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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

TXR No.: 0053515
Date: October 17, 2006

MEMORANDUM

SUBJECT: CLOTHIANIDIN: HED's Response to the Developmental Immunotoxicity Study Waiver Request. PC Code 044309; DP Number 318520.

TO: Venus Eagle, RM 01
Insecticide/Rodenticide Branch
Registration Division (7505P)

FROM: Kelly Schumacher, Biologist
Registration Action Branch 2
Health Effects Division (7509P)

THRU: Richard A. Loranger, Ph.D., Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509P)

CONCLUSION: The Health Effects Division (HED) has reviewed the rationale submitted by Bayer CropScience (BCS) to support the waiver of a developmental immunotoxicity (DIT) study on clothianidin and the removal of the 10X database uncertainty factor (UF_{DB}). The request to waive the submission of the study is denied. Limited data has identified the immune system as a potential target in the young. Thus, HED continues to recommend submission of a developmental immunotoxicity study to fully characterize the potential for clothianidin to adversely impact development of the immune system. Because the developing immune system can be highly sensitive, and in some cases markedly more so than the adult immune system, the DIT study has the potential to result in a more protective (*i.e.*, lower) regulatory endpoint. The 10X UF_{DB} is applied to account for the lack of this study.

I. BACKGROUND

Bayer CropScience (BCS) (27 W. Alexander Drive, RTP, NC 27709), has submitted a request, dated April 27, 2005, to waive the requirement for a developmental immunotoxicity (DIT) study and to remove the 10X database uncertainty factor (UF_{DB}) for the following pesticide technical active ingredient: clothianidin technical, EPA Reg. No. 74207-1, PC Code 044309.

To justify their request to waive the DIT on clothianidin and remove the UF_{DB}, BCS provided a rationale (MRID 46536501) dated April 27, 2005, as well as a guideline immunotoxicity study conducted in adult rats (OPPTS 870.7800; MRID 46536502). As stated in their rationale, BCS "requests a waiver for this study [DIT study on clothianidin] and removal of the 10X database uncertainty factor".

II. RESPONSE

Consistent with the Office of Pesticide Program's FQPA policy (Determination of the Appropriate FQPA Safety Factor(s) in Tolerance Assessment, http://www.epa.gov/pesticides/trac/science/determ.pdf), on November 14, 2002, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) recommended that testing be conducted to assess immune system function in adults and in young animals following exposure to clothianidin during the period of organogenesis. This decision was based on evidence of decreased absolute and adjusted organ weights of the thymus and spleen in multiple studies in the clothianidin data base and on evidence of increased quantitative susceptibility of juvenile rats, compared to adults, in the two-generation reproduction study to these effects. HIARC evaluated the need for a UF_{DB} in the absence of the DIT study, and they determined that there is insufficient data to justify selection of an additional safety factor for the protection of infants and children lower than the default value of 10X for both single and repeated dose exposure scenarios. Therefore, they determined that a UF_{DB} of 10X should be applied to both single and repeated dose exposure scenarios (*i.e.*, acute and chronic RfDs, short- and intermediate-term incidental oral exposures, and short-, intermediate-, and long-term dermal and inhalation exposures resulting from residential uses of clothianidin) to account for the lack of a developmental immunotoxicity study with clothianidin.

A guideline immunotoxicity study conducted in adult rats (MRID 46536502) has now been reviewed by HED and shows no clothianidin-mediated immunotoxicity, in the form of a T-cell dependent anti-SRBC-forming cell response, in adults at doses lower than those resulting in generalized signs of toxicity (*e.g.*, decreases in body weight). While the antibody response was intact in adult rats in the presence of decreased body weights in this study, it cannot be concluded that similar effects will occur in offspring. Immunotoxicity studies in adults are not necessarily predictive of offspring toxicity (Luebke *et al.*, 2006). Organ weight data indicate that maternal exposure to clothianidin in the multi-generation reproduction study (MRID 45422715) altered the mass of two very important lymphoid organs in the F1 generation and that this generation was

affected at a lower dose than were the adults. A developmental immunotoxicity (DIT) study is required to more thoroughly evaluate the immune response of the offspring.

