DATA EVALUATION RECORD

3/16/2001

PYRACLOSTROBIN (BAS 500F)

Study Type: §82-1b, 90-Day Oral Toxicity Study in Mice

Work Assignment No. 3-01-113 A (MRID 45118320)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
John W. Allran, M.S.

Signature: John W. Allran
Date: 3/15/01

Secondary Reviewer
Kelley Van Vreede, M.S.

Signature: Kelley Van Vreede
Date: 3/15/01

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 3/16/01

Quality Assurance
Steve Brecher, Ph.D.

Signature: Steven Brecher
Date: 3/15/01

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EPA Reviewer: Ghazi Dannan, Ph.D.
Registration Action Branch 3/HED (7509C)
EPA Secondary Reviewer: William Greear, M.P.H., D.A.B.T.
Registration Action Branch 3/HED (7509C)
EPA Work Assignment Manager: Sanyvette Williams-Foy, D.V.M.
Registration Action Branch 2/HED (7509C)

Ghazi A. Dannan 7/

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding] - mouse
OPPTS Number: 870.3100 OPP Guideline Number: §82-1[b]

DP BARCODE: D269669; D267732
P.C. CODE: 099100
SUBMISSION CODE: S583112
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Pyraclostrobin Technical (98.5% a.i.)

SYNONYMS: BAS 500 F; Reg. No. 304 428; methyl-N-[[[1-(4-chlorophenyl)pyrazol-3-yl] oxy]-*o*-tolyl]-N-methoxycarbamate

CITATION: Mellert, W., Deckardt, K., Gembardt, Chr., et.al (1999) BAS 500 F - Subchronic Oral Toxicity Study in B6C3F1 crl BR Mice Administration in the Diet for 3 Months. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, Germany. Laboratory Project Id.: 60C0183/96016, November 25, 1999. MRID 45118320. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In this subchronic oral study (MRID 45118320), pyraclostrobin (BAS 500F; 98.5% a.i.; Lot/Batch # CP 025394) was administered in the diet to 10 B6C3F1 Crl BR mice/sex/group at nominal doses of 0, 50, 150, 500, 1000, or 1500 ppm (equivalent to 0, 9.2/12.9, 30.4/40.4, 119.4/162.0, 274.4/374.1, 475.5/634.8 mg/kg/day for the males/females) for 3 months.

No treatment-related differences were observed in mortality, clinical signs, or organ weight data. Body weights were decreased throughout treatment in the 150 ppm males (↓8-11%) and in the 500 ppm (↓6-19%), 1000 ppm (↓7-27%), and 1500 ppm (↓9-34%) animals. Similarly, body weight gains were decreased throughout treatment in the 150 ppm males (↓31-62%) and in the 500 ppm (↓39-122%), 1000 ppm (↓63-233%) and 1500 ppm (↓82-367%) animals. Food consumption was increased sporadically throughout treatment in the 1000 ppm (↑27-49%) and

1500 ppm animals (↑27-58%). Food efficiency was decreased throughout treatment in the 500 ppm, 1000 ppm, and 1500 ppm males (↓62-350%). Despite food spillage problems that were unaccoumted for, it seems that a catabolic state might have been induced by treatment as evidenced by the decreased body weights, body weight gains, and food efficiency, while food consumption was increased. It is uncertain whether pyraclostrobin exerted a direct effect on catabolism or if the catabolic state was a compensatory response to reduced digestion and absorption of food.

Several changes in clinical chemistry indicated a continual catabolic state associated with a compromised nutritional status. Urea was increased in the 150, 500, 1000 and 1500 ppm animals (↑21-82%), indicating increased protein metabolism. Triglycerides were decreased in the 150, 500, 1000, and 1500 ppm animals (↓32-72%). Creatinine was decreased in the 1000 and 1500 ppm females (↓10-13%) due to decreased muscle mass. Globulins were decreased (↓7-16%) and cholesterol was increased (↑19-52%) in the 500, 1000, and 1500 ppm females. Consistent with the clinical chemistry findings and compromised nutritional status, were dose-dependent reduction in lipid vacuoles/content in liver, kidneys, and adrenals.

The mouse hematopoietic and immune systems seem to be target organs. Mild hypochromic, microcytic anemia was evidenced at the highest three doses by decreased hemoglobin in the 1000 ppm females (↓4%) and in the 1500 ppm males (↓10%) and females (↓9%), decreased mean cell volume in the 500, 1000, and 1500 ppm males (↓3-12%), and decreased mean cell hemoglobin in the 500, 1000, and 1500 ppm animals (↓1-12%). Additionally, leukocytes were decreased in the 1000 and 1500 ppm males (↓54-55%), and platelets were increased in the 500 ppm males (↑13%) and in the 1500 ppm males (↑11%) and females (↑18%). In agreement with the large decline in total leukocytes, absolute differential counts were also affected as evidenced by large decrease in neutrophils (up to 50%) in the two top dose female groups and the dose-dependent decrease of lymphocytes among males and females (up to 77% and 46%, respectively). These changes, combined, are more likely due to a direct effect by Pyraclostrobin on erythropoiegenesis than being secondary to the apparent compromised nutritional status and the continued catabolic state. Also, the large reduction in total and differential leukocytes, especially lymphocytes, are consistent with the microscopic findings (below) of thymus atrophy and increased apoptosis (cell death) of mesenteric lymph nodes; collectively, these results clearly indicate that the immune system is a target organ of Pyraclostrobin.

Numerous macroscopic changes indicated insult to the digestive tract, such as: ulcer/erosion of the glandular stomach in the 500, 1000, and 1500 ppm males (2-4/10 treated vs 1/10 control); duodenal wall thickening in the 500, 1000, and 1500 ppm animals (14-20/20 treated vs 0/20 controls); and discoloration of the contents of the jejunum and colon in the 1500 ppm males (1-2/10 treated vs 0/10 controls).

