinical signs associated with increased cholinesterase levels, this effect was considered equivocal. Additionally at 20,000 ppm, alanine aminotransferase (ALT) was increased throughout the study in both males (↑39-211%) and females (↑118-240%); albumin and A/G ratio were decreased by 7-19% in males during Weeks 26-52; and triglycerides were increased (↑100-117%) throughout the study in females.

Sodium levels were significantly increased by 1% in males at ≥1500 ppm during Weeks 13 and 26. Additionally in the 20,000 ppm females, gamma-glutamyl transpeptidase (GGTP) was increased throughout the study; however, levels were similarly increased over controls prior to treatment. These effects were considered to be minor and not adverse. All other differences from controls were either not dose-dependent, transient, and/or minor.

Table 3b. Selected mean (±SD) clinical chemistry values in dogs treated with Flubendiamide in the diet for up to 1 year [a]

Parameter		Dose (ppm)			
		0	100	1500	20,000
Males					
ALP (U/L)	0 weeks	385±38	365±12	401±34	398±95
	13 weeks	255±43	255±37	1493±470 (↑485)	1964±594* (↑670)
	26 weeks	204±22	246±146	1892±666 (↑827)	2566±938* (↑1158)
	39 weeks	175±28	178±31	1930±718 (↑1003)	2825±954* (↑1514)
	52 weeks	191±30	247±136	2123±646 (↑1012)	2888±834* (↑1412)
ALT (U/L)	0 weeks	30±6	23±4	23±3	23±4
	13 weeks	36±9	30±3	38±5	50±19 (↑39)
	26 weeks	38±9	45±41	59±20	118±62 (↑211)
	39 weeks	38±10	26±6	57±30	90±30* (↑137)
	52 weeks	36±9	31±11	50±9	80±28** (↑122)
Albumin (g/dL)	0 weeks	3.23±0.07	3.28±0.11	3.13±0.15	3.12±0.08
	26 weeks	3.29±0.20	3.09±0.10	3.01±0.12* (↓9)	2.91±0.07** (↓12)
	39 weeks	3.24±0.11	3.22±0.13	3.01±0.17	2.96±0.10* (↓9)
	52 weeks	3.28±0.12	3.24±0.06	3.25±0.07	3.06±0.13* (↓7)
A/G ratio	0 weeks	1.36±0.08	1.31±0.09	1.27±0.10	1.27±0.05
	26 weeks	1.13±0.06	1.10±0.09	0.98±0.09	0.92±0.11* (↓19)
	39 weeks	1.12±0.02	1.06±0.06	0.95±0.12* (↓15)	0.94±0.10* (↓16)
	52 weeks	1.16±0.05	1.05±0.06	1.03±0.08	0.94±0.11** (↓19)
Cholinesterase (U/L)	0 weeks	2663±249	2952±417	2959±692	2846±208
	13 weeks	2636±252	3099±287	3369±648 (↑28)	3377±419 (↑28)
	26 weeks	2571±240	3014±283	3213±662 (↑25)	3280±394 (↑28)
	39 weeks	2483±212	3014±288	3264±436* (↑31)	3300±445* (↑33)
	52 weeks	2497±173	3044±294	3299±672 (↑32)	3377±470 (↑35)
Females					
ALP (U/L)	0 weeks	312±28	335±38	331±34	326±14
	13 weeks	187±40	255±37	1209±346 (↑547)	2683±998** (↑1335)
	26 weeks	153±30	261±37	1536±448* (↑904)	3882±1234** (↑2437)
	39 weeks	132±18	213±56	1232±367* (↑833)	3106±967** (↑2253)
	52 weeks	145±32	244±60	1303±319* (↑799)	3985±1706** (↑2648)
ALT (U/L)	0 weeks	29±7	26±4	28±7	27±5
	13 weeks	33±7	28±6	38±2	72±32 (↑118)
	26 weeks	29±6	28±6	33±7	76±45 (↑162)
	39 weeks	25±4	24±5	30±1	85±71* (↑240)
	52 weeks	29±6	28±8	32±10	84±34* (↑190)
Triglycerides (mg/dL)	0 weeks	22±2	27±4	27±4	25±5
	13 weeks	21±3	20±6	32±11	44±21 (↑110)
	26 weeks	22±4	23±2	32±8	44±15 (↑100)
	39 weeks	23±3	26±7	32±7	50±14** (↑117)
	52 weeks	18±7	18±6	28±9	39±16* (↑117)
Cholinesterase (U/L)	0 weeks	3092±509	3354±676	2740±300	3552±616
	13 weeks	3157±628	3352±821	2948±369	4367±1245 (↑38)
	26 weeks	2989±597	3367±904	2984±413	4193±1076 (↑40)
	39 weeks	2875±566	3302±751	2792±332	4202±1353 (↑46)
	52 weeks	2975±597	3452±895	3098±503	4230±1112 (↑42)

a Data obtained from Tables 19-1, 19-2, 19-5, 19-6, 20-1, 20-2, and 20-8 on pages 139-141, 143-44, 150-152, and 157 of the study report.

* Statistically different from controls, $p \leq 0.05$.

** Statistically different from controls, $p \leq 0.01$.

227

F. URINALYSIS: No treatment-related effects were observed on urinalysis.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** At 1500 ppm, absolute (↑7-10%) and relative to body (↑14%) liver weights were slightly increased in both sexes (Table 4). At 20,000 ppm, absolute (↑12% [M], ↑23% [F]) and relative to body (↑26% [M], ↑37% [F]) liver weights were more noticeably increased in both males and females.

   At 20,000 ppm, ovary weights were increased when compared with controls. This was due to one female (# 32) with extremely heavy ovaries. Increased uterine weight was also noted in this animal. In the absence of any histopathological correlate, this effect on the ovaries and uterus was considered to be due to the estrus cycle of this animal and not treatment-related. All other organ weights were similar to controls.

Table 4. Selected mean (±SD) absolute (g) and relative to body (%) organ weights in dogs treated with Flubendiamide in the diet for up to 1 year [a]

Organ	Dose (ppm)			
	0	100	1500	20,000
Males				
Terminal body weight (kg)	12.2±1.8	12.4±0.8	11.4±1.0	10.8±1.0
Liver-absolute (g)	286±42	294±21	305±22 (↑7)	319±12 (↑12)
relative to body (%)	2.35±0.13	2.38±0.11	2.67±0.07* (↑14)	2.97±0.22** (↑26)
Females				
Terminal body weight (kg)	10.9±1.0	10.7±1.8	10.5±1.3	9.8±1.4
Liver-absolute (g)	261±16	263±29	287±18 (↑10)	320±36* (↑23)
relative to body (%)	2.41±0.11	2.55±0.68	2.75±0.24 (↑14)	3.30±0.48 (↑37)

a Data obtained from Tables 21-1 thru 22-2 on pages 161-164 of the study report.
* Statistically different from controls, $p \leq 0.05$.
** Statistically different from controls, $p \leq 0.01$.

2. **Gross pathology:** In the 20,000 ppm females, enlarged liver was observed in 1/4 females (# 29) compared with 0/4 controls. The liver in this animal was also dark in color, which was correlated microscopically with brown pigment deposition in the Kupffer cells.

   All other changes were considered incidental and not related to treatment.

3. **Microscopic pathology:** Selected microscopic findings are presented in Table 5. At 20,000 ppm, incidence of slight deposition of brown pigment in liver Kupffer cells was observed in 2/4 males and 1/4 females compared with 0 respective controls.

At 20,000 ppm, slight dilatation of the cerebral ventricle was observed in 2/4 females compared with 0 controls. However, this effect was also observed in 1/4 males at 0 (control) and 100 ppm; therefore this was not considered treatment-related.

Table 5. Selected microscopic findings (#affected/4 animals) in dogs treated with Flubendiamide in the diet for up to 1 year [a]

Finding	Dose (ppm)			
	0	100	1500	20,000
Males				
Liver brown pigment deposition, Kupffer cell total (slight)	0	0	0	2
Females				
Liver brown pigment deposition, Kupffer cell total (slight)	0	0	0	1

a Data obtained from Tables 25-1 and 26-1 on pages 167 and 169, and Appendices 22-13, 22-14, and 23-13 on pages 355-56, and 371 of the study report; n=4.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: The LOAEL was 1500 ppm based on decreased body weight gain, decreased sodium levels, and increased relative liver weight in males; shortened APTT and increased or increased tendency of ALP in both sexes.

B. REVIEWER COMMENTS: No treatment-related adverse effects were observed on mortality, clinical signs of toxicity, food consumption, ophthalmoscopic examination, or urinalysis.

A gradual decrease (↓5-6%; [NS]) in bodyweights was observed in the 1500 ppm males during Weeks 24-52, resulting in a decrease (↓21%; NS) in overall (Weeks 0-52) bodyweight gain. At 20,000 ppm, bodyweights were decreased in males (↓5-11%; NS) beginning at Week 11 and in females (↓6-10%; NS) beginning at Week 16, resulting in a decrease (↓42-46%; NS) in overall bodyweight gains in both sexes.

The liver appeared to be the target organ. At ≥1500 ppm, ALP activity was increased in both males (↑485-1514%) and females (↑547-2648%) throughout the study. Additionally at 20,000 ppm, ALT was increased throughout the study in both males (↑39-211%) and females (↑118-240%); albumin and A/G ratio were decreased by 7-19% in males during Weeks 26-52; and triglycerides were increased (↑100-117%) throughout the study in females. The increases in liver enzymes were an obvious indication of hepatocellular leakage, but the increases in triglycerides in females and decreases in albumin in males also may suggest an effect on hepatic lipid and protein metabolism.

At 1500 ppm, absolute (↑7-10%) and relative to body (↑14%) liver weights were slightly increased in both sexes. At 20,000 ppm, absolute (↑12% [M], ↑23% [F]) and relative to body (↑26% [M], ↑37% [F]) liver weights were more noticeably increased in both males and females. Upon necropsy, enlarged liver was observed in 1/4 females at 20,000 ppm compared with 0/4 controls. The liver in this animal was also dark in color, which was correlated microscopically with brown pigment deposition in the Kupffer cells. This pigment was determined to be lipofuscin, and not hemosiderin or bile pigment. Additionally at 20,000 ppm, incidence of slight deposition of brown pigment in liver Kupffer cells was also observed in 2/4 males.

At ≥1500 ppm, APTT was shortened by 6-21% in both sexes throughout the study. The decreases observed in APTT may indicate an increase in the pro-coagulant activity in the system.

Serum cholinesterase levels were increased in the ≥1500 ppm males (↑25-35%) and the 20,000 ppm females (↑38-46%). Because there were no biologically adverse systemic clinical signs associated with increased cholinesterase levels, this effect was considered equivocal.

The LOAEL is 1500 ppm (equivalent to 35.2/37.9 mg/kg/day in M/F), based on: decreased bodyweight and bodyweight gain in males; increased ALP in both sexes; and increased absolute and relative liver weights in both sexes. The NOAEL is 100 ppm (equivalent to 2.21/2.51 mg/kg/day in M/F).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

STUDY DEFICIENCIES: No deficiencies were noted.

15

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.4200a [§83-2a]; Carcinogenicity Study in Rats

Work Assignment No. 4-1-124 J; formerly 3-1-124 J (MRID 46817219)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Rd, Bldg. 100, Ste. B
Durham, NC 27713

Primary Reviewer:
Ronnie J. Bever Jr., Ph.D.
Signature: Ronnie J. Bever Jr.
Date: 1/31/07

Secondary Reviewer:
Stephanie E. Foster, M.S.
Signature: Stephanie E. Foster
Date: 1/31/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 1/31/07

Quality Assurance:
Steve Brecher, Ph.D., D.A.B.T.
Signature: Steven Brecher
Date: 1/31/07

Disclaimer

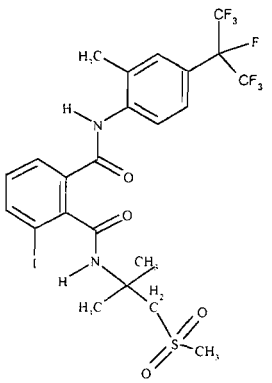
This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

151

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description:	White crystals
Lot Nos.:	1FH0018P; 1FH0019M
Purity:	96.7-97.8% a.i.
Stability of compound:	Stability was confirmed in 20 and 20,000 ppm diets kept under a sealed, cold, and dark condition for 5 weeks, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient air for 8 days.
CAS #:	272451-65-7
Structure:	

2. Vehicle - Diet

3. Test animals

Species:	Rat
Strain:	Fischer (F344/DuCrj); SPF
Age and group mean weight at study initiation:	5 weeks of age; 90-108 g males; 77-93 g females
Source:	Atsugi Breeding Center, Charles River Japan, Inc. (Kanagawa)
Housing:	Housed in wire-mesh stainless steel cages in racks in groups of 5 (same sex and dose group)
Diet:	Certified diet MF Mash (Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	Filtered (sand and charcoal) well water, sterilized with sodium hypochlorite and UV light, *ad libitum*
Environmental conditions	
Temperature:	22-26°C
Humidity:	38-72%
Air changes:	≥10/hour
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	9 (males) or 10 (females) days

B. STUDY DESIGN

1. In life dates: Start: 08/02/01 End: Approximately 08/02/03

Results:

Homogeneity (% CV): 0.3-6.7%, except 12.5% in the 50 ppm pre-study mix

Stability (% nominal): 97-100%

Concentration (% of nominal): 93-103%

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

5. **Statistics**: Significance was indicated at 5 and 1% probability.

Parameter	Statistical procedure
Body weight Food consumption Total and differential leukocyte counts Organ weights	Bartlett's test was conducted. One-way analysis of variance (ANOVA) and Dunnett's test were performed when group variances were homogeneous. The Kruskal-Wallis test and a Dunnett-type mean rank sum test were conducted when group variances were heterogeneous.
Mortality	Life table analysis was performed.
Clinical observations (general) Gross pathology Histopathology	Fisher's Exact probability test (one-tail analysis) was conducted.

The statistical analyses were considered appropriate.

C. METHODS

1. **Observations:** Animals were observed for mortality and moribundity at least twice daily, except once daily on weekends and holidays. Clinical signs of toxicity were recorded once daily. Detailed clinical examinations, including palpation of masses, were performed at least weekly. The detailed clinical examinations included a limited functional observational battery, evaluating the following parameters:

Home cage	Handling	Open field
Excitement	Handling difficulty	Jumping
Sedation	Changes in muscle tone	Circling
Abnormal posture	Tremors	Convulsions
Abnormal behavior	Palpebral closure	Abnormal gait
	Changes in pupil size	Spontaneous motor activity
	Salivation	Respiration
	Lacrimation	Vocalization
	Discharges	Piloerection
	Exophthalmos	Abnormal posture
	Changes in body temperature	Abnormal behavior
	Abnormal respiratory sound	
	Changes in fur	
	Changes in skin and mucous membranes	

6. **Urinalysis:** Urinalysis was not performed and is not required by the guideline (OPPTS 870.4200a).

7. **Sacrifice and pathology:** Animals were killed after 104 weeks of treatment by exsanguination while under deep ether anesthesia. All animals were subjected to a detailed necropsy, and the following CHECKED (X) tissues were collected. The (XX) organs were weighed from 10 animals/sex/dose group selected in order of animal number.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroids* [a]
X	Rectum*	X	Urinary bladder*	XX	Thyroids* [a]
XX	Liver*+	XX	Testes*+	X	Harderian gland
	Gall bladder* (not rat)	XX	Epididymides*+		OTHER
	Bile duct* (rat)	X	Prostate*	X	Bone (sternum and femur)
X	Pancreas*	X	Seminal vesicle*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus*+ (horns and cervix)	X	Knee joint
X	Lung*++	X	Mammary gland*	X	Head (oral mucosa and middle ears)
X	Nose* (nasal cavity)	X	Coagulating glands	X	All gross lesions and masses*
X	Pharynx*	X	Vagina		
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200.
a Thyroids and parathyroids were weighed together
\+ Organ weight required in carcinogenicity studies.
++ Organ weight required if inhalation route.

The collected tissues were fixed in 10% neutral-buffered formalin. The lungs were instilled with formalin before fixation. The fixed tissues were embedded in paraffin, stained with hematoxylin and eosin, routinely prepared, and examined microscopically as follows. The head and tongue were collected, but were not examined microscopically. Histopathological examinations were performed on all other tissues collected from the 0 and 20,000 ppm groups; all tissues from animals killed *in extremis* or found dead; the liver, kidneys, thyroids, spleen, and bone marrow, and all gross lesions from all animals; and skin of all females. Microscopic lesions were graded as slight, moderate, or severe.

TABLE 2. Mean (±SD) body weights and body weight gains (g) at selected intervals in rats treated with NNI-0001 for up to 104 weeks. [a]

Week(s)	Dose (ppm)			
	0	50	1000	20,000
Males				
0	97±4	97±4	97±4	97±4
13	324±17	320±15	319±15	321±15
52	434±24	427±26	435±23	437±23
104	435±32	444±35	453±37	455±27* (↑5)
BWG: 0-13	227	223	222	224
BWG: 13-52	110	107	116	116
BWG: 52-104	1	17	18	18
BWG: 0-104	338	347	356	358 (↑6)
Females				
0	86±4	86±4	86±4	86±4
13	178±7	182±9	182±8	180±9
52	222±11	224±12	226±11	221±12
76	253±21	253±16	251±16	243±19* (↓4)
104	284±23	288±23	276±26	264±31** (↓7)
BWG: 0-13	92	96	96	94
BWG: 13-52	44	42	44	41
BWG: 52-104	62	64	50	43
BWG: 0-104	198	202	190	178 (↓10)

a Data (n=34-50) were obtained from pages 80-85 of MRID 46817219. Percent differences from controls (calculated by reviewers) are included in parentheses. Body weight gain (BWG) was calculated by the reviewers from data presented in this table.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** No adverse effect was observed on food consumption. Increases ($p \leq 0.05$) of 3-15% were noted in the 20,000 ppm group, resulting in an average food consumption increase of 5% in each sex over the controls. Differences ($p \leq 0.05$) were sporadic and minor at other doses. In high dose females, food efficiency appeared to decrease in the second half of the study, primarily in the last 6 months. This is consistent with the observation of emaciation in females at termination.

2. **Compound consumption:** The mean achieved dosages are shown in Table 1.

D. HEMATOLOGY: No adverse effects were noted on total leukocyte count or on differential leukocyte counts (Table 3). Eosinophils were decreased ($p \leq 0.05$) by 30-40% in the ≥1000 ppm males. Leukocytes were decreased ($p \leq 0.05$) by 12-37% in the ≥1000 ppm females; however, variation was high. These effects were considered minor. Data were re-evaluated

TABLE 4. Mean (±SD) for selected organ weights in rats treated with NNI-0001 for up to 104 weeks. [a]

Organ		Dose (ppm)			
		0	50	1000	20,000
Males					
Liver:	absolute (g)	9.74±1.64	9.84±1.00	11.73±1.44* (↑20)	14.09±2.84** (↑45)
	relative to body (%)	2.39±0.50	2.23±0.12	2.63±0.17	3.26±0.56** (↑36)
Testes:	absolute (g)	2.86±0.61	3.33±0.93	3.37±1.15	4.24±0.67** (↑48)
	relative to body (%)	0.70±0.15	0.76±0.20	0.76±0.25	0.98±0.13** (↑40)
Females					
Liver:	absolute (g)	6.64±1.09	6.33±0.85	7.79±1.11* (↑17)	8.89±0.95** (↑34)
	relative to body (%)	2.40±0.37	2.29±0.35	2.98±0.33** (↑24)	3.47±0.30** (↑45)
Kidneys:	absolute (g)	1.61±0.13	1.62±0.07	1.71±0.13	1.79±0.13** (↑11)
	relative to body (%)	0.59±0.04	0.58±0.03	0.66±0.04** (↑12)	0.70±0.06** (↑19)
Thyroid:	absolute (mg)	19.8±12.6	17.6±3.3	18.9±3.1	24.4±5.2** (↑23)
	relative to body (%)	0.0071±0.0045	0.0064±0.0013	0.0072±0.0011	0.0096±0.0020** (↑35)

a Data (n=10) were obtained from pages 97-100 of MRID 46817219. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

2. **Gross pathology:** The incidences of selected gross lesions are reported in Table 5. Increases ($p \leq 0.05$; except when noted) in the incidences (% treated vs % controls) of the following macroscopic lesions were noted in the 20,000 ppm males: (i) liver spot(s) (22% vs 6%); (ii) accentuated lobular pattern in the liver (48% vs 0%); (iii) coarse liver surface (40% vs 16%); (iv) liver enlargement (12% vs 2%; not statistically significant [NS]); (v) liver mass(es) (14% vs 0%); (vi) dark-colored kidney (12% vs 2%; NS); (vii) testicular mass(es) (86% vs 76%; NS); and (viii) dark-colored spleens (20% vs 2%). The dark-color spleens were not considered to be treatment-related since there were no corroborating microscopic lesions or organ weight changes. In the 1000 and 20,000 ppm females, increased ($p \leq 0.05$) incidences of darkly-colored liver (56-72% vs 4%), liver spot(s) (26-32% vs 6%), liver enlargement (14-62% vs 0%), and hair loss (44-56% vs 22%) were observed. Additionally, in the 20,000 ppm females, the incidence of emaciation (24% vs 10%, NS) and uterine masses (30% vs 20%, NS) were increased.

TABLE 6. Incidence (# affected/# examined [%]) of microscopic lesions in rats treated with NNI-0001 for up to 104 weeks.[a]

Microscopic lesion	Dose (ppm)			
	0	50	1000	20,000
Males				
Liver: Fatty change, hepatocyte, periportal	7/50 (14)	9/50 (18)	21/50** (42)	27/50** (54)
Slight	7	5	8	8
Moderate	0	4	13	19
Fatty change, hepatocyte, diffuse	1/50 (2)	4/50 (8)	4/50 (8)	6/50 (12)
Slight	1	4	1	3
Moderate	0	0	1	2
Severe	0	0	2	1
Kidney: Nephropathy, chronic	33/50 (66)	35/50 (70)	42/50* (84)	46/50** (92)
Slight	25	23	16	11
Moderate	8	11	24	32
Severe	0	1	2	3
Bone marrow: Increased hematopoiesis Vertebra	9/50 (18)	4/50 (8)	12/50 (24)	14/50 (28)
Slight	1	1	0	2
Moderate	2	2	2	3
Severe	6	1	10	9
Sternum	10/50 (20)	3/50* (6)	13/50 (26)	15/50 (30)
Slight	1	0	1	2
Moderate	3	2	1	3
Severe	6	1	11	10
Femur	9/50 (18)	5/50 (10)	15/50 (30)	15/50 (30)
Slight	1	3	2	2
Moderate	2	1	3	4
Severe	6	1	10	9
Thyroid: Follicular cell hypertrophy (all Slight)	1/50 (2)	0/50 (0)	1/50 (2)	39/50** (78)

(table continues next page)

incidence of preneoplastic lesions was evident in the testes.

Although increases in liver masses in the 20,000 ppm males (14% treated vs 0% controls) and uterine masses in the 20,000 ppm females (30% treated vs 20% controls) were noted grossly, histology did not support an increase in tumors in these organs. Without further evidence, this compound is not considered a carcinogen.

TABLE 7. Incidence (# affected/# examined [%]) of testicular neoplasia in rats treated with NNI-0001 for up to 104 weeks.[a]

Lesion	Dose (ppm)			
	0	50	1000	20,000
Gross mass(es)	38/50 (76)	38/50 (76)	34/50 (68)	43/50 (86)
Interstitial cell tumors	43/50 (86)	38/47[b] (81)	35/50 (70)	46/50 (92)

a Data were obtained from pages 109 and 122 of MRID 46817219. No statistical difference was found ($p \leq 0.05$).
b Examined for animals with gross lesions and animals killed *in extremis* or found dead

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The LOAEL was 1000 ppm, based on various toxic effects of the test substance detected in both sexes. The Sponsor listed most differences ($p \leq 0.05$) from the controls as toxic effects; however, several of these toxic effects were not considered adverse by the reviewers for reasons provided in the results section. Effects that were considered adverse are detailed below.

B. REVIEWER COMMENTS: No adverse treatment-related effects were observed on mortality, food consumption, leukocyte count, or differential leukocyte counts.

At 1000 and 20,000 ppm, increased ($p \leq 0.05$) incidences of hair loss were observed in females (44-56% treated vs 22% controls) and were first observed during Week 39. Increased ($p \leq 0.05$) incidence of slight folliculitis was noted on the lumbo-dorsal skin in the $\geq$1000 ppm females (42-44% vs 20%). Additionally, gross skin lesions were examined and the number of females with folliculitis was increased at $\geq$1000 ppm (22 affected at each dose vs 10 controls). Increases ($p \leq 0.05$) were observed in absolute and/or relative to body liver weight in both sexes (↑17-45%) and kidney weights in females (↑11-19%). In females, increased ($p \leq 0.05$) incidences of darkly-colored liver (56-72% vs 4%), liver spot(s) (26-32% vs 6%), and liver enlargement (14-62% vs 0%) were observed grossly. Except as noted, increased ($p \leq 0.05$) incidences (% treated vs % controls) in the following microscopic lesions were observed in the 1000 and 20,000 ppm groups: (i) periportal hepatocyte fatty change in males (42-54% vs 14%; slight to moderate severity) and females (34-36% vs 2%; slight to severe); (ii) slight to severe diffuse hepatocyte fatty change in males (12% vs 2%; at 20,000 ppm only and NS) and females (20-32% vs 4%); (iii) slight diffuse hepatocyte hypertrophy in females (44-62% vs 0%); (iv) chronic nephropathy in males (84-92% vs 66%; slight to severe) and females (58-60% vs 18%; slight to moderate); and (v) slight to severe hematopoiesis in vertebra, sternum, and femur in males (24-30% vs 18-20%; NS). Increases

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200a; OECD 451) for a carcinogenicity study in Rats.

C. **STUDY DEFICIENCIES:** Summary severity data for histological findings were not provided, but this deficiency does not affect the conclusions of this review.

(IET 01-0080)

Table 17 - 5 Histopathology - Incidence of microscopic neoplastic lesions in male rats
All animals examined

Site & Lesion	Dose (ppm) / No. of animals examined		0 50	50 50	1000 50	20000 50
Systemic tumor :		[N=]	50	50	50	50
Histiocytic sarcoma[Ma]			0	1	0	0
Mononuclear cell leukemia [Ma]			7	4	6	8
Skin :		[N=]	50	26 b	33 b	50
Papilloma [Be]			1	2	3	1
Keratoacanthoma [Be]			2	1	2	1
Trichoepithelioma [Be]			0	0	1	1
Fibroma [Be]			8	12	7	6
Fibrosarcoma [Ma]			1	0	1	0
Lipoma [Be]			1	2	1	0
Leiomyosarcoma [Ma]			0	0	0	1
Rhabdomyosarcoma [Ma]			0	1	0	0
Schwannoma [Be]			1	1	0	0
Schwannoma, malignant [Ma]			1	0	0	0
Mammary gland :		[N=]	50	5 b	17 b	50
Adenocarcinoma [Ma]			0	0	0	1
Fibroadenoma [Be]			1	0	2	0
Bone :		[N=]	50	5 b	17 b	50
Osteoma [Be]			1	0	0	0
Osteosarcoma [Ma]			0	0	0	1
Nasal cavity :		[N=]	50	5 b	17 b	50
Adenocarcinoma [Ma]			0	0	1	0
Lung :		[N=]	50	12 b	24 b	50
Adenoma [Be]			4	1	5	0
Adenocarcinoma [Ma]			0	1	0	1
Oral cavity :		[N=]	2 a	0 a	0 a	0 a
Papilloma [Be]			1	-	-	-
Basal cell adenoma [Be]			1	-	-	-
Liver :		[N=]	50	50	50	50
Hepatocellular adenoma [Be]			2	1	1	3
Hepatocellular carcinoma [Ma]			0	0	0	1
Pancreas :		[N=]	50	5 b	18 b	50
Islet cell adenoma [Be]			0	0	0	1
Islet cell carcinoma [Ma]			1	0	1	0
Kidney :		[N=]	50	50	50	50
Adenoma [Be]			0	0	0	1
Adenocarcinoma [Ma]			0	1	0	0
Urinary bladder :		[N=]	50	5 b	17 b	50
Transitional cell papilloma [Be]			0	0	0	2
Testis :		[N=]	50	47 b	50 b	50
Interstitial cell tumor [Be]			43	38	35	46 ↑6%

<Continued on next page>

[N=]: Number of animals examined at the site.
[Be]: Benign neoplasm; [Ma]: Malignant neoplasm.
a: Examined on the animals that showed macroscopic lesions. Not subjected to statistical analysis.
b: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.

(IET 01-0080)

Table 18 - 5 Histopathology - Incidence of microscopic neoplastic lesions in female rats
All animals examined

Site & Lesion	Dose (ppm)	0	50	1000	20000
	No. of animals examined	50	50	50	50
Systemic tumor :	[N=]	50	50	50	50
Malignant lymphoma (lymphocytic) [Ma]		0	0	1	0
Histiocytic sarcoma[Ma]		1	0	0	0
Mononuclear cell leukemia [Ma]		9	5	4	3
Skin :	[N=]	50	50	50	50
Papilloma [Be]		0	0	1	1
Keratoacanthoma [Be]		1	0	0	0
Basal cell adenoma [Be]		0	0	1	0
Fibroma [Be]		0	1	0	3
Leiomyosarcoma [Ma]		0	1	0	0
Schwannoma, malignant [Ma]		1	0	0	0
Mammary gland :	[N=]	50	26 b	24 b	50
Adenoma [Be]		1	0	0	0
Adenocarcinoma [Ma]		0	1	0	1
Fibroadenoma [Be]		8	10	12	8
Thymus :	[N=]	49	10 b	11 b	49
Not in section		1	0	0	1
Thymoma [Be]		0	1	0	0
Lung :	[N=]	50	11 b	13 b	50
Adenoma [Be]		2	1	1	1
Adenocarcinoma [Ma]		0	0	1	0
Heart :	[N=]	50	10 b	10 b	50
Schwannoma [Be]		1	1	0	0
Forestomach :	[N=]	50	9 b	11 b	50
Squamous cell carcinoma [Ma]		0	0	1	0
Liver :	[N=]	50	50	50	50
Hepatocellular adenoma [Be]		0	0	1	0
Pancreas :	[N=]	50	9 b	10 b	50
Islet cell adenoma [Be]		0	1	0	0
Kidney :	[N=]	50	50	50	50
Adenoma [Be]		0	0	0	1
Urinary bladder :	[N=]	50	8 b	12 b	50
Not in section		0	1	0	0
Transitional cell papilloma [Be]		1	0	1	2
Ovary :	[N=]	50	13 b	14 b	50
Not in section		0	1	0	0
Granulosa cell tumor, malignant [Ma]		0	0	1	1
Uterine horn:	[N=]	50	19 b	24 b	50
Not in section		0	1	0	0
Adenocarcinoma [Ma]		1	0	0	1
Leiomyoma [Be]		0	1	0	0
Leiomyosarcoma [Ma]		1	0	1	1

<Continued on next page>

[N=]: Number of animals examined at the site.
[Be]: Benign neoplasm; [Ma]: Malignant neoplasm.
b: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis

(IET 01-0080)

Table 18 - 7 Histopathology - Incidence of microscopic neoplastic lesions in female rats
All animals examined

Site & Lesion	Dose (ppm)	0	50	1000	20000
	No. of animals examined	50	50	50	50
<Continued from previous page>					
No. of benign neoplasms		54	48	40	40
No. of malignant neoplasms		20	16	14	12
No. of benign & malignant neoplasms		74	64	54	52
No. of animals with benign neoplasm(s)		31	32	31	32
No. of animals with malignant neoplasm(s)		16	16	13	12
No. of animals with neoplasm(s)		40	36	37	37

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.4200b [§83-2b]; Carcinogenicity Study in Mice

Work Assignment No. 4-1-124 K; formerly 3-1-124 K (MRID 46817220)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Rd, Bldg. 100, Ste. B
Durham, NC 27713

Primary Reviewer:
Ronnie J. Bever Jr., Ph.D.
Signature: Ronnie J. Bever Jr.
Date: 2/2/07

Secondary Reviewer:
Stephanie E. Foster, M.S.
Signature: Michael E Viana FOR STEPHANIE E. FOSTER
Date: 2/2/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 2/2/07

Quality Assurance:
Steve Brecher, Ph.D., D.A.B.T.
Signature: Steven Brecher
Date: 2/2/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Marion Copley, DVM, DABT **Signature**: Marion Copley
Registration Action Branch 3, Health Effects Division (7509P) **Date**: 6/26/07
Work Assignment Manager: Myron Ottley **Signature**: Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date**: 6/24/09
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity, dietary study in mice; OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 027602 **DP BARCODE**: D331553
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 96.7% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Takeuchi, Y. (2004) NNI-0001: Carcinogenicity study in mice. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET -01-0126, May 6, 2004. MRID 46817220. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., 2-5, Nihonbashi 1-Chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY - In this carcinogenicity study (MRID 46817220), NNI-0001 (Flubendiamide; 96.7% a.i.; Lot No.: 1FH0019M) was administered in the diet to (SPF) ICR (Crj: CD-1) mice (52/sex/dose) at nominal concentrations of 0, 50, 1000, or 10,000 ppm (equivalent to 0/0, 4.85/4.44, 94/93, and 988/937 mg/kg/day in males/females) for up to 78 weeks.

No adverse treatment-related effects were observed on mortality, clinical signs of toxicity, body weights, body weight gains, food consumption, leukocyte count, or differential leukocyte counts.

Hepatotoxicity was observed. At 1000 ppm, increased ($p<=0.05$) absolute liver weight was noted in the males (incr 41%). Increases in the incidence of darkened livers was observed in the 1000 and 10,000 ppm males (12-31% treated vs 0% controls; $p<=0.05$). Except as noted, increased ($p<=0.05$) incidences (% treated vs % controls) in the following microscopic lesions were observed in the 1000 and 10,000 ppm groups: (i) slight to severe centrilobular hepatocyte microvesicular fatty change in males (35% vs 14%) and females (27-35% vs 6%); (ii) slight to severe diffuse hepatocyte microvesicular fatty change in females (17-33% vs 2%); (iii) slight to moderate diffuse hepatocyte macrovesicular fatty change in females (23-29% vs 6%); (iv) slight to moderate periportal hepatocyte macrovesicular fatty change in females (27% vs 0%; 10,000 ppm only); (v) slight to moderate centrilobular hepatocyte hypertrophy in males (21-54% vs 0%) and females (10-14% vs 0%; slight severity); and (vi) slight to severe foci of cellular alteration (vacuolated cell type) in males (19% vs 6%; 10,000 ppm only). At 10,000 ppm, increased ($p<=0.01$) absolute and relative to body liver weights were observed in both sexes, and

245

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description: White crystals

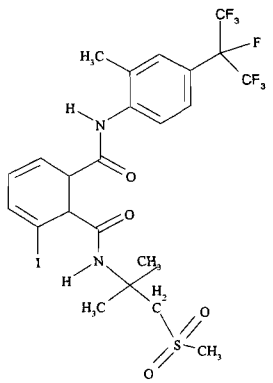
Lot Nos.: 1FH0019M

Purity: 96.7% a.i.

Stability of compound: Stability was confirmed in 20 and 20,000 ppm diets kept under a sealed, cold, and dark condition for 5 weeks, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient air for 8 days.

CAS #: 272451-65-7

Structure:



2. Vehicle - Diet

3. Test animals

Species: Mouse

Strain: (SPF)ICR (Crj: CD-1)

Age and group mean weight at study initiation: 5 weeks of age; 28.0-33.4 g males; 21.8-27.2 g females

Source: Atsugi Breeding Center, Charles River Japan, Inc. (Kanagawa)

Housing: Housed in aluminum cages with wire-mesh floors in stainless steel racks in groups of 4 (same sex and dose)

Diet: Certified diet MF Mash (Oriental Yeast Co., Ltd., Tokyo), *ad libitum*

Water: Filtered (sand and charcoal) well water, sterilized with sodium hypochlorite and UV light, *ad libitum*

Environmental conditions

- **Temperature:** 22-26°C
- **Humidity:** 39-73%
- **Air changes:** ≥10/hour
- **Photoperiod:** 12 hours light/12 hours dark

Acclimation period: 10 (males) or 11 (females) days

B. STUDY DESIGN

1. In life dates: Start: 12/17/01 End: 6/25/03

2. **Animal assignment:** The animals were randomly assigned to the test groups shown in Table 1. After allocation, it was confirmed that there were no statistically significant differences in the mean body weights among groups, and all individual body weights were within ±20% of the mean value of each sex.

TABLE 1. Study design. [a]

Test group	Nominal concentration in diet (ppm)	Dose to animal (mg/kg/day; M/F)	Terminal Sacrifice at Week 78 (# mice/sex)
Control	0	0/0	52
Low (LDT)	50	4.85/4.44	52
Mid (MDT)	1000	94/93	52
High (HDT)	10,000	988/937	52

a Data were obtained from pages 23, 82, and 83 of MRID 46817220.

3. **Dose-selection rationale:** Doses were selected on the basis of results from a concurrently submitted subchronic oral toxicity study (MRID 46817211, separate DER in this TXR) in the same strain of mouse. In this subchronic study, the LOAEL was 1000 ppm, based on slight hepatotoxicity in both sexes. At 1000 and 10,000 ppm, increased absolute and relative to body liver weights were noted in females, and increased incidences of centrilobular hepatocyte fatty change and centrilobular hepatocyte hypertrophy were observed in both sexes. At 10,000 ppm in males, relative liver weights were increased, and an increased incidence of darkened liver was observed. Increased levels of serum triglycerides were noted in both sexes at 10,000 ppm; however, the difference was not statistically significant, and the toxicological relevance was considered equivocal.

4. **Treatment preparation, analysis, and administration:** Dietary formulations were prepared by first making a premix of the appropriate amount of test substance with basal diet for each dose level. The premixes were then diluted with diet to the desired concentration. No adjustment for purity was made. Dietary formulations were sealed in plastic bags and stored in aluminum containers in the dark at 4°C until use. Dietary formulations were prepared once prior to initiation of treatment and once every 4 weeks during the treatment period. Concentrations at each dietary level were measured prior to treatment and at approximately 3, 9, and 15 months. Homogeneity (top, middle, bottom) of the test compound in each dietary formulation was tested in the first and last preparations, and approximately 6 and 12 months after the initiation of treatment. Stability was confirmed in 20 and 20,000 ppm diets kept under a sealed, cold, and dark condition for 5 weeks, further stored under a sealed and dark condition at room temperature for 5 days, and then exposed to ambient air for 8 days (IET 00-0156).

Results:

Homogeneity (% CV): 0.7-8.5%

Stability (% nominal): 94-102%

247

Concentration (% of nominal): 96-105%

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

5. **Statistics** - Significance was indicated at 5 and 1% probability.

Parameter	Statistical procedure
Body weight Food consumption Total and differential leukocyte counts Organ weights	Bartlett's test was conducted. One-way analysis of variance (ANOVA) and Dunnett's test were performed when group variances were homogeneous. The Kruskal-Wallis test and a Dunnett-type mean rank sum test were conducted when group variances were heterogeneous.
Mortality	Life table analysis was performed.
Clinical signs Gross pathology Histopathology	Fisher's exact probability test (one-tail analysis) was conducted.

The statistical analyses were considered appropriate.

C. METHODS

1. **Observations:** Animals were observed for mortality and moribundity at least twice daily, except once daily on weekends and holidays. Clinical signs of toxicity were recorded once daily. Detailed clinical examinations, including palpation of masses, were performed at least weekly. The detailed clinical examinations included a limited functional observational battery, evaluating the following parameters:

Home cage	Handling	Open field
Excitement	Handling difficulty	Jumping
Sedation	Changes in muscle tone	Circling
Abnormal posture	Tremors	Convulsions
Abnormal behavior	Palpebral closure	Abnormal gait
	Salivation	Spontaneous motor activity
	Lacrimation	Respiration
	Discharges	Vocalization
	Exophthalmos	Piloerection
	Changes in body temperature	Abnormal posture
	Abnormal respiratory sound	Abnormal behavior
	Changes in fur	
	Changes in skin and mucous membranes	

Environmental conditions, duration of observation in the open field, details about the observer, and positive control data were not reported. The time of onset, nature, severity, and duration of signs were recorded when any abnormalities were detected.

2. **Body weight:** All animals were weighed prior to treatment, at treatment initiation, weekly for 13 weeks, once every 4 weeks from Week 16 to 76, at week 78, and at termination. Body weight gains were not reported.

3. **Food consumption and compound intake:** The report stated that food consumption was calculated as a mean value (g food/mouse/day) for each cage. Mean food consumption was determined for a period of 3 consecutive days weekly from Week 1 to 13 and every 4 weeks from Week 16. A weighed average of the group mean food consumption during the treatment period was also calculated for each sex. Compound intake (mg/kg bw/day) values were calculated as group mean food consumption x nominal concentration/group mean body weight. A weighed average of the group mean test substance intake during the treatment period was also calculated for each sex.

4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were not performed and are not required by the guideline (OPPTS 870.4200b).

