DATA EVALUATION RECORD

PYRACLOSTROBIN (BAS 500F) 5/24/2001

Study Type: §82-1a, 90-Day Oral Toxicity Study in Rats

Work Assignment No. 3-02-140A (formerly 3-01-113B) (MRIDs 45118321 & 45118322)

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DATA EVALUATION RECORD

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

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

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

SYNONYMS: BAS 500F, Methyl-N-[[[1-(4-chlorophenyl)pyrazol-3-yl]oxy]-o-tolyl]-N-methoxycarbamate

CITATION: Mellert, W., Deckardt, K., Bahnemann, R., et.al. (1999) BAS 500 F- Subchronic Oral Toxicity Study in Wistar Rats Administration in the Diet for 3 Months. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, Germany. Laboratory Project Id.: 50C0183/96015, July 2, 1999. MRID 45118321. Unpublished.

Mellert, W., Deckardt, K., Gembardt, Ch., et.al. (1999) BAS 500 F- Repeated Dose Oral Toxicity Study in Wistar Rats Administration in the Diet for 4 Weeks. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, Germany. Laboratory Project Id.: 30C0376/95083, December 2, 1999. MRID 45118322. Unpublished.

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

EXECUTIVE SUMMARY: In this subchronic oral study, (MRIDs 45118321 & 45118322) pyraclostrobin (BAS 500F; 98.5% a.i.; Lot/Batch # CP 025394) was administered in the diet to 10 Wistar rats/sex/group at doses of 0, 50, 150, 500, 1000, or 1500 ppm (equivalent to 0, 3.5/4.2, 10.7/12.6, 34.7/40.8, 68.8/79.7, and 105.8/118.9 mg/kg/day for males/females) for 3 months.

X

There were no mortalities. No treatment-related differences were observed in clinical signs, ophthalmology, or urinalysis at any dose level. There were no treatment-related findings at 50 ppm, and the only findings noted at 150 ppm was extramedullary hematopoiesis in the spleen of females (3/10 vs 0/10 controls). However, because the finding in the spleen was not corroborated by hematology changes at the same dose, they are not considered to be of toxicological relevance.

Terminal body weights (↓7%) and overall (day 91) body weight gains (↓11%) were decreased in males in the 500 ppm group. At 1000 and 1500 ppm, body weights (↓12-26%) and body weight gains (↓23-75%) were decreased throughout treatment in males. Additionally at 1500 ppm, body weights (↓8-9%) and body weight gains (↓16-65%) were decreased in females. Body weights were decreased in the 1500 ppm females (↓8-9%) on study days 7, 14, and 91. Food consumption was decreased intermittently throughout treatment in both sexes (↓6-17%) in the 500 ppm group. In the 1000 and 1500 ppm groups, food consumption was decreased (↓9-46%) in both sexes throughout the study. Several clinical chemistry parameters differed from controls and reflected the continual catabolic state associated with the reduced nutritional status in these animals. For example, in the 500 ppm group cholesterol was decreased in males (↓19%), and alkaline phosphatase was decreased in females (↓14%). In the 1000 and 1500 ppm groups, alkaline phosphatase (↓14-23%) and globulins (↓8-13%) were decreased in both sexes. Triglycerides (↓50-61%) and cholesterol (↓26-29%) were decreased in males and total bilirubin (↑58-95%) was increased in males. Serum cholinesterase was decreased in the 1000 (↓41%) and 1500 (↓49%) females. [In addition, at 1500 ppm total bilirubin (↑35%) was increased in females.]

The liver, spleen, and duodenum were the target organs of toxicity. Absolute (↑16-58%) and relative (↑22-74%) spleen weights were increased) in the females and/or males at doses of 500 ppm and greater. Relative liver weight was increased in the females 1000 and 1500 ppm groups (↑14-34%). Absolute weights of the liver in females in the 1500 ppm group were increased (↑22%).

Several hematological findings corroborated these effects on the spleen. Mild hemolytic anemia was evidenced by increased reticulocytes in the 1000 ppm males and in the 1500 ppm animals (↑41-94%), decreased erythrocytes in the 1000 and 1500 ppm females (↓7-11%), and increased prothombin time in males (↑11-13%). Likewise, gross and microscopic spleen changes, including spleen discoloration (2-11/20 vs 0/20 controls), sinus distension (18/20 vs 0/20 controls), extramedullary hematopoiesis (9/10 females vs 0/10 controls), and histiocytosis (13-17/20 vs 0/20 controls) corroborated a toxic effect on the spleen, consistent with the hematology.