Microscopically, ulcer/erosion of the glandular stomach was observed in all treated females (3-7/10 treated vs 1/10 control) and in all treated males except for the 50 ppm group (2-8/10 treated vs 1/10 control), and slight to moderate thickening of the duodenal mucosa was observed in the

500, 1000, and 1500 ppm animals (16-20/20 treated vs 0/20 controls) with a mean thickness in the 500, 1000, and 1500 ppm males (0.46-0.49 mm) and females (0.43-0.46 mm) greater than the control males (0.33 mm) and females (0.30 mm). These findings correlated with gross pathology findings in the digestive tract. Additionally, increased apoptosis was observed in the mesenteric lymph nodes in the 150 ppm females (2/10 treated vs 0/10 controls), and in the 500, 1000, and 1500 ppm animals (5-16/20 treated vs 0 controls). Atrophy of the thymus was observed in the 500, 1000, and 1500 ppm males and females and the 150 ppm females (3-8/10 treated vs 0/10 controls). Focal necrosis of the liver was observed in the 1500 ppm females (1/10 treated vs 0 controls).

The LOAEL is 150 ppm (equivalent to 30.4 and 40.4 mg/kg/day for males and females, respectively) based on changes in body weight and body weight gains in the males and changes in clinical chemistry and microscopic pathology in both sexes. The NOAEL for this study is 50 ppm (equivalent to 9.2 and 12.9 mg/kg/day for males and females, respectively).

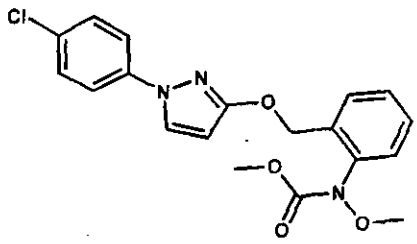
The submitted study is classified as **acceptable/guideline (§82-1b)** and satisfies the requirements for a subchronic oral toxicity study in the mouse.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Pyraclostrobin Technical (BAS 500F)
   Description: Viscous reddish brown liquid
   Lot/Batch #: CP 025394
   Purity (w/w): 98.5% a.i.
   Stability of compound: The compound was stable in the diet for up to 43 days at room temperature.
   CAS #: 175013-18-0
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Mouse
   Strain: B6C3F1 Crl BR
   Age and weight at the start of dosing: 47-49 days old; 20.6-26.5 g, males (group means: 23.4-23.9 g); 17.1-20.9, females (group means: 18.9-19.3 g)
   Source: Charles River GmbH
   Housing: Individually, in type MI Makrolon cages with wire mesh tops
   Diet: Ground Kliba maintenance diet (Klingentalmühle AG, Kaiseraugst, Switzerland), *ad libitum*
   Water: Tap water, *ad libitum*
   Environmental conditions:
   Temperature: 20-24 °C
   Humidity: 30-70%
   Air changes: Not provided
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 9 days

B. STUDY DESIGN

1. In life dates - start: 06/14/96 end: 09/18/96

2. Animal assignment - The mice were randomly assigned (stratified by weight) to the test groups shown in Table 1.

Table 1. Study design [a]

Test Group	Nominal Dose (ppm)	Achieved Dose (mg/kg/day) M/F	Assigned animals	
			Males	Females
Control	0	0	10	10
Low	50	9.2/12.9	10	10
Low-Mid	150	30.4/40.4	10	10
Mid	500	119.4/162.0	10	10
Mid-High	1000	274.4/374.1	10	10
High	1500	475.5/634.8	10	10

a Data obtained from the study report, pages 20 and 41.

3. Dose selection rationale - No dose rationale was provided.

4. Treatment preparation, dosing, and analysis - Test material was frozen, mechanically crushed, and mixed with acetone. A premix was made by spraying this solution onto diet and removing the acetone under partial vacuum by heating to approximately 40°C for 50 minutes. The premix was adjusted to the desired concentrations by dilution with diet and stored. Test material was prepared prior to the study and divided into four portions: the first portion was administered immediately; the second was stored in a cold room until administration; and the third and fourth were frozen until immediately before use. Prior to the study, homogeneity (top, middle, bottom) of the 50, 500, and 1500 mg/kg formulations was determined. Stability was analyzed for a 20 mg/kg diet formulation that was stored at room temperature for up to 43 days. Concentration analyses were performed on all dose formulations once during the study. Samples were kept frozen until analysis. Each sample was analyzed two to four times.

Results -

Homogeneity analysis (range as % of nominal ± SD): 100.5 ± 2.4% - 107.3 ± 2.0%

Stability analysis (% of day 0): 104%

Concentration analysis (range as % of nominal): 98.1-107.3%

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

5. Statistics - Food consumption, food efficiency, body weight, and body weight gain data were analyzed by one-way analysis of variance (ANOVA) followed by pair-wise comparisons of treatment groups with controls by Dunnett's test. Hematology, clinical chemistry, and organ weight data were analyzed by Kruskal-Wallis followed by pair-wise comparisons of treatment groups with controls by Mann-Whitney U-test for clinical chemistry and hematology or by Wilcoxon's test for organ weight data.

C. METHODS

1. Observations - The mice were monitored for mortality and moribundity twice daily (once daily on weekends and holidays). Additionally, detailed clinical examinations were performed weekly.

2. Body weight and body weight gains - Each animal was weighed prior to treatment, weekly throughout the study, and at necropsy. Cumulative group mean body weight gains (g) were calculated weekly.