5. **Hematology and clinical chemistry:** Blood samples were collected for hematology during Week 78 from all surviving animals. Blood was collected from the posterior vena cava while mice were under ether anesthesia. Blood smears were also prepared, and were examined when the large unstained cell count was 0.1 x $10^3/\mu L$ or more or there were any parameters not determined successfully by the hematology analyzer. Blood smears were prepared at Week 52, but were not examined. The CHECKED (X) parameters were examined.

   a. **Hematology**

	Hematocrit (HCT)	X	Leukocyte differential count*
	Hemoglobin (HGB)		Mean corpuscular hemoglobin (MCH)
X	Leukocyte count (WBC)		Mean corpuscular hemoglobin concentration (MCHC)
	Erythrocyte count (RBC)		Mean corpuscular volume (MCV)
	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (Control and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451

   b. **Clinical chemistry:** Clinical chemistry was not performed and is not required by the guideline (OPPTS 870.4200b).

6. **Urinalysis:** Urinalysis was not performed and is not required by the guideline (OPPTS 870.4200b).

7. **Sacrifice and pathology:** Animals were killed after 78 weeks of treatment by exsanguination while animals were under deep ether anesthesia. All animals, including those found dead or killed *in extremis*, were subjected to a detailed necropsy, and the following CHECKED (X) tissues were collected. The (XX) organs were weighed from 10 animals/sex/dose group selected in order of animal number.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroids* [a]
X	Rectum*	X	Urinary bladder*	XX	Thyroids* [a]
XX	Liver*+ [a]	XX	Testes*+	X	Harderian gland
XX	Gall bladder* (not rat) [a]	XX	Epididymides*+		OTHER
	Bile duct* (rat)	X	Prostate*	X	Bone (sternum and femur)
X	Pancreas*	X	Seminal vesicle*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus*+ (horns and cervix)	X	Knee joint
X	Lung*++	X	Mammary gland*	X	Head (oral mucosa; middle ear)
X	Nose* (nasal cavity)	X	Coagulating glands	X	All gross lesions and masses*
X	Pharynx*	X	Vagina		
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200.
a Thyroids and parathyroids were weighed together; liver and gallbladder were weighed together.
\+ Organ weight required in carcinogenicity studies.
++ Organ weight required if inhalation route.

The collected tissues were fixed in 10% neutral-buffered formalin. The lungs were instilled with formalin before fixation. The head and tongue were collected, but were not examined microscopically. The fixed tissues were embedded in paraffin, stained with hematoxylin and eosin, routinely prepared, and examined microscopically as follows: Histopathological examinations were performed on all tissues collected from the 0 and 10,000 ppm groups; all tissues from animals killed *in extremis* or found dead from all dose groups; the liver, kidneys, thyroids, and gross lesions of both sexes and pituitaries of females in all dose groups. Microscopic lesions were graded as slight, moderate, or severe.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** No adverse treatment-related effects were observed on survival. The 1000 ppm males had increased ($p \leq 0.05$) mortality at Weeks 37 and 38, but this response was transient and unrelated to dose. Mortality rates at Week 78 were 38-46% in males and 23-29% in females, and the response was unrelated to dose in both sexes.

2. **Clinical signs of toxicity:** No adverse treatment-related effects were observed on clinical signs of toxicity. Decreased spontaneous motor activity was observed (6/52, 8/52, 5/52, 12/52, controls to high dose, respectively) in the 10,000 ppm males (23%) compared to the controls (12%), but this was considered a minor difference.

B. BODY WEIGHT AND BODY WEIGHT GAINS: No adverse treatment-related effects were observed on body weight and body weight gains (Table 2). Decreased ($p \leq 0.01$) body weight was observed in the 10,000 ppm males (↓7%) during Week 52, but no other statistically significant difference was observed in either sex at any other time point evaluated during the study. At 10,000 ppm, the overall (Weeks 0-78) body weight gain was decreased by 8% in males and by 2% in females, which was considered a minor difference.

TABLE 2. Mean (±SD) body weights and body weight gains (g) at selected intervals in mice treated with NNI-0001 for up to 78 weeks. [a]

Week(s)	Dose (ppm)			
	0	50	1000	10,000
Males				
0	31.0±1.3	31.0±1.3	31.0±1.3	31.0±1.3
13	48.2±4.6	47.7±4.3	48.3±4.2	47.6±4.6
52	55.8±5.6	54.3±5.4	54.3±5.9	52.1±6.7** (↓7)
78	55.5±5.4	54.3±6.5	55.4±5.7	53.6±5.9
BWG: 0-13	17.2	16.7	17.3	16.6
BWG: 13-52	7.6	6.6	6.0	4.5
BWG: 52-78	-0.3	0.0	1.1	1.5
BWG: 0-78	24.5	23.3	24.4	22.6
Females				
0	24.6±1.4	24.6±1.4	24.6±1.4	24.6±1.4
13	38.8±5.0	38.2±4.5	38.5±4.8	39.1±4.9
52	52.8±9.0	52.8±9.1	50.4±7.9	52.2±7.2
78	53.5±10.8	53.8±8.6	50.7±7.7	52.8±8.3
BWG: 0-13	14.2	13.6	13.9	14.5
BWG: 13-52	14.0	14.6	11.9	13.1
BWG: 52-78	0.7	1.0	0.3	0.6
BWG: 0-78	28.9	29.2	26.1	28.2

a Data (n=28-52) were obtained from pages 70-75 of MRID 46817220. Percent differences from controls (calculated by reviewers) are included in parentheses. Body weight gain (BWG) was calculated by the reviewers from data presented in this table.

** Significantly different from controls; $p \leq 0.01$

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** No treatment-related effect was observed on food consumption. Increases ($p \leq 0.05$) were noted in the 10,000 ppm females during Week 36 (↑16%) and 1000 ppm females during Week 60 (↑22%). Average food consumption during the study was increased by 5-6% at 10,000 ppm.

2. **Compound consumption:** The mean achieved dosages are shown in Table 1.

D. HEMATOLOGY: No adverse effects were noted on total leukocyte count or on differential leukocyte counts. Eosinophils were decreased ($p \leq 0.05$) by 43% in the 10,000 ppm males, but this was not considered toxicologically relevant.

252

E. SACRIFICE AND PATHOLOGY

1. **Organ weights:** (Table 3) Increased ($p \leq 0.01$) absolute and relative to body liver weights were observed in the 10,000 ppm males (↑52, 55%, respectively) and females (↑75, 74%, respectively). Increased ($p \leq 0.05$) absolute liver weight was noted in the 1000 ppm males (↑41%). Increased ($p \leq 0.01$) absolute and relative thyroid weights were observed at 10,000 ppm in both sexes (↑167-175%). Increased absolute and relative adrenal weight was observed in the 10,000 ppm males (↑32-44%), but adrenal toxicity was not corroborated by other clinical or pathological data.

TABLE 3. Mean (±SD) for selected organ weights in mice treated with NNI-0001 for up to 78 weeks. [a]

Organ	Dose (ppm)			
	0	50	1000	10,000
Males				
Liver: absolute (g)	2.63±0.69	2.71±0.50	3.71±1.16* (↑41)	4.00±1.08** (↑52)
relative to body (%)	4.68±1.53	5.28±1.20	6.63±2.42	7.27±2.01** (↑55)
Thyroid: absolute (mg)	6.6±2.0	6.5±1.7	8.9±2.2	18.1±6.9** (↑174)
relative to body (%)	0.012±0.003	0.013±0.003	0.016±0.005	0.033±0.014** (↑175)
Females				
Liver: absolute (g)	2.01±0.31	2.19±0.53	2.48±0.25	3.51±1.06** (↑75)
relative to body (%)	4.17±0.92	4.19±0.71	5.17±0.64	7.26±1.79** (↑74)
Thyroid: absolute (mg)	6.7±1.3	7.0±1.6	10.3±3.8	17.9±8.0** (↑167)
relative to body (%)	0.014±0.004	0.014±0.003	0.022±0.008	0.038±0.017** (↑171)

a Data (n=10) were obtained from pages 87-90 of MRID 46817220. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

2. **Gross pathology:** The incidences of selected gross lesions are reported in Table 4. Increases ($p \leq 0.05$; except when noted) in the incidences (% treated vs % controls) of the following macroscopic lesions were noted: darkened livers in the ≥1000 ppm males (12-31% vs 0%) and the 10,000 ppm females (10% vs 0%); and liver spot(s) in the 10,000 ppm males (17% vs 8%). An increased ($p \leq 0.05$) incidence of enlarged thyroid was observed at ≥1000 ppm in both sexes (10-63% treated vs 0% controls). Other differences ($p \leq 0.05$) were unrelated to dose.

TABLE 4. Incidence (# affected/# examined [%]) of gross lesions in mice treated with NNI-0001 for up to 78 weeks. [a]

Gross lesion	Dose (ppm)			
	0	50	1000	10,000
Males				
Liver: Dark in color	0/52 (0)	0/52 (0)	6/52* (12)	16/52** (31)
Spot(s)	4/52 (8)	2/52 (4)	4/52 (8)	9/52 (17)
Thyroid: Enlargement	0/52 (0)	0/52 (0)	5/52* (10)	28/52** (54)
Females				
Liver: Dark in color	0/52 (0)	0/52 (0)	1/52 (2)	5/52* (10)
Thyroid: Enlargement	0/52 (0)	0/52 (0)	10/52** (19)	33/52** (63)

a Data were obtained from pages 97-100 and 106-109 of MRID 46817220.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

3. Microscopic pathology

a. Non-neoplastic: The incidences of selected microscopic lesions are reported in Table 5. Except as noted, increased ($p \leq 0.05$) incidences (% treated vs % controls) in the following microscopic hepatic lesions were observed in the 1000 and 10,000 ppm groups: (i) slight to severe centrilobular hepatocyte microvesicular fatty change in males (35% vs 14%) and females (27-35% vs 6%); (ii) slight to severe diffuse hepatocyte microvesicular fatty change in females (17-33% vs 2%); (iii) slight to moderate diffuse hepatocyte macrovesicular fatty change in females (23-29% vs 6%); (iv) slight to moderate periportal hepatocyte macrovesicular fatty change in females (27% vs 0%; 10,000 ppm only); (v) slight to moderate centrilobular hepatocyte hypertrophy in males (21-54% vs 0%) and females (10-14% vs 0%; slight severity); and (vi) slight to severe foci of cellular alteration (vacuolated cell type) in males (19% vs 6%; 10,000 ppm only). An increased incidence in slight to moderate subcapsular cell hyperplasia in adrenals was noted in the 10,000 ppm females, but the increase was not statistically significant, was slight in magnitude, and an effect was not corroborated by other pathological evidence. Consequently, this effect was not considered adverse. In the thyroid in both sexes, increased ($p \leq 0.01$) incidences were noted of follicular cell hypertrophy with hydropic change at $\geq$1000 ppm, increased large-sized follicles at $\geq$1000 ppm, and altered colloid at 10,000 ppm. Additionally, an increased ($p \leq 0.05$) incidence of thyroid follicular cell hyperplasia was noted in the 10,000 ppm females. Other differences ($p \leq 0.05$) were unrelated to dose or were decreases in abnormality incidence.

TABLE 5. Incidence (# affected/# examined [%]) of microscopic lesions in mice treated with NNI-0001 for up to 78 weeks.[a]

Microscopic lesion	Dose (ppm)			
	0	50	1000	10,000
Males				
Liver: Fatty change, microvesicular, hepatocyte, centrilobular	7/52 (14)	3/52 (6)	18/52* (35)	18/52* (35)
Slight	6	1	12	9
Moderate	1	2	5	8
Severe	0	0	1	1
Hypertrophy, hepatocyte, centrilobular	0/52 (0)	0/52 (0)	11/52** (21)	28/52** (54)
Slight	0	0	9	10
Moderate	0	0	2	18
Foci of cellular alteration (vacuolated cell type)	3/52 (6)	4/52 (8)	4/52 (8)	10/52* (19)
Slight	0	0	1	5
Moderate	1	2	2	4
Severe	2	2	1	1
Thyroid: Follicular cell hypertrophy with hydropic change	0/52 (0)	0/52 (0)	8/52** (15)	30/52** (58)
Increased large-sized follicle	0/52 (0)	0/52 (0)	17/52** (34)	36/52** (69)
Altered colloid	1/52 (2)	1/52 (2)	2/52 (4)	11/52** (21)
Females				
Liver: Fatty change, microvesicular, hepatocyte, centrilobular	3/52 (6)	1/52 (2)	14/52** (27)	18/52** (35)
Slight	2	1	5	6
Moderate	1	0	9	9
Severe	0	0	0	3
Fatty change, microvesicular, hepatocyte, diffuse	1/52 (2)	3/52 (6)	9/52** (17)	17/52** (33)
Slight	1	3	7	8
Moderate	0	0	2	5
Severe	0	0	0	4
Fatty change, macrovesicular, hepatocyte, diffuse	3/52 (6)	5/52 (10)	15/52** (29)	12/52* (23)
Slight	3	5	9	8
Moderate	0	0	6	4
Fatty change, macrovesicular, hepatocyte, periportal	0/52 (0)	1/52 (2)	0/52 (0)	14/52** (27)
Slight	0	1	0	6
Moderate	0	0	0	8

Footnotes continue on next page

Liver:	Hypertrophy, hepatocyte, centrilobular Slight	0/52 (0)	0/52 (0)	5/52* (10)	7/52** (14)
Thyroid:	Follicular cell hypertrophy with hydropic change	0/52 (0)	0/52 (0)	8/52** (15)	16/52** (31)
	Increased large-sized follicle	5/52 (10)	3/52 (6)	25/52** (48)	42/52** (81)
	Altered colloid	3/52 (6)	1/52 (2)	5/52 (10)	32/52** (62)
	Follicular cell hyperplasia	0/52 (0)	0/52 (0)	0/52 (0)	6/52* (12)
Adrenal:	hyperplasia, subcapsular cell	12/52 (23)	2/13 [b]	0/12 [b]	19/52 (37)
	Slight	7	2	0	15
	Moderate	5	0	0	4

a Data were obtained from pages 133-138, 149-154, and 299-554 of MRID 46817220.
b Examined the animals that showed macroscopic lesions at terminal kill and all animals killed *in extremis* or found dead during the study.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

b. Neoplastic: Incidences of neoplastic lesions were reported in the Study Report on pages 114-115 and 120-122 and are included as an Attachment to this DER. No treatment-related effect was observed on the incidence of neoplasia. Earlier occurrence of neoplasms and increases of rare types of tumors were also not observed.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The LOAEL was 1000 ppm, based on various toxic effects on the liver and thyroid detected in both sexes (discussed under reviewer comments). The Sponsor concluded that the test compound has no carcinogenic potential in mice.

B. REVIEWER COMMENTS: The reviewer agrees with the investigators' conclusions. No adverse treatment-related effects were observed on mortality, clinical signs of toxicity, body weights, body weight gains, food consumption, leukocyte count, or differential leukocyte counts.

Hepatotoxicity was observed. Increased ($p \leq 0.05$) absolute liver weight was noted in the 1000 ppm males (↑41%). Increased ($p \leq 0.01$) absolute and relative to body liver weights were observed in the 10,000 ppm males (↑52-55%) and females (↑74-75%). Increases ($p \leq 0.05$; except when noted) in the incidences (% treated vs % controls) of the following macroscopic lesions were noted: darkened livers in the ≥1000 ppm males (12-31% vs 0%) and the 10,000 ppm females (10% vs 0%); and liver spot(s) in the 10,000 ppm males (17% vs 8%). Except as noted, increased ($p \leq 0.05$) incidences (% treated vs % controls) in the following microscopic hepatic lesions were observed in the 1000 and 10,000 ppm groups: (i) slight to severe centrilobular hepatocyte microvesicular fatty change in males (35% vs 14%) and females (27-35% vs 6%); (ii) slight to severe diffuse hepatocyte microvesicular fatty

change in females (17-33% vs 2%); (iii) slight to moderate diffuse hepatocyte macrovesicular fatty change in females (23-29% vs 6%); (iv) slight to moderate periportal hepatocyte macrovesicular fatty change in females (27% vs 0%; 10,000 ppm only); (v) slight to moderate centrilobular hepatocyte hypertrophy in males (21-54% vs 0%) and females (10-14% vs 0%; slight severity); and (vi) slight to severe foci of cellular alteration (vacuolated cell type) in males (19% vs 6%; 10,000 ppm only).

The thyroid was also affected. Increased ($p \leq 0.01$) absolute and relative weights were observed at 10,000 ppm in both sexes (↑167-175%). An increased ($p \leq 0.05$) incidence of enlargement was observed at ≥1000 ppm in both sexes (10-63% treated vs 0% controls). In both sexes, increased ($p \leq 0.01$) incidences were noted of follicular cell hypertrophy with hydropic change at ≥1000 ppm, increased large-sized follicles at ≥1000 ppm, and altered colloid at 10,000 ppm. Additionally, an increased ($p \leq 0.05$) incidence of follicular cell hyperplasia was noted in the 10,000 ppm females.

The LOAEL is 1000 ppm (equivalent to 94/93 mg/kg/day in males/females), based on hepatotoxicity in both sexes. The NOAEL is 50 ppm (equivalent to 4.85/4.44 mg/kg/day in males/females).

At the doses tested, there was no treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate based on hepatotoxicity observed in both sexes at 1000 ppm..

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

C. **STUDY DEFICIENCIES:** Environmental conditions, duration of observation in the open field, details about the observer, and positive control data were not reported. Tabulated severity data for histological findings were not provided, Group mean and standard deviation for body weights should have been calculated for each individual test group at Week 0. However, these deficiencies do not affect the conclusions of this review.

257

ATTACHMENT

The following are pages 114 – 115, 120 - 122 of the study report.

(IET 01-0126)

Table 17 - 5 Histopathology - Incidence of microscopic neoplastic lesions in male mice
All animals examined

Site & Lesion	Dose (ppm)	0	50	1000	10000
	No. of animals examined	52	52	52	52
Systemic tumor :	[N=]	52	52	52	52
Malignant lymphoma (lymphocytic) [Ma]		5	7	7	5
Malignant lymphoma (mixed cell) [Ma]		1	1	0	0
Skin :	[N=]	52	33 b	28 b	52
Papilloma [Be]		1	0	0	0
Fibroma [Be]		1	0	0	0
Liposarcoma [Ma]		0	0	1	0
Hemangiosarcoma [Ma]		0	0	0	1
Schwannoma, malignant [Ma]		1	0	1	0
Spleen :	[N=]	52	31 b	28 b	52
Hemangiosarcoma [Ma]		2	0	0	1
Lung :	[N=]	52	28 b	28 b	52
Adenoma [Be]		16	7	4	15
Adenocarcinoma [Ma]		5	3	3	7
Oral cavity :	[N=]	0 a	0 a	0 a	1 a
Squamous cell carcinoma [Ma]		-	-	-	1
Tongue :	[N=]	1 a	0 a	0 a	0 a
Squamous cell carcinoma [Ma]		1	-	-	-
Forestomach :	[N=]	52	24 b	22 b	52
Papilloma [Be]		0	0	0	2
Small intestine :	[N=]	52	25 b	22 b	52
Adenocarcinoma [Ma]		0	0	0	1
Liver :	[N=]	52	52	52	52
Hepatocellular adenoma [Be]		9	9	13	12
Hepatocellular carcinoma [Ma]		3	6	2	2
Hepatoblastoma [Ma]		0	1	0	0
Hemangiosarcoma [Ma]		3	1	0	4
Kidney :	[N=]	52	52	52	52
Adenoma [Be]		0	0	0	1
Urinary bladder :	[N=]	52	31 b	26 b	52
Transitional cell carcinoma [Ma]		1	1	0	0
Epididymis :	[N=]	52	26 b	23 b	52
Histiocytic sarcoma [Ma]		1	0	0	0
Seminal vesicle :	[N=]	52	41 b	36 b	52
Adenocarcinoma [Ma]		1	0	0	0
Coagulating gland :	[N=]	52	41 b	36 b	52
Adenoma [Be]		2	0	0	0
Adrenal :	[N=]	52	24 b	24 b	52
Cortical adenoma [Be]		0	0	1	1

<Continued on next page>

[N=]: Number of animals examined at the site.
[Be]: Benign neoplasm; [Ma]: Malignant neoplasm.
a: Examined on the animals that showed macroscopic lesions. Not subjected to statistical analysis
b: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.

(IET 01-0126)

Table 17 - 6 Histopathology - Incidence of microscopic neoplastic lesions in male mice
All animals examined

Site & Lesion	Dose (ppm)	0	50	1000	10000
	No. of animals examined	52	52	52	52
<Continued from previous page>					
Adrenal :	[N=]	52	24 b	24 b	52
Subcapsular cell adenoma [Be]		1	0	0	0
Harderian gland :	[N=]	52	24 b	22 b	52
Adenoma [Be]		3	3	0	2
Thoracic cavity :	[N=]	0 a	1 a	0 a	0 a
Hemangiosarcoma [Ma]		-	1	-	-
Abdominal cavity :	[N=]	3 a	0 a	0 a	0 a
Hemangiosarcoma [Ma]		1	-	-	-
Histiocytic sarcoma [Ma]		1	-	-	-

******** End of list ********

No neoplastic lesions were observed at other sites.
See Table 19 for number of animals examined at the sites not listed here.

No. of benign neoplasms	33	19	18	33
No. of malignant neoplasms	26	21	14	22
No. of benign & malignant neoplasms	59	40	32	55
No. of animals with benign neoplasm(s)	26	16	17	24
No. of animals with malignant neoplasm(s)	20	19	12	20
No. of animals with neoplasm(s)	37	30	25	38

[N=]: Number of animals examined at the site.
[Be]: Benign neoplasm; [Ma]: Malignant neoplasm.
a: Examined on the animals that showed macroscopic lesions. Not subjected to statistical analysis.
b: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.

(IET 01-0126)

Table 18 - 5 Histopathology - Incidence of microscopic neoplastic lesions in female mice
All animals examined

Site & Lesion	Dose (ppm) / No of animals examined		0 / 52	50 / 52	1000 / 52	10000 / 52
Systemic tumor :		[N=]	52	52	52	52
Malignant lymphoma (lymphocytic) [Ma]			8	5	9	10
Malignant lymphoma (mixed cell) [Ma]			0	1	0	2
Histiocytic sarcoma[Ma]			0	1	0	0
Skin :		[N=]	52	23 b	20 b	52
Papilloma [Be]			0	0	1	0
Squamous cell carcinoma [Ma]			0	0	0	1
Basal cell carcinoma [Ma]			0	0	1	0
Sebaceous adenoma [Be]			1	0	0	0
Lipoma [Be]			1	0	0	0
Liposarcoma [Ma]			1	0	0	0
Hemangiosarcoma [Ma]			1	0	0	0
Schwannoma, malignant [Ma]			0	0	0	1
Fibrous histiocytoma, malignant [Ma]			0	1	2	1
Mammary gland :		[N=]	52	17 b	12 b	52
Adenosquamous carcinoma [Ma]			0	0	0	1
Adenocarcinoma [Ma]			3	5	1	0
Bone marrow :		[N=]	52	13 b	12 b	52
Hemangiosarcoma [Ma]			0	1	0	0
Spleen :		[N=]	52	22 b	17 b	52
Hemangiosarcoma [Ma]			2	4	1	1
Bone :		[N=]	52	13 b	13 b	52
Osteosarcoma [Ma]			0	0	0	1
Lung :		[N=]	52	22 b	20 b	52
Adenoma [Be]			8	5	6	8
Adenocarcinoma [Ma]			3	4	2	7
Tongue :		[N=]	1 a	0 a	0 a	0 a
Squamous cell carcinoma [Ma]			1	-	-	-
Forestomach :		[N=]	52	13 b	14 b	52
Papilloma [Be]			0	0	0	1
Glandular stomach :		[N=]	52	13 b	15 b	52
Sarcoma, undifferentiated [Ma]			0	0	0	1
Small intestine :		[N=]	52	14 b	12 b	52
Adenoma [Be]			0	1	0	0

<Continued on next page>

[N=]: Number of animals examined at the site.
[Be]: Benign neoplasm; [Ma]: Malignant neoplasm.
a: Examined on the animals that showed macroscopic lesions. Not subjected to statistical analysis.
b: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.

(IET 01-0126)

Table 18 - 6 Histopathology - Incidence of microscopic neoplastic lesions in female mice
All animals examined

Site & Lesion	Dose (ppm)	0	50	1000	10000
	No. of animals examined	52	52	52	52
<Continued from previous page>					
Liver :	[N=]	52	52	52	52
Hepatocellular adenoma [Be]		1	2	0	1
Hepatocellular carcinoma [Ma]		1	0	0	0
Hemangiosarcoma [Ma]		0	4	1	0
Urinary bladder :	[N=]	52	13 b	12 b	52
Submucosal mesenchymal tumor [Be]		1	0	0	0
Ovary :	[N=]	52	40 b	36 b	52
Adenoma [Be]		1	0	0	0
Theca-cell tumor [Be]		0	0	0	1
Luteoma [Be]		1	0	0	1
Granulosa cell tumor [Be]		1	0	0	0
Hemangiosarcoma [Ma]		0	1	0	0
Granular cell tumor, malignant [Ma]		0	0	0	1
Uterine horn:	[N=]	52	23 b	22 b	52
Adenoma [Be]		1	0	0	0
Leiomyoma [Be]		0	0	1	0
Leiomyosarcoma [Ma]		0	0	0	3
Endometrial stromal polyp [Be]		0	0	0	1
Hemangioma [Be]		0	0	1	1
Hemangiosarcoma [Ma]		2	1	1	3
Uterine cervix:	[N=]	52	14 b	12 b	52
Leiomyoma [Be]		1	0	0	0
Leiomyosarcoma [Ma]		2	0	0	0
Vagina :	[N=]	52	13 b	12 b	52
Leiomyoma [Be]		1	0	0	0
Hemangiosarcoma [Ma]		1	0	0	0
Pituitary :	[N=]	52	52	52	52
Anterior adenoma [Be]		1	2	2	3
Thyroid :	[N=]	52	52	52	52
Follicular cell adenoma [Be]		0	1	0	0
Harderian gland :	[N=]	52	13 b	12 b	52
Adenocarcinoma [Ma]		0	0	0	1
Abdominal cavity :	[N=]	1 a	2 a	3 a	2 a
Leiomyosarcoma [Ma]		0	0	1	0
Hemangiosarcoma [Ma]		0	0	1	1

<Continued on next page>

[N=]: Number of animals examined at the site.
[Be]: Benign neoplasm; [Ma]: Malignant neoplasm.
a: Examined on the animals that showed macroscopic lesions. Not subjected to statistical analysis.
b: Examined on the animals that showed macroscopic lesions at terminal kill and on all the animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.

247

(IEI 01-0126)

Table 18 - 7 Histopathology - Incidence of microscopic neoplastic lesions in female mice
All animals examined

Site & Lesion	Dose (ppm)	0	50	1000	10000
	No. of animals examined	52	52	52	52
<Continued from previous page>					
******** End of list ********					
No neoplastic lesions were observed at other sites.					
See Table 20 for number of animals examined at the sites not listed here.					
No. of benign neoplasms		19	11	11	17
No. of malignant neoplasms		25	28	20	35
No. of benign & malignant neoplasms		44	39	31	52
No. of animals with benign neoplasm(s)		15	10	11	15
No. of animals with malignant neoplasm(s)		19	23	18	29
No. of animals with neoplasm(s)		30	27	26	38

EPA Reviewer: Nancy E. McCarroll
Toxicology Branch, Health Effects Division (7509P)
EPA Secondary Reviewer: Gregory Akerman, PhD.
Toxicology Branch, Health Effects Division (7509P)

Signature: Nancy McCarroll
Date: 07/05/07
Signature: Greg Akerman
Date: 07/05/07
EPA Template version 02/06

TXR#: 0054319

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** (Bacterial system, *Salmonella typhimurium; E. coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100[1] [§ 84 2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 027602 **DP BARCODE:** DP 331553

TEST MATERIAL (PURITY): NNI-0001 technical (97.8%, Lot No. 1FH0018P)

SYNONYMS: Flubendiamide; N2- [1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-{2-methyl-4-[1,2,2,2,-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}phthalamide

CITATION: Inagaki, K. (2003). Amendment 1 Bacterial reverse mutation test of NNI-0001. Toxicology & Pharmaceutical Research Center, Nihon Nohyaku Co., Ltd., Osaka, Japan. Final Report No. LSRC-TO2-018A, March 5, 2003. MRID 46817221. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., Osaka, Japan

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 46817221), strains TA1535, TA1537, TA98, TA100 of *Salmonella typhimurium* and strain WP2 (*uvrA*) of *Escherichia coli* were exposed to NNI-0001 technical (97.8%, Lot No. 1FH0018P) in dimethyl sulfoxide (DMSO) at concentrations of 0, 1.22, 4.88, 19.5, 78.1, 313, 1250, or 5000 µg/plate in the absence of mammalian metabolic activation (S9-mix) (Trial1) and 0, 3.86, 11.6, 34.7, 104 or 313µg/plate –S9 or 61.7, 185, 556, 1670, or 5000 µg/plate +S9 (Trial 2). The S9-fraction was obtained from phenobarbital and 5,6-benzoflavone induced male Sprague-Dawley rat liver.

NNI-0001 technical was tested to insoluble concentrations (≥ 104 µg/plate –S9 and ≥ 556 µg/plate +S9) but failed to induce a cytotoxic or mutagenic response. The solvent and positive control values induced the expected responses in the respective strains. **There was no evidence of induced mutant colonies over background.**

[1]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5100[1]; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: NNI-0001 technical

Description: White crystal

Lot/Batch #: 1FH0018P

Purity: 97.8%

CAS # of TGAI: 272451-65-7

Solvent Used: Dimethyl sulfoxide (DMSO)

2. Control materials:

Negative: None

Solvent (final conc.): DMSO / 0.1 mL/plate

Positive: Nonactivation:

Sodium azide	0.5 µg /plate TA1535
2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01 µg /plate TA100
	0.02 µg /plate WP_2 *uvrA*
2-Nitrofluorene	0.1 µg/plate TA198
9-Aminoacridine	80 µg /plate TA1537

Activation:

2-Aminoanthracene (2-Anthramine) 0.5, 1, 2, 10 µg/plate for strains TA98, TA100, TA1535 and TA1537, and WP_2 *uvrA*, respectively.

3. Activation: S9 derived from male 7-week old Sprague-Dawley rats (200-300 g)

x	Induced		Aroclor 1254	x	Rat	x	Liver
	Non-induced	x	Phenobarbitol & 5,6 benzoflavone		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition:

$MgCl_2$	8 µmol
KCl	33µmol
Glucose-6-phosphate	5 µmol
NADH	4 µmol
NADPH	4 µmol
Sodium phosphate buffer (pH 7.4)	100 mM
S9-fraction	10%

4. Test organisms: *S. typhimurium* strains

	TA97	x	TA98	x	TA100		TA102		TA104
x	TA1535	x	TA1537		TA1538		list any others: *E. coli* WP_2 *uvrA*		

Properly maintained?	x	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	x	Yes		No

5. Test compound concentrations used:

Preliminary Dose-range Finding & Mutation Assay (Trial1): (all strains, triplicate plating)
0, 1.22, 4.88, 19.5, 78.1, 313, 1250, or 5000 µg/plate +/-S9

Mutation Assay (Trial 2): (all strains, triplicate plating)

Nonactivated conditions: 0, 3.86, 11.6, 34.7, 104 or 313 µg/plate
Activated conditions: 0, 61.7, 185, 556, 1670, or 5000 µg/plate +S9

B. TEST PERFORMANCE:

1. Type of *Salmonella* assay:

	Standard plate test
X	Pre-incubation (20 minutes)
	"Prival" modification *(i.e. azo-reduction method)*
	Spot test
	Other

2. Protocol: The preincubation test was conducted by incubating 0.1 mL of the appropriate test material solution, positive control or vehicle, 0.1 mL of a bacterial suspension (containing ~ 10^9 cells/mL) and 0.5 mL of either S9-mix or phosphate buffer at 37°C for about 20 minutes. Two mL of soft agar were added and the contents of each tube were mixed and poured onto minimal glucose agar plates. Plates were incubated at 37°C for 48 hours. The number of revertant colonies per plate was counted and means and standard deviations determined. A sterility check of the highest test material concentration and the S9 mix was performed.

3. Statistical analysis: No statistical analysis was conducted.

4. **Evaluation criteria:**
   a. Assay acceptability: The assay was considered acceptable if: the number of revertant colonies in the negative (solvent) control was within the provided historical control range and the positive controls induced an increase in the revertant colony counts of the respective stain that was within the provided historical control range.

   b. Positive response: The test material was considered positive if it induced a "significant" (i.e., ≥ 2-fold), dose-related and reproducible increase in revertant colonies of any strain compared to the historical negative control range.

II. REPORTED RESULTS:

No analytical results were reported.

A. PRELIMINARY CYTOTOXICITY ASSAY:

A dose- finding experiment, designated Trial 1 by our reviewers was conducted. This trial contained all of the elements of a mutagenicity assay and is discussed below.

B. MUTAGENICITY ASSAYS:

NNI-002 480 technical was tested at seven concentrations ranging from 1.22 to 5000 µg/plate with and without S9-mix (Trial 1) or 3.86 to 313 µg/plate –S9 or 61.7 to 5000 µg/plate +S9 (Trial 2) using a 20-minute preincubation assay. Summarized results of the means and standard deviations of mutant colony counts for Trial 2 are presented in Study Report, Tables 3 and 4, pp.24 and 25 (see Attachment). As shown, compound precipitation was seen at ≥ 104 µg/plate – S9 and ≥ 556 µg/plate +S9. No cytotoxicity or mutagenicity was seen at any test material concentration in any tester strain with or without S9-mix in either assay. The solvent and positive controls induced the appropriate responses (i.e., within the laboratory=s historical control ranges) in the respective strains.

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigators concluded that NNI-001 technical was not mutagenic in this test system.

B. REVIEWERS' COMMENTS:

The reviewers agree with the investigator's conclusion. NNI-001 technical was tested to insoluble concentrations (≥ 104 µg/plate –S9 and ≥ 556 µg/plate +S9) and failed to induce either a cytotoxic or mutagenic effect with or without S9-mix using in this test system. By contrast, the positive controls induced marked increases in mutant colonies in the appropriate tester strain. Based on these findings, NNI-001 is considered negative in this acceptable bacterial gene mutation assay.

C. STUDY DEFICIENCIES: None.

D. ATTACHMENT: Study Report (MRID 468172221, Tables 3 and 4, pp 24-25)

ATTACHMENT

The following attachment contains Tables 3 and 4, pp 24-25 of Study Report (MRID 46817221 .

Table 3. Number of revertant colonies without metabolic activation system in main experiment

Test or control substance	Dose (μg/plate)	Number of revertant colonies/plate (Average ± Standard Deviation, n=3)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2 *uvrA*	TA98	TA1537
DMSO	-	132 ± 22	10 ± 3	20 ± 2	16 ± 2	4 ± 1
NNI-0001	3.86	126 ± 6	13 ± 2	16 ± 3	22 ± 5	5 ± 1
	11.6	129 ± 20	8 ± 1	20 ± 2	18 ± 10	4 ± 1
	34.7	132 ± 26	8 ± 3	19 ± 7	20 ± 7	4 ± 1
	104	140 ± 16 C1	8 ± 2 C1	18 ± 4 C1	21 ± 5 C1	4 ± 2 C1
	313	123 ± 6 C2	6 ± 4 C2	20 ± 6 C2	15 ± 1 C2	5 ± 1 C2
AF-2	0.01	512 ± 22	-	-	-	-
	0.02	-	-	390 ± 25	-	-
NaN_3	0.5	-	313 ± 27	-	-	-
2-NF	1	-	-	-	190 ± 9	-
9-AA	80	-	-	-	-	544 ± 39

DMSO: dimethylsulfoxide, AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, NaN_3: sodium azide, 2-NF: 2-nitrofluorene, 9-AA: 9-aminoacridine

-: No data

Precipitation (After incubation of plate):

C1: Although precipitation was observed with the unaided eye, the number of revertant colonies was countable using a colony counter.

C2: Although it was not possible to count the number of revertant colonies using a colony counter, it was countable with the unaided eye.

Table 4. Number of revertant colonies with metabolic activation system in main experiment

Test or control substance	Dose (μg/plate)	Number of revertant colonies/plate (Average ± Standard Deviation, n=3)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2 *uvrA*	TA98	TA1537
DMSO	-	120 ± 17	10 ± 2	19 ± 4	36 ± 13	8 ± 2
NNI-0001	61.7	126 ± 14	6 ± 3	18 ± 4	30 ± 6	7 ± 3
	185	121 ± 15	9 ± 2	13 ± 2	29 ± 3	5 ± 2
	556	124 ± 17 C1	10 ± 4 C1	18 ± 2 C1	30 ± 4 C1	8 ± 3 C1
	1,670	143 ± 16 *,C1	13 ± 5 *,C1	20 ± 8 *,C1	31 ± 7 *,C1	5 ± 2 *,C1
	5,000	129 ± 14 *,C1	9 ± 1 *,C1	17 ± 4 *,C1	24 ± 1 *,C1	8 ± 1 *,C1
2-AA	0.5	-	-	-	241 ± 15	-
	1	920 ± 47	-	-	-	-
	2	-	485 ± 6	-	-	497 ± 61
	10	-	-	730 ± 56	-	-

DMSO: dimethylsulfoxide, 2-AA: 2-aminoanthracene
-: No data
Cytotoxicity:
*: Cytotoxicity could not be observed because of precipitation
Precipitation (After incubation of plate):
C1: Although precipitation was observed with the unaided eye, the number of revertant colonies was countable using a colony counter.

277

18

EPA Reviewer: Nancy E. McCarroll **Signature:** Nancy E. McCarroll
Toxicology Branch, Health Effects Division (7509P) **Date:** 07/05/07
EPA Secondary Reviewer: Gregory Akerman, PhD. **Signature:** Gregory Akerman
Toxicology Branch, Health Effects Division (7509P) **Date:** 07/05/07
EPA Template version 02/06

TXR#: 0054319

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** (Bacterial system, *Salmonella typhimurium; E. coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100[1] [§84 2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 027602 **DP BARCODE:** DP331553

TEST MATERIAL (PURITY): NNI-0001 480 SC [formulation containing 489.54 g/L active ingredient (ai), Batch No. 07846/0031 (0020)]

SYNONYMS: Flubendiamide; N2- [1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-{2-methyl-4-[1,2,2,2,-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}phthalamide

CITATION: Herbold, B. (2004). NNI-0001 480 SC *Salmonella/* microsome test plate incorporation and preincubation method. Bayer HealthCare AG PH-PD Toxicology International, 42096 Wuppertal, Germany. Study No. T 3073247, May 19, 2004. MRID 46817222. Unpublished.

SPONSOR: Bayer CropScience AG, Monheim, Germany

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 46817222), strains TA1535, TA1537, TA98, TA100 and TA102 of *Salmonella typhimurium* were exposed to NNI-0001 480 SC [formulation containing 489.54 g/L ai, Batch No. 07846/0031 (0020)] in deionized water at concentrations of 0, 16, 50, 158, 500, 1581 or 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). Both a standard plate test and a 20-minute preincubation assay were conducted with comparable concentrations. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

NNI-0001 480 SC was tested up to the limit dose of 5000 µg/plate for this test system but failed to induce either a cytotoxic or mutagenic response. The solvent and positive control values induced the expected responses in the respective strains. However, information on test material purity was not included in the report. **There was no evidence of induced mutant colonies over**

[1]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

290

background.

This study is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirement for Test Guideline OPPTS 870.5100[1]; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. However, the study can be upgraded when the missing information is provided. Nevertheless, an acceptable bacterial reverse gene mutation assay (MRID 46817221) on the technical grade of the test material was available.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: NNI-0001 480 SC

Description: White viscous suspension

Lot/Batch #: 07846/0031(0020)

Purity: Not listed; reported as a formulation containing 489.54 g/L ai and said to be ~22% water

CAS # of TGAI: 272451-65-7

Solvent Used: Deionized water (dH_2O)

2. Control materials:

Negative: None

Solvent (final conc.): dH_2O / 0.1 mL/plate

Positive:

Nonactivation:

Sodium azide 10 µg /plate TA1535
Nitrofurantoin 0.2 µg /plate TA100
4-Nitro-o-phenylenediamine 0.5 µg/plate TA98
10 µg /plate TA1537
Mitomycin C 0.2 µg/plate TA102 (plate incorporation trial)
Cumene hydroperoxide 50 µg/plate TA102 (preincubation trial)

Activation:

2-Aminoanthracene (2-anthramine) 3 µg/plate All strains

3. Activation: S9 derived from adult male Sprague-Dawley rats (200-300 g)

x	Induced	x	Aroclor 1254	x	Rat	x	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			Other		Other		

Describe S9 mix composition:

$MgCl_2X\ 6H_2O$	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
Phosphate buffer	100 mM
S9-fraction	10%

280

4. Test organisms: *S. typhimurium* strains

	TA97	x	TA98	x	TA100	x	TA102		TA104
x	TA1535	x	TA1537		TA1538		list any others:		

Properly maintained?	x	Yes	No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	x	Yes	No

5. Test compound concentrations used:

Standard plate test and preincubation test: (all strains, triplicate plating)
Nonactivated conditions: 0, 16, 50, 158, 500, 1581, 5000 µg/plate
Activated conditions: 0, 16, 50, 158, 500, 1581, 5000 µg/plate

B. TEST PERFORMANCE:

1. Type of *Salmonella* assay:

- [X] Standard plate test
- [X] Pre-incubation (20 minutes)
- [ ] "Prival" modification *(i.e. azo-reduction method)*
- [ ] Spot test
- [ ] Other

2. Protocol: The standard plate test was conducted by adding in order: 0.1 mL of the appropriate test material solution, positive control or vehicle, 0.1 mL of the appropriate 17-hour bacterial culture and 0.5 mL of either S9-mix for tests with activation or phosphate buffer for tests without activation to 2 mL of soft agar at 45°C in a test tube. After mixing, the contents were poured onto the surface of solid agar plates and the plates incubated at 37°C for 48 hours. The number of revertant colonies per plate was then counted. No further details were provided in the present study.