Dose-related increases in minimal to slight hepatocyte hypertrophy, predominantly in zone 3, were observed in the 500 (3/10 vs 0/10 controls) and 1000 ppm males (6/10 vs 0/10 controls) and in the 1500 ppm animals (4-10/20 vs 0/20 controls). Dose-related thickening of the duodenal wall was observed macroscopically in the 1000 ppm females (2/10 vs 0/10 controls) and in the 1500 ppm animals (20/20 vs 0/20 controls). Microscopically, dose-related increases were

observed in the incidences of hyperplasia in the duodenal mucosa in the 500 (4/10 vs 2/10 controls) and 1000 ppm males (5/10 vs 2/10 controls) and in the 1500 ppm animals (20/20 vs 4/20 controls).

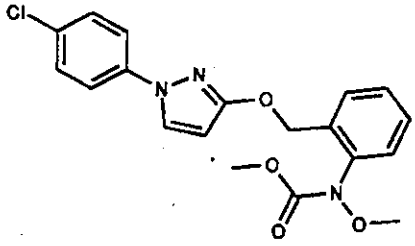
The LOAEL is 500 ppm (equivalent to 34.7/40.8 mg/kg/day for males/females) based on decreased body weights/body weight gains (males), decreased food consumption, increased relative liver weight and absolute and relative spleen weight in females, and histopathology of the duodenum, spleen and liver. The NOAEL for this study is 150 ppm (equivalent to 10.7/12.6 mg/kg/day for males/females).

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Pyraclostrobin Technical (BAS 500F)
   Description: Viscous dark 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: Rat
   Strain: Wistar [Chbb:THOM (SPF)]
   Age and weight at the start of dosing: 42 days old; 163-190 g, males; 119-145 g, females
   Source: Dr. Karl Thomae GmbH, Biberach/Riss, FRG
   Housing: Individually, in type DK III stainless steel wire mesh cages.
   Diet: Ground Kliba maintenance diet 343 meal (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: 11 days

B. STUDY DESIGN

1. In life dates - start: 05/10/96 end: 08/14/96

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

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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	3.5/4.2	10	10
Low-Mid	150	10.7/12.6	10	10
Mid	500	34.7/40.8	10	10
Mid-High	1000	68.8/79.7	10	10
High	1500	105.8/118.9	10	10

a Data obtained from the study report, page 41.

3. Dose selection rationale - Based upon the results of a 28-day range-finding study (MRID 45118322) submitted with the 90-day study, the doses summarized in Table 1 were selected for the 90-day study. See Appendix in this DER for details of the 28-day study.

4. Treatment preparation, dosing, and analysis - The test material was frozen, mechanically crushed, and mixed with acetone. Then, 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. These premixes were adjusted to the desired concentrations by dilution with diet and stored at room temperature. 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. After stability was demonstrated for 43 days, another batch of test material was prepared and was stored at room temperature until use. At the start of the study, homogeneity (top, middle, bottom) of the 50, 500, and 1500 mg/kg formulations was determined. During the study, stability was analyzed for a 20 mg/kg diet formulation at room temperature for up to 43 days. Concentration analyses were performed on all dose formulations at the start of and towards the end of the study. Samples were kept frozen until analysis. Each sample was analyzed two to four times.

Results -

The results of the homogeneity analysis on triplicate samples from feed containing 50, 500, and 1500 ppm pyraclostrobin were (mean % of nominal ± SD): 100.5±2.4, 107±2.0, and 107.3±2.0, respectively.

Stability analysis at day 43 (expressed as % of day 0): 104%

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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, urinalysis, 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, by Fisher's Exact test for urinalysis, or by Wilcoxon test for organ weight data.

C. METHODS

1. Observations - The rats were monitored for mortality and moribundity twice daily (once daily on weekends and holidays). Changes in clinical condition or behavior were recorded daily. Additionally, a more detailed clinical examination was performed once per week.

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 based on individual body weight gain (g/week)/food consumption (g/week) X 100. Compound intake values (mg/kg/day) were calculated using the individual values for food consumption and body weight data and the nominal dose.

4. Water consumption - Water consumption was not measured.

5. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on each animal prior to dosing and on control and high-dose groups on day 90 of treatment.

