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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: September 25, 2023

SUBJECT: **1,2,4-Triazole (a common metabolite of conazole fungicides):** Review of Chronic Toxicity Study in Rats

PC Codes: 600074
Decision No.: 593459, 589417
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0058618
MRID Nos.: 52070901, 52070902

DP Barcodes: D468183, D466929
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: 288-88-0
40 CFR: N/A

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The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

I. CONCLUSIONS/DISCUSSION

As requested by the agency, the U.S. Triazole Task Force submitted a chronic study conducted with the metabolite 1-1,2,4-triazole (MRID 52070901), and an addendum to the same study (MRID 52070902). The HED evaluation of this study is in the attached Data Evaluation Record.

EPA Reviewer: Minerva Mercado-Feliciano PhD DABT **Signature:**
RAB4, Health Effects Division (7509P) **Date:** 08/11/2023
EPA Secondary Reviewer: Jeremy Leonard, PhD **Signature:**
RAB4, Health Effects Division (7509P) **Date:** 08/11/2023

Template version 09/11

TXR#: 0058618

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity, rat; feeding. OPPTS 870.4100a [§83-1a]; OECD 452.

PC CODE: 600074 **DP BARCODE:** D468183, D466929

TEST MATERIAL (PURITY): 1,2,4-Triazole (98.5-99.1% a.i.)

SYNONYMS: None provided in the study report

CITATION: Wahle, B.S. (2010) A Chronic Toxicity Testing Study in the Wistar Rat with 1,2,4-Triazole. Xenometrics, LLC; Stilwell, Kansas, USA. Laboratory report number 07-C72-MD, 3-Dec-2010. MRID 52070901. Unpublished

McCoole, M.D. and Peffer, R.C. (2022) Amendment No. 1: A Chronic Toxicity Testing Study in the Wistar Rat with 1,2,4-Triazole. Bayer CropScience LP, Chesterfield, Missouri, USA. Study ID 07-C72-MD. 16-Dec-2022. MRID 52070902. Unpublished

SPONSOR: Triazole Derivative Metabolite Group (TDMG). Sponsor's Agent: U.S. Triazole Task Force (USTTF).

SCIENTIFIC INTEGRITY: The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 52070901) 1,2,4-triazole (98.5-99.1% a.i., batch/lot #S4317788) was administered to 30 Wistar: Crl:WI(Han) rats/sex/dose in diet at dose levels of 0, 125, 375, 1000, or 2000 ppm (equivalent to 0, 7, 21, 58 or 113 mg/kg/day for males and 0, 8, 26, 71 or 136 mg/kg/day for females) for 52 weeks. In addition to the usual chronic toxicity evaluations using 20 animals/sex/dose (toxicology group), 10 animals/sex/dose were evaluated for neurotoxicity (neurotoxicology group; functional observation battery, motor

activity, *in situ* fixation and detailed neuropathology), with 10 animals/sex/dose from the toxicology group also evaluated for reproductive function (estrous cycle, ovarian follicle counts, sperm analysis).

There were no treatment-related effects on clinical signs, mortality, ocular findings, body weight, hematology, clinical chemistry, or urinalysis parameters. The only significant finding from the functional observation battery was a 17% increase in forelimb strength in high dose males at the 12-month evaluation, compared to controls. However, since the effect on forelimb strength was of a small magnitude and occurred in the absence of any other functional effects, it was considered incidental.

Sperm parameters were similar in treated and control males. As expected, over the last 3 weeks of this 1-year study many females were reaching or had already reached reproductive senescence, as shown by both vaginal cytology (no cycles or very long cycles: 20%, 30%, 40%, 10% and 30% in the control, 8, 26, 71 and 136 mg/kg/day groups, respectively), and by the incidence of females without recent ovulation (67% and 3% in the control and 136 mg/kg/day groups, respectively, had < 2 corpora lutea). Estrous cyclicity was not evaluated at earlier times. By evaluating the available data only, mean cycle length or mean number of cycles was similar in control and treated groups. However, the available data does not allow evaluation of females still cycling separately from females reaching or already in senescence. Combining these two different populations may be a confounder, and not an appropriate procedure to analyze this kind of data. However, even if the data is made available in the future, the low number of animals evaluated will mean that separating into two different population groups will lead to low statistical power (very low N) for each population.

Ovarian follicle counts were provided for individual animals in the control and high dose groups only. The mean number of preantral and antral follicles at the highest dose tested (HDT) was similar to the control. When all females are included (N = 9-10), the mean number of corpora lutea at the HDT was 2X that of the control; however, the increase was not statistically significant due to the high variability in both groups (CV = 90-142%). If the females not ovulating are excluded from the analysis (N = 3-7), there is no significant difference in mean number of corpora lutea between the two groups.

There were no significant dose-dependent effects in organ weights or gross pathology. In the chronic toxicology animals, there was a significant increased incidence of Purkinje cell loss in males (50% compared to 0% control) and females (70% compared to 0% control) at the HDT (lower doses not evaluated). In the neurotoxicity animals, a significant increased incidence of Purkinje cell loss at brain level 7 was detected in males (60% compared to 0% control) and females (70% compared to 0% control) at the HDT but not at lower doses (evaluated at the next 2 lower doses). All other histopathology findings occurred with similar incidence in control and treated groups or were otherwise considered to be incidental.

The LOAEL is 113 mg/kg/day, based on Purkinje cell loss in the brain of both sexes. The NOAEL is 71 mg/kg/day.

This chronic study in the rat is **acceptable (guideline)** and satisfies the guideline requirement for

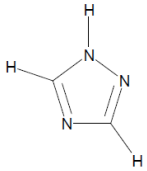
a chronic oral study [OPPTS 870.4100, OECD 452] in rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: 1,2,4-triazole

Description:	White scales (flakes)
Lot/batch #:	S4317788
Purity:	98.5-99.1% a.i.
Compound stability:	Stable at room temperature (~ 22 ºC)
CAS # for TGAI:	288-88-0
Structure:	

2. Vehicle: Rodent diet (see below); test article was dissolved in ethanol (Bayer lot numbers 16-06081, 16-10071, 16-02081, and 16-04081) before being added to rodent diet.

3. Test animals:

Species:		Rat
Strain:		Wistar: Crl:WI(Han)
Age/weight at study initiation:		Toxicology: ~ 8 weeks; 133-255 g males; 153-191 g females Neurotoxicology: ~9 weeks; 237-305 g males; 168-219 g females
Source:		Charles River Laboratories, Inc. (Raleigh, NC)
Housing:		Individually in suspended stainless steel wire-mesh cages containing sanitized cage board in the bedding tray; cage racks rotated monthly.
Diet:		Purina Mills Certified Rodent Diet 5002 in "meal" form; *ad libitum*
Water:		Tap water; *ad libitum*
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	11.52/hr
	Photoperiod:	12 hrs dark/ 12 hrs light (6 am to 6 pm)
Acclimation period:		Toxicology: 14 days (28 Jan to 11 Feb, 2008) Neurotoxicology: 22 days (28 Jan to 19 Feb, 2008)

B. STUDY DESIGN:

1. In life dates: Toxicology: Start: 11 February, 2008; End: 2 February, 2009.
Neurotoxicology: Start: 19 February, 2008; End: 16 February, 2009.

2. Animal assignment - Animals were assigned to the test groups noted in Table 1 using a weight stratification-based computer program.

Table 1. Study design

Test group	Concentration in diet (ppm)	Dose to animal (mg/kg/day)		Chronic Toxicology 52 weeks (1 yr)		Neurotoxicology 52 weeks (1 yr)	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	20	20	10	10
Low (LDT)	125	6.9 ± 2.0	8.3 ± 1.4	20	20	10	10
Mid 1 (MDT1)	375	21 ± 6	26 ± 4	20	20	10	10
Mid 2 (MDT2)	1000	58 ± 16	71 ± 10	20	20	10	10
High (HDT)	2000	113 ± 31	136 ± 22	20	20	10	10

LDT = lowest does tested; MDT1 = first mid dose tested; MDT2 = second mid dose tested; HDT = highest dose tested.
Data from page 29 of MRID 52070901.

3. **Dose selection rationale:** Dose levels for this study were selected on the results a of previous combined subchronic toxicity/neurotoxicity screening study in the Wistar Rat with 1,2,4-triazole (MRID 46467303). In that study, the investigator considered the following effects as treatment-related at 3000 ppm and higher doses (no-observed-adverse-effect level (NOAEL) of 500 ppm): decreases in body weight gain in both sexes; functional observation battery (FOB) findings (tremors and muscle fasciculation, as well as gait incoordination, decreased rearing, and/or ungroomed appearance) in both sexes; decreased motor activity on week 4 only in males; decreased absolute brain weight in both sexes; and neuropathology findings (cellular degeneration/ necrosis in the cerebellum, peripheral nerve fiver degeneration) in both sexes. It was anticipated that respective low and high dietary concentrations of 125 and 2000 ppm would constitute a NOAEL and a maximum tolerated dose (MTD), respectively, with a predictive goal of reaching a 10% decrement in body weight gain at the MTD over the course of this 1-year study. The intermediate dietary levels of 375 and 1000 ppm were to help confirm any dose response relationships that may have emerged.

4. **Diet preparation and analysis:** Dosed diet was prepared weekly by mixing appropriate amounts of test substance (dissolved in ethanol) with the diet indicated above. Adjustments were not made for test article purity. The control diet was prepared the same as the treated diet, excluding only the test chemical. Test diets were mixed at room temperature and stored in a freezer until use. A sample of each batch of feed was retained in the freezer.

   To confirm homogeneity of the test diet, samples were taken from the top, middle, and bottom of all prepared batches of test diet during the first week of the study and analyzed. A homogeneous distribution of the test substance in the feed was defined in terms of a percent relative standard deviation (%RSD; same as CV), derived from the 9 samples taken, which was < 10%. Stability of the test substance in the same rodent diet used in the current study was determined in a previous study (MRID 46616402). Stability in the feed was assessed following 1, 3, and 7 days of room temperature storage (~22 ºC) at 50 or 6000 ppm and 7, 14, and 28 (50 ppm) or 35 days (6000 ppm) of freezer storage (~ -23 ºC). The concentration of 1,2,4-triazole in the various test diets was analytically verified from samples collected within 1 week of each of the first 3 weeks of the study and at monthly intervals thereafter.

