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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: September 25, 2023

SUBJECT: **Triazole Metabolites: Triazole Alanine (PC 600011) and Triazole Acetic Acid (PC 600082)**: Review of Toxicology Studies

PC Codes: 600011, 600082
Decision No.: 591421
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0058589
MRID No.: See table

DP Barcode: D467496
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: 86362-20-1, 28711-29-7
40 CFR: N/A

FROM: Minerva Mercado-Feliciano, PhD, Toxicologist
Risk Assessment Branch IV
Health Effects Division (HED, 7509T)

THROUGH: Shalu Shelat, Branch Chief
Risk Assessment Branch IV
Health Effects Division (HED, 7509T)

TO: Laura Weissenborn, Chemical Review Manager
Khue Nguyen, Team Leader
Cathryn Britton, Branch Chief
Pesticide Re-evaluation Division (PRD, 7509T)

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

Newly submitted studies for triazole alanine and triazole acetic acid were evaluated. Data Evaluation Records are attached, as summarized below:

Chemical	Study Type	Year	MRID	Comments
Triazole Alanine	Prenatal developmental (rabbit)	2010	52154801	Acceptable/guideline
	Chronic oral toxicity with neurotoxicology evaluation (rat)	2012	52154804	Acceptable/guideline
Triazole Acetic Acid	90-Day oral toxicity with neurotoxicology evaluation (rat)	2010	52154805	Acceptable/guideline
	Prenatal developmental (rabbit)	2010	52154803	Acceptable/guideline
	Reproduction and fertility effects (rat)	2010	52154802	Acceptable/guideline

DATA EVALUATION RECORD

TRIAZOLE ALANINE (METABOLITE OF TRIAZOLE)

Study Type: OCSPP 870.3700b, Developmental Toxicity Study in Rabbits

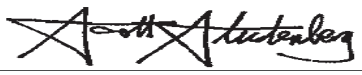
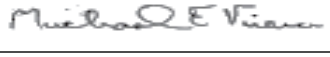
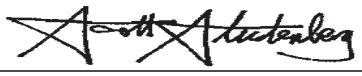
EPA Contract No. 68HERC22D0017
Task Assignment No. 5540-2.1-022 (MRID 52154801)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Prepared by

PB&A/
CSS JV

1151 Lost Creek Boulevard
Austin, TX 78746

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/13/2023
Secondary Reviewer: Michael E. Viana, Ph.D.	Signature: Date:	 05/18/2023
Quality Assurance: Sarah E. Saucier, Ph.D.	Signature: Date:	 05/19/2023
Project Manager: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/19/2023

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by PB&A/CSS Joint Venture personnel. Contractor's role did not include establishing Agency policy.

EPA Reviewer: Minerva Mercado-Feliciano PhD, DABT **Signature:**
Risk Assessment Branch IV, HED (7509C) **Date:** 2 Aug 2023
EPA Secondary Reviewer: Megan Stallard, PhD **Signature:**
Risk Assessment Branch IV, HED (7509C) **Date:** 3 Aug 2023
Template version 02/06

DATA EVALUATION RECORD

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

PC CODE: 600011 **DP BARCODE:** Not provided
TXR#: 0058589

TEST MATERIAL (PURITY): Triazole alanine (98% a.i.)

SYNONYMS: CGA131013; 1*H*-1,2,4-triazole-1-propanoic acid, α-amino

CITATION: Hoberman, A.M. (2010) Triazole alanine - Oral (stomach tube) developmental toxicity study in rabbits. Charles River Laboratories Preclinical Services, Horsham, PA. Laboratory Project No.: BWS00004, December 31, 2010. MRID 52154801. Unpublished.

SPONSOR: Triazole Derivative Metabolite Group, U.S. Triazole Task Force (Agent), c/o McDermott, Will & Emery, 600 13th St., N.W., Washington, D.C.

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 developmental toxicity study (MRID 52154801), groups of 25 presumed-pregnant female New Zealand White rabbits were administered triazole alanine (metabolite of triazole; 98% a.i.; Batch No. MES 133/1) in 0.5% aqueous carboxymethylcellulose via oral gavage (dose volume 10 mL/kg) at dose levels of 0, 30, 100, or 250 mg/kg/day during gestation days (GD) 6-28. On GD 29, all surviving does were euthanized, the uterus and ovaries were removed by cesarean section, and the uterine contents examined. The fetuses were examined for external, visceral, and skeletal malformations and variations.

There were no adverse effects of treatment on maternal mortality; clinical signs, body weight, gravid uterine weight, adjusted GD 29 body weight, or adjusted GD 6-29 body weight gain; food consumption; gross pathology; liver and kidney weights; or cesarean section parameters. The observed decreases in body weight gain and food consumption did not result in significant decreases in absolute body weight and are therefore not considered adverse.

A maternal LOAEL was not detected. The maternal NOAEL is 250 mg/kg/day, the highest dose tested.

At cesarean section, there were no adverse effects of treatment on fetal deaths or resorptions. There were no effects of treatment on fetal malformations. Fetal body weights (male, female, and total) were decreased ($p \leq 0.01$) by 10-12% at 250 mg/kg/day. However, fetal weights are within the dynamic range of the measurement because the decrease is just outside of the coefficient of variation (CV) of the concurrent control (8.5%) and within historical control ranges. The incidence of two variations was increased ($p \leq 0.01$) in the 250 mg/kg/day fetuses: angulated hyoid ala (litter incidence of 52% vs. 8.7% control) and thickened ribs (litter incidence of 12% vs. 0% controls). However, the incidence of these variations was similar to historic controls, and they are not expected to affect function. The non-adverse skeletal findings are not related to a decrease in fetal weight because (1) the hyoid is a very small bone that would not impact fetal weight; and (2) thickened ribs represent an increase in bone deposition that would add to fetal weight, not decrease the weight. No other treatment-related effects were noted in fetuses.

A developmental LOAEL was not detected. The developmental NOAEL is 250 mg/kg/day, the highest dose tested.

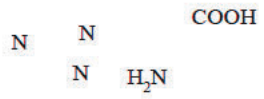
This study is classified **Acceptable/Guideline** and satisfies the guideline requirement (OCSPP 870.3700b; OECD 414) in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triazole alanine

Description:	White solid
Batch #:	MES 133/1
Purity:	98.0% a.i.
Expiration / storage:	February 2012 / 2-8°C, protected from light and humidity
CAS # of TGAI:	86362-20-1
Structure:	

2. Vehicle and/or positive control: Aqueous 0.5% carboxymethylcellulose

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, Hra:(NZW)SPF, time-mated females
Age / weight on GD 0:	Approximately 5.5 months / 2.63-4.34 kg
Source:	Covance Research Products, Inc. (Denver, PA)
Housing:	Individually in stainless steel cages suspended over lined trays
Diet:	Certified Rabbit Chow #5322 (PMI Nutrition International, St. Louis, MO); approximately 150 g/rabbit/day during acclimation and 180-185 g/rabbit/day during treatment; supplemented with timothy cubes (Bio-Serv, Inc., Frenchtown, NJ).
Water:	Reverse osmosis-processed tap water with <1.2 ppm chlorine added, *ad libitum*
Environmental conditions:	**Temperature:** 16-22°C **Humidity:** 30-70% **Air changes:** ≥10/hour **Photoperiod:** 12 hours light / 12 hours dark
Acclimation period:	2-5 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Start: July 19, 2009 End: August 14, 2009

2. Mating: Females were naturally bred (time-mated) with males of the same strain by the Supplier prior to shipment to the testing facility. The day on which mating was detected and females were presumed pregnant was designated as gestation day (GD) 0; mated females arrived at the testing facility on GD 1-4.

3. Animal assignment: Animals were stratified by GD 0 body weight with a weight-ordered randomization procedure and allocated into four groups (Table 1). Details of the procedure were not provided.

TABLE 1. Animal assignment. [a]

Dose (mg/kg/day)	0	30	100	250
Number of Females	25	25	25	25

a Data obtained from page 23 of MRID 52154801.

4. **Dose selection rationale:** Dose levels for the present study were selected based on the results of an oral dose range-finding study with triazole alanine in rabbits (Study No. BWS00003; further reference details not provided) administered 0, 30, 100, 175, or 250 mg/kg/day from GD 6-29. There were no treatment-related effects on gross lesions or liver weights (absolute and relative to body). Scant feces were noted in a generally dose-related manner but considered a treatment-related effect only at 250 mg/kg/day (incidence = 9). Treatment-related decreases in mean overall body weight gain (GD 6-29) were observed at 250 mg/kg/day with no treatment-related effect on mean body weight. Mean gravid uterine weight was decreased by 10% at 250 mg/kg/day with no effect on corrected mean body weight. Mean absolute and relative feed consumption values were also decreased at 250 mg/kg/day. All does were pregnant. Decreases in fetal body weights (approximately ↓16-18%) at 250 mg/kg/day were considered treatment related with no other effects on cesarean-section or litter parameters. Although two gross fetal alterations occurred in two 250 mg/kg/day littermates, neither finding was considered a treatment-related effect. No treatment-related effects were reported at levels of ≤175 mg/kg/day. Therefore, dose levels of 30, 100, and 250 mg/kg/day were selected for the present study.

5. **Dosage preparation and analysis:** Test item suspensions were prepared daily and stored at room temperature. The test substance was considered 98% active/pure for the purpose of dosage calculations. The vehicle was prepared weekly and stored at 2-8°C. Further preparation details were not provided. Test suspensions were stirred continuously during administration. It was stated that stability of formulations was provided by the Sponsor; formulations were stable for up to eight days at room temperature.

   Duplicate samples were taken from the top, middle and bottom of each dose suspension on the first day of preparation and duplicate samples from a single level of each suspension were collected on the final day of preparation for concentration and homogeneity analyses. It was stated that all concentration and homogeneity samples were within the 85-115% acceptance criteria for concentration analyses and within the 5% RSD criteria for homogeneity analyses.

   Results

   Homogeneity (%RSD): 0.73-3.4%

   Stability (% of time 0): Not reported

   Concentration (% of nominal): 94-107%

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual doses was acceptable.

6. **Dosage administration:** The dosing solutions were administered by daily oral gavage (dose volume 10 mL/kg) during GD 6-28, inclusive. Dose volumes were adjusted based on the most recent body weight.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** The animals were examined twice daily for mortality, morbidity, clinical observations, abortion, and premature delivery prior to daily dose administration, within two hours after administration, and prior to scheduled euthanasia on GD 29. Body weights were measured on GD 0 by the Supplier, on the day of arrival at the testing facility, and daily during GD 6-29. Food consumption was measured daily from arrival through GD 29.

   All surviving does were euthanized by i.v. administration of a euthanasia solution consisting of pentobarbital sodium and phenytoin sodium on GD 29, and necropsied. The liver, kidneys, and gravid uteri were excised and weighed, and the stomachs were examined internally for gross lesions. Uteri of apparently non-pregnant does were stained with 10% ammonium sulfide to confirm pregnancy status. The liver, kidneys, and stomach were retained in neutral-buffered 10% formalin.

   The number and distribution of *corpora lutea* were recorded and each uterus was excised and examined for pregnancy status, number and distribution of implantation sites, live and dead fetuses, and early and late resorptions. The following definitions were provided.

   - An early resorption was defined as one in which organogenesis was not grossly evident.
   - A late resorption was defined as one in which the occurrence of organogenesis was grossly evident.
   - A live fetus was defined as a term fetus that responded to stimuli.

   It was stated that dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. The placentae were examined for size, color, and shape. To minimize bias, cesarean-section results, and all fetal observations, were conducted “blind” to the dose group.

   One 30 mg/kg/day rabbit (#89) was euthanized on GD 10 and examined for a cause of death or condition. The rabbit was examined for gross lesions and the lungs, trachea, and esophagus were perfused and stored in neutral-buffered 10% formalin along with the heart, liver, kidneys, stomach, and spleen. Pregnancy status and uterine contents were determined and the conceptuses *in utero* were examined to the extent possible.

2. **Fetal evaluations:** Fetuses were removed individually and identified by litter number and uterine distribution. Each fetus was weighed and examined for gross external alterations. Live fetuses were euthanized by an intraperitoneal injection of the euthanasia solution and examined internally to determine sex. Cavitated organs were evaluated by dissection. The brains of approximately one-half of the fetuses per litter were examined *in situ* after cross-sectioning between the parietal and frontal bones. The heads of the remaining fetuses were removed, fixed in Bouin's solution, and examined for soft tissue alterations according to Wilson's sectioning technique. All fetuses were examined for skeletal alterations after

staining with alizarin red S. Skeletal preparations were retained in glycerin with thymol added as a preservative.

Fetal malformations were defined as irreversible changes that occur at low incidences in this species and strain; fetal variations were defined as reversible delays or accelerations in development that are common findings in this species and strain.

D. DATA ANALYSIS

1. **Statistical analyses:** Group mean values and standard deviations were calculated. All statistical tests were two-sided with minimum significance levels of 5% and 1%. The litter was considered the experimental unit, if appropriate.

Parameter	Statistical test
Maternal body weight and body weight gain, food consumption, percentage male fetuses (litter), percentage resorbed conceptuses, fetal body weight, and fetal anomaly data	Bartlett's test was applied. If Bartlett's test was significant ($p \leq 0.001$), a Kruskal-Wallis test was applied (≤75% ties) and, if significant ($p \leq 0.05$), Dunn's Method of Multiple Comparisons was conducted. If there were >75% ties, Fisher's exact test was used. If Bartlett's test was not significant ($p > 0.001$), an analysis of variance (ANOVA) was applied and, if significant ($p \leq 0.05$), Dunnett's test was applied.
Clinical observations and proportional data	Variance test for homogeneity of the binomial distribution.
Count data	A Kruskal-Wallis test was applied (≤75% ties) and, if significant ($p \leq 0.05$), Dunn's Method of Multiple Comparisons was conducted. If there were >75% ties, Fisher's exact test was used.

The Reviewers considered the statistical analyses appropriate.

2. **Indices:** The following indices were determined from cesarean section and fetal examination data.

$$\text{Pre-implantation loss index (\%)} = \frac{\text{\# of } \textit{corpora lutea} - \text{\# of implantations}}{\text{\# of } \textit{corpora lutea}} \times 100$$

$$\text{Post-implantation loss (\%)} = \frac{\text{\# of implantations - \# of live fetuses}}{\text{\# of implantations}} \times 100$$

$$\text{Sex ratio} = \frac{\text{\# of live male fetuses}}{\text{\# of live fetuses}} \times 100$$

3. **Historical control data:** Historical control data were provided for reproductive indices for full and dose range-finding studies (No. of studies[does] = 48[1030] and 37[192], respectively), necropsy observations (No. of studies[does] = 88[1273]), external alterations (No. of studies[litters] = 48[948] and 37[184], respectively), soft tissue alterations (No. of studies[litters] = 49[936]), and skeletal alterations and fetal ossification sites (No. of studies[litters] = 48[931]).

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations:** All does survived until scheduled euthanasia, except for doe #89 (30 mg/kg/day) that was euthanized on GD 10 because the doe was about to deliver. It was apparent that this doe had been mated at the supplier a few weeks earlier than the other does shipped to the Testing Facility. The premature delivery was not considered related to treatment.

   The number of animals with soft or liquid feces was increased (not statistically significant [NS]) at 250 mg/kg/day (6/25 treated vs. 1/25 control); this was observed only once in each of 6 the high dose animals, and 3 times in only one control animal. This finding was not considered adverse. No other treatment-related effects on clinical signs were observed. Other clinical observations that did not occur in a dose-dependent manner included ungroomed coat, scant feces, sparse hair coat, rales, a scab on the ear, red substance on the ear, red substance in the cage, localized alopecia, red and pink perinasal substance, chromodacryorrhea, tachypnea and a swollen and/or red perivaginal area.

2. **Body weight:** Selected body weight, cumulative body weight gain, and gravid uterine weight data are presented in Table 2. There were no treatment-related effects on absolute body weights or gravid uterine weights. Body weight gains during GD 17-21 were decreased ($p \leq 0.01$) by 38% each in the 100 and 250 mg/kg/day animals. There were no further treatment-related effects on body weight gain at 100 mg/kg/day, but overall (GD 6-29) body weight gain was decreased ($p \leq 0.01$) by 29%. The decreases in body weight gain at 250 mg/kg/day were not considered adverse because they did not result in significant decreases in absolute body weight. Differences in body weight gains at ≤100 mg/kg/day were considered unrelated to treatment.

TABLE 2. Mean (± SD) maternal body weights (kg), body weight gains (kg), and gravid uterus weights (g) in rabbits administered triazole alanine by oral gavage during GD 6-28. [a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (n = 23)	30 (n = 24) [b]	100 (n = 25)	250 (n = 25)
GD 0	3.06 ± 0.22	3.06 ± 0.21	3.09 ± 0.25	3.08 ± 0.24
GD 6	3.22 ± 0.21	3.22 ± 0.22	3.27 ± 0.24	3.22 ± 0.26
GD 15	3.40 ± 0.22	3.40 ± 0.22	3.45 ± 0.25	3.37 ± 0.27 [c]
GD 28	3.65 ± 0.23	3.62 ± 0.24	3.67 ± 0.29	3.51 ± 0.29
GD 29	3.67 ± 0.24	3.65 ± 0.24	3.71 ± 0.30	3.54 ± 0.30
BWG GD 0-6	0.16 ± 0.06	0.16 ± 0.08	0.18 ± 0.07	0.14 ± 0.06
GD 17-21	0.08 ± 0.03	0.08 ± 0.04	0.05 ± 0.05** (↓38)	0.05 ± 0.05** (↓38)
GD 6-29	0.45 ± 0.10	0.43 ± 0.12	0.43 ± 0.13	0.32 ± 0.15** (↓29)
Gravid uterus weight (g)	522.71 ± 109.44 CV = 21%	502.38 ± 67.31	489.89 ± 94.24	451.81 ± 103.90
GD 29 BW (adjusted) [d]	3.14 ± 0.23	3.15 ± 0.23	3.22 ± 0.30	3.09 ± 0.23
GD 6-29 BWG (adjusted) [e]	−0.07 ± 0.11	−0.07 ± 0.13	−0.06 ± 0.15	−0.13 ± 0.12

a Data were obtained from Tables 5-6 on pages 43-45 of MRID 52154801.
b Analysis excluded data for animal #89 that was euthanized on GD 10.
c Excludes an outlier value that appears to be incorrectly recorded (n = 24).
d GD 29 body weight (terminal) – gravid uterine weight
e GD 6-29 BWG – gravid uterine weight
** Significantly different from control; $p \leq 0.01$.

3. **Food consumption:** Selected food consumption data are presented in Table 3. There were decreases ($p<0.05$) during GD 17-23 and for overall (GD 6-29) in absolute (↓19% and ↓11%, respectively) and relative (↓16% and ↓9%, respectively) food consumption at 250 mg/kg/day that were consistent with the observed decreases in body weight gains. There were no effects of treatment on food consumption at ≤100 mg/kg/day.

TABLE 3. Mean (± SD) absolute (g/day) and relative (g/kg/day) food consumption in rabbits administered triazole alanine by oral gavage during GD 6-28. [a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (n = 23)	30 (n = 24) [b]	100 (n = 25)	250 (n = 25)
Absolute consumption				
GD 17-21	158.3 ± 20.4	155.9 ± 25.5	148.0 ± 28.1 [c]	129.0 ± 31.7** (↓19)
GD 6-29	138.3 ± 19.0	143.5 ± 21.2	142.3 ± 20.6	123.0 ± 25.2* (↓11) [c]
Relative consumption				
GD 17-21	45.6 ± 4.8	45.3 ± 7.0	42.4 ± 7.3 [c]	38.3 ± 8.9** (↓16)
GD 6-29	40.1 ± 4.6	41.9 ± 5.9	41.0 ± 5.3	36.4 ± 7.0* (↓9) [c]

a Data were obtained from Table 7 on page 46 of MRID 52154801.
b Analysis excluded data for animal #89 that was euthanized on GD 10.
c Excludes a value associated with spillage.
* Significantly different from control; $p\leq0.05$.
** Significantly different from control; $p\leq0.01$.

4. **Gross pathology:** There were no treatment-related gross pathology findings.

5. **Organ weight:** There were no treatment-related effects on absolute or relative (to body) liver and kidney weights.

6. **Cesarean section data:** Cesarean section data are presented in Table 4. All placentae appeared normal. A statistically significant increase ($p\leq0.05$) in percentage of post-implantation loss for the 30 mg/kg/day dosage group was unrelated to dose and considered unrelated to treatment. Fetal body weights (male, female, and total) were decreased ($p\leq0.01$) by 10-12% at 250 mg/kg/day only (body weights at ≤100 mg/kg/day were similar to control). However, all fetal body weights (all dose groups) were within the Testing Facility historical control data ranges, and the body weight decrease at 250 mg/kg/day (10%) was similar to the control group coefficient of variation (8.5%); therefore, the effect was not considered treatment-related. Additionally, non-adverse skeletal findings (angulated hyoid ala and thickened ribs; discussed below) do not support a decrease in fetal weight.

TABLE 4. Cesarean section observations. [a]

Observation	Dose (mg/kg/day)				Historical Controls [e]
	0	30	100	250	
Animals treated	25	25	25	25	1030
Animals pregnant	23	24 [b]	25	25	966
Pregnancy rate (%)	92	96	100	100	94.1 (83.3-100)
Nonpregnant [c]	2	1	0	0	--
Maternal wastage					
No. removed from study	0	1 [b]	0	0	--
No. died	0	0	0	0	8
No. died pregnant	0	0	0	0	--
No. died nonpregnant	0	0	0	0	--
No. aborted	0	0	0	0	7
No. premature delivery	0	0	0	0	2
Total No. *corpora lutea* [c] **Mean ± SD per doe**	211 9.2 ± 1.9	217 9.4 ± 1.5	238 9.5 ± 1.5	231 9.2 ± 1.7	-- 9.4 (8.1-11.0)
Total No. implantations [c] **Mean ± SD per doe)**	202 8.8 ± 2.1	205 8.9 ± 1.6	216 8.6 ± 2.2	209 8.4 ± 1.9	-- 8.7 (6.9-9.7)
Total No. live litters	23	23	25	25	--
Total No. live fetuses	197	189	207	202	--
No. live fetuses/litter	8.6 ± 2.1	8.2 ± 1.8	8.3 ± 2.2	8.1 ± 1.9	8.2 (5.6-9.5)
Total No. dead fetuses	0	1	0	0	--
Total No. resorptions [c] **Early** **Late**	5 5 0	15 7 8 [d]	9 5 4	7 6 1	-- -- --
Resorptions/litter	0.2 ± 0.05	0.6 ± 0.8	0.4 ± 0.6	0.3 ± 0.5	0.4 (0.1-1.2)
% Litters w/ any resorptions	17.4	47.8	32.0	24.0	29.0 (10.0-73.9)
Litters w/ total resorptions	0	0	0	0	--
Mean fetal weight (g/litter)	44.5 ± 3.8 CV = 8 5%	43.4 ± 5.0	43.1 ± 5.3	39.9 ± 4.2** (↓10)	42.7 (38.3-46.7)
Live males	44.9 ± 3.8 CV = 8 5%	43.5 ± 5.6	44.1 ± 5.4	40.3 ± 4.6** (↓10)	43.2 (38.7-48.0)
Live females	44.6 ± 4.1 CV = 9 2%	42.8 ± 5.5	42.0 ± 5.4	39.4 ± 4.1** (↓12)	41.9 (37.4-45.2)
Sex ratio (mean % male)	44.6 ± 14.1	46.4 ± 22.8	48.9 ± 17.4	47.0 ± 12.3	--
Pre-implantation loss (%)	4.7 ± 14.6	5.5 ± 8.6	9.5 ± 16.1	9.1 ± 12.4	--
Post-implantation loss (%)	2.3 ± 5.3	7.9 ± 9.6* (↑243)	4.2 ± 6.8	3.3 ± 6.2	--

a Data were obtained from Tables 9-10 on pages 48-50 and Table 21 on pages 91-94 of MRID 52154801.
b Analysis excluded data for animal #89 that was euthanized on GD 10 due to early delivery and obvious signs of being mated earlier than other animals in the study.
c Calculated by the Reviewers.
d The mean (± SD) late resorptions/litter (0.3 ± 0.6) was increased ($p \leq 0.05$).
e Historical controls are from 48 full studies conducted between January 2007 and January 2009.
* Significantly different from control; $p \leq 0.05$.
** Significantly different from control; $p \leq 0.01$.

B. DEVELOPMENTAL TOXICITY

1. Multiple malformations/variations: One control group fetus and two 250 mg/kg/day fetuses had multiple malformations and/or variations. Fetus #52-6 (0 mg/kg/day) presented with a gross external finding of forepaws that were flexed downward and skeletal malformations consisting of bent femur, variations of incompletely-ossified (hypoplastic) ribs, large xiphoid, and large lumbar vertebral arches. Fetus #18-1 (250 mg/kg/day) presented with a gross external malformation of a small, misshapen ear, soft tissue malformations of constricted pulmonary artery and distended aorta, a skeletal malformation of an incompletely ossified tympanic ring, and a skeletal variation of unossified hyoid ala.