3. Food consumption/efficiency - Food consumption (g/animal/day) for each animal was recorded weekly throughout the study. Group mean food efficiency (%) was calculated as body weight gain (g/week)/food consumption (g/week) X 100. Compound intake values (mg/kg/day) were calculated using the consumption and body weight data and the nominal dose.

4. Water consumption - Water consumption was not measured.

5. Ophthalmoscopy - Ophthalmoscopic examinations were not performed.

6. Blood - Blood was collected at study termination from the retroorbital venous plexus of fasted, unanesthetized animals or after decapitation from fasted, anesthetized animals. The checked (X) hematology and clinical blood chemistry parameters were examined.

   a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
X	Magnesium	X	Blood urea nitrogen
X	Potassium	X	Total Cholesterol
X	Sodium	X	Globulins
X	Phosphorus	X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (AP)	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase (CPK)		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
X	Serum aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

7. Urinalysis - Urinalyses were not performed (not required in the most recent guidelines).

8. Sacrifice and Pathology - Animals surviving to termination were sacrificed by decapitation under CO_2 anesthesia. All animals (including decedents) were subjected to a detailed necropsy. The following checked (X) tissues were collected from all animals and preserved in 4% formaldehyde. Additionally, the (XX) organs from all animals were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./ HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes
X	Jejunum	X	Thymus		
X	Ileum				GLANDULAR
X	Cecum		UROGENITAL	XX	Adrenal gland
X	Colon	XX	Kidneys	X	Lacrimal gland
X	Rectum	X	Urinary bladder	X	Mammary gland
XX	Liver	XX	Testes	X	Parathyroids
X	Gall bladder	X	Epididymides	X	Thyroids
X	Pancreas	X	Prostate		
		X	Seminal vesicle		OTHER
	RESPIRATORY	XX	Ovaries	X	Bone (with joint)
X	Trachea	X	Uterus	X	Skeletal muscle
X	Lungs	X	Oviducts	X	Skin
	Nose	X	Vagina	X	All gross lesions and masses
	Pharynx				
	Larynx				

The thymus, lungs, liver, kidneys, stomach, duodenum, jejunum, ileum, mesenteric lymph nodes, and all gross lesions and masses were examined histologically from all animals. The adrenal glands were examined in all females. The remaining tissues/organs were examined only in the control and high-dose animals. Additionally, the thickness of the duodenal mucosa (from the tips of the villi to the smooth muscle) was measured microscopically in all animals.

II. RESULTS

A. Observations

1. Mortality - There were no treatment-related mortalities. One 1500 ppm female died at week 8 due to accidental physical trauma.

2. Clinical signs - No treatment-related clinical signs were observed.

B. Body weight and body weight gains - Body weights (Table 2a) were decreased ($p \leq 0.05$ or 0.01) throughout treatment at 150 ppm in the males (↓8-11%), at 500 ppm in the males (↓9-

19%) and females (↓6-13%), at 1000 ppm in the males (↓7-27%) and females (↓9-18%), and at 1500 ppm in the males (↓12-34%) and females (↓9-27%).

Table 2a. Mean body weights ± SD (g) at selected intervals for mice fed pyraclostrobin for up to 3 months.[a]

Study Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	24.4 ± 1.2	23.7 ± 1.0	23.6 ± 1.0	22.8* ± 1.4 (↓7)	21.5** ± 0.9 (↓12)
28	27.4 ± 1.9	25.0* ± 1.9 (↓9)	25.0* ± 1.7 (↓9)	23.3** ± 1.6 (↓15)	20.6** ± 1.6 (↓25)
35	28.9 ± 2.4	26.6* ± 1.3 (↓8)	25.7** ± 1.3 (↓11)	23.4** ± 1.7 (↓19)	20.3** ± 1.1 (↓30)
63	31.7 ± 3.4	28.7* ± 1.4 (↓9)	26.8** ± 1.0 (↓15)	25.1** ± 1.5 (↓21)	22.3** ± 1.5 (↓30)
91	36.0 ± 4.0	31.9** ± 2.1 (↓11)	29.0** ±1.1 (↓19)	26.4** ± 1.1 (↓27)	23.8** ±1.2 (↓34)
Females					
7	19.6 ± 0.6	19.8 ± 0.6	19.2 ± 1.1	18.9 ± 0.6	17.8** ± 0.8 (↓9)
14	21.0 ± 0.5	21.5 ± 0.8	19.7** ± 1.1 (↓6)	19.1** ± 0.7 (↓9)	17.8** ± 0.9 (↓15)
49	24.1 ± 0.9	23.3 ± 1.1	21.9** ± 1.0 (↓9)	20.1** ± 0.6 (↓17)	17.5** ± 1.4 (↓27)
77	26.3 ± 2.0	24.7 ± 1.8	23.0** ± 1.1 (↓13)	21.6** ± 0.9 (↓18)	20.1** ± 1.1 (↓24)
91	26.5 ± 2.5	25.8 ± 2.3	23.4** ± 0.9 (↓12)	22.1** ± 0.6 (↓17)	20.0** ± 1.1 (↓25)

a Data obtained from the study report, Tables IA-007 through IA-010, pages 66-69; percent difference from control is in parentheses; n=10 except for the 1500 ppm female group after day 56 (n=9) when one animal died accidentally.

*, ** Significantly different from controls at $p \leq 0.05$ or 0.01, respectively.

Cumulative body weight gains (Table 2b) were decreased ($p \leq 0.05$ or 0.01) throughout treatment at 150 ppm in the males (↓31-62%), at 500 ppm in the males (↓53-122%) and females (↓39-74%), at 1000 ppm in the males (↓76-233%) and females (↓63-180%), and at 1500 ppm in the males (↓101-367%) and females (↓82-340%). Overall (day 0-91) body weight gains were decreased in the 150 ppm males (↓33%), 500 ppm males (↓60%) and females (↓43%), 1000 ppm males (↓80%) and females (↓63%), and 1500 ppm males (↓101%) and females (↓85%). Additionally, body weight gains were decreased (↓23%; $p \leq 0.05$) at study day 77 in the 150 ppm females.