It is possible to alter antibody response at doses lower than those resulting in lymphoid organ weight changes. This has been shown with perfluorooctanoic acid (PFOA), which suppresses IgM titers at a lower dose than that which resulted in splenic or thymic atrophy. Because data measured in the DIT study could show developmental immunotoxicity at a lower dose than that which resulted in decreased lymphoid organ weights of offspring in the multi-generation reproduction study, a 10X UF_{DB} is applied to account for the lack of this study.

In conclusion, HED has reviewed the rationale submitted by BCS to waive the DIT study on clothianidin and to remove the 10X UF_{DB}. For the reasons stated above, the request to waive the study is denied. Because available data do not fully address the potential for clothianidin to adversely impact development of the immune system, HED recommends the submission of a developmental immunotoxicity study with clothianidin. Because results from the DIT could result in a more protective (*i.e.*, lower) regulatory endpoint, the 10X UF_{DB} is applied to account for the lack of this study.

REFERENCES

Luebke RW, Chen DH, Dietert R, Yang Y, King M, Luster MI. (2006). The comparative immunotoxicity of five selected compounds following developmental or adult exposure. J Toxicol Environ Health B Crit Rev. 2006 Jan-Feb;9(1):1-26.

EPA Reviewer: Kelly Schumacher, M.S. **Signature:**
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 10/17/2006
EPA Secondary Reviewer: Y. Yang, Ph.D. **Signature:**
Toxicology Branch , Health Effects Division (7509P) **Date:** 10/17/2006

TXR #: 0053515

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DATA EVALUATION RECORD

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

PC CODE: 044309

DP BARCODE: D318520

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 19044-1241. 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/dose in the diet at dose levels of 0, 150, 500, or 3000 ppm (0, 13.8, 45.8, or 252.8 mg/kg/day for male rats and 0, 14.0, 46.2, or 253 mg/kg/day for female rats, respectively) for 28 days. A positive control group was given basal diet through the study, but received 50 mg/kg cyclophosphamide monohydrate (CPS) by i.p. injection 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 (SRBC) by tail vein injection once at 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 17-20%. 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, anti-SRBC-forming cells (AFC)/10^6 spleen cells or AFC/spleen. In contrast, CPS, the positive control, decreased the absolute and relative spleen weight by ≥50%, and the anti-SRBC-forming cells response of the spleen by >90%.

The NOAEL for TI-435-mediated T-cell dependent anti-SRBC-forming cells response is >253 mg/kg/day. A LOAEL was not established.

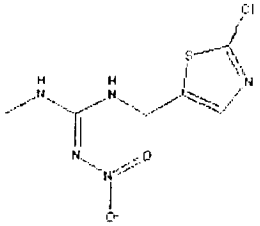
This immunotoxicity study is classified **Acceptable/Guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the rat.

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 in the diet for 21 days at room temperature or refrigerated.
CAS # of TGAI:	210880-92-5; non-stereo: 131748-59-9
Structure:	

2. **Vehicle and/or positive control:** Vehicle – diet; positive control - 50 mg/kg cyclophosphamide monohydrate ISOPAC® (CPS) in PBS, pH 7.2.

3 **Test animals:**

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®	
Age/weight at study initiation:	~2 months; males: 161-216 g; females: 155-183 g	
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:	Males: 7 days; females: 8 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., Virginia BioTechnology Research Park, 800 East Leigh St, Suite 209, Richmond, VA.

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

TABLE 1: Study design				
Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day)	# Male	# Female
I (Negative control)	0	♂ 0, ♀ 0	10	10
II (Low)	150	♂ 13.8, ♀ 14.0	10	10
III (Mid)	500	♂ 45.8, ♀ 46.2	10	10
IV (High)	3000	♂ 252.8, ♀ 253.0	10	10
V (Positive control) [a]	0	♂ 0, ♀ 0	10	10

Data from pp. 9 and 21 of MRID 46536502

[a] Rats in Group V were administered 50 mg/kg CPS by i.p. injection for 4 consecutive days before sacrifice. All rats were administered 0.5 mL (2×10^8) sheep RBC once by i.v. injection four days before sacrifice.