6. Blood - Blood was collected at study termination from the retroorbital venous plexus of non-fasted, unanesthetized 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)		
X	(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	Phosphate	X	Glucose
	ENZYMES		Direct bilirubin
X	Alkaline phosphatase (AP)	X	Total bilirubin
X	Serum Cholinesterase (ChE)	X	Total serum protein (TP)
X	Erythrocyte Cholinesterase	X	Triglycerides
	Creatine phosphokinase (CPK)		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
X	Serum aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

7. Urinalysis - Urine was collected overnight from each animal in metabolism cages on study day 88. Animals were deprived of food and water during the collection period. The checked (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment	X	Nitrite
X	Protein	X	Urobilinogen
		X	Bacteria

8. Sacrifice and Pathology - At study termination, all animals were sacrificed by decapitation and exsanguination under CO_2 anesthesia and were subjected to a detailed necropsy. The following checked (X) tissues were collected from all animals and preserved in 4% formaldehyde. 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	Caecum		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
	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				
	Diaphragm				

The lungs, liver, spleen, kidneys, stomach, duodenum, jejunum, ileum, bone marrow, and any gross lesions were examined histologically from all animals. The remaining tissues/organs were examined only in the control and high-dose animals.

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II. RESULTS

A. Observations

1. Mortality - No mortalities occurred during the study

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) in the males at 500 ppm on study day 91 (↓7%), and at 1000 (↓12-16%) and 1500 (↓17-26%) ppm throughout treatment. Body weights were decreased ($p \leq 0.05$) in the 1500 ppm females on study days 7, 14, and 91 only (↓8-9%).

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

Study Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
0	178.7 ± 7.6	178.5 ± 4.5	176.1 ± 9.1	178.2 ± 6.2	177.1 ± 5.5
7	228.3 ± 7.8	227.3 ± 7.8	220.4 ± 10.5	200.1** ± 5.6 (↓12)	189.3** ± 8.0 (↓17)
49	399.4 ± 19.3	389.6 ± 28.4	380.7 ± 27.5	347.0** ± 13.9 (↓13)	304.4** ± 13.3 (↓24)
91	465.1 ± 23.9	452.4 ± 31.6	432.4* ± 33.6 (↓7)	392.2** ± 24.2 (↓16)	343.6** ± 16.0 (↓26)
Females					
0	133.8 ± 3.6	134.6 ± 8.1	135.7 ± 6.1	133.3 ± 5.1	132.3 ± 4.5
7	153.0 ± 4.8	155.7 ± 9.2	153.1 ± 8.3	147.9 ± 5.7	139.1** ± 5.6 (↓9)
14	171.0 ± 9.1	174.0 ± 9.9	168.5 ± 10.6	166.3 ± 6.6	157.4* ± 8.3 (↓8)
91	247.8 ± 15.8	254.5 ± 18.3	241.6 ± 16.2	234.9 ± 13.9	226.4* ± 13.6 (↓9)

a Data obtained from the study report, Tables IA-007 through IA-010, pages 67-70; n=10. Results of the 50 ppm dose group were similar to those of the control group and therefore are not reproduced here.

*, ** Significantly different from controls at $p \leq 0.05$ or 0.01, respectively; percent difference from control is in parentheses

Cumulative body weight gains (Table 2b) were decreased ($p \leq 0.01$) in the 1000 (↓23-56%) and 1500 (↓40-75%) ppm males throughout treatment. Overall (days 0-91) body weight

gains were decreased ($p \leq 0.05$ or 0.01) in the 500 (↓11%), 1000 (↓25%), and 1500 (↓42%) ppm males. Body weight gains were decreased ($p \leq 0.05$ or 0.01) in the 1500 ppm females intermittently throughout treatment (↓16-65%); however, overall body weight gains were decreased in these animals (↓17%; $p \leq 0.05$).