Results:
Homogeneity analysis: 91-105% of nominal; 2.6-7.0% RSD.
Stability analysis: 95-99% of nominal after 7 days at room temperature; 97.7-98.2% of nominal after ~30 days of freezer storage.
Concentration analysis: 98-99% of nominal.

5. **Statistics:** According to the study report: "Continuous data (i.e., body weight, food consumption, clinical chemistry, hematology, etc.) may have been evaluated for homogeneity of variance using Bartlett's test." Group means were analyzed by a one-way variance analysis (ANOVA). The test article-treated groups were compared to the control group using Dunnett's test. Organ weights were evaluated using an analysis of covariance (ANCOVA), with terminal body weight as the covariate. Frequency data (i.e., incidences) were evaluated using the Chi-Square and/or Fisher's Exact tests. For the Bartlett test, a probability (p) level ≤ 0.001 was considered significant. For all other statistical tests, differences with p values ≤ 0.05 were considered statistically significant.

   For the functional observational battery (FOB), continuous data was first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction between dose group and test week. For weeks on which there was a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different from the control group. Categorical data collected in the FOB were analyzed in a similar manner, using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

   Motor and locomotor activity (total session activity and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data was first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction with test occasion. For weeks on which there was a significant treatment effect, Dunnett's test was used to determine which, if any, groups were significantly different from the control group. Interval data was subjected to a two-way Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by a Repeated Measures ANOVA to determine on which weeks there was a significant treatment by interval interaction. For those weeks, the data for each interval was subjected to analysis using a one-way ANOVA to determine at which intervals there was a significant treatment effect. For those intervals, Dunnett's test was used to determine which groups, if any, were significantly different from the control group.

   Ovarian follicle counts were analyzed using a t-test (Corel Quattro Pro version 8.0.0.709, two samples assuming equal variance).

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected at least once daily (twice daily during

the normal work week and once daily on weekends and holidays) for signs of moribundity and mortality.

1b. Clinical examinations: Detailed physical examinations were conducted weekly and included evaluation of external surface areas (visual inspection and palpation for externally detectable masses), orifices, posture, general behavior, respiration, and excretory products.

1c. Functional observation battery (FOB): Animals were evaluated as described by Moser[1] during the week prior to initiation of exposure to 1,2,4-triazole and approximately 3, 6, 9, and 12 months of exposure. The technicians conducting the FOB were blind with respect to the animal's group assignment. Observations for all animals were routinely performed by the same observer throughout the study. Measurements (e.g., grip strength and foot splay) may have been performed by either the observer or a second person. Inter-observer reliability for this laboratory has been documented (Sheets, L.P., A Verification of Personnel Training to Perform a Functional Observational Battery with Rats, Bayer CropScience LP Report Number G200166, 2004). Studies have been conducted with acrylamide, carbaryl, and untreated rats to establish the sensitivity, reliability, and validity of the test procedures and to serve as a historical control (MRID 42770301). The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*		Reactivity*		Mobility
	Biting		Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*		Salivation*	X	Arousal/ general activity level*
	Tremors*		Piloerection*	X	Convulsions*
	Abnormal Movements*		Fur appearance		Tremors*
X	Palpebral closure*		Palpebral closure*		Abnormal movements*
	Faces consistency		Respiratory rate+	X	Urination / defecation*
X	Piloerection		Red/crusty deposits*		Grooming
X	Salivation		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Lacrimation		Eye prominence*		Gait score*
X	Vocalization	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Rearing	X	Ease of removal		Backing
X	Urination / defecation	X	Ease of handling		Time to first step
X	Gait abnormalities	**X**	**PHYSIOLOGICAL OBSERVATIONS**	X	Posture
X	Arousal			X	Palpebral closure
X	Bizarre / stereotypic behavior	X	Body weight*	X	Piloerection
X	Stains	X	Body temperature+	X	Salivation
X	Respiratory abnormalities	X	Pupil size	X	Lacrimation
X	**SENSORY OBSERVATIONS**	**X**	**NEUROMUSCULAR OBSERVATIONS**	X	Vocalization
X	Approach response+			X	Urination
X	Touch response+		Hindlimb extensor strength	X	Defecation
X	Startle response* (auditory)	X	Forelimb grip strength*	X	Stains
X	Pain response* (tail pinch)	X	Hindlimb grip strength*	X	Respiratory abnormalities

[1] V.C. Moser (1989) Screening Approaches to Neurotoxicity: A Functional Observational Battery. J. Am. Coll. Toxicol. 8 (1) 85-93.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Pupil response*	X	Landing foot splay*		
	Eyeblink response		Rotarod performance	X	OTHER OBSERVATIONS
	Forelimb extension				
	Hindlimb extension				
X	Air righting reflex+				
	Olfactory orientation				

* Required parameters; +Recommended parameters

1c. Motor and locomotor activity: Animals were tested individually for 60 minutes in a figure-eight maze with eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure activity; each time a beam is interrupted, an activity count is registered. Broad-spectrum background noise (74 + 2dB(A)) was provided throughout the test to minimize acoustical variations during testing. Uniformity of light intensity (100+70 Lux) over each of the mazes was verified daily. Studies with untreated animals and with rats treated with reference substances that increase and decrease motor activity established the sensitivity, reliability, and validity of these test procedures (MRID 42770301 and Sheets, L.P., "Motor Activity Assessment (Lab Room 304) - Historical Control and Method Validation Study Using Triadimefon and Chlorpromazine in Wistar Rats," Bayer CropScience LP Division Report Number 110506, 2002). Motor and locomotor activity were examined as activity for the 60-minute session and activity during each ten-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by counting only one interruption of a beam in a given location, with the next measurement recorded once the rat had relocated in the maze and disrupted another beam.

2. **Body weight:** Animals were weighed weekly for the first 13 weeks of the study, every 4 ± 1 weeks thereafter, and prior to necropsy.

3. **Food consumption and compound intake:** Food consumption for each animal was determined weekly for the first 13 weeks of the study and every 4 ± 1 weeks thereafter.

4. **Ophthalmoscopic examination:** Eyes were examined prior to randomization, and only those animals free of ocular abnormalities were placed on study. All surviving control and high-dose animals were examined just prior to study termination.

5. **Hematology and clinical chemistry:** Blood was collected from the first 10 rats/sex/dietary level at approximately 3, 6, and 12 months. All rats were fasted overnight (~12-16 hours) with water available prior to blood collection. Each rat was anesthetized with inhaled isoflurane prior to blood collection. 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 corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Heinz Bodies
X	(Activated Partial Thromboplastin time)	X	Erythrocyte morphology
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.

b. Clinical chemistry:

X	ELECTROLYTES	X	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
X	Lactic acid dehydrogenase (LDH)	X	Uric acid
X	Alanine amino-transferase (ALT/ SGPT)*		
X	Aspartate amino-transferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Sorbitol dehydrogenase*		
	Glutamate dehydrogenase		

* Recommended for chronic studies based on Guideline 870.4100.

6. Urinalysis: Urine was collected from the first 10 rats/sex/dietary level at approximately 3, 6, and 12 months. Animals were housed overnight in cages fitted with urine collection trays (non-fasted). 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)	X	Nitrate
X	Protein*	X	Urobilinogen

* Recommended for chronic studies based on Guideline 870.4100.

7. Reproductive parameters:

7a. Estrous cycle staging: Daily vaginal smears were conducted for the first available 10 females/dietary level, over a three-week period just prior to terminal sacrifice.

7b. Male reproductive function: At termination, sperm was collected from the left testis and epididymis of the first surviving 10 males/dietary level for enumeration of

homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. In addition, an evaluation of the morphology and motility was performed on sperm sampled from the distal portion (closest to the urethra) of the vas deferens. Sperm motility and counts were conducted using IVOS (Integrated Visual Operating Systems, 2005). Motility was examined from all control and treated groups (for any motility/movement and progressive forward motility). Morphology and counts were initially conducted on the control and highest dose group.

8. Sacrifice and pathology:

8a. Chronic effects: The first 20 surviving rats/sex/dose were euthanized via inhaled carbon dioxide and exsanguinated. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for possible histological examination. The (XX) organs, in addition, were weighed. Representative sections of target organs and gross lesions were processed and examined microscopically for all animals. Representative sections of other organs were processed and examined microscopically for control and high dose animals only (except the vagina, and the exorbital/lacrimal, clitoral, preputial, and Zymbal glands). Those tissues that showed compound-related effects were further investigated at lower doses to establish no-observed-effect levels if necessary.

X	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*
X	**RESPIRATORY**
X	Trachea*
X	Lung*++
X	Nose*
X	Pharynx*
X	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta, thoracic*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
X	Thymus
X	**UROGENITAL**
XX	Kidneys*+
X	Urinary bladder*
X	Testes*+ (only 1)
XX	Epididymides*+ (only 1)
XX	Prostate*
X	Seminal vesicle*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland*
X	Cervix
X	Clitoral gland
X	Preputial gland

X	NEUROLOGIC
XX	Brain (multiple sections)*+
X	Periph. nerve (sciatic)*
X	Spinal cord (3 levels)*
XX	Pituitary*
X	Eyes (retina, optic nerve)*
X	**GLANDULAR**
XX	Adrenal gland*+
X	Lacrimal gland
X	Parathyroids*
XX	Thyroids*
X	**OTHER**
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*
X	Tooth
X	Oral structure
X	Vagina
X	Harderian gland
X	Zymbal gland

* Required for chronic studies based on Guideline 870.4100.
+ Organ weight required in chronic studies.
++ Organ weight required if inhalation route.

8b. Ovarian follicle and corpora lutea counts: A quantitative evaluation of follicles and corpora lutea was conducted on the same females subjected to estrous cycle staging with the following exception. Animal MD0111 was used for cycle staging in place of MD0101

(did not have a vaginal opening). However, MD0101 was used for follicle and corpora lutea counts. Multiple sections (5) taken approximately 300 microns apart were evaluated for each ovary. Counts were made for combined primordial follicles plus preantral follicles, antral follicles, and corpus lutea.