The preincubation test was conducted by incubating 0.1 mL of the appropriate test material solution, positive control or vehicle, 0.1 mL of the appropriate 17-hour bacterial suspension and 0.5 mL of either S9-mix or phosphate buffer at 37°C for about 20 minutes. Two mL of soft agar was then added, the contents mixed and poured onto solid agar plates and the test then continued as described for the standard plate test.

All plating for both protocols was in triplicate; bacterial counts were determined for each S9-activated concentration.

3. Statistical analysis: No statistical analysis was conducted.

4. **Evaluation criteria:** The number of revertant colonies per plate was counted and the mean number of revertant colonies per plate and the standard deviation for each dose and control group were calculated. All plating was in triplicate. Cytotoxicity was detected as a decrease in the number of revertants per plate, thinning or elimination of the background lawn of bacteria, or by a reduction in the bacterial titer.

   Criteria for an acceptable assay were vehicle and positive control values within the historical control ranges of the performing laboratory and sufficient bacterial density in each bacterial suspension (a titer of viable bacteria $\geq 10^8$/mL).

   Criteria for a positive response were a reproducible, dose-related increase in the number of revertants per plate in a least one tester strain with or without S9-mix. Increase should be ≥2-fold for strains TA1535, TA100 and TA98; ≥3-fold for strain TA1537 or an increase of about 100 revertant colonies for strain TA 102.

II. REPORTED RESULTS:

No analytical results were reported; however, the investigator stated that the batch used in this assay was "analytically examined prior to study initiation and was approved for use until December 15, 2004."

A. PRELIMINARY CYTOTOXICITY ASSAY:

No preliminary cytotoxicity assay was conducted.

B. MUTAGENICITY ASSAYS:

NNI-002 480 SC was tested at six concentrations ranging from 16 to 5000 µg/plate using all five tester strains with and without S9-mix, in both a standard plate test and a 20-minute preincubation assay. Summarized results of the average mutant colony counts for the standard plate test trial (µg/plate) and the preincubation trial (µg/tube) are presented in Study Report, Table 5.2, pp.25 and 26 (see Attachment). As shown, no cytotoxicity or mutagenicity was seen at any test material concentration in any tester strain with or without S9-mix in either assay. The solvent and positive controls induced the appropriate responses (within the laboratory's historical control ranges) in the respective strains.

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigators concluded that NNI-001 480 SC was not mutagenic in this test system.

B. REVIEWERS' COMMENTS:

The reviewers agree with the investigator's conclusion. NNI-001 480 SC was tested to the limit dose for this test system and failed to induce either a cytotoxic or mutagenic effect with or without S9-mix using either the standard plate assay or the preincubation test. By contrast, the positive controls induced marked increases in mutant colonies in the appropriate tester strain. However, no information on the purity of the test material was provided. Accordingly, this is an unacceptable study but can be upgraded when the study sponsor provides the missing information. It should be noted that an acceptable bacterial reverse gene mutation assay (MRID 46817221) on the technical grade of the test material was available.

C. STUDY DEFICIENCIES:

Purity information on the test material was not provided (see above).

D. ATTACHMENT: Study Report (MRID 468172222, Table 5.2, pp 25-26)

Abbreviations for Table 5.2
NF = Nitrofurantoin
9-AA = 9-Aminoacridine
4-NPDA = 4-Nitro-o-phenylendiamine
MMC = Mitomycin C
2-AA = 2-Aminoanthracene

ATTACHMENT

The following attachment contains Table 5.2, pp 25-26 of Study Report (MRID 46817222.

,)

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix					
Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	14	140	9	21	230
16	18	142	6	24	220
50	15	126	9	21	216
158	15	122	7	22	225
500	17	114	7	23	218
1581	17	136	6	24	241
5000	25	145	7	32	227
Na-azide	591				
NF		321			
4-NPDA			101	138	
MMC					706
6-10 µg/Tube					
0	34	152	10	24	243
16	31	152	10	28	200
50	30	157	10	25	221
158	26	177	12	23	252
500	29	172	12	28	242
1581	33	179	13	27	221
5000	24	196	10	27	237
Na-azide	618				
NF		586			
4-NPDA			150	189	
Cumene					609

NNI-0001 480 SC
Salmonella/Microsome Test

26

Study No. T 3073247
Bayer HealthCare AG

Summary of Mean Values With S9 Mix					
Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	13	142	11	34	248
16	10	135	11	37	276
50	11	124	11	30	269
158	8	140	13	34	262
500	10	145	9	41	284
1581	11	111	11	44	289
5000	12	121	16	41	272
2-AA	100	1437	268	1272	679
6-10 µg/Tube					
0	19	173	17	39	255
16	15	162	13	37	293
50	19	172	12	46	258
158	17	184	12	35	277
500	14	179	13	54	298
1581	15	167	13	44	274
5000	12	146	15	40	262
2-AA	180	1665	375	1399	691

EPA Reviewer: Nancy E. McCarroll
Toxicology Branch, Health Effects Division (7509P)
EPA Secondary Reviewer: Gregory Akerman, PhD.
Toxicology Branch, Health Effects Division (7509P)

Signature: Nancy E. McCarroll
Date: 07/05/07
Signature: Gregory Akerman
Date: 07/05/07

TXR#: 0054319

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cell Gene Mutation Assay in V79 Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 027602 **DP BARCODE:** DP331553

TEST MATERIAL (PURITY): NNI-0001 technical (97.3%, Lot No. 1FH0019M)

SYNONYMS: Flubendiamide; N2- [1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-{2-methyl-4-[1,2,2,2,-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}phthalamide

CITATION: Herbold, B. (2003). NNI-001 V79/HPRT In Vitro for the Detection of Induced Forward Mutations. Bayer HealthCare, PH-PD P Health Care toxicology, Molecular and Genetic Toxicology, Wuppertal, Germany. Final Report No. AT00460, June 12, 2003. MRID 46817224. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Monhelm, Germany

EXECUTIVE SUMMARY - In independent trials of a mammalian cell gene mutation assay at the HGPRT locus (MRID 46817224), V79 (Chinese hamster lung fibroblast) cells cultured *in vitro* were exposed to NNI-0001 technical (97.3%, Lot No. 1FH0019M) in dimethyl sulfoxide (DMSO) at concentrations of 0, 7.5, 15, 30, 60, 120, or 240 µg/mL in the presence and absence of S9-activation for 5 hours.

NNI-001 precipitated at 240 µg/mL+/-S9. No clear or reproducible evidence of cytotoxicity was observed at any concentration in any trial (+/-S9). No appreciable increase in the mutant frequency was observed in any trial at any concentration in the presence or absence of S9-activation. The positive controls induced the appropriate response in both trials (+/-S9). **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

280

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material** - NNI-001

Description:	White powder
Batch #:	1FH0019M
Purity:	97.3%
CAS # of TGAI:	272451-65-7

2. **Control materials**

Negative control:	The solvent control alone served as the negative control.
Solvent control (final concentration):	DMSO, 1% (v/v)
Positive control:	Nonactivation: Ethyl methanesulfonate (EMS, 900 µg/mL) Activation: Dimethylbenzanthracene in DMSO (DMBA, 20 µg/mL)

3. **Activation** - S9 derived from male Sprague-Dawley rats (age, weight, and supplier were not reported).

[X]	Induced	[X]	Aroclor 1254	[X]	Rat	[X]	Liver
[ ]	Non-induced	[ ]	Phenobarbital	[ ]	Mouse	[ ]	Lung
[ ]		[ ]	None	[ ]	Hamster	[ ]	Other
[ ]		[ ]	Other	[ ]	Other	[ ]	

The S9 fraction was prepared in-house and stored at approximately -80°C until used. It was not reported if the efficacy of the batch was checked before use. The protein content of the S9 fraction was 24.7 mg/mL. The S9 mix consisted of 40% (v/v) S9 fraction and 60% (v/v) cofactor solution (1 mM NADP, 8 mM $MgCl_2X6\ H_2O$, 33 mM KCl, 5 mM glucose-6-phosphate, and 150 mM sodium phosphate buffer, pH 7.4). The final S9 culture concentration was approximately 2% (v/v).

4. **Test cells** - mammalian cells in culture

[ ]	Mouse lymphoma L5178Y cells	[X]	V79 cells (Chinese hamster lung fibroblasts)
[ ]	Chinese hamster ovary (CHO) cells	[ ]	list any others

Properly maintained?	[X]	Yes	[ ]	No
Periodically checked for Mycoplasma contamination?	[X]	Yes	[ ]	No
Periodically checked for karyotype stability?	[X]	Yes	[ ]	No
Periodically "cleansed" against high spontaneous background?	[X]	Yes	[ ]	No

Media: The culture medium contained hypoxanthine-free Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum (FCS), L-glutamine (2 mM), non-essential amino acids, vitamins, $NaHCO_3$, penicillin (100 units/mL), and streptomycin (100 µg/mL). The treatment medium was the culture medium with 2% FCS. The selection medium was culture medium containing 6-thioguanine (10 µg/mL).

the presence or absence of S9-activation. The positive controls induced the appropriate response in both trials (+/-S9). Accordingly, NNI-001 is negative in an acceptable assay in this test system.

C. STUDY DEFICIENCIES – None.

D. ATTACHMENT - Tables 3 -6 from pages 32 - 35 of MRID 46817224

ATTACHMENT

The following attachment contains Tables 3 -6 from pages 32 - 35 of MRID 46817224.

NNI-0001
HPRT-Test

32

Study No. T 2071518
Bayer AG

Table 3

V79/HPRT MUTAGENESIS ASSAY RESULTS: WITHOUT ACTIVATION

BAYER AG

Study Number: T2071518 Batch Number: 1F1800190 Test Date: January 21, 2003

Test Substance: NNI 0001 Vehicle: DMSO Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 X 10E5 / Dish for mutant selection

Concentration[1] Concentration[2]	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION ABSOLUTE x 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES/ DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY x 10E-6 (a)
Negative Control (b)	110.0	+/- 7.8	103.1	105.50	98.5	1	1	1	0	0	0	0	0	3	98.5	+/- 7.5	1.3
Negative Control (b)	120.5	+/- 4.9	75.2	104.13	95.8	1	0	0	0	0	0	0	0	1	99.2	+/- 5.2	0.5
Vehicle Control (c)	106.7	+/- 12.5	100.0	105.13	100.0	1	1	0	0	0	0	0	0	2	81.5	+/- 18.9	1.2
Vehicle Control (c)	160.3	+/- 6.4	100.0	106.69	100.0	2	1	0	0	0	0	0	0	3	88.0	+/- 15.2	1.4
Positive Control EMS 900 µg/ml	18.0	+/- 5.3	14.9	42.00	44.0	69	69	58	89	78	48	96	69	596	41.3	+/- 13.2	600.8
Positive Control EMS 900 µg/ml	2.3	+/- 0.6	1.5	47.50	43.7	102	108	147	100	98	89	76	102	816	53.7	+/- 2.1	633.5
TEST SUBSTANCE																	
7.5 µg/ml	113.0	+/- 21.6	105.9	98.00	85.6	1	1	0	0	0	0	0	0	2	44.5	+/- 12.7	1.9
7.5 µg/ml	174.7	+/- 29.3	108.9	68.25	62.8	1	0	0	0	0	0	0	0	1	94.2	+/- 13.8	0.4
15 µg/ml	134.3	+/- 22.2	125.9	44.56	42.4	1	0	0	0	0	0	0	0	1	57.3	+/- 10.9	0.7
15 µg/ml	99.7	+/- 14.0	62.2	41.25	38.0	1	1	0	0	0	0	0	0	3	42.3	+/- 11.9	2.0
30 µg/ml	92.3	+/- 4.2	86.6	87.50	83.2	1	0	0	0	0	0	0	0	1	82.0	+/- 7.9	0.6
30 µg/ml	94.0	+/- 3.6	58.6	56.50	53.8	1	0	0	0	0	0	0	0	1	75.0	+/- 6.1	0.6
60 µg/ml	122.0	+/- 17.5	114.4	11.38	10.8	1	1	0	0	1	0	0	0	2	104.8	+/- 13.4	0.8
60 µg/ml	122.0	+/- 25.2	76.1	11.38	10.5	1	1	0	0	1	0	0	0	2	115.8	+/- 14.2	1.8
120 µg/ml	107.7	+/- 11.6	100.9	14.00	13.3	1	1	0	0	0	0	0	0	2	102.7	+/- 26.4	0.8
120 µg/ml	197.0	+/- 8.5	122.9	19.69	18.1	1	3	0	0	0	0	0	0	4	116.3	+/- 6.3	1.4
240 µg/ml 1	220.3	+/- 0.6	206.4	3.94	3.7	1	0	0	0	0	0	0	N	1	112.8	+/- 10.6	0.4
240 µg/ml 1	164.0	+/- 32.2	102.3	1.88	*	1	0	0	0	0	0	0	N	1	140.8	+/- 23.0	0.3

291

NNI-0001 33 Study No. T 2071518
HPRT-Test Bayer AG

Table 4

V79/HPRT MUTAGENESIS ASSAY RESULTS: WOHACTIVATION

BAYER AG

Study Number: T2071518 Batch Number: 1FH0010W Test Date: February 18, 2003

Test Substance: NNI 0001 Vehicle: DMSO Selective Agent: 10 Ag/ml 6-thioguanine

Cells seeded for analysis: 200 cells / dish for C.E.; 3 x 10E5 / dish for mutant selection

Concentration[1] Concentration[2]	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT MEN.CONTROL	POPULATION ABSOLUTE x 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY x 10E-6 Val
Negative Control Ba	123.7	+/- 22.8	105.4	196.88	65.6	1	0	0	0	0	0	0	0	1	106.8	+/- 0.8	0.4
Negative Control Bb	152.7	+/- 20.6	135.1	328.13	160.3	1	1	0	0	0	0	0	0	2	63.5	+/- 5.3	1.3
Vehicle Control Bc	117.3	+/- 7.6	100.0	302.81	100.0	1	0	0	0	0	0	0	0	1	74.3	+/- 8.9	0.5
Vehicle Control Bc	113.0	+/- 42.5	100.0	204.75	100.0	1	0	0	0	0	0	0	0	1	68.5	+/- 9.2	0.5
Positive Control EMS 900 Ag/ml	39.0	+/- 11.3	33.2	175.31	57.9	89	99	102	100	111	134	99	106	820	37.2	+/- 7.3	919.3
Positive Control EMS 900 Ag/ml	72.5	+/- 14.8	64.2	184.00	89.9	147	112	102	98	103	115	116	108	893	111.3	+/- 10.2	334.2
TEST SUBSTANCE																	
7.5 Ag/ml	143.7	+/- 26.4	122.4	259.63	76.2	1	0	0	0	0	0	0	0	1	113.2	+/- 10.3	0.4
7.5 Ag/ml	108.3	+/- 17.2	95.9	206.75	98.8	2	1	1	0	0	0	0	0	4	63.7	+/- 8.5	2.0
15 Ag/ml	94.0	+/- 9.2	80.1	268.13	88.5	1	2	2	1	0	0	0	0	6	90.5	+/- 6.1	2.8
15 Ag/ml	161.0	+/- 32.1	142.5	199.13	97.3	1	1	0	0	0	0	0	0	2	108.2	+/- 14.3	0.8
30 Ag/ml	100.7	+/- 17.6	85.8	320.63	105.9	1	2	1	0	0	0	0	0	4	86.3	+/- 10.5	1.9
30 Ag/ml	70.0	+/- 5.3	61.9	277.88	135.7	1	0	0	0	0	0	0	0	1	96.3	+/- 11.4	0.4
60 Ag/ml	94.0	+/- 15.7	80.1	384.25	126.9	1	0	0	0	0	0	0	0	1	108.3	+/- 9.9	0.4
60 Ag/ml	130.3	+/- 32.0	115.3	272.88	133.3	2	1	1	0	0	0	0	0	4	61.7	+/- 1.0	2.0
120 Ag/ml	113.3	+/- 18.0	96.6	361.00	119.2	1	0	0	0	0	0	0	0	1	45.5	+/- 6.3	0.9
120 Ag/ml	95.7	+/- 23.5	84.7	305.00	149.0	1	1	2	1	0	0	0	0	5	66.8	+/- 10.4	3.1
240 Ag/ml 1	120.0	+/- 17.0	102.3	129.63	42.8	3	0	1	2	1	1	0	0	9	121.8	+/- 12.1	3.1
240 Ag/ml 1	140.3	+/- 13.4	124.2	120.75	59.0	1	0	0	0	0	0	0	0	1	127.7	+/- 14.2	0.3

192

NNI-0001 34 Study No. T 2071518
HPRT-Test Bayer AG

Table 5

V79/HPRT MUTAGENESIS ASSAY RESULTS: ACTIVATION

BAYER AG

Study Number: T2071518 Batch Number: 1FH0019W Test Date: January 28, 2003

Test Substance: NNI 0001 Vehicle: DMSO Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 X 10E5 / Dish for mutant selection

Concentration[1] / Concentration[2]	SURVIVAL TO TREATMENT: MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION: ABSOLUTE X 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER: 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY X 10E-6 [a]
Negative Control [b]	112.3	+/- 26.0	106.3	100.50	156.7	1	1	0	0	0	0	0	0	2	91.7	+/- 6.5	0.9
Negative Control [b]	103.0	+/- 10.5	86.6	89.38	67.8	1	1	0	0	0	0	0	0	2	73.2	+/- 12.5	1.3
Vehicle Control [c]	105.7	+/- 13.7	100.0	44.13	100.0	1	0	0	0	0	0	0	0	1	56.3	+/- 4.2	0.7
Vehicle Control [c]	119.0	+/- 18.1	100.0	131.75	100.0	1	1	1	0	0	0	0	0	3	57.3	+/- 11.5	2.2
Positive Control DMBA 20 µg/ml	123.3	+/- 37.6	114.7	27.00	42.1	8	9	6	9	9	10	7	8	66	47.8	+/- 1.8	57.6
Positive Control DMBA 20 µg/ml	108.3	+/- 14.5	91.8	26.00	21.3	5	9	10	12	7	9	8	5	65	46.0	+/- 4.9	58.9
TEST SUBSTANCE																	
7.5 µg/ml	169.3	+/- 9.1	160.3	25.00	39.0	1	0	0	0	0	0	0	0	1	76.5	+/- 13.9	0.5
7.5 µg/ml	213.7	+/- 19.8	179.6	24.94	18.9	1	1	0	0	0	0	0	0	2	58.8	+/- 7.2	1.6
15 µg/ml	174.5	+/- 29.0	165.1	31.88	49.7	1	1	0	0	0	0	0	0	2	53.8	+/- 6.0	1.5
15 µg/ml	157.5	+/- 10.6	132.4	11.00	8.3	1	0	0	0	0	0	0	0	1	80.0	+/- 12.5	0.5
30 µg/ml	214.7	+/- 18.7	203.2	50.63	78.9	1	4	2	0	0	0	0	0	7	63.5	+/- 21.9	3.5
30 µg/ml	185.7	+/- 19.1	156.0	45.50	34.5	1	2	0	0	0	0	0	0	3	69.2	+/- 14.0	1.8
60 µg/ml	169.7	+/- 23.7	160.6	39.00	60.8	1	1	1	1	2	2	0	0	8	79.0	+/- 14.8	4.2
60 µg/ml	237.3	+/- 30.1	199.4	37.19	28.2	1	1	3	0	0	0	0	0	5	35.3	+/- 6.2	5.9
120 µg/ml	190.3	+/- 16.6	160.1	15.75	24.6	2	2	0	0	0	0	0	0	4	27.0	+/- 5.3	6.2
120 µg/ml	209.7	+/- 22.3	176.2	21.94	16.7	2	2	2	1	1	1	0	0	9	66.3	+/- 11.8	5.7
240 µg/ml 1	211.7	+/- 20.6	208.3	-	-	-	-	-	-	-	-	-	-	-	-	-	---
240 µg/ml 1	259.0	+/- 31.2	217.6	6.25	4.7	1	1	0	0	0	0	0	0	2	64.3	+/- 11.4	1.3

293

NNI-0001 35 Study No. T 2071518
HPRT-Test Bayer AG

Table 6

V79/HPRT MUTAGENESIS ASSAY RESULTS: ACTIVATION

BAYER AG

Study Number: T2071518 Batch Number: VFN00184 Test Dates: March 4, 2003

Test Substance: NNI 0001 Vehicle: DMSO Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 x 10E5 / Dish for mutant selection

Concentration(1) Concentration(2)	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION ABSOLUTE X 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY X 10E-6 (a)
Negative Control (b)	113.0	+/- 8.9	82.7	265.50	157.3	1	0	0	0	0	0	0	0	1	65.3	+/- 10.2	0.7
Negative Control (b)	118.3	+/- 21.1	77.0	174.80	107.7	1	0	0	0	0	0	0	0	1	66.2	+/- 18.8	0.6
Vehicle Control (c)	136.7	+/- 40.7	100.0	168.75	108.0	1	0	0	0	0	0	0	0	1	59.5	+/- 2.8	0.7
Vehicle Control (c)	153.7	+/- 13.6	100.0	161.56	108.0	1	1	0	0	0	0	0	0	2	92.7	+/- 0.6	0.9
Positive Control DMBA 20 µg/ml	115.7	+/- 24.8	84.4	28.08	16.6	18	13	14	18	25	19	29	11	167	72.5	+/- 16.3	84.3
Positive Control DMBA 20 µg/ml	72.0	+/- 11.3	46.9	40.50	25.1	36	35	25	29	34	34	28	26	267	29.8	+/- 3.3	345.0
TEST SUBSTANCE																	
20 µg/ml	141.7	+/- 24.8	103.7	81.94	48.6	1	0	0	0	0	0	0	0	1	64.0	+/- 10.0	0.7
20 µg/ml	138.3	+/- 5.8	90.0	77.00	47.7	1	1	0	0	0	0	0	0	2	59.8	+/- 1.9	1.4
40 µg/ml	148.0	+/- 19.5	108.3	91.88	54.4	1	1	1	0	0	0	0	0	3	39.7	+/- 13.3	3.2
40 µg/ml	127.0	+/- 27.5	82.6	51.56	31.9	1	1	0	0	0	0	0	0	2	42.2	+/- 5.5	2.0
80 µg/ml	155.7	+/- 5.1	113.9	92.25	54.7	1	1	0	0	0	0	0	0	2	75.8	+/- 13.0	1.1
80 µg/ml	111.3	+/- 5.9	72.5	70.31	43.5	1	1	0	0	0	0	0	0	2	48.8	+/- 6.0	1.2
120 µg/ml	132.0	+/- 12.2	96.6	67.06	39.7	1	0	0	0	0	0	0	0	1	20.7	+/- 2.8	2.0
120 µg/ml	120.3	+/- 33.3	78.3	34.25	22.4	1	0	0	0	0	0	0	0	1	67.5	+/- 4.9	0.6
160 µg/ml 1	133.7	+/- 30.1	97.8	68.25	40.4	1	0	0	0	0	0	0	0	1	76.3	+/- 3.3	0.5
160 µg/ml 1	161.7	+/- 9.9	118.2	62.00	36.4	1	0	0	0	0	0	0	0	1	45.8	+/- 9.3	0.9
200 µg/ml 1	289.3	+/- 18.9	211.7	16.88	10.0	3	2	1	0	0	0	0	0	6	51.3	+/- 4.6	4.9
200 µg/ml 1	155.7	+/- 63.5	190.0	15.88	9.3	1	0	0	1	0	0	0	0	1	30.7	+/- 7.8	1.4
240 µg/ml 1	292.0	+/- 43.1	266.3	5.25	3.1	1	1	2	1	0	0	0	0	5	91.5	+/- 16.3	2.3
240 µg/ml 1	366.7	+/- 30.4	268.9	2.19	*	1	1	1	1	1	2	0	0	7	89.0	+/- 30.3	3.3
	322.5																

294

EPA Reviewer: Nancy McCarroll. **Signature:** Nancy McCarroll
Toxicology Branch, Health Effects Division (7509P) **Date:** 07/05/07
EPA Secondary Reviewer: Gregory Akerman, Ph.D **Signature:** Gregory Akerman
Toxicology Branch, Health Effects Division (7509P) **Date:** 07/05/07

Template version 02/06

TXR#: 0054319

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Mammalian Cytogenetics** Chromosome Aberration Test in Chinese Hamster Lung (CHL) cells; OPPTS 870.5375 [§84-2]; OECD 473.

PC CODE: 027602 **DP BARCODE:** DP 331553

TEST MATERIAL (PURITY): NNI-0001 technical (97.8%, Lot No. 1FH0018P)

SYNONYMS: Flubendiamide; N2- [1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-{2-methyl-4-[1,2,2,2,-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}phthalamide

CITATION: Miyahana, K. (2004). *In Vitro* Chromosome Aberration Test of NNI-0001 in Cultured Chinese Hamster Cells. Toxicology & Pharmaceutical Research Center, Nihon Nohyaku Co., Ltd., Osaka, Japan. Final Report No. LSRC-TO3-047A, March 4, 2004. MRID 46817223. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd., Osaka, Japan

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (chromosome aberration) (MRID 46817223), cultured Chinese hamster lung (CHL) cells were exposed to NNI-0001 technical (97.8%, Lot No. 1FH0018P) in dimethyl sulfoxide (DMSO) at concentrations of 0, 550, 1100 or 2200 µg/mL with or without metabolic activation for 6 hours, 0, 300, 600 or 1200 µg/mL –S9 for 20 hours, or 0, 125, 250 or 500 µg/mL –S9 for 40 hours. Cells were harvested at 20 hours for the 6-hour treatment or immediately after the continuous 20- or 40-hour exposures. The S9 was derived from the livers of phenobarbital/5,6-benzoflavone induced male Sprague Dawley rats.

In agreement with the preliminary results, compound precipitation of NNI-001 technical was observed at ≥ 550 µg/mL (6-hr treatment) or ≥125µg/mL (20- and 40-hr treatments). Also in agreement with the preliminary data, ≤50% of the cells survived treatment ≥ 2200µg/mL +/-S9 (6-hr treatment) or ≥ 500µg/mL -S9 (40-hr treatment). Following treatment for 20 hours, 50% of the cells were recovered at 1200 µg/mL -S9. NNI-001 was not clastogenic or increased the incidence of polyploidy cells. The solvent and positive control values were appropriate and within the testing laboratory's historical control ranges in both experiments. **Accordingly, NNI-001 did not increase the incidence of cells with structural chromosome aberrations at any concentration, harvest time or assay condition.**

1 of 76

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *In vitro* mammalian cytogenetics (chromosome aberrations) OPPTS 870.5375; OECD 473.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: NNI-0001 technical

Description: White crystal

Lot/Batch #: 1FH0018P

Purity: 97.8%

CAS # of TGAI: 272451-65-7

Solvent Used: Dimethyl sulfoxide (DMSO)

2. Control materials:

Negative control: None

Solvent control (final conc'n): DMSO / 1%

Positive controls: Nonactivation: Mitomycin C (MMC) 0.10 µg/mL (6-hr exposure); 0.07 µg/mL (20- and 40-hrs exposure). Solvent was physiological saline

Activation: Cyclophosphamide (CP) 10 µg/mL / solvent was physiological saline

3. Activation: S9 derived from Sprague-Dawley rats

[x]	Induced	[ ]	Aroclor 1254	[X]	Rat	[x]	Liver
[ ]	Non induced	[X]	Phenobarbital/5,6 benzoflavone	[ ]	Mouse	[ ]	Lung
[ ]		[ ]	None	[ ]	Hamster	[ ]	Other
[ ]		[ ]	Other	[ ]	Other		

Describe S9 mix composition: The S9 was prepared in-house and contained the following components:

30% S9
40 mM nicotinamide adenine dinucleotide phosphate (NADP)
50 mM glucose-6-phosphate
330 mM KCl
50 mM $MgCl_2$
20mM Hepes buffer, pH 7.2

4. Test cells: mammalian cells in culture

Chinese hamster lung (CHL) were obtained from the Japan Health Sciences Foundation. Cells were maintained in Eagle's minimal essential medium (EMEM) supplemented with 20% fetal bovine serum (FBS). Cells used in the assay were cultured in EMEM with 10% FBS.

Media:

Properly maintained?	[x]	Yes	[ ]	No
Periodically checked for *Mycoplasma* contamination?	[x]	Yes	[ ]	
Periodically checked for karyotype stability?	[ ]	Yes	[x]	No or not stated

5. Test compound concentrations used:

Preliminary cytotoxicity: 0, 313, 625, 1250, 2500, or 5000 µg/mL for the 6-, 20- or 40-hour exposures – S9 or 6-hour +S9

Cytogenetic Assay:

Nonactivated conditions: 0, 550, 1100, 2200 µg/mL for the 6-hour exposure
0, 300, 600, 1200 µg/mL for the continuous 20- exposure
0, 125, 250, 500 µg/mL for the continuous 40-hour exposure

S9-activated conditions: 0, 550, 1100, 2200 µg/mL for the 6-hour exposure

B. TEST PERFORMANCE:

1. **Preliminary cytotoxicity assay:** Cytotoxicity was determined prior to the cytogenetic assays. Prepared cultures were treated with the solvent or the selected test material concentrations (313 –5000 µg/mL +/-S9) for 6 hours or for 20 or 40 hours without S9 activation. At the end of the 6-hour exposure, cells were washed, reincubated for an additional 14 hours, and stained with 0.3% trypan blue. Cells treated without S9 activation continuously for 20 or 40 hours were washed and similarly stained with 0.3% trypan blue. The concentrations at which ≥50% of cells was killed (as indicated by trypan blue exclusion) were selected for the chromosome aberration assay.

2. **Cytogenetic assay:**

a. **Cell exposure time:**

	Test material	Solvent control	Positive control
Non-activated:	6, 20 or 40 h	6, 20 or 40 h	6, 20 or 40 h
Activated:	6 h	6 h	6 h

b. **Spindle inhibition:**

Inhibition used/concentration: Colcemid® / 0.1 µg/mL

Administration time: 2 hours (before cell harvest)

c. **Cell harvest time after termination of treatment:**

	Test material	Solvent control	Positive control
Non-activated:	20 or 0 h	20 or 0 h	20 or 0 h
Activated:	20 h	20 h	20 h

d. **Details of slide preparation:** At harvest, culture medium was removed and each culture was treated with 0.25% trypsin for mitotic shake-off. Recovered cells were centrifuged; the supernatant was discarded and pelleted cells were treated with hypotonic 0.075 M KCl, fixed in methanol:acetic acid (3:1), dropped onto 2 slides and stained with 6% Giemsa.

e. **Metaphase analysis**

No. of cells examined per dose: 200 (100 per duplicate culture)

Scored for structural?	x	Yes		No
Scored for numerical?	x	Yes, polyploidy		No
Coded prior to analysis?	x	Yes		No

3. **Evaluation criteria:** The test material was considered positive if it induced 1) a significant ($p<0.01$) increase in the frequency of metaphases with aberrant aberrations (excluding gaps) and 2) the increase was concentration-related and reproducible. Historical solvent and positive control data from the performing laboratory were provided.

4. **Statistical analysis:** The data were analyzed for significance using Fisher's exact test.

II. REPORTED RESULTS:

The test material was reported to be insoluble in water. Therefore, DMSO was selected as the solvent of choice for the preliminary cytotoxicity tests.

A. **PRELIMINARY CYTOTOXICITY ASSAY:** Cells were exposed for 6 hours to five concentrations ranging from 313 to 5000µg/mL +/-S9 or 20 or 40 hours to comparable nonactivated concentrations. Compound precipitation was seen at ≥ 625 µg/mL +/-S9 (6-hr treatment) and at all tested nonactivated levels (20 or 40 hours of exposure). As shown in Study Report Table 1, page 22 (see Attachment), ≤50% of the cells survived treatment ≥ 2500 µg/mL +/-S9 (6-hr treatment), ≥ 625µg/mL -S9 (20-hr treatment) or, ≥ 313µg/mL -S9 (40-hr treatment). Compound precipitation occurred at concentrations ≥ 625 µg/mL (6-hr treatment) or ≥313µg/mL (20- and 40-hr treatments). Based on the results, starting concentrations used in the chromosome aberration assays were:

2200 µg/mL +/-S9 (6-hr exposure, 20 hr harvest)
1200 µg/mL -S9 (20-hr exposure, harvest immediately after treatment)
500 µg/mL -S9 (40-hr exposure, harvest immediately after treatment)

B. **CYTOGENETIC ASSAY:** Summarized data from the cytotoxicity phases of the cytogenetic assay are presented in Study Report Table 2, page 23 (see Attachment). In agreement with the preliminary assay results, compound precipitation was observed at ≥ 550 µg/mL (6-hr treatment) or ≥125µg/mL (20- and 40-hr treatments). Also in agreement with the preliminary data, ≤50% of the cells survived treatment with ≥ 2200 µg/mL +/-S9 (6-hr treatment) or ≥ 500µg/mL -S9 (40-hr treatment). Following treatment for 20 hours, 50% of the cells were recovered at 1200 µg/mL -S9. Data from the analysis of metaphase plates are summarized in Study Report Tables 3-6, pages 24-27 (see Attachment). As shown, no significant increases in either cells with aberrant morphology or number was observed at any concentration following a 6-hour treatment with or without S9 or following a 20- or 40-hour treatment without S9 activation. By contrast, the positive controls (0.1 or 0.07 µg/mL, MMC –S9 or 10 µg/mL, CP +S9) induced significant ($p<0.001$) clastogenic activity.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATOR'S CONCLUSIONS:** The investigators concluded that NNI-001Technical did not show evidence of a clastogenic activity in this *in vitro* test system.

B. **REVIEWERS' COMMENTS:** The reviewers agree with the investigators' conclusions. NNI-001Technical was tested to cytotoxic concentrations (≤50% reduction in cell survival) for either a 6-hour exposure in the absence or presence of S9 activation or following a continuous 20 –or 40- hour exposure without S9 activation but failed to induce either a numerical or structural change in treated Chinese hamster lung cells. Although compound precipitation was seen at all concentrations tested in the cytogenetic assay, the evidence of cytotoxicity at the highest nonactivated or S9-activated concentrations for the 6- and 40-hour

exposures indicates that the test material penetrated the cell membrane and interacted with the DNA of the target cells. The sensitivity of the test system to detect a clastogenic response was adequately demonstrated by the findings with the positive controls. Accordingly, NNI-001 technical is negative in an acceptable/guideline *in vitro* cytogenetic test.

C. **STUDY DEFICIENCIES:** None.

ATTACHMENT

The following attachment contains Tables 1-6, pages 22-27 of MRID 46817223.

Table 1. Preliminary cytotoxicity test

Concentration (μg/ml)	Relative the number of viable cell (%)			
	With S9 mix	Without S9 mix		
	6 hours exposure	6 hours exposure	20 hours exposure	40 hours exposure
0	100.0	100.0	100.0	100.0
313	-	-	89.5[a)]	60.0[a)]
625	94.1[a)]	100.0[a)]	84.2[a)]	37.1[a)]
1250	100.0[a)]	88.2[a)]	42.1[a)]	11.4[a)]
2500	29.4[a)]	29.4[a)]	15.8[a)]	2.9[a)]
5000	17.6[a)]	17.6[a)]	5.3[a)]	0[a)]

- : Not set
a) : The precipitation was observed at the beginning and the end of the exposure.

Table 2. Cytotoxicity in chromosome aberration test

Concentration (μg/ml)	Relative the number of viable cell (%)			
	With S9 mix	Without S9 mix		
	6 hours exposure	6 hours exposure	20 hours exposure	40 hours exposure
0	100.0	100.0	100.0	100.0
125	-	-	-	94.3[a)]
250	-	-	-	68.6[a)]
300	-	-	100.0[a)]	-
500	-	-	-	48.6[a)]
550	100.0[a)]	105.6[a)]	-	-
600	-	-	95.0[a)]	-
1100	77.8[a)]	83.3[a)]	-	-
1200	-	-	50.0[a)]	-
2200	44.4[a)]	44.4[a)]	-	-

- : Not set
[a)] : The precipitation was observed at the beginning and the end of the exposure.