Fetus #21-1 (250 mg/kg/day) presented with external malformations of gastroschisis and no tail; soft tissue examination confirmed the gastroschisis with protruding organs, including liver, stomach, spleen, pancreas, and intestines. Other soft tissue findings included a thick liver, an absent right kidney, and a small, misshapen bladder. Skeletal variations consisted of flat ribs, a misaligned lumber vertebral arch, fused sacral vertebral arches and centra, and fused caudal vertebrae with only six present. Because there were no consistent patterns amongst these animals that suggested a relationship with treatment, they were not considered related to treatment.

A summary of the overall incidences of fetal alterations is presented in Table 5. The numbers of fetuses and litters with any alteration(s) were increased ($p \leq 0.01$) at 250 mg/kg/day by approximately 5- and 3-fold, respectively. The percentage of fetuses with any alteration(s) per litter was increased ($p \leq 0.01$) at 250 mg/kg/day by 145%.

TABLE 5. Summary of fetal alterations. [a]

Observations	Dose (mg/kg/day)			
	0	30	100	250
Number of fetuses (litters) examined	**197 (23)**	**190 (23)**	**207 (25)**	**202 (25)**
Fetuses (litters) with any alteration(s) observed	7 (6)	12 (10)	14 (10)	34 (17)**
% Fetuses with any alteration(s)/litter	6.6 ± 20.8	6.3 ± 8.1	6.9 ± 10.8	16.2 ± 15.1** (↑145)

a Data were obtained from Table 11 on page 51 of MRID 52154801.
** Significantly different from control; $p \leq 0.01$.

2. **External examinations:** External malformations are presented in Table 6 and consist of the findings noted previously in single animals with multiple malformations and/or variations. Malformations included forepaws that were flexed downward (Fetus #52-6; 0 mg/kg/day), a small, misshapen ear (Fetus #18-1; 250 mg/kg/day), and gastroschisis and no tail (Fetus #21-1; 250 mg/kg/day). Except for the finding in the control fetus, all other findings were in 1 fetus vs. 0 fetus control; therefore, they were considered incidental. There were no external variations present.

TABLE 6. External examination. [a]

Observation	Dose (mg/kg/day)			
	0	30	100	250
Number of fetuses (litters) examined	**197 (23)**	**190 (23)**	**207 (25)**	**202 (25)**
Malformations				
Fore and/or hindlimb(s), flexed	1 (1)	0 (0)	0 (0)	0 (0)
Ear(s), small	0 (0)	0 (0)	0 (0)	1 (1)
Ear(s), misshapen	0 (0)	0 (0)	0 (0)	1 (1)
Gastroschisis	0 (0)	0 (0)	0 (0)	1 (1)
Tail, absent	0 (0)	0 (0)	0 (0)	1 (1)

a Data were obtained from Table 12 on page 52 of MRID 52154801.

3. **Visceral examination:** Visceral malformations and variations are presented in Table 7. Malformations (noted previously in animals with multiple findings) included a constricted pulmonary artery and distended aorta (Fetus #18-1; 250 mg/kg/day) and confirmation of the observed gastroschisis (external malformation) with protruding liver, stomach, spleen, pancreas, and intestines (Fetus #21-1; 250 mg/kg/day) vs. 0 fetuses control. Additional malformations in this animal included a thick liver, an absent right kidney, and a small, misshapen bladder. An additional finding of distended aorta was observed in 1 fetus at 30

mg/kg/day. The absence of an intermediate lobe of the lung was observed in single animals at 0 and 250 mg/kg/day. This finding is a common variation in the rabbit. All other visceral variations occurred in a single fetus and/or in a manner unrelated to dose; therefore, they were considered incidental.

TABLE 7. Visceral examination. [a]

Observation	Dose (mg/kg/day)			
	0	30	100	250
Number of fetuses (litters) examined	**197 (23)**	**190 (23)**	**207 (25)**	**202 (25)**
Malformations				
Pulmonary artery, constricted	0 (0)	0 (0)	0 (0)	1 (1)
Aorta, distended	0 (0)	1 (1)	0 (0)	1 (1)
Liver, protruding through abdominal opening	0 (0)	0 (0)	0 (0)	1 (1)
Liver, thick	0 (0)	0 (0)	0 (0)	1 (1)
Stomach, protruding through abdominal opening	0 (0)	0 (0)	0 (0)	1 (1)
Kidneys, absent	0 (0)	0 (0)	0 (0)	1 (1)
Intestines, protruding through abdominal opening	0 (0)	0 (0)	0 (0)	1 (1)
Bladder, small	0 (0)	0 (0)	0 (0)	1 (1)
Bladder, misshapen	0 (0)	0 (0)	0 (0)	1 (1)
Spleen, protruding through abdominal opening	0 (0)	0 (0)	0 (0)	1 (1)
Pancreas, protruding through abdominal opening	0 (0)	0 (0)	0 (0)	1 (1)
Variations				
Lung, intermediate lobe absent	1 (1)	0 (0)	0 (0)	1 (1)

a Data were obtained from Table 13 on pages 53-54 of MRID 52154801.

4. **Skeletal examination:** Skeletal malformations and variations are presented in Table 8. Malformations noted previously include bent femur (Fetus #52-6; 0 mg/kg/day) and an incompletely ossified tympanic ring (Fetus #18-1; 250 mg/kg/day). Additional malformations observed in fetus #67-8 (0 mg/kg/day) and fetus #78-4 (30 mg/kg/day) were fused ribs with no additional alterations. Previously-noted skeletal variations include incompletely ossified (hypoplastic) ribs, large xiphoid, and large lumbar vertebral arches (Fetus #52-6; 0 mg/kg/day); an unossified hyoid ala (Fetus #18-1; 250 mg/kg/day); and flat ribs, a misaligned lumber vertebral arch, fused sacral vertebral arches and centra, and fused caudal vertebrae with only six present (Fetus #21-1; 250 mg/kg/day).

Increased ($p \leq 0.01$) incidences of two common variations were observed in the 250 mg/kg/day fetuses. Angulated hyoid ala occurred at a litter incidence of 52% (24 fetuses, 13 litters) and thickened ribs occurred at a litter incidence of 12% (3 fetuses, 3 litters); these incidence levels were slightly outside of the Testing Facility historical control ranges of 0-50% and 0-10%, respectively. Angulated hyoid is due to delayed ossification and typically resolves with continued growth (DeSesso and Scialli, 2018, Birth Defects Research 110 (15) pp 1157-1187). Thickened ribs are a benign variation, not expected to affect the animals functionally. These variations are not considered adverse, due to their incidence being similar to historic controls and not expected to affect function.

There were no treatment-related or biologically relevant differences in the average number of ossification sites per fetus. There were no treatment-related effects on fetal alterations at ≤100 mg/kg/day.

TABLE 8. Skeletal examination. [a]

Observation	Dose (mg/kg/day)				Historical Controls
	0	30	100	250	
Number of fetuses (litters) examined	**197 (23)**	**190 (23)**	**207 (25)**	**202 (25)**	
Malformations					
Hindlimb, femur, bent	1 (1)	0 (0)	0 (0)	0 (0)	
Skull, tympanic ring, incompletely ossified	0 (0)	0 (0)	0 (0)	1 (1)	
Ribs, fused	1 (1)	1 (1)	0 (0)	0 (0)	
Variations					
Ribs, incompletely ossified (hypoplastic)	1 (1)	0 (0)	0 (0)	0 (0)	
Ribs, thickened	0 (0)	0 (0)	0 (0)	3 (3)** 12%	1.5% (0-10%)
Ribs, flat	0 (0)	0 (0)	0 (0)	1 (1)	
Xyphoid, large	1 (1)	0 (0)	0 (0)	0 (0)	
Hyoid, ala, angulated	2 (2) 8.7%	2 (2)	5 (4)	24 (13)** 52%	19.1% (0-50%)
Hyoid ala, not ossified	0 (0)	0 (0)	0 (0)	1 (1)	
Lumbar vertebrae, arch, large	1 (1)	0 (0)	0 (0)	0 (0)	
Lumbar vertebrae, arch, misaligned	0 (0)	0 (0)	0 (0)	1 (1)	
Sacral vertebrae, arches, fused	0 (0)	0 (0)	0 (0)	1 (1)	
Sacral vertebrae, centra, fused	0 (0)	0 (0)	0 (0)	1 (1)	
Caudal vertebrae, fused	0 (0)	0 (0)	0 (0)	1 (1)	
Caudal vertebrae, six present	0 (0)	0 (0)	0 (0)	1 (1)	

a Data were obtained from Table 14 on pages 55-57 of MRID 52154801.
** Significantly different from control; $p<0.01$.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: The maternal no-observable adverse effect level (NOAEL) for triazole alanine was 100 mg/kg/day based on adverse clinical signs and reductions in body weight, body weight gain, and feed consumption in the 250 mg/kg/day dose group. The developmental NOAEL was also 100 mg/kg/day based on reduced fetal weights and slight increases in two common skeletal variations in the 250 mg/kg/day dose group. Based on these data, triazole alanine should not be identified as a selective developmental toxicant.

B. REVIEWER COMMENTS: The Reviewers only partially agree with the Investigators' Conclusions. As explained below, the effects used by the Investigators to set the maternal NOAEL are not considered adverse by the agency, and instead represent a Lowest Observed Effect Level (LOEL) which would not be appropriate as the basis of a point of departure (POD) for risk assessment.

1. **Maternal toxicity:** There were no adverse effects of treatment on maternal mortality; clinical signs, body weight, gravid uterine weight, adjusted GD 29 body weight, or adjusted GD 6-29 body weight gain; food consumption; gross pathology; liver and kidney weights; or cesarean section parameters.

   At 250 mg/kg/day, body weight gain during GD 17-21 was decreased ($p\leq0.01$) by 38% and overall (GD 6-29) body weight gain was decreased ($p\leq0.01$) by 29%. There were decreases ($p<0.05$) during GD 17-21 and for overall food consumption (GD 6-29) in absolute (↓19% and ↓11%, respectively) and relative (↓16% and ↓9%, respectively) food consumption at 250 mg/kg/day that were consistent with the decreases in body weight gain. The observed

decreases in body weight gain and food consumption did not result in significant decreases in absolute body weight and are therefore not considered adverse.

A maternal LOAEL was not detected. The maternal NOAEL is 250 mg/kg/day, the highest dose tested.

2. **Developmental toxicity:** At cesarean section, there were no adverse effects of treatment on fetal deaths or resorptions. There were no effects of treatment on fetal malformations. Fetal body weights (male, female, and total) were decreased ($p \leq 0.01$) by 10-12% at 250 mg/kg/day. However, fetal weights are within the dynamic range of the measurement because the decrease is just outside of the coefficient of variation (CV) of the concurrent control (8.5%) and within historical control ranges. The incidence of two variations was increased ($p \leq 0.01$) in the 250 mg/kg/day fetuses: angulated hyoid ala (litter incidence of 52% vs. 9% control) and thickened ribs (litter incidence of 12% vs. 0% controls). However, the incidence of these variations was similar to historic controls, and they are not expected to affect function. The non-adverse skeletal findings are not related to a decrease in fetal weight because (1) the hyoid is a very small bone that would not impact fetal weight; and (2) thickened ribs represent an increase in bone deposition that would add to fetal weight, not decrease the weight. No other treatment-related effects were noted in fetuses.

A developmental LOAEL was not detected. The developmental NOAEL is 250 mg/kg/day, the highest dose tested.

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement (OCSPP 870.3700b; OECD 414) in the rabbit.

C. **STUDY DEFICIENCIES:** There were no study deficiencies.

DATA EVALUATION RECORD

TRIAZOLE ALANINE (METABOLITE OF TRIAZOLE)

Study Type: OCSPP 870.4100a; Chronic Oral Toxicity Study in Rats

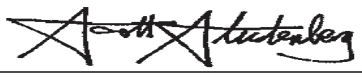
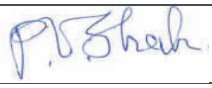
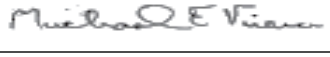
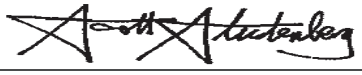
EPA Contract No. 68HERC22D0017
Task Assignment No. 5540-2.1-022 (MRID 52154804)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Prepared by

PB&A/
CSS JV

1151 Lost Creek Boulevard
Austin, TX 78746

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/22/2023
Secondary Reviewer: P.V. Shah, Ph.D.	Signature: Date:	 06/01/2023
Quality Assurance: Michael E. Viana, Ph.D.	Signature: Date:	 06/02/2023
Project Manager: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 06/04/2023

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by PB&A/CSS Joint Venture personnel**.** Contractor's role did not include establishing Agency policy.

EPA Reviewer: Jeremy Leonard, PhD **Signature:**
Risk Assessment Branch IV, HED (7509T) **Date:** 6/27/2023
EPA Secondary Reviewer: Minerva Mercado-Feliciano PhD, DABT **Signature:**
Risk Assessment Branch IV, HED (7509T) **Date:** 7/14/2023
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Chronic oral (feeding) toxicity study in rats; OCSPP 870.4100a [§83-1a]; OECD 452.

PC CODE: 600011 **DP BARCODE:** Not provided
TXR #: 0058589

TEST MATERIAL (PURITY): Triazole alanine (98% a.i.)

SYNONYMS: CGA131013; 1*H*-1,2,4-triazole-1-propanoic acid, α-amino

CITATION: Wahle, B.S. (2012) A chronic toxicity testing study in the Wistar rat with triazole alanine (TA). Xenometrics, LLC, Stilwell, Kansas. Study Number: 07-C72-MG, January 5, 2012. MRID 52154804. Unpublished.

SPONSOR: Triazole Derivative Metabolite Group, U.S. Triazole Task Force (Agent), c/o McDermott, Will & Emery, 600 13th St., N.W., Washington, D.C.

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 52154804), groups of 20 rats/sex (Toxicology group) were administered triazole alanine (98% a.i.; Batch MES133/1) in the diet at target dose levels of 0, 30, 100, 300, and 1000 mg/kg/day (concentrations were adjusted during the initial three months of treatment, with Month 4-12 concentrations of 600, 2000, 6000, and 20,000 ppm; overall intake equivalent to 28/36, 93/120, 278/375, or 916/1273 mg/kg/day in males/females) for up to 12 months. The Toxicology group rats were evaluated for body weight and body weight gain, food consumption, ophthalmoscopy, serum chemistry, hematology, urinalysis, organ weights, and macroscopic and microscopic pathology. Separate groups of 10 rats/sex (Neurotoxicology group) were administered the test item at the same dietary concentrations and underwent neurological evaluations (*i.e.*, functional observational battery [FOB] and motor/locomotor activity assessments), determination of absolute and relative (to body) brain weights, and macroscopic and microscopic evaluation of selected neurological tissues.

No treatment-related effects were observed on mortality, clinical observations, neurological evaluations (FOB and motor/locomotor activity), body weight and body weight gain, food consumption, ophthalmoscopic evaluation, serum chemistry, hematology, urinalysis, organ weights, and macroscopic and microscopic pathology.

The LOAEL was not determined. The NOAEL is 20,000 ppm (equivalent to 916/1273 mg/kg/day in males/females, approximating the limit dose).

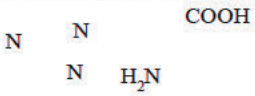
This study is classified **Acceptable/Guideline** for a chronic oral toxicity study (OCSPP 870.4100a; OECD 452) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triazole alanine

Description:	White solid
Batch #:	MES 133/1
Purity:	98%
CAS # of TGAI:	86362-20-1
Expiration/Storage:	February 2012/<10°C (protected from light)
Structure:	

2. Vehicle: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar: Crl:WI(Han)
Age/weight at study initiation:	8 weeks old / 197-266 g (males) and 147-196 g (females) - Toxicology group 9 weeks old / 203-305 g (males) and 165-204 g (females) - Neurotoxicology group
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually housed in stainless steel, wire-mesh cages.
Diet:	Certified Rodent diet 5002 (Purina Mills), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** ≥12.63/hour **Photoperiod:** 12 hours light / 12 hours dark
Acclimation period:	Eight days

B. STUDY DESIGN

1. In-life dates: Start: April 21, 2009 End: April 21, 2010 (Toxicology group); May 4, 2010 (Neurotoxicology group)

2. Animal assignment/ dose levels: After Day 8 of acclimation, animals were assigned to their respective dose groups shown in Table 1 with a body weight stratification-based program.

TABLE 1. Study design. [a]

Nominal Concentration (ppm)	Month 1 (ppm)	Month 2 (ppm)	Month 3 (ppm)	Month 4-12 (ppm)	Target dose (mg/kg/day)	Average test substance intake		No. Rats/sex (Toxicology group)	No. Rats/sex (Neurotoxicology group)
						Male (mg/kg/day)	Female (mg/kg/day)		
0	0	0	0	0	0	0	0	20	10
600	339	468	531	571	30	28 ± 3	36 ± 3	20	10
2000	1171	1497	1874	1948	100	93 ± 10	120 ± 12	20	10
6000	3419	4684	5539	6026	300	278 ± 32	375 ± 31	20	10
20,000	11615	15516	19441	19408	1000	916 ± 121	1273 ± 131	20	10

a Data obtained from Text Tables B on page 16 and J on page 30 of MRID 52154804.

3. Dose selection rationale: Dose levels for this study were selected based on the results of a previously performed 90-day feeding study in the Wistar Rat with triazole alanine (MRID 00164107). Decreases in absolute body weight and body weight gain of 8% and 11%,

respectively, were observed in the 20,000 ppm males (approximating a limit dose of 1000 mg/kg/day). Based on these results, it was anticipated that a low and high dose of 600 and 20,000 ppm (approximating doses of 30 and 1000 mg/kg/day) would constitute a clear no-observed-effect level (NOEL) and a maximum tolerated dose, respectively, with the latter expected to induce a 10% decrease in absolute body weight over the course of the 1-year study. The intermediate dietary levels of 2000 and 6000 ppm (approximating doses of 100 and 300 mg/kg/day, respectively) were selected to serve as confirmation of dose response relationships.

4. **Diet preparation and analysis:** Test diets were prepared weekly by combining the required amounts of test item (not corrected for purity) with basal diet to achieve each dose level. Control diet was prepared in a similar manner, excluding the test item. The prepared test diets were stored at −15°C to −25°C.

Test item concentrations in the diet were adjusted monthly during the first three months of the study to achieve the target doses. Dietary concentrations used in the current study were based on male-only rat data from a previous study. Concentrations during the first month were based on the mean weekly food consumption by control male Wistar rats of similar age/weight from that previous study. Concentrations during Months 2 and 3 were determined from mean food consumption over the first three weeks of the preceding month. Additional adjustments of ± 5-10% from the calculated nominal dietary concentrations were allowed, if deemed necessary, to account for changes in growth rate or slight deviations from nominal values. Beginning at Month 4, the test item was administered at constant concentrations in the diet (0, 600, 2000, 6000, and 20,000 ppm).

Homogeneity and stability analyses with dietary preparations containing triazole alanine were conducted previously (Jensen, 2010; included as an appendix to the MRID)[1]. The distribution and homogeneity of the test substance in rodent diet was evaluated at nominal concentrations of 200 and 22,000 ppm. Triplicate samples were collected from the top, middle, and bottom of each prepared diet. Homogeneous distribution of the test item in the diet was defined as a coefficient of variation (%CV) of <10%. The stability of the test item in the diet preparations was evaluated after 7 days at room temperature (approximately 22°C) and after 7, 14, and 28 days frozen at approximately −23°C. Chemical stability in diet preparations was defined as the time that ≥80% of test item, relative to the initial concentration, was recoverable.

The test item concentrations in the various test diets were analytically verified from samples collected within one week of each of the first three weeks of the study and at approximately one-month intervals during the remainder of the in-life phase.

Results

Homogeneity (%CV): 1.6-6.1%

Stability (% of time 0): 90.4-100.7% (room temperature, 7 days); 97.0-100.6% (frozen, 28 days)

[1] Jensen, T.L. (2010) A homogeneity and stability study of triazole alanine in rodent ration. Unpublished report, Laboratory ID No. 09-H72-RH, Xenometrics LLC, Toxicology, Stilwell, KS.

Concentration (% of nominal): 94-105%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual doses was acceptable.

5. **Statistics:** Continuous data (*i.e.*, body weight, food consumption, clinical pathology, organ weight, etc.) were evaluated for homogeneity of variance by using Bartlett's test. Group means were generally analyzed with a one-way analysis of variance (ANOVA) followed by Dunnett's test; organ weights were evaluated by using an analysis of covariance (ANCOVA), with terminal body weight as the covariate. Frequency data (*i.e.*, clinical observational incidence, etc.) were evaluated with a Chi-Square test and/or Fisher's Exact test. For Bartlett's test, significance was denoted at a probability (p) level of <0.001. For all other statistical tests, significance was denoted at a probability (p) level of <0.05.

For the Functional Observational Battery (FOB) data, continuous data were first analyzed with a repeated-measures ANOVA, followed by a one-way ANOVA, if a significant interaction between dose group and test week was observed. For weeks with a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different relative to control. Categorical data collected in the FOB were analyzed in a similar manner by General Linear Modeling and Categorical Modeling (CATMOD) procedures, with *post-hoc* comparisons that used Dunnett's test and an Analysis of Contrasts, respectively.

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

C. METHODS

1. Observations

a. Cageside observations: Cageside evaluations for moribundity and mortality were conducted twice daily on weekdays and once daily on weekends and holidays, except for a single day (October 31, 2009) when no cageside observations were conducted.

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

2. Neurological evaluations: A functional observational battery (FOB) and motor/locomotor activity evaluations were conducted the week prior to treatment and during Months 3, 6, 9, and 12 on ten rats/sex/dose level. It was reported that the rats were observed without the observer having knowledge of the treatment group. Although observations were routinely conducted by the same observer throughout the study, conduct of some measurements (*e.g.*, grip strength and foot splay) occasionally involved a second technician. Inter-observer reliability was established previously to ensure the consistency of the results between technicians.

a. Functional observational battery (FOB): The durations of the home cage and open field observation periods were not reported. The FOB parameters were scored for severity as follows: not observed, slight, moderate to severe, and unknown. Males and females were tested separately, and the open field arenas were cleaned to reduce the residual scent from the opposite sex. Historical control data determined with treated and untreated rats to establish FOB sensitivity, reliability, and validity have been reported but were not provided. The CHECKED (X) parameters were examined.

	HOME CAGE AND OPEN FIELD OBSERVATIONS		MANIPULATIVE OBSERVATIONS		PHYSIOLOGIC OBSERVATIONS
X	Posture*	X	Ease of removal*	X	Temperature+
X	Involuntary clonic and tonic*	X	Ease of handling*	X	Body weight*
X	Palpebral closure*	X	Muscle tone	X	Pupil response*
X	Piloerection*	X	Approach response+	X	Pupil size*
X	Salivation*	X	Touch response		
X	Lacrimation*	X	Auditory response*		
X	Vocalizations+	X	Tail pinch response*		
X	Rearing+	X	Righting response+		
X	Urination*	X	Landing foot splay*		
X	Defecation*	X	Forelimb grip strength*		
X	Gait abnormalities*	X	Hindlimb grip strength*		
X	Arousal*		Proprioceptive response+		
X	Stereotypy*				
X	Bizarre behavior*				
X	Stains*				
X	Respiratory abnormalities+				
X	Exophthalmus*				
X	Tremors*				

* Required parameters; +Recommended parameters (based on EPA 870.6200, Neurotoxicity Screening Battery)

b. Motor/locomotor activity: Motor and locomotor activities were determined for individual animals over 60-minute periods, consisting of six 10-minute intervals, in a figure-eight maze. Each maze consisted of a series of inter-connected alleys converging on a central arena with eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure motion (each beam interruption was registered as an activity count). Broad-spectrum background noise was provided during testing to minimize acoustical variations. Uniformity of the light intensity over each of the mazes was verified daily.

Motor activity was measured as the number of beam interruptions that occurred during the test session, and locomotor activity was calculated by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated and interrupted another beam. Habituation was evaluated as a

decrement in activity during the test session. Activity assessments were conducted without the observer having knowledge of the treatment group. Animals were allowed to acclimate to the testing room with minimal disturbance prior to evaluation. Males and females were tested separately, and mazes were cleaned to reduce the residual scent from the opposite sex.

3. **Body weight and body weight gain:** Body weights were determined weekly during the first 13 weeks of treatment, every 4 ± 1 weeks for the remainder of the study, and prior to necropsy for calculation of organ (to body) weight ratios.

4. **Food consumption and compound intake:** Individual food consumption determinations were conducted weekly during the first 13 weeks of treatment and every 4 ± 1 weeks for the remainder of the study. Food consumption data were corrected, if necessary, for spilled food or clogged feeders.

   Mean daily intake of the test item was determined from food consumption, body weight, and diet analysis data. Intake was determined separately for Months 1, 2, and 3 and then for Months 4-12 due to the changing dietary concentrations for these periods.

5. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were conducted in a semi-darkened room. Examinations were conducted on all animals prior to randomization, and animals with ocular abnormalities were excluded. All surviving control and 20,000 ppm animals were examined just prior to study termination. Pupillary reflex was determined with a Finnoff transilluminator, and a mydriatic agent was applied to each eye. The cornea, aqueous humor, and lens were examined with a slit lamp microscope, and the vitreous humor, retina, choroid, and optic disc were examined with an indirect ophthalmoscope and a condensing lens.

6. **Hematology and clinical chemistry:** Blood samples were collected from the first ten fasted rats/sex/dose level at approximately 3, 6, and 12 months, and the same animals were sampled at each time point, if possible. Each animal was anesthetized with isoflurane and blood was collected from the retro-orbital sinus as appropriate (with or without anticoagulant). Differential blood counts were determined on all animals euthanized due to moribundity. 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	Heinz bodies
X	(Activated partial thromboplastin time)	X	Erythrocyte morphology
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for chronic rodent studies based on Guideline 870.4100.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Inorganic phosphorus	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose (fasting)*
	ENZYMES (more than 2 hepatic enzymes e.g., *)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase	X	Uric acid
X	Lactic acid dehydrogenase (LDH)		A/G ratio
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transpeptidase (GGTP)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for chronic rodent studies based on Guideline 870.4100.

7. Urinalysis: Urine samples were collected from the first ten non-fasted rats/sex/dose level at approximately 3, 6, and 12 months, and the same animals were sampled at each time point, if possible. Animals were housed overnight in cages fitted with urine collection trays. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin (bile pigments)
X	pH*	X	Blood / blood cells* (hemoglobin)
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Leukocytes
	Reducing substances	X	Urobilinogen

* Recommended for chronic rodent studies based on Guideline 870.4100.

8. Euthanasia and pathology

a. Toxicology group: After approximately 52 weeks of dosing, the first 20 surviving rats/sex/dose level were euthanized by carbon dioxide inhalation and exsanguination. Animals were subjected to a complete gross examination and gross lesions were saved from all animals, including those found dead or euthanized due to moribundity. The following CHECKED (X) tissues were collected for possible histological examination and the (XX) organs were also weighed. Paired organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach* [a]		Lymph nodes* [b]	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (w/optic nerve)*
X	Jejunum*	X	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland^
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+		
X	Tooth	XX	Epididymides*+		OTHER
X	Pancreas*	XX	Prostate*	X	Bone (sternum, rib, and femur)
	Gall bladder (not rat)*	X	Preputial gland^	X	Joint (femur/tibia)
	RESPIRATORY	X	Seminal vesicle*	X	Skeletal muscle
X	Trachea*	X	Cervix	X	Skin*
X	Lung*	X	Mammary gland*	X	All gross lesions and masses*
X	Nasal structure	XX	Ovaries*+	X	Harderian gland
X	Oral structure	X	Clitoral gland^	X	Zymbal's gland^
X	Nasopharynx*	XX	Uterus*+		
X	Larynx*	X	Vagina^		

* Required for chronic studies based on Guideline 870.4100.
\+ Organ weight required in chronic rodent studies.
^ Preserved for possible microscopic evaluation only.
a Glandular and non-glandular
b Cervical and mesenteric

Representative sections of collected tissues from the control and the 20,000 ppm animals were processed routinely and stained with hematoxylin and eosin (H&E) for examination by light microscopy; target organs and gross lesions were examined for all animals. Exceptions were the vagina and the exorbital/lacrimal, clitoral, preputial, and Zymbal's glands (excised and preserved for possible microscopic evaluation). Tissues in which compound-related effects were detected were examined from the 600, 2000, and 6000 ppm dose groups, as necessary, to establish no-observed-effect levels. Intestinal tissue samples were further investigated by staining with Von Kossa stain to identify mineralization/calcification.

b. **Neurotoxicology group:** All remaining animals (nominally 10/sex/dose level; the ten animals subjected to FOB/motor activity evaluations, if possible) were selected for perfusion and tissue collection for neurological evaluation. Animals were anesthetized with pentobarbital and perfused via the left ventricle with a sodium nitrite:phosphate buffer flush followed by a universal fixative. Representative sections of the tissues shown in the following table were processed for all control and 20,000 ppm animals. Tissues from control and high dose animals were evaluated microscopically, which were collected from the same animals used in the FOB and motor activity evaluations when possible. Tissues from animals treated at ≤6000 ppm were not evaluated, as treatment-related effects were not observed in the high-dose animals).

Tissue	Section	Method	Stain
Brain (levels 1-8)	Coronal	Paraffin	H&E
Spinal cord			
Cervical	Cross and longitudinal	Paraffin	H&E
Thoracic	Cross and longitudinal	Paraffin	H&E
Lumbar	Cross and longitudinal	Paraffin	H&E
Cauda Equina	Longitudinal	Paraffin	H&E
Spinal nerve root fiber and ganglia			
Dorsal and ventral, cervical (bilateral)	Longitudinal	Glycol methacrylate	Modified Lee's
Dorsal and ventral, lumbar (bilateral)	Longitudinal	Glycol methacrylate	Modified Lee's
Gasserian ganglion	Longitudinal	Glycol methacrylate	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 and longitudinal	Glycol methacrylate	Modified Lee's
Tibial (bilateral)	Cross/transverse and longitudinal	Glycol methacrylate	Modified Lee's
Sural (bilateral)	Cross/transverse and longitudinal	Glycol methacrylate	Modified Lee's

II. RESULTS

A. OBSERVATIONS

1. Mortality: There were no effects of treatment on mortality. Survival ratios in the combined Toxicology and Neurotoxicology groups (n = 30) were 30/30, 29/30, 27/30, 29/30, and 29/30 for males and 29/30, 29/30, 28/30, 29/30, and 29/30 for females at 0, 600, 2000, 6000, and 20,000 ppm, respectively.

2. Clinical signs of toxicity: There were no treatment-related effects on clinical signs.

B. NEUROLOGICAL EVALUATIONS

1. FOB: There were no treatment-related effects on any FOB parameters. Significant differences from the control were seen sporadically with posture, reaction to handling, approach response, and body temperature. However, as these observations were not consistent at each measured time point and often did not demonstrate a dose response, they are considered spurious findings.

2. Motor/locomotor activity: Summary motor and locomotor activity data are presented in Table 2, and interval motor and locomotor activity data are presented in Appendices I-IV at the end of this DER. There were no adverse treatment-related effects on motor or locomotor activity. The results showed decreases in activity for all groups over the course of the study that were attributed to normal decreases in activity with age and/or habituation.

TABLE 2. Summary mean (± SD) total (0-60 minutes) motor and locomotor activity count data in rats treated with triazole alanine in the diet for up to 12 months. [a]

Interval	Dose level (ppm and mg/kg/day [M/F])				
	0	600	1000	6000	20,000
	0/0	28/36	93/120	278/375	916/1273
Males – total motor activity					
Pretreatment	587 ± 210	559 ± 152	510 ± 114	559 ± 109	578 ± 125
Month 3	286 ± 175	252 ± 104	242 ± 102	255 ± 111	298 ± 108
Month 6	221 ± 71	230 ± 88	224 ± 86	276 ± 80	255 ± 63
Month 9	122 ± 60	127 ± 56	137 ± 49	151 ± 83	152 ± 84
Month 12	151 ± 47	160 ± 73	184 ± 53	172 ± 63	182 ± 87
Males – total locomotor activity					
Pretreatment	395 ± 160	363 ± 120	333 ± 86	396 ± 104	407 ± 102
Month 3	169 ± 93	160 ± 60	148 ± 62	157 ± 72	186 ± 63
Month 6	131 ± 38	137 ± 50	139 ± 62	160 ± 53	159 ± 40
Month 9	71 ± 36	74 ± 36	74 ± 31	83 ± 51	85 ± 49
Month 12	76 ± 30	81 ± 28	92 ± 23	95 ± 43	85 ± 38
Females – total motor activity					
Pretreatment	563 ± 137	579 ± 162	551 ± 131	497 ± 110	617 ± 240
Month 3	333 ± 71	393 ± 76	371 ± 66	368 ± 128	490 ± 255
Month 6	310 ± 130	371 ± 120	378 ± 91	343 ± 112	349 ± 100
Month 9	292 ± 88	286 ± 43	304 ± 62	266 ± 83	308 ± 110
Month 12	217 ± 91	240 ± 33	247 ± 96	234 ± 88	262 ± 77
Females – total locomotor activity					
Pretreatment	353 ± 94	374 ± 84	358 ± 92	306 ± 101	392 ± 160
Month 3	189 ± 42	238 ± 66	209 ± 49	205 ± 74	281 ± 128
Month 6	179 ± 73	228 ± 82	214 ± 59	202 ± 74	203 ± 60
Month 9	159 ± 47	190 ± 46	167 ± 47	174 ± 71	189 ± 71
Month 12	120 ± 62	154 ± 36	137 ± 51	139 ± 68	159 ± 63

a Data were obtained from pages 177-178 and 189-190 of MRID 52154804; n = 10.

C. **BODY WEIGHTS AND BODY WEIGHT GAINS:** Selected mean body weight and body weight gain data for the Toxicology and Neurotoxicology group rats are presented in Tables 3a and 3b, respectively. There were no treatment-related effects on body weights or body weight gains in the Toxicology or Neurotoxicology group rats. The increase ($p<0.05$) in overall body weight gain of 21% in the 600 ppm females (36 mg/kg/day) was unrelated to dose and not considered adverse.

TABLE 3a. Selected mean (± SD) body weights (g) and mean body weight gains (g) in the Toxicology group rats treated with triazole alanine in the diet for up to 12 months [a]

Study Day	Dose level (ppm and mg/kg/day [M/F])				
	0	600	1000	6000	20,000
	0/0	28/36	93/120	278/375	916/1273
Males					
0	237 ± 16	235 ± 17	236 ± 16	237 ± 18	236 ± 13
168	482 ± 45	479 ± 48	486 ± 37	486 ± 34	493 ± 40 [b]
350	566 ± 59	562 ± 60 [b]	565 ± 57	577 ± 46 [b]	574 ± 51 [b]
Overall BWG (0-350)	328 ± 48	327 ± 51 [b]	329 ± 49	339 ± 35 [b]	339 ± 44 [b]
Females					
0	168 ± 9	168 ± 8	168 ± 8	167 ± 9	166 ± 12
168	258 ± 19 [b]	263 ± 22	265 ± 18	254 ± 16	251 ± 21
350	292 ± 32 [b]	318 ± 41	312 ± 42 [b]	285 ± 31	290 ± 35
Overall BWG (0-350)	124 ± 27 [b]	150 ± 38* (↑21)	144 ± 39 [b]	119 ± 26	124 ± 31

a Data were obtained from pages 207-210 and 215-218 and pages 232 and 240 of MRID 52154804; n = 20, unless otherwise noted. Percentage differences from control (calculated by the Reviewers) are presented in parentheses.
b n = 19.
* Significantly different from control; $p<0.05$.

TABLE 3b. Selected mean (± SD) body weights (g) and mean body weight gains (g) in Neurotoxicology group rats treated with triazole alanine in the diet for up to 12 months [a]

Study Day	Dose level (ppm and mg/kg/day [M/F])				
	0	600	1000	6000	20,000
	0/0	28/36	93/120	278/375	916/1273
Males					
0	271 ± 21	268 ± 31	278 ± 18	268 ± 19	271 ± 18
161	484 ± 47	489 ± 67	512 ± 58	466 ± 37	472 ± 47
357	561 ± 57	565 ± 79	624 ± 98 [b]	537 ± 49	553 ± 53
Overall BWG (0-357)	290 ± 44	297 ± 52	345 ± 79 [b]	269 ± 37	283 ± 42
Females					
0	188 ± 11	185 ± 11	183 ± 11	187 ± 7	179 ± 9
161	266 ± 28	271 ± 27	262 ± 21	257 ± 18	252 ± 18
357	331 ± 52	326 ± 52	313 ± 41 [c]	292 ± 24	297 ± 20
Overall BWG (0-357)	144 ± 46	141 ± 45	129 ± 34 [c]	105 ± 20	118 ± 17

a Data were obtained from 211-214 and 219-222 and pages 236 and 244 of MRID 52154804; n = 10, unless otherwise noted.
b n = 8
c n = 9

D. FOOD CONSUMPTION

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

2. **Compound intake:** Compound intake estimates are reported in Table 1. The calculated values generally approximated the target doses of 30, 100, 300, and 1000 mg/kg/day.

E. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related effects on ophthalmoscopic findings.

F. BLOOD ANALYSES

1. **Hematology:** There were no treatment-related effects on hematology or coagulation parameters. Any observed significant ($p<0.05$) changes were within historical control ranges, did not occur in a manner related to dose, or were not considered toxicologically or biologically relevant.

2. **Clinical chemistry:** Two changes in clinical chemistry parameters may have been related to treatment but were not considered adverse or biologically relevant because they only occurred in males and were not consistent over time. Serum potassium concentrations were decreased ($p<0.05$) by 6 to 16% in the ≥600 ppm males (≥28 mg/kg/day) at the 6-month interval with no dose response from 1000 to 20,000 ppm (93 mg/kg/day to 916 mg/kg/day). These decreases were considered transient because there was no dose response, and these changes did not occur at the 3- or 12-month intervals. In addition, serum glucose concentrations were increased ($p<0.05$) by 14-18% in the ≥1000 ppm males (≥93 mg/kg/day) at the 6-month interval. Although these values exceeded the historical control range, they were considered transient because the increases occurred in a manner unrelated to dose and there were no changes at the 3- or 12-month intervals.

   All other observed significant ($p<0.05$) changes were within historical control ranges, did not occur in a manner related to dose, or were not considered toxicologically or biologically relevant.

G. URINALYSIS: There were no treatment-related effects on urinalysis parameters.

H. EUTHANASIA AND PATHOLOGY

1. Organ weight: There were no treatment-related effects on absolute or relative (to body) organ weights in the Toxicology or Neurotoxicology group animals. Absolute brain weight was increased ($p<0.05$) by 4% in the 20,000 ppm Toxicology group males (916 mg/kg/day) with no changes in relative (to body) brain weight. Relative (to body) liver weights were increased ($p<0.05$) by 11% and 10% in the 6000 and 20,000 ppm Toxicology group females (375 mg/kg/day and 1273 mg/kg/day), respectively, but these changes demonstrated no relationship to dose. Therefore, these changes were not considered treatment related. All other statistically significant ($p<0.05$) changes in organ weights demonstrated no relationship to dose.

2. Gross pathology: There were no treatment-related effects on gross pathology in the Toxicology or Neurotoxicology group animals.

3. Microscopic pathology: No treatment-related differences in microscopic findings were observed in the Toxicology or Neurotoxicology group animals.

Intestinal mucosal mineralization (calcification; confirmed by staining with Von Kossa stain) was observed in the cecum of both sexes in the Toxicology group, with similar incidences in the control and 20,000 ppm animals (916/1273 mg/kg/day). Mineralization in the colon was increased ($p<0.05$) in the20,000 ppm (916 mg/kg/day) males (8/20 treated vs. 1/20 control) but severity was decreased (1.1 treated vs. 2.0 control); incidence in females was similar to that of the controls. Additionally, mineralization was not increased in the ileum or rectum of treated animals and was not present in the duodenum or jejunum of the control and 20,000 ppm animals. The statistically significant changes in males are not considered adverse or biologically relevant because (i) severity was decreased compared to controls; (ii) there were no concomitant clinical signs of intestinal disturbance/function; and (iii) mineralization is a common background lesion in the aging rat.

Optic nerve degeneration was increased (8/20 treated vs. 3/20 control; $p<0.05$) with similar severity in the 20,000 ppm females (1273 mg/kg/day). However, the degeneration was always unilateral, with no similar change in the optic nerves of neurotoxicity group females that were not bled. Therefore, this microscopic lesion was considered associated with the retro-orbital bleeding technique and not related to treatment.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Through approximately one year of continuous and repeated dietary exposure to the test substance up to the limit dose (achieved at approximately 916 and 1273 mg/kg/day for males and females, respectively), there were no adverse effects observed for either sex in any parameter measured in this study (which included mortality, clinical observations, body weight, body weight change, food consumption, functional observational battery, motor activity, ophthalmology exams, serum chemistry, hematology, urinalysis, gross pathology, organ weights, and microscopic pathology). Therefore, based on a lack of adverse compound-related effects, the systemic chronic toxicity and neurotoxicity no-observed-adverse-effect level (NOAEL) in this study

was the target dose of 1000 mg/kg/day (20,000 ppm), which corresponded to achieved dose levels of 916 and 1273 mg/kg/day for male and female rats, respectively.

B. REVIEWER COMMENTS: The Reviewers agree with the Investigators' conclusion.

No treatment-related effects were observed on mortality, clinical observations, neurological evaluations (FOB and motor/locomotor activity), body weight and body weight gain, food consumption, ophthalmoscopic evaluation, serum chemistry, hematology, urinalysis, organ weights, and macroscopic and microscopic pathology.

The LOAEL was not determined. The NOAEL is 20,000 ppm (equivalent to 916/1273 mg/kg/day in males/females, approximating the limit dose).

C. STUDY DEFICIENCIES: There were no study deficiencies.

Appendix I. Interval motor activity data for male rats administered triazole alanine in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	121 ± 32	118 ± 40	120 ± 53	102 ± 59	67 ± 43	61 ± 53
30 MG/KG	125 ± 37	114 ± 46	117 ± 41	89 ± 29	68 ± 32	45 ± 32
100 MG/KG	115 ± 26	98 ± 21	100 ± 21	83 ± 31	66 ± 36	48 ± 30
300 MG/KG	135 ± 29	119 ± 21	106 ± 26	87 ± 29	60 ± 35	52 ± 32
1000 MG/KG	125 ± 18	123 ± 30	106 ± 25	107 ± 33	68 ± 36	49 ± 36

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	97 ± 32	67 ± 27	45 ± 43	35 ± 37	25 ± 34	17 ± 30
30 MG/KG	80 ± 24	69 ± 34	30 ± 24	30 ± 27	22 ± 23	20 ± 23
100 MG/KG	82 ± 26	54 ± 18	39 ± 24	31 ± 22	14 ± 16	22 ± 24
300 MG/KG	103 ± 22	62 ± 26	37 ± 29	24 ± 23	19 ± 22	10 ± 12
1000 MG/KG	105 ± 19	71 ± 21	45 ± 27	39 ± 26	22 ± 21	16 ± 15

b) Month 3

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	86 ± 31	60 ± 27	32 ± 17	22 ± 22	13 ± 13	9 ± 11
30 MG/KG	93 ± 32	58 ± 28	37 ± 25	23 ± 16	12 ± 15	8 ± 10
100 MG/KG	91 ± 34	55 ± 21	32 ± 19	23 ± 12	12 ± 11	11 ± 13
300 MG/KG	110 ± 43	56 ± 22	36 ± 14	30 ± 14	25 ± 19	19 ± 16
1000 MG/KG	105 ± 27	60 ± 17	38 ± 14	25 ± 12	16 ± 15	12 ± 9

c) Month 6

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	60 ± 17	22 ± 17	14 ± 16	9 ± 11	7 ± 9	10 ± 15
30 MG/KG	65 ± 21	26 ± 16	14 ± 14	11 ± 12	7 ± 12	5 ± 8
100 MG/KG	68 ± 14	24 ± 17	19 ± 16	9 ± 11	11 ± 12	6 ± 9
300 MG/KG	73 ± 40	24 ± 19	18 ± 15	18 ± 13	11 ± 10	8 ± 12
1000 MG/KG	80 ± 28	29 ± 27	16 ± 17	17 ± 20	4 ± 6	7 ± 11

d) Month 9

Appendix I. Interval motor activity data for male rats administered triazole alanine in the diet (continued).

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	56 ± 21	37 ± 14	23 ± 13	13 ± 7	13 ± 8	9 ± 12
30 MG/KG	67 ± 19	37 ± 16	17 ± 15	18 ± 23	13 ± 11	7 ± 10
100 MG/KG	76 ± 18	39 ± 17	27 ± 9	18 ± 11	14 ± 14	11 ± 12
300 MG/KG	66 ± 23	33 ± 13	23 ± 13	19 ± 11	11 ± 12	21 ± 20
1000 MG/KG	81 ± 33	37 ± 17	21 ± 14	22 ± 26	13 ± 17	8 ± 11

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 04/21/2009
Number of Rats/Group: 10/0 MG/KG 10/30 MG/KG 8 /100 MG/KG 10/300 MG/KG 10/1000 MG/KG

e) Month 12

Data copied from pages 179-183 of MRID 51254804

Appendix II. Interval locomotor activity data for male rats administered triazole alanine in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	90 ± 27	82 ± 31	86 ± 44	63 ± 47	41 ± 30	35 ± 35
30 MG/KG	86 ± 23	80 ± 31	80 ± 31	57 ± 24	37 ± 28	23 ± 22
100 MG/KG	81 ± 16	69 ± 22	68 ± 19	53 ± 22	37 ± 26	25 ± 22
300 MG/KG	98 ± 24	88 ± 22	77 ± 22	61 ± 28	44 ± 29	28 ± 26
1000 MG/KG	91 ± 13	90 ± 28	78 ± 26	75 ± 27	47 ± 27	27 ± 26

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	61 ± 20	42 ± 14	28 ± 28	18 ± 20	13 ± 18	8 ± 12
30 MG/KG	56 ± 17	47 ± 24	18 ± 15	16 ± 14	12 ± 14	11 ± 13
100 MG/KG	55 ± 16	33 ± 11	24 ± 15	17 ± 12	8 ± 10	11 ± 12
300 MG/KG	65 ± 18	39 ± 19	22 ± 16	14 ± 13	11 ± 13	6 ± 8
1000 MG/KG	70 ± 15	45 ± 12	28 ± 16	23 ± 14	12 ± 11	9 ± 10

b) Month 3

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	58 ± 10	34 ± 14	17 ± 11	12 ± 14	6 ± 7	4 ± 6
30 MG/KG	60 ± 21	34 ± 18	21 ± 13	13 ± 10	6 ± 9	4 ± 6
100 MG/KG	58 ± 23	33 ± 11	19 ± 13	14 ± 9	8 ± 9	8 ± 10
300 MG/KG	67 ± 24	33 ± 14	19 ± 10	17 ± 8	13 ± 11	10 ± 9
1000 MG/KG	71 ± 17	36 ± 10	24 ± 9	14 ± 7	8 ± 7	7 ± 6

c) Month 6

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	40 ± 15	12 ± 9	7 ± 8	4 ± 5	5 ± 6	3 ± 4
30 MG/KG	40 ± 16	14 ± 10	7 ± 8	6 ± 7	4 ± 7	2 ± 4
100 MG/KG	40 ± 12	12 ± 10	9 ± 7	4 ± 5	6 ± 6	3 ± 5
300 MG/KG	43 ± 28	13 ± 10	10 ± 8	7 ± 6	7 ± 6	4 ± 6
1000 MG/KG	51 ± 19	16 ± 14	8 ± 10	8 ± 8	1 ± 1	3 ± 4

d) Month 9

Appendix II. Interval locomotor activity data for male rats administered triazole alanine in the diet (continued).

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	36 ± 13	18 ± 9	10 ± 7	4 ± 2	5 ± 5	3 ± 4
30 MG/KG	38 ± 9	20 ± 7	7 ± 6	6 ± 6	8 ± 6	3 ± 5
100 MG/KG	41 ± 9	20 ± 8	12 ± 2	9 ± 5	5 ± 3	5 ± 4
300 MG/KG	40 ± 15	17 ± 8	11 ± 8	11 ± 7	6 ± 6	10 ± 10
1000 MG/KG	41 ± 17	17 ± 9	9 ± 7	9 ± 9	5 ± 5	4 ± 4

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 04/21/2009
Number of Rats/Group: 10/0 MG/KG 10/30 MG/KG 8 /100 MG/KG 10/300 MG/KG 10/1000 MG/KG

e) Month 12

Data copied from pages 184-188 of MRID 51254804

Appendix III. Interval motor activity data for female rats administered triazole alanine in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	129 ± 25	105 ± 28	104 ± 34	94 ± 31	80 ± 31	51 ± 34
30 MG/KG	128 ± 39	116 ± 35	101 ± 30	98 ± 21	79 ± 42	57 ± 39
100 MG/KG	121 ± 17	105 ± 23	94 ± 30	87 ± 29	89 ± 38	57 ± 39
300 MG/KG	116 ± 24	85 ± 19	81 ± 26	82 ± 40	72 ± 34	62 ± 37
1000 MG/KG	134 ± 59	114 ± 44	108 ± 59	96 ± 39	87 ± 44	78 ± 46

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	99 ± 27	75 ± 24	58 ± 20	44 ± 13	34 ± 19	23 ± 12
30 MG/KG	110 ± 18	85 ± 19	64 ± 26	59 ± 22	34 ± 30	42 ± 26
100 MG/KG	96 ± 13	78 ± 14	61 ± 20	57 ± 21	49 ± 33	30 ± 19
300 MG/KG	103 ± 25	72 ± 28	53 ± 26	73 ± 22	39 ± 33	29 ± 28
1000 MG/KG	129 ± 60	90 ± 54	86 ± 46	83 ± 46	59 ± 46	43 ± 36

b) Month 3

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	102 ± 39	64 ± 35	44 ± 21	37 ± 25	35 ± 16	29 ± 19
30 MG/KG	112 ± 29	61 ± 18	52 ± 23	50 ± 27	54 ± 31	42 ± 28
100 MG/KG	100 ± 18	64 ± 19	53 ± 14	57 ± 26	56 ± 35	49 ± 21
300 MG/KG	108 ± 37	65 ± 20	51 ± 18	43 ± 15	44 ± 26	33 ± 22
1000 MG/KG	105 ± 27	65 ± 24	58 ± 21	39 ± 25	48 ± 18	33 ± 12

c) Month 6

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	99 ± 31	62 ± 16	43 ± 18	33 ± 19	30 ± 13	25 ± 17
30 MG/KG	94 ± 17	59 ± 14	37 ± 7	37 ± 18	26 ± 13	34 ± 13
100 MG/KG	97 ± 20	50 ± 13	55 ± 14	45 ± 25	27 ± 18	30 ± 19
300 MG/KG	98 ± 18	48 ± 16	36 ± 17	35 ± 22	27 ± 19	23 ± 16
1000 MG/KG	101 ± 31	55 ± 14	46 ± 21	41 ± 25	36 ± 21	29 ± 32

d) Month 9

Appendix III. Interval motor activity data for female rats administered triazole alanine in the diet (continued).

