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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: November 28, 2023

SUBJECT: **1,2,4-Triazole (a common metabolite of difenoconazole, prothioconazole, tebuconazole, triadimefon and other conazoles):** Review of Toxicology Study Submitted by a Registrant Pursuant to §6(a)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

PC Codes: 600074, 128847, 113961, 128997, 109901
Decision No.: 585749
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0058617

MRID No.: 51917801

DP Barcode: D465871
Registration No.: 264-824
Regulatory Action: Adverse Data §6(a)(2)
Case No.: N/A
CAS No.: 288-88-0, 119446-68-3, 178928-70-6, 107534-96-3, 43121-43-3
40 CFR: N/A

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

I. CONCLUSIONS/DISCUSSION

The registrant (Bayer Crop Science LP) for Prothioconazole Technical Fungicide (Registration 264-824) submitted an additional prenatal developmental study in rabbit (MRID 51917801) conducted with the metabolite 1-1,2,4-triazole, as information which may be required under Section 6(a)(2) of FIFRA. HED has reviewed the study and generated the attached Data Evaluation Record.

EPA Reviewer: Minerva Mercado-Feliciano, PhD., DABT **Signature:**
RAB4, Health Effects Division (7509P) **Date:** 14 Aug 2023
EPA Reviewer: Jessica Kidwell **Signature:** Jessica Kidwell
RAB4, Health Effects Division (7509P) **Date:** 14 Aug 2023
Template version 11/01

TXR#: 0053214

DATA EVALUATION RECORD

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

PC CODE: 600074 **DP BARCODE:** D465871

TEST MATERIAL (PURITY): 1,2,4-Triazole (99.9% a.i.)

SYNONYMS: None provided

CITATION: Moxon, M.E. (2004) 1H-[1, 2, 4] Triazole: Prenatal Developmental Toxicity Study in the Rabbit. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire UK. Laboratory study number RB1011, 25-June-2004. MRID 51917801. Unpublished.

SPONSOR: Triazole Derivatives Metabolite Group.

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 51917801) the initial study design was to administer 1H-1,2,4-Triazole (99.9% a.i., lot # Kl7096/l) to 24 timed-mated female New Zealand White (Interfauna) rabbits/dose in aqueous 0.5% (w/v) carboxymethyl cellulose by gavage at dose levels of 0, 15, 30, 60 or 80 mg/kg/day on gestation days (GD) 5 through 26, inclusive. Following the premature humane kill of 4/12 rabbits given 80 mg/kg/day, the study design was modified to include a 60 mg/kg/day dose group with 12 rabbits. After additional morbidity, the 80 and 60 mg/kg/day groups were terminated early on GD 9-12 (on the same date but different GDs because of the staggered dosing). Therefore, findings may not be exactly comparable to the control, 15 or 30 mg/kg/day groups, which were terminated GD 20-29. All animals were weighed up to and including day 16. Uterine content was evaluated only in humanely killed does. Individual fetuses were not evaluated.

Survival in the 15 mg/kg/day group was the same as in controls (96%). Survival was decreased by 1,2,4-triazole treatment in a dose-dependent manner at 30, 60 and 80 mg/kg/day (71%, 58% and 17%, respectively) due to severe clinical signs that required humane sacrifice. Severe clinical signs (abnormal gait, ataxia, abnormal breathing, cold to touch, hunched posture moribundity, shaking, and subdued behavior) were observed only on the same day as humane sacrifice, and all animals humanely killed showed at least 2 severe clinical signs. Salivation and piloerection were observed mostly on the same day as humane sacrifice, and in a few animals on the day before. In general, less severe clinical signs (aggressive behavior, few feces, diarrhea) were observed sporadically and with increasing incidence over time, especially at higher doses. The earliest humane kill was in the 80 mg/kg/day group on GD 9 (treatment day 5). In the 30 mg/kg/day group, the earliest humane kill was on GD 13 (treatment day 9).

The mean body weights of treatment groups were not significantly different from the control group. However, animals with severe clinical signs showed consistent weight loss at all doses, and sudden (1-day) weight loss at 80 mg/kg/day. Mean food consumption was decreased in a dose-dependent manner at 30 mg/kg/day and higher doses. This is consistent with the consistent and/or sporadic weight loss in rabbits that were humanely killed. Gross findings of colon distended with gas and/or empty small intestine at 80 mg/kg/day are consistent with the decrease in food intake at the same dose.