Table 3. Chromosome aberration test (6 hours exposure with S9 mix)

Test substance	Concen-tration (μg/ml)	Number of meta-phases	Number of chromosome aberration							Number of aberrant cells		Poly-ploid cells
			gap	Chromatid type		Chromosome type		frg	mul			
			g	ctb	cte	csb	cse			+g	-g	
DMSO	-	100	0	2	1	0	0	0	0	3	3	2
		100	0	3	0	1	0	0	0	4	4	2
		(Mean)	0.0	2.5	0.5	0.5	0.0	0.0	0.0	3.5	3.5	2.0
NNI-0001	550	100	0	5	0	0	0	0	0	4	4	0
		100	0	3	2	0	0	0	0	5	5	1
		(Mean)	0.0	4.0	1.0	0.0	0.0	0.0	0.0	4.5	4.5	0.5
NNI-0001	1100	100	0	2	0	0	0	0	0	2	2	2
		100	0	3	2	0	0	0	0	4	4	2
		(Mean)	0	2.5	1.0	0.0	0.0	0.0	0.0	3.0	3.0	2.0
NNI-0001	2200	100	0	0	1	0	0	0	0	1	1	0
		100	0	5	0	0	0	0	0	5	5	2
		(Mean)	0.0	2.5	0.5	0.0	0.0	0.0	0.0	3.0	3.0	1.0
Cyclophos-phamide	10	100	0	7	9	0	0	0	0	15	15	3
		100	0	9	8	0	2	0	0	18	18	1
		(Mean)	0.0	8.0	8.5	0.0	1.0	0.0	0.0	16.5	16.5***	2.0

Abbreviations : g, gap ; ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; frg, fragmentation ; mul, Multiple aberration , +g, including gaps ; -g, excluding gaps, DMSO, dimethylsulfoxide

*** : Significantly different from the solvent control at $p<0.001$(Fisher Exact Probability Test)

Table 4. Chromosome aberration test (6 hours exposure without S9 mix)

Test substance	Concen-tration (μg/ml)	Number of meta-phases	Number of chromosome aberration							Number of aberrant cells		Poly-ploid cells
			gap	Chromatid type		Chromosome type		frg	mul			
			g	ctb	cte	csb	cse			+g	-g	
DMSO	-	100	0	1	0	0	0	0	0	1	1	1
		100	0	4	0	0	0	0	0	3	3	3
		(Mean)	0.0	2.5	0.0	0.0	0.0	0.0	0.0	2.0	2.0	2.0
NNI-0001	550	100	0	1	0	0	0	0	0	1	1	1
		100	0	3	0	0	0	0	0	3	3	2
		(Mean)	0.0	2.0	0.0	0.0	0.0	0.0	0.0	2.0	2.0	1.5
NNI-0001	1100	100	0	2	0	0	1	0	0	3	3	2
		100	0	0	1	0	0	0	0	1	1	3
		(Mean)	0	1.0	0.5	0.0	0.5	0.0	0.0	2.0	2.0	2.5
NNI-0001	2200	100	0	2	0	0	0	0	0	2	2	2
		100	0	1	0	1	0	0	0	2	2	2
		(Mean)	0.0	1.5	0.0	0.5	0.0	0.0	0.0	2.0	2.0	2.0
Myto-micin C	0.10	100	0	13	10	0	1	0	1	19	19	1
		100	0	20	22	0	4	0	0	27	27	2
		(Mean)	0.0	16.5	16.0	0.0	2.5	0.0	0.5	23.0	23.0***	1.5

Abbreviations : g, gap ; ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; frg, fragmentation ; mul, Multiple aberration , +g, including gaps ; -g, excluding gaps, DMSO, dimethylsulfoxide

*** : Significantly different from the solvent control at $p<0.001$(Fisher Exact Probability Test)

Table 5. Chromosome aberration test (20 hours exposure without S9 mix)

Test substance	Concen-tration (μg/ml)	Number of meta-phases	Number of chromosome aberration							Number of aberrant cells		Poly-ploid cells
			gap	Chromatid type		Chromosome type		frg	mul			
			g	ctb	cte	csb	cse			+g	-g	
DMSO	-	100	0	2	1	0	0	0	0	3	3	1
		100	2	4	0	0	0	0	0	6	4	0
		(Mean)	1.0	3.0	0.5	0.0	0.0	0.0	0.0	4.5	3.5	0.5
NNI-0001	300	100	0	1	0	3	0	0	0	3	3	0
		100	1	2	0	0	0	0	0	3	2	2
		(Mean)	0.5	1.5	0.0	1.5	0.0	0.0	0.0	3.0	2.5	1.0
NNI-0001	600	100	0	1	0	0	1	0	0	2	2	0
		100	0	3	0	0	0	0	0	3	3	2
		(Mean)	0	2.0	0.0	0.0	0.5	0.0	0.0	2.5	2.5	1.0
NNI-0001	1200	100	2	1	0	0	1	0	0	4	2	3
		100	1	4	0	0	0	0	0	5	4	1
		(Mean)	1.5	2.5	0.0	0.0	0.5	0.0	0.0	4.5	3.0	2.0
Myto-micin C	0.07	100	1	7	15	1	0	0	0	20	19	0
		100	1	12	12	4	2	0	0	20	20	2
		(Mean)	1.0	9.5	13.5	2.5	1.0	0.0	0.0	20.0	19.5***	1.0

Abbreviations : g, gap ; ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; frg, fragmentation ; mul, Multiple aberration , +g, including gaps ; -g, excluding gaps, DMSO, dimethylsulfoxide

*** : Significantly different from the solvent control at $p<0.001$ (Fisher Exact Probability Test)

Table 6. Chromosome aberration test (40 hours exposure without S9 mix)

Test substance	Concen-tration (μg/ml)	Number of meta-phases	Number of chromosome aberration							Number of aberrant cells		Poly-ploid cells
			gap	Chromatid type		Chromosome type		frg	mul			
			g	ctb	cte	csb	cse			+g	-g	
DMSO	-	100	0	2	0	0	0	0	0	2	2	1
		100	1	2	0	1	1	0	0	5	4	1
		(Mean)	0.5	2.0	0.0	0.5	0.5	0.0	0.0	3.5	3.0	1.0
NNI-0001	125	100	0	3	0	1	0	0	0	4	4	1
		100	0	3	1	0	0	0	0	4	4	1
		(Mean)	0.0	3.0	0.5	0.5	0.0	0.0	0.0	4.0	4.0	1.0
NNI-0001	250	100	0	2	1	0	0	0	0	3	3	0
		100	0	1	0	3	0	0	0	3	3	0
		(Mean)	0	1.5	0.5	1.5	0.0	0.0	0.0	3.0	3.0	0.0
NNI-0001	500	100	0	2	1	0	0	0	0	3	3	3
		100	1	1	0	0	1	0	0	3	2	2
		(Mean)	0.5	1.5	0.5	0.0	0.5	0.0	0.0	3.0	2.5	2.5
Myto-micin C	0.07	100	1	15	17	0	2	0	1	27	26	1
		100	0	19	31	3	2	0	1	26	26	0
		(Mean)	0.5	17.0	24.0	1.5	2.0	0.0	1.0	26.5	26.0***	0.5

Abbreviations : g, gap ; ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; frg, fragmentation ; mul, Multiple aberration , +g, including gaps ; -g, excluding gaps, DMSO, dimethylsulfoxide

*** : Significantly different from the solvent control at $p<0.001$(Fisher Exact Probability Test)

EPA Reviewer: Nancy McCarroll
Toxicology Branch, Health Effects Division (7509P)
EPA Secondary Reviewer: Gregory Akerman, Ph.D.
Toxicology Branch, Health Effects Division (7509P)
TXR#: 0054319

Signature: Nancy McCarroll
Date: 07/05/07
Signature: Gregory Akerman
Date: 07/05/07

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

PC CODE: 0274602 **P BARCODE:** D331553

TEST MATERIAL (PURITY): NNI-0001 technical (97.8%, Lot No. 1FH0018P)

SYNONYMS: Flubendiamide; N2- [1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-{2-methyl-4-[1,2,2,2,-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}phthalamide

CITATION: Miyahana, K. (2003). Micronucleus test of NNI-0001 in mice. Toxicology & Pharmaceutical Research Center, Research Division, Nihon Nohyaku Co., Ltd. Osaka, Japan. Study No. GA-08, 02-0020, Final Report No. LSRC-T02-089A, December 9, 2003. MRID 46817225. Unpublished.

SPONSOR: Nihon Nohyaku Co., Ltd. Osaka, Japan

EXECUTIVE SUMMARY - In a bone marrow micronucleus assay (MRID 46817225), young adult male and female ICR mice (5/sex/dose) were treated once via oral gavage with 0, 500, 1000 or 2000 mg/kg NNI-0001 technical (97.8%, Lot No. 1FH0018P) suspended in 1% carboxylmethylcellulose. Bone marrow cells were harvested at 24 and 48 hours following dosing. Dose selection was based on the preliminary findings showing no effects at 500, 1000 or 2000 mg/kg.

No deaths or clinical signs of toxicity occurred at any dose in the treated animals. There was no cytotoxic effect on the bone marrow. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time in any treatment group.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material - NNI-0001 technical

Description: White crystals
Batch/Lot #: 1FH0018P
Purity: 97.8%
CAS # of TGAI: 272451-65-7
Vehicle: 1% carboxymethylcellulose (CMC)

2. Control materials

Negative control:	The vehicle alone served as the negative control
Vehicle:	1% CMC, oral gavage 10 ml/kg
Positive control:	Mitomycin C (Mit C 3 mg/kg, i.p.)

3. Test animals

Species:	Mouse			
Strain/ Sex	ICR /male & female			
Age/weight at initiation of micronucleus assay	8 weeks; 32.84-38.03 g (M); 28.73-31.68 g (F)			
Source:	Japan SLC Inc., (Kotoh-cho, Shizuka, Japan)			
No. animals used per dose	[5] Males		[5] Females	
Properly Maintained?	[X] Yes		[ ] No	

4. Test compound administration

	Dose levels	Final volume	Route
Pilot study	0, 1000 and 2000 mg/kg	10 mL/kg	Oral gavage (3♂, 3♀/dose)
Main study	0, 500, 1000 and 2000 mg/kg	10 mL/kg	Oral gavage (5♂, 5♀/dose)

B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[X] once		[ ] twice (24 hrs apart)		[ ] Other				
Sampling (after last dose):	[ ] 6 hr		[ ] 12 hr	[X] 24 hr	[X] 48 hr		[ ] 72 hr		

b. Positive control

Dosing:	[X] once		[ ] twice (24 hrs apart)		[ ] Other				
Sampling (after last dose):	[ ] 6 hr		[ ] 12 hr	[X] 24 hr	[ ] 48 hr		[ ] 72 hr		

2. Tissues and cells examined

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	Per 200 PCEs & NCEs

108

3. **Details of slide preparation** - Immediately after sacrifice, the marrow from both femurs was aspirated and flushed with fetal bovine serum. The cells were collected by centrifugation and two bone marrow smears per animal were prepared and air-dried overnight. The slides were stained with Giemsa solution, mounted and coded prior to evaluation.

4. **Evaluation criteria** -The test article was considered to be positive if a statistically significant and dose-related increase in micronucleated polychromatic erythrocyte (MPCE) frequency was observed for at least one test level. Historical negative and positive controls from the performing laboratory were provided.

5. **Statistical methods** - The MPCE and NCE data from the treated and positive control animals were compared to the vehicle controls using the tables of Kastenbaum and Bowman and the Wilcoxon rank sum test. Significance was indicated at $p<0.05$.

II. REPORTED RESULTS: Dose formulations were not analyzed for actual concentrations or stability in the vehicle. The test material was listed as stable at 5 °C in the dark.

A. PRELIMINARY TOXICITY ASSAY – In the pilot study, no deaths or clinical signs of toxicity were seen 48 hours after oral gavage treatment of male and female mice with ≥1000 mg/kg. Based on these results, 2000 mg/kg was chosen as the high dose in the micronucleus assay.

B. MICRONUCLEUS ASSAY - The results of the micronucleus assay for male and female mice are summarized in Study Report Table 1, pages 20 and 21 (see Attachment). No unscheduled deaths or clinical signs were reported at any dose (0, 500, 1000 or 2000 mg/kg). No significant increases in the number of NCE/2000 PCE or the MPCE frequency were observed in any treatment group when compared to controls. The positive control (Mit C at 3 mg/kg) induced a marked increase in MPCEs compared to the concurrent vehicle controls.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigators concluded that NNI-0001 did not induce micronuclei in bone marrow cells of male or female mice at doses up to 2000 mg/kg.

B. REVIEWERS' COMMENTS – No mortality or clinical signs of toxicity were noted in the treated animals at doses up to the limit dose for this test system. The sensitivity of the test system to detect a genotoxic response was clearly demonstrated by the marked increase in MPCEs in both sexes that was induced by the positive control (Mit C, 3 mg/kg). Based on these findings, NNI-0001 is neither clastogenic nor aneugenic in this acceptable *in vivo* cytogenetic assay.

C. STUDY DEFICIENCIES - None.

ATTACHMENT

The following attachment contains Table 1, pages 20 and 21 of MRID 46817225.

Table 1. Summary of results and statistical analysis

(Male)

Treatment	Dose (mg/kg)	No. of mice	MNIE (%)		IE/(IE+ME) (%)	
			Mean ± SD (range)	S^K	Mean ± SD (range)	S^W
(24 Hours)						
1%CMC	-	5	0.11 ± 0.02 (0.10~0.15)	-	50.7 ± 6.6 (42.5~59.5)	-
NNI-0001	500	5	0.09 ± 0.02 (0.05~0.10)	N.S.	54.7 ± 4.2 (49.0~60.0)	N.S.
NNI-0001	1,000	5	0.11 ± 0.07 (0.00~0.15)	N.S.	53.1 ± 4.0 (48.0~59.0)	N.S.
NNI-0001	2,000	5	0.10 ± 0.05 (0.05~0.15)	N.S.	49.8 ± 7.3 (40.0~58.0)	N.S.
Mitomycin C	3	5	2.41 ± 1.14 (1.25~4.05)	#	41.2 ± 10.4 (31.5~53.5)	N.S.
(48 Hours)						
1%CMC	-	5	0.06 ± 0.04 (0.00~0.10)	-	44.2 ± 6.7 (40.0~56.0)	-
NNI-0001	500	5	0.08 ± 0.08 (0.00~0.15)	N.S.	49.1 ± 5.0 (44.0~56.0)	N.S.
NNI-0001	1,000	5	0.05 ± 0.06 (0.00~0.15)	N.S.	46.3 ± 4.8 (40.5~52.0)	N.S.
NNI-0001	2,000	5	0.05 ± 0.07 (0.00~0.15)	N.S.	48.4 ± 7.9 (40.5~59.5)	N.S.

1%CMC : 1% (w/v) carboxymethylcellulose sodium salt solution
MNIE : Frequency of micronucleated immature erythrocytes
IE/(IE+ME) : Ratio of immature erythrocytes to total erythrocytes.
SD : Standard deviation.
(Range) : The range from the lowest value to the highest value
S^K and S^W : Statistical analysis using the table of Kastenbaum-Bowman and Wilcoxon's sum of rank test, respectively
N.S. : Not statistically significant from corresponding negative control (1% CMC) group at $p<0.05$
: Statistical analysis was not performed because the frequency of the micronucleated immature erythrocytes was out of the range listed in the tables of Kastenbaum & Bowman. However, frequency of micronucleated immature erythrocytes increased clearly

Table 1. (continued) Summary of results and statistical analysis

(Female)

Treatment	Dose (mg/kg)	No. of mice	MNIE (%) Mean ± SD (range)	S^K	IE/(IE+ME) (%) Mean ± SD (range)	S^W
(24 Hours)						
1%CMC	-	5	0.09 ± 0.07 (0.00~0.15)	-	50.5 ± 2.3 (48.5~53.5)	-
NNI-0001	500	5	0.09 ± 0.04 (0.05~0.15)	N.S.	50.2 ± 2.8 (46.5~53.5)	N.S.
NNI-0001	1,000	5	0.04 ± 0.02 (0.00~0.15)	N.S.	50.0 ± 6.2 (43.0~59.5)	N.S.
NNI-0001	2,000	5	0.09 ± 0.07 (0.00~0.15)	N.S.	52.8 ± 4.2 (48.0~59.0)	N.S.
Mitomycin C	3	5	2.50 ± 0.59 (2.15~3.50)	#	43.4 ± 5.3 (37.5~50.5)	N.S.
(48 Hours)						
1%CMC	-	5	0.05 ± 0.05 (0.00~0.10)	-	49.8 ± 3.7 (49.5~52.5)	-
NNI-0001	500	5	0.07 ± 0.04 (0.05~0.15)	N.S.	49.2 ± 1.4 (48.0~51.5)	N.S.
NNI-0001	1,000	5	0.03 ± 0.03 (0.00~0.05)	N.S.	50.9 ± 4.9 (44.0~57.0)	N.S.
NNI-0001	2,000	5	0.09 ± 0.02 (0.05~0.10)	N.S.	48.8 ± 3.2 (46.0~53.0)	N.S.

1%CMC : 1% (w/v) carboxymethylcellulose sodium salt solution
MNIE : Frequency of micronucleated immature erythrocytes
IE/(IE+ME) : Ratio of immature erythrocytes to total erythrocytes.
SD : Standard deviation.
(Range) : The range from the lowest value to the highest value
S^K and S^W : Statistical analysis using the table of Kastenbaum-Bowman and Wilcoxon's sum of rank test, respectively
N.S. : Not statistically significant from corresponding negative control (1% CMC) group at $p<0.05$
: Statistical analysis was not performed because the frequency of the micronucleated immature erythrocytes was out of the range listed in the tables of Kastenbaum & Bowman. However, frequency of micronucleated immature erythrocytes increased clearly.

312

EPA Reviewer: Nancy McCarroll **Signature:** Nancy McCarroll
Toxicology Branch, Health Effects Division (7509P) **Date:** 07-05-07
EPA Secondary Reviewer: Gregory Akerman, Ph.D. **Signature:** Gregory Akerman
Toxicology Branch, Health Effects Division (7509P) **Date:** 07/05/07
TXR#: 0054319

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

PC CODE: 027602 **DP BARCODE:** D331553

TEST MATERIAL (PURITY): NNI-0001 technical (98.9 %, Lot No. 3FH0032M)

SYNONYMS: Flubendiamide; N2- [1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-{2-methyl-4-[1,2,2,2,-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl}phthalamide

CITATION: Herbold, B. (2005). NNI-0001 Micronucleus– Test on the male mice TXAMX034. Study Report NO. AT01775; Bayer HealthCare AG PH-PD Toxicology International, 42096 Wuppertal, Germany. Study No. T 0073947, January 10, 2005. MRID 46817226. Unpublished.

SPONSOR: Bayer CropScience AG, Monheim, Germany

EXECUTIVE SUMMARY - In a bone marrow micronucleus assay (MRID 46817226), young adult male Hsd/Win: NMRI male mice (5/dose) were treated twice (24 hours apart) by intraperitoneal injection (i.p, 20 mL/kg) with NNI-0001 technical (98.9 %, Lot No. 3FH0032M) in 0.5% aqueous Cremophor as a suspension at doses of 0, 1000, 2000, or 4000 mg/kg. Bone marrow cells were harvested at 24 hours following the final dosing. Dose selection was based on the preliminary findings of a pilot in male and female mice administered 0, 1000, 2000 or 4000 mg/kg. Clinical signs in both sexes included: apathy, roughened fur, weight loss, sternal recumbency, spasm, and difficulty breathing. Since no substantial difference in toxicity was noted in males or females, only males were used in the micronucleus assay.

No deaths occurred at any dose; clinical signs of toxicity, similar to those seen in the preliminary testing, (i.e., apathy, roughened fur, weight loss, sternal recumbency, spasm, and difficulty breathing) were noted in the treated animals. There was no cytotoxic effect on the bone marrow. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time in any treatment group.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test

3/3

Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material - NNI-0001

Description:	Fine white powder
Batch #:	3FH 00 32 M
Purity:	98.9% a.i.
CAS # of TGAI:	272451-65-7

2. Control materials

Negative control:	The vehicle alone served as the negative control
Vehicle:	0.5% aqueous Cremophor (20 mL/kg, i.p.)
Positive control:	Cyclophosphamide in physiological saline (CP, 20 mg/kg, i.p.)

3. Test animals

Species:	Mouse			
Strain/ Sex	Hsd/Win: NMRI/male			
Age/weight at arrival:	6-12 weeks; 37-44 g			
Source:	Harlan Winkelmann GmbH, Borchen			
No. animals used per dose	[5] Males [a]	[0] Females		
Properly Maintained?	[X] Yes	[ ] No		

a An additional 5 males were dosed at 4000 mg/kg to serve as replacements as needed.

4. Test compound administration

	Dose levels	Final volume	Route
Pilot study	0, 1000, 2000 and 4000 mg/kg	20 mL/kg	i.p. (3♂, 3♀/dose)
Main study	0, 1000, 2000 and 4000 mg/kg	20 mL/kg	i.p. (5♂/dose)

B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[ ] Once	[X] twice (24 hrs apart)			[ ] Other				
Sampling (after last dose):	[ ] 6 hr		[ ] 12 hr		[X] 24 hr		[ ] 48 hr		[ ] 72 hr

b. Positive control

Dosing:	[X] Once		[ ] twice (24 hrs apart)			[ ] Other			
Sampling (after last dose):	[ ] 6 hr		[ ] 12 hr		[X] 24 hr		[ ] 48 hr		[ ] 72 hr

2. Tissues and cells examined

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	Per 2000 PCEs

3. Details of slide preparation - Immediately after sacrifice, the marrow from at least one

femur was aspirated and flushed with fetal calf serum. The cells were collected by centrifugation and marrow smears (number of slides/animal was not reported) were prepared and air-dried overnight. The slides were stained automatically using an Ames Hema-Tek Slide Stainer (Miles Company). Slides were 'destained' with methanol, rinsed with deionized water, and air-dried. The slides were cleared in xylene, mounted with coverslips, and coded prior to evaluation.

4. Evaluation criteria

a. Assay validity - The assay was considered valid if the following criterion was met: The number of micronucleated polychromatic erythrocytes (MPCE) in the positive and negative controls was within the provided historical control range.

b. Positive result - The test article was considered to be positive if a statistically significant, biologically relevant increase in MPCE frequency was observed compared to the negative control.

5. Statistical methods - The MPCE and NCE data from the treated and positive control animals were compared to the vehicle controls using the Wilcoxon non-parametric rank sum test. Significance was indicated at $p<0.05$.

II. REPORTED RESULTS: Dose formulations were not analyzed for actual concentrations. However, stability in the vehicle was reported to be 92-98% of the nominal dose for at least 4 hours.

A. PRELIMINARY TOXICITY ASSAY – In the pilot study, 3 male and 3 female mice received two i.p. doses of the test material at 1000, 2000 or 4000 mg/kg separated by 24 hours. Male and females treated with NN1-0001 at ≥1000 mg/kg displayed the following clinical signs for up to 24 hours after the second application: apathy, roughened fur, weight loss, sternal recumbency, spasm, high-stepping gait, difficulty breathing and silted eyes. No animals died at any dose. Based on these results, a maximum tolerated dose (MTD) of 4000 mg/kg was chosen as the high dose in the micronucleus assay. Since no substantial difference in toxicity was noted in males or females, only males were used in the micronucleus assay.

B. MICRONUCLEUS ASSAY - The results of the micronucleus assay are summarized in Study Report Table 6, page 49 (see Attachment). No unscheduled deaths occurred. The following clinical signs of toxicity (similar to those seen in the preliminary toxicity assay) were noted in the treated animals following the second dosing: apathy, roughened fur, weight loss, sternal recumbency, spasm, and difficulty breathing. No significant increases in the number of NCE/2000 PCE or the MPCE frequency were observed in any treatment group when compared to controls. The positive control (cyclophosphamide) induced statistically significant increases ($p<0.01$) in MPCEs compared to the concurrent vehicle controls.

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that NNI-0001 did not induce micronuclei in bone marrow cells of male mice at doses up to 4000 mg/kg.

B. REVIEWERS' COMMENTS – While mortality was not observed at any dose, clinical signs of toxicity were noted in the treated animals following the second dosing and included: apathy, roughened fur, weight loss, sternal recumbency, spasm, and difficulty breathing. However, the test material failed to induce either a cytotoxic, clastogenic or aneugenic effect at doses well in excess of the limit dose of 2000 mg/kg. The sensitivity of the test system to detect a genotoxic response was clearly demonstrated by the significant ($p<0.01$) increase in MPCEs compared to the concurrent vehicle controls that was induced by the positive control (Cyclophosphamide, 20 mg/kg). Based on these findings, NNI-0001 is neither clastogenic nor aneugenic in this acceptable *in vivo* cytogenetic assay.

C. STUDY DEFICIENCIES - None.

D. ATTACHMENT – Study Report (MRID 46817226), Table 6, page 49.

ATTACHMENT

The following attachment contains Table 6 from page 49 of MRID 46817226.

NNI-0001 49 Study No. T 0073947
Micronucleus test Bayer HealthCare AG

Table 6

Summary of Results of the Micronucleus Test with NNI-0001

experimental groups	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
negative control	10,000	2106 ± 1555	3.7 ± 3.4	2.6 ± 0.9
NNI-0001 2x 1000 mg/kg	10,000	2555 ± 529	3.2 ± 1.3	2.8 ± 1.5
NNI-0001 2x 2000 mg/kg	10,000	3609 ± 1308	5.8 ± 2.9	5.2 ± 3.0
NNI-0001 2x 4000 mg/kg	10,000	3374 ± 1251	3.8 ± 1.2	5.4 ± 4.8
positive control CP 20 mg/kg	10,000	1896 ± 692	5.7 ± 2.8	17.0* ± 4.6

*P < 0.01 in non-parametric Wilcoxon ranking test

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.6200 [§81-8a]; Neurotoxicity Screening Battery in Rats

Work Assignment No. 4-1-124 W; formerly 3-1-124 W (MRID 46817227)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer David A. McEwen, B.S.	Signature: David A. McEwen Date: 1/19/07
Secondary Reviewer John W. Allran, M.S.	Signature: John W. Allran Date: 01-19-07
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 1/19/07
Quality Assurance: Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 1/19/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Mary Ko Manibusan **Signature:** MK
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
EPA Work Assignment Manager: Myron Ottley, Ph. D. **Signature:** Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8]; OECD 424.

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR# 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 97.0-97.4% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Gilmore, R.G. and S.G. Lake (2003) An acute oral neurotoxicity screening study with technical grade NNI-0001 in Fischer 344 rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 02-N12-LC, April 17, 2003. MRID 46817227. Unpublished.

SPONSOR: Bayer CropScience LP, 2 T.W. Alexander Dr, Research Triangle Park, NC

EXECUTIVE SUMMARY - In an acute neurotoxicity study (MRID 46817227), groups of non-fasted Fischer 344 rats (12/sex/dose) were given a single oral (gavage; 10 mL/kg) dose of NNI-0001 (Flubendiamide; 97.0-97.4% a.i., Batch # 1FH0019M) in aqueous 0.5% methyl cellulose/0.4% Tween 80 at doses of 0, 200, 700, or 2000 mg/kg (limit dose) and observed for 14-15 days. A functional observational battery (FOB) and motor activity testing were performed on all animals during pre-exposure, Day 0 (at 8 hours post-dosing, the estimated time-of-peak effect), and Days 7 and 14. At study termination, 6 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. The brain and peripheral nervous system tissues collected from the perfused animals in the control and 2000 mg/kg groups were subjected to histopathological evaluation. Positive control data were not provided; however, data previously reviewed by the Agency have been included in this DER.

No compound-related effects on mortality, clinical signs of toxicity, body weight, body weight gain, FOB parameters, motor activity, brain weight, gross pathology, or neuropathology were observed at any dose in either sex.

No evidence of neurotoxicity was observed at any dose.

The LOAEL was not observed. The NOAEL is 2000 mg/kg (limit dose).

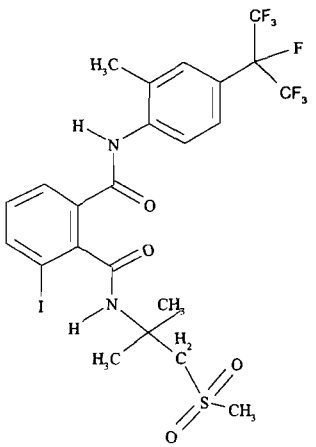
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (870.6200; OECD 424) for an acute neurotoxicity study in rats.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** NNI-0001

Description:	White powder
Batch #:	1FH0019M
Purity:	97.0-97.4% a.i.
Stability:	The test material was shown to be stable in the vehicle for up to 8 days refrigerated.
CAS # of TGAI:	272451-65-7
Structure:	

2. **Vehicle** – 0.5% methyl cellulose/0.4% Tween 80 in deionized water

3. **Test animals**

Species:	Rat
Strain:	Fischer 344 CDF(F-344)CRLBR
Age/group mean weight at dosing:	Approximately 9 weeks/ 186-190 g males; 128-133 g females
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Individually in suspended, stainless steel wire-mesh cages
Diet:	Rodent Lab Chow #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during neurobehavioral testing
Water:	Tap water, *ad libitum*, except during neurobehavioral testing
Environmental conditions:	**Temperature:** 19-25EC **Humidity:** 30-70% **Air changes:** 10-15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	At least 6 days

B. STUDY DESIGN

1. **In-life dates** - Start: 08/12/2002 End: 08/29/2002

2. **Animal assignment, treatment, and dose rationale** - Animals were randomly assigned to the test groups noted in Table 1. The body weights of all animals were within 20% of the mean weight for each sex. The animals were given a single gavage dose (10 mL/kg) of NNI-0001 in 0.5% methyl

cellulose/0.4% Tween 80 in deionized water then observed daily for 14-15 days. Dose levels for the current study were chosen based on the results of an acute oral toxicity study (LD_{50}, report number LSRC-T02-026A) in which NNI-0001 was administered once via gavage to adult Sprague-Dawley rats at the limit dose (2000 mg/kg). No clinical signs of toxicity were observed in any animal. Additionally, an acute oral toxicity study in mice revealed no clinical signs of toxicity at 2000 or 5000 mg/kg. Based on the results of the acute studies and a blood/plasma kinetics study (study number not reported), the time of peak-effect was determined to be approximately 8 hours post-dosing.

TABLE 1. Study design [a]

Experimental parameter	Dose (mg/kg)			
	0	200	700	2000
Total number of animals/sex/group	12/sex	12/sex	12/sex	12/sex
Behavioral testing (FOB, Motor Activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	6/sex	6/sex	6/sex	6/sex

a Data were extracted from pages 17-18 of the study report.

3. **Test Substance preparation and analysis** – Dose formulations were prepared prior to dosing by mixing the appropriate amount of NNI-0001 with 0.5% methyl cellulose/0.4% Tween 80 in deionized water. Dose formulations were stored refrigerated until dosing. Homogeneity and stability were verified at concentrations of 10 and 200 mg/mL (equivalent to 100 and 2000 mg/kg) which bracketed the concentrations in the current study. Each dosing suspension was also analyzed for actual content.

Results

Homogeneity analysis (range as % CV): 1.1-4.0%

Stability analysis (% of initial after 8 days refrigerated): 93.4-102%

Concentration analysis (range as % of nominal): 104-111%

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

4. **Statistics** - The data were analyzed using the following statistical methods, and significance was defined at p#0.05 for all tests except Bartlett's test (p#0.001):

Parameter	Statistical Method
Continuous pathology data	Bartlett's test to analyze for homogeneity of variances. Data with homogenous variances were further analyzed using ANOVA followed by Dunnett's for pairwise comparisons. Data with non-homogeneous variances were further analyzed using Kruskal-Wallis test followed by a Mann-Whitney U test for pairwise comparisons.
Micropathology frequency data	Chi-square test followed by a one-tailed Fisher's Exact test
Motor and locomotor activity (session data)	Repeated-measures ANOVA followed by one-way ANOVA if significant treatment x week interaction. For weeks in which there was a significant treatment effect, Dunnett's was used to determine significance from controls.
Motor and locomotor activity (interval data)	Two-way repeated measures ANOVA (using interval x week) followed by a Repeated-measures ANOVA to determine which weeks had a significant treatment x interval interaction. For those weeks, each interval was analyzed using an one-way ANOVA followed by Dunnett's as necessary.
Continuous FOB data	Repeated-measures ANOVA followed by one-way ANOVA and Dunnett's as necessary
Categorical FOB data	General Linear Modeling (GLM) followed by Dunnett's or Categorical Modeling (CATMOD) followed by Analysis of Contrasts
Terminal body weight Brain weight	Bartlett's test for homogeneity of variances (brain weight) or Levene test (terminal body weight). Homogeneous data were further analyzed using ANOVA followed by Dunnett's. Non-homogeneous data were analyzed using the non-parametric Kruskal-Wallis test followed by a Mann-Whitney U test.

The reviewers consider the statistical methods to be appropriate.

C. METHODS / OBSERVATIONS

1. **Mortality and clinical observations** - Animals were observed at least once daily for mortality and clinical signs of toxicity. Detailed physical examinations were performed daily following dosing.

2. **Body weight** - Animals were weighed weekly as part of the FOB.

3. **Food consumption** – Food consumption was not recorded.

4. **Cholinesterase determination** - Cholinesterase activity was not evaluated.

5. Neurobehavioral assessment

a. Functional Observational Battery (FOB) – All animals were subjected to a FOB during pre-exposure (baseline), at Day 0 (8 hours post-dosing, time-of-peak effect), and Days 7 and 14. The FOB was conducted by an observer who was 'blind' to the treatment status of the animal, and the same observer performed all of the evaluations. The scoring criteria for the FOB were provided on pages 139-145 of the study report. The time in the open-field was 2 minutes. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
	Biting	X	Ease of removal	X	Rearing+
X	Convulsions*	X	Lacrimation*/chromodacryorrhea	X	Arousal/ general activity level*
X	Tremors*	X	Salivation*	X	Convulsions*
X	Abnormal movements*		Piloerection*	X	Tremors*
	Palpebral closure*	X	Fur appearance	X	Abnormal movements*
	Feces consistency	X	Palpebral closure*	X	Urination / defecation*
X	Piloerection	X	Nasal discharge	X	Piloerection
X	Gait abnormalities		Respiratory rate+	X	Respiratory abnormalities
X	Vocalizations	X	Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*		Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*	X	Pupil size		Backing
X	Pain response*				Time to first step
X	Pupil response*		**PHYSIOLOGICAL OBSER.**	X	Vocalizations
	Eye blink response	X	Body weight*		**NEUROMUSCULAR OBSER.**
	Forelimb extension	X	Body temperature+		Hindlimb extensor strength
	Hindlimb extension		**OTHER OBSERVATIONS**	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
					Rotarod performance

* Required parameters; + Recommended parameters

b. Locomotor activity - Locomotor activity was evaluated following the FOB pre-exposure (baseline) and on Days 0, 7, and 14. An automated Columbus Instruments Universal Maze Monitoring System was used with a figure-eight maze for motor activity data collection. Each test session consisted of six 10-minute epochs, totaling 60 minutes of testing per animal per test session. Motor activity was measured as the number of beam breaks that occurred during the test session. Locomotor activity was measured by elimination consecutive counts for a given beam. Habituation was evaluated as a decrement in activity during the test session. Each week testing was staggered over 2 days for each sex to accommodate the schedule for behavioral testing. Males and females were tested on separate

days, and the open-field and mazes were cleaned during the ensuing interval to remove any residual scent from the other sex. The order of testing and assignment to mazes was done in semi-random order such that no animal was tested more than once in the same maze. Broad spectrum background noise (74 dBA) was provided to minimize acoustical variations, and light intensity (100±70 lux) over each maze was verified daily.

6. **Sacrifice and pathology** – Animals not selected for neuropathological evaluation were sacrificed via CO_2 asphyxiation and subjected to gross necropsy. Animals selected for neuropathological evaluation (6 rats/sex/dose) were anesthetized using pentobarbital (50 mg/kg i.p.), and perfused via the left ventricle with a phosphate buffered sodium nitrite flush followed by *in situ* fixation with 10% buffered formalin. The entire brain, spinal cord, eyes (with optic nerves), selected bilateral peripheral nerves (sciatic, tibial, and sural), gasserian ganglion, gastrocnemius muscle, and both forelimbs were post-fixed in 10% buffered formalin. The brain was weighed prior to fixing, and the brain:body weight ratio was calculated.

The tissues from the control and 2000 mg/kg animals were further processed for microscopic evaluation. The brain, spinal cord sections, cauda equina, eyes, optic nerve, and gastrocnemius muscle were embedded in paraffin and stained with hematoxylin-eosin. The dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings, Gasserian ganglia, and peripheral nerves (sciatic, tibial, and sural) were embedded in glycol methacrylate, sectioned (2-3 µm), and stained with a modified Lee's stain. The sciatic nerve was also sectioned longitudinally at approximately 1 µm and stained with toluidine-blue. The following CHECKED (X) tissues from the control and 2000 mg/kg animals, as well as all gross lesions were examined microscopically:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Olfactory bulbs		Mid-thigh
X	Cerebral cortex		Sciatic Notch
X	Hippocampus		
X	Thalamus		
X	Hypothalamus		
X	Midbrain		**OTHER**
X	Cerebellum	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Cervical dorsal root ganglion
X	Thoracic	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER	X	Lumbar dorsal root ganglion
X	Gasserian ganglion	X	Lumbar dorsal root fibers
X	Optic nerve	X	Lumbar ventral root fibers
X	Eyes		
X	Gastrocnemius muscle		

7. **Positive controls** – It was stated that four studies (MRIDs 42770301, 45464601, 45464602, and 43656301, performed in 1993, 1999, 2000, and 1994; respectively) were previously performed to generate positive control data and validate the procedures and observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity, behavioral effects, and neuropathological lesions. However, data from these studies were not provided. Below are summaries of seven positive control studies (MRIDs 45540501 through 45540507) previously reviewed by the Agency. Exposure to **3,3-Iminodipropionitrile** (2000 mg/kg, single i.p. dose) induced the following in both sexes: (i) decreased body weight; (ii) FOB effects (eg. ataxia, females only); (iii) decreased fore- and hindlimb grip strength; (iv) corneal opacities; (v) blood lacunae in the iris; (vi) anisocoria; and (vii) hematobulbus. Additionally, the following histopathological effects were noted: (i) axonal atrophy in the distal segments of the tibial, sural, and sciatic nerves; (ii) intraoccular hemorrhage; (iii) retinal degeneration with atrophy; and (iv) degeneration and atrophy of the optic nerve. **Acrylamide** (40 mg/kg, 11 daily gavage doses in 2 weeks) induced the following in both sexes: (i) abnormal gait (ataxia, splay of toes of the hindlimbs and/or splay of the hind limbs); (ii) decreased fore- and hindlimb grip strength; and (iii) increased hindlimb foot splay. Additionally in the males, body weight and body weight gains were decreased, and decreased activity, reduced tail pinch response, and increased reaction time to hot-plate test were observed. In addition to decreased brain weight in both sexes, the following histopathological effects were noted: (i) selective Purkinje cell necrosis and vacuolation of the molecular layer of the cerebellar cortex; (ii) cytoplasmic remodeling in the lumbar spinal ganglia cells which resembles chromatolysis; (iii) Wallerian-like axonal degeneration of the sciatic, sural, tibial, and plantar nerves; (iv) neurofilament accumulation, decrease in or loss of synaptic vesicles, and swelling of synaptic terminals in the gastrocnemius muscle; and (v) neuronal necrosis in the mesencephalic trigeminal nucleus region of the midbrain in one male. In addition to the effects given above, acrylamide (30 mg/kg, daily gavage doses up to 4 weeks) induced mortality in both sexes. **Trimethyltin chloride** (6, 9, or 12 mg/kg, single i.p. dose) induced ataxia, tremors, convulsions, decreased grip strength, increased foot splay, and increased motor activity. Additionally, the following neuropathological effects were noted: (i) neuronal necrosis of the olfactory bulbs and midbrain; (ii) axonal degeneration of the cervical ganglia and peripheral nerves; (iii) hydrocephalus internus of the frontal and parietal lobes; (iv) Purkinje cell necrosis in the pons with cerebellar cortex, mid-cerebellum, and medulla oblongata; (v) chromatolysis of alpha motor neurons in the cervical and lumbar spinal cord; and (vi) vacuolar degeneration of the lumbar ganglia. Inter-observer reliability was demonstrated using **carbaryl** (10 or 30 mg/kg, single i.p. dose), **nomifensin** (10 mg/kg, gavage on 2 days), and **diazepam** (3 mg/kg, i.p. on 2 days). All observers detected the FOB effects from carbaryl (abnormal body posture, tremors, repetitive chewing, impaired gait, and reduced rearing), the increased motor activity from nomifensin, and the decreased motor activity from diazepam.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs** – No treatment-related clinical signs were observed at any dose in either sex.

2. **Mortality** – Two control group females (#s LC0104 and LC0105) were found dead or sacrificed *in extremis* on Days 1 or 2. It was determined that each animal had a perforated esophagus, caused by a dosing injury. All other animals survived to scheduled sacrifice.

B. BODY WEIGHT AND BODY WEIGHT GAIN – No treatment-related effects on body weight or overall (0-14) body weight gain (calculated by reviewers) were observed at any dose (Table 2).

TABLE 2. Mean (±SD) body weight and body weight gain (g) in rats exposed to NNI-0001 once via gavage. [a]

Days	Dose (mg/kg)			
	0	200	700	2000
Males				
0	186±8	190±10	187±9	190±7
14	231±11	238±13	233±13	243±14
Overall (0-14) weight gain [b]	45	48	46	53
Females				
0	130±3	129±4	133±4	128±5
14	149±4	149±6	158±8	152±6
Overall (0-14) weight gain [b]	19	20	25	24

a Data were obtained from Table 3 on pages 103, 106, 107, and 110; n=12, except in the control females on Day 14, where n=10.

b Calculated by reviewers from data contained within this table.

C. FOOD CONSUMPTION – Food consumption was not recorded.

D. CHOLINESTERASE ACTIVITIES – Cholinesterase activity was not evaluated.

E. NEUROBEHAVIORAL RESULTS

1. **FOB findings** – No treatment-related effects were noted in any FOB parameter at any dose.

2. **Motor activity** – No treatment-related effects on total session motor or locomotor activity were observed at any dose in either sex (Tables 3a and 3b). Interval motor and locomotor activity were reported on pages 118-134 of the study report. Locomotor activity was increased ($p \leq 0.05$) by 200% at Interval 6 on Day 14 in the 700 mg/kg males; however, this finding was considered incidental as it was not dose-dependent. Habituation was unaffected by treatment.

TABLE 3a. Mean (±SD) total session motor activity in rats exposed to NNI-0001 once via gavage. [a]

Test day	Dose (mg/kg)			
	0	200	700	2000
Males				
Pretest	360±110	292±119	297±143	287±86
Day 0	189±67	155±58	229±89	201±77
Day 7	458±159	406±126	504±194	424±97
Day 14	469±131	362±105	559±133	415±100
Females				
Pretest	378±169	430±176	519±186	465±215
Day 0	267±98	301±110	375±94	361±90
Day 7	512±214	601±170	647±131	643±136
Day 14	545±168	575±125	589±87	599±131

a Data were obtained from Table 4 on pages 112-113 of the study report; n=12, except in the control females on Days 0-14, where n=10.

TABLE 3b. Mean (±SD) total session locomotor activity in rats exposed to NNI-0001 once via gavage. [a]

Test day	Dose (mg/kg)			
	0	200	700	2000
Males				
Pretest	200±43	165±77	173±82	171±63
Day 0	96±39	72±16	115±31	103±37
Day 7	258±76	242±75	298±110	231±63
Day 14	277±56	219±62	319±88	263±69
Females				
Pretest	239±132	242±104	326±104	269±138
Day 0	146±60	170±58	215±49	208±73
Day 7	301±120	360±90	395±86	418±95
Day 14	314±120	347±69	359±95	382±100

a Data were obtained from Table 5 on pages 115-116 of the study report; n=12, except in the control females on Days 0-14, where n=10.

F. SACRIFICE AND PATHOLOGY

1. **Gross pathology** - No treatment-related gross lesions were observed at any dose.

2. **Brain weight** – Absolute and relative (to body) brain weights were similar to controls on Day 14/16 in both sexes (Table 4).