8b. Neuropathology: Animals (the same 10/sex/dose level that received FOB/motor activity evaluations when possible) were deeply anesthetized (i.p. pentobarbital) and perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by universal fixative. Tissues were collected for possible histological examination and processed as indicated in the table below. Initially, tissues from control (0 ppm) and high dose (2,000 ppm) animals were evaluated microscopically. Tissues in which treatment-related neuropathology was detected were examined at lower doses, as necessary, to establish no-observed-effect levels.

Tissue	Section	Method[b]	Stains[c]
Brain (Levels 1-8) [a]	Coronal	Paraffin	H&E
Spinal Cord			
Cervical	Cross & Longitudinal	Paraffin	H&E
Thoracic	Cross & Longitudinal	Paraffin	H&E
Lumbar	Cross & Longitudinal	Paraffin	H&E
Cauda Equina	Longitudinal	Paraffin	H&E
Spinal Nerve Root Fiber & Ganglia			
Dorsal & Ventral, Cervical (bilateral)	Longitudinal	GMA	Modified Lee's
Dorsal & Ventral, Lumbar (bilateral)	Longitudinal	GMA	Modified Lee's
Gasserian Ganglion	Longitudinal	GMA	Modified Lee's
Eyes (bilateral)	Cross	Paraffin	H&E
Optic Nerves (bilateral)	Cross	Paraffin	H&E
Gastrocnemius Muscle	Cross	Paraffin	H&E
Peripheral Nerves			
Sciatic (bilateral)	Cross/Transverse & Longitudinal	GMA	Modified Lee's
Tibial (bilateral)	Cross/Transverse & Longitudinal	GMA	Modified Lee's
Sural (bilateral)	Cross/Transverse & Longitudinal	GMA	Modified Lee's

[a] Terminal body and fixed brain weights were determined and a brain/body weight ratio calculated.
[b] GMA - glycol methacrylate
[c] H&E - hematoxylin and eosin

Copied from page 27 of MRID 52070901.

II. RESULTS:

A. OBSERVATIONS:

1. Clinical signs and mortality: There were no treatment-related clinical signs. The incidence of mortality was similar in control and treated groups. A few animals were found dead or killed for humane reasons. In the control, female #0126 was found dead on day 346 after previously displaying hunched back, shaking of the head and shoulders, paleness, thin and rough coat, red discharge from the nose, and retinal degeneration in both eyes. At 125 ppm (7

mg/kg/day), male #1024 was found dead on study day 20 with no observed clinical signs other than a sore on its thorax-dorsal region. At 375 ppm (21/26 mg/kg/day), male #2004 showed hunched back, repetitive chewing, swollen limbs, limping, rough coat, and was killed on study day 239; male #2008 showed splayed hind limbs, hunched back, decreased activity, cold to touch and pale eyes, rough coat, red discharge from the nose, and was killed on study day 168; male #2023 showed red discharge from the right eye and a palpable mass, and was killed on study day 261; female #2103 showed hunched back, dehydration, rough coat, red staining around the mouth, and labored breathing and was found dead on study day 260; female #2110 was found dead on study day 355 without showing significant clinical signs. At 1000 ppm (58 mg/kg/day), male #3006 showed dragging of limbs and a sore on its tail, red discharge and staining of the nose and was killed on study day 259. At 2000 ppm (136 mg/kg/day), female #4103 was found dead on study day 81 without showing significant clinical signs; female #4120 showed red stained perianal area and was found dead on study day 224.

Table 2. Incidence of mortality [a]

ppm	0	125	375	1000	2000
Males – mg/kg/day	**0**	**6.9**	**21**	**58**	**113**
Toxicology	0/20	0/20	2/20	1/20	0/20
Neurotoxicology	0/10	1/10	1/10	0/10	0/10
Total	0/30	1/30	3/30	1/30	0/30
Females – mg/kg/day	**0**	**8.3**	**26**	**71**	**136**
Toxicology	0/20	0/20	2/20	0/20	2/20
Neurotoxicology	1/10	0/10	0/10	0/10	0/10
Total	1/30	0/30	2/30	0/30	2/30

a Data obtained from pages 46-55 in MRID 52070901.

2. **FOB**: Selected parameters that showed some statistical differences between control and treated groups are summarized in tables 3a, 3b and 4. Most statistically significant changes were not dose- or time-dependent, and therefore are not considered adverse. Forelimb strength in males at the 12-month evaluation was increased by 17% at the high dose compared to the concurrent control. However, because the change was only of small magnitude and occurred in the absence of any other functional effects, it was considered incidental.

Table 3a. Incidence of selected FOB observations for males [a]

Observation – Finding A | Finding B	Time	mg/kg/day				
		0	6.9	21	58	113
During handling						
Ease of removal – Minimal resistance | Minimal resistance w/ vocalization	Pre-treatment	6|4	7|3	4|6	2|8	3|7
	3 months	1|9	2|7	3|7	1|9	3|7
	6 months	0|10	2|7	3|7*	5|5*	2|8
	9 months	3|7	5|4	1|8	2|8	1|9
	12 months	4|6	6|3	3|6	5|5	4|6
Reaction to handling – Minimal resistance | Minimal resistance w/ vocalization	Pre-treatment	9|1	10|0	7|3	7|3	9|1
	3 months	7|3	8|1	7|3	5|5	8|2
	6 months	3|7	8|1*	7|3	8|2*	9|1*
	9 months	7|3	9|0*	7|2	8|2	6|4
	12 months	6|4	9|0*	8|1	6|4	7|3
Stains, lacrimal – None of any color | Red slight | Red moderate	Pre-treatment	10|0|0	10|0|0	10|0|0	10|0|0	10|0|0
	3 months	10|0|0	9|0|0	10|0|0	10|0|0	10|0|0
	6 months	10|0|0	9|0|0	9|0|1	10|0|0	9|0|1
	9 months	10|0|0	9|0|0	9|0|0	9|0|1	9|0|1
	12 months	10|0|0	8|1|0	9|0|0	9|0|1	9|0|1
Reflex/Physiological						
Approach response – No reaction | Slight reaction	Pre-treatment	0|10	0|10	0|10	0|10	0|10
	3 months	3|7	4|5	2|8	3|7	1|9
	6 months	5|5	5|4	2|8	2|8	3|7
	9 months	3|7	2|7	3|6	0|10*	2|8
	12 months	4|6	5|4	5|4	5|5	5|5

a Data obtained from pages 84-129 in MRID 52070901.
* Statistically different ($p < 0.05$) from the control.

Table 3b. Incidence of selected FOB observations for females [a]

Observation – Finding A | Finding B	Time	mg/kg/day				
		0	8.3	26	71	136
Home cage						
Posture – Standing normally | Rearing	Pre-treatment	9|1	7|3	7|3	10|0	9|1
	3 months	6|4	6|4	9|1	8|2	6|4
	6 months	8|2	9|1	7|3	10|0	8|2
	9 months	4|6	8|2	7|3	6|4	5|5
	12 months	9|0	9|1	6|4*	9|1	6|4*
During handling						
Ease of removal – Minimal resistance | Minimal resistance w/ vocalization	Pre-treatment	3|7	4|6	2|8	5|5	5|5
	3 months	3|7	0|10*	5|5	3|7	3|7
	6 months	3|7	2|8	6|4	5|5	3|7
	9 months	3|7	5|5	5|5	6|4	7|3
	12 months	2|7	2|8	2|8	5|5	8|2*
Reaction to handling – Minimal resistance | Minimal resistance w/ vocalization	Pre-treatment	10|0	7|3*	9|1	7|3*	8|2
	3 months	7|3	6|4	8|2	7|3	7|3
	6 months	7|3	8|2	9|1	9|1	7|3
	9 months	7|3	9|1	9|1	10|0	9|1
	12 months	6|3	7|3	7|3	10|0*	9|1
Reflex/Physiological						
Approach response – No reaction | Slight reaction	Pre-treatment	0|10	0|10	0|10	0|10	0|10
	3 months	0|10	0|10	0|10	0|10	0|10
	6 months	4|6	4|6	3|7	5|5	2|8
	9 months	2|8	0|10	3|7	2|8	1|9
	12 months	5|4	2|8	0|10*	1|9*	2|8

a Data obtained from pages 130-174 in MRID 52070901.
* Statistically different ($p < 0.05$) from the control.

Table 4. Forelimb grip strength (kg) [a]

ppm	0	125	375	1000	2000
Males – mg/kg/day	**0**	**6.9**	**21**	**58**	**113**
Pre-treatment	1.06 ± 0.13	1.15 ± 0.15	1.15 ± 0.14	1.14 ± 0.14	0.99 ± 0.13
3 months	1.55 ± 0.30	1.58 ± 0.20	1.46 ± 0.22	1.51 ± 0.15	1.50 ± 0.20
6 months	1.47 ± 0.13	1.55 ± 0.23	1.51 ± 0.19	1.53 ± 0.18	1.49 ± 0.20
9 months	1.57 ± 0.18	1.70 ± 0.13	1.59 ± 0.20	1.54 ± 0.16	1.61 ± 0.14
12 months	1.49 ± 0.16 CV = 11%	1.59 ± 0.15	1.52 ± 0.21	1.60 ± 0.16	1.74 ± 0.11* ↑17%
Females – mg/kg/day	**0**	**8.3**	**26**	**71**	**136**
Pre-treatment	0.88 ± 0.08	0.87 ± 0.10	0.90 ± 0.11	0.92 ± 0.09	0.91 ± 0.08
3 months	1.42 ± 0.15	1.40 ± 0.18	1.37 ± 0.19	1.37 ± 0.15	1.33 ± 0.13
6 months	1.33 ± 0.15	1.32 ± 0.13	1.37 ± 0.16	1.28 ± 0.19	1.36 ± 0.20
9 months	1.42 ± 0.17	1.34 ± 0.14	1.37 ± 0.19	1.33 ± 0.19	1.45 ± 0.19
12 months	1.30 ± 0.07	1.33 ± 0.16	1.32 ± 0.19	1.28 ± 0.13	1.43 ± 0.14

a Data obtained from pages 175-55 in MRID 52070901.