Table 2b. Cumulative mean body weight change ± SD (g) at selected intervals for mice fed pyraclostrobin for up to 13 weeks.[a]

Study Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	0.9 ± 0.6	0.2 ± 0.6	-0.2** ± 0.7 (↓122)	-1.2 ± 0.6** (↓233)	-2.4** ± 0.6 (↓367)
28	3.9 ± 1.5	1.5* ± 2.5 (↓62)	1.1** ± 1.9 (↓72)	-0.7 ± 0.7** (↓118)	-3.3** ± 1.4 (↓185)
42	5.5 ± 2.1	3.7* ± 1.1 (↓33)	2.6** ± 1.5 (↓53)	-0.1 ± 0.7** (↓102)	-2.7** ± 1.4 (↓149)
56	7.5 ± 2.8	5.2* ± 1.6 (↓31)	3.1** ± 1.7 (↓59)	1.5 ± 0.7** (↓80)	-1.4** ± 1.5 (↓119)
70	8.9 ± 3.1	6.0* ± 1.9 (↓33)	4.1** ± 1.7 (↓54)	2.1 ± 0.5** (↓76)	-0.4** ± 1.4 (↓104)
91	12.6 ± 3.3	8.4** ± 2.1(↓33)	5.1** ± 1.5 (↓60)	2.5 ± 0.5** (↓80)	-0.1** ± 1.1 (↓101)
Females					
7	0.5 ± 0.4	0.7 ± 0.8	0.1 ± 0.5	-0.4** ± 0.2 (↓180)	-1.2** ± 0.5 (↓340)
14	1.9 ± 0.5	2.4 ± 1.0	0.5** ± 0.6 (↓74)	-0.2** ± 0.5 (↓111)	-1.1** ± 0.5 (↓158)
63	5.9 ± 1.5	5.1 ± 1.2	3.6** ± 0.9 (↓39)	1.9** ± 0.5 (↓68)	0.5** ± 1.8 (↓92)
77	7.3 ± 2.3	5.6* ± 1.6 (↓23)	3.8** ± 0.7 (↓48)	2.3** ± 0.6 (↓68)	1.3** ± 0.8 (↓82)
91	7.5 ± 2.7	6.7 ± 2.0	4.3** ± 0.4 (↓43)	2.8** ± 0.5 (↓63)	1.1** ± 0.7 (↓85)

a Data obtained from the study report, Tables IA 011 through IA-014, pages 70-73; percent difference from controls is listed in parentheses; n=10 except for the 1500 ppm female group after day 56 (n=9) when one animal died accidentally.

*, ** Significantly different from controls at p≤0.05 or 0.01, respectively.

C. Food consumption and compound intake:

1. Food consumption - Food consumption (Table 3a) was increased (p≤0.05 or 0.01) intermittently throughout treatment in the 1000 ppm males (↑35-49%) and females (↑27-39%) and in the 1500 ppm males (↑30-49%) and females (↑27-58%). However, according to the study report (p. 40 of MRID 45118320), all groups had food spilling problems that were unaccounted for. This renders the data on food consumption, compound consumption, and food efficiency unreliable.

Table 3a. Mean food consumption ± SD (g/animal/day) at selected intervals in mice fed pyraclostrobin for up to 3 months.[a]

Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	6.3 ± 2.0	5.8 ± 0.8	6.3 ± 1.2	6.9 ± 1.5	7.1 ± 2.6
14	5.9 ± 1.1	6.3 ± 2.2	7.9 ± 2.3	8.8** ± 1.8 (↑49)	8.8** ± 2.4 (↑49)
21	6.1 ± 0.4	6.2 ± 1.2	6.8 ± 1.3	7.4 ± 1.2	7.9** ± 2.0 (↑30)
56	5.4 ± 0.7	5.2 ± 0.6	6.5 ± 2.1	7.3* ± 1.6 (↑35)	7.2* ± 2.0 (↑33)
91	5.7 ± 0.7	5.7 ± 0.9	6.0 ± 1.4	5.9 ± 1.6	7.0 ± 1.9
Females					
7	5.7 ± 1.4	7.3 ± 3.6	8.8 ± 3.3	8.5 ± 2.3	9.0* ± 2.7 (↑58)
21	6.4 ± 1.6	7.0 ± 1.7	7.7 ± 2.0	8.9** ± 1.9 (↑39)	8.6* ± 1.3 (↑34)
84	5.6 ± 0.5	6.3 ± 1.1	6.5 ± 0.7	7.1* ± 1.6 (↑27)	7.1* ± 1.2 (↑27)
91	6.2 ± 1.4	6.3 ± 0.9	7.2 ± 2.0	6.8 ± 1.5	7.9 ± 1.7

a Data were obtained from the study report, Tables IA 003 through IA-006, pages 62-65; n=10 except for the 1500 ppm female group after day 56 (n=9) when one animal died accidentally; percent difference from controls is included in parentheses.

*, ** Significantly different from controls $p \leq 0.05$ or 0.01, respectively.

2. Compound consumption - The mean achieved dosages are presented in Table 1.

3. Food efficiency - Treatment-related decreases ($p \leq 0.01$) in food efficiency (Table 3b) were observed throughout dosing in the 500 ppm (↓65-123%), 1000 ppm (↓62-218%), and 1500ppm (↓77-350%) males. Additionally, food efficiency was decreased ($p \leq 0.05$) on study day 49 in the 150 ppm males (↓43%). In the first three weeks of the study, treatment-related decreases in food efficiency were observed in the 1000 ppm (↓86-154%), and 1500 ppm (↓79-254%) females. Food efficiency was also decreased ($p \leq 0.01$) in the 500 ppm females on study day 14 (↓68%).