TABLE 4. Mean (±SD) absolute and relative (to body) brain weights (g) in rats exposed to NNI-0001 once via gavage. [a]

Parameter	Dose (mg/kg)			
	0	200	700	2000
Males				
Terminal BW (g)	228.5±12.3	232.4±12.1	232.1±16.1	242.4±13.0
Absolute Brain	1.676±0.086	1.719±0.038	1.748±0.059	1.709±0.040
Relative Brain	0.734±0.019	0.741±0.039	0.755±0.037	0.707±0.046
Females				
Terminal BW (g)	148.0±4.6	147.1±5.8	158.7±6.0* (↑7)	153.5±5.2
Absolute Brain	1.618±0.040	1.637±0.036	1.657±0.065	1.637±0.029
Relative Brain	1.094±0.048	1.114±0.044	1.046±0.064	1.067±0.031

a Data were extracted from Table OW1K-SUM on pages 397 and 398 of the study report; n=6.
* Statistically significantly different from controls at $p \leq 0.05$

3. **Neuropathology** – No treatment related neuropathological lesions were observed. Axonal degeneration of individual nerve fibers was noted in several peripheral nerves. There was no indication of an effect of treatment, as these isolated lesions were minimal to slight in severity and were found at approximately the same frequency in the control and 2000 mg/kg animals.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS** – The Sponsor concluded that a single oral gavage dose of NNI-0001 caused no adverse systemic or neuropathological effects in rats at the limit dose. The NOAEL was 2000 mg/kg.

B. **REVIEWER COMMENTS** – No compound-related effects on mortality, clinical signs of toxicity, body weight, body weight gain, FOB parameters, motor activity, brain weight, gross pathology, or neuropathology were observed at any dose in either sex.

No evidence of neurotoxicity was observed at any dose.

The LOAEL was not observed. The NOAEL is 2000 mg/kg (limit dose).

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (870.6200; OECD 424) for an acute neurotoxicity study in rats.

C. **STUDY DEFICIENCIES** – None

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870.7485 [§85-1]; Metabolism Study in Rats

Work Assignment No. 4-01-124 M; formerly 3-1-124 M
(MRIDs 46817229, 46817230, and 46817231)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 01/19/07

Secondary Reviewer:
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J. Bever Jr
Date: 01/19/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 01/19/07

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 01/19/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Mary K. Manibusan **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/26/09

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 [§85-1]; OECD 417.

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (RADIOCHEMICAL PURITY): NNI-0001 (Flubendiamide; >98%)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Motoba, K. (2005) Absorption, distribution, metabolism, and excretion of radiolabeled NNI-0001 following a single oral administration to male and female rats. Toxicological & Pharmaceutical Research Center, Research Division, Nihon Nohyaku Co., Ltd., Osaka, Japan. Laboratory Study Report Number: LSRC-M04-115A (Amendment I), September 16, 2005. MRID 46817229. Unpublished

Motoba, K. (2005) Absorption, distribution, metabolism, and excretion of [phthalic ring-(U)-^{14}C] NNI-0001 following 14 repetitive oral administrations to male and female rats. Toxicological & Pharmaceutical Research Center, Research Division, Nihon Nohyaku Co., Ltd., Osaka, Japan. Laboratory Study Report Number: LSRC-M04-114A (Amendment I), September 16, 2005. MRID 46817230. Unpublished

Motoba, K. (2004) Biliary excretion study of [phthalic ring-(U)-^{14}C] NNI-0001 following a single oral administration to male and female rats. Toxicological & Pharmaceutical Research Center, Research Division, Nihon Nohyaku Co., Ltd., Osaka, Japan. Laboratory Study Report Number: LSRC-M04-107A, May 14, 2004. MRID 46817231. Unpublished

SPONSOR: Bayer CropScience LP, Toxicology, 17745 South Metcalf Avenue, Stilwell, KS

EXECUTIVE SUMMARY: In a series of metabolism studies (MRIDs 46817229, 46817230, and 46817231), [phthalic ring-(U)-^{14}C]-NNI-0001 (Lot #s 0FH0001S-R and CP-2761; radiochemical purity >98%) or [aniline ring-(U)-^{14}C]-NNI-0001 (Lot # 1FH0002S-R; radiochemical purity >99.5%) in aqueous 2% (w/v) sodium carboxymethylcellulose containing 0.4% Tween 80 was administered by oral gavage to groups of Wistar rats at doses of 2 or 200 mg/kg. For the pharmacokinetic studies, four rats/sex were used. For the ADME studies, 12

rats/sex were used, and 4 rats/sex were killed at 9, 24, and 168 h post-dosing. For biliary excretion studies, three males and six females were used, and biliary stability was determined using two males and one female. In the [aniline ring-(U)-^{14}C] studies, two or four rats/sex were used in preliminary excretion and ADME studies, respectively. Finally, 30 male and 60 female rats were dosed with non-radioactive NNI-0001 for analysis of biliary metabolites. The concentration time-courses of radioactivity in blood and plasma were plotted, the concentrations of radioactivity in tissues and excreta were determined, and metabolites were identified and quantified in the urine, feces, and bile.

Radioactivity was detected in blood and plasma of the 2 mg/kg group and in the blood of the 200 mg/kg group at 1 h post-dosing. In animals given a single 2 mg/kg dose of [phthalic ring-U-^{14}C]-NNI-0001, males and females had approximately equal maximum radioactivity concentrations (C_{max}) in blood and plasma, but males demonstrated longer times to maximum concentration (t_{max}) than females. Females demonstrated longer terminal elimination half-lives than males. In the 200 mg/kg animals, the observed C_{max} did not increase in proportion to dose, suggesting saturation of the absorption process. Pharmacokinetic parameters were not analyzed for aniline ring-labeled NNI-0001. In animals given daily 2 mg/kg doses of phthalic ring-labeled NNI-0001, blood and plasma concentrations 24 h after doses 6, 13, and 14 were similar to each other, and were comparable to concentrations observed 24 h after a single dose when compared by sex. Maximum blood and plasma concentrations were observed 9 h after the 14th dose. Concentrations were consistently higher in females than in males. Radioactivity was still detectible in both the blood and plasma of females 168 h after dose 14, but was near or below the limit of quantitation in males.

The greatest concentrations of radioactivity were observed in the tissues and organs at 9 h post-dosing. Generally, the liver and kidneys contained the greatest percentage of the administered dose.

Total recoveries ranged from 94.1-104.7% of the administered doses, with no differences observed between sexes, dose levels, or position of the radiolabel. In the single and multiple dose studies, the majority of the radioactivity was recovered in the feces, with minor amounts recovered in the urine. Males excreted more radioactivity in the urine than females. Females excreted the test compound at a slower rate than the males. Generally, the majority of the radioactivity was recovered at the first 24 h collection point. It was stated that no significant excretion of radioactivity was detected in expired air at 24 h post-dosing. In the bile duct-cannulated rats, the majority of radioactivity was associated with the gastrointestinal contents at 48 h post-dosing. Absorption was estimated from radioactivity present in the bile, urine, gastrointestinal tract, liver, and carcass, and was determined to be 23.5% in the males and 34.1% in the females. Again, females appeared to excrete radioactivity more slowly than males.

The majority of radioactivity in urine and fecal extract samples was present as parent and up to three metabolites. Metabolic profiles were qualitatively similar for both radiolabeled forms. Oxidation of the methyl moiety on the aniline ring was determined to be the main metabolic pathway in both sexes. In excreta, parent and identified metabolites accounted for the majority of the administered dose. The total administered dose accounted for in the excreta was 80.1-98.6%. Parent compound was excreted almost exclusively in the feces. The 2 mg/kg single dose males

eliminated smaller amounts of parent than the 2 mg/kg females, while the 200 mg/kg males and females eliminated larger amounts. The metabolite found in the greatest quantity was NNI-0001-benzyl alcohol, with the 2 mg/kg males eliminating more than the females. The 200 mg/kg males eliminated a small amount (0.2%); this metabolite was not quantifiable in the 200 mg/kg females. The 2 mg/kg males excreted greater quantities of NNI-0001-benzoic acid than the females. NNI-0001-benzoic acid was not detected in the excreta of the 200 mg/kg rats. Only one other identified metabolite (NNI-0001-benzaldehyde) was found in the urine and feces of the single dose animals. In animals receiving daily doses of [^{14}C]-NNI-0001, NNI-0001-benzyl alcohol was the only metabolite present at >5% of the administered dose. One minor metabolite, NNI-0001-iodophthalimide, was the primary metabolite found in the fat of female rats. Additional minor (<5%) metabolites were identified in the bile. These metabolites were also produced in greater amounts by males than by females. The liver of male rats dosed with [aniline ring-(U)-^{14}C] contained several metabolites, while female livers contained almost solely unchanged parent. This finding may reflect the slower metabolism of the compound in female rats.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound:

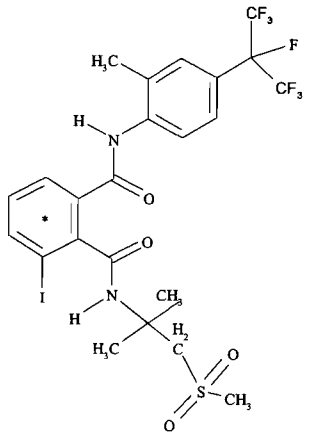
Radiolabeled test material 1: [Phthalic ring-(U)-^{14}C] NNI-0001

Radiochemical purity: >98% (TLC, HPLC)

Specific activity: 3.44[1] or 3.85[2] GBq/mmol

Lot number: [1]0FH0001S-R or [2]CP-2761

Structure



* = position of ^{14}C

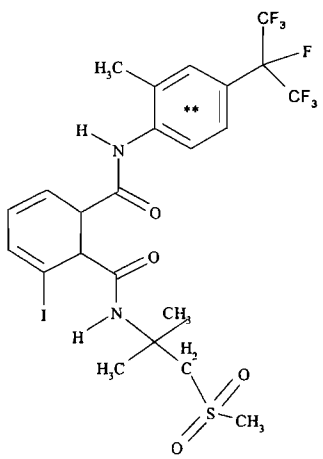
Radiolabeled test material 2: [Aniline ring-(U)-^{14}C] NNI-0001

Radiochemical purity: >99.5% (TLC, HPLC)

Specific activity: 2.48 GBq/mmol

Lot number: 1FH0002S-R

Structure



** = position of ^{14}C

Non-Radiolabeled test material 1: NNI-0001

Description: Not provided

Lot number: 1FH0013P; 1FH0019M

Purity: 96.7-99.6%

CAS # of TGAI: 272451-65-7

2. Vehicle: Aqueous 2% (w/v) sodium carboxymethylcellulose containing 0.4% Tween 80

3. **Test animals:**

Species:	Rat
Strain:	Fischer (F344/DuCrj)
Age/weight at study initiation:	6 to 8 weeks; 84.5-176.7 g males, 75.1-138.3 g females
Source:	Japan Charles River Co., Ltd. (Tokyo, Japan)
Housing:	Individually in glass metabolic cages, bollman cages, or suspended, stainless steel wire mesh cages
Diet:	Lab MR Stock® (Nihon Nousan Kogyo, Co., Ltd.) *ad libitum*, except 16-18 h prior to dosing in the single oral dose ADME study. 10% sucrose in distilled water was fed after bile duct cannulation in the biliary excretion study.
Water:	Tap water *ad libitum*
Environmental conditions:	
Temperature:	22±3EC
Humidity:	50±20%
Air changes:	At least 10/hr
Photoperiod:	12 h light/12 h dark
Acclimation period:	At least 6-7 days

4. **Preparation of dosing solutions:** Radiolabeled test substance was isotopically diluted with non-radiolabeled material in acetone to yield the following concentrations: 0.4 mg/0.5 MBq/mL (for the 2 mg/kg dose) or 40 mg/0.5 MBq/mL (for the 200 mg/kg dose) in the single dose ADME and biliary excretion studies (MRIDs 46817229 and 46817231); and 0.4 mg/0.2MBq/mL in the multiple dose ADME study (MRID 46817230). In all cases, the acetone solution was combined with an equal volume of 2% (w/v) sodium carboxymethylcellulose solution containing 0.4% Tween 80. The acetone was evaporated under a nitrogen gas stream, and distilled water was added to the remaining mixture to restore the original concentration. The specific activities of the dosing solutions were 2.5 MBq/mg and 0.025 MBq/mg for the 2 mg/kg and 200 mg/kg doses in the single dose ADME study, and 0.5 MBq/mg for the 2 mg/kg dose in the multiple dose ADME study. All dose solutions were prepared the day prior to dosing and stored below 4°C. It was also stated that all dose solutions were analyzed for stability by TLC and HPLC.

   The test compound was found to be stable in the vehicle for at least 15 days (MRID 46817229).

B. STUDY DESIGN AND METHODS

1. **Group arrangements:** In the single and multiple dose ADME studies, animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. Animals were not randomized in the biliary excretion study. It was stated that in a 13-week oral toxicity study in rats, the maximum non-toxic dose level was approximately 2.5 mg/kg/day, and the minimum toxic dose level was approximately 10 mg/kg/day. Therefore, animals were dosed at 2 mg/kg (expected to be a non-toxic dose) and 200 mg/kg (expected to be a toxic dose). Animals were weighed at study initiation. In the single dose studies, animals were observed for mortality, moribundity, and clinical signs of toxicity just after administration, 1 and 3 h post-dosing, and at sample collection (every 24 h). In the multiple dose study, animals were observed 1 h post-dosing and at sample collection. The results of these observations were not reported.

TABLE 1: Dose groups for [^{14}C]-NNI-0001 metabolism studies [a]

Test group	Nominal dose (mg/kg)	Actual dose (mg/kg)	# animals/ group	Comments
[Phthalic ring-(U)-^{14}C]-NNI-0001				
Single low dose pharmacokinetics	2	1.57 (M) 1.55 (F)	4/sex	Blood and plasma samples were collected at regular intervals up to 168 h.
Single high dose pharmacokinetics	200	190.95 (M) 193.29 (F)		
Single low dose ADME	2	1.82 (M) 1.83 (F)	12/sex	Four rats/sex/dose were killed at 9 and 24 h post-dosing and tissues were collected. In the remaining 4 rats/sex/dose, urine, feces, and cage wash were collected at regular intervals for up to 168 h, at which time the animals were killed and tissues collected. Expired air was trapped for 24 h. Urine, feces, and liver were analyzed for metabolites.
Single high dose ADME	200	205.63 (M) 215.03(F)		
Multiple low dose ADME	2	2.17 (M) 2.23 (F)	12/sex	Animals received 14 consecutive daily doses of test compound. Blood was sampled just before the seventh and the last doses. Four rats/sex were killed at 9 and 24 h post-dosing, and blood and tissues were collected. In the remaining 4 rats/sex/dose, urine, feces, and cage wash were collected at regular intervals for up to 168 h, at which time the animals were killed and tissues collected. Urine, feces, and fat were analyzed for metabolites.
Biliary excretion	2	1.87 (M) 1.97 (F)	3 male 6 female	Bile, urine, and feces were collected at regular intervals up to 48 h post-dosing. Animals were then killed and liver, gastrointestinal tract (with contents), and residual carcass were collected.
Biliary stability	2	Not provided	2 male 1 female	Bile was analyzed for metabolites within 14 d after collection, then frozen at -20°C for 183 d and reanalyzed.
[Aniline ring-(U)-^{14}C]-NNI-0001				
Preliminary single low dose	2	2.26 (M) 2.21 (F)	2/sex	Urine, feces, and expired air were collected up to 24 h post-dosing.
Single low dose ADME	2	1.88 (M) 1.92 (F)	4/sex	Urine, cage rinse, and feces were collected at regular intervals up to 168 h post-dosing, at which time the animals were killed and tissues were collected.
[Non-radioactive]-NNI-0001				
Biliary metabolites	20	Not applicable	30 male 60 female	The animals were dosed twice with the test compound (approximately 16 h prior to and just after bile duct cannulation), and bile only was collected.

a Data were obtained from pages 37 and Appendix VI on pages 108-111 of MRID 46817229, page 25 and Appendix VI on pages 67-68 of MRID 46817230, and pages 31, 32, 35, and 38 and Appendix VI on page 149 of MRID 46817231.

2. **Dosing and sample collection:** Animals were dosed by oral gavage with dose amounts based on individual body weights and a dosing volume of 5 mL/kg. In the single dose studies, rats were starved for 16-18 h prior to dosing; animals were not food-deprived in the multiple dose study. It was not stated if animals were starved in the biliary excretion study. The actual amount administered was calculated by subtracting the remaining amount of radioactivity post-dosing from the initial amount. The amount of test substance administered was then calculated by dividing the administered amount of radioactivity by the specific activity. The actual mean administered doses and the number of animals treated in each study are reported in Table 1.

a. **Pharmacokinetic studies:** In the single dose pharmacokinetic studies, blood samples were collected from the retroorbital plexus in heparinized glass capillaries at 1, 3, 6, 9, 12, and 24 h, and then every 24 h until 168 h post-dosing. In the multiple dose study, blood samples were collected 24 h following the sixth and thirteenth doses. In both studies, a portion of these samples was centrifuged to obtain plasma, and hematocrit values were obtained and recorded. Blood was not collected from the biliary excretion animals.

b. **Absorption, distribution, metabolism, and excretion (ADME) studies:** Excreta were collected up to the time of scheduled termination. In the single and multiple dose ADME studies, urine and feces were collected in cooled (<10°C) traps. Urine was collected at 6, 12, and 24 h, and then at 24 h intervals in the single dose studies, and at 24 h intervals in the multiple dose study. The cages were rinsed with water at every collection and combined with the corresponding urine sample. In the biliary excretion study, bile and urine were collected at 0-6, 6-12, 12-24, and 24-48 h. Feces were collected at 24 h intervals in all studies. The method of termination used was not specified. In the single dose ADME studies, animals were killed at 168 h post-dosing; in the multiple dose study, animals were killed at 168 h after the last dose. In the biliary excretions study, animals were killed at 48 h post-dosing.

Expired air was analyzed in the single dose ADME studies by trapping the exhaust air from the cages of two rats/sex/dose with 500 mL of 20% aqueous monoethonolamine at 24 h intervals.

The following tissues were collected at study termination of the single and multiple dose ADME studies:

eyes	liver	stomach
brain	kidneys	small intestine
pituitary	adrenals	large intestine
salivary gland	spleen	testes
thyroid	pancreas	prostate
thymus	fat	ovaries
heart	muscle	uterus
lung	urinary bladder	bone marrow/bone [a]

a Bone was collected and analyzed in the multiple dose study only.

In the biliary excretion study, liver, gastrointestinal tract, and residual carcass were collected at termination. In all studies, the gastrointestinal tract was incised and rinsed with distilled water to obtain the contents. In the single dose studies and the biliary excretion study, urine and/or bile were stored at 4°C until their initial radioanalysis. All other samples in these studies and all samples in the multiple dose study were stored below -20°C.

Weighed aliquots of blood were lysed by adding 20% aqueous methanol, solubilized with Soluene 350® and bleached with benzoylperoxide in toluene. Other weighed liquid samples (such as bile and urine) were analyzed directly. Solid or solid-containing samples (feces, gastrointestinal contents, and organs/tissues except bone) were homogenized, solubilized with Soluene 350® overnight at 50°C, and bleached with benzoylperoxide in toluene. Bone samples were combusted. Residual carcasses (including skin and fur) were incubated in 0.5*N* aqueous sodium hydroxide at 60°C for approximately two days. In some cases, weighed

carcass samples (without skin and fur) were minced with a meat grinder and then processed as described for solid samples.

Radioactivity in all prepared samples was quantitated by liquid scintillation counting (LSC). Scintillation counting data were automatically converted to disintegrations per minute (dpm) with an instrument-installed external standard and quenching library. Prior to counting the first samples, ten blank vials were counted, and the mean of the obtained data was designated as background. This mean plus three to four standard deviations was defined as the detection minimum. Recovery of radioactivity following combustion was verified prior to use as >95%.

c. **Metabolite characterizations:** Portions of urine and bile samples collected at each interval were pooled by sex and dose and directly analyzed by two-dimensional TLC. Samples of feces, gastrointestinal tract contents, and liver were extracted twice with a 10-fold volume of 1:1 (v/v) methanol/acetone. The extracts were combined and subjected to radioanalysis by LSC and two-dimensional TLC, and HPLC (except liver). Residual radioactivity in the post-extraction solids were solubilized as previously described. Fat samples were homogenized in *n*-hexane, and the *n*-hexane was then partitioned with acetonitrile. The acetonitrile phase was then analyzed by TLC.

Additionally in the bile, major metabolites were isolated from the bile of rats dosed twice with non-radioactive NNI-0001 by solvent extraction and chromatography. Isolated metabolites were analyzed by LC/MS, NMR, and TLC. When metabolite standards were available, comparison of spectral data and co-chromatography were done to confirm metabolite identity. Isolated metabolites were also hydrolyzed with β-D-glucuronidase, a mixture of γ-glutamyltranspeptidase and carboxypeptidase A, or carboxypeptidase A alone. Samples hydrolyzed with β-D-glucuronidase were extracted with ethyl acetate and reconstituted with methanol.

3. **Statistics:** Statistical analyses were limited to calculations of mean, standard deviation, and relative standard deviation. All calculations were performed with Microsoft Excel 2002. Maximum concentration (C_{max}) and time to maximum concentration (T_{max}) were determined directly from the average values of the groups. The area under the curve (AUC) was calculated by the trapezoid method. The elimination half-life ($T_{½}$) was calculated from the elimination rate constant (k), which was obtained by regression analysis of the elimination phase data.

II. RESULTS

A. **PHARMACOKINETIC STUDIES:** Single dose pharmacokinetic parameters for blood and plasma are presented in Table 2a. In animals given a single 2 mg/kg dose of [phthalic ring-U-^{14}C]-NNI-0001, males and females had approximately equal maximum radioactivity concentrations (C_{max}) in blood and plasma, but males demonstrated longer times to maximum concentration (t_{max}) than females. At 2 mg/kg, the C_{max} were 0.14-0.23 µg equiv./g, observed at 12 and 6 h in the males and females, respectively. Females demonstrated longer terminal

elimination half-lives (37.6 and 41.1 h in plasma and blood, respectively) than males (12.6 and 28.7 h in plasma and blood, respectively). In the 200 mg/kg animals, the observed C_{max} (0.4-0.5 µg equiv./g) did not increase in proportion to dose, suggesting saturation of the absorption process. T_{max} occurred at 48 h in the males, but C_{max} was observed at 6 and 48 h in the females. No other conclusions could be derived from the 200 mg/kg group data, as the concentration of radioactivity in many of the samples was below the limit of quantitation. The dosing solution should have been formulated with a higher specific activity. Pharmacokinetic parameters were not analyzed for aniline ring-labeled NNI-0001.

TABLE 2a. Pharmacokinetic parameters for the blood and plasma of rats following a single oral dose of [phthalic ring-U-^{14}C]-NNI-0001 [a]

Parameter [b]	2 mg/kg		200 mg/kg	
	Male	Female	Male	Female
Blood				
C_{max} (µg equiv./g)	0.18	0.14	0.5	0.5
t_{max} (h)	12	6	48	6 and 48[c]
$t_{½}$ (h)	28.7	41.1	NA	NA
AUC (µg•h/g; 0-168 h)	5.45	7.62	NA	NA
Plasma				
C_{max} (µg equiv./g)	0.23	0.20	0.5	0.4
t_{max} (h)	12	6	12	12
$t_{½}$ (h)	12.6	37.6	NA	NA
AUC (µg•h/g; 0-168 h)	5.58	9.18	NA	NA

a Data were obtained from Tables 2 and 3 on pages 56 and 57 of MRID 46817229; n=4 rats/group.

b Parameters C_{max} = maximum radioactivity concentration
t_{max} = time to maximum concentration
$t_{½}$ = terminal elimination half-life
AUC = area under concentration-time curve

c Equal C_{max} were observed at 6 and 48 h.

NA Not calculable because radioactivity in 2 blood and 7 plasma samples were below the limit of quantitation

In animals given daily 2 mg/kg doses of phthalic ring-labeled NNI-0001 (Table 2b), blood and plasma concentrations 24 h after doses 6, 13, and 14 were similar to each other, and were comparable to concentrations observed 24 h after a single dose when compared by sex. Maximum blood and plasma concentrations were observed 9 h after the 14th dose. Concentrations were consistently higher in females than in males. Radioactivity was still detectible in both the blood and plasma of females 168 h after dose 14 (0.028-0.034 µg equiv./g), but was near or below the limit of quantitation in males.

TABLE 2b. Radioactivity concentration (µg equiv./g) for the blood and plasma of rats following a multiple oral doses of [phthalic ring-U-^{14}C]-NNI-0001 [a]

Time/dose	Multiple 2 mg/kg dosing	
	Male	Female
Blood		
24 h post dose 1[b]	0.077	0.101
24 h post dose 6[c]	0.082	0.123
24 h post dose 13[c]	0.109	0.119
9 h post dose 14[d]	0.157	0.135
24 h post dose 14[d]	0.094	0.108
168 h post dose 14[d]	0.009	0.028
Plasma		
24 h post dose 1[b]	0.081	0.117
24 h post dose 6[c]	0.078	0.159
24 h post dose 13[c]	0.103	0.144
9 h post dose 14[d]	0.173	0.168
24 h post dose 14[d]	0.089	0.137
168 h post dose 14[d]	ND	0.034

a Data were obtained from Tables 2 and 3 on page 42 of MRID 46817230; data are the mean of 4 rats/group.
b Data from Tables 2 and 3 on pages 56 and 57 of MRID 46817229; n=4
c n=12
d n=4
ND Not detected

B. ADME studies

1. **Absorption:** Radioactivity was detected in blood and plasma of the 2 mg/kg group and in the blood of the 200 mg/kg group at 1 h post-dosing.

2. **Tissue distribution:** In the 2 mg/kg males dosed with phthalic ring-labeled NNI-0001, the liver (5.6% of the administered dose) and kidneys (0.59%) contained the highest mean levels of radioactivity at 9 h post-dosing. No other tissue exceeded 0.21% of the administered dose. In the 2 mg/kg females dosed with phthalic ring-labeled NNI-0001, the liver (1.6%) contained the highest mean levels of radioactivity at 9 h post-dosing. No other tissue exceeded 0.03% of the administered dose. In the 200 mg/kg animals dosed with phthalic ring-labeled NNI-0001, the liver (0.05-0.07%) contained the highest mean levels of radioactivity at 9 h post-dosing. No other tissue exceeded 0.005% of the administered dose. In bile duct-cannulated rats dosed with 2 mg/kg of phthalic ring-labeled NNI-0001, the liver contained 3.3% (males) and 5.5% (females) of the administered dose at 48 h post-dosing. In the 2 mg/kg animals dosed with aniline ring-labeled NNI-0001, the liver contained 0.05% (males) and 1.3% (females) of the administered dose at 168 h post-dosing. All other tissues were ≤0.003% in the males and ≤0.04 in the females.

   The concentrations of radioactivity measured in selected tissues (expressed as µg equiv./g tissue) are presented in Tables 3a and b. In animals dosed with phthalic ring-labeled NNI-0001 at 2 mg/kg, the highest concentrations of radioactivity were detected in the liver, fat, adrenals, and kidneys; animals dosed with 200 mg/kg had the highest concentrations in the fat, liver, adrenals, and kidneys. In animals dosed with aniline ring-labeled NNI-0001, the highest concentrations of radioactivity were detected in the liver, fat, adrenals, and kidneys of the females, and in the liver, fat, and kidneys of the males. Radioactivity did not partition

into the RBC. Radioactivity decreased in all tissues over time, but females retained higher concentrations at 168 h post-dosing than males.

TABLE 3a. Mean concentration of radioactivity (µg equiv./g) in selected tissues of rats at specified times post-dosing following a single dose of [^{14}C]-NNI-0001 [a]

Matrix	Phthalic ring-(U)-^{14}C												Aniline ring (U)-^{14}C	
	2 mg/kg						200 mg/kg						2 mg/kg	
	Males			Females			Males			Females			M	F
	9 h	24 h	168 h	9 h	24 h	168 h	9 h	24 h	168 h	9 h	24 h	168 h	168 h	
Blood	0.208	0.042	0.004	0.021	0.013	0.006	0.5	ND	ND	0.4	0.4	ND	0.003	0.012
Plasma	0.275	0.043	ND	0.027	0.016	0.009	0.3	ND	ND	ND	ND	ND	ND	0.015
Liver	2.416	0.213	0.031	0.657	0.473	0.407	2.2	0.9	0.1	3.8	3.8	0.3	0.016	0.555
Kidney	1.073	0.131	0.005	0.178	0.111	0.059	1.1	0.5	ND	1.3	1.4	0.1	0.006	0.074
Adrenal	1.903	0.105	0.007	0.463	0.287	0.137	2.4	0.9	ND	3.4	3.1	ND	ND	0.208
Fat	1.419	0.111	0.009	0.536	0.447	0.331	2.6	0.9	0.1	4.8	6.6	0.4	0.006	0.440

a Data were obtained from Summary Tables 2, 3, and 4 on pages 23-25 of MRID 46817229.
ND Not detected

In animals given daily 2 mg/kg doses of phthalic ring-labeled NNI-0001, the highest concentrations of radioactivity were detected in the liver, fat, adrenals, and kidneys in the males, and in the fat, liver, adrenals, and kidneys in the females. Again, radioactivity decreased in all tissues over time, but females retained higher concentrations at 168 h post-dosing than males.

TABLE 3b. Mean concentration of radioactivity (µg equiv./g) in selected tissues of rats at specified times post-dosing following 14 daily 2 mg/kg doses of phthalic ring-(U)-^{14}C-NNI-0001 [a]

	Males			Females		
	9 h	24 h	168 h	9 h	24 h	168 h
Blood	0.157	0.094	0.009	0.135	0.108	0.028
Plasma	0.173	0.089	ND	0.168	0.137	0.034
Liver	1.594	0.551	0.038	6.454	5.308	1.636
Kidney	0.622	0.270	0.017	1.122	0.906	0.248
Adrenal	0.776	0.201	0.018	2.568	2.134	0.603
Fat	1.043	0.323	0.048	6.771	5.875	1.708

a Data were obtained from Summary Table 2 on page 16 of MRID 46817230.
ND Not detected

3. **Excretion:** Total recoveries (Tables 4a-c) ranged from 94.1-104.7% of the administered doses, with no differences observed between sexes, dose levels, or position of the radiolabel. The majority of the radioactivity was recovered in the feces, with minor amounts recovered in the urine. Males excreted more radioactivity in the urine than females. Females excreted the test compound at a slower rate than the males. Generally, the majority of the radioactivity was recovered at the first 24 h collection point. It was stated that no significant excretion of radioactivity was detected in expired air at 24 h post-dosing. In the single dose 2 mg/kg animals, the majority of the radioactivity was recovered in the feces (91.4-96.2% of the administered dose). Urine accounted for 1.6-1.7% in the males, and 0.4-0.6% in the females. In the 200 mg/kg animals, feces accounted for 93.6-99.6%; urine accounted for 0.13% in the

males and 0.07% in the females. Carcass and cage wash each accounted for ≤4.8%, with more radioactivity observed in the 2 mg/kg female carcasses (4.3-4.8%) than in the males (0.6-0.9%). Radioactivity remaining in the 200 mg/kg carcasses approached the limits of quantitation.

TABLE 4a. Mean recovery of radioactivity (% administered dose) from rats following a single dose of [^{14}C]-NNI-0001 [a]

Matrix		Phthalic ring-(U)-^{14}C				Aniline ring (U)-^{14}C	
		2 mg/kg		200 mg/kg		2 mg/kg	
		Male	Female	Male	Female	Male	Female
Urine	0-24 h	1.30	0.16	0.09	0.06	1.23	0.28
	24-168 h [b]	0.37	0.21	0.04	0.01	0.35	0.28
Total urine		**1.67**	**0.37**	**0.13**	**0.07**	**1.58**	**0.56**
Feces	0-24 h	76.97	58.20	89.92	98.54	79.03	54.52
	24-168 h [b]	19.25	33.20	3.69	1.08	14.58	36.94
Total feces		**96.22**	**91.40**	**93.61**	**99.62**	**93.61**	**91.46**
Cage wash		0.11	0.19	0.37	0.51	0.15	0.51
Carcass		0.93	4.32	<0.01	0.01	0.59	4.83
Total recovery		**98.92**	**96.29**	**94.12**	**100.22**	**95.94**	**97.24**

a Data were obtained from Summary Tables 5 and 6 on page 26 of MRID 46695729. n=4
b Calculated by reviewers

In the bile duct-cannulated rats, the majority of radioactivity was associated with the gastrointestinal contents at 48 h post-dosing (50.6-60.4% of the administered dose). Radioactivity was detected in the feces (11.0-12.8% in both sexes), bile (11.1% in the males; 3.3% in the females), urine (0.8% in the males; 0.2% in the females), gastrointestinal tract (2.4-2.5%), liver (3.3-5.5%), and carcass (5.9% in the males; 22.8% in the females). Absorption was estimated from radioactivity present in the bile, urine, gastrointestinal tract, liver, and carcass, and was determined to be 23.5% in the males and 34.1% in the females. Again, females appeared to excrete radioactivity more slowly than males.

TABLE 4b. Mean recovery of radioactivity (% administered dose) from bile duct cannulated rats 48 h after a single dose of 2 mg/kg phthalic ring-(U)-^{14}C-NNI-0001 [a]

Matrix		Male (n=3)	Female (n=6)
Urine	0-24 h	0.17	0.03
	24-48 h [b]	0.58	0.12
Total urine		**0.75**	**0.15**
Feces	0-24 h	1.42	0.24
	24-48 h [b]	11.35	10.75
Total feces		**12.77**	**10.99**
Bile	0-24 h	2.74	0.56
	24-48 h	8.32	2.72
Total bile		**11.06**	**3.28**
Gastrointestinal contents		60.36	50.61
Gastrointestinal tract		2.49	2.42
Liver		3.28	5.50
Carcass		5.92	22.78
Absorption [c]		23.49	34.13
Total recovery		**96.62**	**95.73**

a Data were obtained from Summary Table 1 on page 22 of MRID 46817231.
b Calculated by reviewers
c Absorbed = bile + urine + gastrointestinal tract + liver + carcass

In animals receiving daily doses of NNI-0001, the majority of radioactivity was recovered in the feces (103.5-104.0% of the administered dose), with urine acting as a minor route of excretion (0.2-0.5%). Cage wash and carcass accounted for ≤0.4% each, with females (0.4%) retaining more radioactivity in the carcass than males (0.02%).

TABLE 4c. Mean cumulative recovery of radioactivity (% administered dose) from rats following daily 2 mg/kg doses of phthalic ring-(U)-^{14}C-NNI-0001 [a]

Time after initial dosing (day)	Male		Female	
	Urine	Feces	Urine	Feces
1	0.07	4.75	0.01	4.25
7	0.25	46.11	0.09	46.76
13	0.44	93.02	0.19	92.24
14 (1 day after final dose)	0.48	102.11	0.20	100.65
17 (4 days after final dose)	0.49	103.39	0.22	102.95
20 (7 days after final dose)	0.50	103.46	0.23	103.97
Cage wash [b]	0.07		0.08	
Carcass [c]	0.02		0.42	
Total recovery	**104.06**		**104.71**	

a Data were obtained from Summary Table 3 on page 17 of MRID 46817230.
b Collected on Day 20 (7 days after final dose)
c Includes GI contents

C. **METABOLITE CHARACTERIZATION STUDIES:** The majority of radioactivity in urine and fecal extract samples was present as parent and up to three metabolites. Metabolic profiles were qualitatively similar for both radiolabeled forms. Oxidation of the methyl moiety on the aniline ring was determined to be the main metabolic pathway in both sexes (Figures 1 and 2). In excreta, parent and identified metabolites accounted for 70.0-98.1% of the administered dose, while unidentified metabolites accounted for 0.3-13.6% of the

administered dose (Table 5). The total administered dose accounted for in the excreta was 80.1-98.6%.

Parent compound was excreted almost exclusively in the feces. The 2 mg/kg single dose males eliminated smaller amounts of parent (15.4-30.5%) than the 2 mg/kg females (65.9% in both radiolabeled forms), while the 200 mg/kg males and females eliminated larger amounts (89.1-97.8% dose). The metabolite found in the greatest quantity was NNI-0001-benzyl alcohol, with the 2 mg/kg males eliminating more (31.3-37.7%) than the females (5.5-5.8%). The 200 mg/kg males eliminated a small amount (0.2%); this metabolite was not quantifiable in the 200 mg/kg females. The 2 mg/kg males excreted greater quantities (15.0-16.4%) of NNI-0001-benzoic acid than the females (0.01-0.1%). NNI-0001-benzoic acid was not detected in the excreta of the 200 mg/kg rats. Only one other identified metabolite (NNI-0001-benzaldehyde) was found in the urine and feces of the single dose animals (0.2-0.5%). In animals receiving daily doses of [^{14}C]-NNI-0001, NNI-0001-benzyl alcohol was the only metabolite present at >5% of the administered dose. One minor metabolite, NNI-0001-iodophthalimide, was the primary metabolite found in the fat of female rats (1.0% of the administered dose).

TABLE 5. Metabolite profile (% administered dose) in excreta of rats dosed with [^{14}C]-NNI-0001 [a]

Compound	Phthalic ring-(U)-^{14}C				Aniline ring (U)-^{14}C	
	2 mg/kg		200 mg/kg		2 mg/kg	
	Male	Female	Male	Female	Male	Female
Identified metabolites						
Parent	15.41	65.88	89.10	97.84	30.49	65.92
NNI-0001-benzyl alcohol [b]	37.70	5.48	0.21	ND	31.25	5.78
NNI-0001-benzaldehyde	0.45	ND	0.25	0.24	0.34	ND
NNI-0001-benzoic acid	16.44	0.01	ND	ND	14.97	0.14
Total Identified	**70.00**	**71.37**	**89.56**	**98.08**	**77.05**	**71.84**
Total unidentified [c]	**13.57**	**4.82**	**0.29**	**0.35**	**10.24**	**4.24**
Total accounted	83.57	76.19	89.85	98.43	87.29	76.08
Unextractable	12.82	5.08	0.17	0.18	6.84	4.01
Total % dose in excreta	**96.39**	**81.27**	**90.02**	**98.61**	**94.13**	**80.09**

a Data were obtained from Summary Tables 7 and 8 on pages 27 and 28 of MRID 46817229.
b May contain NNI-0001-hydroxy due to incomplete separation by TLC
c No individual component exceeded 0.25% in urine or 3.35% in feces.
ND Not detected

Additional minor (<5%) metabolites were identified in the bile: NNI-0001-cyclic acetal; NNI-0001-carboxy-benzyl alcohol; NNI-0001 benzyl alcohol glucuronide; NNI-0001-hydroxy-benzyl alcohol/NNI-0001 hydryoxy glucuronide; NNI-0001 glutathione conjugate; NNI-0001 cysteinyl-glycine conjugate; and NNI-0001 cysteine conjugate. These metabolites were also produced in greater amounts by males than by females. The liver of male rats dosed with [aniline ring-(U)-^{14}C] contained several metabolites, while female livers contained almost solely unchanged parent. This finding may reflect the slower metabolism of the compound in female rats.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS**: The fate of NNI-0001 following a single oral administration was characterized by slow and limited absorption of radioactivity from the gastrointestinal tract. Radioactivity was fairly equally distributed within the blood and most organs and tissues, with preference to the liver, as the main metabolizing organ also responsible for excretion, and fat. The highest concentrations of radioactivity was detected in the tissues and organs at 9 h post-dosing, approximately C_{max} in blood and plasma. A steady decline in radioactivity concentrations in the organs and tissues was observed out to 168 h post-dosing. Radioactivity was rapidly excreted into the feces via biliary excretion. The main metabolic pathway observed in both sexes was oxidation of the methyl moiety on the aniline ring followed by glucuronidation of the hydroxyl moiety in males or direct glutathione conjugation of the phthalic acid ring in females. These metabolites were solely excreted in the bile. Sex related differences noted included slow excretion, less metabolites, and higher distribution of radioactivity in females compared to males. However, qualitatively identical metabolism between sexes was observed. Repetitive dosing did not affect metabolism of NNI-0001, and a slight accumulation was observed only in the females.

B. **REVIEWER COMMENTS**: Radioactivity was detected in blood and plasma of the 2 mg/kg group and in the blood of the 200 mg/kg group at 1 h post-dosing. In animals given a single 2 mg/kg dose of [phthalic ring-U-^{14}C]-NNI-0001, males and females had approximately equal maximum radioactivity concentrations (C_{max}) in blood and plasma, but males demonstrated longer times to maximum concentration (t_{max}) than females. At 2 mg/kg, the C_{max} were 0.14-0.23 µg equiv./g, observed at 12 and 6 h in the males and females, respectively. Females demonstrated longer terminal elimination half-lives (37.6 and 41.1 h in plasma and blood, respectively) than males (12.6 and 28.7 h in plasma and blood, respectively). In the 200 mg/kg animals, the observed C_{max} (0.4-0.5 µg equiv./g) did not increase in proportion to dose, suggesting saturation of the absorption process. T_{max} occurred at 48 h in the males, but C_{max} was observed at 6 and 48 h in the females. No other conclusions could be derived from the 200 mg/kg group data, as the concentration of radioactivity in many of the samples was below the limit of quantitation. The dosing solution should have been formulated with a higher specific activity. Pharmacokinetic parameters were not analyzed for aniline ring-labeled NNI-0001. In animals given daily 2 mg/kg doses of phthalic ring-labeled NNI-0001, blood and plasma concentrations 24 h after doses 6, 13, and 14 were similar to each other, and were comparable to concentrations observed 24 h after a single dose when compared by sex. Maximum blood and plasma concentrations were observed 9 h after the 14th dose. Concentrations were consistently higher in females than in males. Radioactivity was still detectible in both the blood and plasma of females 168 h after dose 14 (0.028-0.034 µg equiv./g), but was near or below the limit of quantitation in males.