Table 2b. Mean cumulative body weight gains (g) ± SD at selected intervals for rats fed pyraclostrobin for up to 3 months.[a]

Study Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	49.7 ± 2.9	48.8 ± 4.7	44.3 ± 7.5	21.9** ± 3.5 (↓56)	12.3** ± 6.0 (↓75)
70	267.6 ± 25.5	257.0 ± 29.5	245.8 ± 30.0	205.9** ± 18.6 (↓23)	157.7** ± 12.5 (↓41)
84	293.5 ± 29.3	279.2 ± 30.9	264.5 ± 33.7	222.5** ± 20.9 (↓24)	177.4** ± 14.6 (↓40)
Overall (91)	286.4 ± 23.5	273.8 ± 30.5	256.3* ± 30.6 (↓11)	214.0** ± 21.9 (↓25)	166.5** ± 15.7 (↓42)
Females					
7	19.2 ± 4.6	21.1 ± 3.7	17.3 ± 6.8	14.6 ± 5.9	6.7** ± 6.0 (↓65)
70	107.9±16.1	109.9±10.5	100.0±10.4	95.0 ±12.3	90.3* ±12.2 (↓16)
Overall (91)	114.0 ± 17.0	119.9 ± 10.9	105.8 ± 13.4	101.6 ± 13.2	94.1* ± 12.0 (↓17)

a Data obtained from the study report, Tables IA-011 through IA-014, pages 71-74; n=10. Results of the 50 ppm dose group were similar to those of the control group and therefore are not reproduced here.

*, ** Significantly different from controls at $p \leq 0.05$ or 0.01, respectively; percent difference from control is in parentheses

C. Food consumption, food efficiency, and compound intake:

1. Food consumption - Food consumption (Table 3a) was decreased ($p \leq 0.01$) in the 1000 (↓10-40%) and 1500 (↓13-46%) ppm males throughout the study. Food consumption was intermittently decreased ($p \leq 0.05$ or 0.01) throughout treatment in the 500 ppm males (↓6-17%) and in the 500 (↓8-13%), 1000 (↓10-32%), and 1500 (↓9-43%) ppm females.

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

Study Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	25.4 ± 1.1	24.4 ± 0.7	21.0** ± 1.2 (↓17)	15.2** ± 1.1 (↓40)	13.7** ± 1.5 (↓46)
14	26.8 ± 1.2	26.2 ± 1.2	25.1* ± 1.7 (↓6)	21.6** ± 0.8 (↓19)	18.3** ± 1.3 (↓32)
56	26.6 ± 1.5	25.5 ± 1.7	24.7* ± 1.6 (↓7)	23.2** ± 1.6 (↓13)	21.1** ± 1.2 (↓13)
77	25.8 ± 1.9	25.9 ± 1.8	24.2 ± 2.1	22.7** ± 1.4 (↓10)	21.4** ± 0.8 (↓18)
91	22.6 ± 1.6	22.4 ± 1.1	20.7** ± 1.3 (↓8)	19.4** ± 1.0 (↓14)	18.1** ± 1.3 (↓20)
Females					
7	17.3 ± 0.9	17.6 ± 2.1	15.1** ± 1.4 (↓13)	11.8** ± 0.8 (↓32)	9.8** ± 1.2 (↓43)
63	18.6 ± 1.6	18.4 ± 1.2	17.1* ± 0.8 (↓8)	16.8* ± 0.8 (↓10)	17.0* ± 1.3 (↓9)
91	16.1 ± 1.2	16.8 ± 1.1	15.7 ± 0.8	15.5 ± 0.7	14.9 ± 1.2

a Data obtained from the study report, Tables IA-003 through IA-006, pages 63-66; n=10; percent difference from control is included in parentheses. Results of the 50 ppm dose group were similar to those of the control group and therefore are not reproduced here.

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

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

3. Food efficiency - Food efficiency (Table 3b) was decreased ($p \leq 0.05$ or 0.01) in the 1000 ppm males (↓26%) and 1500 ppm males (↓54%) and females (↓41%) on study day 7 and in the 1000 (↓72%) and 1500 (↓66%) ppm females on study day 63. However, these decreases were incidental and considered to be of minimal toxicological importance.

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

Study Day	Dose (ppm)				
	0	150	500	1000	1500
Males					
7	27.9 ± 1.8	28.6 ± 2.5	30.1 ± 4.8	20.6** ± 2.3 (↓26)	12.7** ± 5.9 (↓54)
91	-4.6 ± 5.6	-3.5 ± 3.7	-5.7 ± 4.8	-6.3 ± 3.1	-8.8 ± 5.1
Females					
7	15.7 ± 3.4	17.2 ± 3.0	16.1 ± 5.4	17.3 ± 6.2	9.2* ± 8.0 (↓41)
63	8.3 ± 4.5	6.5 ± 2.4	4.5 ± 4.5	2.3* ± 4.5 (↓72)	2.8* ± 3.1 (↓66)
91	0.2 ± 3.9	0.0 ± 3.0	-4.6 ± 5.4	-2.4 ± 4.1	-3.8 ± 3.9

a Data obtained from the study report, Tables IA-015 through IA-018, pages 75-78; n=10; percent difference from control is included in parentheses. Results of the 50 ppm dose group were similar to those of the control group and therefore are not reproduced here.