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	73 ± 21	43 ± 19	27 ± 19	29 ± 20	19 ± 12	26 ± 21
30 MG/KG	84 ± 15	48 ± 12	29 ± 10	30 ± 12	23 ± 11	27 ± 17
100 MG/KG	79 ± 30	42 ± 17	39 ± 23	32 ± 20	30 ± 21	25 ± 14
300 MG/KG	84 ± 23	46 ± 17	31 ± 18	26 ± 20	24 ± 15	23 ± 15
1000 MG/KG	82 ± 23	47 ± 13	40 ± 16	32 ± 19	32 ± 14	29 ± 15

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 04/21/2009
Number of Rats/Group: 10/0 MG/KG 10/30 MG/KG 8 /100 MG/KG 10/300 MG/KG 10/1000 MG/KG

e) Month 12

Data copied from pages 191-195 of MRID 51254804

Appendix IV. Interval locomotor activity data for female rats administered triazole alanine in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	85 ± 13	67 ± 18	66 ± 29	62 ± 23	47 ± 22	27 ± 17
30 MG/KG	87 ± 17	71 ± 17	65 ± 18	65 ± 16	50 ± 28	37 ± 26
100 MG/KG	88 ± 14	68 ± 17	58 ± 24	57 ± 25	53 ± 27	34 ± 23
300 MG/KG	75 ± 14	51 ± 16	49 ± 28	51 ± 28	44 ± 25	36 ± 24
1000 MG/KG	89 ± 35	73 ± 27	67 ± 44	61 ± 34	52 ± 30	49 ± 34

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	62 ± 11	44 ± 14	31 ± 18	24 ± 9	16 ± 11	12 ± 8
30 MG/KG	72 ± 12	53 ± 18	38 ± 18	33 ± 17	19 ± 20	24 ± 18
100 MG/KG	66 ± 9	47 ± 10	35 ± 18	30 ± 15	18 ± 14	14 ± 13
300 MG/KG	62 ± 12	42 ± 16	30 ± 17	36 ± 17	21 ± 19	14 ± 15
1000 MG/KG	72 ± 18	54 ± 31	53 ± 27	46 ± 30	32 ± 28	25 ± 20

b) Month 3

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	63 ± 20	37 ± 21	25 ± 11	22 ± 16	19 ± 10	13 ± 7
30 MG/KG	75 ± 13	40 ± 15	31 ± 14	31 ± 20	31 ± 24	20 ± 14
100 MG/KG	66 ± 9	35 ± 7	29 ± 11	32 ± 19	28 ± 23	24 ± 13
300 MG/KG	67 ± 19	39 ± 15	30 ± 14	26 ± 14	22 ± 17	17 ± 13
1000 MG/KG	69 ± 14	39 ± 13	31 ± 14	23 ± 14	27 ± 13	14 ± 8

c) Month 6

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	59 ± 11	30 ± 9	22 ± 11	20 ± 14	15 ± 8	13 ± 9
30 MG/KG	70 ± 11	38 ± 10	24 ± 5	23 ± 14	17 ± 11	18 ± 10
100 MG/KG	64 ± 6	28 ± 9	26 ± 13	21 ± 15	14 ± 10	14 ± 11
300 MG/KG	68 ± 18	30 ± 12	24 ± 13	22 ± 20	15 ± 11	16 ± 13
1000 MG/KG	68 ± 16	33 ± 7	26 ± 14	26 ± 18	21 ± 15	17 ± 23

d) Month 9

Appendix IV. Interval locomotor activity data for female rats administered triazole alanine in the diet (continued).

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 MG/KG	47 ± 13	22 ± 15	15 ± 10	14 ± 13	11 ± 8	12 ± 14
30 MG/KG	57 ± 8	30 ± 5	16 ± 6	18 ± 8	15 ± 9	18 ± 14
100 MG/KG	50 ± 13	24 ± 9	21 ± 10	16 ± 14	16 ± 12	10 ± 6
300 MG/KG	54 ± 17	29 ± 16	17 ± 14	14 ± 12	13 ± 9	12 ± 12
1000 MG/KG	57 ± 18	30 ± 11	21 ± 8	17 ± 13	18 ± 13	15 ± 10

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 04/21/2009
Number of Rats/Group: 10/0 MG/KG 10/30 MG/KG 8 /100 MG/KG 10/300 MG/KG 10/1000 MG/KG

e) Month 12

Data copied from pages 196-200 of MRID 51254804

DATA EVALUATION RECORD

TRIAZOLE ACETIC ACID (METABOLITE OF TRIAZOLE)

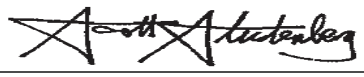
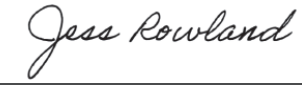
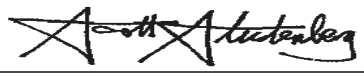
Study Type: OCSPP 870.3100/870.6200; Subchronic Oral Toxicity/Neurotoxicity Screening Study in Rats

EPA Contract No. 68HERC22D0017
Task Assignment No. 5540-2.1-022 (MRID 52154805)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Prepared by

1151 Lost Creek Boulevard
Austin, TX 78746

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/24/2023
Secondary Reviewer: Jess Rowland, M.S.	Signature: Date:	 06/02/2023
Quality Assurance: Michael E. Viana, Ph.D.	Signature: Date:	 06/05/2023
Project Manager: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 06/06/2023

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by PB&A/CSS Joint Venture personnel**.** Contractor's role did not include establishing Agency policy.

EPA Reviewer: Jeremy Leonard, PhD **Signature:**
Risk Assessment Branch IV, HED (7509P) **Date:** 06/28/2023
EPA Secondary Reviewer: Megan Stallard, PhD **Signature:**
Risk Assessment Branch IV, HED (7509P) **Date:** 06/29/2023
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral (feeding) toxicity and screening neurotoxicity study in rats; OCSPP 870.3100 [§82-1a] and 870.6200 [§81-8]; OECD 408 & 424.

PC CODE: 600082 **DP BARCODE:** Not provided

TXR #: 0058589

TEST MATERIAL (PURITY): Triazole acetic acid (98.5% a.i.)

SYNONYMS: Amitrole-triazolylacetic acid; 1,2,4-triazol-1-yl-acetic acid

CITATION: Wahle, B.S. (2010) A combined subchronic toxicity / neurotoxicity screening study in the Wistar rat with triazole acetic acid (TAA). Xenometrics, LLC, Stilwell, Kansas. Study Number: 07-S72-MF, December 17, 2010. MRID 52154805. Unpublished.

SPONSOR: Triazole Derivative Metabolite Group, U.S. Triazole Task Force (Agent), c/o McDermott, Will & Emery, 600 13th St., N.W., Washington, D.C.

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 combined subchronic oral toxicity and subchronic neurotoxicity screening study (MRID 51254805), groups of 16 rats/sex were administered triazole acetic acid (98.5% a.i.; Batch #RDL 211-8-2) in the diet at target dose levels of 0, 100, 500, and 1000 mg/kg/day (concentrations were adjusted monthly during the study; overall intake equivalent to 94/119, 495/627, or 1002/1181 mg/kg/day in males/females) for up to 13 weeks. All rats were evaluated for body weight and body weight gain, food consumption, and ophthalmoscopy. Rats selected for toxicology evaluations (typically the first ten rats/sex/dose level were evaluated for serum chemistry, hematology, urinalysis, organ weights, and macroscopic and microscopic pathology). The last ten rats/sex underwent neurological evaluations (*i.e.*, functional observational battery [FOB] and motor/locomotor activity assessments). These were considered the Toxicology group and Neurotoxicology group,

respectively. At the end of the study, animals from the Neurotoxicology group were also used to determine absolute and relative (to body) brain weights and to undergo macroscopic and microscopic evaluation of selected neurological tissues.

There were no treatment-related effects on mortality, clinical observations, neurological evaluations (FOB and motor/locomotor activity), body weight and body weight gain, food consumption, ophthalmoscopic evaluation, serum chemistry, hematology, urinalysis, organ weights, and macroscopic and microscopic pathology.

The LOAEL was not determined. The NOAEL is the nominal dose of 1000 mg/kg/day (equivalent to 1002/1181 mg/kg/day in males/females, achieving the limit dose).

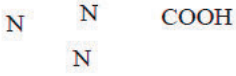
This study is classified **Acceptable/Guideline** for a subchronic oral toxicity and neurotoxicity screening battery (OCSPP 870.3100 and 870.6200; OECD 408 and 424) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triazole acetic acid

Description:	Light beige solid
Batch #:	RDL 211-8-2
Purity:	98.5%
CAS # of TGAI:	28711-29-7
Expiration/Storage:	January 29, 2010 / 18-25°C, protected from light
Structure:	

2. Vehicle: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar: Crl:WI(Han)
Age/weight at study initiation:	Approximately 9 weeks old / 122-278 g (males)* and 140-179 g (females) *(211-278 [males] if the male weighing 122 g was excluded)
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually housed in stainless steel, wire-mesh cages.
Diet:	Certified Rodent diet 5002 (Purina Mills), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** Approximate minimum of 10/hour **Photoperiod:** 12 hours light / 12 hours dark
Acclimation period:	Seven days (receipt to release for study; 10 additional days prior to study initiation)

B. STUDY DESIGN

1. In-life dates: Start: May 21, 2009 End: August 19, 2009

2. Animal assignment/ dose levels: After Day 7 of acclimation, animals were assigned to their respective dose groups shown in Table 1 with a body weight stratification-based program.

TABLE 1. Study design. [a]

Target dose (mg/kg/day)	Month 1 (ppm)	Month 2 (ppm)	Month 3 (ppm)	Average test substance intake[b]		No. Rats/sex
				Male (mg/kg/day)	Female (mg/kg/day)	
0	0	0	0	0	0	16
100	1205	6273	12220	94 ± 8	119 ± 10	16
500	1474	7327	14975	495 ± 52	627 ± 64	16
1000	1799	9499	17309	1002 ± 71	1181 ± 83	16

a Data obtained from Text Tables A on page 16 and J on page 29 of MRID 51254805.
b Active ingredient intake results are presented as the grand mean of average weekly intake for Weeks 1-13. Each dose calculation represents the grand mean + SD of 12 separate calculations using average food consumption determinations taken each week.

Within each treatment group, all surviving animals were evaluated for clinical observations, body weight, food consumption, and ophthalmoscopic parameters. Functional observational battery (FOB) and motor/locomotor activity evaluations were conducted on the last ten

surviving animals/sex, and hematology, clinical chemistry, and urinalysis evaluations were conducted on the first ten surviving animals/sex at each dose level. At scheduled euthanasia, the first ten surviving animals were considered the Toxicology group, with determination of organ weights and microscopic pathology evaluations. The remaining animals per dose level (n = 5 or 6) were considered the Neurotoxicology group, with evaluation of brain weights and microscopic evaluations on selected neurological tissues only. It was noted that, as two of the nominal 100 mg/kg/day females died, the surviving animals were allocated such that there were nine animals in the Toxicity group and five animals in the Neurotoxicology group.

3. **Dose selection rationale:** Dose levels for this study were selected on the results of a previous 28-day toxicity study in the Wistar rat with triazole acetic acid (Wahle, 2010)[1]. Treatment-related effects were not observed up to a dietary concentration of 13,000 ppm (approximating the limit dose of 1000 mg/kg/day). Therefore, a target high-dose level of 1000 mg/kg/day was selected, with lower target dose levels of 100 and 500 mg/kg/day selected to evaluate any dose-response relationships.

4. **Diet preparation and analysis:** Test diets were prepared weekly by combining the required amounts of test item (not corrected for purity) with basal diet to achieve each dose level. Control diet was prepared in a similar manner, excluding the test item. The prepared test diets were stored frozen until presented to the animals the following week.

   Test item concentrations in the diet were adjusted monthly to achieve the target dose levels. Dietary concentrations were based on male rat data only, from a previous study using male Wistar rats of similar age and weight. Concentrations during the first month were based on the mean weekly food consumption by control rats. Concentrations during Months 2 and 3 were determined from mean food consumption over the first three weeks of the preceding month. Additional adjustments of ± 5-10% from the calculated nominal dietary concentrations were allowed, if deemed necessary, to account for changes in growth rate or slight deviations from nominal values.

   Homogeneity and stability analyses with dietary preparations containing triazole acetic acid were conducted previously (Moore, 2010; included as an appendix to the MRID)[2]. The distribution and homogeneity of the test item in rodent diet was evaluated at nominal concentrations of 1000 and 18,000 ppm. Triplicate samples were collected from the top, middle, and bottom of each prepared diet. Homogeneous distribution of the test item in the diet was defined as a relative standard deviation (%RSD) of <10%. The stability of the test item in the diet preparations was evaluated after 7 days at room temperature and after 7, 14, and 28 days frozen. Chemical stability in diet preparations was defined as the time that ≥85% of test item, relative to the initial concentration, was recoverable.

   The test item concentrations in the various test diets were analytically verified from samples collected within one week of each of the first three weeks of the study and at approximately

[1] Wahle, B.S. (2010) A 28-day toxicity testing study in the wistar rat with triazole acetic acid (TAA). Unpublished Report, Laboratory ID No. 07-S72-ME, Xenometrics LLC, Stilwell, KS. No MRID available.

[2] Moore, S.M. (2010) Homogeneity and stability of triazole acetic acid (TAA) mixed in rodent ration diet. Unpublished report No. TXAAY457A, Bayer CropScience LP, Bioanalytical, Residue and Environmental Chemistry Group, Stilwell, KS.

1 ± 0.5 months of the experimental midpoint and end of the in-life phase (Weeks 10 and 14, respectively).

Results

Homogeneity (%RSD): 2.1-3.3%

Stability (% of time 0): 95-96% (room temperature, 7 days); 97-100% (frozen, 28 days)

Concentration (% of nominal): 98-105%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual doses was acceptable.

5. Statistics: Continuous data (*i.e.*, body weight, food consumption, clinical pathology, organ weight, etc.) were evaluated for homogeneity of variance by using Bartlett's test. Group means were generally analyzed with a one-way analysis of variance (ANOVA) followed by Dunnett's test. Frequency data (*i.e.*, clinical observational incidence, etc.) were evaluated with a Chi-Square test and/or Fisher's Exact test. For Bartlett's test, significance was denoted at a probability (p) level of <0.001. For all other statistical tests, significance was denoted at a probability (p) level of <0.05.

For the FOB data, continuous data were first analyzed with a repeated-measures ANOVA, followed by a one-way ANOVA, if a significant interaction between dose group and test week was observed. For weeks with a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different relative to control. Categorical data collected in the FOB were analyzed in a similar manner by General Linear Modeling and Categorical Modeling (CATMOD) procedures, with *post-hoc* comparisons that used Dunnett's test and an Analysis of Contrasts, respectively.

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

C. METHODS

1. Observations

a. Cageside observations: Cageside evaluations for moribundity and mortality were conducted twice daily on weekdays and once daily on weekends and holidays.

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

2. **Neurological evaluations:** A functional observational battery (FOB) and motor/locomotor activity evaluations were conducted the week prior to treatment and during Weeks 2, 4, 8, and 13 on the last ten rats/sex/dose level. It was reported that the rats were observed without the observer having knowledge of the treatment group. Although observations were routinely conducted by the same observer throughout the study, conduct of some measurements (*e.g.*, grip strength and foot splay) occasionally involved a second technician. Inter-observer reliability was established previously to ensure the consistency of the results between technicians.

a. **Functional observational battery (FOB):** The durations of the home cage and open field observation periods were not reported. The FOB parameters were scored for severity as follows: not observed, slight, moderate to severe, and unknown. Males and females were tested separately, and the open field arenas were cleaned to reduce the residual scent from the opposite sex. Historical control data determined with treated and untreated rats to establish FOB sensitivity, reliability, and validity have been reported but were not provided. The CHECKED (X) parameters were examined.

	HOME CAGE AND OPEN FIELD OBSERVATIONS		MANIPULATIVE OBSERVATIONS		PHYSIOLOGIC OBSERVATIONS
X	Posture*	X	Ease of removal*	X	Temperature+
X	Involuntary clonic and tonic*	X	Ease of handling*	X	Body weight*
X	Palpebral closure*	X	Muscle tone	X	Pupil response*
X	Piloerection*	X	Approach response+	X	Pupil size*
X	Salivation*	X	Touch response		
X	Lacrimation*	X	Auditory response*		
X	Vocalizations+	X	Tail pinch response*		
X	Rearing+	X	Righting response+		
X	Urination*	X	Landing foot splay*		
X	Defecation*	X	Forelimb grip strength*		
X	Gait abnormalities*	X	Hindlimb grip strength*		
X	Arousal*		Proprioceptive response+		
X	Stereotypy*				
X	Bizarre behavior*				
X	Stains*				
X	Respiratory abnormalities+				
X	Exophthalmos*				
X	Tremors*				

* Required parameters; +Recommended parameters (based on EPA 870.6200, Neurotoxicity Screening Battery)

b. **Motor/locomotor activity:** Motor and locomotor activities were determined for individual animals over 60-minute periods, consisting of six 10-minute intervals, in a figure-eight maze. Each maze consisted of a series of inter-connected alleys converging on a central arena with eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure motion (each beam interruption was registered as an activity count). Broad-spectrum background noise was provided during testing to minimize acoustical variations. Uniformity of the light intensity over each of the mazes was verified daily.

Motor activity was measured as the number of beam interruptions that occurred during the test session, and locomotor activity was calculated by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated and interrupted another beam. Habituation was evaluated as a decrement in activity during the test session. Activity assessments were conducted without the observer having knowledge of the treatment group. Animals were allowed to acclimate to the testing room with minimal disturbance prior to evaluation. Males and females were tested separately, and mazes were cleaned to reduce the residual scent from the opposite sex.

3. **Body weight and body weight gain:** Body weights were determined weekly during treatment and prior to necropsy for calculation of organ (to body) weight ratios.

4. **Food consumption and compound intake:** Individual food consumption determinations were conducted weekly during treatment. Food consumption data were corrected, if necessary, for spilled food or clogged feeders.

   Mean daily intake of the test item was determined from food consumption, body weight, and diet analysis data. Intake was determined separately for Months 1, 2, and 3 due to the changing dietary concentrations for these periods.

5. **Ophthalmoscopic examination:** Examinations were conducted on all animals prior to randomization, and animals with ocular abnormalities were excluded, with one exception. All surviving control and high-dose animals were examined just prior to study termination. Further details regarding the ophthalmoscopic examinations were not provided.

   Temporary animal #19 presented with an eye lesion during the pre-exposure eye examination but was included on study (#MF3014) to have the required number of males to conduct the study. The same lesion was observed for #MF3014 during the examination at the end of the study.

6. **Hematology and clinical chemistry:** Blood samples were collected from the first ten fasted rats/sex/dose level prior to study termination. Each animal was anesthetized with isoflurane and blood was collected from the retro-orbital sinus as appropriate (with or without anticoagulant). Differential blood counts were determined on all animals euthanized due to moribundity. 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	Heinz bodies
X	(Activated partial thromboplastin time)	X	Erythrocyte morphology
	(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	Inorganic phosphorus	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose (fasting)*
	ENZYMES (more than 2 hepatic enzymes e.g., *)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase	X	Uric acid
X	Lactic acid dehydrogenase (LDH)		A/G ratio
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transpeptidase (GGTP)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

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

7. Urinalysis: Urine samples were collected from the first ten non-fasted rats/sex/dose level prior to study termination. Animals were housed overnight in cages fitted with urine collection trays. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin (bile pigments)
X	pH*	X	Blood / blood cells* (hemoglobin)
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Leukocytes
		X	Urobilinogen

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

8. Euthanasia and pathology

a. Toxicology group: After 13 weeks of dosing, the first ten surviving rats/sex/dose level were euthanized by carbon dioxide inhalation and exsanguination. Animals were subjected to a complete gross examination, and gross lesions were saved from all animals, including those found dead or euthanized due to moribundity. The following CHECKED (X) tissues were collected for possible histological examination (preserved in 10% formalin), and the (XX) organs were also weighed. Paired organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain*+[c]
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach* [a]		Lymph nodes* [b]	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (w/optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland^
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+		
X	Tooth	XX	Epididymides*+		OTHER
X	Pancreas*	XX	Prostate*	X	Bone (sternum, rib, and femur)
	Gall bladder (not rat)*	X	Preputial gland^	X	Joint (femur/tibia)
	RESPIRATORY	X	Seminal vesicle*	X	Skeletal muscle
X	Trachea*	X	Cervix	X	Skin*
XX	Lung*	X	Mammary gland*	X	All gross lesions and masses*
X	Nasal structure	XX	Ovaries*+	X	Harderian gland
		X	Clitoral gland^	X	Zymbal's gland^
X	Nasopharynx*	XX	Uterus*+		
X	Larynx*	X	Vagina^		

* Required for 90-day oral rodent studies based on Guideline 870.3100.
\+ Organ weight required in rodent studies.
^ Preserved for possible microscopic evaluation only.
a Glandular and non-glandular
b Cervical and mesenteric
c Cerebellum, cerebrum-midbrain, and medulla/pons

Representative sections of collected tissues from the control and the high-dose animals were processed routinely and stained with hematoxylin and eosin (H&E) for examination by light microscopy; target organs and gross lesions were examined for all animals. Exceptions were the vagina and the exorbital/lacrimal, clitoral, preputial, and Zymbal's glands (excised and preserved for possible microscopic evaluation). Tissues in which compound-related effects were detected were examined from the low- and mid-dose groups, as necessary, to establish no-observed-effect levels. Intestinal tissue samples were further investigated by staining with Von Kossa stain to identify mineralization/calcification.

b. **Neurotoxicology group:** All remaining animals (nominally the remaining 6/sex/dose level; the animals subjected to FOB/motor activity evaluations, if possible) were selected for perfusion and tissue collection for neurological evaluation. Animals were anesthetized with pentobarbital and perfused via the left ventricle with a sodium nitrite:phosphate buffer flush followed by a universal fixative. Representative sections of the tissues shown in the following table were processed for all control and high-dose animals. Tissues from control and high-dose animals were evaluated microscopically. Tissues in which compound-related effects were detected were examined from the low- and mid-dose groups, as necessary, to establish no-observed-effect levels.

Tissue	Section	Method	Stain
Brain (levels 1-8)	Coronal	Paraffin	H&E
Spinal cord			
Cervical	Cross and longitudinal	Paraffin	H&E
Thoracic	Cross and longitudinal	Paraffin	H&E
Lumbar	Cross and longitudinal	Paraffin	H&E
Cauda Equina	Longitudinal	Paraffin	H&E
Spinal nerve root fiber and ganglia			
Dorsal and ventral, cervical (bilateral)	Longitudinal	Glycol methacrylate	Modified Lee's
Dorsal and ventral, lumbar (bilateral)	Longitudinal	Glycol methacrylate	Modified Lee's
Gasserian ganglion	Longitudinal	Glycol methacrylate	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 and longitudinal	Glycol methacrylate	Modified Lee's
Tibial (bilateral)	Cross/transverse and longitudinal	Glycol methacrylate	Modified Lee's
Sural (bilateral)	Cross/transverse and longitudinal	Glycol methacrylate	Modified Lee's

II. RESULTS

A. OBSERVATIONS

1. Mortality: There were no effects of treatment on mortality. One low-dose female (#MF1101) was found dead on Day 49 but the cause of death was not determined. A second low-dose female (#MF1107) was found dead on Day 90; the cause of death was attributed to the blood collection procedure. All other animals survived until scheduled euthanasia.

