DATA EVALUATION RECORD

CLOTHIANIDIN/044309
[OPPTS (§870.7800)]

STUDY TYPE: IMMUNOTOXICITY - RAT
MRID 46536502

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 113-2005

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DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [feeding]-[rat]; OPPTS 870.7800

PC CODE: 044309

DP BARCODE: D318520
SUBMISSION NO.: Not provided

TEST MATERIAL (PURITY): TI-435 (Clothianidin, 98.8% a.i.)

SYNONYMS: [C(E)]-N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N"-nitroguanidine

CITATION: Hoberman, A.L. (2004). Oral (diet) repeated dose 28-day toxicity/immunotoxicity study of TI-435 in rats. CR-DDS Argus Division, 905 Sheehy Drive, Building A, Horsham, PA 17044-1241. Study No. RLF00001. September 3, 2004. MRID 46536502. Unpublished.

SPONSOR: Sumitomo Chemical Takeda Agro Co., Ltd., Technical Division, Development Dept., Sumitomofudousan Kayabachou Building, 16-3 Shinkawa, 1-Chome, Chuo-ku, Tokyo, 104-0033 Japan.

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 46536502), TI-435 (clothianidin, 98.8% a.i.) was administered to ten Crl:CD®(SD)IGS BR VAF/Plus® rats/sex/group in the diet at concentrations of 0, 150, 500, or 3000 ppm (equivalent to 0, 13.8, 45.8, and 252.8 mg/kg/day for male rats and 0, 14.0, 46.2, and 253 mg/kg/day for female rats) for 28 days. A positive control group was given basal diet through the study, but received 50 mg/kg cyclophosphamide monohydrate (CPS) in pH 7.2 PBS for four consecutive days before study termination. All rats in the study were sensitized with 0.5 mL 2×10^8 sheep red blood cells by tail vein injection four days before study termination.

None of the rats died during the study. The total body weight of TI-435 high-dose male and female rats was decreased ~13% by the end of the 28-day study. Total body weight gain of TI-435 high-dose male and female rats was decreased 25-45% and food consumption was decreased ~18%. No other TI-435 treatment-related effects were noted. No TI-435-related decrease was found in absolute or relative spleen weight and no treatment-related effects were found in the total number of spleen cells, IgM antibody forming cells (AFC)/10^6 spleen cells or IgM AFC/spleen ($\times10^3$). In contrast, CPS, the positive control, decreased the absolute and relative spleen weight by ~50%, and the humoral response of the spleen by >90%. Based on the results of this study, TI-435 in not a humoral immune suppressant when given to male and female rats in the diet in concentrations up to 3000 ppm.

The NOAEL for TI-435-mediated immunotoxicity is >253 mg/kg/day. A immunotoxicity LOAEL was not established.

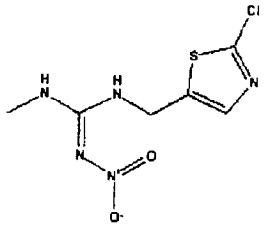
This immunotoxicity study is Classified **Acceptable/Guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the rat.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** TI 435

Description:	Yellow powder
Lot/Batch #:	30037120
Purity:	98.8 % a.i.
Compound Stability:	Stable refrigerated
CAS # of TGAI:	205510-53-8
Structure:	

2. **Vehicle:** Diet

3. **Positive Control:** 50 mg/kg cyclophosphamide monohydrate (CPS) in pH 7.2 PBS

4. **Test animals:**

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®	
Age/weight at study initiation:	~2 months; males - 161-216; females 155-183	
Source:	Charles River Laboratory, Raleigh, NC	
Housing:	individually in stainless steel cages with wire bottoms	
Diet:	Purina Certified Rodent Diet ® #5002, *ad libitum*	
Water:	filtered tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12 hours light/dark
Acclimation period:	~10 days	

B. STUDY DESIGN:

1. **In life dates:** Start: July 7, 2004; End: August 5, 2004. The in-life portion of the study was done by CR-DDS Argus Division, 905 Sheehy Drive, Building A, Horsham, PA. The immunotoxicity study was conducted at ImmunoTox, Inc., Richmond, VA.