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

D. Ophthalmoscopic examination - There were no treatment-related observations.

E. Blood analyses

1. Hematology - The following treatment-related differences were observed (Table 4): (i) increased reticulocytes in the 1000 ppm males (↑41%) and in the 1500 ppm males (↑94%) and females (↑64%); (ii) increased absolute neutrophils count in the 1000 (↑89%) and 1500 (↑120%) ppm males; (iii) increased prothrombin time in the 1000 (↑11%) and 1500 (↑13%) ppm males; (iv) increased leukocytes in the 1000 (↑71%) and 1500 (↑68%) ppm females with increased absolute neutrophils (↑56 and 84%, respectively) and increased absolute lymphocytes (↑76 and 70%, respectively); and (v) decreased erythrocytes in the 1000 (↓7%) and 1500 (↓11%) ppm females.

   Several other hematological parameters attained significance (p≤0.05, 0.02, or 0.002) but the differences were minor and/or not dose-related. For example, in the 500, 1000, and 1500 ppm female groups, there were increases in mean corpuscular hemoglobin (MCH, ↑3-4%) and in mean corpuscular volume (MCV, ↑4-6%), while the 1000 and 1500 ppm female dose groups had decreased hemoglobin (HGB, ↓5 and 7%, respectively) and decreased mean corpuscular hemoglobin concentration (MCHC, ↓2% each). Also, hematochrit (HCT) was decreased (↓5%) in the 1500 ppm female dose group. Finally, males had increased MCV (↑3-5%) in the 1000 and 1500 ppm groups and decreased MCHC (↓3-4%) in the top three dose groups.

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

Parameter	Dose (ppm)			
	0	500	1000	1500
Males				
Reticulocytes (10^{-3} erythrocytes)	17 ± 5	19 ± 4	24** ± 5 (↑41)	33*** ± 5 (↑94)
Neutrophils[b] (10^9/L)	0.66 ± 0.16	0.78 ± 0.30	1.25 ± 0.52 (↑89)	1.445 ± 0.52 (↑120)
Prothrombin time (seconds)	26.0 ± 1.4	27.1 ± 2.2	28.9*** ± 1.6 (↑11)	29.4*** ± 1.6 (↑13)
Females				
Reticulocytes (10^{-3} erythrocytes)	14 ± 3	13 ± 2	15 ± 3	23*** ± 8 (↑64)
Leukocytes (10^9/L)	3.90 ± 1.46	4.69 ± 1.22	6.65*** ± 1.52 (↑71)	6.55** ± 2.07 (↑68)
Neutrophils[b] (10^9/L)	0.68 ± 0.54	0.71 ± 0.21	1.06 ± 0.32 (↑56)	1.25 ± 0.50 (↑84)
Lymphocytes[b] (10^9/L)	2.84 ± 0.98	3.58 ± 1.01	5.00 ± 1.31 (↑76)	4.83 ± 1.61 (↑70)
Erythrocytes (10^{12}/L)	7.95 ± 0.39	7.70 ± 0.22	7.36*** ± 0.28 (↓7)	7.10*** ± 0.22 (↓11)

a Data were obtained from the study report, Tables IB-001 through IB-010, pages 85-94; n=10; numbers listed parenthetically represent the percent difference from controls. Results of the 50 and 150 ppm dose groups were similar to those of the control group and therefore are not reproduced here.

b Statistics not performed.

,* Significantly different from controls $p \leq 0.02$, or 0.002, respectively.

2. Clinical chemistry - The following treatment-related differences ($p \leq 0.05$, 0.02, or 0.002) were observed (Table 5): (i) decreased alkaline phosphatase in the 500 ppm females (↓14%), in the 1000 ppm males (↓23%) and females (↓14%), and in the 1500 ppm males (↓22%) and females (↓20%); (ii) increased total bilirubin in the 1000 ppm males (↑58%) and in the 1500 ppm males (↑95%) and females (↑35%); (iii) decreased globulins in the 1000 ppm males (↓8%) and females (↓13%) and 1500 ppm males (↓13%) and females (↓12%); (iv) slightly increased albumin (↑5-6%) in the male groups 500, 1000, and 1500 ppm; (v) decreased triglycerides in the 1000 (↓50%) and 1500 (↓61%) ppm males; and (vi) decreased cholesterol in the 500 (↓19%), 1000 (↓26%), and 1500 (↓29%) ppm males. In addition, serum cholinesterase was decreased ($p \leq 0.002$) in the 1000 (↓41%) and 1500 (↓49%) females.