2. Clinical signs of toxicity: There were no treatment-related effects on clinical signs. Males at the high dose tended to spill food more often (12, 6, 25, 37% incidence at 0, 100, 500, and 1000 mg/kg/day, respectively). However, neither food consumption nor body weight was affected, and so this behavior is not considered adverse.

B. NEUROLOGICAL EVALUATIONS

1. FOB: There were no treatment-related effects on any FOB parameters.

2. Motor/locomotor activity: Summary motor and locomotor activity data are presented in Table 2, and interval motor and locomotor activity data are presented in Appendices I-IV at the end of this DER. There were no adverse treatment-related effects on motor or locomotor activity. The interval data demonstrated habituation, and the results showed decreases in activity for all groups over the course of the study.

TABLE 2. Summary mean (± SD) total (0-60 minutes) motor and locomotor activity count data in rats treated with triazole acetic acid in the diet for up to 13 weeks. [a]

Interval	Nominal dose level (mg/kg/day)			
	0	100	500	1000
Males – total motor activity				
Pretreatment	485 ± 210	538 ± 166	498 ± 114	513 ± 125
Week 2	473 ± 97	488 ± 154	443 ± 117	498 ± 143
Week 4	480 ± 75	406 ± 98	403 ± 82	383 ± 87
Week 8	405 ± 92	363 ± 86	343 ± 55	348 ± 83
Week 13	335 ± 77	316 ± 58	328 ± 47	305 ± 99
Males – total locomotor activity				
Pretreatment	327 ± 166	379 ±136	347 ± 89	364 ± 105
Week 2	337 ± 75	325 ± 111	319 ± 84	337 ± 112
Week 4	320 ± 61	274 ± 74	274 ± 57	250 ± 66
Week 8	254 ± 54	233 ± 59	239 ± 49	218 ± 57
Week 13	215 ± 48	215 ± 45	226 ± 33	206 ± 71
Females – total motor activity				
Pretreatment	666 ± 161	658 ± 204	729 ± 266	527 ± 141
Week 2	568 ± 226	487 ± 128	598 ± 206	495 ± 109
Week 4	578 ± 256	293 ± 129	530 ± 159	400 ±123
Week 8	624 ± 144	465 ± 180	581 ± 180	474 ± 201
Week 13	474 ± 155	427 ± 176	474 ± 201	357 ± 71
Females – total locomotor activity				
Pretreatment	436 ± 108	444 ± 183	452 ± 144	310 ± 95
Week 2	383 ± 165	321 ± 95	418 ± 158	319 ± 102
Week 4	361 ±130	174 ± 75	353 ± 116	269 ± 96
Week 8	420 ± 86	291 ± 160	358 ± 107	295 ± 69
Week 13	299 ± 84	253 ± 120	314 ± 158	230 ± 38

a Data were obtained from pages 151-152 and 168-169 of MRID 52154805; n = 10.

C. **BODY WEIGHTS AND BODY WEIGHT GAINS:** Selected mean body weight and body weight gain data are presented in Table 3. There were no treatment-related effects on body weights or body weight gains.

TABLE 3. Selected mean (± SD) body weights (g) and mean body weight gains (g) in rats treated with triazole acetic acid in the diet for up to 13 weeks [a]

Study Day	Nominal dose level (mg/kg/day)			
	0	100	500	1000
Males				
0	243 ± 15	243 ± 16	235 ± 33	242 ± 14
42	373 ± 30	375 ± 27	359 ± 33	371 ± 32
84	423 ± 39	430 ± 35	409 ± 40	420 ± 39
Overall BWG (0-84) [b]	180	187	174	178
Females				
0	165 ± 8	163 ± 8	161 ± 8	165 ± 10
42	223 ± 12	216 ± 17	213 ± 18	218± 15
84	246 ± 13	241 ± 15 [c]	233 ± 18	238 ± 17
Overall BWG (0-84) [b]	81	78	72	73

a Data were obtained from pages 186-189 of MRID 51254805; n = 16, unless otherwise noted.
b Calculated by the Reviewers from the mean data in this table.
c n = 15.

D. **FOOD CONSUMPTION**

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

2. **Compound intake:** Compound intake estimates are reported in Table 1. The calculated values generally approximated the target doses of 100, 500, and 1000 mg/kg/day.

E. OPHTHALMOSCOPIC EXAMINATION: There were no adverse effects noted during the ophthalmoscopic examinations. A dose-related increase in hazy eyes was seen in females (25, 37, 43, 50% incidence at 0, 100, 500, and 1000 mg/kg/day, respectively) and males (37, 43, 50, 62% incidence at 0, 100, 500, and 1000 mg/kg/day, respectively). However, this increase was not statistically significant, there were no changes in condition otherwise, and there was a high background level in both sexes. Additionally, other ophthalmology measures were normal, and so while dose-related, these findings are not considered adverse.

F. BLOOD ANALYSES

1. **Hematology:** There were no treatment-related effects on the hematology or coagulation parameters. Total white blood cell count was increased ($p<0.05$) by 26% in the high-dose males with concomitant increases ($p<0.05$) in absolute lymphocyte, monocyte, and basophil counts in these animals of 40-53%. All increased values were within historical control data ranges and were not observed in females, and there were no differences from control values for relative leukocyte counts in males; therefore, they were not considered adverse or biologically relevant. Any other observed significant ($p<0.05$) changes were within historical control ranges and/or did not occur in a manner related to dose.

2. **Clinical chemistry:** There were no treatment-related effects on the clinical chemistry parameters. Potassium concentration was decreased ($p<0.05$) by 8% in the high-dose males. Mean values were within the historical control data range, and no additional changes in potassium concentrations were noted in the females or in any other electrolyte concentration in either sex. Therefore, this change was considered incidental.

   All other observed significant ($p<0.05$) changes were within historical control ranges, did not occur in a manner related to dose, or were not considered toxicologically or biologically relevant.

G. URINALYSIS: There were no treatment-related effects on the urinalysis parameters.

H. EUTHANASIA AND PATHOLOGY

1. **Organ weight:** There were no treatment-related effects on absolute or relative (to body) organ weights. All significant ($p<0.05$) changes in organ weights demonstrated no relationship to dose.

2. **Gross pathology:** There were no treatment-related effects on gross pathology.

3. **Microscopic pathology:** No treatment-related differences in microscopic findings were observed in the animals selected for microscopic toxicology or neurotoxicology evaluation.

   Intestinal mucosal mineralization (calcification) observed in control and high-dose Toxicology group animals from both sexes was confirmed by staining with Von Kossa stain. Increased (not significant [NS]) incidences were observed in the cecum (9/10 treated vs.

6/10 control in both males and females) with slight increases in severity in both sexes. There were no differences in the incidences of mineralization in the colon, but the severity was increased in females (2.0 treated vs. 1.0 control). There was also an increased incidence and severity in mineralization of the rectum in high-dose females (6/10 at 1.3 and 6/10 at 2.7, respectively). These changes were not considered adverse or biologically relevant because: (i) the overall incidences of intestinal mineralization were generally similar 10/10 treated vs. 8/10 control [males] and 9/10 treated vs. 8/10 control [females]); (ii) there were no concomitant clinical signs of intestinal disturbance/function; and (iii) mineralization is a common background lesion in the aging rat.

All other noted microscopic findings with minimal incidence and/or are considered common to the Wistar rat and, therefore, not related to treatment.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Effects attributable to exposure to triazole acetic acid were not observed in this study. Based on the lack of adverse toxicological findings after 13 weeks of continuous and repeated dietary exposure, the no-observed-adverse-effect level (NOAEL) was the nominal high dose level of 1000 mg/kg/day that corresponded to achieved test item dose levels of 1002 and 1181 mg/kg/day for male and female rats, respectively. This is a limit dose for studies of this type.

B. REVIEWER COMMENTS: The Reviewers agree with the Investigators' conclusion.

There were no treatment-related effects on mortality, clinical observations, neurological evaluations (FOB and motor/locomotor activity), body weight and body weight gain, food consumption, ophthalmoscopic evaluation, serum chemistry, hematology, urinalysis, organ weights, and macroscopic and microscopic pathology.

The LOAEL was not determined. The NOAEL is 1000 mg/kg/day (equivalent to 1002/1181 mg/kg/day in males/females, achieving the limit dose).

C. STUDY DEFICIENCIES: There were no study deficiencies.

Appendix I. Interval motor activity data for male rats administered triazole acetic acid in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	122 ± 30	131 ± 52	103 ± 44	71 ± 46	42 ± 39	17 ± 27
100 mg/kg	118 ± 23	111 ± 49	109 ± 31	91 ± 30	67 ± 38	42 ± 45
500 mg/kg	120 ± 17	116 ± 39	110 ± 22	84 ± 27	45 ± 32	24 ± 27
1000 mg/kg	115 ± 26	113 ± 31	105 ± 41	81 ± 35	59 ± 39	40 ± 34

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	116 ± 29	96 ± 22	90 ± 24	79 ± 32	56 ± 23	35 ± 32
100 mg/kg	115 ± 38	94 ± 32	86 ± 30	75 ± 28	60 ± 30	58 ± 56
500 mg/kg	110 ± 38	85 ± 27	82 ± 19	79 ± 29	45 ± 27	42 ± 35
1000 mg/kg	115 ± 22	101 ± 35	102 ± 47	77 ± 18	60 ± 37	42 ± 22

b) Week 2

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	126 ± 31	95 ± 11	84 ± 16	66 ± 17	55 ± 20	55 ± 39
100 mg/kg	107 ± 30	78 ± 23	70 ± 26	64 ± 21	48 ± 17	39 ± 27
500 mg/kg	114 ± 31	73 ± 18	73 ± 21	59 ± 20	52 ± 31	32 ± 18
1000 mg/kg	113 ± 24	78 ± 20	60 ± 21	56 ± 22	39 ± 21	38 ± 23

c) Week 4

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	122 ± 43	87 ± 20	64 ± 19	52 ± 16	41 ± 18	39 ± 22
100 mg/kg	102 ± 31	66 ± 25	58 ± 18	46 ± 18	46 ± 25	45 ± 20
500 mg/kg	106 ± 26	60 ± 11	62 ± 21	50 ± 15	40 ± 15	26 ± 17
1000 mg/kg	116 ± 24	63 ± 18	54 ± 22	45 ± 21	40 ± 18	29 ± 14

d) Week 8

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	100 ± 30	62 ± 18	59 ± 23	43 ± 22	34 ± 16	37 ± 22
100 mg/kg	91 ± 19	63 ± 24	49 ± 16	43 ± 12	37 ± 15	32 ± 15
500 mg/kg	104 ± 23	64 ± 12	57 ± 15	38 ± 10	32 ± 11	35 ± 18
1000 mg/kg	102 ± 27	61 ± 24	49 ± 20	31 ± 24	34 ± 20	28 ± 21

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 05/21/2009
Number of Rats/Group: 10/0 mg/kg10/100 mg/kg 10/500 mg/kg 10/1000 mg/kg

e) Week 13

Data copied from pages 153-157 of MRID 51254805

Appendix II. Interval locomotor activity data for male rats administered triazole acetic acid in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	89 ± 28	87 ± 48	69 ± 33	48 ± 35	25 ± 29	10 ± 17
100 mg/kg	82 ± 18	82 ± 42	83 ± 31	64 ± 25	44 ± 30	23 ± 30
500 mg/kg	90 ± 13	85 ± 37	80 ± 23	56 ± 20	26 ± 21	10 ± 11
1000 mg/kg	90 ± 24	78 ± 28	73 ± 31	62 ± 27	37 ± 26	24 ± 23

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	88 ± 24	69 ± 18	65 ± 16	57 ± 24	37 ± 18	23 ± 23
100 mg/kg	83 ± 28	64 ± 25	57 ± 25	49 ± 20	35 ± 19	38 ± 40
500 mg/kg	89 ± 32	64 ± 22	59 ± 17	58 ± 21	29 ± 20	20 ± 20
1000 mg/kg	89 ± 23	73 ± 25	68 ± 37	53 ± 18	38 ± 24	16 ± 12

b) Week 2

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	94 ± 19	67 ± 10	54 ± 14	43 ± 12	30 ± 16	32 ± 27
100 mg/kg	78 ± 20	54 ± 20	44 ± 16	42 ± 17	31 ± 14	25 ± 17
500 mg/kg	88 ± 23	52 ± 15	49 ± 19	39 ± 12	28 ± 20	17 ± 14
1000 mg/kg	82 ± 17	53 ± 14	39 ± 14	36 ± 19	21 ± 11	19 ± 13

c) Week 4

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	85 ± 25	55 ± 14	38 ± 14	31 ± 9	24 ± 12	21 ± 13
100 mg/kg	74 ± 19	44 ± 19	35 ± 13	28 ± 11	26 ± 16	26 ± 15
500 mg/kg	80 ± 23	42 ± 9	43 ± 19	35 ± 11	25 ± 11	14 ± 13
1000 mg/kg	81 ± 14	39 ± 11	34 ± 14	25 ± 13	22 ± 14	17 ± 10

d) Week 8

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	68 ± 21	40 ± 12	38 ± 12	25 ± 15	22 ± 15	22 ± 15
100 mg/kg	68 ± 12	44 ± 18	32 ± 11	27 ± 11	24 ± 9	20 ± 11
500 mg/kg	77 ± 16	47 ± 9	37 ± 11	25 ± 7	20 ± 9	21 ± 11
1000 mg/kg	75 ± 21	42 ± 18	32 ± 15	19 ± 16	22 ± 15	16 ± 12

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05/21/2009
Number of Rats/Group: 10/0 mg/kg10/100 mg/kg 10/500 mg/kg 10/1000 mg/kg

e) Week 13

Data copied from pages 158-162 of MRID 51254805

Appendix III. Interval motor activity data for female rats administered triazole acetic acid in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	132 ± 28	117 ± 35	124 ± 37	116 ± 37	100 ± 37	77 ± 34
100 mg/kg	123 ± 21	128 ± 34	125 ± 46	109 ± 44	88 ± 42	85 ± 42
500 mg/kg	135 ± 39	132 ± 50	135 ± 56	110 ± 62	106 ± 41	110 ± 50
1000 mg/kg	126 ± 17	100 ± 23	91 ± 29	87 ± 31	69 ± 36	54 ± 36

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	145 ± 41	113 ± 49	102 ± 44	80 ± 49	70 ± 52	57 ± 44
100 mg/kg	124 ± 24	94 ± 22	88 ± 18	65 ± 35	68 ± 36	48 ± 33
500 mg/kg	137 ± 34	125 ± 52	99 ± 40	94 ± 43	74 ± 36	69 ± 48
1000 mg/kg	120 ± 25	98 ± 27	86 ± 29	73 ± 29	57 ± 27	62 ± 24

b) Week 2

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	156 ± 52	125 ± 56	118 ± 54	87 ± 46	52 ± 46	41 ± 50
100 mg/kg	109* ± 44	63* ± 30	34* ± 22	26* ± 16	37 ± 22	24 ± 22
500 mg/kg	145 ± 23	111 ± 36	88 ± 28	79 ± 37	61 ± 37	47 ± 42
1000 mg/kg	122 ± 22	78* ± 18	63* ± 25	59 ± 29	41 ± 30	37 ± 29

c) Week 4

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	167 ± 35	121 ± 49	113 ± 40	90 ± 41	72 ± 38	62 ± 26
100 mg/kg	129 ± 34	99 ± 36	83 ± 41	67 ± 43	44 ± 27	43 ± 32
500 mg/kg	154 ± 39	106 ± 27	108 ± 44	83 ± 41	62 ± 30	68 ± 34
1000 mg/kg	132 ± 29	87 ± 27	65* ± 31	59 ± 27	44 ± 15	47 ± 17

d) Week 8

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	142 ± 48	97 ± 39	76 ± 30	58 ± 26	59 ± 20	42 ± 16
100 mg/kg	127 ± 31	73 ± 36	63 ± 38	56 ± 33	59 ± 33	49 ± 32
500 mg/kg	141 ± 35	90 ± 38	69 ± 38	68 ± 47	49 ± 33	57 ± 42
1000 mg/kg	117 ± 19	68 ± 13	53 ± 26	42 ± 12	40 ± 14	38 ± 19

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05/21/2009
Number of Rats/Group: 10/0 mg/kg10/100 mg/kg 10/500 mg/kg 10/1000 mg/kg

e) Week 13

Data copied from pages 170-174 of MRID 51254805

Appendix IV. Interval locomotor activity data for female rats administered triazole acetic acid in the diet.

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	88 ± 16	79 ± 28	88 ± 30	78 ± 31	60 ± 27	44 ± 20
100 mg/kg	85 ± 21	87 ± 38	88 ± 41	71 ± 33	60 ± 36	53 ± 31
500 mg/kg	85 ± 14	79 ± 29	88 ± 37	67 ± 33	70 ± 33	64 ± 37
1000 mg/kg	79 ± 7	63 ± 21	58 ± 23	48 ± 19	36 ± 23	26 ± 24

a) Pretreatment

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	102 ± 29	78 ± 37	72 ± 38	51 ± 30	47 ± 40	32 ± 28
100 mg/kg	92 ± 20	60 ± 21	57 ± 16	45 ± 29	40 ± 20	29 ± 19
500 mg/kg	103 ± 25	88 ± 42	70 ± 34	67 ± 31	46 ± 28	44 ± 36
1000 mg/kg	81 ± 17	65 ± 23	54 ± 24	47 ± 29	35 ± 17	38 ± 20

b) Week 2

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	107 ± 31	81 ± 28	78 ± 37	51 ± 25	21 ± 22	22 ± 27
100 mg/kg	74* ± 27	39* ± 20	19* ± 14	13* ± 10	19 ± 13	11 ± 13
500 mg/kg	107 ± 16	76 ± 29	60 ± 24	50 ± 26	33 ± 21	27 ± 27
1000 mg/kg	88 ± 13	49* ± 13	44* ± 21	40 ± 24	26 ± 21	22 ± 23

c) Week 4

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	122 ± 20	83 ± 30	76 ± 29	58 ± 22	47 ± 34	35 ± 18
100 mg/kg	93 ± 30	63 ± 30	49 ± 36	41 ± 40	21* ± 20	24 ± 25
500 mg/kg	105 ± 31	69 ± 22	69 ± 29	52 ± 30	31 ± 17	33 ± 19
1000 mg/kg	98 ± 19	61 ± 20	45 ± 25	40 ± 21	23* ± 8	28 ± 13

d) Week 8

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 mg/kg	104 ± 25	65 ± 23	44 ± 18	32 ± 16	33 ± 18	22 ± 11
100 mg/kg	85 ± 14	43 ± 26	35 ± 31	34 ± 28	30 ± 23	26 ± 16
500 mg/kg	99 ± 26	61 ± 33	46 ± 29	42 ± 38	33 ± 25	33 ± 31
1000 mg/kg	84 ± 10	43 ± 6	30 ± 13	26 ± 11	24 ± 12	22 ± 12

Mean ± S.D for 1:00:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05/21/2009
Number of Rats/Group: 10/0 mg/kg10/100 mg/kg 10/500 mg/kg 10/1000 mg/kg

e) Week 13

Data copied from pages 175-179 of MRID 51254805

DATA EVALUATION RECORD

TRIAZOLE ACETIC ACID (METABOLITE OF TRIAZOLE)

Study Type: OCSPP 870.3700b; Prenatal Developmental Toxicity Study in Rabbits

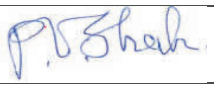
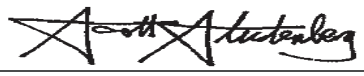
EPA Contract No. 68HERC22D0017
Task Assignment No. 5540-2.1-022 (MRID 52154803)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Prepared by

PB&A/
CSS JV

1151 Lost Creek Boulevard
Austin, TX 78746

Primary Reviewer: Michael E. Viana, Ph.D.		Signature: Date:	 05/25/2023
Secondary Reviewer: Scott D. Studenberg, Ph.D., DABT		Signature: Date:	 05/29/2023
Quality Assurance: P. V. Shah, Ph.D.		Signature: Date:	 06/04/2023
Project Manager: Scott D. Studenberg, Ph.D., DABT		Signature: Date:	 06/04/2023

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by PB&A/CSC Joint Venture personnel. Contractor's role did not include establishing Agency policy.

EPA Reviewer: Minerva Mercado-Feliciano PhD, DABT **Signature:**
Risk Assessment Branch IV, HED (7509P) **Date:** 14-Jul-2023
EPA Secondary Reviewer: Megan Stallard, PhD **Signature:**
Risk Assessment Branch IV, HED (7509P) **Date:** 14-Jul-2023
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study in Rabbits; OCSPP 870.3700b [§83-3b]; OECD 414.

PC CODE: 600082 **DP BARCODE:** Not provided
TXR#: 0058589

TEST MATERIAL (PURITY): Triazole acetic acid (98.4% a.i.)

SYNONYMS: AE C619102; Amitrole-triazolylacetic acid; 1,2,4-triazol-1-yl-acetic acid

CITATION: Hoberman, A.M. (2010) Triazole acetic acid – oral (stomach tube) developmental toxicity study in rabbits. Charles River Laboratories, Preclinical Services, Horsham, PA. Laboratory Project ID: YJK00003, December 17, 2010. MRID 52154803. Unpublished.

SPONSOR: Triazole Derivative Metabolite Group, U.S. Triazole Task Force, McDermott, Will, & Emery, 600 13th St, N.W., Washington, D.C.

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 developmental toxicity study (MRID 52154803), groups of 25-27 New Zealand White [Hra:(NZW)SPF] rabbits were administered triazole acetic acid (98.4% a.i.; Batch/Lot # CH-476108) in reverse-osmosis deionized water via daily oral gavage (dose volume 10 mL/kg) at dose levels of 0, 100, 750, or 1000 (limit dose) mg/kg/day during gestation days (GD) 6-28. On GD 29, all surviving does were euthanized, the uterus and ovaries were removed by cesarean section, and the uterine contents examined. The fetuses were examined for external, visceral, and skeletal anomalies, malformations, and variations.

There were no effects of treatment on gross pathology, organ weights, or cesarean section

parameters. One 100 mg/kg/day doe, five 750 mg/kg/day does, and two 1000 mg/kg/day does were found dead or euthanized prior to scheduled termination due to causes unrelated to treatment (one adverse lenticular opacity, the rest gavage errors). Treatment related mortalities included one 750 mg/kg/day doe delivered that its litter prematurely on GD 29 and was euthanized, and eight 1000 mg/kg/day does found dead. These rabbits had necropsy findings of stomach lesions described as numerous discolored (black or red) areas or ulcerations ranging in size from pinpoint to 1.0 cm diameter. These stomach lesions were attributed to the low pH (pH 1.9-2.0) of the test formulations, resulting in irritation, decreased food consumption, decreased body weight gains, morbidity, and death.

At 1000 mg/kg/day, increased incidences of scant feces (beginning on GD 14) were observed in six of the eight does that died prematurely with treatment-related changes. These findings were generally consistent with decreases in food consumption and increases in the incidence of stomach lesions. Additionally, at 1000 mg/kg/day, body weight gains were decreased during GD 12-15 (0.00 kg treated vs. 0.10 kg control) and GD 24-29 (0.01 kg treated vs. 0.09 kg control), resulting in decreased body weight gains during treatment (GD 6-29; ↓31%) and overall (GD 0-29; ↓25%). At 750 mg/kg/day, absolute food consumption was decreased by 11% during GD 12-15, by 14-19% during GD 21-21, and by 10% during treatment (GD 6-29); relative food consumption was decreased by 11% during GD 12-15, by 13-18% during GD 21-21, and by 9% during treatment (GD 6-29). At 1000 mg/kg/day, absolute food consumption was decreased by 8-33% during GD 9-29 and by 12% during treatment (GD 6-29); relative food consumption was decreased by 8-33% during GD 9-29 and by 11% during treatment (GD 6-29).

A systemic maternal LOAEL could not be determined. The systemic maternal NOAEL is 1000 mg/kg/day, the highest dose tested.

The portal of entry maternal LOAEL is 1000 mg/kg/day based on mortality and stomach lesions. The portal of entry maternal NOAEL is 750 mg/kg/day.

There were no dead fetuses and no effects on fetal resorptions. There were no treatment related external, visceral, or skeletal malformations or variations. Fetal weights were decreased by 9-11% in the males, females, and combined sexes at 750 and 1000 mg/kg/day. This apparent effect was not considered significant because (1) the small magnitude of the change, and (2) means for control and treated groups are within the historical control ranges.

A developmental LOAEL was not detected. The developmental NOAEL is 1000 mg/kg/day, the highest dose tested.