2. **Animal assignment:** The rats were assigned to the test groups noted in Table 1 using a computer-generated (weight-ordered) randomization procedure.

TABLE 1: Study design

Test group	TI-435 Conc. in diet (ppm)	Dose to animal (mg/kg/day)	# Male	# Female
I (Negative Control)	0	♂ 0, ♀ 0	10	10
II	150	♂ 13.8, ♀ 14.0	10	10
III	500	♂ 45.8, ♀ 46.2	10	10
IV	3000	♂ 252.8, ♀ 253.0	10	10
V (Positive control)*	0	♂ 0, ♀ 0	10	10

Data from ppages 9 and 21 of MRID 46536502
*Rats in Group V were administered 50 mg/kg CPS for 4 consecutive days before sacrifice. All rats were administered 0.5 mL 2×10^8 sheep RBC i.v. four days before sacrifice.

3. **Dose selection:** The dietary concentrations used in this study were selected by the study sponsor based on the results of a previously conducted subchronic study.

4. **Diet preparation and analysis:** The test diet mixtures were prepared by the Diet Preparation Lab at Bayer CropScience LP, Toxicology, Stilwell, KS, and were used as received. The prepared diets were stored frozen when received and at room temperature during each week of use. The test diets were received by the performing laboratory on July 1 and 15, 2004.

   At the diet preparation laboratory, the diets were prepared by adding the appropriate amount of test material to ~200 mL acetone. Once throughly mixed, the acetone mixture was added to Certified Rodent Diet ® #5002 and mixed for 10 minutes in a Hobart mixer. Each test diet was prepared at two week intervals. A sample of each batch of mixed diet was taken and retained at refrigerator temperatures. Samples for analysis were taken and stored in a similar manner. Stability of the diets at room temperature and at refrigerator temperatures and homogeneity of the test substance in the diet at the low and high doses were tested. Homogeneity was checked from the top, middle and bottom of the mixing bowl.

Results:

Homogeneity analysis: The mean concentration of the low-dose diet was 158 ppm (105% nominal, RSD = 2.02%) and the high-dose diet was 3158 ppm (105% nominal, RSD = 1.01%).

Stability analysis: After 21 days of storage at refrigerator temperature, a 4% decline in concentration was found for the low-dose diet, and a 2% decline was found in the high-dose diet. The diet was considered stable for 21 days at refrigerator temperature.

Concentration analysis: The concentrations of the three diet mixtures were within 103-105% of the nominal concentration. The percent relative standard deviations of the diets were within 0.459-1.56%

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

5. **Statistics:** Continuous data were analyzed by Bartletts test to determine if homogeneous. If homogeneous, the data were analyzed by ANOVA followed by Dunnett's test to determine differences between groups. If the data were heterogeneous, the data were analyzed by the Kruskal-Wallis test followed by Dunn's Method of Multiple Comparisons to determine differences between groups. Incidence data were analyzed by Fisher's Exact Test.

C. **METHODS:**

1. **Observations:** The animals were observed twice daily on study days 1-3 and at least daily thereafter through the 28-day study.

2. **Body weight:** The rats were weighed weekly throughout the study.

3. **Food/water consumption and compound intake:** Food consumption and compound intake were recorded weekly throughout the study. Uneaten food was recorded at the end of the study.

4. **Sacrifice and pathology:** After 28 days, all rats in the study were sacrificed under CO_2 anesthesia and gross necropsy of the thoracic, abdominal and pelvic viscera was done. The necropsy included an initial physical examination of external surfaces, and all orifices, as well as an internal examination of tissues and organs. In addition, the cranial, thoracic and abdominal cavities were examined.

   The spleen of each rat was removed, weighed, placed in Earle's Balanced Salt Solution and shipped on wet ice to ImmunoTox, Inc. Gross splenic lesions were retained in 10% neutral formalin for possible histological examination.