Several other clinical chemistry parameters attained 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. For example, chloride was increased in the 500, 1000, and 1500 ppm males (↑1-2%), chloride was decreased (↓2%) in the 1000 and 1500 ppm female groups, and glucose was decreased in the 1000 ppm females (↓12%).

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

Chemistry Parameter	Dose (ppm)			
	0	500	1000	1500
Males				
ALP (μkat/L)	5.55 ± 0.42	5.29 ± 0.69	4.28** ± 0.97 (↓23)	4.34*** ± 0.72 (↓22)
Total Bilirubin (μMol/L)	1.69 ± 0.56	2.20 ± 0.62	2.67*** ± 0.59 (↑58)	3.29*** ± 1.11 (↑95)
Globulin (g/L)	31.62 ± 1.61	31.21 ± 2.01	29.24** ± 2.18 (↓8)	27.50*** ± 2.28 (↓13)
Albumin (g/L)	35.48 ± 1.25	37.33** ± 1.33 (↑5)	37.78** ± 1.83 (↑6)	37.76** ± 1.03 (↑6)
Triglycerides (mMol/L	3.83 ± 1.31	2.89 ± 1.20	1.92** ± 1.03 (↓50)	1.51*** ± 0.70 (↓61)
Cholesterol (mMol/L)	2.26 ± 0.50	1.84* ± 0.22 (↓19)	1.67*** ± 0.20 (↓26)	1.60*** ± 0.33 (↓29)
Females				
ALP (μkat/L)	4.45 ± 0.39	3.82** ± 1.21 (↓14)	3.83** ± 0.86 (↓14)	3.55*** ± 0.58 (↓20)
Serum Cholinesterase (μkat/L)	47.94 ± 10.50	41.20 ± 7.56	28.52*** ± 4.23 (↓41)	24.34*** ± 5.54 (↓49)
Total Bilirubin (μMol/L)	2.17 ± 0.49	1.93 ± 0.68	2.69 ± 0.55	2.94** ± 0.66 (↑35)
Globulin (g/L)	30.55 ± 2.87	29.35 ± 2.38	26.69** ± 2.18 (↓13)	26.84** ± 1.38 (↓12)

a Data obtained from the study report, Table IB, pages 95-100; percent difference from control is listed in parentheses; n =10. Results of the 50 and 150 ppm dose groups were similar to those of the control group and therefore are not reproduced here.

*, **, or *** Significantly different from controls at $p \leq 0.05$, 0.02, or 0.002, respectively.

F. Urinalysis - There were no treatment-related findings in any urinalysis parameter. Masses of bacteria were observed in the urine of the 1500 ppm males (7/10 treated vs 0 controls); however, this finding was considered incidental and unrelated to treatment.

G. Sacrifice and Pathology:

1. Organ weight - The following treatment-related increases ($p \leq 0.05$ or 0.01) were observed in the absolute and relative (to body) organ weights (Table 6): (i) absolute liver in the 1500 ppm females (↑22%); (ii) relative liver in the 500 (↑5%), 1000 (↑14%), and 1500 (↑34%) ppm females; (iii) absolute spleen in the 500 (↑18%), 1000 (↑33%), and 1500 ppm females (↑58%) and in the 1500 ppm males (↑16%); and (iv) relative spleen in the 500 ppm females (↑22%) and in the 1000 ppm males (↑29%) and females (↑41%) and 1500 ppm males (↑61%) and females (↑74%).

The following decreases ($p \leq 0.05$ or 0.01) compared to concurrent controls were observed in absolute organ weights but were considered not directly related to treatment because they were either correlated with the decreased terminal body weights of these animals and/or were not corroborated by gross or microscopic pathology: (i) liver, in the 150 (↓12%), 500 (↓13%), 1000 (↓18%), and 1500 (↓20%) ppm males; (ii) kidneys, in the 1500 ppm males (↓17%); (iii) adrenals, in the 500 ppm males (↓17%) and females (↓12%), 1000 ppm males (↓16%) and females (↓15%), and 1500 ppm males (↓17%) and females (↓23%).