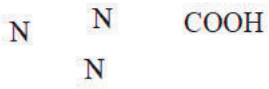
This study is classified A**cceptable/Guideline** and satisfies the guideline requirements (OCSPP 870.3700b; OECD 414) for a developmental toxicity study in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triazole acetic acid

Description:	Light beige solid
Lot/batch #:	CH-476108
Purity:	98.4% a.i.
Expiration date / storage:	March 22, 2009 / Room temperature
CAS #of TGAI:	28711-29-7
Structure:	

2. Vehicle and/or positive control: Reverse-osmosis deionized water.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White [Hra:(NZW)SPF]
Age/weight at study initiation:	Approximately six months / 2.72-3.72 kg
Source:	Covance Research Products (Denver, PA)
Housing:	Individually-housed in stainless steel cages with lined cage pans.
Diet:	Certified Rabbit Chow #5322 (PMI® Nutrition International, St. Louis, MO); 150 g/day during acclimation to study initiation, 180-185 g/day during treatment. Timothy hay cubes were provided if necessary because of reduced food consumption.
Water:	Reverse-osmosis deionized tap water (<1.2 ppm chlorine added), *ad libitum*
Environmental conditions:	**Temperature:** 16-22°C **Humidity:** 30-70% **Air changes:** ≥10 / hour **Photoperiod:** 12 hours light / 12 hours dark
Acclimation period:	2-5 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Start: November 9, 2008 End: December 5, 2008

2. Mating: The females were naturally bred by the Supplier with male breeder rabbits of the same source and strain. The day of mating was designated as gestation day (GD) 0. No additional information was provided.

3. Animal assignment: Animals were randomly assigned (stratified by body weight) to the dose groups presented in Table 1.

TABLE 1: Study design [a]

Group	Dose (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg)	# of rabbits
Control	0	0	10	25
Low	100	10	10	26
Mid	750	75	10	27
High	1000	100	10	27

a Data obtained from page 24 of MRID 52154803.

4. **Dose selection rationale:** It was stated that the doses used in the present study were selected based on the results of a previously performed dose range-finding study (Study YJK00002[1]); however, a summary of this study was not provided. The low dose was expected to be a no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity; the mid dose was expected to cause a slight decrease in maternal body weight; and the high dose was expected to cause decreases in both maternal and fetal weights. The high dose (1000 mg/kg/day) represented the limit dose for a developmental toxicity study in rabbits (OCSPP 870.3700b).

5. **Dosage preparation and analysis:** It was stated that the test suspensions were prepared at least once weekly and stored at room temperature. The appropriate amount of the test substance (not corrected for purity) was weighed and moistened with a small amount of vehicle to form a paste/slurry. Additional vehicle was added to yield the desired concentration. The suspension was stirred (with sonication, if necessary) until visually homogeneous. The suspensions were portioned and stored until required. Portions were stirred continuously prior to and during administration. Unused suspensions were discarded after the daily dosing. It was stated that stability of the formulations was provided by the Sponsor; formulations in distilled water were stable for up to ten days at room temperature. Concentration and homogeneity (top, middle, and bottom) analyses of the suspensions were performed on duplicate samples from the first day of preparation; concentration analyses were also performed on all suspensions from the last preparation.

   Results

   Homogeneity (%RSD): 0.90-4.9%

   Stability (% of time 0): Not reported

   Concentration (% nominal): 95-109%

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

6. **Dosage administration:** All doses were administered once daily by oral gavage on GD 6-28 at a dose volume of 10 mL/kg. Dosing was based on the body weight of the most recent body weight determination.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** The does were observed for mortality at least twice daily and for general appearance at least once prior to dose initiation. Clinical observations, abortion or premature delivery, and mortality were also recorded prior to dose administration, between one to two hours post-dosing, and on the day of euthanasia. Body weights were recorded on GD 0 by the Supplier, on the day of arrival at the performing

[1] Hoberman, A.M., (2008) Oral (stomach tube) dosage-range developmental toxicity study of triazole acetic acid in rabbits. Charles River Laboratories Study YJK00002 Letter Report, September 25, 2008.

laboratory, and daily during dosing and the post-dosing period. Food consumption was recorded daily after arrival. On GD 29, all surviving does were euthanized by an i.v. injection of a euthanasia solution (390 mg pentobarbital sodium and 50 mg phenytoin sodium), the reproductive organs and gravid uterus removed by cesarean section, the gravid uterus was weighed, and a complete necropsy was performed. The liver, kidneys, and any gross lesions (except parovarian cysts) were excised, the organs weighed, and all fixed in neutral-buffered 10% formalin for possible future examination. The uterus of any non-pregnant does were stained with 10% ammonium sulfide to visualize any implantation sites. The number and distribution of *corpora lutea* were recorded, and the uterus was examined for pregnancy, the number and distribution of implantation sites, live and dead fetuses, and early and late resorptions. The following definitions were reported.

- Early resorption: organogenesis was not grossly evident.
- Late resorption: organogenesis was grossly evident.
- Live fetus: a term fetus that responded to stimuli. Non-responding term fetuses were considered dead (it was stated there were no dead fetuses).

Rabbits that died or were euthanized prior to scheduled termination were examined to determine the cause of death or reason for morbidity on the day the observation was made. Rabbits were examined grossly. The lungs, trachea, and esophagus were perfused and excised; the heart, stomach, and spleen were excised; and the livers and kidneys were excised and weighed. All tissues were fixed in neutral-buffered 10% formalin and retained for possible future histopathological evaluation. Pregnancy status and uterine contents were recorded, gravid uterine weights were recorded, and conceptuses *in utero* and/or delivered pups were examined to the extent possible by using the methods previously described for term fetuses. The uterus of each apparently non-pregnant doe was stained with 10% ammonium sulfide as previously stated.

Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Placentae were examined for size, color, and shape.

2. **Fetal evaluations:** The fetuses were removed from the uterus, weighed, and examined externally. It was stated that late resorptions were examined for gross external alterations to the extent possible. Live fetuses were euthanized by an i.p. injection of euthanasia solution (same as administered to the does) and sexed by internal examination. Cavitated organs were dissected for examination. The brain was examined *in situ* in approximately one-half of the fetuses by a single cross-section cut between the parietal and frontal bones of the skull; the remaining fetuses were decapitated, the heads fixed in Bouin's solution, and the soft tissues (including the eyes, brain, nasal passages, and tongue) examined with Wilson's sectioning technique. All fetuses were fixed in alcohol, eviscerated, cleared, stained with Alizarin red S, and examined for skeletal alterations. The skeletal preparations were retained in glycerol with thymol for possible additional examinations.

Fetal alterations were defined as follows.

- Malformations: irreversible changes that occur at low incidences in this species and strain; or
- Variations: reversible delays or accelerations in development that are common findings in this species and strain.

D. DATA ANALYSIS

1. **Statistical analyses:** Means and standard deviations were calculated for all continuous data. Significance was noted at $p \leq 0.05$ or $p \leq 0.01$. Maternal body weight, body weight gain, food consumption, organ weights, litter mean sex ratio (% males), percentage resorptions, fetal body weight, and fetal anomaly data were analyzed with Bartlett's test of the homogeneity of variances. If Bartlett's test was not significant ($p > 0.001$), an analysis of variance (ANOVA) was performed; if the ANOVA was significant ($p \leq 0.05$), Dunnett's test was used to compare the treated groups to control. If Bartlett's test was significant ($p \leq 0.001$), a Kruskal-Wallis test was performed (if ≤75% ties); if the Kruskal-Wallis test was significant ($p \leq 0.05$), Dunn's method of multiple comparisons was used to compare the treated groups to control. If there were >75% ties, the data were analyzed with Fisher's Exact Test. Count data were analyzed with the method described for the Kruskal-Wallis test. Clinical observations and other proportional data were analyzed with a variance test for the homogeneity of the binomial distribution.

   The Reviewers considered the statistical analyses used appropriate.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study.

$$\text{Pre-implantation loss (\%)} = \frac{\text{\# of corpora lutea} - \text{\# of implantations}}{\text{\# of corpora lutea}} \times 100$$

$$\text{Post-implantation loss (\%)} = \frac{\text{\# of implantations} - \text{\#live fetuses}}{\text{\# of implantations}} \times 100$$

3. **Historical control data:** Historical control data were provided for the period June 2006 to June 2008. Reproductive indices from 47 full studies on 984 rabbits and 43 dose range-finding studies on 280 rabbits, necropsy observations from 92 studies on 1308 rabbits, fetal external alterations from 50 full studies on 7989 fetuses (974 litters) and 40 dose range-finding studies on 1664 fetuses (198 litters), and fetal visceral and skeletal alterations and fetal ossification site data from 49 studies on 7655 fetuses (934 litters) were reported.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical observations:** All study mortalities are presented in Table 2.

TABLE 2. Mortalities in rabbits administered triazole acetic acid via oral gavage during GD 6-28. [a]

Rabbit #	Removal day	Mode of death	Treatment related	Description
100 mg/kg/day				
7725	GD 6 [b]	Euthanasia	No	Clinical observations: adverse lenticular opacity (unrelated to treatment). Necropsy observations: all tissues appeared normal. Replacement: #5849.
750 mg/kg/day				
7779	GD 9	Found dead	No	Clinical observations: appeared normal. Necropsy observations: apparent intubation error. Lungs: spongy and red. Esophagus: thin area. Body weight: Unremarkable. Food consumption: 0 g on GD 6-7. Replacement: #5850.
7781	GD 7	Euthanasia	No	Clinical observations: salivation, hyperpnea, rales (DG 7). Necropsy observations: apparent intubation error. Trachea: perforation. Body weight loss GD 7. Food consumption: 0 g on GD 6-7. Replacement: #4400.
7790	GD 9	Found dead	No	Clinical observations: appeared normal. Necropsy observations: apparent intubation error. Heart: white band encompassing ventricles. Bronchus (right): blocked with firm white material; unable to perfuse right diaphragmatic lobe of lungs. Body weight loss GD 8. Food consumption: reduced GD 7-8.
7792	GD 27	Found dead	No	Clinical observations: abrasion on the nose GD 14-17; rales GD 24-26; scant feces GD 25. Necropsy observations: diaphragmatic hernia. This congenital abnormality contributed to death. Other observations: medially and right side – portion of cecum and right lateral lobe of the liver were protruding into the thoracic cavity. Body weight loss GD 22-26. Food consumption: severely reduced GD 22-26.
7794	GD 29	Premature delivery	**Yes**	Clinical observations: scant feces GD 25, 27, 29; ungroomed coat GD 29. Necropsy observations: Stomach: numerous black ulcerations on mucosal surface. Liver: small. Kidneys: large. Adipose: surrounding kidney friable. Body weight loss GD 21-29. Food consumption: severely reduced GD 23-29.
7798	GD 10	Euthanasia	No	Clinical observations: clear perinasal substance, pale; rales; hyperpnea; dyspnea; gray mucous membranes; red perioral substance; GD 10. Necropsy observations: apparent intubation error. Lungs: mottled red and dark red. Body weight: unremarkable. Food consumption: unremarkable.

TABLE 2. Mortalities in rabbits administered triazole acetic acid via oral gavage during GD 6-28. [a]

Rabbit #	Removal day	Mode of death	Treatment related	Description
1000 mg/kg/day				
7751	GD 6 [b]	Found dead	No	Clinical observations: appeared normal. Necropsy observations: apparent intubation error. Lungs: perforation in right diaphragmatic lobe; right lobes light brown. Thoracic cavity: 15 mL of brown fluid present. Body weight: Unremarkable. Replacement: #4399.
4399	GD 29	Found dead	**Yes**	Clinical observations: appeared normal. Necropsy observations: Stomach: numerous black ulcerations on mucosal surface (pinpoint to 0.5 cm in diameter). Body weight loss GD 24-28. Food consumption: severely reduced GD 24-28.
7756	GD 13	Found dead	**Yes**	Clinical observations: scant feces GD 10. Necropsy observations: Stomach: numerous black ulcerations on mucosal surface too numerous to count (0.1 cm to 1.1 cm × 0.4 cm × 0.1 cm). Abdominal organs/adipose tissue: pale. Kidney: right ran area. Trachea: epithelial lining numerous pinpoint black areas, cartilage rings brown. Heart: pale. Body weight loss GD 9-12. Food consumption: severely reduced GD 9-12.
7759	GD 17	Found dead	**Yes**	Clinical observations: clear perinasal substance, excess salivation, brown or red perioral substance, hyperpnea GD 12; rales GD 12-16; decreased motor activity GD 13; scant feces GD 14-16); miosis GD 15. Necropsy observations: Stomach: numerous red areas on cardiac and fundic regions too numerous to count (pinpoint to 0.2 cm diameter). Small intestines: pale. Spleen: small (0.63 g). Body weight loss GD 9-16. Food consumption: severely reduced GD 11-16.
7761	GD 17	Found dead	**Yes**	Clinical observations: scant feces GD 15; mydriasis, labored breathing GD 16. Necropsy observations: Stomach: numerous black areas too numerous to count (pinpoint to 0.2 cm diameter) on cardiac and fundic regions. Body weight loss GD 9-16. Food consumption: severely reduced GD 12-16.
7765	GD 8	Found dead	No	Clinical observations: appeared normal. Necropsy observations: apparent intubation error. Esophagus: perforation proximal to thyroid. Body weight: unremarkable. Food consumption: unremarkable. Replacement: #9763.
7769	GD 9	Found dead	**Yes**	Clinical observations: decreased motor activity; cyanosis; red perioral substance; bradypnea; gasping; ungroomed coat GD 9. Necropsy observations: Liver: tan area on caudal lobe. Heart: pale. Stomach: numerous black ulcerations too numerous to count (pinpoint to 1.0 cm diameter), mucosal surface visible through serosal surface.

TABLE 2. Mortalities in rabbits administered triazole acetic acid via oral gavage during GD 6-28. [a]

Rabbit #	Removal day	Mode of death	Treatment related	Description
				Intestines: pale, with brown striations on entire length of the duodenum. Body weight loss GD 7-9. Food consumption: severely reduced GD 6-9.
7772	GD 16	Found dead	**Yes**	Clinical observations: scant feces GD 14-15. Necropsy observations: all tissues appeared normal. Body weight loss GD 11-15. Food consumption: severely reduced GD 11-15.
7773	GD 27	Found dead	**Yes**	Clinical observations: scant feces GD 24, 26; mild dehydration GD 26. Necropsy observations: Stomach: numerous black ulcerations on mucosal surface too numerous to count (pinpoint to 0.5 cm diameter). Body weight loss GD 22-26. Food consumption: severely reduced GD 22-26.
7775	GD 19	Found dead	**Yes**	Clinical observations: scant feces GD 14-17. Necropsy observations: Thymus: numerous red areas. Stomach: numerous black areas on mucosal surface (fundic and cardiac regions) too numerous to count (pinpoint to 1.0 cm diameter). Body weight loss GD 13-18. Food consumption: severely reduced GD 13-18.

a Data were obtained from Appendix 6 on pages 242-244 of MRID 52154803.
b Removed prior to dosing.

The treatment-related mortalities were attributed to the acidic pH of the test formulations (pH 1.9-2.0; compared to normal rat stomach pH 3.2-3.9[2]), resulting in the numerous black (or red) ulcerations of the mucosal surface in the stomach, associated with decreased food consumption and body weight loss. The remainder of mortalities were considered apparent intubation errors unrelated to the test substance.

At ≥750 mg/kg/day, increases ($p \leq 0.05$) in the number of does and the number of incidences of scant feces generally occurred in does that did not survive until scheduled euthanasia. Incidents first occurred during the second half of the treatment period (GD 25 at 750 mg/kg/day and GD 14 at 1000 mg/kg/day). At 1000 mg/kg/day, scant feces were observed in six of the eight does that died prematurely with treatment-related changes. These findings were generally consistent with decreases in food consumption and stomach lesions and were considered adverse treatment-related effects in the 1000 mg/kg/day does.

There were no clinical signs attributed to treatment in the does that survived to scheduled euthanasia. All observations were sporadic, were noted in a single rabbit, and/or were seen in a manner unrelated to dose.

2. **Body weight:** Selected maternal body weight, body weight gain, and gravid uterus weight data are presented in Table 3. Body weights, gravid uterus weights, and GD 29 body weights (corrected for gravid uterus weight) were unaffected by treatment in all groups. At 1000 mg/kg/day, body weight gains were decreased ($p \leq 0.05$) during GD 12-15 (0.00 kg

[2] McConnell et al. 2008; J Pharm Pharmacol 60(1):63-70. https://doi.org/10.1211/jpp.60.1.0008.

treated vs. 0.10 kg control) and GD 24-29 (0.01 kg treated vs. 0.09 kg control), resulting in decreased (p≤0.05) body weight gains during treatment (GD 6-29; ↓31%) and overall (GD 0-29; ↓25%).

TABLE 3. Selected mean (± SD) maternal body weights (kg), body weight gains (kg), and gravid uterus weights (g) in pregnant rabbits administered triazole acetic acid via oral gavage during GD 6-28. [a]

Day	Dose (mg/kg/day)			
	0 (n = 23)	100 (n = 23)	750 (n = 21)	1000 (n = 21)
GD 0	3.17 ± 0.24	3.17 ± 0.24	3.19 ± 0.23	3.20 ± 0.25
GD 6	3.27 ± 0.24	3.24 ± 0.21	3.29 ± 0.21	3.29 ± 0.24
GD 14	3.47 ± 0.26	3.45 ± 0.21	3.44 ± 0.21 [b]	3.36 ± 0.26 [c]
GD 21	3.58 ± 0.27	3.59 ± 0.22	3.55 ± 0.25 [b]	3.52 ± 0.28 [d]
GD 29	3.72 ± 0.28	3.76 ± 0.22	3.61 ± 0.23 [e]	3.60 ± 0.32 [f]
BWG GD 6-29	0.45 ± 0.11	0.51 ± 0.13	0.35 ± 0.20 [e]	0.31 ± 0.14** (↓31) [f]
BWG GD 0-29	0.55 ± 0.12	0.59 ± 0.14	0.45 ± 0.23 [e]	0.41 ± 0.10* (↓25) [f]
Gravid uterus weight	491.82 ± 70.14	505.70 ± 75.31	477.76 ± 102.55 [g]	432.79 ± 101.10 [f]
GD 29 (adjusted)	3.23 ± 0.26	3.25 ± 0.20	3.14 ± 0.21 [g]	3.16 ± 0.34 [f]
GD 6-29 BWG (adjusted)	−0.04 ± 0.12	0.01 ± 0.11	−0.09 ± 0.14 [g]	−0.13 ± 0.14 [f]
GD 0-29 BWG (adjusted)	0.05 ± 0.14	0.08 ± 0.14	0.01 ± 0.18 [g]	−0.02 ± 0.11 [f]

a Data were obtained from pages 51-53 of MRID 52154803. Percentage differences from control (calculated by the Reviewers) are presented in parentheses.
b n = 19.
c n = 20.
d n = 16.
e n = 18.
f n = 14.
g n = 17.
* Significantly different from control; p≤0.05.
** Significantly different from control; p≤0.01.

3. **Food consumption:** Absolute (g/rabbit/day) and relative (g/kg/day) food consumption data are presented in Table 4. At 750 mg/kg/day, absolute food consumption was decreased (p≤0.05) by 11% during GD 12-15, by 14-19% during GD 21-29, and by 10% during treatment (GD 6-29); relative food consumption was decreased (p≤0.05) by 11% during GD 12-15, by 13-18% during GD 21-29, and by 9% during treatment (GD 6-29). At 1000 mg/kg/day, absolute food consumption was decreased (p≤0.05) by 8-33% during GD 9-29 and by 12% during treatment (GD 6-29); relative food consumption was decreased (p≤0.05) by 8-33% during GD 9-29 and by 11% during treatment (GD 6-29).

TABLE 4. Mean (± SD) maternal absolute (g/rabbit/day) and relative (g/kg/day) food consumption in rabbits administered triazole acetic acid via oral gavage during GD 6-28. [a]

Day	Dose (mg/kg/day)			
	0 (n = 23)	100 (n = 23)	750 (n = 21)	1000 (n = 21)
Absolute (g/rabbit/day)				
GD 6-9	168.3 ± 23.6	175.4 ± 11.5	162.2 ± 35.7	158.9 ± 23.1
GD 9-12	168.2 ± 27.4	163.8 ± 27.8	155.0 ± 35.5 [b]	142.4 ± 31.7* (↓15)
GD 12-15	166.4 ± 26.9	166.5 ± 20.1 [c]	147.4 ± 24.7* (↓11) [d]	110.7 ± 57.2** [e] (↓33)
GD 15-18	171.6 ± 21.1	175.9 ± 9.5	164.1 ± 22.1 [b]	143.8 ± 42.0** (↓16) [f]
GD 18-21	172.9 ± 13.1	173.7 ± 14.6	163.8 ± 22.7 [b]	158.5 ± 25.0* (↓8) [g]
GD 21-24	160.3 ± 24.7	162.4 ± 22.0	137.4 ± 41.0* (↓14) [b]	135.1 ± 37.7* (↓16) [g]
GD 24-29	128.8 ± 34.4	133.6 ± 29.7	103.8 ± 37.4* (↓19) [f]	104.4 ± 35.6* (↓19) [h]
GD 6-29	159.5 ± 18.2	161.7 ± 14.2	143.2 ± 22.1* (↓10) [f]	140.3 ± 19.0** (↓12) [h]
Relative (g/kg/day)				
GD 6-9	50.9 ± 7.4	53.1 ± 4.2	48.9 ± 10.3	48.1 ± 7.8
GD 9-12	49.8 ± 7.8	48.8 ± 8.4	46.2 ± 10.3 [b]	42.5 ± 9.4* (↓15)
GD 12-15	48.3 ± 7.6	48.6 ± 5.8 [c]	43.2 ± 7.5* (↓11) [d]	32.4 ± 16.2**(↓33) [e]
GD 15-18	49.0 ± 5.8	50.4 ± 3.8	47.1 ± 5.5 [b]	41.5 ± 11.4** (↓15) [f]
GD 18-21	48.8 ± 3.0	48.9 ± 4.2	46.4 ± 5.8 [b]	45.0 ± 6.2* (↓8) [g]
GD 21-24	44.5 ± 6.3	44.8 ± 5.8	38.7 ± 11.6* (↓13) [b]	38.4 ± 10.1* (↓14) [g]
GD 24-29	35.2 ± 9.4	36.1 ± 8.2	28.9 ± 10.2* (↓18) [f]	28.7 ± 8.2* (↓18) [h]
GD 6-29	45.5 ± 5.0	46.1 ± 4.5	41.3 ± 6.1* (↓9) [f]	40.4 ± 3.8** (↓11) [h]

a Data were obtained from pages 54-55 of MRID 52154803. Percentage differences from control (calculated by the Reviewers) are presented in parentheses.
b n = 19.
c n = 22.
d n = 18.
e n = 20.
f n = 17.
g n = 15.
h n = 14.
* Significantly different from control; p≤0.05.
** Significantly different from control; p≤0.01.

4. **Gross pathology**: There were no effects of treatment noted at necropsy in the does that survived to scheduled euthanasia. Necropsy findings for the rabbits that were euthanized prior to schedule, delivered prematurely, or were found dead were presented in Table 2.

5. **Organ weights**: There were no effects of treatment noted at necropsy on terminal body weight or liver and kidney weights in the does that survived to scheduled euthanasia.

6. **Cesarean section data**: Cesarean section data are presented in Table 5. There were no effects on the cesarean section parameters (increased mortality was previously discussed). All placentae appeared normal.

Fetal weights were decreased (p≤0.05) by 9-11% in males, females, and combined sexes at 750 and 1000 mg/kg/day. This apparent effect was not considered significant because (1)

the small magnitude of the change was similar to the coefficient of variation of the control group, and (2) means for control and treated groups are within the historical control ranges.