5. **Immunotoxicity: Antibody plaque-forming cell (AFC) assay, day 4 response:** All animals were exposed to the test material for 28 days. The positive control rats received a 50 mg/kg IP injection of CPS once per day for 4 days before sacrifice. Four days before sacrifice, all rats were sensitized with sheep red blood cells (sRBC, 2×10^8) by tail vein injection. The primary response to sheep erythrocytes was measured using a modified hemolytic plaque assay (Jerne, N.K., et al., Plaque forming cells: Methodology and Theory. Transpl. Rev. 18:130-191, 1974). Cell counts were performed and the number of cells/spleen, AFC/spleen and AFC/10^6 spleen cells were determined.

II. RESULTS:

A. OBSERVATIONS:

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

2. **Mortality:** All animals survived to study termination.

B. BODY WEIGHT and BODY WEIGHT GAIN:

As shown in Table 2, the average body weight and body weight gain of high-dose male and female rats was statistically decreased by dietary treatment with TI-435 over the 28-day study period. Likewise, the average body weight and body weight gain of male and female positive control rats were decreased four days after treatment with CPS.

TABLE 2. Average body weight and body weight gain of rats exposed to TI-435 for 28 days

Exposure conc. (ppm)	Body weights (g ± SD)		Total weight gain	
	Day 1	Day 29	g	% of control[a]
Males				
0	189.9 ± 15.7	385.5 ± 33.7	195.6 ± 22.1	–
150	190.0 ± 15.0	388.6 ± 43.0	198.6 ± 35.4	101
500	188.2 ± 16.0	378.0 ± 26.7	189.8 ± 21.7	97
3000	189.9 ± 17.8	336.2** ± 30.2	146.3** ± 22.2	75
CPS (50 mg/kg)	189.1 ± 15.5	327.9** ± 32.2	138.8** ± 30.4	71
Females				
0	175.8 ± 7.6	237.2 ± 16.0	61.4 ± 11.0	-
150	174.8 ± 6.9	233.0 ± 19.8	58.2 ± 14.8	95
500	174.2 ± 4.4	236.6 ± 15.0	62.4 ± 11.6	102
3000	173.4 ± 7.5	207.4** ± 14.3	34.0** ± 10.6	55
CPS (50 mg/kg)	175.0 ± 5.8	212.9** ± 12.5	37.9** ± 9.4	62

Data from Tables 3-6, pages 38-41 of MRID 46536502
[a]% of Control calculated by reviewer
** = $p \leq 0.01$ (N=10 for all groups)

C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Average food consumption of high-dose rats was statistically decreased ~20% in male rats and 17% in female rats. Food consumption was also slightly decreased in CPS treated rats. No treatment-related effects were noted for rats fed ≤500 ppm.

2. **Compound consumption**: Average compound consumption is in Table 1 above.

D. GROSS NECROPSY:

No treatment-related effects were noted at necropsy.

1. **Organ weight**: Dietary treatment for 28 days with TI-435 had no effect on the absolute or relative spleen weight of male and female rats (Table 3). Treatment with the CPS positive control decreased the absolute and relative spleen weights of male and female rats >50%.

TABLE 3. Absolute (g) and relative to body weight (%) spleen weights of rats exposed to TI-435.

Parameter	Control	Dietary Concentration (ppm)			CPS (50 mg/kg)
		150	500	3000	
Male					
Body Weight (g)	385.5 ± 33.7	388.6 ± 43.0	378.0 ± 26.7	336.2** ± 30.2	327.9** ± 32.2
Spleen					
Absolute	0.81 ± 0.14	0.83 ± 0.12	0.87 ± 0.15	0.71 ± 0.18	0.31** ± 0.05
Relative	0.209 ± 0.024	0.212 ± 0.026	0.232 ± 0.040	0.213 ± 0.059	0.095** ± 0.019
Female					
Body Weight (g)	237.2 ± 16.0	233.0 ± 19.8	236.6 ± 15.0	207.4** ± 14.3	212.9** ± 12.5
Spleen					
Absolute	0.58 ± 0.09	0.57 ± 0.05	0.57 ± 0.07	0.55 ± 0.07	0.26** ± 0.03
Relative	0.245 ± 0.039	0.245 ± 0.026	0.238 ± 0.027	0.265 ± 0.036	0.123** ± 0.015