The following increases ($p \leq 0.05$ or 0.01) in relative (to body) organ weights were observed but their relevance to treatment is questionable due to the absence of gross or microscopic pathology: (i) kidneys in the 500 ppm females (↑6%) and in the 1000 ppm males (↑10%) and females (↑9%) and in the 1500 ppm males (↑15%) and females (↑8%); (ii) testes at 1000 (↑24%) and 1500 (↑41%) ppm; (iii) ovaries at 1500 ppm (↑29%); (iv) adrenals in the 1500 ppm males (↑10%); and (v) brain in the 1000 (↑16%) and 1500 (↑32%) ppm males. The incresed relative brain weights is most likely due the large decrease in body weights in both male groups.

Additionally, the absolute brain weights were different ($p \leq 0.05$) from controls in the 150 (↑3%), 1000 (↓3%) and 1500 (↓3%) ppm females. The relative adrenal weights were decreased ($p \leq 0.05$ or 0.01) in the 50 (↓14%) and 1500 (↓16%) ppm females. However, these changes were not dose-related. All other absolute and relative organ weights were comparable to controls.

Table 6. Selected organ weights (g) in rats fed pyraclostrobin for up to 3 months.[a]

Organ		Dose (ppm)			
		0	500	1000	1500
Males					
Spleen-	absolute	0.874 ± 0.111	0.848 ± 0.113	0.946 ± 0.111	1.018* ± 0.108 (↑16)
	relative	0.199 ± 0.024	0.21 ± 0.033	0.256** ± 0.027 (↑29)	0.32** ± 0.039 (↑61)
Females					
Liver-	absolute	6.772 ± 0.646	6.931 ± 0.467	7.274 ± 0.617	8.253** ± 0.744 (↑22)
	relative	2.942 ± 0.161	3.095* ± 0.151 (↑5)	3.34** ± 0.194 (↑14)	3.935** ± 0.218 (↑34)
Spleen-	absolute	0.555 ± 0.102	0.654* ± 0.091 (↑18)	0.74** ± 0.136 (↑33)	0.878** ± 0.132 (↑58)
	relative	0.241 ± 0.036	0.293* ± 0.043 (↑22)	0.34** ± 0.058 (↑41)	0.42** ± 0.064 (↑74)

a Data were obtained from the study report, pages 106-109
*, ** Significantly different from controls at $p \leq 0.05$ or 0.01, respectively

2. Gross pathology - Several macroscopic changes (Table 7) were observed (data presented as number of affected animals vs 0 controls, unless otherwise noted). Thickening of the

duodenal wall was observed in the 1000 ppm females (2/10) and in the 1500 ppm animals (20/20). Spleen discoloration was observed in the 1000 ppm males (1/10) and females (1/10) and in the 1500 ppm males (6/10) and females (5/10).

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

Observation	Dose (ppm)					
	0	50	150	500	1000	1500
Males						
Duodenum- thickening of wall	0	0	0	0	0	10
Spleen- discoloration	0	0	0	0	1	6
Females						
Duodenum- thickening of wall	0	0	0	0	2	10
Spleen- discoloration	0	0	0	0	1	5

a Data obtained from the study report, Tables IC5 through 6, pages 110-111; n=10

3. Microscopic pathology - The following treatment-related microscopic changes (Table 8) were observed (data presented as number of affected animals vs 0 controls, unless otherwise noted; n=10): (i) minimal to slight hyperplasia of the duodenal mucosa in the 500 (4 treated vs 2 controls) and 1000 (5 treated vs 2 controls) males and 1500 ppm males (10 treated vs 2 controls) and females (10 treated vs 2 controls); (ii) hepatocyte hypertrophy (minimal to slight and predominantly in zone 3) in the liver of the 500 (3) and 1000 (6) ppm males and 1500 ppm males (10) and females (4); (iii) sinus distension in the spleen in the 500 ppm males (1) and females (2), 1000 ppm males (10) and females (8), and 1500 ppm males (8) and females (10); (iv) extramedullary hematopoiesis in the spleen in the 150 (3), 500 (3), 1000 (9), and 1500 (9) ppm females; and (v) histiocytosis in the spleen in the 150 ppm males (1) and females (1), 500 ppm males (3) and females (2), 1000 ppm males (6) and females (7), and 1500 ppm males (10) and females (7).