3. **Motor activity:** Motor activity data is summarized in Table 5a, and locomotor activity data is summarized in Table 5b. There was a tendency in males to have higher mean motor activity at the HDT (113 mg/kg/day) and higher mean locomotor activity at the HDT and at the next lower dose; however, the apparent differences from the control group were not statistically significant. Females had similar motor activity in control and treated groups. To explore the tendency for higher motor activity in males, mean interval data for males only is summarized in an attachment, and selected intervals are shown in Table 6 below. Mean motor and locomotor activity were similar in control and treated groups during intervals 1 and 2. Mean motor activity for intervals 3-6 was sporadically increased in a dose-dependent manner, but not in a time dependent manner. Mean locomotor activity at the last two intervals measured (5 and 6) was significantly increased at times for the top 2 doses (58 and 113 mg/kg/day) at the 9- and 12-month evaluations. Overall, the statistical changes in motor activity were sporadic and did not show a significant dose- or time-dependent pattern, therefore are considered incidental.

Table 5a. Mean total motor activity [a] and [Relevant historical controls] [b]

ppm	0	125	375	1000	2000
Males – mg/kg/day	**0**	**6.9**	**21**	**58**	**113**
Pre-treatment [585-688, 4 studies]	500 ± 120 CV = 24%	552 ± 156	465 ± 112	556 ± 152	511 ± 129
3 months	274 ± 86 CV = 31%	327 ± 80	342 ± 117	357 ± 102	362 ± 78 ↑32%
6 months	207 ± 69 CV = 33%	174 ± 75	185 ± 90	208 ± 83	247 ± 86
9 months	143 ± 30 CV = 21%	164 ± 56	182 ± 74	176 ± 56	202 ± 52 ↑41%
12 months	160 ± 56 CV = 35%	164 ± 72	183 ±120	213 ± 51	243 ± 89 ↑52%
Females – mg/kg/day	**0**	**8.3**	**26**	**71**	**136**
Pre-treatment [682-830, 4 studies]	604 ±156 CV = 26%	478 ±139	454 ± 70	454 ±121	469 ±132
3 months	419 ±155 CV = 37%	336 ± 90	352 ± 60	400 ±132	386 ± 88
6 months	314 ± 74 CV = 24%	230 ± 75 ↓27%	316 ± 63	325 ± 58	329 ± 86
9 months	269 ± 96 CV = 36%	237 ± 48	274 ± 80	269 ± 88	275 ± 70
12 months	265 ± 67 CV = 25%	230 ± 71	256 ± 56	255 ±100	263 ± 89

a Data obtained from pages 185-186 in MRID 52070901. Apparent change indicated only if larger than control CV.
b Historical control data from MRID 42770301. Only groups that are similar to the current study in strain, age and days in study (0, 3, 6, 9 or 12 months) are included, if available. **Note: all control studies measured motor activity for 90 min; the current study measured 60 min**.

Table 5b. Mean total locomotor activity [a]

ppm	0	125	375	1000	2000
Males – mg/kg/day	**0**	**6.9**	**21**	**58**	**113**
Pre-treatment	351 ± 99 CV = 28%	381 ±127	313 ± 82	370 ±124	340 ± 94
3 months	157 ± 45 CV = 29%	189 ± 64	201 ± 82	221 ± 81 ↑41%	211 ± 68 ↑34%
6 months	103 ± 34 CV = 33%	95 ± 56	101 ± 47	111 ± 45	131 ± 47
9 months	69 ± 21 CV = 30%	85 ± 42	93 ± 37 ↑35%	84 ± 34	97 ± 33 ↑41%
12 months	70 ± 28 CV = 40%	70 ± 37	80 ± 42	102 ± 41 ↑46%	117 ± 49 ↑67%
Females – mg/kg/day	**0**	**8.3**	**26**	**71**	**136**
Pre-treatment	381 ± 127 CV = 33%	308 ± 118	273 ± 70	288 ± 110	327 ± 121
3 months	240 ± 126 CV = 53%	193 ± 63	204 ± 36	242 ±103	258 ± 71
6 months	172 ± 44 CV = 26%	138 ± 51	183 ± 33	182 ± 41	216 ± 68
9 months	157 ± 38 CV = 24%	133 ± 41	171 ± 54	162 ± 75	168 ± 37
12 months	150 ± 31 CV = 21%	131 ± 72	154 ± 43	170 ± 63	149 ± 40

a Data obtained from pages 187-188 in MRID 52070901. Apparent change indicated only if larger than control CV.

Table 6. Mean motor activity for selected intervals – males [a]

mg/kg/day	0	6.9	21	58	113	0	6.9	21	58	113
Interval 3	**Motor**					**Locomotor**				
3 months	41 ± 17 CV = 41%	64 ± 24 ↑56%	63 ± 26 ↑54%	68 ± 31 ↑66%	52 ± 27	21 ± 12 CV = 57%	36 ± 15 ↑71%	36 ± 18 ↑71%	44 ± 21 ↑110%	32 ± 18
6 months	32 ± 15 CV = 47%	25 ± 18	24 ± 21	27 ± 14	33 ± 23	15 ± 7 CV = 47%	11 ± 11	11 ± 9	14 ± 7	17 ± 11
9 months	17 ± 10 CV = 59%	20 ± 15	30 ± 19	20 ± 13	29 ± 14	7 ± 6 CV = 86%	11 ± 10	13 ± 8	6 ± 6	15 ± 9 ↑114%
12 months	19 ± 17 CV = 89%	18 ± 13	20 ± 15	25 ± 15	40 ± 20* ↑111%	9 ± 9 CV = 100%	8 ± 6	8 ± 7	12 ± 12	19 ± 11 ↑111%
Interval 4	**Motor**					**Locomotor**				
3 months	26 ± 15 CV = 58%	38 ± 27	53 ± 29* ↑104%	52 ± 23 ↑100%	56 ± 21* ↑115%	14 ± 9 CV = 64%	21 ± 16	29 ± 23 ↑107%	30 ± 17 ↑114%	32 ± 13 ↑129%
6 months	17 ± 11 CV = 65%	27 ± 21	18 ± 17	20 ± 15	25 ± 16	7 ± 8 CV = 114%	12 ± 9	9 ± 9	10 ± 8	13 ± 9
9 months	13 ± 6 CV = 46%	20 ± 10 ↑54%	16 ± 15	21 ± 11 ↑62%	19 ± 10	5 ± 3 CV = 60%	8 ± 5	8 ± 9	10 ± 7 ↑100%	8 ± 5
12 months	17 ± 14 CV = 82%	17 ± 19	26 ± 26	21 ± 17	28 ± 18	5 ± 5 CV = 100%	8 ± 13	10 ± 9	12 ± 10 ↑140%	13 ± 8 ↑160%
Interval 5	**Motor**					**Locomotor**				
3 months	19 ± 18 CV = 95%	26 ± 23	33 ± 21	31 ± 25	39 ± 22 ↑105%	9 ± 10 CV = 111%	14 ± 14	17 ± 12	18 ± 15	23 ± 15 ↑156%
6 months	14 ± 16 CV = 114%	13 ± 9	10 ± 14	16 ± 14	24 ± 20	6 ± 8 CV = 133%	6 ± 5	5 ± 7	5 ± 6	11 ± 10
9 months	3 ± 3 CV = 100%	10 ± 11 ↑233%	17 ± 16 ↑467%	20 ± 16* ↑567%	19 ± 16* ↑533%	2 ± 3 CV = 150%	5 ± 6	8 ± 6 ↑300%	11 ± 9 ↑450%	10 ± 9 ↑400%

mg/kg/day	0	6.9	21	58	113	0	6.9	21	58	113
12 months	11 ± 13 CV = 118%	11 ± 15	11 ± 10	22 ± 12	24 ± 15	3 ± 6 CV = 200%	5 ± 7	4 ± 4	9 ± 6	11 ± 8* ↑267%
Interval 6	**Motor**					**Locomotor**				
3 months	18 ± 17 CV = 94%	24 ± 23	32 ± 26	18 ± 10	41 ± 31 ↑128%	9 ± 8 CV = 89%	12 ± 11	18 ± 16 ↑100%	11 ± 7	20 ± 14 ↑122%
6 months	11 ± 16 CV = 145%	6 ± 7	9 ± 9	16 ± 16	22 ± 13	3 ± 4 CV = 133%	3 ± 4	5 ± 5	7 ± 7	10 ± 8 ↑233%
9 months	7 ± 11 CV = 157%	9 ± 7	15 ± 15	19 ± 12 ↑171%	20 ± 19 ↑186%	3 ± 5 CV = 167%	4 ± 5	6 ± 7	10 ± 5* ↑233%	10 ± 8 ↑233%
12 months	8 ± 10 CV = 125%	12 ± 12	17 ± 22	25 ± 19 ↑213%	24 ± 20 ↑200%	3 ± 3 CV = 100%	5 ± 7	5 ± 7	10 ± 7 ↑233%	12 ± 10* ↑300%

a Data obtained from pages 189-193 199-203 and in MRID 52070901. Apparent change indicated only if larger than control CV.

B. <u>BODY WEIGHT AND FOOD CONSUMPTION</u>: Table 7 summarizes mean body weight data. Mean food consumption data is available in study report pages 246-253. There were no significant differences in body weight or food consumption between control and treated groups.