Table 3b. Mean food efficiency (%) ± SD at selected intervals in mice fed pyraclostrobin for up to 3 months.[a]

Days	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	2.2 ± 1.5	0.5 ± 1.4	-0.5** ± 1.8 (↓123)	-2.6** ± 1.6 (↓218)	-5.5** ± 2.5 (↓350)
21	3.4 ± 2.6	2.0 ± 0.9	1.2** ± 0.8 (↓65)	0.5** ± 0.4 (↓85)	0.8** ± 0.7 (↓77)
49	5.8 ± 1.5	3.3* ± 2.4 (↓43)	1.8** ± 1.0 (↓69)	2.2** ± 0.8 (↓62)	0.2** ± 1.1 (↓97)
91	3.5 ± 3.0	3.3 ± 2.2	1.8 ± 1.3	-0.1** ± 1.1 (↓103)	-0.1** ± 0.9 (↓103)
Females					
7	1.3 ± 1.1	1.7 ± 1.8	0.0 ± 1.0	-0.7** ± 0.3 (↓154)	-2.0** ± 1.1 (↓254)
14	3.1 ± 0.8	3.4 ± 1.0	1.0** ± 1.1 (↓68)	0.4** ± 0.7 (↓87)	0.1** ± 0.9 (↓97)
21	2.8 ± 0.7	2.2 ± 1.2	1.8 ± 1.1	0.4** ± 0.9 (↓86)	0.6** ± 0.5 (↓79)
91	1.1 ± 2.0	2.0 ± 2.4	1.3 ± 1.3	1.7 ± 0.9	0.9 ± 1.0

a Data were obtained from the study report, Tables IA-015 through IA-018, pages 74-77; n=10 except for the 1500 ppm female group after day 56 (n=9) when one animal died accidentally; numbers listed parenthetically represent the percent difference from controls.

*, ** Significantly different from controls $p \leq 0.05$ or 0.01, respectively.

D. Blood analyses

1. Hematology - The following treatment-related differences ($p \leq 0.05$, 0.02, or 0.002) were observed (Table 4): (i) decreased leukocytes in the 1000 ppm (↓54%) and 1500 ppm (↓55%) males; (ii) decreased hemoglobin in the 1000 ppm females (↓4%) and in the 1500 ppm males (↓10%) and females (↓9%); (iii) decreased mean cell volume in the 500 ppm (↓3%), 1000 ppm (↓3%), and 1500 ppm (↓12%) males; (iv) decreased mean cell hemoglobin in the 500 ppm females (↓2%), 1000 ppm males (↓1%) and females (↓4%), and 1500 ppm males and females (↓12% each); and (v) increased platelets in the 500 ppm males (↑13%) and in the 1500 ppm males (↑11%) and females (↑18%).

   Several other hematological parameters attained significance ($p \leq 0.05$, 0.02, or 0.002) but the differences were minor and/or not dose-related, such as decreased hematocrit in the 150 ppm (↓4%), 500 ppm, 1000 ppm, and 1500 ppm males (↓5% each); and decreased mean corpuscular hemoglobin concentration in the female groups 500 ppm (↓3%), 1000 ppm (↓3%), and 1500 ppm (↓4%).

In agreement with the treatment-related decreased total leukocytes, absolute differential counts were also affected even though the changes were not analyzed for statistical significance. For instance, neutrophils were decreased by up to 50% in the two top dose female groups while lymphocytes were dose-dependently decreased in males and females by up to 77% and 46%, respectively (Table 4).

Table 4. Selected hematology values (mean ± SD) for mice fed pyraclostrobin for up to 3 months.[a]

Parameter	Dose (ppm)			
	0	500	1000	1500
Males				
Leukocytes (10^9/L)	5.92 ± 1.57	6.36 ± 5.37	2.72*** ± 1.11 (↓54)	2.67*** ± 1.19 (↓55)
Hemoglobin (mmol/L)	11.8 ± 0.5	11.4 ± 0.5	11.4 ± 0.4	10.6*** ± 0.9 (↓10)
MCV (10^{-15} L)	48.3 ± 1.4	47.0* ± 0.9 (↓3)	46.7** ± 1.0 (↓3)	42.6*** ± 3.5 (↓12)
MCH (10^{-15} mol)	0.99 ± 0.01	0.99 ± 0.01	0.98* ± 0.02 (↓1)	0.87*** ± 0.07 (↓12)
Platelets (10^9/L)	1120 ± 94	1271** ± 81 (↑13)	1137 ± 233	1247** ± 114 (↑11)
Neutrophils (10^9/L)	1.35 ± 0.59	2.40 ± 2.21	1.19 ± 0.65	1.71 ± 1.08
Lymphocytes (10^9/L)	4.01 ± 0.94	3.88 ± 3.63 (↓3)	1.51 ± 0.92 (↓62)	0.94 ± 0.40 (↓77)
Females				
Leukocytes (10^9/L)	6.04 ± 3.44	3.72 ± 1.86	3.25 ± 2.93	3.17 ± 2.37
Hemoglobin (mmol/L)	11.4 ± 0.3	11.0 ± 0.7	10.9** ± 0.5 (↓4)	10.4** ± 1.1 (↓9)
MCH (10^{-15} mol)	1.02 ± 0.02	1.00* ± 0.02 (↓2)	0.98** ± 0.03 (↓4)	0.90*** ± 0.12 (↓12)
Platelets (10^9/L)	1048 ± 103	1086 ± 87	1112 ± 86	1236** ± 233 (↑18)
Neutrophils (10^9/L)	2.18 ± 2.09	1.41 ± 0.72	1.05 ± 0.65 (↓52)	1.08 ± 0.51 (↓50)
Lymphocytes (10^9/L)	3.68 ± 2.67	2.26 ± 1.87 (↓39)	2.16 ± 2.42 (↓41)	2.00 ± 1.89 (↓46)

a Data were obtained from the study report, Tables IB-001, IB-002, IB-004, and IB-006, pages 82, 83, 85, and 87; n=10 except for the 1500 ppm female group where n=9 after one animal died accidentally on day 56; numbers listed parenthetically represent the percent difference from controls.