The percent of does with live fetuses was significantly decreased at 30 mg/kg/day (67%) compared to the control and 15 mg/kg/day dose groups (95% and 96%, respectively). Fetuses could not be evaluated in the 60 and 80 mg/kg/day because those groups were terminated early. In the pregnant does suffering for severe clinical signs, the number of resorptions was low, suggesting that the maternal effects did not directly affect the embryos.

The maternal LOAEL is 30 mg/kg/day, based on severe clinical signs (abnormal gait, ataxia, abnormal breathing, cold to touch, hunched posture moribundity, shaking, and subdued behavior) that required humane sacrifice, a decreased percentage of females with live fetuses, decreased food intake and consistent body weight loss. The maternal NOAEL is 15 mg/kg/day.

A developmental LOAEL could not be determined because the fetal lifestage was not examined due to maternal mortality.

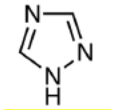
The developmental toxicity study in the rabbit is classified **acceptable/non-guideline** and can be used for risk assessment.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: 1H-[1,2,4]-Triazole

Description:	White solid
Lot #:	Kl7096/1
Purity:	99.9% a.i.
Compound Stability:	Store in refrigerator;1 February 2008.
CAS #of TGAI:	288-88-0
Structure:	

2. Vehicle and/or positive control: The vehicle was aqueous 0.5% (w/w) carboxymethylcellulose (CMC), Glassware and Media, Astrazeneca Pharmaceuticals, Alderley Park

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White (Interfauna)	
Age/weight at study initiation:	Requested weight range 3.3 - 4.0 kg; actual mean ~ 3.6 kg	
Source:	Harlan UK. Limited	
Housing:	Individually, cage type not indicated	
Diet:	Harlan Teklad TRB Rabbit Diet 9603 *ad libitum*	
Water:	Source not indicated, analyzed periodically for contaminants, *ad libitum*	
Environmental conditions:	**Temperature:**	18 ± 3°C
	Humidity:	30-70%
	Air changes:	at least 15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	2-3 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: 18 February 2004; End: 16 March 2004

2. **Mating:** Time-mated females were supplied by the vendor on day 2 or 3 of gestation. The day on which breeding occurred was designated as gestation day (GD) 1.

3. **Animal assignment:** The rabbits were randomly assigned an animal number on arrival. The range of animal numbers determined which treatment each animal received, as indicated in Table 1. Animals on GD 2 or 3 were received over a 9-day period, they started treatment of different dates. Actual receipt dates were the 18, 20, 25 and 27 of February 2004. For example, 24 animals were received on 18-Feb-2004, and were assigned numbers 73, 25, 1, 49, 50, 2, 74, 26, 27, 75, 51, 3, 4, 52, 28, 76, 77, 5, 29, 53, 30, 54, 78 and 6 (i.e. the first 6 animals for each treatment group), and since they were all on GD 3, treatment started 2 days later (GD 5). Sisters and females which had been mated with the same male were distributed across the groups.

- Initially, rabbits were assigned to groups as indicated in the top section of Table 1. The study report indicates that, following the premature termination of several rabbits given 80 mg/kg/day the design of the study was modified as indicated in the bottom section of Table 1. The report does not state if the animals receiving 60 mg/kg/day had receive 80 mg/kg/day previously. Animals were received and dosed in a staggered manner over a 9-day period, and the report appendixes allow to determine the exact dates each animal started treatment and died. Review of this data indicates that, for the group of 12 animals in the final 80 mg/kg/day group, the last death occurred on 27-Feb-2004. This was the same start of treatment (GD 5) for 3 of the animals in the final 60 mg/kg/day group. Therefore, it is possible that 3 of the animals in the 60 mg/kg/day received 80 mg/kg/day on GD 5 only.

TABLE 1: Animal assignment

Group	1	2	3	4	
Initial					
Dose (mg/kg bw/day)	0	15	30	80	--
Number of females	24	24	24	24	--
Animal numbers	1-24	25-48	49-72	73-96	--
After Morbidity in Group 4					
Dose (mg/kg bw/day)	0	15	30	80	60
Number of females	24	24	24	11	11
Animal numbers	1-24	25-48	49-72	73-84	85-96
Days dosed (GD)	5-26	5-26	5-26	5-9	5-11

Data taken from text table, pages 13 and 15-16 in study report.