3. Dose selection

The dose levels were selected based on the results from a previously conducted subchronic toxicity study in rats.

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 thoroughly 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: The test article was considered stable in the diet for 21 days refrigerated and at room temperature. 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. After 21 days of storage at room temperature, the low-dose and high-dose mixtures were 99% and 100% of initial values, respectively.

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 Bartlett's 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 throughout 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 and placed in a tared tube with Earle's Balanced Salt Solution in HEPES medium with gentamycin. The tubes were then weighed and shipped on wet ice to ImmunoTox, Inc. Gross splenic lesions were retained in 10% neutral buffered formalin for possible histological examination.

5. Immunotoxicity - Antibody plaque-forming cell (PFC) assay, day 4 response:

All animals were exposed to the test material via diet for 28 days. The positive control rats received a 50 mg/kg i.p. 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 T-dependent 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 treatment-related clinical signs of toxicity were observed.

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

B. Body weight and weight gain: As shown in Table 2, the average body weights of high-dose male and female rats were both statistically decreased 13% following dietary treatment with TI-435 over the 28-day study period. Body weight gains of high-dose males and females were decreased 25% and 45%, respectively, over 28 days of treatment. These decreases were noticed throughout the study, beginning on day 8, the date of the first body weight measurement.

The average body weight and body weight gain of male and female positive control rats were decreased four days after treatment with CPS. These decreases were only noted on day 29, following administration of 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	102
500	188.2 ± 16.0	378.0 ± 26.7	189.8 ± 21.7	97
3000	189.9 ± 17.8	336.2** ± 30.2 (87)	146.3** ± 22.2	75
CPS (50 mg/kg)	189.1 ± 15.5	327.9** ± 32.2 (85)	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 (87)	34.0** ± 10.6	55
CPS (50 mg/kg)	175.0 ± 5.8	212.9** ± 12.5 (90)	37.9** ± 9.4	62

Data from Tables 3-6, pages 38-41 of MRID 46536502
[a] % of control calculated by reviewer
** Statistically different ($p < 0.01$) from the control. (N= 10 for all groups)

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C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption**: Average food consumption of high-dose rats over days 1-29 was statistically decreased ~20% in male rats and 17% in female rats from controls. Food consumption was also slightly decreased in CPS treated rats due to a lower intake over days 22-29 following CPS administration. 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 in males or females treated with TI-435. Changes in the left lateral lobe were noted in 1/10 males and 1/10 females treated with CPS.

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 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 (38)[a]
Relative	0.209 ± 0.024	0.212 ± 0.026	0.232 ± 0.040	0.213 ± 0.059	0.095** ± 0.019 (45)
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 (45)
Relative	0.245 ± 0.039	0.245 ± 0.026	0.238 ± 0.027	0.265 ± 0.036	0.123** ± 0.015 (50)

Data from Tables 13 and 14, pages 48 and 49, MRID 46536502
[a] % of control, in parentheses, calculated by reviewer
** Statistically different ($p < 0.01$) from the control. (N = 10 for all groups)

E. IMMUNOTOXICITY TESTS:

a. **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

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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/106 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 significantly alter the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes.

TABLE 4: Results of antibody plaque-forming cell (PFC) assay on rats orally exposed to TI-435 for 28 days [a]

Test group (ppm; n=10)	Spleen cells (x 10^7)	Specific activity (IgM AFC/10^6 spleen cells)	Total Spleen activity (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)[b]	26** ± 10 (4)	3** ± 1 (0.6)
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 (1)	2** ± 1 (0.2)

[a] Data obtained from Tables 3 and 4, page 178 and 179 of MRID 46536502.
[b] % of control, in parentheses, calculated by reviewer.
** Statistically different ($p < 0.01$) from the control.

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 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 weights of high-dose male and female rats were each 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 17-20%. 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. In contrast, CPS, the positive control, decreased the absolute and relative spleen weight by 50%, and the anti-SRBC forming cells response of the spleen by >90%. Based on the results of this study, TI-435 did not significantly alter the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes when given to adult 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.

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