Table 7. Mean body weights (grams) [a]

ppm	0	125	375	1000	2000
CHRONIC TOXICOLOGY (N = 20)					
Males – mg/kg/day	**0**	**7**	**21**	**58**	**113**
Day 0	225 ± 13	226 ± 13	226 ± 14	221 ± 13	218 ± 23
Day 28	330 ± 26	344 ± 25	338 ± 19	326 ± 24	330 ± 20
Day 91	422 ± 34	448 ± 36*	432 ± 31	411 ± 31	408 ± 31
Day 175	480 ± 46	494 ± 45	479 ± 47	463 ± 35	459 ± 36
Day 343	543 ± 57	558 ± 57	545 ± 62	514 ± 43	512 ± 44
Females – mg/kg/day	**0**	**8**	**26**	**71**	**136**
Day 0	176 ± 10	175 ± 9	174 ± 8	176 ± 8	176 ± 8
Day 28	220 ± 15	214 ± 15	209 ± 15	211 ± 14	212 ± 13
Day 91	258 ± 22	251 ± 22	245 ± 21	245 ± 19	244 ± 17
Day 175	279 ± 25	277 ± 28	269 ± 22	268 ± 22	266 ± 19
Day 343	320 ± 45	314 ± 64	297 ± 37	292 ± 34	291 ± 29
NEUROTOXICOLOGY (N = 10)					
Males – mg/kg/day	**0**	**7**	**21**	**58**	**113**
Day 0	269 ± 20	270 ± 19	273 ± 18	267 ± 16	271 ± 19
Day 28	359 ± 31	376 ± 34	365 ± 31	361 ± 25	361 ± 25
Day 84	438 ± 44	451 ± 40	445 ± 43	439 ± 39	429 ± 36
Day 168	497 ± 56	519 ± 48	509 ± 55	495 ± 41	481 ± 48
Day 350	578 ± 68	596 ± 53	595 ± 65	568 ± 46	532 ± 56
Females – mg/kg/day	**0**	**8**	**26**	**71**	**136**
Day 0	194 ± 16	192 ± 12	189 ± 11	192 ± 11	195 ± 10
Day 28	229 ± 20	221 ± 15	216 ± 15	219 ± 13	222 ± 15
Day 84	255 ± 22	246 ± 16	242 ± 18	240 ± 13	241 ± 17
Day 168	282 ± 22	273 ± 17	271 ± 21	266 ± 17	265 ± 18
Day 350	314 ± 43	304 ± 21	318 ± 53	298 ± 36	299 ± 37

a Data obtained from pages 210-213 in MRID 52070901.
* Statistically different ($p < 0.05$) from the control.

C. OPHTHALMOSCOPIC EXAMINATION: Evaluation of the control and high dose groups right before termination did not find treatment-related ocular lesions.

D. BLOOD ANALYSES:

1. **Hematology**: There were no significant changes in leukocyte or platelet parameters at any time point tested, and no significant changes in clotting parameters at 3 or 6 months.

   Sporadic decreases in hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) (4-9% compared to control; $p < 0.05$) were seen at the top 2 doses; however, the changes were not always dose-dependent, and at most time points tested there was no significant change in related erythrocyte parameters (erythrocyte counts, hemoglobin concentration, and mean corpuscular hemoglobin concentration). Overall, the changes in hemoglobin and related parameters in either sex were not considered treatment-related because of their small magnitude, not always following a dose-dependent trend, and lack of correlation with other erythrocyte parameters.

   In males and females at termination, activated partial thromboplastin time (APTT) was statistically increased 12-13% at the HDT (see Table 8). In females only, prothrombin time was statistically decreased 5% compared to controls, also at the HDT. Overall, the changes in clotting parameters in either sex were not considered treatment-related because of their small magnitude and overall variability.

Table 8. Clotting hematology findings in both sexes after 12 months of treatment (N = 9-10) [a]

Males – mg/kg/day	**0**	**7**	**21**	**58**	**113**
APTT, s	16.1 ± 1.6 CV = 10%	14.6 ± 1.9	15.9 ± 1.4	16.6 ± 1.0	14.2 ± 2.0* ↓12%
Prothrombin Time, s	15.1 ± 0.5 CV = 3%	15.2 ± 0.3	14.7 ± 0.8	15.2 ± 0.7	15.1 ± 0.7
Females – mg/kg/day	**0**	**8**	**26**	**71**	**136**
APTT, s	16.5 ± 2.0 CV = 2%	14.0 ± 1.3* ↓15%	15.2 ± 1.0	14.7 ± 1.3	14.4 ± 1.6* ↓13%
Prothrombin Time, s	15.3 ± 0.4 CV = 3%	15.3 ± 0.4	15.3 ± 0.2	15.2 ± 0.6	14.6 ± 0.5* ↓5%

a Data obtained from pages 1057-1068 in MRID 52070901.
* Statistically different ($p < 0.05$) from the control.

2. **Clinical chemistry:** Clinical chemistry findings are summarized in pages 1039-1056 of the study report. A few parameters showed sporadic statically significant differences from control that were not dose- or time-dependent. The only exception was a 36% decrease in triglycerides (110 ± 38 vs. control of 172 ± 53 mg/dL) detected in males dosed with the HDT (136 mg/kg/day) at termination. This finding is considered incidental in the absence of other clinical chemistry changes.

E. URINALYSIS: Urinalysis findings are summarized in pages 1087-1098 of the study report. There were no significant findings in females. At the top two doses males showed decreased mean protein (61-69% compared to control) and specific gravity (2-3%) and increased mean urine volume (125-202%), but only at the 6-month evaluation. Because these effects were not detected at the 3- or 12-month evaluations, and there were no other corroborating findings (kidney histopathology and body weights), the urinalysis findings in males were considered incidental.

F. REPRODUCTIVE FUNCTION:

a. Vaginal cytology and ovarian follicle cell counts: Results from the evaluation of vaginal smears and ovarian follicle counts are summarized in Table 9 and attachment tables A-2, A-3 and A-4. As expected, over the last 3 weeks of this 1-year study many females were reaching or had already reached reproductive senescence. This was indicated by the incidence of females with either no cycles or very long cycles detected by vaginal cytology (20%, 30%, 40%, 10%, and 30% in the control, 8, 26, 71, and 136 mg/kg/day groups, respectively), and by the incidence of females with < 2 corpora lutea (i.e. no recent ovulation) detected by ovarian histology (67% and 3% in the control and 136 mg/kg/day groups, respectively). These findings indicating senescence were not dose-dependent, and therefore were not related to treatment.

The study report reported summarized vaginal cytology data but did not provide individual animal data regarding estrous stages (i.e., if the animal was in diestrus, proestrus, estrus, or metestrus at a given time). By evaluating the available data only, mean cycle length or mean number of cycles was similar in control and treated groups. However, the available data does not allow to evaluate females still cycling separately from females reaching or already in senescence. Combining these two different populations may be a confounder, and not an appropriate procedure to analyze this kind of data. However, even if the data is made available in the future, the low number of animals evaluated will mean that separating into the two different population groups will lead to low statistical power (very low N) for each population.

Ovarian follicle counts were provided for individual animals in the control and high dose groups only. The mean number of preantral and antral follicles at the HDT was similar to the control. When all females are included (N = 9-10) The mean number of corpora lutea at the HDT was 2X that of the control; however, the increase was not statistically significant due to the high variability in both groups (CV = 90-142%). If the females not ovulating are excluded from the analysis (N = 3-7), there is no significant difference in mean number of corpora lutea between the two groups.

Table 9. Estrous evaluation (3 weeks before termination) and ovarian follicle counts [a]

mg/kg/day	0	8	26	71	136
Vaginal Cytology					
Number evaluated	*10*	*10*	*10*	*10*	*10*
Num. with pre-senescence cycle length [b]	5	4	5	7	5
Num. with very long cycle [c]	2	0	0	1	0
Num. not cycling (no cycles detected)	0	3	4	1	3
Num. with short cycle [d]	0	0	0	0	0
Mean cycle length over 3-week period	6.6 ± 0.9	5.1 ± 0.4	4.9 ± 0.5*	5.7 ± 0.9	5.2 ± 0.6
Mean number of cycles over 3-week period	2.1 ± 0.3	1.4 ± 0.4	1.6 ± 0.5	2.3 ± 0.4	1.6 ± 0.5
Ovarian Follicle Counts – All Females					
Number evaluated	*9* [e]	*0*	*0*	*0*	*10*
Num. not ovulating (0-1 corpora lutea)	6	--	--	--	3
Mean number of pre-antral follicles [f]	31.3 ± 10.5	--	--	--	34.9 ± 10.9
Mean number of antral follicles	31.9 ± 4.6	--	--	--	35.1 ± 10.7
Mean number of corpora lutea	10.7 ± 15.2	--	--	--	22.2 ± 19.9
Ovarian Follicle Counts – Excluding Females Not Ovulating					
Number evaluated	*3* [e]	*0*	*0*	*0*	*7*
Mean number of pre-antral follicles [f]	30.0 ± 3.3	--	--	--	39.1 ± 7.7
Mean number of antral follicles	34.7 ± 1.7	--	--	--	39.1 ± 10.0
Mean number of corpora lutea	31.3 ± 7.3	--	--	--	31.7 ± 16.2

a Data obtained from pages 280-281 and 1193-1194 in MRID 52070902.
b Before reproductive senescence, the rat estrous cycle usually lasts about 4 days. Here we consider the range 3.5-5.5 days as pre-senescence.
c > 9 days, usually indicates the rat is approaching reproductive senescence,
d < 3.5 days.
e An additional animal was evaluated (#0101) however it was excluded from all calculations by the EPA reviewers because it had a reproductive tract malformation (no vaginal opening) and it was a statistical outlier per Grubbs tests ($p < 0.05$) for the number of antral follicles (11 vs. group range of 25-37).
f Includes primordial and other stages of pre-antral follicles.
* Statistically different ($p < 0.05$) from the control.

b. <u>Sperm measures</u>: Results from the evaluation of sperm are summarized in Table 10. All parameters were similar in treated and control groups. Apparent increases in number of sperm in the epididymides and with detached head are due to high variability in both the control and HDT.

Table 10. Sperm analysis [a]

mg/kg/day	0	7	21	58	113
Sperm Motility					
Number evaluated	*10*	*10*	*10*	*10*	*10*
Mean sperm motility (%)	84.5 ± 5.3	83.5 ± 3.2	85.4 ± 2.6	86.1 ± 2.8	82.3 ± 6.5
Mean progressive sperm (%)	58.5 ± 7.2	59.1 ± 8.4	62.1 ± 4.4	62.9 ± 5.1	57.4 ± 8.5
Sperm Counts					
Number evaluated	*10*	*0*	*0*	*0*	*10*
Mean sperm/gram, testis	34.7 ± 9.2	--	--	--	37.2 ± 7.3
Mean sperm/gram, epididymides	79.7 ± 22.6	--	--	--	103.7 ± 57.5
Sperm Morphology					
Number evaluated	*10*	*0*	*0*	*0*	*10*
Mean number normal	197.9 ± 1.6	--	--	--	196.3 ± 1.5
Mean number abnormal	2.0 ± 1.5	--	--	--	3.0 ± 1.3
Mean number w/ detached head	0.1 ± 0.3	--	--	--	0.7 ± 0.6

a Data obtained from page 283 in MRID 52070901.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight**: Selected findings are summarized in Table 11. There were no significant dose-dependent effects in the chronic toxicology group or the neurotoxicology group.