4. **Dose selection rationale:** The dose levels for this study were selected on the basis of the results of a preliminary study in the New Zealand White rabbit (report number CTL/RB 1010/Technical Toxicology/Report). No additional information was provided.

5. **Dosage preparation and analysis:** Dosing suspensions were prepared weekly by adding the vehicle to an appropriate (weighed) quantity of test material. No adjustment for purity of the test substance was made. Dose preparations were stored at 4°C until use. The first batch of dose preparation made for each group was analyzed prior to being used for dosing. The chemical stability of the lowest and highest concentrations of dose preparation, under the conditions of storage used, were established for the period of use. No determination of homogeneity was made because the dose preparations were solutions.

   Results: Homogeneity: Not determined
   Stability analysis: 100-104% after 0-8 days at 4°C
   Concentration analysis: 99.3-105% of nominal.

6. **Dosage administration:** The rabbits were dosed orally, by gavage, at a constant dose volume of 1 ml/kg according to their daily individual bodyweights.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** Detailed clinical observations were recorded daily, as soon as possible after dosing, and towards the end of each working day. The bodyweight of each rabbit was recorded on arrival, on day 4 and immediately prior to dosing from day 5. All animals were weighed up to and including day 16. Food consumption was recorded on days 5, 8, 11, 14, 17, 20, 23, 26 and 30. All rabbits requiring euthanasia during the study, were examined, including external observation and an examination of the thoracic and abdominal viscera and uterine contents. All macroscopic findings were recorded.

2. **Uterine evaluation:** The pregnancy status of each animal was determined. Uterine contents were evaluated only in does that were humanely killed (died before 15 March 2004). Where there was no clear evidence of implantation, the uterus was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. The following details were recorded: number of corpora lutea, number of implantations, position of implantations, number of live fetuses, and number of intra-uterine deaths (early and late).

2. **Fetal evaluations:** Not conducted

D. DATA ANALYSIS: The investigators analyzed data as indicated below, however most calculations were redone by the reviewer due to inconsistencies between the individual data and the summary tables.

Maternal bodyweights during the dosing period were evaluated by analysis of covariance on day 5 bodyweight (day of initial dosing) up to day 16 (before deaths started to occur in some groups). Maternal food consumption during the dosing period was considered by analysis of variance (the last day when all animals were represented). All analyses were carried out in SAS (1999). Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided.

Indices were not calculated. Historical control data was not provided.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality:** Mortality data is summarized in Table 2. Three does receiving 80 mg/kg/day (#76, #82 and #84; Group 4) were humanely killed on GD 9 (treatment day 5) due to severe clinical signs on the same day; none of them displayed significant clinical signs before GD 9, however #82 and #84 had lost a significant amount of body weight by GD 8 (178 and 108 g, respectively). At the same dose, 5 rabbits (#73, #78, #79, #80, #81) were humanely killed on

GD 10 (treatment day 6), and 2 others on GD 11 (#74) and GD 12 (#75), respectively. The group was terminated on 26-Feb-2004, thus the remining 2 does (which had shown body weight loss but no significant clinical signs) were killed on GD 9 (#83) and GD 11 (#77), respectively.

The investigators decided to reduce the dose, for half of the does in Group 4, to 60 mg/kg/day. However, given the staggered study design, it is not clear if a few of the rabbits treated at 60 mg/kg/day had been treated at 80 mg/kg/day for 1-2 days before the switch. Four does treated at 60 mg/kg/day (#86, #87, #89, #90) were humanely killed on GD 10 (treatment day 6) and one additional doe (#85) was humanely killed on GD 12 (treatment day 8). These animals showed consistent weight loss starting GD 6-9 and severe clinical signs on the day of humane kill.

Seven does treated with 30 mg/kg/day were humanely killed on GD 13 (#59), GD 14 (#54, #57), GD 19 (#58) or GD 20 (#55, #60, #62). In addition to severe clinical signs, 6 of these rabbits had sporadic or consistent body weight loss starting GD 6-9. All other animals in 30 mg/kg/day groups survived to study termination (GD 20-29).