*,**,*** Significantly different from controls $p \leq 0.05$, 0.02, or 0.002, respectively. Differential counts were not analyzed statistically.

2. Clinical chemistry - The following treatment-related differences ($p \leq 0.05$, 0.02, or 0.002) were observed (Table 5): (i) increased urea in the 150 ppm males (↑21%) and females (↑49%), in the 500 ppm males (↑46%) and females (↑82%), in the 1000 ppm males (↑66%) and females (↑79%), and in the 1500 ppm males (↑65%) and females (↑62%); (ii) decreased triglycerides in the 150 ppm males (↓32%) and females (↓37%), in the 500

ppm males (↓54%) and females (↓62%), in the 1000 ppm males (↓65%) and females (↓61%), and in the 1500 ppm males (↓72%) and females (↓62%); (iii) decreased creatinine in the 1000 ppm (↓10%) and 1500 ppm (↓13%) females; (iv) decreased globulin in the 500 ppm (↓7%), 1000 ppm (↓15%) and 1500 ppm (↓16%) females; and (v) increased cholesterol in the 500 ppm (↑19%), 1000 ppm (↑28%) and 1500 ppm (↑52%) females. Additionally, urea was increased in the 50 ppm males (↑9%).

Several other clinical chemistry parameters attained statistical significance ($p \leq 0.05$, 0.02, or 0.002) but were deemed unrelated to treatment because the differences were minor, and/or not dose-related, such as: (i) increased chloride (↑2-3%) in the 500 ppm, 1000 ppm, and 1500 ppm females; (ii) decreased potassium (↓9%) in the 1500 ppm males; and (iii) alkaline phosphatase in males was decreased (↓13-18%) at the low and mid-doses and increased (↑12-20%) at the top two doses; (iv) decreased calcium (↓3-6%) in both sexes of the 1000 and 1500 ppm groups; and (v) decreased total protein (↓4-11%) in the 1000 and 1500 ppm males and females.

Table 5. Selected clinical chemistry values (mean ± SD) for mice fed pyraclostrobin for up to 3 months.[a]

Parameter	Dose (ppm)				
	0	150	500	1000	1500
Males					
Urea (mmol/L)	7.25 ± 0.71	8.75*** ± 0.64 (↑21)	10.55*** ± 1.65 (↑46)	12.01*** ± 1.92 (↑66)	11.97*** ± 2.42 (↑65)
Triglycerides (mmol/L)	1.70 ± 0.46	1.15** ± 0.47 (↓32)	0.78*** ± 0.18 (↓54)	0.59*** ± 0.20 (↓65)	0.47*** ± 0.24 (↓72)
Females					
Urea (mmol/L)	6.09 ± 0.91	9.07** ± 2.91 (↑49)	11.06*** ± 3.97 (↑82)	10.89*** ± 2.77 (↑79)	9.85*** ± 2.60 (↑62)
Triglycerides (mmol/L)	1.53 ± 0.55	0.96* ± 0.45 (↓37)	0.58*** ± 0.34 (↓62)	0.59*** ± 0.27 (↓61)	0.58*** ± 0.16 (↓62)
Creatinine (µmol/L)	38.9 ± 3.[illegible]	38.8 ± 3.8	36.8 ± 4.3	35.0* ± 3.4 (↓10)	33.7** ± 5.0 (↓13)
Globulins (g/L)	22.03 ± 1.19	22.37 ± 2.02	20.56** ± 1.91 (↓7)	18.68*** ± 1.28 (↓15)	18.47*** ± 1.86 (↓16)
Cholesterol (mmol/L)	2.50 ± 0.37	2.76 ± 0.51	2.98** ± 0.26 (↑19)	3.20*** ± 0.27 (↑28)	3.79*** ± 0.61 (↑52)

a Data were obtained from the study report, Tables IB-009 through IB-012, pages 90-93; n=10 except for the 1500 ppm female group where n=9 after one animal died accidentally on day 56; numbers listed parenthetically represent the percent difference from controls.

*,**,*** Significantly different from controls p≤0.05, 0.02, or 0.002, respectively.

E. Sacrifice and Pathology:

1. Organ weight - The absolute weight of liver, kidneys, spleen, ovaries, and adrenals were statistically significantly decreased (↓6-48%) at one or more doses above 150 ppm. However, in most cases, the decreases were offset by proportionate decrease in absolute body weight. The following changes in organ weight relative to body weight were observed. Males had a dose-related increase in the relative weights of liver (↑8-25%), testes (↑29-43%), and adrenals (↑44-89%). Females, on the other hand, had relative to body weight decreases in ovaries (↓25-28%) at the two top doses and in adrenals at the 500 (↓16%), 1000 (↓27%), and 1500 ppm (↓20%).