Data from Tables 13 and 14, pages 48 and 49, MRID 46536502
**p ≤ 0.01 (N=10 for all groups)

E. IMMUNOTOXICITY TESTS:

Antibody plaque-forming cell (PFC) assay: Immunotoxicity findings for the antibody plaque-forming cell assay are summarized in Table 4. Treatment of male and female rats with up to 3000 ppm TI-435 did not decrease the number of spleen cells while the CPS positive control decreased the number of spleen cells by ~85%. The apparent increase in IgM antibody observed in male rats is predominately from the abnormally low response of the vehicle control rats. This is not considered an adverse immune response. In the current study, the responses of the control and treated male rat groups for IgM AFC/10^6 spleen cells and IgM AFC/spleen (x 10^3) were within the established normal range at ImmunoTox, Inc. In contrast, the positive control, CPS, decreased by >90% the IgM AFC/10^6 spleen cells and IgM AFC/spleen (x 10^3), indicating severe suppression of the humoral immune response.

The data suggest that under the conditions of this study, TI-435 did not suppress the humoral immune response in a dose-dependent manner in that it did not significantly alter the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes.

TABLE 4: Results of antibody plaque-forming cell assay on rats orally exposed to TI-435 for 28 days[a]			
Test group (ppm)	**Spleen cells (x 10^7)**	**IgM AFC/10^6 spleen cells**	**IgM AFC/spleen (x 10^3)**
Male			
0	92.36 ± 7.83	616 ± 171	542 ± 152
150	112.07 ± 6.71	838 ± 164	949* ± 216
500	105.00 ± 7.49	1583** ± 268	1665** ± 311
3000	108.56 ± 8.50	1243 ± 389	1384 ± 452
CPS, 50 mg/kg	12.11** ± 0.85 (13)	26** ± 10	3** ± 1
Female			
0	62.68 ± 4.27	1284 ± 420	817 ± 267
150	60.38 ± 2.63	1243 ± 212	761 ± 137
500	61.17 ± 3.84	1603 ± 287	989 ± 207
3000	68.87 ± 4.85	1269 ± 221	854 ± 152
CPS, 50 mg/kg	9.42** ± 0.73 (15)	14** ± 9	2** ± 1

Data from Tables 3 and 4, page 178 and 179 of MRID 46536502
**$p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Based on the study results, the study author concluded that the NOEL for general toxicity in male and female rats was 500 ppm (45.8 mg/kg/day and 46.2 mg/kg/day in male and female rale rats, respectively). Exposure to 3000 ppm of TI-435 in the diet produced reductions in body weight, body weight gain, and food consumption. The NOEL for immunotoxicity was >3000 ppm (252.8 mg/kg/day for males and 253.0 mg/kg/day for females). Exposure to TI-435 in the diet did not adversely affect the functional ability of the humoral component of the immune system.

B. REVIEWER COMMENTS: Male and female SD rats were fed diets containing up to 3000 ppm TI-435 to determine if the test material was immunotoxic. None of the rats died during the study. The total body weight of high-dose male and female rats was decreased ~13% by the end of the 28-day study. Total body weight gain of high-dose male and female rats was decreased 25-45% and food consumption was decreased ~18%. No other TI-435 treatment-related effects were noted.

No decrease was found in absolute or relative spleen weights and no treatment-related effects were found in the total number of spleen cells, IgM antibody forming cells (AFC)/10^6 spleen cells or IgM AFC/spleen ($\times 10^3$). In contrast, CPS, the positive control, decreased the absolute and relative spleen weight by 50%, and the humoral response of the spleen by >90%. Based on the results of this study, TI-435 in not a humoral immune suppressant when given to male and female rats in the diet at concentrations up to 3000 ppm.

C. STUDY DEFICIENCIES: None that would affect the interpretation of the study.