Only one doe was humanely killed in the control and in the 15 mg/kg/day. Control group doe #7 had scabs in the anterior thorax starting day 5, sporadic decreases in body weight starting day 6, and sporadic malocclusion starting day 7; all signs lasted until death. In the 15 mg/kg/day group, doe #31 lost weight consistently starting day 6, until death. All other animals in the Control and 15 mg/kg/day groups survived to study termination (GD 20-29).

TABLE 2. Mortality and pregnancy status of rabbits treated with 1,2,4-triazole [a]

mg/kg/day	0	15	30	60	80
Number examined (N)	*24*	*24*	*24*	*12*	*12*
Number killed for clinical signs		0	7[d]	5	10
Number killed for other reasons	1[b]	1[c]	0	0	0
Gestational (treatment) day	20 (16)	21 (17)	13-20 (9-16)	10-12	9-12 (5-8)
Percent survival to termination	96	96	71	58 [e]	17 [e]

a Data from pages 28 and 46-57 in the study report.
b Animal #7 had scabs in the anterior thorax starting day 5, sporadic decreases in body weight starting day 6, and sporadic malocclusion starting day 7; all signs lasted until death.
c Animal #31 lost weight consistently starting day 6, until death.
d In addition to the clinical signs indicated in Table 3 below: 6 animals, showed sporadic (#55 and #58) or consistent (#54, #57, #59 and #60) weight loss starting day 6; animal #62 showed weight loss on day 19.
e The top two dose groups were terminated early, by March 2-5, which was GD 9-12 (treatment days 5-8). Therefore, percent survival is not comparable to the control, 15 mg/kg/day or 30 mg/kg/day groups (terminated GD 20-29). In other words, if the top 2 groups had been terminated later, the survival could have been lower.

2. **Clinical observations**: The incidence of clinical signs is summarized on Table 3. No significant clinical signs were observed in the control and 15 mg/kg/day groups. Severe clinical signs (abnormal gait, ataxia, abnormal breathing, cold to touch, hunched posture moribundity, shaking, and subdued behavior) were observed at 30 mg/kg/day and higher doses, but only on the same day as humane sacrifice. All animals humanely killed in groups dosed at 30 mg/kg/day and higher doses showed at least 3 severe clinical signs (Attachment 1). Salivation and piloerection were also observed on the same day as humane sacrifice. Most

animals with severe clinical signs also had consistent (across several days) or sudden weight loss. In general, less severe clinical signs (aggressive behavior, few feces, diarrhea) were observed sporadically and with increasing incidence over time, especially at higher doses.

TABLE 3. Number of animals (percent) with selected clinical observations and days observed in female rabbits treated with 1,2,4-triazole [a]

mg/kg/day	0	15	30	60	80
Number examined (N)	*24*	*24*	*24*	*12*	*12*
Abnormal gait	0	0	0	0	9 (75)***
Days observed					9-12
Aggressive	0	2 (8)	0	0	0
Days observed		13-23			
Ataxia	0	0	3 (13)	4 (33)**	1
Days observed			13-20	10-12	10
Breathing irregular	0	0	3 (13)	3 (25)*	0
Days observed			19-20	10-12	
Breathing decreased rate	0	0	1 (4)	1 (8)	1 (8)
Days observed			13	10	10
Breathing increased rate	0	0	2 (8)	1 (8)	9 (75)***
Days observed			14	10	9-12
Cold	0	0	4 (17)	5 (42)**	7 (58)***
Days observed			13-20	10-12	9-12
Hunched	0	0	5 (20)*	2 (17)	6 (50)***
Days observed			13-20	10-12	9-11
Moribund	0	0	0	1 (8)	1 (8)
Days observed				10	10
Piloerection	0	0	3 (13)	2 (17)	7 (58)***
Days observed			13-20	10-12	9-12
Salivation	0	0	5 (20)*	4 (33)**	9 (75)***
Days observed			13-20	10	9-11
Shaking	0	0	0	1 (8)	2 (17)
Days observed				10	9
Subdued	1 (4)	0	7 (29)**	5 (42)	6 (50)
Days observed	20		13-20	9-12	9-12

a Data from study report pages 29-31.
Statistical significance per Fisher's exact test by reviewer: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

2. **Body weight:** As summarized in Table 4, the mean body weights of treatment groups were not significantly different from the control group. However, animals with severe clinical signs showed consistent weight loss at all doses, and sudden (1-day) weight loss at 80 mg/kg/day.