Table 11. Mean absolute (grams or mg) and relative (%body) weights of selected organs [a]

ppm		0	125	375	1000	2000
CHRONIC TOXICOLOGY (N = 20)						
Males – mg/kg/day		**0**	**7**	**21**	**58**	**113**
Terminal body, g		546 ± 55	562 ± 61	552 ± 61	517 ± 39	516 ± 42
Brain	g	2.16 ± 0.08	2.16 ± 0.07	2.15 ± 0.11	2.10 ± 0.11	2.11 ± 0.07
	%	0.40 ± 0.05	0.39 ± 0.04	0.39 ± 0.05	0.41 ± 0.03	0.41 ± 0.04
Kidney	g	3.02 ± 0.33	3.11 ± 0.29	3.19 ± 0.35	2.93 ± 0.20	3.11 ± 0.33
	%	0.56 ± 0.05	0.56 ± 0.05	0.58 ± 0.06	0.57 ± 0.04	0.61 ± 0.06
Liver	g	16.0 ± 2.3	16.7 ± 2.5	16.3 ± 1.9	15.5 ± 1.2	16.1 ± 1.8
	%	2.93 ± 0.33	2.98 ± 0.35	2.96 ± 0.21	3.02 ± 0.36	3.12 ± 0.34
Testis	g	4.09 ± 0.37	4.10 ± 0.36	4.00 ± 0.37	3.78 ± 0.67	4.03 ± 1.29
	%	0.75 ± 0.07	0.74 ± 0.10	0.73 ± 0.10	0.73 ± 0.12	0.78 ± 0.24
Epididymides	g	1.66 ± 0.25	1.54 ± 0.14	1.57 ± 0.21	1.57 ± 0.26	1.62 ± 0.18
	%	0.31 ± 0.05	0.28 ± 0.03	0.29 ± 0.05	0.30 ± 0.05	0.32 ± 0.04
Prostate	g	1.02 ± 0.21	1.03 ± 0.23	1.04 ± 0.15	1.02 ± 0.20	1.07 ± 0.20
	%	0.19 ± 0.04	0.19 ± 0.04	0.19 ± 0.03	0.20 ± 0.05	0.21 ± 0.04
Females – mg/kg/day		**0**	**8**	**26**	**71**	**136**
Terminal body		324 ± 51	326 ± 70	306 ± 41	303 ± 35	300 ± 31
Brain	g	2.00 ± 0.09	2.00 ± 0.09	2.01 ± 0.11	2.00 ± 0.09	1.97 ± 0.08
	%	0.63 ± 0.09	0.63 ± 0.09	0.67 ± 0.08	0.66 ± 0.06	0.66 ± 0.07
Kidney	g	2.11 ± 0.19	2.16 ± 0.30	2.11 ± 0.19	2.12 ± 0.23	2.17 ± 0.22
	%	0.66 ± 0.07	0.67 ± 0.09	0.70 ± 0.06	0.70 ± 0.07	0.73 ± 0.08

ppm		0	125	375	1000	2000
CHRONIC TOXICOLOGY (N = 20)						
Liver	g	10.0 ± 1.6	10.1 ± 1.7	9.4 ± 1.5	9.5 ± 1.0	9.7 ± 0.8
	%	3.10 ± 0.21	3.12 ± 0.26	3.07 ± 0.23	3.15 ± 0.24	3.24 ± 0.20
Ovary	mg	168 ± 86	131 ± 25	177 ± 154	141 ± 38	132 ± 43
	%	0.06 ± 0.04	0.04 ± 0.01	0.06 ± 0.06	0.05 ± 0.01	0.05 ± 0.02
Uterus	mg	687 ± 162	711 ± 182	733 ± 227	680 ± 201	752 ± 333
	%	0.22 ± 0.06	0.23 ± 0.07	0.24 ± 0.08	0.23 ± 0.08	0.25 ± 0.11
NEUROTOXICOLOGY (N = 10)						
Males – mg/kg/day		**0**	**7**	**21**	**58**	**113**
Terminal body		581 ± 67	601 ± 51	602 ± 67	576 ± 48	540 ± 58
Brain	g	1.97 ± 0.10	2.02 ± 0.10	2.06 ± 0.09	2.00 ± 0.09	1.98 ± 0.09
	%	0.34 ± 0.04	0.34 ± 0.03	0.35 ± 0.04	0.35 ± 0.03	0.37 ± 0.05
Females – mg/kg/day		**0**	**8**	**26**	**71**	**136**
Terminal body		326 ± 42	311 ± 28	327 ± 56	300 ± 36	307 ± 37
Brain	g	1.85 ± 0.09	1.90 ± 0.10	1.85 ± 0.08	1.88 ± 0.08	1.86 ± 0.04
	%	0.58 ± 0.07	0.62 ± 0.07	0.58 ± 0.11	0.63 ± 0.07	0.61 ± 0.08

a Data obtained from pages 1160-1169 in MRID 52070901.

2. **Gross pathology:** Selected findings are summarized in Table 12. There were no significant dose-dependent effects in the chronic toxicology group or the neurotoxicology group.

Table 12. Gross pathology [a]

	Males					Females				
mg/kg/day	**0**	**7**	**21**	**58**	**113**	**0**	**8**	**26**	**71**	**136**
CHRONIC TOXICOLOGY										
Number evaluated	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*20*
Epididymis, reduced size	0	0	0	1	0	--	--	--	--	--
Prostate, reduced size	0	0	0	0	1	--	--	--	--	--
Testis, abnormal consistency	0	0	1	0	2	--	--	--	--	--
Testis, enlarged	0	0	0	0	1	--	--	--	--	--
Testis, reduced size	0	0	0	1	1	--	--	--	--	--
Uterus, mass	--	--	--	--	--	0	0	0	0	1
Kidney, calculus	0	0	0	0	0	0	0	1	0	1
Urinary bladder, discoloration	0	0	0	0	0	0	0	0	0	1
NEUROTOXICOLOGY										
Number evaluated	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Testis, abnormal consistency	0	0	1	0	0	--	--	--	--	--

a Data obtained from page 1099- in MRID 52070901.

3. **Microscopic pathology:** Selected findings are summarized in Table 13. Dose-dependent findings were confined to the nervous system, eyes, cecum, and male reproductive system.

Nervous system: In the chronic toxicology animals, there was a significant increased incidence of Purkinje cell loss in males (50% compared to 0% control) and females (70% compared to 0% control) at the HDT (lower doses not evaluated). In the neurotoxicology

animals, a significant increased incidence of Purkinje cell loss at brain level 7 was detected in males (60% compared to 0% control) and females (70% compared to 0% control) at the HDT but not at lower doses (evaluated at the next 2 lower doses). In the neurotoxicology animals, there was also a significant increased incidence of right sural nerve fiber degeneration in females (60% compared to 11% control) at the HDT (lower doses not evaluated). The incidence of right sural neve fiber degeneration was also increased in males (60%); however, the control group had a higher background than females (30%), and therefore the change was not statistically significant. The incidence and severity of left sural nerve fiber degeneration were similar between the control and HDT. The sural nerve was not evaluated in the chronic toxicity animals.

Eyes: In the chronic toxicology animals, an increase incidence (compared to control) of degeneration of the retina was detected in males only at the HDT (20% vs. 5%); however, it was not statistically significant, and the mean severity at the high dose was lower than in controls. The same tissue was evaluated in the neurotoxicology animals, and there was no increased incidence compared to the concurrent control; therefore, the finding in the toxicology animals was considered incidental.

Cecum: In the chronic toxicology animals, an increase incidence (compared to control) of mucosa mineralization was detected in females only at the top 2 doses (55% and 70% vs. 35% in the control); however, it was not statistically significant, and the mean severity at the high dose was similar to controls. The same tissue was not evaluated in the neurotoxicology animals. Considering the high background in concurrent controls, this finding in the toxicology animals is considered incidental.

Male reproductive system: In the chronic toxicology animals, an increase incidence of epididymis aspermia together with testicular degeneration were detected in 2 males at 113 mg/kg/day (10% incidence) but not in controls. Evaluation of 1 male in each of the next 2 lower dose groups also found testicular degeneration in both males and epididymis aspermia in one of the males. The reason for the evaluations at the lower doses seems to be the gross findings in the testis (see Table 12). These tissues were not evaluated in the neurotoxicology animals. Because of the low incidence and the absence of adverse effects in the sperm evaluation (see Table 10), these findings are considered incidental and probably age-related.

Table 13. Incidence of microscopic pathology (mean severity) [a]

	Males					Females				
mg/kg/day	**0**	**7**	**21**	**58**	**113**	**0**	**8**	**26**	**71**	**136**
CHRONIC TOXICOLOGY										
Brain, *num. evaluated*	*20*	*0*	*0*	*0*	*20*	*20*	*0*	*0*	*0*	*20*
Atrophy	0	--	--	--	1 (2)	0	--	--	--	0
Nerve fiber, degeneration	0	--	--	--	1 (1)	0	--	--	--	0
Purkinje cell loss, total	0	--	--	--	10*(3.3)	0	--	--	--	14*(2.9)
slight					2					5
moderate					3					1
marked					5					3
Cecum, *num. evaluated*	*20*	*0*	*0*	*0*	*20*	*20*	*0*	*0*	*20*	*20*
Mucosa, mineralization	4 (1.3)	--	--	--	3 (1.0)	7 (2.9)	--	--	11 (1.8)	14 (2.8)
minimal	3				3				5	2
slight	1					3			3	2
moderate						2			3	7
marked						2				3
Epididymis, *num. evaluated*	*20*	*0*	*0*	*1*	*20*	--	--	--	--	--
Aspermia	0	--	--	1 (2.0)[b]	2 (4.0)[b]	--	--	--	--	--
Thrombosis	0	--	--	0	1 (3.0)	--	--	--	--	--
Eye, *num. evaluated*	*20*	*0*	*1*	*0*	*20*	*20*	*0*	*0*	*0*	*20*
Retina, degeneration	1 (3.0)	--	1 (4.0)	--	4 (2.3)	3 (2.7)	--	--	--	2 (2.0)
Pituitary, *num. evaluated*	*18*	*0*	*0*	*0*	*20*	*20*	*1*	*0*	*0*	*20*
Hyperplasia	0	--	--	--	0	2 (2.5)	0	--	--	4 (2.0)
Adenoma	0	--	--	--	3	4	1	--	--	0
Carcinoma	0	--	--	--	0	1	0	--	--	1
Neurilemmoma, malignant	1	--	--	--	0	0	0	--	--	0
Testis, *num. evaluated*	19	0	1	1	20	--	--	--	--	--
Degeneration	0	--	1 (3.0)	1 (4.0)[b]	2 (4.0)[b]	--	--	--	--	--
NEUROTOXICOLOGY										
Brain, L7, *num. evaluated*	*10*	*0*	*10*	*10*	*10*	*9*	*0*	*10*	*10*	*10*
Purkinje cell loss	0	--	0	0	6*(2.7)	0	--	0	0	7*(3.4)
Eye, *num. evaluated*	*10*	*0*	*0*	*0*	*10*	*10*	*0*	*0*	*0*	*10*
Retina, degeneration	0	--	--	--	1 (2.0)	1 (3.0)	--	--	--	1 (1.0)
Sural nerve, *num. evaluated*	*10*	*0*	*0*	*0*	*10*	*9*	*0*	*0*	*0*	*10*
Right nerve fiber degeneration	3 (1.0)	--	--	--	6 (1.0)	1 (1.0)	--	--	--	6*(1.0)
Left nerve fiber degeneration	7 (1.1)	--	--	--	8 (1.0)	5 (1.0)	--	--	--	5 (1.0)