2. Gross pathology - The following macroscopic changes (Table 6) were observed (data presented as number of affected animals vs. controls (dose group 0); n = 10 except for the 1500 ppm female group where n = 9): (i) ulcer/erosion of the glandular stomach at 500

ppm (2 treated vs 1 control), 1000 ppm (2 treated vs 1 control), and at 1500 ppm (4 treated vs 1 control) in the males; (ii) duodenal wall thickening in the 500 ppm males (8) and females (6), in the 1000 ppm males (10) and females (10), and in the 1500 ppm males (10) and females (9); and (iii) discoloration of the contents of the jejunum (2) and colon (1) in the 1500 ppm males.

Table 6. Selected macroscopic findings (# of affected animals) in mice fed pyraclostrobin for up to 3 months.[a]

Observation	Dose (ppm)					
	0	50	150	500	1000	1500
Males						
Glandular stomach- ulcer/erosion	1	0	1	2	2	4
Duodenum- thickening of wall	0	0	0	8	10	10
Jejunum- discoloration of contents	0	0	0	0	0	2
Colon- discoloration of contents	0	0	0	0	0	1
Females						
Duodenum- thickening of wall	0	0	0	6	10	9

a Data obtained from the study report, Tables IC5 through IC6, pages 98-99; n=10 except for the 1500 ppm female group where n=9 due to an accidental death and tissue autolysis of one animal on day 56.

3. Microscopic pathology - The following treatment-related microscopic changes (Table 7) were observed (data presented as number of affected animals vs. controls (dose group 0); n=10 except for the 1500 ppm female group where n = 9): (i) ulcer/erosion of the glandular stomach in the 50 ppm females (3 treated vs 1 control), 150 ppm males (2 treated vs 1 control) and females (5 treated vs 1 control), 500 ppm males (4 treated vs 1 control) and females (7 treated vs 1 control), 1000 ppm males (5 treated vs 1 control) and females (6 treated vs 1 control), and 1500 ppm males (8 treated vs 1 control) and females (6 treated vs 1 control); (ii) slight to moderate thickening of the duodenal mucosa in the 500 ppm males (10) and females (6), 1000 ppm males (10) and females (10), and 1500 ppm males (10) and females (9), with a mean thickness in the 500, 1000, and 1500 ppm males (0.46-0.49 mm) and females (0.43-0.46 mm) greater than the control males (0.33 mm) and females (0.30 mm); (iii) minimal to slight increased apoptosis in the mesenteric lymph nodes in the 150 ppm females (2), 500 ppm males (1) and females (4), 1000 ppm males (1) and females (6), and 1500 ppm males (9) and females (7); (iv) slight to severe atrophy of the thymus in the 150 ppm females (6), the 500 ppm males (3) and females (4), the 1000 ppm males (6) and females (8), and the 1500 ppm males (8) and females (4); and (v) focal necrosis of the liver in the 1500 ppm females (1). In addition, fatty vacuoles were dose-dependently decreased in livers and kidneys, especially among males; also, the treated females had diminished lipid content in the adrenal cortex (MRID 45118320; not

reproduced in Table 7). These findings are consistent with the apparent diminished nutritional status.

Table 7. Selected microscopic findings (# of animals) in mice fed pyraclostrobin for up to 3 months.[a]

Observation	Dose (ppm)					
	0	50	150	500	1000	1500
Males						
Glandular stomach- ulcer/erosion	1	1	2	4	5	8
Duodenum- thickening of mucosa	0	0	0	10	10	10
slight[b]	0	0	0	6	1	0
moderate[b]	0	0	0	4	9	10
Thymus- atrophy (total)	0	0	0	3	6	8
slight[b]	0	0	0	2	4	1
moderate[b]	0	0	0	1	1	2
severe[b]	0	0	0	0	1	5
Lymph node- increased apoptosis (total)	0	0	0	1	1	9
minimal[b]	0	0	0	1	1	0
slight[b]	0	0	0	0	0	9
Females						
Glandular stomach- ulcer/erosion	1	3	5	7	6	6
Duodenum- thickening of mucosa	0	0	0	6	10	9
slight[b]	0	0	0	6	10	7
moderate[b]	0	0	0	0	0	2
Thymus- atrophy (total)	0	0	6	7	8	4
slight[b]	0	0	3	2	3	0
moderate[b]	0	0	2	5	4	2
severe[b]	0	0	1	0	1	2
Liver- focal necrosis	0	0	0	0	0	1
Lymph node- increased apoptosis	0	0	2	4	6	7
minimal[b]	0	0	0	0	5	5
slight[b]	0	0	2	4	1	2

a Data obtained from the study report, Tables IC7 through IC12, pages 100-105; n=10 except for the 1500 ppm female group where n=9 due to an accidental death and tissue autolysis of one animal on day 56.

b Severity was graded from 1 - 5 for minimal, slight, moderate, severe, and extreme, respectively.

III. DISCUSSION

A. Investigator's conclusions - It was concluded that oral administration of pyraclostrobin altered hematological parameters by inducing leukopenia, thrombocytosis, and mild hypochromic microcytic anemia. These hematological changes, as well as differences in clinical chemistry indicative of a catabolic state, were related to the reduced body weights and food efficiency. The NOAEL for this study was 50 ppm.

B. Reviewer's discussion/conclusions - In this subchronic oral study, pyraclostrobin was administered in the diet to 10 mice/sex/group at doses of 0, 50, 150, 500, 1000, or 1500 ppm (equivalent to 0, 9.2/12.9, 30.4/40.4, 119.4/162.0, 274.4/374.1, and 475.5/634.8 mg/kg/day for males/females) for 3 months. The analytical data indicated that the variation between nominal and actual dosage to the study animals was acceptable.

There were no treatment-related mortalities. One 1500 ppm female died at week 8 due to accidental physical trauma. No treatment-related differences were observed in clinical signs or organ weights.