TABLE 5. Cesarean section observations. [a]

Observation	Dose (mg/kg/day)			
	0	100	750	1000
Animals treated	25	26	27	27
Animals pregnant	23	24	23	22
Pregnancy rate (%)	92.0	92.3	85.2	81.5
Nonpregnant [b]	2	2	4	5
Maternal wastage	0	1	6 [c]	10 [d]
Found dead	0	0	3	8**
Unscheduled euthanasia	0	1	2	0
No. aborted	0	0	0	0
No. premature delivery	0	0	1	0
Total No. *corpora lutea* [e]	198	207	160	124
Mean (± SD) *corpora lutea*/dam	8.6 ± 1.6	9.0 ± 1.3	9.4 ± 1.4	8.8 ± 2.3
Total No. implantations [e]	190	198	154	114
Mean (± SD) implantations/dam	8.3 ± 1.7	8.6 ± 1.5	9.0 ± 2.1	8.1 ± 2.1
Total No. live litters	23	23	17	14
Total No. live fetuses	184	190	147	110
Mean (± SD) No. live fetuses/litter	8.0 ± 1.8	8.3 ± 1.4	8.6 ± 2.3	7.8 ± 2.1
Total No. dead fetuses	0	0	0	0
Total No. resorptions [f]	6	8	7	4
Early	3	6	3	4
Late	3	2	4	0
Mean (± SD) resorptions/litter	0.3 ± 0.7	0.3 ± 0.6	0.4 ± 1.0	0.3 ± 0.5
Early	0.1 ± 0.4	0.3 ± 0.5	0.2 ± 0.4	0.3 ± 0.5
Late	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.8	0.0 ± 0.0
Litters with total resorptions	0	0	0	0
Mean fetal weight (g) [g]	44.5 ± 4.6 CV=10%	44.0 ± 4.0	40.1 ± 4.2** (↓10)	40.2 ± 3.9** (↓10)
Live males	45.1 ± 5.0	44.5 ± 4.4	40.2 ± 5.1** (↓11)	41.2 ± 4.3* (↓9)
Live females	43.6 ± 4.8	43.6 ± 4.1	39.4 ± 4.3** (↓10)	39.1 ± 3.3** (↓10)
Sex ratio (mean ± SD % male)	53.0 ± 18.4	51.7 ± 14.8	60.3 ± 17.8	46.3 ± 23.2
Pre-implantation loss (%)	4.1 ± 6.4	4.6 ± 6.7	4.3 ± 15.2	7.0 ± 9.6
Post-implantation loss (%)	3.1 ± 8.4	3.9 ± 6.3	5.4 ± 11.7	3.6 ± 5.9

a Data were obtained from Tables 9 and 10 on pages 56-58 of MRID 52154803. Percentage differences from control (calculated by the Reviewers) are presented in parentheses.
b Calculated by the Reviewers from data presented in this table.
c Includes one rabbit found dead and one rabbit euthanized due to apparent intubation errors and replaced on study.
d Includes two rabbits found dead from apparent intubation errors and replaced on study.
e Calculated by the Reviewers from individual data in Table 21 on pages 102-105.
f Calculated by the Reviewers from data presented in this table.
g Historical controls (mean, range): fetal 42.7, 38.3-46.7; live males 43.3, 38.7-48.0; live females 41.8, 37.4-45.5.
* Significantly different from control; p≤0.05.
** Significantly different from control; p≤0.01.

B. DEVELOPMENTAL TOXICITY

1. **External examinations**: External findings are presented in Table 4. One control fetus (#7746-4) had missing first digits on both forepaws, a malformation. There were no other external malformations and no external variations.

TABLE 4. External malformations (# fetuses/# litters). [a]				
Observations	**Dose (mg/kg/day)**			
	0	**100**	**750**	**1000**
No. Fetuses (litters) examined	**184 (23)**	**190 (23)**	**147 (17)**	**110 (14)**
Forepaws: first digits absent, bilateral	1 (1) [b]	0 (0)	0 (0)	0 (0)

a Data were obtained from Table 12 on page 60 of MRID 52154803.
b Fetus #7746-4 also included in skeletal malformations.

2. **Visceral examination:** Visceral findings are presented in Table 5. There were no visceral malformations. Folded retina (eyes) was observed in two fetuses (two litters) at 100 mg/kg/day and one fetus at 750 mg/kg/day, and an additional 100 mg/kg/day fetus was noted with the left carotid artery arising from the innominate artery, all compared to 0 control. These isolated variations were considered incidental. There were no other visceral variations.

TABLE 5. Visceral variations (# fetuses/# litters). [a]				
Observations	**Dose (mg/kg/day)**			
	0	**100**	**750**	**1000**
No. Fetuses (litters) examined	**184 (23)**	**190 (23)**	**147 (17)**	**110 (14)**
Eyes: retina folded	0 (0)	2 (2)	1 (1)	0 (0)
Vessels: left carotid arises from the innominate artery	0 (0)	1 (1)	0 (0)	0 (0)

a Data were obtained from Table 13 on page 61 of MRID 52154803.

3. **Skeletal examination:** Skeletal findings are presented in Table 6. Three fetuses were found with multiple skeletal malformations. One control fetus (#7746-4) was observed with missing first digits on both forepaws (previously cited in External examination) and not ossified metacarpals, proximal phalanges, and distal phalanges (with phalanx absent). One 100 mg/kg/day fetus (#7707-6) was noted with hemivertebrae present as the 13th thoracic vertebrae, right arch and centrum with attached rib, fused centra of the 13th thoracic and 1st lumbar vertebrae, and bifid centrum of the 1st lumbar vertebrae. Finally, one 1000 mg/kg/day fetus (#7758-5) had fused centra of the 1st and 2nd and the 2nd and 3rd vertebrae, unilateral ossification of the centrum of the 1st lumbar vertebra, and bifid centrum of the 2nd lumbar vertebra. Because these malformations were confined to single fetuses with no relationship to dose, they were considered incidental. There were no other skeletal malformations.

Three 750 mg/kg/day fetuses were found with multiple skeletal variations. One fetus (#7780-9) was observed with an irregular frontal nasal suture (skull) and fused sternal centra; one fetus (#7784-4) was noted with incompletely ossified manubrium and sternal centra, scapulae ala irregularly shaped, and small phalanx of the forelimb; and one fetus (#7795-9) was seen with misaligned caudal vertebrae and fused sternal centra. Because these variations were confined to single fetuses with no relationship to dose, they were considered incidental. All other skeletal variations were observed at low incidence (maximum of 4 fetuses; 3 litters) and/or in a manner unrelated to dose and were considered incidental. Fetal ossification sites were unaffected by treatment.

TABLE 6. Skeletal malformations and variations (# fetuses/# litters). [a]

Observations	Dose (mg/kg/day)			
	0	100	750	1000
No. Fetuses (litters) examined	**184 (23)**	**190 (23)**	**147 (17)**	**110 (14)**
Malformations				
Multiple malformations: forepaws with bilateral first digits absent; not ossified metacarpals, proximal phalanges, and distal phalanges (phalanx absent)	1 (1 [b])	0 (0)	0 (0)	0 (0)
Multiple malformations: hemivertebrae present as 13th thoracic vertebra; right arch and centrum with attached rib; fused centra of 13th thoracic and 1st lumbar vertebrae; and bifid centrum of 1st lumbar vertebrae	0 (0)	1 (1 [c])	0 (0)	0 (0)
Multiple malformations: fused centra of 1st and 2nd, and 2nd and 3rd vertebrae; unilateral ossification of centrum of 1st lumbar vertebra; and bifid centrum of 2nd lumbar vertebra	0 (0)	0 (0)	0 (0)	1 (1 [d])
Variations				
Multiple variations: skull, nasal – frontal, suture irregular; sternal centra, fused	0 (0)	0 (0)	1 (1)	0 (0)
Multiple variations: manubrium and sternal centra, incompletely ossified; scapulae, ala irregularly shaped; forelimb, small phalanx	0 (0)	0 (0)	1 (1)	0 (0)
Multiple variations: caudal vertebrae, misaligned; sternal centra, fused	0 (0)	0 (0)	1 (1)	0 (0)

a Data were obtained from Table 14 on pages 62-64 of MRID 52154803.
b Fetus #7746-4; also included in external malformations.
c Fetus #7707-6.
d Fetus #7758-5.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The maternal NOAEL for triazole acetic acid is 100 mg/kg/day based on effects on clinical signs, food consumption, body weight gain, and mortality at 750 and 1000 mg/kg/day dosages. These effects were likely due to localized gastrointestinal tract disturbance including stomach mucosal surface erosions/ulcerations related to the strong acidic property (pH 1.9-2.0) of the test substance.

The developmental NOAEL is also 100 mg/kg/day based on decreased mean fetal weights at 750 and 1000 mg/kg/day dosage groups. No gross, soft tissue or skeletal alterations related to the test substance occurred at dosages up to 1000 mg/kg/day.

B. REVIEWERS COMMENTS: The Reviewers generally agree with the Investigators' conclusions, except including observed clinical signs and effects on body weight gain in the determination of the NOAEL. These findings occurred only in the 1000 mg/kg/day animals and are not considered adverse.

1. Maternal toxicity: There were no effects of treatment on gross pathology, organ weights, or cesarean section parameters.

One 100 mg/kg/day doe, five 750 mg/kg/day does, and two 1000 mg/kg/day does were found dead or euthanized prior to scheduled termination due to causes unrelated to treatment (one adverse lenticular opacity, the rest gavage errors). Treatment related mortalities included one 750 mg/kg/day doe that delivered its litter prematurely on GD 29 and was euthanized, and eight 1000 mg/kg/day does found dead. These rabbits had necropsy findings of stomach lesions described as numerous discolored (black or red) areas or ulcerations ranging in size from pinpoint to 1.0 cm diameter. These stomach lesions were attributed to the low pH (pH 1.9-2.0) of the test formulations, resulting in irritation, decreased food consumption, decreased body weight gains, morbidity, and death.

At 1000 mg/kg/day, increased ($p \leq 0.01$) incidences of scant feces (beginning on GD 14) were observed in six of the eight does that died prematurely with treatment-related changes. These findings were generally consistent with decreases in food consumption and increases in the incidence of stomach lesions.

Additionally, at 1000 mg/kg/day, body weight gains were decreased ($p \leq 0.05$) during GD 12-15 (0.00 kg treated vs. 0.10 kg control) and GD 24-29 (0.01 kg treated vs. 0.09 kg control), resulting in decreased ($p \leq 0.05$) body weight gains during treatment (GD 6-29; ↓31%) and overall (GD 0-29; ↓25%). At 750 mg/kg/day, absolute food consumption was decreased ($p \leq 0.05$) by 11% during GD 12-15, by 14-19% during GD 21-21, and by 10% during treatment (GD 6-29); relative food consumption was decreased ($p \leq 0.05$) by 11% during GD 12-15, by 13-18% during GD 21-21, and by 9% during treatment (GD 6-29). At 1000 mg/kg/day, absolute food consumption was decreased ($p \leq 0.05$) by 8-33% during GD 9-29 and by 12% during treatment (GD 6-29); relative food consumption was decreased ($p \leq 0.05$) by 8-33% during GD 9-29 and by 11% during treatment (GD 6-29).

A systemic maternal LOAEL could not be determined. The systemic maternal NOAEL is 1000 mg/kg/day, the highest dose tested.

The portal of entry maternal LOAEL is 1000 mg/kg/day based on mortality and stomach lesions due to the low pH of the dosing formulation. The portal of entry maternal NOAEL is 750 mg/kg/day.

2. Developmental toxicity

a. Deaths/resorptions: There were no dead fetuses and no effects on fetal resorptions.

b. Altered growth: Fetal weights were decreased ($p \leq 0.05$) by 9-11% in, males females, and combined sexes at 750 and 1000 mg/kg/day. This apparent effect was not considered significant because (1) the small magnitude of the change, and (2) means for control and treated groups are within the historical control ranges.

c. Developmental variations: There were no treatment-related variations.

d. Developmental malformations: There were no treatment-related malformations.

A developmental LOAEL was not detected. The developmental NOAEL is 1000 mg/kg/day, the highest dose tested.

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement (OCSPP 870.3700b; OECD 414) in the rabbit.

C. STUDY DEFICIENCIES: The following deficiency was noted:

- Stability data were not reported.

DATA EVALUATION RECORD

TRIAZOLE ACETIC ACID (METABOLITE OF TRIAZOLE)

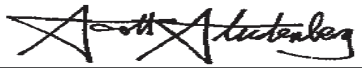
Study Type: OCSPP 870.3800; Reproduction and Fertility Effects in Rats

EPA Contract No. 68HERC22D0017
Task Assignment No.: 5540-2.1-022 (MRID 52154802)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Prepared by

1151 Lost Creek Boulevard
Austin, TX 78746

Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 05/19/2023
Secondary Reviewer: Sarah E. Saucier, Ph.D.	Signature: Date:	 05/26/2023
Quality Assurance: Michael E. Viana, Ph.D.	Signature: Date:	 06/01/2023
Project Manager: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 06/01/2023

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by PB&A/CSS Joint Venture personnel. Contractor's role did not include establishing Agency policy.

EPA Reviewer: Minerva Mercado-Feliciano PhD, DABT **Signature:**
Risk Assessment Branch IV, HED (7509P) **Date:** 14-Jul-2023
EPA Secondary Reviewer: Megan Stallard, PhD **Signature:**
Risk Assessment Branch IV, HED (7509P) **Date:** 14-Jul-2023
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study – Rat; OCSPP 870.3800 [§83-4]; OECD 415/416.

PC CODE: 600082 **DP BARCODE:** Not provided
TXR#: 0058589

TEST MATERIAL (PURITY): Triazole acetic acid (98.5% a.i.)

SYNONYMS: Amitrole-triazolylacetic acid; 1,2,4-triazol-1-yl-acetic acid

CITATION: Schneider, S. (2010) Triazole acetic acid: enhanced one-generation reproduction toxicity study in Wistar rats - administration via the diet. Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany. Project No.: 75R0330/08062, December 14, 2010. MRID 52154802. Unpublished.

SPONSOR: Triazole Derivative Metabolite Group, c/o McDermott, Will & Emery, 600 13th St., N.W., Washington, D.C.

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 an extended one-generation reproduction toxicity study (MRID 52154802), groups of 25 Han Wistar (Crl:WI[HAN]) rats/sex were administered triazole acetic acid (98.5% a.i.; Batch #RDL211-8-2) via the diet at nominal dose levels of 0, 100, 300, or 1000 mg/kg/day (equivalent to 0/0, 96/98, 287/293, and 959/976 mg/kg/day in males/females during pre-mating) continuously throughout the entire study, which included at least 73 days prior to mating to produce the F1 generation. Dietary concentrations were adjusted weekly for each group and sex based on body weight and food consumption from the preceding week. During lactation, the females were administered dietary concentrations adjusted to 50% of the concentrations fed during the final week of pre-mating (to account for increased food intake by

dams during lactation). On post-natal day (PND) 4, litters with more than eight offspring were randomly culled to eight offspring (four pups/sex/litter, if possible). On PND 21, 25 F1 pups/sex (one pup/sex/litter, if possible) were selected and administered the same nominal dose levels as their parents until attainment of sexual maturation.

There were no effects of treatment on mortality, clinical signs, organ weights, or macroscopic or microscopic pathology.

At 1000 mg/kg/day (nominal dose and the limit dose), male body weights were decreased by 5-7% from Weeks 6-16; however, this magnitude of body weight change is not considered adverse. Female body weights were similar to controls during pre-mating, gestation and lactation.

A LOAEL for parental toxicity was not detected. The NOAEL for parental toxicity is 1000 mg/kg/day (actual doses of 959/976 mg/kg/day in males/females during pre-mating), the highest dose tested.

There were no adverse effects of treatment on offspring for litter parameters, viability, clinical signs, body weights and body weight gains, sexual maturation, organ weights, or gross pathology.

The LOAEL for offspring toxicity was not observed. The NOAEL for offspring toxicity is 1000 mg/kg/day (actual doses of 959.0/976.3 mg/kg/day in male/female parents during pre-mating; 925.7/770.1 mg/kg/day in F1 offspring).

There were no adverse effects of treatment on estrous cyclicity or periodicity, sperm parameters, or reproductive performance.

The LOAEL for reproductive toxicity was not observed. The NOAEL for reproductive toxicity is 1000 mg/kg/day (actual doses of 959.0/976.3 mg/kg/day in males/females during pre-mating).

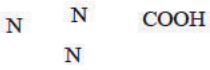
This study is classified **Acceptable/Guideline** and satisfies the guideline requirements (OCSPP 870.3800; OECD 416) for an extended one-generation reproduction toxicity study in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Triazole acetic acid

Description:	Light beige solid
Batch #:	RDL211-8-2
Purity:	98.5% a.i.
Expiration/Storage:	January 29, 2010 / 25 ± 5°C
CAS # of TGAI:	28711-29-7
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Han Wistar; Crl:WI(Han)
Age at study initiation:	(P) 34-36 days; (F1) PND 21
Weight at study initiation:	(P) Males: 124.9-158.8 g; Females: 110.6-138.5 g (F1 [mean]) Males: 48.8-49.3 g; Females: 47.1-48.0 g
Source:	Charles River Laboratories Research Models and Services, Germany GmbH
Housing:	Individually in Makrolon type M III cages with Lignocel FS 14 fibres dust-free bedding (Ssniff, Soest, Germany), except during overnight matings (male and female mating partners were housed together) and late gestation through lactation (dams and litters were housed together). Nesting material was provided and wooden gnawing blocks for environmental enrichment.
Diet:	Ground Kliba maintenance diet mouse/rat "GLP" meal (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*.
Water:	Drinking water from water bottles, *ad libitum*.
Environmental conditions:	**Temperature:** 20-24°C **Humidity:** 30-70% **Air changes:** 15/hour **Photoperiod:** 12 hours light / 12 hours dark
Acclimation period:	7 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Start: February 19, 2009 End: July 15, 2009

2. Mating procedure: During the mating period, each female was paired with a preselected male from the same dose group for up to a maximum of two weeks. Each male was placed in the cage of its female mating partner overnight for a period of approximately 15-17 hours. A vaginal smear was prepared the morning after each pairing and examined for the presence of sperm. If sperm was detected, pairing was discontinued, and the day of detection was considered gestation day (GD) 0.

3. Study schedule: The P were fed the test diets for at least 73 days prior to mating. The test diets were provided to the males during mating and up to necropsy (after F1 pups were weaned on post-natal day [PND] 21) and to the females during mating, gestation, lactation, and up to necropsy (PND 4 or 21). The females were allowed to litter naturally and rear their pups. On PND 4, litters with more than eight pups were randomly culled to eight pups

total (four pups/sex, if possible; if not possible, the most even distribution possible with a total of eight pups was selected). On PND 21, 25 pups/sex from each dose level were selected as F1 animals (one pup/sex/litter, if possible). If fewer than 25 litters were available, additional pups were selected from the available litters. The selected F1 pups were fed diets at the same dose levels as their parents until attainment of sexual maturity and then euthanized.

4. **Animal assignment:** The P rats were assigned to test groups presented in Table 1, which also shows nominal doses (target) and estimated doses (calculated per food consumption and body weights) for P and F1 animals. Rats with the lowest and highest body weights were excluded and animals were assigned to the test groups with a randomization program according to body weights three days prior to the first day of test item administration.

TABLE 1. Animal assignment and mean test compound intake (mg/kg/day) . [a]

Group	Nominal dose (mg/kg/day) [b]				Animals per dose group	
	0	100	300	1000	Males	Females
P generation males (overall)	0	95.7	287.2	959.0	25	25
F1 generation males (Weeks 0-3)	0	93.2	280.4	925.7	25	25
P generation females, pre-mating	0	97.8	293.1	976.3	25	25
F1 generation females (Weeks 0-1)	0	77.6	245.7	770.1	25	25
P generation females, gestation	0	105.1	315.2	1073.1	25	25
P generation females, lactation	0	115.3	353.1	1226.5	25	25

a Data were obtained from pages 32 60 and 127-128 of MRID 52154802.
b Dietary concentrations were decreased by 50% for females during lactation.

5. **Dose selection rationale:** Dose levels were selected based on the results of a dose range-finding study (Project No. 91R0330/08042; details and complete citation not provided). The selected nominal dose levels were 100, 300, and 1000 mg/kg/day (the limit dose).

6. **Dosage preparation and analysis:** Dose formulations were prepared by mixing the required quantity of test substance with a small amount of food to create a premix (adjustment for purity not stated). For each dose formulation, additional amounts of food were added to the premix to obtain the desired concentration. The dietary formulations were prepared at intervals that maintained test item stability. The actual diet concentrations (ppm) were not reported; diet concentrations were adjusted according to body weights and/or study period to maintain the nominal dose levels. The initial diet preparation concentrations (Week 1 of premating) were calculated from body weights determined at randomization and historical food consumption data/sex (18 g/day for males and 15 g/day for females). Diet concentrations during the remainder of premating were adjusted weekly based on measured body weights and food consumption values determined from the previous week. Test diet concentrations during mating were maintained at the levels used during the final week of premating. After successful mating, test diet concentrations for males were adjusted weekly until the end of the study. Diet concentrations for females during gestation were maintained at the levels used during the final week of premating, and diet concentrations during lactation were decreased to 50% of the respective concentrations during the final week of premating. This adjustment was made to maintain the dams at their

respective nominal dose levels. Diet concentrations for post-weaning dams were returned to the levels used during the final week of premating. Diet concentrations fed to pups selected to continue in the study were based on historical body weight and food consumption data for rats of a similar age. During the first week of the rearing period of F1 animals, diet concentrations were determined based on Day 0 body weights and historical food consumption data from the previous week. Subsequent diet concentrations for F1 animals were adjusted as described for P animals.

Stability of the test item in diet preparations was verified previously after storage for up to 21 days at room temperature (data reported on pages 559-564 of the MRID). Diet samples for analyses were collected at study initiation and towards the end of the premating period. Additional analyses of diet preparations for females were conducted during the gestation and lactation periods.

Results

Homogeneity (%CV): 2-14% (mean); 1.03-19.3% (individual)

Stability (% of time 0): 90.4-98.8%

Concentration (% of nominal): 77-109%

The initial sample analyses for the high-dose diet preparations from the Week 1 analyses yielded concentrations for two of the three samples that deviated by approximately 20% from the nominal value. As the repeat analyses yielded values that also differed approximately 20% from the nominal values but in the opposite directions to the original values, the means of all values were reported. The analytical data indicated that the mixing procedure was generally adequate and that the variance between nominal and actual doses to the animals was acceptable.

C. OBSERVATIONS

1. **Parental animals:** All parental rats were checked for mortality and morbidity twice daily by visual inspection (or once daily on weekends and holidays). Cageside clinical examinations were conducted on all rats daily; parturition and lactation behaviors for dams were generally evaluated during the daily cageside examination and twice daily on Monday through Friday. Body weights were determined on the first day of test diet administration, weekly thereafter, and at euthanasia. Exceptions included: P females were weighed on GD 0, 7, 14, and 20 and females that delivered litters were weighed on lactation day (LD) 1, 4, 7, 14, and 21. Body weight gains were calculated for each weighing interval. In addition, females were not weighed during pairing until GD 0 was established. Food consumption was determined on a weekly basis (6-day period) for P animals and F1 animals post-weaning, except for pregnant females and females with litters. Food consumption during gestation and lactation was determined for GD 0-7, 7-14, and 14-20 and for LD 1-4, 4-7, 7-14, and 14-21. Food consumption was not determined during the mating period, for females without positive evidence of sperm, or females without litters. Actual test item intake was

calculated for males (overall) and for females during the premating, gestation, and lactation periods. The following equation was used to calculate the achieved doses.

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{mean daily food consumed (g/rat)} \times \text{concentration(ppm)}}{\text{body weight (g)on last day before the end of the period}}$$

Estrous cycle length was determined daily for a minimum of three weeks prior to pairing, continuing during the pairing period until evidence of mating was observed. An additional final vaginal smear was collected at necropsy to determine the stage of estrus at euthanasia.

2. **Litter observations:** Litter observations (X) are presented in Table 2.

TABLE 2. F1 litter observations [a]

Observation	Time of observation						
	PND 0	PND 1	PND 4 [b]	PND 4 [c]	PND 7	PND 14	PND 21
Number of live pups	X	X	X	X	X	X	X
Pup weight	---	X	X	---	X	X	X
External alterations	X	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X	X
Sex of each pup (M/F)	X	---	---	---	---	---	X

a Data were obtained from pages 43-44 of MRID 52154802.
b Before standardization (culling)
c After standardization (culling)
--- Not examined

The live pups were examined daily for clinical symptoms (including gross-morphological findings) during the clinical inspection of the dams. On PND 4, litters were randomly standardized to a maximum of eight pups/litter (four pups/sex, if possible); litters with fewer than eight pups were not culled. All culled pups were euthanized and necropsied; culled pups with notable findings were evaluated further depending on the finding(s).

3. **Post weaning F1 animals:** The study report does not indicate how mortality, cage side clinical observations or food consumption observations were made for the F1 animals after weaning. Data for these endpoints was reported for weeks 0, 1 and 2 in males (about PND 22-42), and for weeks 0 and 1 for females (about PND 22-35). Body weights were determined post-weaning on the first day of test diet administration, weekly thereafter, and at euthanasia.

Sexual maturation was assessed in all selected F1 males and females. Preputial separation was determined in males by daily examination beginning on PND 38 and vaginal patency was determined in females by daily examination beginning on PND 27. Body weights on the day of attainment of criterion were recorded.

4. **Postmortem observations**

a. **Parental animals:** All parental rats were euthanized by exsanguination after decapitation under isoflurane anesthesia, weighed, and subjected to a complete necropsy, with a focus on the reproductive organs. The following tissues (X) were collected and the (XX) tissues were weighed.

XX	Adrenals	XX	Prostate
XX	Brain	XX	Seminal vesicles (w/coagulation glands)
XX	Cauda epididymis [a]	XX	Spleen
XX	Epididymides	XX	Testes
XX	Kidneys	XX	Thyroid (w/parathyroids)
XX	Liver	XX	Uterus (w/cervix and oviducts)
XX	Ovaries	X	Vagina
XX	Pituitary	X	Gross lesions

a Weight only

Tissues were fixed in 4% neutral-buffered formaldehyde, except the left epididymis, left testis, and ovaries (modified Davidson's fluid). All tissues, except the brain, spleen, and thyroids (w/parathyroids), were examined microscopically as follows:

- all gross lesions in affected animals;
- all control and high-dose rats, except for the liver and kidneys for which only males were examined; and
- all organs in mating pairs of any low- or mid-dose rats with decreased fertility.