In the 2 mg/kg males dosed with phthalic ring-labeled NNI-0001, the liver (5.6% of the administered dose) and kidneys (0.59%) contained the highest mean levels of radioactivity at 9 h post-dosing. No other tissue exceeded 0.21% of the administered dose. In the 2 mg/kg females dosed with phthalic ring-labeled NNI-0001, the liver (1.6%) contained the highest mean levels of radioactivity at 9 h post-dosing. No other tissue exceeded 0.03% of the administered dose. In the 200 mg/kg animals dosed with phthalic ring-labeled NNI-0001, the

liver (0.05-0.07%) contained the highest mean levels of radioactivity at 9 h post-dosing. No other tissue exceeded 0.005% of the administered dose. In bile duct-cannulated rats dosed with 2 mg/kg of phthalic ring-labeled NNI-0001, the liver contained 3.3% (males) and 5.5% (females) of the administered dose at 48 h post-dosing. In the 2 mg/kg animals dosed with aniline ring-labeled NNI-0001, the liver contained 0.05% (males) and 1.3% (females) of the administered dose at 168 h post-dosing. All other tissues were ≤0.003% in the males and ≤0.04 in the females. In animals dosed with phthalic ring-labeled NNI-0001 at 2 mg/kg, the highest concentrations of radioactivity were detected in the liver, fat, adrenals, and kidneys; animals dosed with 200 mg/kg had the highest concentrations in the fat, liver, adrenals, and kidneys. In animals dosed with aniline ring-labeled NNI-0001, the highest concentrations of radioactivity were detected in the liver, fat, adrenals, and kidneys of the females, and in the liver, fat, and kidneys of the males. Radioactivity did not partition into the RBC. Radioactivity decreased in all tissues over time, but females retained higher concentrations at 168 h post-dosing than males. In animals given daily 2 mg/kg doses of phthalic ring-labeled NNI-0001, the highest concentrations of radioactivity were detected in the liver, fat, adrenals, and kidneys in the males, and in the fat, liver, adrenals, and kidneys in the females. Again, radioactivity decreased in all tissues over time, but females retained higher concentrations at 168 h post-dosing than males.

Total recoveries ranged from 94.1-104.7% of the administered doses, with no differences observed between sexes, dose levels, or position of the radiolabel. The majority of the radioactivity was recovered in the feces, with minor amounts recovered in the urine. Males excreted more radioactivity in the urine than females. Females excreted the test compound at a slower rate than the males. Generally, the majority of the radioactivity was recovered at the first 24 h collection point. It was stated that no significant excretion of radioactivity was detected in expired air at 24 h post-dosing. In the single dose 2 mg/kg animals, the majority of the radioactivity was recovered in the feces (91.4-96.2% of the administered dose). Urine accounted for 1.6-1.7% in the males, and 0.4-0.6% in the females. In the 200 mg/kg animals, feces accounted for 93.6-99.6%; urine accounted for 0.13% in the males and 0.07% in the females. Carcass and cage wash each accounted for ≤4.8%, with more radioactivity observed in the 2 mg/kg female carcasses (4.3-4.8%) than in the males (0.6-0.9%). Radioactivity remaining in the 200 mg/kg carcasses approached the limits of quantitation. In the bile duct-cannulated rats, the majority of radioactivity was associated with the gastrointestinal contents at 48 h post-dosing (50.6-60.4% of the administered dose). Radioactivity was detected in the feces (11.0-12.8% in both sexes), bile (11.1% in the males; 3.3% in the females), urine (0.8% in the males; 0.2% in the females), gastrointestinal tract (2.4-2.5%), liver (3.3-5.5%), and carcass (5.9% in the males; 22.8% in the females). Absorption was estimated from radioactivity present in the bile, urine, gastrointestinal tract, liver, and carcass, and was determined to be 23.5% in the males and 34.1% in the females. Again, females appeared to excrete radioactivity more slowly than males. In animals receiving daily doses of NNI-0001, the majority of radioactivity was recovered in the feces (103.5-104.0% of the administered dose), with urine acting as a minor route of excretion (0.2-0.5%). Cage wash and carcass accounted for ≤0.4% each, with females (0.4%) retaining more radioactivity in the carcass than males (0.02%).

The majority of radioactivity in urine and fecal extract samples was present as parent and up to three metabolites. Metabolic profiles were qualitatively similar for both radiolabeled forms. Oxidation of the methyl moiety on the aniline ring was determined to be the main metabolic pathway in both sexes (Figures 1 and 2). In excreta, parent and identified metabolites accounted for 70.0-98.1% of the administered dose, while unidentified metabolites accounted for 0.3-13.6% of the administered dose. The total administered dose accounted for in the excreta was 80.1-98.6%. Parent compound was excreted almost exclusively in the feces. The 2 mg/kg single dose males eliminated smaller amounts of parent (15.4-30.5%) than the 2 mg/kg females (65.9% in both radiolabeled forms), while the 200 mg/kg males and females eliminated larger amounts (89.1-97.8% dose). The metabolite found in the greatest quantity was NNI-0001-benzyl alcohol, with the 2 mg/kg males eliminating more (31.3-37.7%) than the females (5.5-5.8%). The 200 mg/kg males eliminated a small amount (0.2%); this metabolite was not quantifiable in the 200 mg/kg females. The 2 mg/kg males excreted greater quantities (15.0-16.4%) of NNI-0001-benzoic acid than the females (0.01-0.1%). NNI-0001-benzoic acid was not detected in the excreta of the 200 mg/kg rats. Only one other identified metabolite (NNI-0001-benzaldehyde) was found in the urine and feces of the single dose animals (0.2-0.5%). In animals receiving daily doses of [^{14}C]-NNI-0001, NNI-0001-benzyl alcohol was the only metabolite present at >5% of the administered dose. One minor metabolite, NNI-0001-iodophthalimide, was the primary metabolite found in the fat of female rats (1.0% of the administered dose). Additional minor (<5%) metabolites were identified in the bile: NNI-0001-cyclic acetal; NNI-0001-carboxy-benzyl alcohol; NNI-0001 benzyl alcohol glucuronide; NNI-0001-hydroxy-benzyl alcohol/NNI-0001 hydryoxy glucuronide; NNI-0001 glutathione conjugate; NNI-0001 cysteinyl-glycine conjugate; and NNI-0001 cysteine conjugate. These metabolites were also produced in greater amounts by males than by females. The liver of male rats dosed with [aniline ring-(U)-^{14}C] contained several metabolites, while female livers contained almost solely unchanged parent. This finding may reflect the slower metabolism of the compound in female rats.

C. **STUDY DEFICIENCIES:** The following minor deficiency was noted but does not alter the conclusions of this review:

- The specific activity of the 200 mg/kg dosing solution was too low to allow accurate quantitation in the pharmacokinetic studies.

Figure 1. Urinary and fecal metabolite pathways

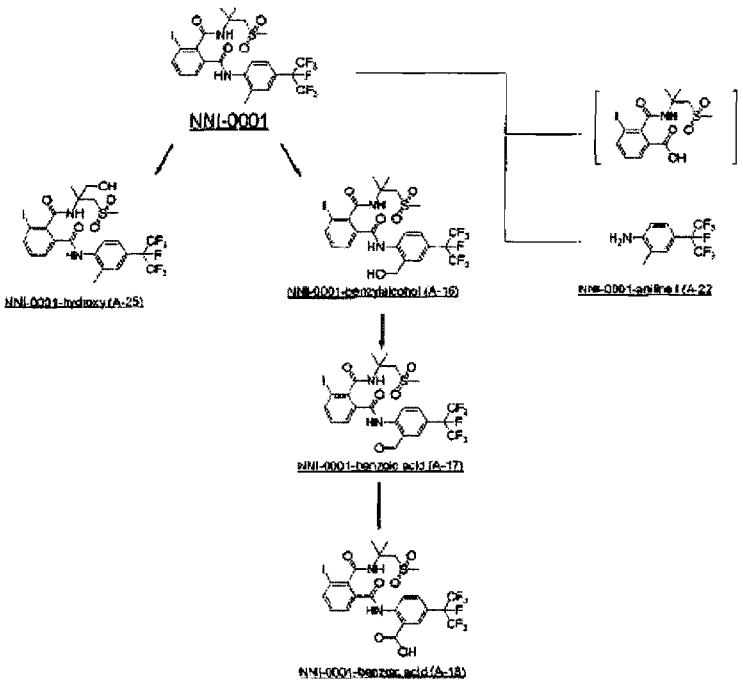
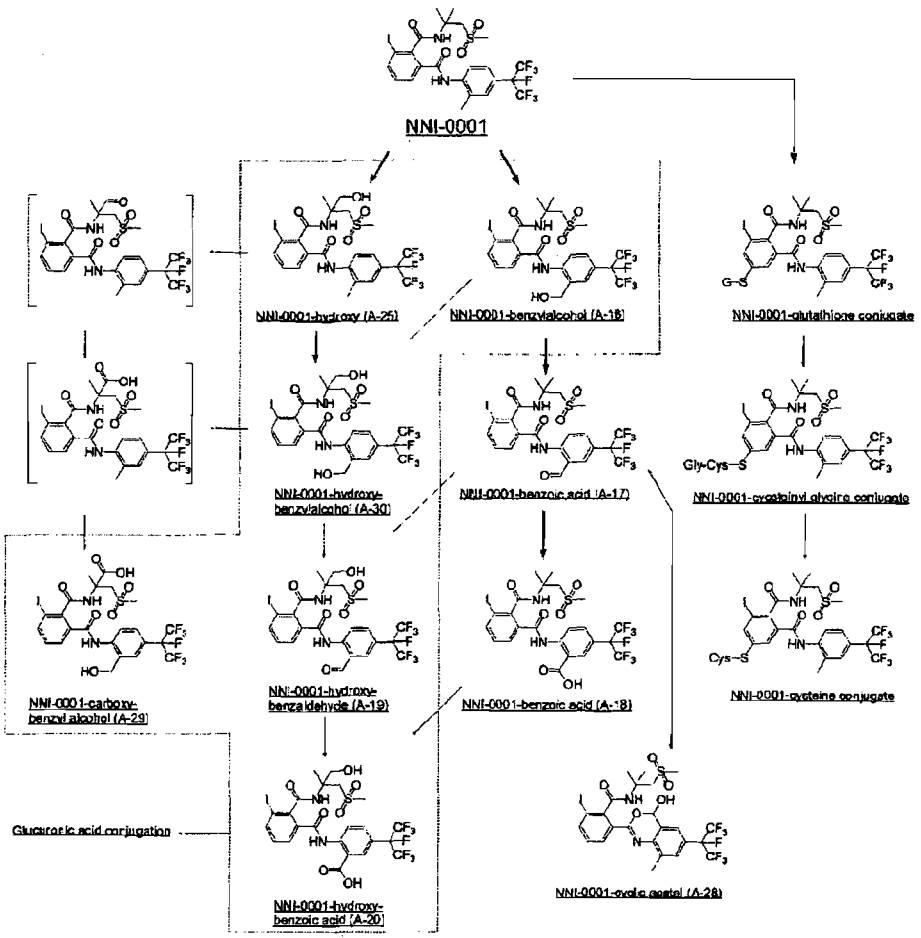


Figure 2. Biliary metabolite pathways



DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: OPPTS 870 6300 [§83-6], Developmental Neurotoxicity Study in Rats

Work Assignment No. 4-1-124 L; formerly 3-1-124 L (MRID 46817228)

Prepared for

Health Effects Division
Office of Pesticide Programs
U S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer:
David A. McEwen, B.S.
Signature: David A. McEwen
Date: 01/19/07

Secondary Reviewer:
Ronnie J. Bever Jr., Ph.D.
Signature: Ronnie J Bever Jr
Date: 01/19/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.I.
Signature: Michael E Viana
Date: 01/19/07

Quality Assurance:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 01/19/07

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Stanley Gross, for **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
EPA Work Assignment Manager: Myron Ottley, Ph. D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study – Rat; OPPTS 870.6300 ('83-6); OECD 426 (draft)

PC CODE: 027602 **DP BARCODE:** D 331553 (SB)
TXR# 0054319

TEST MATERIAL (PURITY): NNI-0001 (Flubendiamide; 97.3% a.i.)

SYNONYMS: N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Sheets, L.P., R.G. Gilmore, and H.E. Hoss (2006) A developmental neurotoxicity screening study with technical grade NNI-0001 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 04-D72-VK, February 17, 2006. MRID 46817228. Unpublished.

SPONSOR: Bayer CropScience LP, 2 T.W. Alexander Dr, Research Triangle Park, NC

EXECUTIVE SUMMARY - In a developmental neurotoxicity study 2006 (MRID 46817228) technical grade NNI-0001 (Flubendiamide; 97.3% a.i., Lot/Batch # 1FH0019M) was administered to approximately 30 mated female Wistar rats per dose in the diet at nominal dose levels of 0, 120, 1200, or 12,000 ppm from gestation day (GD) 6 through lactation day (LD) 21. Doses were adjusted during lactation to achieve a more consistent dosage throughout exposure. The mean daily intake during gestation and lactation was 0, 9.9, 99.5, and 979.6 mg/kg/day. Dams were allowed to deliver naturally and were killed on LD 21. On postnatal day (PND) 4, litters were standardized to 8 pups/litter. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) were allocated to subsets for functional observational battery (FOB), motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination.

Maternal Effects:
All dams survived to scheduled sacrifice. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point.

During gestation, slight increases ($p \leq 0.05$) were noted in body weight (↑4-5%) and body weight gains (GD 0-20, ↑11%) at 12000 ppm. During lactation, body weights

were increased ($p \leq 0.05$) by 4% at 120 and 1200 ppm on LD 0 and by 4-6% at 1200 and above on LD 21. Body weight gains (LD 0-21, calculated by reviewers) were also increased by 17-18% at 1200 ppm and above. Food consumption in the treated groups was similar to controls during gestation and lactation. These differences in body weight and body weight gain were not considered to be treatment-related as the body weights of the treated animals were slightly higher than controls at the beginning of the study and minor increases in weight are not considered to be adverse. In the LD21 dams, increases ($p \leq 0.05$) in absolute (↑26-34%) and relative (to body, ↑20-28%) liver weight were observed at 1200 ppm and above.

Reproductive parameters were similar to controls in all dose groups.

Pup effects.:
Offspring pre-weaning body weights were decreased ($p \leq 0.05$) at 12,000 ppm (↓9% both sexes) at PND 21. Body weight gains were decreased ($p \leq 0.05$) during several pre-weaning intervals at 12,000 ppm (↓12-20%, males and ↓11-20%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 11% at 12,000 ppm.

Sexual maturation – The day of preputial separation was delayed ($p \leq 0.01$) in the 1200 and 12,000 ppm males (47.5 and 48.7 treated vs. 44.9 controls). The day of vaginal patency was delayed ($p \leq 0.01$) in the 12,000 ppm females (35.3 treated vs. 32.6 controls). Only 2 pups in the 12,000 ppm group did not display pupil constriction on PND 21.

Functional observational battery – Treatment-related FOB effects were limited to ocular lesions (corneal opacity, dark red eyes, and/or enlarged eyes) at 12,000 ppm (1-2 males and 1-4 females). The lesions were observed beginning on PND 21 and persisted until PND 60 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

At 12,000 ppm, treatment-related ocular lesions were noted in pups of both sexes as follows. During pre-weaning (PND 0-21), the following effects on the eyes (# of litters affected/20-29 vs. 0 controls) were observed: (i) enlarged eyeball (1-9 litters on PND 15-21); (ii) corneal opacity (2-3 litters on PND 16-21); (iii) dark red (1-6 litters on PND 15-21); and (iv) exophthalmia (1 litter on PND 20-21). Throughout post-weaning (PND 22-72), the following effects on the eyes (# of animals affected/65-66 vs. 0 controls) were noted: (i) enlarged (9 males/12 females); (ii) general opacity (8 males/10 females); (iii) red (4 males/8 females; and (iv) exophthalmia (2 males). Additionally in the 1200 ppm males, one pup displayed enlarged eye, general opacity, and exophthalmia. No compound-related effects were noted at 1200 ppm in the females or at 120 ppm in either sex.

Microscopic examination – At 12,000 ppm, the following compound-related microscopic effects (# affected/10 vs. 0 controls, unless otherwise stated) were noted in the eye and optic nerve: (i) retinal degeneration (2 males and 1 female); (ii) hemorrhage (3 males vs. 1 control); (iii) cataract (2 males and 1 female); and (iv) atrophy of the optic nerve (3 males and 1 female). No other treatment-related microscopic lesions were noted at any dose in either sex.

Maternal LOAEL = 99.5 mg/kg/day based on increased liver weights. Increased liver weight in isolation is not considered an "adverse" effect, but considering the consistent observation of liver toxicity (e.g., centrilobular hepatocyte fatty change, hypertrophy, increase in liver enzymes, foci of cellular alterations) demonstrated across multiple durations and species at similar doses, the weight-of-evidence supports this effect as an "adverse" finding and thus, a firm basis for the LOAEL. Maternal NOAEL= 9.9 mg/kg/day.

Offspring LOAEL = 99.5 mg/kg/day based on delayed balanopreputial separations. The Offspring NOAEL = 9.9 mg/kg/day.

This study is classified (**acceptable/non-guideline**) and satisfies the guideline requirement; OPPTS 870.6300, '83-6, OECD 426 (draft) for a developmental neurotoxicity study in rats.

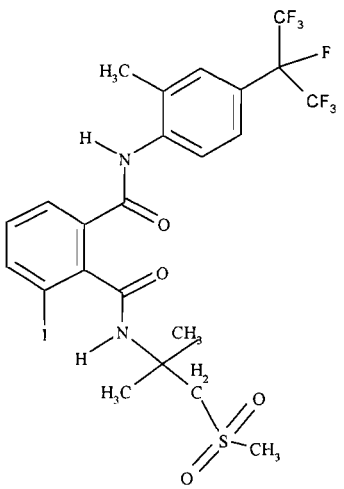
COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001

Description: White powder
Lot/Batch #: 1FH0019M
Purity: 97.3% a.i.
Stability: The test material was shown to be stable in the diet for up to 7 days at room temperature or 28 days frozen.
CAS # of TGAI: 272451-65-7
Structure:



2. Vehicle – Diet

3. Test animals (P)

Species:	Rat	
Strain:	Wistar HAN CRL:WI (GLX/BRL/HAN) IGSBR	
Age at study initiation:	Approximately 12 weeks at cohabitation	
Mean group weights on GD 0:	230.2-237.9 g females only	
Source:	Charles River Laboratories, Inc. (Raleigh, NC)	
Housing:	During gestation and lactation, individual dams and litters were kept together in plastic cages with corn cob bedding. The week following weaning, the remaining F_1 pups were kept individually in stainless steel wire-mesh cages.	
Diet:	Rodent Lab Chow #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during neurobehavioral testing	
Water:	Tap water, *ad libitum*, except during neurobehavioral testing	
Environmental conditions	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN

1. In-life dates - Dams received the test material starting on 6/20/04 and ending on approximately 7/25/04.

2. **Study schedule** - The maternal animals were mated and assigned to study. The test substance was administered to the dams from gestation day (GD) 6 through lactation day (LD) 21. Pups were weaned on postnatal day (PND) 21, after which time all animals received untreated diet. On PND 4, the litters were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. All litters not selected for further observations and all P females without a litter were sacrificed, and were discarded without further examinations. F_1 pups remained on study until PND 75 (±5 days, study termination).

3. **Mating procedure** - Females were paired 1:1 with males of the same strain and source for a maximum of four consecutive days. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day (GD) 0, and each female was housed individually in a plastic nesting box.

4. **Animal assignment** - Time-mated females were randomly assigned to test groups as shown in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 4. Dams were assigned to functional observation testing as shown.

TABLE 1. Study design [a]

Experimental Parameter	Subset	Dose (ppm)			
		0	120	1200	12,000
Maternal Animals					
No. of dams assigned	NA	30	30	30	30
Mean daily intake (mg/kg/day)	NA	0	9.9	99.5	979.6
FOB (GD 13 and 20)	NA	30	30	30	30
FOB (LD 11 and 21)	NA	10	9	10	10
Offspring (F_1) [b]					
Motor activity (PND 13, 17, 21, 60±2)	A	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Acoustic startle habituation (PND 22, 60±2)	B	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Passive avoidance (PND 22 and 29)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Water maze (PND 60±2 and 7 days later)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
FOB (PND 4, 11, 21, 35±1, 45±1, 60±2)	C	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)	1 pup/litter (~16/sex)
Perfusion, neuropathology, and morphometric analysis (PND 21)	D	10/sex	10/sex	10/sex	10/sex
Brain weight (PND 75±5)	A, B, C	10/sex	10/sex	10/sex	10/sex
Ophthalmologic examination (PND 50-60)	A, B, C	~10/sex	~10/sex	~10/sex	~10/sex
Perfusion and neuropathology (PND 75±5)	A, B, C	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination	same animals selected for ophthalmologic examination

a Data obtained from pages 19, 20, and 41 of the study report.

b Unless otherwise indicated, 1 male or female pup/litter was used (~16 [minimum of 10]/sex/dose, representing at least 20 litters).

NA Not applicable

5. **Dose selection rationale** - Dose levels were chosen based on the results of a two-generation reproduction study in Wistar rats (MRID 46817216; reviewed concurrently), in which NNI-0001 was administered in the diet at nominal concentrations of 20, 50, 2000, or 20,000 ppm. At 2000 ppm and above, treatment-related effects noted in the parental generation females included increased liver and thyroid weights as well as histopathological effects in both organs. Based on these results, doses of 120, 1200, and 12,000 ppm were chosen for the current study.

6. **Dosage preparation, administration, and analysis** - Formulations were prepared weekly by mixing appropriate amounts of test substance with the diet. The test diets were stored frozen (-20°C) until use. Dietary formulations were provided to the dams for *ad libitum* consumption weekly throughout the exposure period (GD 6 through LD 21). Dietary concentrations were adjusted (reduced) during lactation, relative to gestation, to maintain a more constant level of exposure (mg/kg/day) throughout the treatment period. F_1 animals were not directly supplied with the test diets. Prior to initiation of the study, stability for up to 7 days at room temperature or for up to 28 days frozen and homogeneity (top, middle, bottom) at concentrations of 40 and 12,000 ppm (which bracketed those in the current study) were determined. Actual concentration at each dose was tested for each batch of dietary formulations used in the current study.

Results

Homogeneity analysis (% relative standard deviation): 1.98-5.56%

Stability analysis (range as % of Day 0)
At room temperature for 7 days: 93-100%
Frozen for 28 days: 94-99%

Concentration analysis (% of nominal):

Dose (ppm)	% Nominal
120	108
1200	106
12,000	108

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

C. OBSERVATIONS

1. In-life observations

a. **Maternal animals** – Cage-side checks for mortality, moribundity, and clinical signs of toxicity were conducted at least once daily for maternal animals. Detailed clinical examinations were performed daily during exposure (GD 6 through LD 21).

Animals presumed to be pregnant (approximately 30/dose) were observed on GD 13 and 20 and a minimum of 10 dams/dose were observed on LD 11 and 21 as part of a functional observational battery (FOB). The FOB included, but was not limited to, the following observations (with severity scoring).

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking or degree of lacrimation and salivation 2) Presence or absence of piloerection and exophthalmos, 3) Ranking or count of urination and defecation 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

The technicians performing the FOB were 'blind' as to the animal's treatment group. Several technicians were used during the FOB; however, it was stated that evidence of inter-observer reliability (positive control studies) is maintained at the laboratory. No further information concerning the performance of the FOB was provided.

Individual maternal body weight and food consumption data were recorded weekly throughout gestation (GD 6, 13, and 20), on the day of delivery (LD 0), and on LD 4, 7, 14, and 21.

b. Offspring

1. **Litter observations** - The day of completion of parturition was designated as PND 0. Live pups were counted, sexed and weighed individually for each litter on PND 0, 4, 11, 17, and 21. At least once daily, all surviving offspring were examined cage-side for gross signs of toxicity, mortality, or morbidity.

   On PND 4, litters were standardized (using random procedures) to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible). Pups not chosen for the F_1 groups and dams that had insufficient pups were killed and discarded without further examination.

2. **Developmental landmarks** - Beginning on PND 38, male offspring were examined daily for preputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The age of onset was recorded, and the pups were weighed when vaginal patency or preputial separation was first noted. In addition, all pups were tested for the presence of pupil constriction on PND 21.

3. **Post-weaning observations** - After weaning on PND 21, offspring were examined twice daily for mortality and clinical observations were recorded daily. In addition, detailed clinical observations were recorded weekly during post-weaning. Body weights were recorded weekly.

4. **Neurobehavioral evaluations** - Observations and the schedule for those observations are summarized as follows from the report.

i. **Functional observational battery (FOB)** - On PND 4, 11, 21, 35 (±1 day), 45 (±1 day), and 60 (±2 days), selected pups (approximately 16/sex/dose; Subset C) were observed outside the home cage according to procedures outlined for the dams. The neonates were not evaluated in the open field on PND 4 and 11.

ii. **Motor activity testing** - Activity was evaluated in approximately 16 pups/sex/dose (Subset A) on PND 13, 17, 21, and 60 (±2 days). Motor and locomotor activity were measured by testing animals in figure eight mazes using a Columbus Instruments Universal Maze Monitoring System (Columbus, OH). Broad-spectrum background noise [74±2dB(A)] was provided, and the light intensity (100±70 Lux) over each maze was verified daily. Each test session was 60 minutes in duration, and consisted of 6 ten-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii. **Acoustic startle habituation** - Acoustic startle habituation testing was performed on approximately 16 pups/sex/dose (Subset B) on PNDs 22 and 60 (±2 days). A Coulbourn Instruments Integrated Startle Response Test System (Allentown, PA) was used to conduct the test and collect the data. The test session consisted of 50 trials that began following a 5 minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus (50 msec burst [0 msec rise/fall] of broad-spectrum 'white' noise [~118 dB (lin)]) at 10-second intervals. The response amplitude was recorded (maximum value on the curve) and the baseline (animal's body weight) was subtracted. The latency to peak is the time (msec) following the onset of the stimulus when the peak response amplitude occurs.

iv. **Learning and memory testing** - Learning and memory testing was performed on approximately 16 pups/sex/dose (Subset C). Passive avoidance testing was performed on PNDs 22 and 29; water maze testing was performed on PND 60 (±2 days) and again seven days later. For both tests, only animals that demonstrated acquisition on the first day were tested for retention seven days later.

Passive avoidance test - The test was conducted using a Coulbourn Instruments Shuttle Cage System (Allentown, PA). Each shuttle cage consisted of two equal sized compartments separated by a wall that supported a (guillotine-type) door. The walls of one compartment were covered with black film (dark-side), and the other compartment was illuminated with a high-intensity lamp. The floor of the dark-side consisted of a grid of stainless-steel bars.

Movement of the animal across the doorway was detected with a photocell system. A Coulbourn solid state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) shock to the grid floor when the animal crossed into the dark compartment. After adaptation, individual animals were placed into the lighted compartment of a conditioning apparatus facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the dark-side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light was switched off, signaling the end of the trial. At that time the animal was returned to the holding cage to await the next trial. If the rat failed to cross within 180 seconds, it was returned to the holding cage and the latency assigned an arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For the second trial, rats were placed in the illuminated side of the apparatus, given a 20 second acclimation period, and the latency to enter the dark side was recorded. Animals that either failed to reach criterion within 15 trials, or failed to cross during the first two trials during acquisition, were excluded from the retention phase of the experiment.

Water maze - A Plexiglas M-maze containing 7.5 inches of water (22±1EC) was used. On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors during each trial. Animals that satisfied the above criteria within the 15 trial limit were tested for retention seven days following acquisition. Animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment. The correct goal and criterion were the same in both sessions.

5. **Ophthalmology** - Animals that were selected for perfusion (minimum of 10/sex/dose) were subjected to ophthalmoscopic examinations at approximately 50-60 days of age. The eyes of each animal were examined with a slit lamp microscope and an indirect ophthalmoscope equipped with a condensing lens.

6. **Cholinesterase determination** – Cholinesterase activity was not determined.

360

7. Postmortem observations

a. **Maternal animals** - Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 24 (rats that did not deliver) or LD 21 (following weaning). The liver and thyroid were weighed and the dams were discarded without further necropsy.

b. **Offspring** - The offspring selected for perfusion on PND 21 (subset D) and at study termination (subsets A-C), as well as those selected for fresh brain weight determinations (approximately 10/sex/group from subsets A-C) were examined grossly.

The animals selected for perfusion on PND 21 (Subset D) and at termination (Subsets A-C) were anaesthetized with pentobarbital (50 mg/kg i.p.), and then perfused with a buffered sodium nitrite flush followed by *in situ* fixation with 1.0% (w/v) glutaraldehyde and 4% (w/v) formaldehyde in phosphate buffer. Only the brain (with olfactory bulbs) was collected from the perfused animals on PND 21. Upon study termination, the brain and spinal cord, eyes (with optic nerves), selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected. All tissues were post-fixed in 10% buffered formalin. The brain from each animal was weighed, sectioned (8 coronal sections), and examined microscopically. The brain, spinal cord, cauda equina, eyes, optic nerves, and gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All other collected tissues were embedded in resin, sectioned, and stained with modified Lee's stain. Additionally, the brain sections selected for morphometric measurements were stained with Luxol fast blue/cresyl violet. The following (CHECKED X) tissues, to be examined microscopically, were collected from perfused animals at study termination:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Olfactory bulb region		Proximal
X	Olfactory bulb section		Distal
X	Forebrain (optic nerve) section		
X	Forebrain (optic chiasma) section		**OTHER**
X	Midbrain section	X	Sural nerve
X	Mesencephalon	X	Tibial nerve
X	Cerebellum/Pons		Peroneal nerve
X	Medulla oblongata	X	Cervical dorsal root ganglion
	SPINAL CORD	X	Cervical dorsal root fibers
X	Cervical swelling	X	Cervical ventral root fibers
X	Thoracic swelling		Thoracic dorsal root ganglion
X	Lumbar swelling		Thoracic dorsal root fibers
	OTHER		Thoracic ventral root fibers
X	Gasserian ganglion	X	Lumbar dorsal root ganglion
	Pituitary gland	X	Lumbar dorsal root fibers
X	Eyes (with optic nerve)	X	Lumbar ventral root fibers
X	Skeletal muscle (gastrocnemius)		
X	Cauda equina		

Only tissues from the control and 12,000 ppm groups were subjected to microscopic examination and morphometric analysis. Prior to sectioning, the anterior to posterior length of the cerebrum and cerebellum were measured. The following brain sections were measured: 1) frontal cortex thickness; 2) parietal cortex thickness; 3) caudate putamen horizontal width; 4) hippocampal gyrus thickness; and 5) cerebellum height.

Additionally, the entire head was collected from animals culled on PND 4 and preserved in Bouin's fixative. In animals not selected for perfusion (Subset D) that were at least 21 days of age, the eyes were collected and preserved in 10% formalin for possible microscopic evaluation.

D. DATA ANALYSIS

1. **Statistical analyses** – In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test). The level of significance was set at p#0.05, with the exception of Bartlett's test which was set at p#0.001. The following data sets were analyzed by specific statistical procedures:

Parameter	Statistical Procedure
FOB continuous data, motor and locomotor total session activity, acoustic startle response amplitude data (peak amplitude), water maze (latency data)	ANOVA followed by Dunnett's, as necessary
FOB categorical data	General Linear Modeling, Categorical Modeling, Dunnett's test, and Analysis of Contrasts
Interval motor and locomotor activity data	Repeated measures ANOVA (test interval and test occasion) followed by ANOVA and Dunnett's, as necessary
Acoustic startle response amplitude, block data	Repeated measures ANOVA (test block) followed by Dunnett's, as necessary
Passive avoidance (latency data)	Wilcoxon Test for time to failure
Passive avoidance (number of trials-to-criterion) Water maze (number of trials-to-criterion and number of errors)	Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention
Brain weight, gross brain measurements	ANOVA or Kruskal-Wallis
Microscopic brain measurements	ANOVA and/or 2-tailed T-test
Micropathology	Chi-Square and One-tailed Fisher's Exact test

The reviewers consider the statistical methods to be appropriate.

2. **Indices**

a. **Reproductive indices** - The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating index = # inseminated females/# females co-housed with males x 100

Fertility index = # pregnant females/# inseminated females x 100

b. **Offspring viability indices** - The following viability (survival) indices were calculated from lactation records of litters in the study:

Live birth index = # live pups born per litter/total # pups per litter x 100

Viability index = # live pups on PND 4 pre-culling per litter/# live pups born per litter x 100

Lactation index = # live pups on PND 21 per litter/# live pups on PND 4 post-culling per litter x 100

3. **Positive and historical control data** – This study did not include concurrent positive controls, but references were made to previous studies to serve that purpose. It was stated that previous studies (MRID 42770301 [1993] and Bayer Report No. 110506 [2002]) with untreated animals and rats treated with substances that increase (triadimefon) and decrease (chlorpromazine) motor activity have established the sensitivity, reliability, and validity of the test procedures. Additional studies (MRID 45441302 [2001]) have been performed to establish test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages. Further studies (MRID 45441303 [2001]) were performed using reference substances (8OHDPAT, mCPP, and scopolamine) to validate the procedures and observers of the performing lab to conduct the FOB, auditory startle, passive avoidance, and water maze tests. Evidence of inter-observer reliability (MRID 42770301 [1993] and Bayer Report No. G200166 [2004]) is maintained for those individuals performing the observations.

The data from these referenced studies were not provided with the current study; however, summaries of the data from MRIDs 45464601, 45464602, 45441302, and 45441303, previously submitted to the Agency, were obtained and reviewed, and are included as Appendix I to this DER. It should be noted that the positive control data have been determined, by the Agency, to be marginal to inadequate.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations** - No treatment-related mortalities were observed during the study, all dams survived to scheduled sacrifice. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point.

2. **Body weight and food consumption** - Selected group mean body weights and body weight gains for pregnant and nursing dams are summarized in Table 2. During gestation, slight increases ($p \leq 0.05$) were noted in body weight (↑4-5%) and body weight gains (GD 0-20, ↑11%) at 12000 ppm. During lactation, body weights were increased ($p \leq 0.05$) by 4% at 120 and 1200 ppm on LD 0 and by 4-6% at 1200 and above on LD 21. Body weight gains (LD 0-21, calculated by reviewers) were also increased by 17-18% at 1200 and above. Food consumption in the treated groups was similar to controls during gestation and lactation. These differences in body weight and body weight gain were not considered to be treatment-related as the body weights of the treated animals were slightly higher than controls at the beginning of the study and minor increases in weight are not considered to be adverse.

TABLE 2. Selected mean (±SD) body weight and body weight gain in dams exposed to NNI-0001 in the diet from GD 6 through LD 21. [a]

Observations	Dose (ppm)			
	0	120	1200	12,000
Gestation (n=27-29)				
Body weight (g)				
GD 0	230.2±2.26	235.9±2.20	237.9±2.59	234.8±2.29
GD 13	268.3±2.37	276.7±2.96	279.5±2.99*(↑4)	278.2±2.64*(↑4)
GD 20	333.0±3.45	342.1±4.27	349.3±3.94**(↑5)	348.7±3.79**(↑5)
Body weight gain (g)				
GD 0-20	102.8±2.32	106.2±3.04	111.4±2.62*(↑8)	113.9±2.93*(↑11)
Lactation (n=20-29)				
Body weight (g)				
LD 0	259.7±2.38	268.9±2.84*(↑4)	270.7±2.99*(↑4)	266.3±2.75
LD 14	300.1±3.00	309.8±4.33	315.4±4.15	307.2±3.69
LD 21	290.4±3.30	301.3±3.64	306.6±3.43**(↑6)	302.5±3.63*(↑4)
Body weight gain (g)				
LD 0-21[b]	30.7	32.4	35.9 (↑17)	36.2 (↑18)

a Data were extracted from Tables 3 and 6 on pages 74 and 80 of the study report. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Calculated by reviewers from data within this table
* Statistically significantly different from controls at $p \leq 0.05$
** Statistically significantly different from controls at $p \leq 0.01$

3. **Test substance intake** - Mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3. Based on these results the average intake during gestation and lactation was 0, 9.9, 99.5, and 979.6 mg/kg/day. Intake was based on maternal food consumption, body weight, and nominal dose.

TABLE 3. Test substance intake (mg/kg/day) in dams exposed to NNI-0001 in the diet from GD 6 through LD 21. [a]

Interval	Dose (ppm)			
	0	120	1200	12,000
Gestation (n=27-29)				
GD 6-13	0.0±0.00	10.4±0.22	102.1±1.91	1059.5±25.8
GD 13-20	0.0±0.00	10.7±0.26	102.9±1.49	1074.9±16.75
Lactation (n=19-23)				
LD 0-7	0.0±0.00	10.2±0.35	97.4±4.06	964.5±28.88
LD 7-14	0.0±0.00	9.2±0.17	97.9±1.73	882.0±19.37
LD 14-21	0.0±0.00	8.8±0.11	97.0±1.69	917.1±27.06

a Data were extracted from Table 8 on pages 84-85 of the study report.

4. **Reproductive performance** – Reproductive parameters were similar to controls in all dose groups (Table 4).

TABLE 4. Reproductive performance [a]

Observation	Dose (ppm)			
	0	120	1200	12,000
Number mated	30	30	30	30
Mating index (%)[b]	100.0	100.0	100.0	100.0
Fertility index (%)[b]	93.3	96.7	90.0	96.7
Gestation length (median # of days)	22.0	22.0	22.0	22.0

a Data were extracted from Table 1 on page 70 of the study report.

b **Mating index** = # inseminated females/# females co-housed with males x 100 and **fertility index** = # pregnant females/# inseminated females x 100

5. **Organ weight** - In the LD 21 dams, increases ($p \leq 0.05$) in absolute (↑26-34%) and relative (to body, ↑20-28%) liver weight were observed at 1200 ppm and above (Table 5). No treatment-related effect was noted in thyroid weight in the 12,000 ppm dams.

TABLE 5. Mean (±SD) liver weights in PND 21 dams [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
Absolute (g)	14.205±1.011	15.107±1.278	17.943±1.807* (↑26)	19.050±1.132* (↑34)
Relative (to body, %)	4.991±0.282	5.064±0.244	5.978±0.646* (↑20)	6.388±0.172* (↑28)

a Data were extracted from Text Table 19 on page 62 of the study report; n=10. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

* Statistically significantly different from controls at $p \leq 0.05$

B. OFFSPRING

1. **Viability and clinical signs** - Litter size and viability results from pups during lactation are

summarized in Table 6. No compound related effects on any litter parameter were noted at any dose. Live birth, viability, and lactation indices were similar to controls at all doses. It was stated that the relatively high incidence of mortality at 12,000 ppm was due to the loss of an entire litter and was not considered to be related to treatment.

TABLE 6. Litter size and viability [a]

Observation	Dose (ppm)			
	0	120	1200	12,000
No. of litters	20	22	21	23
Total number of pups born	230	255	245	274
Number born dead	1	0	0	2
Sex Ratio Day 0 (% %)	NR	NR	NR	NR
Mean litter size:				
PND 0	11	12	12	12
PND 4 [b]	11	12	12	12
PND 4 [c]	8	8	8	8
PND 21	8	8	8	8
# Deaths Days 4-24 [d]	4	1	3	7
Live birth index (%) [e]	99.7	100.0	100.0	99.3
Viability index (%) [e]	99.1	99.5	98.6	98.3
Lactation index (%) [e]	98.8	99.4	98.2	96.7

a Data were extracted from pages 43 and 45 and Table 9 on pages 75-76 of the study report.
b Pre-culling
c Post-culling
d Found dead, moribund, or missing
e **Live birth index** = # live pups born per litter/total # pups per litter x 100; **viability index** = # live pups on PND 4 pre-culling per litter/# live pups born per litter x 100; and **lactation index** = # live pups on PND 21 per litter/# live pups on PND 4 post-culling per litter x 100
NR Not reported

At 12,000 ppm, treatment-related ocular lesions were noted in pups of both sexes as follows. During pre-weaning (PND 0-21), the following effects on the eyes (# of litters affected/20-29 vs. 0 controls) were observed: (i) enlarged eyeball (1-9 litters on PND 15-21); (ii) corneal opacity (2-3 litters on PND 16-21); (iii) dark red (1-6 litters on PND 15-21); and (iv) exophthalmia (1 litter on PND 20-21, Table 6a). Throughout post-weaning (PND 22-72), the following effects on the eyes (# of animals affected/65-66 vs. 0 controls) were noted: (i) enlarged (9 males/12 females); (ii) general opacity (8 males/10 females); (iii) red (4 males/8 females; and (iv) exophthalmia (2 males, Table 6b). Additionally in the 1200 ppm males, one pup displayed enlarged eye, general opacity, and exophthalmia. No compound-related effects were noted at 1200 ppm in the females or at 120 ppm in either sex.