a Data obtained from page 1170- in MRID 52070901. Severity grades: 1 (minimal), 2 (slight), 3 (moderate), 4 (marked), 5 (severe). L = level

b Males with aspermia in the epididymides also had testicular degeneration.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Through approximately 1 year of continuous and repeated dietary exposure to the test substance, the toxicological response of the rat was principally characterized by a decrease in body weight gain (1000- and 2000-ppm males and females) and structural changes in the brain (2000-ppm males and females). Based on these findings, the LOAEL in this study was 1000 ppm, which was equivalent to 58 and 71 mg 1,2,4-triazole/kg body wt/day for male and female rats, respectively. Based on a lack of adverse compound-related effects, the systemic Chronic toxicity NOAEL in this study was 375 ppm which was equivalent to 21 and 26 mg 1,2,4-triazole/kg body wt/day for male and female rats, respectively.

B. REVIEWER COMMENTS: There were no treatment-related effects on clinical signs, mortality, ocular findings, body weight, hematology, clinical chemistry or urinalysis parameters. The only treatment-related significant finding from the functional observation battery was a 17% increase in forelimb strength in males at the 12-month evaluation, compared to controls. However, as there were no other functional changes, and the increase was slight, the change in forelimb grip strength is not considered adverse. There was a tendency in males to have higher motor activity at the HDT (113 mg/kg/day) and higher locomotor activity at the HDT and at the next lower dose; however, the apparent differences from the control group were not statistically significant. Mean motor activity for intervals 3-6 was sporadically increased in a dose-dependent manner, but not in a time dependent manner. Mean locomotor activity at the last two intervals measured (5 and 6) was significantly increased at the top 2 doses 58 and 113 mg/kg/day) at the 9- and 12-month evaluations. However, changes in male motor activity were sporadic, did not show a significant dose- or time-dependent pattern, and are considered incidental. Females had similar motor activity in control and treated groups.

Sperm parameters were similar in treated and control males. As expected, over the last 3 weeks of this 1-year study many females were reaching or had already reached reproductive senescence, as shown by both vaginal cytology (no cycles or very long cycles: 20%, 30%, 40%, 10% and 30% in the control, 8, 26, 71 and 136 mg/kg/day groups, respectively), and by the incidence of females without recent ovulation (67% and 3% in the control and 136 mg/kg/day groups, respectively, had < 2 corpora lutea). By evaluating the available data only, mean cycle length or mean number of cycles was similar in control and treated groups. However, the available data does not allow to evaluate females still cycling separately from females reaching or already in senescence. Combining these two different populations may be a confounder, and not an appropriate procedure to analyze this kind of data. However, even if the data is made available in the future, the low number of animals evaluated will mean that separating into population will lead to low statistical power (very low N) for each population.

Ovarian follicle counts were provided for individual animals, in the control and high dose groups only. The mean number of preantral and antral follicles at the HDT was similar to control. When all females are included (N = 9-10) The mean number of corpora lutea was at the HDT was 2X that of control, however the increase was not statistically significant due to

the high variability in both groups (CV = 90-142%). If the females not ovulating are excluded from the analysis (N = 3-7) there is no significant difference in mean number of corpora lutea between the two groups.

There were no significant dose-dependent effects in organ weights or gross pathology. In the chronic toxicology animals, there was a significant increased incidence of Purkinje cell loss in males (50% compared to 0% control) and females (70% compared to 0% control) at the HDT (lower doses not evaluated). In the neurotoxicity animals, a significant increased incidence of Purkinje cell loss at brain level 7 was detected in males (60% compared to 0% control) and females (70% compared to 0% control) at the HDT but not at lower doses (evaluated at the next 2 lower doses). All other histopathology findings occurred with similar incidence in control and treated groups or were otherwise considered to be incidental.

C. STUDY DEFICIENCIES: Individual animal data was not provided from the vaginal cytology evaluation. This is a minor deficiency and does not prevent the use of this study for risk assessment purposes.

Attachment:

Table A-1: Mean Interval Motor and Locomotor Activity for Males

mg/kg/day	0	6.9	21	58	113	0	6.9	21	58	113
Interval 1	**Motor**					**Locomotor**				
Pre-treatment	109 ± 19 CV = 17%	123 ± 24	101 ± 16	121 ± 21	119 ± 20	81 ± 15 CV = 19%	90 ± 23	74 ± 12	83 ± 16	86 ± 14
3 months	103 ± 32 CV = 31%	102 ± 20	95 ± 13	107 ± 30	110 ± 19	68 ± 15 CV = 22%	68 ± 18	61 ± 8	68 ± 18	65 ± 19
6 months	93 ± 30 CV = 32%	66 ± 20	77 ± 22	82 ± 26	92 ± 38	52 ± 15 CV = 29%	41 ± 20	49 ± 12	50 ± 19	53 ± 16
9 months	74 ± 22 CV = 30%	70 ± 21	67 ± 33	64 ± 26	80 ± 34	40 ± 12 CV = 30%	39 ± 17	40 ± 17	34 ± 21	39 ± 17
12 months	69 ± 15 CV = 30%	67 ± 33	69 ± 46	79 ± 23	76 ± 26	36 ± 9 CV = 25%	32 ± 14	36 ± 20	41 ± 10	40 ± 10
Interval 2	**Motor**					**Locomotor**				
Pre-treatment	103 ± 26 CV = 25%	109 ± 29	89 ± 21	110 ± 30	102 ± 31	73 ± 25 CV = 34%	75 ± 26	61 ± 19	74 ± 23	70 ± 25
3 months	68 ± 25 CV = 37%	72 ± 19	67 ± 18	80 ± 31	64 ± 17	37 ± 12 CV = 32%	38 ± 10	40 ± 14	50 ± 26 ↑35%	40 ± 15
6 months	41 ± 18 CV = 44%	37 ± 28	45 ± 26	47 ± 32	51 ± 17	20 ± 12 CV = 60%	21 ± 20	22 ± 14	25 ± 16	28 ± 13
9 months	29 ± 15 CV = 52%	34 ± 20	37 ± 17	31 ± 23	35 ± 20	12 ± 7 CV = 58%	18 ± 14	18 ± 9	14 ± 10	16 ± 9
12 months	37 ± 11 CV = 52%	39 ± 22	41 ± 30	41 ± 14	51 ± 26	14 ± 5 CV = 36%	14 ± 8	17 ± 11	19 ± 9	22 ± 14 ↑57%
Interval 3	**Motor**					**Locomotor**				
Pre-treatment	104 ± 27 CV = 26%	115 ± 39	92 ± 17	101 ± 35	96 ± 21	76 ± 23 CV = 30%	81 ± 32	60 ± 15	69 ± 31	68 ± 17
3 months	41 ± 17 CV = 41%	64 ± 24 ↑56%	63 ± 26 ↑54%	68 ± 31 ↑66%	52 ± 27	21 ± 12 CV = 57%	36 ± 15 ↑71%	36 ± 18 ↑71%	44 ± 21 ↑110%	32 ± 18
6 months	32 ± 15 CV = 47%	25 ± 18	24 ± 21	27 ± 14	33 ± 23	15 ± 7 CV = 47%	11 ± 11	11 ± 9	14 ± 7	17 ± 11
9 months	17 ± 10 CV = 59%	20 ± 15	30 ± 19	20 ± 13	29 ± 14	7 ± 6 CV = 86%	11 ± 10	13 ± 8	6 ± 6	15 ± 9 ↑114%
12 months	19 ± 17 CV = 89%	18 ± 13	20 ± 15	25 ± 15	40 ± 20* ↑111%	9 ± 9 CV = 100%	8 ± 6	8 ± 7	12 ± 12	19 ± 11 ↑111%
Interval 4	**Motor**					**Locomotor**				
Pre-treatment	89 ± 19 CV = 21%	92 ± 38	78 ± 25	84 ± 34	83 ± 32	63 ± 14 CV = 22%	65 ± 30	55 ± 18	59 ± 29	50 ± 24
3 months	26 ± 15 CV = 58%	38 ± 27	53 ± 29* ↑104%	52 ± 23 ↑100%	56 ± 21* ↑115%	14 ± 9 CV = 64%	21 ± 16	29 ± 23 ↑107%	30 ± 17 ↑114%	32 ± 13 ↑129%
6 months	17 ± 11 CV = 65%	27 ± 21	18 ± 17	20 ± 15	25 ± 16	7 ± 8 CV = 114%	12 ± 9	9 ± 9	10 ± 8	13 ± 9
9 months	13 ± 6 CV = 46%	20 ± 10 ↑54%	16 ± 15	21 ± 11 ↑62%	19 ± 10	5 ± 3 CV = 60%	8 ± 5	8 ± 9	10 ± 7 ↑100%	8 ± 5
12 months	17 ± 14 CV = 82%	17 ± 19	26 ± 26	21 ± 17	28 ± 18	5 ± 5 CV = 100%	8 ± 13	10 ± 9	12 ± 10 ↑140%	13 ± 8 ↑160%
Interval 5	**Motor**					**Locomotor**				
Pre-treatment	59 ± 37 CV = 63%	68 ± 34	62 ± 31	83 ± 30	66 ± 36	35 ± 27 CV = 77%	43 ± 23	40 ± 21	54 ± 27	36 ± 29
3 months	19 ± 18 CV = 95%	26 ± 23	33 ± 21	31 ± 25	39 ± 22 ↑105%	9 ± 10 CV = 111%	14 ± 14	17 ± 12	18 ± 15	23 ± 15 ↑156%
6 months	14 ± 16 CV = 114%	13 ± 9	10 ± 14	16 ± 14	24 ± 20	6 ± 8 CV = 133%	6 ± 5	5 ± 7	5 ± 6	11 ± 10