TABLE 3. Mean ± SD maternal body weights in kilograms [a] and incidence of weight loss [b]

Dose in mg/kg/day	0	15	30	60	80
Number evaluated	*22*	*23*	*21*	*11* [c]	*10* [c]
GD 5	3.64 ± 0.27	3.55 ± 0.26	3.57 ± 0.26	3.64 ± 0.28	3.60 ± 0.17
GD 7	3.66 ± 0.29	3.56 ± 0.26	3.60 ± 0.25	3.58 ± 0.28	3.55 ± 0.15
GD 9	3.67 ± 0.26	3.57 ± 0.27	3.60 ± 0.26	3.48 ± 0.31	3.43 ± 0.18
GD 11	3.68 ± 0.25	3.58 ± 0.28	3.57 ± 0.30	3.24 ± 0.24 [c]	3.39 ± 0.13 [c]
GD 13	3.71 ± 0.24	3.59 ± 0.32	3.55 ± 0.33	[e]	[e]
GD 15	3.76 ± 0.24	3.62 ± 0.34	3.64 ± 0.31 [f]	[e]	[e]
Weight loss, consistent	1 (4)	1 (4)	6 (25)	5 (42)*	8 (67)***
Days observed	14-20	6-21	6-20	6-12	6-12
Weight loss, sudden	0	0	1 (4)	0	6 (50)***
Days observed			19-20		7-11

a Calculated from individual animal data in study report pages 159-166; only pregnant animals are included. There were no statistical significance differences from control, by ANOVA with Tukey's post-test calculated by the reviewer.
b Data from study report pages 29-31. Statistical significance per Fisher's exact test by reviewer: * $p < 0.05$; *** $p < 0.001$.
c Except as noted due to mortality.
d N = 2
e N = 0
f N = 18

3. **<u>Food consumption</u>:** As summarized in Table 5, the mean food consumption was decreased in a dose-dependent manner at 30 mg/kg/day and higher doses. This is consistent with the consistent and/or sporadic weight loss in rabbits that were humanely killed.

TABLE 5. Maternal food consumption in grams (mean ± standard deviation) a

mg/kg/day	0	15	30	60	80
Number evaluated	*22*	*23*	*21* [b]	*11* [b]	*10* [b]
GD 5-8	158 ± 32	142 ± 63	129 ± 45* ↓18%	100 ± 61* ↓37%	62 ± 56*** ↓61%
GD 8-11	148 ± 33	129 ± 47	108 ± 53 ↓27%	77 ± 41** [c] ↓48%	53 ± 35* [c] ↓64%
GD 11-14	143 ± 38	117 ± 47	101 ± 52** [d] ↓29%	[e]	[e]

a Calculated from individual animal data in study report pages 168-172; only pregnant animals are included.
b Data from study report pages 29-31. Statistical significance per Fisher's exact test by reviewer: * $p < 0.05$; *** $p < 0.001$.
c N = 2
d N = 20
e N = 0
Significantly different from control: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

4. **<u>Gross pathology</u>:** The only significant gross pathology findings were a few incidences of colon distended with gas and/or empty small intestine at 80 mg/kg/day. These findings are consistent with the decrease in food intake at the same dose.

TABLE 5. Incidence (percent) of microscopic findings in adult female rabbits [a]

Dose in mg/kg/day	0	15	30	60	80
Number examined	*24*	*24*	*24*	*12*	*12*
Colon, distended with gas	0	0	0	0	2
Ileum, empty	0	0	0	0	3
Jejunum, empty	0	0	0	0	3

a Data taken from pages 39-40 in study report.

5. **Cesarean section data:** The available cesarean data is summarized in Table 6. The percent of does with live fetuses was significantly decreased at 30 mg/kg/day (67%) compared to the control and 15 mg/kg/day dose groups (95% and 96%, respectively). Fetuses could not be evaluated in the 60 and 80 mg/kg/day because those groups were terminated prematurely due to severe clinical signs. The lower part of Table 6 presents uterine data for the humanely killed does. Even while suffering for severe clinical signs, the number of resorptions was low, suggesting that the maternal effects did not directly affect the embryos.