TABLE 6a. Incidence (# of liters affected) of ocular lesions in F_1 pups during pre-weaning (PND 0-21). [a]

Observation	Dose (ppm)			
	0	120	1200	12,000
Enlarged	0	0	0	9

Corneal opacity	0	0	0	3
Dark Red	0	0	0	6
Exophthalmia	0	0	0	1

a Data were extracted from Table 10 on page 90 of the study report; n=20-29.

TABLE 6b. Incidence (# affected) of ocular lesions in F_1 pups during post-weaning (PND 22-72). [a]

Observation	Dose (ppm)							
	0	120	1200	12,000	0	120	1200	12,000
	Males				Females			
Enlarged [b]	0	0	1	9	0	0	0	12
General opacity [b]	0	0	1	8	0	0	0	10
Red [b]	0	0	0	4	0	0	0	8
Exophthalmia	0	0	1	2	1	0	0	0

a Data were extracted from Table 11 on pages 93-94 and Appendix XII on pages 310-376 of the study report; n=65-66.

b Observed either unilaterally in the left or right eye, or bilaterally in both eyes.

2. **Body weight** – Offspring pre-weaning body weights were decreased ($p \leq 0.05$) at 12,000 ppm (↓9% both sexes) at PND 21 (Table 7a). Body weight gains (Table 7b) were decreased ($p \leq 0.05$) during several pre-weaning intervals at 12,000 ppm (↓12-20%, males and ↓11-20%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 11% at 12,000 ppm.

TABLE 7a. Mean (±SD) pre-weaning F_1 pup body weights (g) [a]

Postnatal Day	Dose (ppm)			
	0	120	1200	12,000
	Males			
0	5.9±0.10	6.0±0.09	6.2±0.10	6.3±0.10
4 [b]	9.9±0.27	10.1±0.22	9.9±0.29	9.9±0.23
4 [c]	9.9±0.27	10.0±0.23	9.9±0.29	9.9±0.25
17	38.4±0.68	39.1±0.75	38.0±0.73	36.1±1.02
21	49.7±0.88	50.0±0.91	47.9±0.79	45.1±1.25* (↓9)
	Females			
0	5.6±0.10	5.7±0.09	5.8±0.10	5.9±0.10
4 [b]	9.5±0.26	9.8±0.23	9.6±0.26	9.6±0.26
4 [c]	9.6±0.26	9.8±0.23	9.5±0.26	9.6±0.26
17	37.5±0.62	38.4±0.63	37.0±0.70	35.3±1.11
21	48.4±0.86	49.1±0.85	46.8±0.77	44.0±1.39* (↓9)

a Data were extracted from Table 12 on pages 96-98 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).

b Pre-culling

c Post-culling

* Statistically significantly different from controls at $p \leq 0.05$

TABLE 7b. Selected mean (±SD) pre-weaning F_1 pup body weight gains (g) [a]				
Interval (PND)	Dose (ppm)			
	0	120	1200	12,000
Males				
0-4	3.9±0.20	4.1±0.15	3.7±0.22	3.7±0.17
4-21	39.8±0.70	40.0±0.79	38.0±0.60	35.1±1.03**(↓12)
11-21	24.7±0.51	24.6±0.52	23.2±0.31*(↓6)	21.4±0.56**(↓13)
17-21	11.3±0.37	10.9±0.40	9.9±0.32*(↓12)	9.0±0.37**(↓20)
Females				
0-4	3.9±0.20	4.1±0.17	3.7±0.21	3.7±0.21
4-21	38.8±0.69	39.3±0.72	37.3±0.58	34.4±1.17**(↓11)
11-21	23.9±0.46	23.9±0.47	22.8±0.40	20.7±0.68**(↓13)
17-21	10.9±0.38	10.6±0.37	9.8±0.32	8.7±0.38**(↓20)
Combined				
Overall (0-21) gain [b]	43.1	43.5	41.2	38.3 (↓11)

a Data were extracted from Table 13 on pages 100-103 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
b Calculated by reviewers using combined data from Table 12 on pages 96-98 of the study report
* Statistically significantly different from controls at $p \leq 0.05$
** Statistically significantly different from controls at $p \leq 0.01$

Offspring post-weaning body weights remained decreased throughout the study at 12,000 ppm in both sexes; however, they only attained statistical significance ($p \leq 0.05$) in the males on Days 28 and 35 (↓7-8%; Table 8).

TABLE 8. Selected mean (±SD) post-weaning F_1 pup body weights (g) [a]				
Post-natal Day	Dose (ppm)			
	0	120	1200	12,000
Males				
28	79.5±6.8	81.8±7.9	77.6±7.6	72.8±10.3* (↓8)
35	127.5±8.6	130.3±12.0	124.9±10.7	118.1±14.9* (↓7)
42	173.3±10.2	176.7±14.3	171.3±13.4	163.0±18.7
70	325.0±14.9	330.2±21.6	327.5±23.1	310.4±33.3
Females				
29	78.6±7.8	80.3±6.3	76.1±6.6	74.0±7.1
50	158.9±9.6	158.8±9.9	156.5±11.7	155.2±8.8
71	200.1±12.3	200.3±11.2	199.0±16.3	198.3±11.1

a Data were extracted from Table 15 on pages 107-108 of the study report; n=20-23 litters. Numbers presented parenthetically are percent difference from controls (calculated by reviewers).
* Statistically significantly different from controls at $p \leq 0.05$

3. Developmental landmarks

a. Sexual maturation – The day of preputial separation was delayed greater than 2 days ($p \leq 0.01$) in the 1200 and 12,000 ppm males (47.5 and 48.7 treated vs. 44.9 controls). The day of vaginal patency was delayed ($p \leq 0.01$) greater than 2 days in the 12,000 ppm females (35.3 treated vs. 32.6 controls). Only 2 pups in the 12,000 ppm group did not display pupil

constriction on PND 21.

TABLE 9. Sexual maturation (mean ±SD day of onset) in F_1 pups [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
Preputial separation	44.9±0.50	45.6±0.48	47.5±0.59**	48.7±0.73**
Vaginal patency	32.6±0.59	33.1±0.52	33.2±0.65	35.3±0.63**

a Data were extracted from Table 14 on page 105 of the study report; n=20-23 litters.
** Statistically significantly different from controls at $p \leq 0.01$

Reduced weight gain that resulted in decreased body weight during lactation persisted in high-dose males until study termination and in high-dose females for the first week measured after weaning. These differences from control were only statistically significant in males for the first two weeks measured after weaning. Lower body weights at the highest dietary level averaged 4-7% less than controls for males and 6% less than controls for females. Body weight was not affected by treatment in males or females at the two lower dietary levels. Therefore, it does not appear that body weight reduction is associated with the delay in sexual maturation.

b. Physical landmarks – Evaluation of physical landmarks (eye opening, pinna unfolding, incisor erupting) was not performed.

4. Behavioral assessments

a. Functional observational battery – Treatment-related FOB effects were limited to ocular lesions (corneal opacity, dark red eyes, and/or enlarged eyes) at 12,000 ppm (1-2 males and 1-4 females, Table 10). The lesions were observed beginning on PND 21 and persisted until PND 60 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

TABLE 10. Incidence (# affected [% incidence]) of ocular lesions in F_1 pups [a]

Observation	PND	Dose (ppm)							
		0	120	1200	12,000	0	120	1200	12,000
		Males (n=14-16)				Females (n=13-16)			
Corneal opacity	4	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	21	0	0	0	1 (6)	0	0	0	2 (13)
	35	0	0	0	2 (13)	0	0	0	2 (13)
	45	0	0	0	1 (7)	0	0	0	3* (21)
	60	0	0	0	0	0	0	0	3* (23)
Dark red	4	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	21	0	0	0	1 (6)	0	0	0	1 (7)
	35	0	0	0	1 (6)	0	0	0	2 (13)
	45	0	0	0	0	0	0	0	1 (7)
	60	0	0	0	0	0	0	0	0
Enlarged	4	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0
	21	0	0	0	2 (13)	0	0	0	2 (13)
	35	0	0	0	2 (13)	0	0	0	4* (27)
	45	0	0	0	1 (7)	0	0	0	3* (21)
	60	0	0	0	1 (7)	0	0	0	3* (23)

a Data were extracted from Table 17 on pages 135-194 of the study report.
* Statistically significantly different from controls at $p \leq 0.05$

b. Motor activity – No treatment-related differences in total session motor or locomotor activity were observed at any dose in either sex (Tables 11a and 11b). In the females, total session motor activity was increased ($p \leq 0.05$) by 92% at 12,000 ppm on PND 17 and by 28% at 120 ppm on PND 21. However, these findings were not considered to be related to treatment as they were not dose-dependent and/or transient. Interval motor and locomotor activity levels were similar to controls in both sexes at all time points. Levels of activity progressively increased with age compared to the levels on PND 13. Habituation was evident in both sexes at all ages.

TABLE 11a. Mean (±SD) total session motor activity (counts) in F_1 pups [a]

Interval (PND)	Dose (ppm)			
	0	120	1200	12,000
Males				
13	104±112	62±49	82±54	61±50
17	203±89	234±166	160±111	207±165
21	301±76	312±101	320±101	342±132
60	500±92	502±108	502±126	529±129
Females				
13	59±45	59±66	52±34	60±57
17	162±138	165±121	202±137	312±161* (↑92)
21	282±91	361±90* (↑28)	358±94	308±84
60	680±137	683±205	703±186	667±106

a Data were extracted from Text Table 12 on page 52 of the study report; n=15-16 pups.

TABLE 11b. Mean (±SD) total session locomotor activity (counts) in F_1 pups [a]

Interval (PND)	Dose (ppm)			
	0	120	1200	12,000
Males				
13	11±11	5±4	5±4	6±7
17	51±25	58±55	36±31	42±45
21	90±27	91±24	88±30	96±42
60	352±88	345±86	351±116	375±110
Females				
13	7±10	5±11	4±4	7±13
17	37±30	48±42	53±42	82±44
21	84±30	100±36	95±23	103±29
60	424±106	449±151	412±124	453±116

a Data were extracted from Text Table 13 on page 52 of the study report; n=15-16 pups.

c. Auditory startle reflex habituation – While for PND60 males there appeared to be a dose-response increase in acoustic startle peak amplitude in the blocks, when individual animal data was graphed onto a scatter plot (see appendix 1), the individual variability was clearly evident; based on results from the individual animal data visualized on the scatter plot, the increase in amplitude was judged not treatment-related but was largely driven by individual outliers. Therefore, no treatment-related effect on total session peak

amplitude, latency, or habituation were observed in either sex at PND 22 or 60±2 (Table 12). The amplitude of the startle response increased with age in both sexes. At both ages, habituation was apparent in the control animals as a decrease in amplitude during the course of the test session.

TABLE 12. Mean (±SD) overall (Blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 rats [a]

Dose (ppm)	Parameter	Males		Females	
		PND 22	PND 60±2	PND 22	PND 60±2
0	Peak Amp.	38±17	162±87	26±8	108±63
	Latency	37±3	40±3	37±3	41±3
120	Peak Amp.	29±12	219±121	29±11	94±58
	Latency	38±6	40±3	38±3	38±4
1200	Peak Amp.	30±11	213±144	26±9	66±37
	Latency	37±2	39±3	38±4	40±3
12,000	Peak Amp.	33±11	228±145	35±18	95±56
	Latency	39±5	39±2	38±5	39±3

a Data were extracted from Text Table 14 on pages 54-55 of the study report; n=14-16.

d. Learning and memory testing – No compound-related effects were noted at any dose in either sex in the passive avoidance or M-maze tests (Tables 13 and 14). In the passive avoidance test, acquisition was evident in both sexes as a marked increase in the latency to cross for the second trial compared to the first trial. Retention was evident as a protracted delay to cross within the 180-sec time limit of the first trail compared to the first trial on the first test day, and a reduced number of trials-to-criterion on the second test day compared to the first day.

TABLE 13. Mean (±SD) passive avoidance performance in F_1 rats [a]

Session/Parameter		Dose (ppm)			
		0	120	1200	12,000
Males (n=14-16)					
Session 1 (PND 22) Learning	Trials to Criterion	3.1±1.1	3.1±0.3	3.0±0.0	3.3±0.8
	Latency Trial 1 (sec)	60.7±59.6	33.8±27.5	26.1±11.8	38.8±42.3
	Latency Trial 2 (sec)	180.0±0.0	174.8±20.7	180.0±0.0	176.7±9.8
	Failed to Meet Criterion	0	0	0	0
Session 2 (PND 29) Retention	Trials to Criterion	2.2±0.6	2.0±0.0	2.3±0.8	2.3±0.6
	Latency Trial 1 (sec)	179.2±2.9	180.0±0.0	169.4±28.9	158.7±47.4
	Latency Trial 2 (sec)	177.8±8.3	180.0±0.0	180.0±0.0	179.0±3.9
Females (n=15-16)					
Session 1 (PND 22) Learning	Trials to Criterion	3.1±0.3	3.0±0.4	3.0±0.0	3.2±0.5
	Latency Trial 1 (sec)	53.8±48.2	43.6±55.2	30.2±21.4	28.6±23.2
	Latency Trial 2 (sec)	176.8±12.7	174.4±22.3	180.0±0.0	177.4±10.2
	Failed to Meet Criterion	0	0	0	0
Session 2 (PND 29) Retention	Trials to Criterion	2.0±0.0	2.5±0.8	2.4±0.8	2.3±0.6
	Latency Trial 1 (sec)	180.0±0.0	174.7±20.3	175.0±20.2	163.7±38.5
	Latency Trial 2 (sec)	180.0±0.0	168.0±30.4	163.0±39.5	178.0±8.1

a Data were extracted from Text Table 16 on page 57 of the study report.

In the water maze test, acquisition was evident in both sexes as a progressive decrease in the average time to escape over successive trials. Retention was evident as a reduction in the number of trials-to-criterion and a shorter trial duration for the first trial compared to the first trial of acquisition. The increased ($p \leq 0.05$) second trial duration during acquisition noted in the 12,000 ppm males was considered incidental and unrelated to treatment as the difference was small and within the range of historical controls (11.3 to 21.4 seconds).

TABLE 14. Mean (±SD) water maze performance in F_1 rats [a]

Session/Parameter		Dose (ppm)			
		0	120	1200	12,000
Males					
Session 1 (PND 60±2) Learning	Trials to Criterion	5.9±1.2	6.4±1.8	6.9±2.5	7.8±3.4
	Trial 1 Errors	0.6±0.9	0.7±0.9	0.4±0.8	0.8±1.1
	Latency Trial 1 (sec)	15.7±16.1	14.9±9.8	19.7±14.4	18.1±14.2
	Trial 2 Errors	0.2±0.4	0.4±0.6	0.6±0.9	0.8±1.1
	Latency Trial 2 (sec)	9.7±5.4	9.8±5.4	11.4±5.5	17.3±13.1*
	Failed to Meet Criterion	0	0	0	1
Session 2 (PND 67±2) Retention	Trials to Criterion	5.5±0.8	5.6±1.4	6.3±2.1	6.0±1.7
	Trial 1 Errors	0.6±1.0	0.1±0.5	0.6±1.0	0.9±1.5
	Latency Trial 1 (sec)	12.3±11.3	6.8±4.4	10.6±14.1	11.4±13.7
	Trial 2 Errors	0.0±0.0	0.0±0.0	0.3±0.7	0.1±0.5
	Latency Trial 2 (sec)	4.0±1.7	3.8±1.2	5.3±4.3	4.0±2.0
Females					
Session 1 (PND 60±2) Learning	Trials to Criterion	7.4±3.1	7.0±2.2	8.8±3.1	7.9±2.2
	Trial 1 Errors	1.1±1.3	0.9±1.2	1.1±1.1	1.5±1.1
	Latency Trial 1 (sec)	19.6±14.5	18.0±14.3	17.8±11.2	24.9±15.4
	Trial 2 Errors	0.7±1.6	0.6±0.9	1.0±1.0	0.8±0.7
	Latency Trial 2 (sec)	12.9±10.9	13.0±9.1	15.6±13.9	14.4±9.1
	Failed to Meet Criterion	1	0	1	0
Session 2 (PND 67±2) Retention	Trials to Criterion	7.3±3.3	7.0±2.5	6.5±1.8	7.7±3.6
	Trial 1 Errors	0.3±0.7	0.2±0.5	0.5±0.8	0.5±0.7
	Latency Trial 1 (sec)	8.1±8.7	9.1±10.5	7.8±5.1	9.7±6.1
	Trial 2 Errors	0.4±0.9	0.5±1.2	0.2±0.6	0.2±0.4
	Latency Trial 2 (sec)	7.5±9.6	9.2±11.6	6.4±5.8	6.2±3.6

a Data were extracted from Text Table 17 on pages 58-59 of the study report; n=14-16.

5. **Ophthalmology** – Although corneal opacities were noted on PND 50 (M: 2, 1, 0, 3; F: 1, 1, 1, 1; in the control, 120, 1200, and 12,000 ppm groups, respectively), it was stated that no compound-related ocular lesions were noted in either sex. These results differ from the results for clinical observations, FOB, and gross pathology, where compound-related corneal opacities and exophthalmos were evident in the 12,000 ppm pups. It was stated that these differences, along with other ocular lesions, were not attributed to treatment, because of the low incidence (1 female and 3 males account for all the findings at 12,000 ppm).

6. Postmortem results

a. Organ weights – No treatment-related effects were noted in terminal body weights or absolute and relative (to body) brain weights on PNDs 21 or 75±5 in either sex (Table 15).

TABLE 15. Mean (±SD) brain weight data from perfused and non-perfused F_1 rats [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
Males				
PND 21 (Perfused)				
Terminal body weight (g)	48.5±5.7	47.6±3.2	50.1±4.0	46.9±3.3
Brain weight (g)	1.428±0.057	1.378±0.083	1.483±0.086	1.394±0.049
Brain-to-body weight ratio (%)	2.985±0.388	2.903±0.168	2.974±0.295	2.987±0.257
PND 75±5 (Termination – Perfused)				
Terminal body weight (g)	362.4±28.9	349.0±29.7	351.4±32.1	333.9±33.8
Brain weight (g)	1.918±0.091	1.856±0.073	1.933±0.110	1.842±0.089
Brain-to-body weight ratio (%)	0.531±0.039	0.535±0.045	0.553±0.041	0.556±0.049
PND 75±5 (Termination – Non-perfused)				
Terminal body weight (g)	339.0±20.1	342.2±42.5	354.4±28.6	334.9±22.7
Brain weight (g)	2.022±0.058	1.973±0.089	2.006±0.054	1.980±0.099
Brain-to-body weight ratio (%)	0.598±0.029	0.585±0.079	0.569±0.045	0.594±0.051
Females				
PND 21 (Perfused)				
Terminal body weight (g)	48.4±3.7	51.6±4.4	46.7±3.6	44.6±5.3
Brain weight (g)	1.364±0.085	1.408±0.036	1.386±0.092	1.363±0.066
Brain-to-body weight ratio (%)	2.829±0.207	2.747±0.243	2.977±0.213	3.084±0.280
PND 75±5 (Termination – Perfused)				
Terminal body weight (g)	206.8±19.1	209.7±15.9	206.1±17.4	208.2±14.6
Brain weight (g)	1.757±0.074	1.822±0.078	1.754±0.086	1.795±0.041
Brain-to-body weight ratio (%)	0.857±0.099	0.872±0.055	0.857±0.087	0.866±0.060
PND 75±5 (Termination – Non-perfused)				
Terminal body weight (g)	206.3±11.6	211.5±15.3	209.1±22.7	207.4±12.1
Brain weight (g)	1.773±0.143	1.827±0.133	1.831±0.131	1.886±0.079
Brain-to-body weight ratio (%)	0.861±0.069	0.866±0.068	0.882±0.086	0.911±0.050

a Data were extracted from Text Table 18 on page 61 of the study report; n=10.

b) Neuropathology

1. **Macroscopic examination** – No gross lesions or significant differences in cerebellum and cerebrum lengths were observed on PND 21 or at termination in either sex.

2. **Microscopic examination** – At 12,000 ppm, the following compound-related microscopic effects (# affected/10 vs. 0 controls, unless otherwise stated) were noted in the eye and optic nerve (Table 16): (i) retinal degeneration (2 males and 1 female); (ii) hemorrhage (3 males vs. 1 control); (iii) cataract (2 males and 1 female); and (iv) atrophy of the optic nerve (3 males and 1 female). No other treatment-related microscopic lesions were noted at any dose in either sex.

TABLE 16. Incidence of microscopic lesions (# affected/10) in F_1 rats [a]

Observation		Dose (ppm)			
		0	12,000	0	12,000
		Males		Females	
Eye	Retinal degeneration	0	2	0	1
	Hemorrhage	1	3	0	0
	Cataract	0	2	0	1
Optic nerve, Atrophy		0	3	0	1

a Data extracted from Table MP2-SUM on pages 884-887.

No treatment-related differences in morphometric brain measurements were noted in the 12,000 ppm animals compared to controls on PND 21 or 75±5 (Tables 17a and 17b).

TABLE 17a. Mean (±SD) morphometric brain measurements (mm) in male F_1 rats [a]

Parameter	Dose (ppm)			
	0	120	1200	12,000
PND 21				
Cerebrum Length	13.56±0.27	13.29±0.36	13.86±0.37	13.51±0.24
Cerebellum Length	7.37±0.31	7.30±0.40	7.36±0.39	7.22±0.40
Frontal Cortex	1.72±0.01	NM	NM	1.75±0.01
Parietal Cortex	1.90±0.00	NM	NM	1.89±0.00
Caudate Putamen	3.13±0.02	NM	NM	3.01±0.02
Hippocampal Gyrus	1.62±0.00	NM	NM	1.69±0.01
Cerebellum Height	4.12±0.06	NM	NM	4.15±0.04
PND 75±5 (Termination)				
Cerebrum Length	14.76±0.37	14.62±0.33	14.60±0.32	14.48±0.40
Cerebellum Length	7.79±0.39	7.73±0.41	7.77±0.23	7.74±0.18
Frontal Cortex	1.71±0.02	NM	NM	1.72±0.01
Parietal Cortex	1.86±0.01	NM	NM	1.88±0.00
Caudate Putamen	3.46±0.01	NM	NM	3.37±0.04
Hippocampal Gyrus	1.77±0.01	NM	NM	1.84±0.01
Cerebellum Height	4.56±0.10	NM	NM	4.59±0.22

a Data were extracted from Text Table 20 on pages 63-64; n=9-10.
NM Not measured

TABLE 17b. Mean (±SD) morphometric brain measurements (mm) in female F_1 rats [a]				
Parameter	Dose (ppm)			
	0	120	1200	12,000
PND 21				
Cerebrum Length	13.40±0.36	13.54±0.32	13.54±0.29	13.23±0.29
Cerebellum Length	7.26±0.36	7.10±0.52	7.44±0.39	7.12±0.43
Frontal Cortex	1.73±0.00	NM	NM	1.73±0.01
Parietal Cortex	1.86±0.01	NM	NM	1.87±0.01
Caudate Putamen	2.95±0.02	NM	NM	3.02±0.02
Hippocampal Gyrus	1.66±0.02	NM	NM	1.69±0.02
Cerebellum Height	4.08±0.02	NM	NM	4.20±0.07
PND 75±5 (Termination)				
Cerebrum Length	14.18±0.34	14.26±0.40	14.17±0.21	14.25±0.41
Cerebellum Length	7.86±0.46	7.89±0.40	7.69±0.29	7.71±0.38
Frontal Cortex	1.68±0.01	NM	NM	1.68±0.01
Parietal Cortex	1.78±0.00	NM	NM	1.80±0.00
Caudate Putamen	3.37±0.01	NM	NM	3.29±0.04
Hippocampal Gyrus	1.67±0.02	NM	NM	1.68±0.04
Cerebellum Height	4.49±0.17	NM	NM	4.55±0.04

a Data were extracted from Text Table 20 on pages 63-64; n=10.
NM Not measured

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS –

The investigators concluded that dietary administration of NNI-0001 from GD 6 through LD 21 induced the following effects at 1200 ppm and above: (i) increased absolute and relative (to body) liver weight in the dams; (ii) ocular lesions (enlarged eyeball, exophthalamus, ocular opacities) in the pups; (iii) decreased pre-weaning body weight gain in both sexes; and (iv) delayed balanopreputial separation. Additional effects noted at 12,000 ppm included decreased post-weaning body weight and body weight gain in both sexes, and delayed vaginal patency. No evidence of developmental neurotoxicity was observed at any dose in either sex.

B. REVIEWER COMMENTS –

Mean daily mg test substance/kg body weight during the gestation and lactation periods Averaged 0, 9.9, 99.5, and 979.6 mg/kg/day. based on maternal food consumption, body weight, and nominal dose.

Maternal Effects:
No treatment-related mortalities were observed during the study, all dams survived to scheduled sacrifice. No treatment-related clinical signs were observed during gestation or lactation. No adverse effects were observed in any FOB parameter at any time point.

During gestation, slight increases ($p \leq 0.05$) were noted in body weight (↑4-5%) and body weight gains (GD 0-20, ↑11%) at 12000 ppm. During lactation, body weights

were increased ($p \leq 0.05$) by 4% at 120 and 1200 ppm on LD 0 and by 4-6% at 1200 and above on LD 21. Body weight gains (LD 0-21, calculated by reviewers) were also increased by 17-18% at 1200 and above. Food consumption in the treated groups was similar to controls during gestation and lactation. These differences in body weight and body weight gain were not considered to be treatment-related as the body weights of the treated animals were slightly higher than controls at the beginning of the study and minor increases in weight are not considered to be adverse. In the LD21 dams, increases ($p \leq 0.05$) in absolute (↑26-34%) and relative (to body, ↑20-28%) liver weight were observed at 1200 ppm and above.

Reproductive parameters were similar to controls in all dose groups.

Pup effects.:
Offspring pre-weaning body weights were decreased ($p \leq 0.05$) at 12,000 ppm (↓9% both sexes) at PND 21. Body weight gains were decreased ($p \leq 0.05$) during several pre-weaning intervals at 12,000 ppm (↓12-20%, males and ↓11-20%, females). Overall combined pup body weight gains (calculated by reviewers) were decreased by 11% at 12,000 ppm.

Sexual maturation – The day of preputial separation was delayed ($p \leq 0.01$) in the 1200 and 12,000 ppm males (47.5 and 48.7 treated vs. 44.9 controls) greater than 2 days. The day of vaginal patency was delayed ($p \leq 0.01$) in the 12,000 ppm females (35.3 treated vs. 32.6 controls) greater than 2 days. Only 2 pups in the 12,000 ppm group did not display pupil constriction on PND 21.

Functional observational battery – Treatment-related FOB effects were limited to ocular lesions (corneal opacity, dark red eyes, and/or enlarged eyes) at 12,000 ppm (1-2 males and 1-4 females). The lesions were observed beginning on PND 21 and persisted until PND 60 in both sexes. All other FOB findings were considered incidental and unrelated to treatment.

At 12,000 ppm, treatment-related ocular lesions were noted in pups of both sexes as follows. During pre-weaning (PND 0-21), the following effects on the eyes (# of litters affected/20-29 vs. 0 controls) were observed: (i) enlarged eyeball (1-9 litters on PND 15-21); (ii) corneal opacity (2-3 litters on PND 16-21); (iii) dark red (1-6 litters on PND 15-21); and (iv) exophthalmia (1 litter on PND 20-21). Throughout post-weaning (PND 22-72), the following effects on the eyes (# of animals affected/65-66 vs. 0 controls) were noted: (i) enlarged (9 males/12 females); (ii) general opacity (8 males/10 females); (iii) red (4 males/8 females; and (iv) exophthalmia (2 males). Additionally in the 1200 ppm males, one pup displayed enlarged eye, general opacity, and exophthalmia. No compound-related effects were noted at 1200 ppm in the females or at 120 ppm in either sex.

Microscopic examination – At 12,000 ppm, the following compound-related microscopic effects (# affected/10 vs. 0 controls, unless otherwise stated) were noted in the eye and optic nerve: (i) retinal degeneration (2 males and 1 female); (ii) hemorrhage (3 males vs. 1 control); (iii) cataract (2 males and 1 female); and (iv) atrophy of the optic nerve (3 males and 1 female). No other treatment-related microscopic lesions were noted at any dose in either sex.

Maternal LOAEL = 99.5 mg/kg/day based on increased liver weights. Increased liver weight in isolation is not considered an "adverse" effect, but considering the consistent observation of liver toxicity (e.g., centrilobular hepatocyte fatty change, hypertrophy, increase in liver enzymes, foci of cellular alterations) demonstrated across multiple durations and species at similar doses, the weight-of-evidence supports this effect as an "adverse" finding and thus, a firm basis for the LOAEL. Maternal NOAEL= 9.9 mg/kg/day.

Offspring LOAEL = 99.5 mg/kg/day based on delayed balanpreputial separation. The Offspring NOAEL = 9.9 mg/kg/day.

This study is classified (**acceptable/non-guideline**) and satisfies the guideline requirement; OPPTS 870.6300, ' 83-6, OECD 426 (draft) for a developmental neurotoxicity study in rats.

C. **STUDY DEFICIENCIES** - None

APPENDIX I

BAYER CORP

MAIN FORM

Laboratory		Bayer Corp., Stilwell, KS		
Study No.	**MRID**	**TRX**	**Year**	**Citation**
1	45441302		2001	1. Sheets,L.P. and Lake,S.G. (2001) Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal Methimazole Treatment in Wistar Rats. Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-982-RR, Feb 9, 2001. 973 p. MRID 45441302.
2	45441303		2001	2. Sheets,L.P. (2001) Historical Control and Method Validation Studies in rats for a Developmental Neurotoxicity Screening Battery (Auditory Startle Habituation and Cognitive Function (Passive Avoidance and Water Maze Conditioning). Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-992-VV, 98-992-UM, 98-992-WC, 99-D82-AF, Feb 9, 2001. 191 p. MRID 45441303.
3	45464601		1999	3. Sheets,L.P. and Gilmore,R.G. (1999) Verification of Personnel Training to Perform a Functional Observational Battery with Rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-962-LG. September 16, 1999. 94 p. MRID 4544601
4	45464602		2000	4. Sheets,L.P. and Armintrout,G.L. (2000) A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpromazine in Wistar rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-482-OU. June 19, 2000. 56 p. MRID 45464602

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	1. Sheets,L.P. and Lake,S.G. (2001) Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal Methimazole Treatment in Wistar Rats. Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-982-RR, Feb 9, 2001. 973 p. MRID 45441302.	
Positive Control	Methimazole (d)		
Date of Positive Control Data	2001		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PDX X)	PS,VO,EO,SR,PI, ASR	raw, means	yes	var	1	m,f	20	y	
FOB		yes	yes	4.1e+10	1	m,f	15	n	
Motor Activity	PH (Columbus)	yes	yes	13-17, 60	1	m,f	20	y	
Startle	CO	raw, means	yes	223860	1	m,f	20	y	
Learn/Memory	PA MZ	raw, means	yes	24-31 60-67	1	m,f	20	n y	
Std Histopath		yes	yes	11	1	m,f	10	n	
Morphometrics		raw, means	yes	11, 70	1	m,f	10	y, y	
Thyroid Hormone and Histopath		yes	yes	1170	1	m,f	10	y hormone y histopath	

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data
Methods/Results	**Methods:** One dose of methimazole from GD16 to PND10 at 0.1 mg/ml in the drinking water. Standard methods used in the Bayer lab for DNT studies. **Results:** 14% decrease in maternal body weight and decrease in pup weights postnatally recovery in males not in females by PND~60. Most endpoints were affected. Exceptions included: no change in FOB, no change in PND24 passive avoidance, and no evidence of histopathology from standard subjective assessments. Motor activity was only affected on PND 13 and no other ages. No effect on startle on PND23, increases on PND 38 and 60 only in males. Only effect in males on learning phase in water maze test. No effects in females. There was an 84% decrease in T4 and a 16% decrease in T3.
Summary	**Summary**: It appears to be adequate data to support proficiency for developmental exposure to one agent. Problems include: only decrease seen in MA testing and only at one age, no increases; no effects on FOB measures, PND24 learning/memory testing or std histopath. Only effects in one sex in learning portion of water maze, no effects on retention testing. Note that other data from this lab support proficiency with adult motor activity. **Overall Conclusion**: Proficiency = marginal.

382

3/134

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	2. Sheets,L.P. (2001) Historical Control and Method Validation Studies in rats for a Developmental Neurotoxicity Screening Battery (Auditory Startle Habituation and Cognitive Function (Passive Avoidance and Water Maze Conditioning). Bayer Corporation, Stilwell, KS, Laboratory Study Number 98-992-VV, 98-992-UM, 98-992-WC, 99-D82-AF, Feb 9, 2001. 191 p. MRID 45441303.	
Positive Control	1. 8-hydroxy-(di-n-propylamino) piperazine HBr (8OH-DPAT) 2. 1-m-chlorophenyl piperazine HCl (mCPP) 3. Scopolamine HBr		
Date of Positive Control Data	2001		
Species/Strain	Male and female Crl:Wistar(HAN,BR) rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB									
Startle	CO	yes	no	30	5 mg/kg	m	8	n	mCPP
Startle	CO	yes	no	32	0.5, 1.0 mg/kg	f	10	y	8OH-DPAT
Startle	CO	yes	no	30	0.25 mg/kg	m	8	n	8OH-DPAT
Learn/Memory	PA MZ	yes yes	n y	3556		m m,f	1212	yes no	scopolamine, MZ=water m-maze
Std Histo									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Data presentation is adequate.
Methods/Results	**Methods:** For startle used a fairly standard 50 trial habituation paridigm. Recorded peak amplitude. Testing was immediately post-dosing. Cognitive testing used an M-maze and one dose of scopolamine, 1.0 mg/kg administered between 30 and 60 minutes prior to testing. Training consisted of 15 trials. Retention testing was conducted 24 hours later. Variables were trials to criterion, number of errors and latency to goal. Passive avoidance used a standard paridigm: trials to criterion was maxed out at 15. **Results:** For startle there was no effect of the mCPP 8-OHDPAT caused an increase at the highest dose. Results for the water maze testing are not very good. There were no statistically significant effects on any measure in males, in females there was a decrease in latency (??), and an increase in the number that failed to meet criterion (controls = 2; scoploamine = 5). For passive avoidance the data are not very impressive. For the learning phase there was a small increase in the trials to criterion and a small decrease in the latency on trial 2, but not trial 1. For retention testing there was a small increase in trials to criterion, a decrease in latency on trial 1 and no effect on trial 2. Not very big effects compared to published literature on scopolamine and passive avoidance.
Summary	**Summary:** These data alone are marginal to non-acceptable as evidence of proficiency. There are only males for the PA testing and the effects of 1.0 mg/kg scopolamine are rather small. M-maze performance was not affected at all in males and affected only slightly in females. Startle shown to be increased, but not decreased by reference compounds used previously by the author. **Overall Conclusion**: Proficiency = not demonstrated for startle, PA, or M-maze.

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	3. Sheets,L.P. and Gilmore,R.G. (1999) Verification of Personnel Training to Perform a Functional Observational Battery with Rats. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-962-LG. September 16, 1999. 94 p. MRID 45464601	
Positive Control	Carbaryl		
Date of Positive Control Data	1999		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB		yes	yes/no	63	2 (15,30 mg/kg)	m	6	yes	carbaryl
Motor Activity									
Startle									
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data
Methods/Results	**Methods:** Standard FOB with ranking scales plus grip strength and footsplay. Carbaryl administered ip, 15-70 min prior to testing. Five technicians rated each animal and interobserver reliability was assessed. **Results:** Dose response was apparent. A variety of endpoints were affected as would be expected with carbaryl. Some endpoints were not affected. Good overall agreement between observers.
Summary	**Summary:** Data for FOB are inadequate: only data for males, small group size (n=6), one dose, only adults. Only one compound and not all endpoints affected. Same data as was submitted for adult neurotox studies. **Overall Conclusion:** unacceptable

33/34

352

Positive Control Review Form

Testing Laboratory	Bayer Co., Stilwell, KS	4. Sheets,L.P. and Armintrout,G.L. (2000) A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpromazine in Wistar rats.. Unpublished study prepared by Bayer Corporation, Stilwell, KS. Laboratory Study Number 97-482-OU. June 19, 2000. 56 p. MRID 45464602	
Positive Control	triadimefon, chlorpromazine		
Date of Positive Control Data	2000		
Species/Strain	Wistar rats		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Age (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB									
Motor Activity	F8	yes	yes/no	70	0, 200 mg/kg	m	12	yes	triadimefon
	F8	yes	yes/no	70	0, 2 mg/kg	m	12	yes	chlorpromazine
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Good example of data reporting. Separate submission in standard format. QA, summaries and raw data.
Methods/Results	**Methods:** Triadimefon (90 min prior to testing, po) and chlorpromazine (60 min prior, ip) were administered to 70 day old Wistar rats. Testing in figure-8 mazes lasted 90 min, summed in 10 min bins. Data analyzed by SAS. **Results:** Triadimefon resulted in increased activity of about 300% and a decrease in habituation. Chlorpromazine resulted in decreased activity, about 50% for total counts.
Summary	**Summary:** Triadimefon and chlorpromazine data are inadequate due to males only and only adults at 70 days of age. Data demonstrate ability to detect decreases and increases, as well as decreased habituation. Sensitivity is unknown due to lack of dose response. **Overall Conclusion:** Marginal.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; Dermal Penetration Study in Monkeys

Work Assignment No 4-1-124 P; formerly 3-1-124 P (MRID 46817234)

Prepared for
Health Effects Division
Office of Pesticide Programs
U S Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B
Durham, NC 27713

Primary Reviewer:
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 1/31/07

Secondary Reviewer:
Michael E. Viana, Ph.D., D.A.B.I.

Signature: Michael E Viana
Date: 1/31/07

Program Manager:
Michael E. Viana, Ph.D., D.A.B.I.

Signature: Michael E Viana
Date: 1/31/07

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 1/31/07

Disclaimer
This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Mary Ko Manibusan **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline Dermal Penetration Study in Monkeys

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (RADIOCHEMICAL PURITY): NNI-0001 (99.6%)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Sebesta, C. (2005) A study to determine the dermal absorption of NNI-0001 SC 480 when administered dermally to male Rhesus monkeys. Charles River Laboratories, Discovery and Developmental Services, Worcester MA. Laboratory Report No.: VCBZ-0111-03-742, January 31, 2005. MRID 46817234. Unpublished.

SPONSOR: Bayer CropScience LP, Toxicology, 17745 South Metcalf Avenue, Stilwell, KS

EXECUTIVE SUMMARY: In a non-guideline dermal penetration study (MRID 46817234), [aniline-UL-^{14}C]-NNI-0001 (Flubendiamide; 99.6% radiochemical purity; Synthesis No. KML 3120-1) was applied in an aqueous dilution of a commercial formulation, SC 480, as a single dermal dose (2.7 µg/cm^2) to a shaved area (24 cm^2) on each of 5 male rhesus monkeys for an 8-hour exposure period. Following the exposure interval, the application area was washed, and urine, feces, and cage debris/rinse were collected for up to 120 hours post-dose.

Recovery of the applied dose (mass balance) was 105%. The majority of the dose was recovered in the skin swabs (98% of the applied dose). Dermal absorption (based on the sum of residues in urine, feces, and cage wash) was 0.02% of the applied dose. The absorbed dose was found exclusively in the urine during the first time interval (0-4 h).

This study is classified as **acceptable/non-guideline**.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:

[Aniline-UL-^{14}C]-NNI-0001

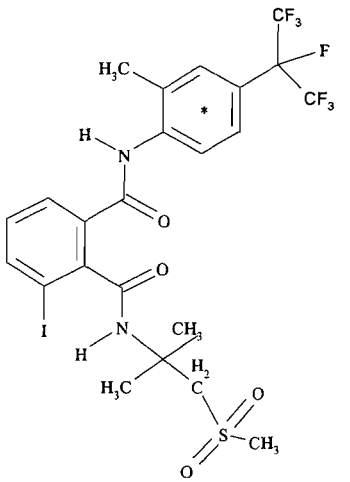
Description: Liquid

Synthesis No. KML 3120-1

Compound Stability: Radiochemical purity was stable in the formulation for 7 days at 40°C.

Vehicle/Solvent used: SC 480 formulation diluted with water

Radiolabelling:



* denotes the position of [^{14}C]-labeled atoms

Specific Activity: 3.96 MBq/mg (~107 µCi/mg)

Radiochemical Purity: 99.6%

Source: Bayer CropScience LP, Stilwell, KS

2. Relevance of test material to proposed formulation(s): An aqueous dilution of a commercial formulation, SC 480, was used.

3. Test animals:

Species: Monkey (male)

Strain: Rhesus

Age/weight at study initiation: Age was not reported; naïve males weighed 3.06-3.48 kg and the one non-naïve male weighed 5.29 kg

Source: Charles River Laboratories, Worcester, MA

Housing: Individually in primate chairs for an 8-hour post-dose period and then in stainless steel metabolism cages

Diet: LabChows® Certified Primate Chow Brand Animal Diet 5048 twice daily, supplemenented with washed fresh produce, except during fasting intervals

Water: Filtered tap water, *ad libitum*

Environmental conditions:

Temperature:	18-29EC
Humidity:	30-70%
Air changes:	≥10/hr
Photoperiod:	12 hours dark/ 12 hours light

Acclimation period: Animals were previously acclimated to primate chair and manual restraints. Further details were not provided.