mg/kg/day	0	6.9	21	58	113	0	6.9	21	58	113
9 months	3 ± 3 CV = 100%	10 ± 11 ↑233%	17 ± 16 ↑467%	20 ± 16* ↑567%	19 ± 16* ↑533%	2 ± 3 CV = 150%	5 ± 6	8 ± 6 ↑300%	11 ± 9 ↑450%	10 ± 9 ↑400%
12 months	11 ± 13 CV = 118%	11 ± 15	11 ± 10	22 ± 12	24 ± 15	3 ± 6 CV = 200%	5 ± 7	4 ± 4	9 ± 6	11 ± 8* ↑267%
Interval 6	**Motor**					**Locomotor**				
Pre-treatment	37 ± 31 CV = 84%	47 ± 38	44 ± 42	56 ± 34	45 ± 33	22 ± 23 CV = 105%	27 ± 26	23 ± 21	32 ± 24	30 ± 24
3 months	18 ± 17 CV = 94%	24 ± 23	32 ± 26	18 ± 10	41 ± 31 ↑128%	9 ± 8 CV = 89%	12 ± 11	18 ± 16 ↑100%	11 ± 7	20 ± 14 ↑122%
6 months	11 ± 16 CV = 145%	6 ± 7	9 ± 9	16 ± 16	22 ± 13	3 ± 4 CV = 133%	3 ± 4	5 ± 5	7 ± 7	10 ± 8 ↑233%
9 months	7 ± 11 CV = 157%	9 ± 7	15 ± 15	19 ± 12 ↑171%	20 ± 19 ↑186%	3 ± 5 CV = 167%	4 ± 5	6 ± 7	10 ± 5* ↑233%	10 ± 8 ↑233%
12 months	8 ± 10 CV = 125%	12 ± 12	17 ± 22	25 ± 19 ↑213%	24 ± 20 ↑200%	3 ± 3 CV = 100%	5 ± 7	5 ± 7	10 ± 7 ↑233%	12 ± 10* ↑300%

Table A-2: Corrected preantral follicle counts by the reviewers (study report summary data is incorrect)

Control		Slide 20 Levels					Slide 20A Levels					Totals		
Num.	**Notes**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**All**	**Ovulating**	**Senescent**
MD0101	Malformation [a]	2	2	3	4	4	2	5	2	2	1	*27* [a]		
MD0102		3	4	4	7	1	1	2	1	3	4	30	30	
MD0103	Not ovulating [b]	4	3	1	1	1	0	2	0	1	2	15		15
MD0104		0	1	2	3	1	3	6	3	6	1	26	26	
MD0105	Not ovulating [b]	3	2	3	4	2	4	3	5	5	1	32		32
MD0106	Not ovulating [b]	2	5	4	10	6	6	6	7	3	5	54		54
MD0107	Not ovulating [b]	3	3	0	0	1	2	4	2	5	0	20		20
MD0108	Not ovulating [b]	1	5	6	7	3	3	4	0	3	1	33		33
MD0109	Not ovulating [b]	4	2	6	4	1	8	6	3	2	2	38		38
MD0110		4	2	4	2	3	2	4	4	5	4	34	34	
										Mean		**31.3**	**30.0**	**32.0**
										SD		**10.5**	**3.3**	**12.6**
										N		**9**	**3**	**6**

2000 ppm		Slide 20 Levels					Slide 20A Levels					Totals		
Num.	**Notes**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**All**	**Ovulating**	**Senescent**
MD4101	Not ovulating [b]	3	1	2	2	2	2	2	2	1	3	20		20
MD4102	Not ovulating [b]	2	2	1	2	1	2	0	4	1	0	15		15
MD4103		1	1	4	9	4	6	7	10	6	7	55	55	
MD4104		2	4	7	3	5	3	2	6	7	2	41	41	
MD4105		10	5	2	3	2	3	2	0	4	3	34	34	
MD4106		4	3	6	2	1	8	5	1	2	5	37	37	
MD4107		7	5	2	4	2	0	3	4	9	3	39	39	
MD4108		4	5	3	3	2	3	3	1	1	3	28	28	
MD4109		1	6	2	11	3	5	9	3	0	0	40	40	
MD4110	Not ovulating [b]	7	8	8	1	0	2	4	3	2	5	40		40
										Mean		**34.9**	**39.1**	**25.0**
										SD		**10.9**	**7.7**	**10.8**
										N		**10**	**7**	**3**

Calculated from individual animal data in pages 1193-1194 MRID 52070902.
a: Animal number MD0101 had no vaginal opening; excluded from calculations.
b: Based on number of number of corpora lutea ≤ 1.

Table A-3: Antral follicle counts by the reviewers.

Control		Slide 20 Levels					Slide 20A Levels					Totals		
Num.	Notes	1	2	3	4	5	1	2	3	4	5	All	Ovulating	Senescent
MD0101	Malformation [a]	0	0	0	1	0	2	1	5	0	2	*11* [a]		
MD0102		5	4	1	3	4	4	6	6	3	1	37	37	
MD0103	Not ovulating [b]	2	2	2	3	5	6	3	3	2	4	32		32
MD0104		4	6	3	0	1	6	7	4	2	1	34	34	
MD0105	Not ovulating [b]	6	2	4	3	0	3	0	3	1	3	25		25
MD0106	Not ovulating [b]	4	4	2	4	3	4	4	2	5	3	35		35
MD0107	Not ovulating [b]	2	7	7	2	2	3	3	3	5	4	38		38
MD0108	Not ovulating [b]	1	3	4	6	3	2	2	1	3	3	28		28
MD0109	Not ovulating [b]	0	1	2	3	4	5	3	3	2	2	25		25
MD0110		2	3	3	3	1	1	5	5	5	5	33	33	
										Mean		**31.9**	**34.7**	**30.5**
										SD		**4.6**	**1.7**	**4.9**
										N		**9**	**3**	**6**

2000 ppm		Slide 20 Levels					Slide 20A Levels					Totals		
Num.	Notes	1	2	3	4	5	1	2	3	4	5	All	Ovulating	Senescent
MD4101	Not ovulating [b]	1	4	4	4	4	5	4	4	1	1	32		32
MD4102	Not ovulating [b]	4	3	2	1	2	3	4	2	2	1	24		24
MD4103		4	4	6	7	7	6	7	5	7	10	63	63	
MD4104		2	3	2	1	4	4	3	4	6	9	38	38	
MD4105		3	6	5	8	4	3	2	3	2	2	38	38	
MD4106		1	2	3	2	4	3	3	4	6	4	32	32	
MD4107		6	5	2	3	9	0	1	1	4	1	32	32	
MD4108		3	6	5	5	4	1	1	0	6	3	34	34	
MD4109		2	2	3	4	3	8	4	9	2	0	37	37	
MD4110	Not ovulating [b]	3	2	3	0	0	2	3	1	4	3	21		21
										Mean		**35.1**	**39.1**	**25.7**
										SD		**10.7**	**10.0**	**4.6**
										N		**10**	**7**	**3**

Calculated from individual animal data in pages 1193-1194.
a: Animal number MD0101 had no vaginal opening and was an outlier per Grubb's test at $p < 0.05$; excluded from calculations.
b: Based on number of number of corpora lutea ≤ 1.

Table A-4: Corpora lutea counts and statistical analysis by the reviewers.

Control		Slide 20 Levels					Slide 20A Levels					Totals		
Num.	**Notes**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**All**	**Ovulating**	**Senescent**
MD0101	Malformation [a]	5	4	3	3	3	3	3	3	3	2	*32* [a]		
MD0102		4	7	4	5	5	2	3	3	3	1	37	37	
MD0103	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
MD0104		2	2	3	4	0	2	2	1	2	3	21	21	
MD0105	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
MD0106	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
MD0107	Not ovulating [b]	0	0	1	0	0	0	0	0	0	0	1		1
MD0108	Not ovulating [b]	0	0	0	1	0	0	0	0	0	0	1		1
MD0109	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
MD0110		4	3	1	3	4	5	4	4	5	3	36	36	
										Mean		**10.7**	**31.3**	**0.3**
										SD		**15.2**	**7.3**	**0.5**
										N		**9**	**3**	**6**

2000 ppm		Slide 20 Levels					Slide 20A Levels					Totals		
Num.	**Notes**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**All**	**Ovulating**	**Senescent**
MD4101	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
MD4102	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
MD4103		5	5	6	7	7	4	2	2	7	7	52	52	
MD4104		1	2	2	2	0	3	1	2	1	5	19	19	
MD4105		1	4	6	2	1	1	1	1	0	1	18	18	
MD4106		3	2	1	1	0	5	6	4	4	2	28	28	
MD4107		6	8	4	7	6	2	8	4	8	7	60	60	
MD4108		4	2	3	1	4	3	3	3	2	4	29	29	
MD4109		2	0	0	1	3	4	2	1	3	0	16	16	
MD4110	Not ovulating [b]	0	0	0	0	0	0	0	0	0	0	0		0
										Mean		**22.2**	**31.7**	**0.0**
										SD		**19.9**	**16.2**	**0.0**
										N		**10**	**7**	**3**

Calculated from individual animal data in pages 1193-1194.
a: Animal number MD0101 had no vaginal opening; excluded from calculations.
b: Based on number of number of corpora lutea ≤ 1.