TABLE 6. Cesarean section observations [a]

Observation	Dose (mg/kg/day)				
	0	15	30	60	80
# Animals assigned (mated)	24	24	24	12	12
# Animals pregnant	22	23	21	11	10
Pregnancy Rate (%)	92	96	88	92	83
Maternal wastage: # Died	1	1	6	5	10
% Died	5	4	29	45	100
# (%) Does with live fetuses	21 (95)	22 (96)	14 (67)**	0	0
The following parameters were evaluated only in humanely killed does					
Number evaluated	*1*	*1*	*6*	*5*	*10*
Corpora lutea/Doe	12	11	9.8 ± 1.0	9.9 ± 2.0	10.6 ± 1.5
Implantations/Doe	10	10	9.0 ± 1.8	9.1 ± 2.1	9.6 ± 1.6
# Does with total resorptions	0	0	1	0	0
# Does with any resorptions	1	1	5	2	1
Resorptions/Doe Early	2	0	0.3 ± 0.5	0.3 ± 0.5	0.1 ± 0.3
Late	4	2	3.7 ± 4.3	0.0 ± 0.0	0.0 ± 0.0
Embryos/Doe [b]	4	8	5.0 ± 3.2	8.8 ± 2.1	9.5 ± 1.5

a Calculated by reviewer, using individual data from study report pages. Values are given as Mean ± Standard Deviation where appropriate.
b It was not indicated if the embryos were alive or dead.
** Statistically different from control at $p < 0.01$ by Fisher's Exact Test calculated by reviewer.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** Following the premature termination of 4/12 rabbits given 80 mg/kg/day, due to clinical signs and loss of bodyweight, dosing of this dose level was discontinued. A further 12 rabbits were given 60 mg/kg/day from day 5 of gestation. Following the premature termination of 5/12 rabbits given 60 mg/kg/day, due to clinical signs and loss of bodyweight, dosing of this dose level was discontinued. Following

the premature termination of 7/24 rabbits given 30 mg/kg/day, 1/24 rabbits given 15 mg/kg/day and 1/24 rabbits in the control group, the study was terminated. This decision was taken by the sponsor, because the objectives of the study could not be met nor a fully compliant guideline study be produced.

B. REVIEWER COMMENTS: Survival in the 15 mg/kg/day group was the same as in controls (96%). Survival was decreased by 1,2,4-triazole treatment in a dose-dependent manner at 30, 60 and 80 mg/kg/day (71%, 58% and 17%, respectively) due to severe clinical signs that required humane sacrifice. Severe clinical signs (abnormal gait, ataxia, abnormal breathing, cold to touch, hunched posture moribundity, shaking, and subdued behavior) were observed only on the same day as humane sacrifice, and all animals humanely killed showed at least 2 severe clinical signs. Salivation and piloerection were observed mostly on the same day as humane sacrifice, and in a few animals on the day before. In general, less severe clinical signs (aggressive behavior, few feces, diarrhea) were observed sporadically and with increasing incidence over time, especially at higher doses. The earliest humane kill was in the 80 mg/kg/day group on GD 9 (treatment day 5). In the 30 mg/kg/day group, the earliest humane kill was on GD 13 (treatment day 9).

The mean body weights of treatment groups were not significantly different from the control group. However, animals with severe clinical signs showed consistent weight loss at all doses, and sudden (1-day) weight loss at 80 mg/kg/day. Mean food consumption was decreased in a dose-dependent manner at 30 mg/kg/day and higher doses. This is consistent with the consistent and/or sporadic weight loss in rabbits that were humanely killed. Gross findings of colon distended with gas and/or empty small intestine at 80 mg/kg/day are consistent with the decrease in food intake at the same dose.

The percent of does with live fetuses was significantly decreased at 30 mg/kg/day (67%) compared to the control and 15 mg/kg/day dose groups (95% and 96%, respectively). Fetuses could not be evaluated in the 60 and 80 mg/kg/day because those groups were terminated early. In the pregnant does suffering for severe clinical signs, the number of resorptions was low, suggesting that the maternal effects did not directly affect the embryos.

The maternal LOAEL is 30 mg/kg/day, based on severe clinical signs that required humane sacrifice, a decreased percentage of females with live fetuses, decreased food intake and consistent body weight loss. The maternal NOAEL is 15 mg/kg/day.

A developmental LOAEL could not be determined because the fetal lifestage was not examined due to maternal mortality.

C. STUDY DEFICIENCIES: The selected doses did not allow for evaluation of fetal anomalies. No other study deficiencies were noted.