B. STUDY DESIGN: A single dermal dose (4 $\mu g/cm^2$ nominal) was applied to a shaved area on the back of each of 5 male rhesus monkeys for an 8-hour exposure period. The application area was washed, and urine, feces, and cage debris/rinse were collected up to 120 hours post-dose.

1. Dose

Rationale: The Sponsor stated that the dermal dose level used was selected to reflect human exposure in the field. The test substance was shown to be non-toxic at this dose.

Nominal doses: 4 $\mu g/cm^2$ skin

Actual doses: 2.70 $\mu g/cm^2$ skin

Dose volume: 4.2 $\mu l/cm^2$

Duration of exposures (time from dose to skin wash): 8 hours

Termination periods (time from dose to end of study): 120 hours; animals were not sacrificed

Number of animals: 5

2. Animal preparation: Animals were obtained from the Test Facility's colony. One non-naïve male and 4 naïve males were used. Approximately 2 weeks prior to treatment, 2 skin swabs were taken of the non-naïve animal confirming that radioactivity was below twice background. A physical examination including a complete blood count and serum chemistry analysis was performed one week prior to treatment. The animals were previously acclimated to primate chair restraint and manual restraint by rope/pole and collar. One day prior to treatment, the animals were anesthetized with ketamine HCl. An area on the back (4x6 cm) was shaved with clippers, avoiding skin irritation, and the area was gently cleaned with water and patted dry. The animals were fasted for approximately 13-14 hours prior to treatment.

3. Dose preparation, administration and quantification:

Preparation: The formulation was prepared by the Sponsor and provided to the Test Facility ready for administration. [Aniline-UL-^{14}C]NNI-0001 was formulated as an SC 480 formulation and the SC formulation was diluted with water. The radiochemical purity was 99.6% with a specific activity of 3.96 MBq/mg.

Application: Animals were anesthetized with ketamine HCl. The dose formulation was continually stirred during treatment. Duoderm® patches were placed around the dosing sites, the dose formulations (100 µL) were applied with pipettes, and the doses were evenly

distributed with the pipette. The dosing site was covered with a 4-inch perforated aluminum pie pan and secured with Cover-Roll®, adhesive tape, and Co-Flex® bandages. Each animal was then secured upright in a primate chair. A pan (with a screen) was placed under each animal to capture excreta. Food was offered to the animals approximately 3.5-4 hours post-dose for 25-30 minutes, and then the animals were fasted again until after the 8 hour exposure period. Water was not withheld.

Quantification: The concentration of radioactivity in the dose formulation was determined in triplicate samples of the dosing solution taken pre-dose, mid-dose, and post-dose. Using these values as a measure of homogeneity, the coefficient of variation was 7.75%. Residual radioactivity in the dosing pipette tip was also determined. Actual dose (2.70 mg/cm^2) differed from nominal dose (4 mg/cm^2; Table 1). Although this seems a substantial inaccuracy, an estimation for dermal penetration was still achieved.

TABLE 1. Dosing[a]

Nominal dose level (µg/cm^2)	Specific activity (µCi/kg)	Actual dose (µg/cm^2)
4	1.984±0.466	2.698±0.173

a Data (mean of 5 males) were calculated by reviewers from values reported on page 31 of MRID 46817234. Nominal dose was reported on page 16.

4. **Skin wash (pre-sacrifice):** After the 8 hour exposure period, the animals were anesthetized with ketamine HCl and the protective dome, securing material, and the Duoderm® patch were collected. During the exposure period, the patch for one of the animals (No. 1003) moved down the animals back because it did not properly adhere. Although samples were collected from Animal No. 1003 and radioassayed, the results were excluded from calculation of group means. The surface of the test site (and surrounding skin for Animal No. 1003) was washed with a series of 20 soapy cotton swabs (aqueous 1% Ivory™ dishwashing liquid), 2 dry swabs, 2 isopropanol swabs, and 4 soapy swabs. Four additional isopropanol swabs were collected from the skin outside of the original dosing site for Animal No. 1003. Animals were then placed individually into metabolism cages, and allowed food twice daily and water *ad libitum*.

5. **Sample collection:** During the exposure period, excreta were collected in a pan at room temperature at 0-4 and 4-8 hours. The pan and screen were washed at each collection period using 50% aqueous ethanol, and wiped with gauze pads. After the exposure period, the protective dome, securing material, and the Duoderm® patch were collected, and the treatment site was washed as described above. The primate chairs were thoroughly wiped with gauze pads soaked with isopropanol. Urine and feces were collected at 8-12 h, 12-24 h, and 24 h intervals up to 120 h post-dose. At the end of each collection interval, cage debris was collected, and the cages were rinsed with 50% aqueous ethanol. After the final excreta collection at 120 hours, each cage was washed with 50% aqueous ethanol, and then wiped with gauze pads. The animals were not killed.

6. **Sample preparation and analysis:** When samples were not analyzed immediately, urine, feces, cage debris, and the various pan/chair/cage rinse samples were stored at -20±5°C; other samples were stored at room temperature. Details of sample preparation are provided in Table 2. Triplicate weighed aliquots of each sample were added to liquid scintillation fluid and assayed for radioactivity using liquid scintillation counting (LSC). Scintillation counter data were automatically corrected for counting efficiency using an external standardization technique and an instrument-stored quench curve generated from a series of sealed quench standards. Amounts of radioactivity in combusted samples were adjusted for efficiency of the combustions system, which was determined from combustion efficiency standards included with the experimental samples. Samples containing radioactivity (dpm) less than or equal to twice background for the system were considered to contain no radioactivity. Total amounts of radioactivity in samples were reported as a percentage of the total dose applied.

TABLE 2. Sample preparation details [a]

Sample type	Preparation method
Dosing pipette tip, Duoderm® patch, aluminum pie pan, dermal swabs, urine pan/screen wipe, and primate chair wash/wipe	Extracted with isopropanol.
Urine	Direct analysis
Feces	Homogenized in Milli-Q water and combusted.
Cage debris/rinse, cage wash, and urine pan/screen wash	Homogenized and combusted.
Cage wipe	Extracted in reagent-grade denatured ethanol.

a Information was obtained from pages 22-23 of MRID 46817234.

II. RESULTS

A. SIGNS AND SYMPTOMS OF TOXICITY: It was stated that redness was noted on Animal Nos. 1003 (neck) and 1004 (shoulder).

B. SUMMARY TABLES: Results are summarized in Table 3.

TABLE 3. Amount of [^{14}C]-NNI-0001 in each matrix [a]

Matrix analyzed	Residues in matrix (% of applied dose; n=4) at 120 h
Urine	0.02±0.02
Feces	0.00±0.00
Cage wash [b]	0.00±0.00
Duoderm® patch/securing material	4.68±3.70
Dome	2.94±3.77
First 4 soapy water swabs [c]	81.02±5.97
Remaining soapy water swabs	14.39±3.37
Dry swabs	0.15±0.08
Alcohol swabs	1.96±0.93
Recovery (sum of above)	105.14±2.28
Dermal absorption (based on urine, feces, and cage wash)	0.02±0.02

a Data were obtained from Tables 3-4 on pages 32-33 of MRID 46817234. Because the dermal patch on Animal No. 1003 did not properly adhere, sample results from this animal were excluded from calculation of group means; so, n=4 rather than n=5.

b Radioactivity in cage debris/rinse, urine pan wash/wipe (4 hours), chair/urine pan wash/wipe (8 hours), cage wash, and cage wipe were each measured to be 0.00±0.00% of applied dose.

c This value includes radioactivity found in the first 4 soapy water swabs, swab extracts, and the residual extracts.

C. TOTAL ABSORBED DOSE: Recovery of the applied dose (mass balance) was 105%. The majority of the dose was recovered in the skin swabs (98% of the applied dose). Dermal absorption (based on the sum of residues in urine, feces, and cage wash) was 0.02% of the applied dose. The absorbed dose was found exclusively in the urine during the first time interval (0-4 h).

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS= CONCLUSIONS: Minimal dermal absorption of the compound occurs, resulting in a low degree of systemic exposure. Complete excretion of the absorbed radioactivity occurred.

B. REVIEWER COMMENTS: Recovery of the applied dose (mass balance) was 105%. The majority of the dose was recovered in the skin swabs (98% of the applied dose). Dermal

absorption (based on the sum of residues in urine, feces, and cage wash) was only 0.02% of the applied dose. The absorbed dose was found exclusively in the urine during the first time interval (0-4 h).

This study is classified as **acceptable/non-guideline**.

DATA EVALUATION RECORD

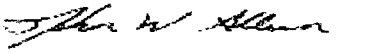
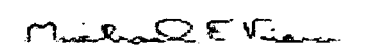
NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; *In Vitro* Metabolism in Rat, Mouse, Dog and Human Microsomes

Work Assignment No. 4-01-124 N; formerly 3-1-124 N (MRID 46817232)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 02/02/07
Secondary Reviewer: John W. Allran, M.S.	Signature:  Date: 02/02/07
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 02/02/07
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature:  Date: 02/02/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Mary K. Manibusan **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 7/1/09
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; *In Vitro* Metabolism in Rat, Mouse, Dog and Human Microsomes

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (RADIOCHEMICAL PURITY): NNI-0001 (>98% radiochemical purity)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Motoba, K. (2004) *In Vitro* Metabolism of NNI-0001. Toxicological & Pharmaceutical Research Center, Research Division, Nihon Nohyaku Co., Ltd., Osaka, Japan. Laboratory Study Report Number: LSRC-M04-184A, August 27, 2004. MRID 46817232. Unpublished

SPONSOR: Bayer CropScience LP, Toxicology, 17745 South Metcalf Avenue, Stilwell, KS

EXECUTIVE SUMMARY: As sex-related differences were previously observed in the *in vivo* metabolism of NNI-0001 in rats (MRIDs 46817229, 46817230, and 46817231), the current study was performed to exam the mechanism of sex-related differences and species specificity of the metabolism of NNI-0001 in mammals. In this non-guideline study (MRID 46817232), the *in vitro* metabolism of [phthalic ring-(U)-^{14}C]-NNI-0001 (Lot # CP-2761; radiochemical purity >98%) was examined using commercially available liver microsomes from rat, mouse, dog, and human, and recombinant microsomes expressing specific cytochrome P-450 isoforms from rat and human. The production of metabolites, especially NNI-0001-benzyl alcohol, was determined.

Microsomes from every species oxidized NNI-0001 to NNI-0001-benzyl alcohol. In all species except rat, microsomes from both sexes produced comparable amounts of NNI-0001-benzyl alcohol; microsomes from female rats did not produce NNI-0001-benzyl alcohol. β-NADPH was required for metabolism, indicating there was not abiotic degradation of the test compound in the test systems. Human microsomes also produced small but significant quantities of NNI-0001-hydroyxbenzoic acid. In the rat recombinant microsomes, only rat CYP3A2 slowly produced NNI-0001-benzyl alcohol metabolite in detectable quantities. In the recombinant human microsomes, only CYP3A4 produced NNI-0001-benzyl alcohol, as well as NNI-0001-hydroxybenzoic acid. In the rat liver microsomes, only anti-CYP2C11 appreciably inhibited production of NNI-0001-benzyl alcohol, while anti-CYP3A2 had no effect on production of

NNI-0001-benzyl alcohol. These results suggest that the metabolism of NNI-0001 in rats is mediated by a cytochrome P-450 that is recognized and partially inhibited by the anti-CYP2C11 antiserum used, but is a distinctly different P-450 isoform. In human liver microsomes, both anti-human CYP3A4 and anti-rat CYP3A2 inhibited production of NNI-0001-benzyl alcohol. However, it was stated that the anti-rat CYP3A2 serum is known to cross-react with and inhibit human CYP3A4, so this result is expected. Since CYP3A4 is known to be expressed in equivalent amounts in human males and females, NNI-0001 should be metabolized similarly in both sexes of humans.

In summary, the slow metabolism and excretion of the test compound observed in females in the *in vivo* rat metabolism study (MRIDs 46817229, 46817230, and 46817231) appears to be unique to rats out of the various species tested *in vitro*. The results suggest that sex-dependent differences in the metabolism and excretion of the test compound should not be observed in the other mammalian species examined, including humans.

This study is classified **acceptable/non-guideline**.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound:

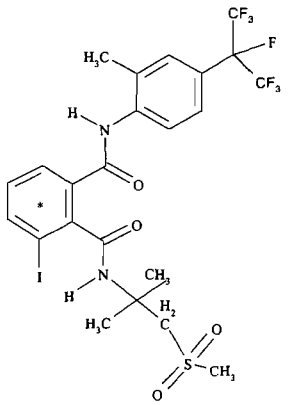
Radiolabeled test material 1: [Phthalic ring-(U)-^{14}C] NNI-0001

Radiochemical purity: >98% (TLC, HPLC)

Specific activity: 3.85 GBq/mmol

Lot number: CP-2761

Structure



* = position of ^{14}C

2. Solvent: The test compound was dissolved in acetonitrile.

3. Microsomes:

Species:	Rat (male and female)
Strain:	Fischer (F344/DuCrj)
Lot #:	HCN
Source:	Japan Charles River Co., Ltd. (Tokyo, Japan)
Species:	Dog (male and female)
Strain:	Beagle
Lot #:	108
Source:	Japan Charles River Co., Ltd. (Tokyo, Japan)
Species:	Mouse (male and female)
Strain:	CD-1
Lot #:	MIC255001 (male); MIC261001 (female)
Source:	Kacnet Co., Ltd. (Tokyo, Japan)
Species:	Human (male and female)
Strain:	Pooled from 10 donors/sex
Lot #:	HHM-0258 (male); HHM-0626 (female)
Source:	Kacnet Co., Ltd. (Tokyo, Japan)
Species:	Recombinant rat
Isoforms:	Control, CYP3A2, CYP2C11, CYP2C12, and CYP2C13
Source:	BD Gentest Co., Ltd. (Edison, NJ, USA)
Species:	Recombinant human
Isoforms:	Control, CYP1A2, CYP2C9*1, CYP2C19, CYP2D6*1, and CYP3A4
Source:	Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan)

B. STUDY DESIGN

1. **Purpose:** This study was performed to examine the mechanism of sex-related differences and species specificity of the metabolism of NNI-0001 in mammals. Because the toxicological studies concurrently submitted with this study used Fischer F344 rats, CD-1 mice, and beagle dogs as test animals, these specific strains were selected as the source of liver microsomes used in this study. As CYP2C11 and CYP2C12 are male- and female-specific rat isoforms, respectively, and CYP3A2 and CYP2C13 are male rat-predominant isoforms, recombinant microsomes expressing these genes were included in the experiments. In humans, CYP3A4, CYP2C9, CYP2D6, CYP1A2, and CYP2C19 are the major hepatic isoforms and were included as recombinant microsomes in the experiments.

2. **Preparation of test compound:** The radiochemical purity of the test compound was confirmed by TLC and HPLC to be >98% before and after experimental use. It was stated that the test compound was dissolved in acetonitrile such that the final concentration used in the incubation mixtures was <1.0% (v/v).

3. **Test systems:** *In vitro* metabolism of the test compound was examined by employing commercially available rat, mouse, dog, and human microsomes or recombinant microsomes expressing specific rat or human cytochrome P-450 isozymes. The recombinant rat microsomes were prepared from a baculovirus-infected insect cell line; the recombinant human microsomes were prepared from a human B-lymphoblast cell line. The metabolites produced were then identified for comparison to the results of the *in vivo* studies.

C. METHODS

1. ***In vitro* metabolism:** The incubation mixture consisted of either 0.2 nmole P-450 equivalents of liver microsomes, 50 pmole P-450 equivalents of the rat recombinant microsomes, or 37-290 pmole P-450 equivalents (in 1 mg total protein) of the human recombinant microsomes, combined with 0.2 nmole (0.77 kBq) of the test compound in 100 mM potassium phosphate buffer (pH 7.4) at a final volume of 0.9mL. The mixture was pre-incubated for 5 min at 37°C, after which the reaction was initiated by adding 0.1 mL of 10 mM β-nicotinamide adenine dinucleotide phosphate reduced (β-NADPH; final concentration 1 mM). The mixture was then incubated for 60 min and stopped by adding 1 mL of (1:1, v/v) acetone/methanol. Control incubations without liver microsomes and/or β-NADPH were performed concurrently.

2. **Inhibition of *in vitro* metabolism by antibodies:** Male rat liver microsomes were incubated with either goat anti-CYP2C11 serum, rabbit anti-CYP3A2 serum, or the corresponding control serum. Human liver microsomes were incubated with either rabbit anti-CYP3A2, control rabbit serum, monoclonal anti-CYP3A4, or control buffer. The incubations were carried out for 30 min at room temperature. The microsomes were then used in the experiments described above.

393

3. **Extraction of metabolites from the reaction mixtures:** After termination of the reactions, the mixtures were centrifuged. The resulting pellet was resuspended with 2 mL of acetone/methanol (1:1, v/v) and centrifuged again. The supernatants were combined and brought up to a final volume of 5 mL. Aliquots of both the supernatant and pellet (dissolved in methanol) were analyzed for radioactivity by liquid scintillation counting (LSC).

   Scintillation counting data were automatically converted to disintegrations per minute (dpm) with an instrument-installed external standard and quenching library. Prior to counting the first samples, ten blank vials were counted, and the mean of the obtained data was designated as background. The mean plus four standard deviations was defined as the detection minimum.

4. **Metabolite characterizations:** Samples were analyzed by one- or two-dimensional TLC followed by radioluminography, and also by HPLC. Reference standards of parent and four metabolites were also analyzed for metabolite identification. It was stated that all of the reference standards were separated from each other under the chromatography conditions used in these experiments.

5. **Statistics:** Statistical analyses were limited to calculations of mean. All calculations were performed with Microsoft Excel 98. HPLC data were processed with System Gold® software (version 8.10, Beckman Instrument Co., Ltd). Inhibition rates were calculated as follows:

   Inhibition rate (%) = $(P_c - P_i)/P_c \times 100$, where:

   P_i = production of metabolite in the presence of antiserum or antibody (%)
   P_c = production of metabolite in the presence of control serum or buffer (%)

II. RESULTS

A. *IN VITRO* METABOLISM BY LIVER MICROSOMES: Results of the *in vitro* liver microsome metabolism experiments are reported in Table 1. Microsomes from every species oxidized NNI-0001 to NNI-0001-benzyl alcohol. In all species except rat, microsomes from both sexes produced comparable amounts of NNI-0001-benzyl alcohol; microsomes from female rats did not produce NNI-0001-benzyl alcohol. β-NADPH was required for metabolism, indicating there was not abiotic degradation of the test compound in the test systems. Human microsomes also produced small but significant quantities of NNI-0001-hydroyxbenzoic acid (data not shown).

TABLE 1. *In vitro* metabolism of NNI-0001 by liver microsomes [a]

Source of microsomes			Production (% of total radioactivity)			
Species	Sex	NADPH [b]	NNI-0001 (parent)	NNI-0001-benzyl alcohol	Others	Unextractable
Rat	Male	+	71.24	26.07	2.60	0.09
		–	97.67	ND	2.27	0.07
	Female	+	97.42	ND	2.53	0.05
		–	97.75	ND	2.14	0.10
Mouse	Male	+	93.40	3.67	2.44	0.49
		–	96.03	ND	3.76	0.21
	Female	+	92.53	2.74	4.39	0.34
		–	95.81	ND	3.93	0.26
Dog	Male	+	92.67	3.53	3.69	0.11
		–	96.97	ND	2.95	0.08
	Female	+	91.97	3.85	4.07	0.10
		–	96.60	ND	3.33	0.07
Human	Male	+	86.54	8.88	4.36	0.22
		–	97.72	ND	2.13	0.15
	Female	+	78.04	12.17	9.12	0.66
		–	97.04	ND	2.80	0.16
Buffer control [c]	–	+	97.29	ND	2.71	–
		–	97.88	ND	2.13	–

a Data were obtained from Table 1 on page 33 of the study report.
b + and – represent the presence or absence of β-NADPH, respectively.
c Incubation without microsomes
ND Not detected

B. *IN VITRO* METABOLISM BY RECOMBINANT MICROSOMES: Results of the *in vitro* recombinant microsome metabolism experiments are reported in Table 2. In the rat recombinant microsomes, only rat CYP3A2 produced NNI-0001-benzyl alcohol metabolite in detectable quantities. It was stated that the rate of production was slow, with only 0.003 pmol NNI-0001-benzyl alcohol/min/pmol P-450 being produced. When testosterone was used as a substrate, the activity of this cytochrome P-450 was measured to be 33 pmol product/min/pmol P-450. In the recombinant human microsomes, only CYP3A4 produced NNI-0001-benzyl alcohol, as well as NNI-0001-hydroxybenzoic acid.

TABLE 2. *In vitro* metabolism of NNI-0001 by recombinant microsomes [a]

P-450 Isoform	Production (% of total radioactivity)			
	NNI-0001 (parent)	NNI-0001-benzyl alcohol	Others	Unextractable
Rat recombinant microsomes				
CYP3A2 (Male predominant)	90.52	4.75	4.51	0.22
CYP2C11 (Male specific)	98.59	ND	0.98	0.44
CYP2C12 (Female specific)	98.14	ND	1.68	0.18
CYP2C13 (Male specific)	97.82	ND	1.98	0.20
Control microsome	97.98	ND	1.81	0.22
Buffer control	96.72	ND	3.28	<0.01
Human recombinant microsomes				
CYP1A2	96.64	ND	1.32	4.04
CYP2C9	94.37	ND	1.62	4.01
CYP2C19	94.87	ND	1.77	3.36
CYP2D6	98.14	ND	1.53	0.33
CYP3A4	78.33	10.06	6.97	4.64
Control microsome	94.88	ND	1.37	3.75
Buffer control	97.86	ND	2.14	NA

a Data were obtained from Tables 3 and 5 on pages 34 and 36 of the study report.
ND Not detected

C. **INHIBITION OF MICROSOMAL ACTIVITY BY ANTIBODIES:** Results of the antiserum inhibition experiments are presented in Table 3. In the rat liver microsomes, only anti-CYP2C11 appreciably inhibited production of NNI-0001-benzyl alcohol (53.5% inhibition), while anti-CYP3A2 had no effect on the production of NNI-0001-benzyl alcohol. In human liver microsomes, both anti-human CYP3A4 (71.2-77.3% inhibition) and anti-rat CYP3A2 (81.0-87.1% inhibition) inhibited the production of NNI-0001-benzyl alcohol. However, it was stated that the anti-rat CYP3A2 serum is known to cross-react with and inhibit human CYP3A4, so this result is expected.

TABLE 3. Inhibition of microsomal cytochrome P-450 activity by antibodies [a]

Antiserum	Production (% of total radioactivity)				Inhibition (%)
	NNI-0001 (parent)	NNI-0001-benzyl alcohol	Others	Unextractable	
Male rat microsomes					
Control	72.35	24.84	2.23	0.58	–
Anti-CYP3A2	61.76	32.77	2.51	2.95	-4.5
Control rabbit serum	64.24	31.35	3.33	1.07	–
Anti-CYP2C11	80.38	14.12	1.94	3.55	53.5
Control goat serum	65.15	30.36	3.21	1.28	–
Male human microsomes					
Control	90.14	5.02	4.76	0.08	–
Anti-human CYP3A4	97.16	0.65	2.12	0.07	71.16
Control buffer	89.31	5.93	4.70	0.06	–
Anti-rat CYP3A2	98.53	0.39	1.04	0.04	87.11
Control rabbit serum	88.59	6.46	4.86	0.09	–
Female human microsomes					
Control	82.90	9.78	7.24	0.08	–
Anti-human CYP3A4	96.12	0.98	2.91	<0.01	77.31
Control buffer	86.16	7.95	5.86	0.02	–
Anti-rat CYP3A2	97.58	1.00	1.25	0.16	81.00
Control rabbit serum	87.27	8.16	4.56	0.01	–

a Data obtained from Tables 2 and 4 on pages 34 and 35 of the study report.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: The rat was the only species that showed sex-related differences in metabolism. Females of all species tested other than rat metabolized the test compound similar to males. The sex-related quantitative difference in metabolism is attributable to differences in hepatic microsomal P-450 isoforms. Further, the metabolism of the test compound in rat may be mediated by P-450 isoforms that are immunologically indistinguishable from CYP2C11. In humans, CYP3A4 is the major isoform responsible for hydroxylation of NNI-0001; therefore, no sex-dependent quantitative differences in metabolism are expected.

B. REVIEWER COMMENTS: Microsomes from every species oxidized NNI-0001 to NNI-0001-benzyl alcohol. In all species except rat, microsomes from both sexes produced comparable amounts of NNI-0001-benzyl alcohol; microsomes from female rats did not produce NNI-0001-benzyl alcohol. β-NADPH was required for metabolism, indicating there was not abiotic degradation of the test compound in the test systems. Human microsomes also produced small but significant quantities of NNI-0001-hydroyxbenzoic acid. In the rat recombinant microsomes, only rat CYP3A2 slowly produced NNI-0001-benzyl alcohol metabolite in detectable quantities. In the recombinant human microsomes, only CYP3A4 produced NNI-0001-benzyl alcohol, as well as NNI-0001-hydroxybenzoic acid. In the rat liver microsomes, only anti-CYP2C11 appreciably inhibited production of NNI-0001-benzyl alcohol, while anti-CYP3A2 had no effect on production of NNI-0001-benzyl alcohol. These results suggest that the metabolism of NNI-0001 in rats is mediated by a cytochrome P-450 that is recognized and partially inhibited by the anti-CYP2C11 antiserum used, but is a distinctly different P-450 isoform. In human liver microsomes, both anti-human CYP3A4

and anti-rat CYP3A2 inhibited production of NNI-0001-benzyl alcohol. However, it was stated that the anti-rat CYP3A2 serum is known to cross-react with and inhibit human CYP3A4, so this result is expected. As CYP3A4 is known to be expressed in equivalent amounts in human males and females, NNI-0001 should be metabolized similarly in both sexes of humans.

In summary, the slow metabolism and excretion of the test compound observed in females in the *in vivo* rat metabolism study (MRIDs 46817229, 46817230, and 46817231) appears to be unique to rats out of the various species tested *in vitro*. The results suggest that sex-dependent differences in the metabolism and excretion of the test compound should not be observed in the other mammalian species examined, including humans.

This study is classified **acceptable/non-guideline**.

C. **STUDY DEFICIENCIES:** No deficiencies were noted.

DATA EVALUATION RECORD

NNI-0001 (FLUBENDIAMIDE)

Study Type: Non-guideline; Toxicokinetic Study in Rats and Mice

Work Assignment No. 4-01-124 O; formerly 3-1-124 O (MRID 46817233)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 02/02/07
Secondary Reviewer: John W. Allran, M.S.	Signature: John W Allran Date: 02/02/07
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 02/02/07
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Steven Brecher Date: 02/02/07

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Mary K. Manibusan **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/9/09
Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/21/09

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Toxicokinetic Study in Rats and Mice.

PC CODE: 027602 **DP BARCODE:** D331553 (SB)
TXR#: 0054319

TEST MATERIAL (PURITY): NNI-0001 (96.7% a.i.)

SYNONYMS: Flubendiamide; N^2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

CITATION: Motoba, K. (2005) Toxicokinetics of NNI-0001: concentration in selected organs, tissues, and plasma following repetitive daily administration to rats and mice. Research Center, Nihon Nohyaku Co., Ltd., Osaka, Japan. Laboratory Study Report Number: LSRC-M05-248A, December 5, 2005. MRID 46817233. Unpublished

SPONSOR: Bayer CropScience LP, Toxicology, 17745 South Metcalf Avenue, Stilwell, KS

EXECUTIVE SUMMARY: In the definitive rat metabolism study (MRIDs 46817229, 46817230, and 46817231), sex-related differences were observed in the *in vivo* metabolism of NNI-0001. Parent and the metabolite NNI-0001-iodo phthalimide were the most prominent radioactive residues found in the fat of female rats given daily doses of NNI-0001 (MRID 46817230). In a concurrently reviewed *in vitro* metabolism study, liver microsomes from female rats showed almost no NNI-0001 metabolizing activity, while liver microsomes from male rats and male and female mice demonstrated potent NNI-0001 metaboizing activity. Therefore, the present study was performed to attempt to clarify the mechanism of sex-related differences and species specificity of the metabolism of NNI-0001 in mammals. In this non-guideline study (MRID 46817233), NNI-0001 (Lot # 1FH0019M; purity 96.7%) in corn oil was administered by daily oral gavage to groups of twelve Fischer (F344/DuCrj) rats/sex or twelve CD-1 (Crlj:CD1[ICR]) mice/sex at a dose of 200 mg/kg for up to 14 days. Groups of four animals were killed 24 h following the first, seventh, or 14th dose and necropsied. Samples of fat, liver, and plasma were taken and examined for the presence of parent and NNI-0001-iodo-phthalimide.

Greater concentrations of parent were found in liver and fat than in plasma in both sexes of both species. High concentrations of parent were found in the liver and fat of female rats (19.0-68.0 mg/L), compared to male rats (0.7-8.9 mg/L) and male and female mice (1.9-8.2 mg/L). In female rats, parent concentration in liver and fat increased from Dose 1 to 7, and then appeared to

405

plateau to Dose 14, while parent concentration appeared to remain relatively steady from Dose 1 on in male rats and male and female mice. Minor concentrations of parent were found in the plasma of female rats (0.9-1.4 mg/L), male rats (<0.1-0.1 mg/L), and male and female mice (0.1-0.2 mg/L). NNI-0001-iodo phthalimide was detected in small concentrations in the fat of male and female rats after Doses 7 and 14 (2.8-3.7 mg/L) and in the plasma of male mice after Dose 7 (0.2 mg/L). NNI-0001-iodo phthalimide was below the level of quantitation in all other samples.

In summary, female rats display greater concentrations of NNI-0001 in liver and fat following daily dosing than male rats; however, male and female mice displayed similar low concentrations of parent in liver and fat. The results of this study were similar to those observed in a concurrently submitted *in vitro* metabolism study (MRID 46817232), in which microsomes from male rats and male and female mice were capable of metabolizing the test compound, while microsomes from female rats were deficient.

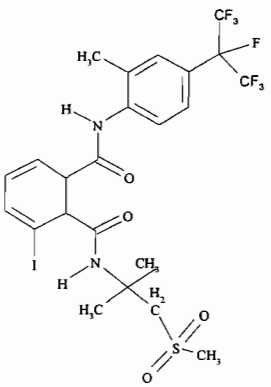
This study is classified **acceptable/non-guideline**.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: NNI-0001 (Flubendiamide)

Description	White crystals
Lot number:	1FH0019M
Purity:	96.7% a.i.
Compound Stability:	Stable in the vehicle for up to 14 days refrigerated
CAS of TGAI:	272451-65-7
Structure	

2. Vehicle: Corn oil

3. Test animals:

Species:	Rat
Strain:	Fischer (F344/DuCrj)
Age/weight at study initiation:	10 weeks; 215.1-222.5 g males, 144.3-151.3 g females
Species:	Mouse
Strain:	Crlj:CD1(ICR)
Age/weight at study initiation:	10 weeks; 37.3-42.3 g males, 28.3-31.8 g females
Source:	Japan Charles River Co., Ltd. (Tokyo, Japan)
Housing:	Suspended, stainless steel wire mesh cages, number/cage not provided
Diet:	Lab MR Stock® (Nihon Nousan Kogyo, Co., Ltd.), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	22±3EC
Humidity:	50±20%
Air changes:	Not provided
Photoperiod:	12 h light/12 h dark
Acclimation period:	At least seven days

4. Purpose: In the definitive rat metabolism study (MRIDs 46817229, 46817230, and 46817231), sex-related differences were observed in the *in vivo* metabolism of NNI-0001. Parent and the metabolite NNI-0001-iodo phthalimide were the most prominent radioactive residues found in the fat of female rats given daily doses of NNI-0001 (MRID 46817230). In a concurrently reviewed *in vitro* metabolism study, liver microsomes from female rats showed almost no NNI-0001 metabolizing activity, while liver microsomes from male rats and male and female mice demonstrated potent NNI-0001 metaboizing activity. Therefore,

the present study was performed to attempt to clarify the mechanism of sex-related differences and species specificity of the metabolism of NNI-0001 in mammals.

5. **Preparation of dosing solutions:** The dosing solution was prepared by suspending a precisely weighed aliquot of the test substance in corn oil. The final volume was adjusted to yield a nominal final concentration of 40 mg/mL. The dosing solution was stored in the dark below 10°C. The dose solutions were analyzed for concentration accuracy and stability by HPLC.

   The dosing solution was found to have concentrations of 49.7 mg/mL prior to dosing and 45.9 mg/mL post-dosing, and was considered to be stable in the vehicle for at least 15 days.

B. STUDY DESIGN AND METHODS

1. **Group arrangements:** Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. In the concurrently reviewed single and multiple dose ADME studies (MRIDs 46817229 and 46817230), rats were dosed at 2 and 200 mg/kg. Therefore, animals were dosed at 200 mg/kg (expected to be a toxic dose).

TABLE 1: Dose groups for NNI-0001 toxicokinetic studies [a]

Species	Nominal dose (mg/kg)	# animals/group	Time of sacrifice
Rat	200	4/sex	24 h after dose 1
			24 h after dose 7
			24 h after dose 14
Mouse			24 h after dose 1
			24 h after dose 7
			24 h after dose 14

a Data were obtained from page 20 of the study report.

2. **In-life observations:** Rats were weighed prior to study initiation and then daily (just prior to administration) until scheduled termination. The animals were observed daily for mortality, moribundity, and clinical signs of toxicity just after administration and at sample collection.

3. **Dosing and sample collection:** Animals were dosed daily by oral gavage with dose amounts based on individual body weights and a dosing volume of 5 mL/kg. Animals were not food-deprived prior to administration of the test compound. At the times designated in Table 1, the animals were killed (method not described) and necropsied. Samples of fat, liver, and plasma were collected for analysis. All samples were stored below 4°C until analyzed.

4. **Metabolite characterizations:** Plasma samples were extracted by shaking with an equal volume of acetonitrile, centrifuging, and removing the supernatant. Liver and fat samples were minced and homogenized with ten volumes of acetonitrile. These homogenates were centrifuged and diluted with distilled water as needed. Extracts were analyzed by HPLC.

Parent and NNI-0001-iodo-phthalimide standards were quantifiable down to 0.05 mg/L by HPLC. In samples fortified with standards, the recoveries of NNI-0001-iodo-phthalimide were limited to <1.0 mg/L in plasma. Overall, in any combination of matrices, target analyte, and fortification level, average recoveries were 90.6-113.2%.

5. **Statistics:** Statistical analyses were limited to calculations of mean, standard deviation, and relative standard deviation. All calculations were performed with Microsoft Excel 2002 (Japanese Version 10.2614.2625).

II. RESULTS

A. GENERAL OBSERVATIONS

1. **Rats:** One male rat in the group scheduled to receive seven doses and one male rat in the group scheduled to receive 14 doses were killed for humane reasons, both after the third dosing. These animals lost body weight, and were said to display poor general condition. At necropsy, dosing suspension was found in the trachea of both animals, suggesting either aspiration of dosing solution or gavage error. All female rats survived to scheduled termination.

2. **Mice:** One male mouse in the group scheduled to receive 14 doses was killed for humane reasons after the tenth dosing. This animal lost weight, and was said to have a poor prognosis from clinical signs (not detailed). At necropsy, this mouse was found to have a damaged esophagus, suggesting gavage error. All female mice survived to scheduled termination.

B. NNI-0001 AND NNI-0001-IODO-PHTHALIMIDE CONCENTRATIONS:

Concentration data for parent and NNI-0001-iodo phthalimide are presented in Table 2. Greater concentrations of parent were found in liver and fat than in plasma in both sexes of both species. High concentrations of parent were found in the liver and fat of female rats (19.0-68.0 mg/L), compared to male rats (0.7-8.9 mg/L) and male and female mice (1.9-8.2 mg/L). In female rats, parent concentration in liver and fat increased from Dose 1 to 7, and then appeared to plateau to Dose 14, while parent concentration appeared to remain relatively steady from Dose 1 on in male rats and male and female mice. Minor concentrations of parent were found in the plasma of female rats (0.9-1.4 mg/L), male rats (<0.1-0.1 mg/L), and male and female mice (0.1-0.2 mg/L). NNI-0001-iodo phthalimide was detected in small concentrations in the fat of male and female rats after Doses 7 and 14 (2.8-3.7 mg/L) and in the plasma of male mice after Dose 7 (0.2 mg/L). NNI-0001-iodo phthalimide was below the level of quantitation in all other samples.

TABLE 2. Mean (±SD) concentrations of NNI-0001 and NNI-0001-iodo-phthalimide in the plasma, liver, and fat of rats and mice following daily 200 mg/kg oral doses of NNI-0001 [a]

Species	Sex	Tissue	Dosing duration (days)	Concentration (mg/L)	
				NNI-0001	NNI-0001-iodo phthalimide
Rat	Male	Plasma	1 [b]	0.1±0.1	<0.1
			7 [b]	0.1±0.1	<0.1
			14 [b]	<0.1	<0.1
		Liver	1	1.8±1.1	<1.0
			7 [b]	1.3±1.3	<1.0
			14 [b]	0.7±0.6	<1.0
		Fat	1	7.9±2.9	<1.0
			7 [b]	8.9±3.2	2.9±0.5
			14 [b]	5.4±0.7	2.8±0.1
	Female	Plasma	1	0.9±0.3	<0.1
			7	1.2±0.1	<0.1
			14	1.4±0.1	<0.1
		Liver	1	19.0±8.5	<0.1
			7	26.7±3.6	<0.1
			14	27.4±5.4	<0.1
		Fat	1	46.5±28.1	<1.0
			7	68.0±8.6	2.9±1.0
			14	64.7±16.5	3.7±0.6
Mouse	Male	Plasma	1	0.2±0.1	<0.1
			7	0.1±<0.1	0.2±0.3
			14 [b]	0.2±<0.1	<0.1
		Liver	1	2.8±0.7	<1.0
			7	2.3±0.4	<1.0
			14 [b]	3.5±0.4	<1.0
		Fat	1	8.2±3.0	<1.0
			7	3.4±1.3	<1.0
			14 [b]	4.8±0.7	<1.0
	Female	Plasma	1	0.1±0.2	<0.1
			7	0.1±0.1	<0.1±0.1
			14	0.1±0.1	<0.1
		Liver	1	1.9±0.1	<1.0
			7	2.4±0.6	<1.0
			14	3.3±1.5	<1.0
		Fat	1	1.9±0.6	<1.0
			7	1.9±1.1	<1.0±0.4
			14	3.1±2.3	<1.0±0.4

a Data were obtained from the Summary Table on page 16 of the study report. n=4, except where indicated
b n=3

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS= CONCLUSIONS:** Levels of NNI-0001 were similar for male rats and mice, whereas the levels in female mice were much lower than those in female rats. Female rats showed higher NNI-0001 levels after a single oral administration in comparison with those for male rats. Furthermore, increased systemic levels of NNI-0001 were noted in females following repetitive administration; however, a plateau was achieved within 7 days. In both sexes of both animal species, higher concentrations of NNI-0001 were detected in the

liver and fat than those in plasma. The comparative kinetic profiles well represented those predicted from *in vitro* metabolism studies. In both sexes of both animal species, NNI-0001-iodo phthalimide was detected only in fat; however, the levels were quite low, even in female rats.

B. REVIEWER COMMENTS: Greater concentrations of parent were found in liver and fat than in plasma in both sexes of both species. High concentrations of parent were found in the liver and fat of female rats (19.0-68.0 mg/L), compared to male rats (0.7-8.9 mg/L) and male and female mice (1.9-8.2 mg/L). In female rats, parent concentration in liver and fat increased from Dose 1 to 7, and then appeared to plateau to Dose 14, while parent concentration appeared to remain relatively steady from Dose 1 on in male rats and male and female mice. Minor concentrations of parent were found in the plasma of female rats (0.9-1.4 mg/L), male rats (<0.1-0.1 mg/L), and male and female mice (0.1-0.2 mg/L). NNI-0001-iodo phthalimide was detected in small concentrations in the fat of male and female rats after Doses 7 and 14 (2.8-3.7 mg/L) and in the plasma of male mice after Dose 7 (0.2 mg/L). NNI-0001-iodo phthalimide was below the level of quantitation in all other samples.

In summary, female rats display greater concentrations of NNI-0001 in fat and liver following daily dosing than male rats; however, male and female mice displayed similar low concentrations of parent in tissues. The results of this study were similar to those observed in a concurrently submitted *in vitro* metabolism study (MRID 46817232), in which microsomes from male rats and male and female mice were capable of metabolizing the test compound, while microsomes from female rats were deficient.

C. STUDY DEFICIENCIES: No deficiencies were noted.

13544

R172115

Chemical Name: Flubendiamide

PC Code: 027602
HED File Code: 13000 Tox Reviews
Memo Date: 6/1/2009
File ID: 00000000
Accession #: 000-00-0130

HED Records Reference Center
7/7/2009