The right testis and cauda epididymis were collected from P males of all dose groups for sperm parameter evaluations. Sperm motility and morphology, and separate sperm head counts for the cauda epididymis and testis were evaluated for the control and high-dose males.

Five sections were prepared from the proximal and the distal part of both ovaries of control and high-dose P females (≥100 µm apart from the inner third of the ovary). All ovarian sections were prepared and evaluated for differential ovarian follicle count (DOFC). The numbers of primordial follicles and growing follicles were determined by light microscopy. Only follicles with an oocyte with visible chromatin on the slide were counted. The number of each type of follicle was recorded individually for each left/right ovary of the pair on all slide levels (1-10) to determine the incidences of each type of follicle. As primordial follicles develop continuously into growing follicles, the assessment of follicles was extended to the combined incidence of primordial plus growing follicles. Generally, the fifth slide prepared from the left and right ovary of each pair was evaluated for histological evaluation.

b. **Pre-weaning F1 animals:** Unselected F1 offspring on PND 4 or 21 were euthanized by carbon dioxide asphyxiation and subjected to a complete necropsy. Pups that were stillborn or died prematurely were subjected to a complete necropsy. Pups displaying notable findings were evaluated on a case-by-case basis; pups without notable findings were discarded. Pups euthanized on PND 21 were necropsied and abnormal tissues were fixed and retained. The brain, spleen, and thymus from one pup/sex/litter (generally the first per litter) were excised and weighed after the scheduled euthanasia on PND 21. The PND 21 body weights were used for calculation of relative (to body) organ weights.

c. **Post-weaning F1 animals:** The study report does not indicate how F1 animals were killed or necropsied after sexual maturation, however necropsy findings were reported.

D. DATA ANALYSIS

1. **Statistics:** Group means and standard deviations (SD) were calculated as appropriate. All analyses were performed separately on males and females with individual rats as the basic experimental unit. For litter/fetal findings, the litter was considered the treated unit and the basis for analysis. The following statistical analyses were performed, with significance denoted at the 5% or 1% levels.

Parameters	Statistical analysis
Food consumption (P animals), body weight and body weight change (P animals and F1 pups; litter means were used for the pup weights), estrous cycle duration, number of mating days, duration of gestation, number of implantation sites, post-implantation loss and % post-implantation loss, number of pups delivered per litter, and duration of sexual maturation (days to vaginal opening or preputial separation)	Simultaneous comparison of all dose groups to control with a two-sided Dunnett's test to evaluate the hypothesis of equal means.
Male and female mating and fertility indices, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups euthanized moribund, viability index, lactation index, number of litters with affected pups at necropsy, sexual maturation data (vaginal opening, preputial separation), and males with abnormal sperm (cutoff value: 90% of control group)	Pairwise comparison of each dose group to control by using Fisher's Exact test for the hypothesis of equal proportions.
Proportions of affected pups per litter with necropsy observations, total spermatids/g testis, and total sperm/g cauda epididymides	Pairwise comparison of each dose group to control by using a one-sided Wilcoxon test for the hypothesis of equal medians.
Sperm motility (%)	Pairwise comparison of each dose by using a one-sided Wilcoxon test with the Bonferroni-Holm adjustment for the hypothesis of equal medians.
Absolute and relative (to body) pup organ weights and terminal body weight parameters	Non-parametric one-way analysis of variance (ANOVA) by using a two-sided Kruskal-Wallis test. If the resulting p-value was ≤0.05, a pairwise comparison of each dose group to control was conducted with a two-sided Wilcoxon test for the hypothesis of equal medians.
Follicles: a) primordial, b) growing, and c) primordial + growing	Pairwise comparison of the high-dose group to control by using a one-sided Wilcoxon test for the hypothesis of equal medians.

The Reviewers considered the statistical analyses appropriate.

2. Indices

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

$$\text{Percentage mating (\% male and female)} = \frac{\text{\# rats mating}}{\text{\# rats paired}} \times 100$$

$$\text{Fertility index (\% male and female)} = \frac{\text{\# pregnant females}}{\text{\# males or females mated}} \times 100$$

$$\text{Gestation index (\%)} = \frac{\text{\# females with live pups born}}{\text{\# pregnant females}} \times 100$$

$$\text{Live birth index (\%)} = \frac{\text{\# liveborn pups at birth}}{\text{\# pups born}} \times 100$$

$$\text{Post} - \text{implantation loss (\%)} = \frac{\text{\# implanatations} - \text{\# pups delivered}}{\text{\# implantations}} \times 100$$

Offspring indices: The following viability indices were calculated from lactation records of litters in the study.

$$\text{Viability index (\%)} = \frac{\text{total \# live pups on PND 4 (pre-culling)}}{\text{total \# live pups on PND 1}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{total \# live pups on PND 21}}{\text{total \# live pups on PND 4 (post-culling)}} \times 100$$

$$\text{Sex ratio (\%)} = \frac{\text{total \# males or females/litter}}{\text{total \# pups/litter}} \times 100$$

3. **Historical control data:** Historical reproductive parameter control data from 51 different studies conducted between April 17, 2000 and September 29, 2008 were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality:** All P animals survived to scheduled euthanasia.

2. **Clinical signs:** There were no treatment-related clinical signs of toxicity in the P animals. During the lactation period, single P dams in the control and 300 mg/kg/day dose groups (#103 and #166, respectively) did not nurse their pup(s) properly resulting in the deaths of most of the pups of dam #103 and all pups of dam #166. Neither of these observations were related to treatment.

3. **Body weights and body weight gains**

a. **Pre-mating:** Selected body weight and body weight gain data for the P animals during pre- and post-mating are presented in Table 3a. In the high-dose males, body weights were decreased ($p \leq 0.05$) by 5-7% from Weeks 6-16; however, this magnitude of body weight change is not considered adverse. Pre-mating body weight gains were decreased ($p \leq 0.05$) during Weeks 1-2 (↓7%) and Weeks 3-6 (↓11-20%), and there were decreases ($p \leq 0.05$) in post-mating body weight gains during Weeks 14-15 by ↓47%, by 10% during Weeks 0-10 (not statistically evaluated), and by 10% for the overall administration period (Weeks 0-16).

Intermittent decreases in mean body weights and weight gain in the mid-dose males were not significant (NS), except for a non-dose-related decrease ($p \leq 0.05$) of 32% in body weight gain during Week 11-12. This was not considered relevant. There were no treatment-related effects on body weight or body weight gain in the low-dose P males and any treated P females during pre-mating for any dose group.

TABLE 3a. Selected mean (± SD) body weights and body weight gains (g) in the P generation rats administered triazole acetic acid in the diet during pre-mating and post-mating (males only). [a]

Observation/Study Day			Nominal dose (mg/kg/day)			
			0	100	300	1000
Males						
Body weight	Pre-mating	Week 0	142 ± 7	142 ± 7	142 ± 8	142 ± 8
		Week 6	329 ± 19	323 ± 16	321 ± 22	313 ± 19* (↓5)
		Week 10	380 ± 24	375 ± 25	368 ± 31	357 ± 25** (↓6)
	Post-mating	Week 13	403 ± 26	398 ± 28	388 ± 33	378 ± 24** (↓6)
		Week 16	427 ± 28	423 ± 30	409 ± 34	399 ± 27** (↓7)
Body weight gain		Week 5-6	21 ± 5	20 ± 4	19 ± 5	17 ± 5** (↓20)
		Weeks 0-10 [b]	238	233	227	215 (↓10)
		Week 14-15	8.6 ± 5.9	9.5 ± 4.9	7.9 ± 6.2	4.6 ± 4.0* (↓47)
		Weeks 0-16	285 ± 24	281 ± 29	267 ± 31	257 ± 24** (↓10)
Females						
Body weight	Pre-mating	Week 0	122 ± 6	124 ± 7	124 ± 5	124 ± 5
		Week 10	215 ± 16	219 ± 15	220 ± 13	216 ± 13.4
Body weight gain		Weeks 0-10	93± 14	95 ± 13	96 ± 13	92 ± 12

a Data were obtained from pages 87-93 of MRID 52154802; n = 25/sex. Percentage differences from control (calculated by the Reviewers) are included in parentheses.
b Calculated by the Reviewers from mean data in this table.
* Significantly different from control; $p \leq 0.05$
** Significantly different from control; $p \leq 0.01$

b. Gestation: Body weight and body weight gain data during gestation are presented in Table 3b. There were no treatment-related effects on body weight or body weight gain in the P females during gestation.

TABLE 3b. Mean (± SD) body weights and body weight gains (g) in females administered triazole acetic acid in the diet during gestation. [a]

Observation/Gestation Day		Nominal dose (mg/kg/day)			
		0	100	300	1000
P Females					
Body weight	GD 0	221 ± 15	233 ± 14	222 ± 12	222 ± 13
	GD 7	243± 17	244 ± 14	242 ± 13	243 ± 16
	GD 14	265 ± 18	266 ± 16	262 ± 13	266 ± 18
	GD 20	323 ± 24	325 ± 21	316 ± 19	322 ± 25
Body weight gain	GD 0-20	101 ± 12	101 ± 11	94 ± 12	100 ± 14

a Data were obtained from pages 94-95 of MRID 52154802; n = 25/sex.

c. **Lactation:** Body weight and body weight gain data during lactation are presented in Table 3c. There were no treatment-related effects on body weight or body weight gain in the P females during lactation.

TABLE 3c. Mean (± SD) body weights and body weight gains (g) in females administered triazole acetic acid in the diet during lactation. [a]

Observation/Lactation Day		Nominal dose (mg/kg/day) [b] 0	100	300	1000
P Females					
Body weight	LD 1	241 ± 18	238 ± 16	237 ± 14	238 ± 15
	LD 4	256 ± 16	257 ± 17	249 ± 13	255 ± 16
	LD 7	262 ± 16	263 ± 16	257 ± 12	261 ± 16
	LD 14	277 ± 18	278 ± 18	271 ± 13	278 ± 17
	LD 21	269 ± 17	269 ± 15	263 ± 9	267 ± 14
Body weight gain	LD 1-21	28 ± 13	30 ± 8	26 ± 12	29 ± 11

a Data were obtained from pages 96-97 of MRID 52154802; n = 25/sex. Percentage differences from control (calculated by the Reviewers) are included in parentheses.
b Diet concentrations were decreased by 50% for females during lactation.

4. Food consumption

a. **Pre-mating:** Selected food consumption data for the P males during pre- and post-mating are presented in Table 4. In the P males, food consumption was decreased ($p \leq 0.05$) at the high dose during Weeks 4-6 by 5%, Weeks 7-8 by 6%, Weeks 9-10 by 7%, and Weeks 14-16 by 6-7%, and were generally correlated with the observed decreases in body weights and body weight gains in these animals. Food consumption was decreased ($p \leq 0.05$) at the mid-dose during Week 9-10, Week 11-12, and Week 15-16 by 6% each but these decreases were generally unrelated to dose. There were no adverse effects of treatment on food consumption in the low-dose P males and any treated P females during pre-mating.

TABLE 4. Selected mean (± SD) food consumption (g/animal/day) in the P generation rats administered triazole acetic acid in the diet. [a]

Observation/Study Day		Nominal dose (mg/kg/day) 0	100	300	1000
Males					
Pre-mating	Week 4-5	21.6 ± 1.2	21.1 ± 1.1	20.8 ± 1.6	20.5 ± 1.5* (↓5)
	Week 5-6	22.1 ± 1.4	21.6 ± 1.4	21.2 ± 1.8	21.0 ± 1.6* (↓5)
	Week 7-8	22.3 ± 1.1	21.9 ± 1.5	21.2 ± 1.8	20.9 ± 2.0** (↓6)
	Week 9-10	21.9 ± 1.5	21.3 ± 1.2	20.5 ± 1.8** (↓6)	20.4 ± 1.8** (↓7)
Post-mating	Week 14-15	21.6 ± 1.7	21.0 ± 1.5	21.0 ± 2.4	20.1 ± 1.4* (↓7)
	Week 15-16	21.6 ± 1.5	21.1 ± 1.5	20.3 ± 1.7* (↓6)	20.4 ± 1.6* (↓6)
Overall	Week 0-16 [b]	20.7 ± 2.1	20.4 ± 1.9	20.0 ± 1.7	20.0 ± 1.7

a Data were obtained from pages 82-83 of MRID 52154802; n = 25/sex. Percentage differences from control (calculated by the Reviewers) are included in parentheses.
b Mean of means; n = 16
* Significantly different from control; $p \leq 0.05$
** Significantly different from control; $p \leq 0.01$

b. Gestation and Lactation: There were no effects of treatment on food consumption in the P females during gestation or lactation.

6. Reproductive function

a. Estrous cycle length and periodicity: There were no effects of treatment on estrous cycle length or periodicity in the P females. Mean days per cycle were 3.9 ± 0.2, 3.9 ± 0.2, 4.6 ± 1.7 and 5.1 ± 3.6 for the 0, 100, 300 and 1000 mg/kg/day groups, respectively.

b. Sperm measures: There were no effects of treatment on sperm counts (96 ± 16 per g testis high dose vs. 95 ± 15 per g testis in control), motility (89 ± 5 % high dose vs. 87 ± 7 % control), or morphology (94% normal high dose and control) in the P males.

7. Reproductive performance: Reproductive performance data are presented in Table 5. There were no effects of treatment on reproductive performance in the P or F1 generation.

TABLE 5. Reproductive performance in P rats administered triazole acetic acid in the diet. [a]

Observation	Nominal dose (mg/kg/day)			
	0	100	300	1000
Males paired	25	25	25	25
Females paired	25	25	25	25
Male mating index (%)	100	100	100	100
Female mating index (%)	100	100	100	100
Male fertility index (%)	100	100	100	100
Female fertility index (%)	100	100	100	100
Mean (± SD) pre-coital interval (days)	2.8 ± 1.04	2.4 ± 0.95	2.2 ± 1.22	2.7 ± 1.10
Mean (± SD) gestation length (days)	22.0 ± 0.35	22.0 ± 0.20	22.0 ± 0.35	21.9 ± 0.49
Gestation index (%)	100	100	100	100

a Data were obtained from pages 103, 105, and 106 of MRID 52154802.

8. Parental post-mortem results

a. Organ weights: Selected absolute and relative (to body) organ weight data are presented in Table 67. There were no effects of treatment on organ weights in the P females. In the high-dose P males, terminal body weight was decreased ($p \leq 0.01$) by 7%. Relative (to body) kidney and liver weights were increased ($p \leq 0.01$) by 11% and 8%, respectively. Relative (to body) kidney weight was also increased ($p \leq 0.01$) by 6% in the mid-dose males. As there were no changes in absolute kidney and liver weights and no corroborating changes in microscopic findings, increases in relative weights were considered secondary to the decreases in body weight and body weight gain at these dose levels and not adverse. The decrease in terminal body weight is considered related to treatment.

TABLE 6. Mean (± SD) absolute and relative (to body) organ weights in P males administered triazole acetic acid in the diet. [a]

Organ/tissue	Nominal dose (mg/kg/day)			
	0	100	300	1000
Terminal body weight (g)	407 ± 28	402 ± 29	391 ± 35	381 ± 26** (↓7)
Absolute kidney (g)	2.44 ± 0.24	2.50 ± 0.22	2.47 ± 0.30	2.52 ± 0.24
Relative kidney (%)	0.60 ± 0.05	0.62 ± 0.04	0.63 ± 0.05* (↑6)	0.66 ± 0.06** (↑11)
Absolute liver (g)	8.75 ± 0.82	8.82 ± 0.97	8.62 ± 0.91	8.83 ± 1.03
Relative liver (%)	2.15 ± 0.13	2.19 ± 0.12	2.20 ± 0.12	2.32 ± 0.18** (↑8)

a Data were obtained from pages 133-134 and 137-138 of MRID 52154802. Percentage differences from control are included in parentheses.
* Significantly different from control; $p<0.05$
** Significantly different from control; $p<0.01$

b. Pathology

i. Macroscopic examination: There were no effects of treatment on macroscopic findings in the P animals. Findings in a single animal and in a single group (no dose response) included adipose tissue necrosis, glandular stomach erosion/ulcer, kidney cysts, liver focal constriction, ovary cyst, pancreas focus, and uterus cyst.

ii. Microscopic examination: There were no effects of treatment on microscopic findings in the P animals. Findings that occurred with similar incidence in control and high dose groups included adrenal cortex accessory tissue, kidney basophilic tubules and/or eosinophilic droplets, kidney cysts, liver lymphoid infiltration and/or necrosis, pituitary cysts and/or dilation, and prostate lymphocytic infiltration. Findings in 1-2 animals in a single group (no dose response) included adipose tissue inflammation, adrenal cortex fatty change, glandular stomach erosion/ulcer, kidney tubular cast, liver fatty change or constriction, ovary cyst, pancreas hyperplasia, pituitary fatty change and/or hyperplasia, prostate inflammation and/or infiltration, and uterus cyst.

iii. Differential ovarian follicle count: There were no effects of treatment on DOFC results in the P females. Mean number of primordial follicles was 177.6 and 191.1 in the control and high dose groups, respectively. Mean number of growing follicles was 20.1 and 18.8 in the control and high dose groups, respectively.

B. OFFSPRING

1. Viability and clinical signs: Litter parameters are presented in Table 7. There were no effects of treatment on any F1 litter parameters.

TABLE 7. Litter parameters for F1 generation. [a]

Observation		Nominal dose (mg/kg/day) [b]			
		0	100	300	1000
No. of implantations		324	326	297	319
Mean (± SD) implantations		13.0 ± 2.01	13.0 ± 1.72	11.9 ± 2.45	12.8 ± 1.61
Number born		308	311	281	297
Mean (± SD) born		12.3 ± 2.04	12.4 ± 1.73	11.2 ± 2.62	11.9 ± 2.05
No. liveborn		301	310	279	293
No. stillborn		7	1	2	4
No. died		9	2	8	3
Sex ratio (% males)	PND 0	46.5	50.3	50.9	48.1
	PND 21	49.7	51.5	51.4	49.2
Mean (± SD) litter size	PND 1	11.3 ± 2.54	12.4 ± 1.66	11.1 ± 2.59	11.6 ± 2.14
	PND 4 [c]	11.3 ± 2.49	12.4 ± 1.73	10.9 ± 2.67	11.5 ± 2.16
	PND 4 [d]	7.8 ± 1.00	8.0 ± 0.00	7.7 ± 0.89	8.0 ± 0.20
	PND 7	7.8 ± 1.00	8.0 ± 0.00	7.4 ± 1.78	8.0 ± 0.20
	PND 14	7.8 ± 1.00	7.9 ± 0.28	7.4 ± 1.78	7.9 ± 0.28
	PND 21	7.8 ± 1.00	7.9 ± 0.28	7.4 ± 1.78	7.9 ± 0.33
Post-implantation loss (%)		5.0 ± 6.11	4.6 ± 4.99	5.8 ± 7.34	7.2 ± 8.21
Live birth index (%)		98	100	99	99
Viability index (%)		94	100	97	98
Lactation index (%)		100	99	96	99

a Data were obtained from pages 105-109 of MRID 52154802.
b Diet concentrations were decreased by 50% for females during lactation.
c Before standardization (culling)
d After standardization (culling)

2. Body weight: Pup body weight and body weight gain data are presented in Table 8. There were no treatment-related effects on pup body weights or PND 4-21 body weight gains in either sex.

TABLE 8. Mean (± SD) F1 pup body weights and overall (PND 4-21) body weight gains (g) after administration of triazole acetic acid in the diet. [a]

Parameter			Nominal dose (mg/kg/day) [b]			
			0	100	300	1000
Body weight	PND 1	Males	6.1 ± 0.63	6.1 ± 0.55	6.3 ± 0.43	6.3 ± 0.62
		Females	5.8 ± 0.60	5.8 ± 0.49	6.1 ± 0.50	6.0 ± 0.57
	PND 4 [c]	Males	9.3 ± 1.09	9.1 ± 1.06	9.1 ± 1.31	9.4 ± 1.27
		Females	9.1 ± 1.06	8.8 ± 0.96	9.0 ± 1.47	9.1 ± 1.25
	PND 4 [d]	Males	9.3 ± 1.06	9.1 ± 1.02	9.1 ± 1.34	9.4 ± 1.30
		Females	9.1 ± 1.09	8.8 ± 0.95	9.0 ± 1.46	9.1 ± 1.25
	PND 7	Males	15.4 ± 1.64	15.4 ± 1.31	15.4 ± 1.19	15.6 ± 1.70
		Females	15.0 ± 1.54	14.9 ± 1.37	15.0 ± 1.42	14.9 ± 1.64
	PND 14	Males	31.8 ± 2.22	31.7 ± 2.03	31.2 ± 1.50	31.8 ± 2.53
		Females	31.2 ± 1.96	30.8 ± 2.12	31.0 ± 2.63	30.8 ± 2.26
	PND 21	Males	49.3 ± 3.90	49.8 ± 3.25	48.9 ± 2.28	48.8 ± 4.19
		Females	48.0 ± 3.79	47.9 ± 3.35	47.9 ± 3.05	47.1 ± 3.51
Body weight gain PND 4-21		Males	39.9 ± 3.64	40.7 ± 2.96	39.6 ± 2.15	39.4 ± 3.59
		Females	38.9 ± 3.56	39.1 ± 3.08	38.8 ± 2.61	38.0 ± 3.04

a Data were obtained from pages 110, 111, and 113 of MRID 52154802; n = 24-25 litters

b Diet concentrations were decreased by 50% for P females during lactation.
c Before standardization (culling)
d After standardization (culling)

3. Sexual maturation: There were no adverse effects of treatment on sexual maturation in the F1 offspring. Mmean number of days to attainment of vaginal opening was 31.9, 32.2, 32.2 and 32.2 in control, low, mid and high dose groups, respectively. Mean number of days to attainment of preputial separation was 42.5, 41.6, 42.2 and 42.5 in control, low, mid and high dose groups, respectively was . Body weight at attainment of each criterion was similar to control for each parameter. All values were within historical control data ranges.

4. Offspring postmortem results

a. Organ weights: There were no adverse effects of treatment on organs weights of the F1 offspring. In the high-dose male pups, relative (to body) thymus weight was increased ($p \leq 0.01$) by 12%; however, this minor change had no corroborating pathology findings and was within the historical control data range. Therefore, it was considered incidental.

b. Macroscopic examination: There were no treatment-related findings at necropsy in the F1 offspring. Hydroureter was detected in one high dose PND 21 pup. Dilated renal pelvis, hydronephrosis and hydroureter were detected in one high dose PND 48 female. Diaphragmatic hernia was detected in one high dose PND 40 female. There were no other macroscopic findings in offspring.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Under the conditions of the present enhanced one-generation reproduction toxicity study, the no-observed-effect level (NOEL) for the P male parental rats for general, systemic toxicity is 100 mg/kg/day based on the small magnitude of food consumption and body weight gain decreases at 300 mg/kg/day. The no-observed-adverse-effect level (NOAEL) for the F0 male parental rats is 300 mg/kg/day based on significant reductions in food consumption and body weight gain observed at the lowest-observed-adverse-effect level (LOAEL) of 1000 mg/kg/day in the P parental males. The NOEL for fertility and reproductive performance for the P parental rats is 1000 mg/kg/day, the highest dose tested. The NOEL for developmental toxicity in the F1 progeny is 1000 mg/kg/day, the highest dose tested.

B. REVIEWER COMMENTS: The Reviewers do not completely agree with the Investigators' Conclusions.

1. Parental animals: There were no effects of treatment on mortality, clinical signs, organ weights, or macroscopic or microscopic pathology. At 1000 mg/kg/day (nominal dose and the limit dose), male body weights were decreased by 5-7% from Weeks 6-16; however, this magnitude of body weight change is not considered adverse. Female body weights were similar to controls during pre-mating, gestation and lactation.

A LOAEL for parental toxicity was not detected. The NOAEL for parental toxicity is

1000 mg/kg/day (actual doses of 959/976 mg/kg/day in males/females during pre-mating), the highest dose tested.

2. **Offspring:** There were no adverse effects of treatment on offspring for litter parameters, viability, clinical signs, body weights and body weight gains, sexual maturation, organ weights, or gross pathology.

   The LOAEL for offspring toxicity was not observed. The NOAEL for offspring toxicity is 1000 mg/kg/day (actual doses of 959.0/976.3 mg/kg/day in male/female parents during pre-mating; 925.7/770.1 mg/kg/day in F1 offspring).

3. **Reproductive performance:** There were no adverse effects of treatment on estrous cyclicity or periodicity, sperm parameters, or reproductive performance.

   The LOAEL for reproductive toxicity was not observed. The NOAEL for reproductive toxicity is 1000 mg/kg/day (actual doses of 959.0/976.3 mg/kg/day in males/females during pre-mating).

   This study is **Acceptable/Guideline** and satisfies the guideline requirements for an extended one-generation reproduction toxicity study (OCSPP 870.3800; OECD 416) in rats.

C. **STUDY DEFICIENCIES:** There were no study deficiencies.