Attachment 1: Clinical signs in animals that were humanely killed. Summarized from pages 46-57 of the study report. Body weight decreases are compared to body weight on GD 5 unless otherwise noted; data supplemented by individual animal data on pages 159-166.

#	GD	Findings
		0 mg/kg/day
07	5-20	Scabs in anterior thorax and wet sore on GD 9; sporadic malocclusion; few feces GD 14
	14-20	Consistent weight loss, -479 g
	17-20	Thin
		15 mg/kg/day
31	6-21	Consistent weight loss, -660 g
	9-16	Few feces
	19-21	Thin, sporadic diarrhea
		30 mg/kg/day
54	5-14	Consistent weight loss, -517 g
	12-14	Few feces
	13	Decreased breathing rate, subdued
	14	Increased breathing rate, subdued, ataxia, hunched, cold, flaccid, sides pinched in, salivation, piloerection
55	13-20	Consistent weight loss, -504 g
	13-19	Sporadic diarrhea, sporadic few feces
	20	Irregular breathing, subdued, hunched, unable to support head, salivation, no feces
57	6-14	Consistent weight loss, -450 g
	8-13	Few feces, sporadic diarrhea
	12-14	Thin
	14	Increased breathing rate, subdued, hunched, cold, sides pinched, slight vaginal bleeding
58	15-19	Consistent weight loss, -361g
	19	Irregular breathing, subdued, hunched, unable to support head, salivation, few feces
59	7-13	Consistent weight loss, -370 g
	13	Subdued, ataxia, hunched, cold, sides pinched, thin, salivation, piloerection, diarrhea
60	7-20	Consistent weight loss, -590 g
	19	Irregular breathing, subdued, thin, slight blood on tray, salivation, few feces, diarrhea
62	19-20	Sudden weight loss, -495 g from maximum weight on day 9.
	20	Subdued, ataxia, cold, sides pinched, piloerection, few feces
		60 mg/kg/day
85	9-12	Consistent weight loss, -325 g
	12	Irregular breathing, subdued, hunched, ataxia, cold, piloerection, diarrhea
86	6-10	Consistent weight loss, -347 g
	10	Decreased breathing rate, subdued, ataxia, cold, salivation, no feces
87	5-10	Few or no feces
	6-10	Consistent weight loss, -666 g
	10	Moribund, increased breathing rate, subdued, cold, salivation
89	6-10	Consistent weight loss, -372 g
	10	Irregular breathing, hunched, ataxia, thin, cold, salivation, piloerection
90	6-10	Consistent weight loss, -415 g
	8	Few feces
	10	Irregular breading, subdued, shaking, ataxia, thin, cold, salivation
		80 mg/kg/day
73	6-9	Consistent weight loss, -157 g
	9	No feces
	10	Sudden weight loss -294 from GD 9, moribund, decreased breathing rate, cold, salivation

#	GD	Findings
74	6-10 11	Consistent weight loss, -112 g Sudden weight loss -199 g from GD 10, Increased breathing rate, subdued, abnormal gait, hunched, cold, salivation, eye discharge
75	9-12 10-11 12	Consistent weight loss, -112 g Diarrhea, sporadic few feces Increased breathing rate, abnormal gait, cold, piloerection,
76	6-9 8-9 9	Consistent weight loss, -134 g Few feces Increased breathing rate, shaking, subdued, abnormal gait, hunched, salivation, piloerection, eye discharge
78	7 8-9 10	Sudden weight loss -118 g from GD 6 Consistent weight loss, -109 g from GD 7 Sudden weight loss -157 g from GD 9, Increased breathing rate, hunched, subdued, abnormal gait, reluctant to move, cold, salivation, no feces
79	6-9 10	Consistent weight loss, -224 g Sudden weight loss -180 g from GD 9, increased breathing rate, ataxia, abnormal gait, salivation, piloerection
80	5-7 8 9 10	Sporadic few or no feces, consistent weight loss -43 g Sudden weight loss -138 g from GD 7 Weight loss -16 g from GD 8 Sudden weight loss -152 g from GD 9, increased breathing rate, hunched, subdued, abnormal gait, cold, salivation, piloerection, no feces
81	7-8 9 10	Consistent weight loss, -30 g Sudden weight loss -140 g from GD 8 Increased breathing rate, subdued, hunched, abnormal gait, cold, salivation, piloerection, no feces, additional -60g from GD 9