Body weights were decreased ($p \leq 0.05$ or 0.01) throughout treatment in the 150 ppm males (↓8-11%) and in the 500 ppm (↓6-19%), 1000 ppm (↓7-27%), and 1500 ppm (↓9-34%) animals. Similarly, body weight gains were decreased ($p \leq 0.05$ or 0.01) throughout treatment in the 150 ppm males (↓31-62%) and in the 500 ppm (↓39-122%), 1000 ppm (↓63-233%) and 1500 ppm (↓82-367%) animals. Additionally, body weight gains were decreased (↓23%; $p \leq 0.05$) at study day 77 in the 150 ppm females. Food efficiency was decreased ($p \leq 0.05$ or 0.01) throughout treatment in the 500 ppm, 1000 ppm, and 1500 ppm males (↓62-350%), at study day 49 in the 150 ppm males (↓43%), in the first three weeks of the study in the 1000 ppm and 1500 ppm females (↓79-254%), and in the 500 ppm females at study day 14 (↓68%). Food consumption was increased ($p \leq 0.05$, 0.01) sporadically throughout treatment in the 1000 ppm (↑27-49%) and 1500 ppm animals (↑27-58%). Despite food spillage problems that were unaccoumted for, it seems that a catabolic state might have been induced by treatment as evidenced by the decreased body weights, body weight gains, and food efficiency, while food consumption was increased. It is uncertain whether pyraclostrobin exerted a direct effect on catabolism or if the catabolic state was a compensatory response to reduced digestion and absorption of food.

Several changes in clinical chemistry ($p \leq 0.05$, 0.02, or 0.002) indicated a continual catabolic state associated with a compromised nutritional status. Urea was increased in the 150, 500, 1000 and 1500 ppm animals (↑21-82%), indicating increased protein metabolism. Triglycerides were decreased in the 150, 500, 1000, and 1500 ppm animals (↓32-72%). Creatinine was decreased in the 1000 and 1500 ppm females (↓10-13%) due to decreased muscle mass. Globulins were decreased (↓7-16%) and cholesterol was increased (↑19-52%) in the 500, 1000, and 1500 ppm females. Consistent with the clinical chemistry findings and compromised nutritional status, were dose-dependent reduction in lipid vacuoles/content in liver, kidneys, and adrenals.

The mouse hematopoietic and immune systems seem to be target organs. Mild hypochromic, microcytic anemia was evidenced at the highest three doses by decreased hemoglobin in the 1000 ppm females (↓4%) and in the 1500 ppm males (↓10%) and females (↓9%), decreased mean cell volume in the 500, 1000, and 1500 ppm males (↓3-12%), and decreased mean cell hemoglobin in the 500, 1000, and 1500 ppm animals (↓1-12%). Additionally, leukocytes were decreased in the 1000 and 1500 ppm males (↓54-55%), and platelets were increased in the 500 ppm males (↑13%) and in the 1500 ppm males (↑11%) and females (↑18%). In agreement with the large decline in total leukocytes, absolute differential counts were also affected as evidenced by large decrease in neutrophils (up to 50%) in the two top dose female groups and the dose-dependent decrease of lymphocytes among males and females (up to 77% and 46%, respectively). These changes, combined, are more likely due to a direct effect by Pyraclostrobin on erythropoiegenesis than being secondary to the apparent compromised nutritional status and the continued catabolic state. Also, the large reduction in total and differential leukocytes, especially lymphocytes, are consistent with the microscopic findings (below) of thymus atrophy and increased apoptosis (cell death) of mesenteric lymph nodes; collectively, these results clearly indicate that the immune system is a target organ of Pyraclostrobin.

Numerous macroscopic changes indicated insult to the digestive tract, such as: ulcer/erosion of the glandular stomach in the 500, 1000, and 1500 ppm males (2-4/10 treated vs 1/10 control); duodenal wall thickening in the 500, 1000, and 1500 ppm animals (14-20/20 treated vs 0/20 controls); and discoloration of the contents of the jejunum and colon in the 1500 ppm males (1-2/10 treated vs 0/10 controls).

Microscopically, ulcer/erosion of the glandular stomach was observed in all treated females (3-7/10 treated vs 1/10 control) and in all treated males except for the 50 ppm group (2-8/10 treated vs 1/10 control), and slight to moderate thickening of the duodenal mucosa was observed in the 500, 1000, and 1500 ppm animals (16-20/20 treated vs 0/20 controls) with a mean thickness in the 500, 1000, and 1500 ppm males (0.46-0.49 mm) and females (0.43-0.46 mm) greater than the control males (0.33 mm) and females (0.30 mm). These findings correlated with gross pathology findings in the digestive tract. Additionally, increased apoptosis was observed in the mesenteric lymph nodes in the 150 ppm females (2/10 treated vs 0/10 controls), and in the 500, 1000, and 1500 ppm animals (5-16/20 treated vs 0 controls). Atrophy of the thymus was observed in the 500, 1000, and 1500 ppm males and females and the 150 ppm females (3-8/10 treated vs 0/10 controls). Focal necrosis of the liver was observed in the 1500 ppm females (1/10 treated vs 0 controls).

The LOAEL is 150 ppm (equivalent to 30.4 and 40.4 mg/kg/day for males and females, respectively) based on changes in body weight and body weight gains in the males and changes in clinical chemistry and microscopic pathology in both sexes. The NOAEL for this study is 50 ppm (equivalent to 9.2 and 12.9 mg/kg/day for males and females, respectively).

The submitted study is classified as **acceptable/guideline (§82-1b)** and satisfies the requirements for a subchronic oral toxicity study in the mouse.

C. Study deficiencies - The following deficiencies were noted but do not change the conclusions of this DER:

- No dose rationale was provided; however, the NOAEL and LOAEL were determined for both sexes within the range of doses tested.
- No ophthalmoscopic examinations were performed.