Table 8. Selected microscopic findings (# of animals) in rats fed pyraclostrobin for up to 3 months.[a]

Observation	Dose (ppm)					
	0	50	150	500	1000	1500
Males						
Duodenum- mucosal hyperplasia (total)	2	1	1	4	5	10
minimal	2	1	1	3	4	1
slight	0	0	0	1	1	9
Liver- hepatocyte hypertrophy	0	0	0	3	6	10
Spleen-						
sinus distension	0	0	0	1	10	8
histiocytosis	0	0	1	3	6	10
Females						
Duodenum- mucosal hyperplasia (total)	2	1	2	1	1	10
minimal	2	1	1	1	0	6
slight	0	0	1	0	1	4
Liver- hepatocyte hypertrophy	0	0	0	0	0	4
Spleen-						
sinus distension	0	0	0	2	8	10
extramedullary hematopoiesis	0	0	3	3	9	9
histiocytosis	0	0	1	2	7	7

a Data obtained from the study report, Tables IC-7 through IC-12, pages 112-117; n=10.

III. DISCUSSION

A. Investigator's conclusions - It was concluded that oral administration of pyraclostrobin for 3 months altered hematological parameters by inducing leukocytosis coupled with neutrophilia, mild hemolytic anemia, and changes in clinical chemistry associated with a decreased nutritional status. Histopathology showed that the duodenum, spleen, and liver were the target organs. Because of decreased absolute liver weights in the males and increased extramedullary hematopoiesis and histiocytosis in the spleen in both sexes, 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 rats/sex/group at doses of 0, 50, 150, 500, 1000, or 1500 ppm (equivalent to 0, 3.5/4.2, 10.7/12.6, 34.7/40.8, 68.8/79.7, and 105.8/118.9 mg/kg/day for males/females) for 3 months. The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the study animals was acceptable.

There were no mortalities. No treatment-related differences were observed in clinical signs, ophthalmology, or urinalysis.

Body weights were decreased ($p \leq 0.05$ or 0.01) in the males at 500 ppm on study day 91 (↓7%), and at 1000 (↓12-16%) and 1500 (↓17-26%) ppm throughout treatment. Body weights were decreased ($p \leq 0.05$) in the 1500 ppm females on study days 7, 14, and 91 (↓8-9%). Cumulative body weight gains were continually decreased ($p \leq 0.01$) in the 1000 (↓23-56%) and 1500 (↓40-75%) ppm males and intermittently decreased ($p \leq 0.05$ or 0.01) in the 1500 ppm females throughout treatment (↓16-65%). Overall (days 0-91) body weight gains were decreased ($p \leq 0.05$ or 0.01) in the 500 (↓11%), 1000 (↓25%), and 1500 (↓42%) ppm males and in the 1500 ppm females (↓17%). Food consumption was decreased (↓10-46%; $p \leq 0.01$) in the 1000 and 1500 ppm males throughout the study. Food consumption was intermittently decreased ($p \leq 0.05$ or 0.01) throughout treatment in the 500 ppm animals (↓6-17%) and in the 1000 (↓10-32%) and 1500 (↓9-43%) ppm females.

Several clinical chemistry parameters differing ($p \leq 0.05$, 0.02, or 0.002) from controls were indicative of the reduced nutritional status. These findings were probably not evidence of direct toxicity, but were secondary to reduced food consumption and declines in body weight and body weight gains in the treated animals. For example, alkaline phosphatase was decreased in the 500 ppm females (↓14%) and in the 1000 ppm (↓14-23%) and 1500 ppm (↓20-22%) animals. Globulins were decreased in the 1000 ppm (↓8-13%) and 1500 ppm (↓12-13%) animals. Triglycerides were decreased in the 1000 (↓50%) and 1500 (↓61%) ppm males, and cholesterol was decreased in the 500 (↓19%), 1000 (↓26%), and 1500 (↓29%) ppm males.

The relatively large decrease in serum cholinesterase ($p \leq 0.002$) in the 1000 (↓41%) and 1500 (↓49%) females is not easy to explain in the absence of observed or known neurotoxic effects associated with pyraclostrobin. Similar findings were seen in the 1500 ppm female dose group in the 28-day dose-range finding study (MRID 445118322) which is summarized as an Appendix with this DER. Other available guideline studies, especially the acute and subchronic oral neurotoxicity studies in rats (MRIDs 45118337 and 45118401), will be monitored for possible neurotoxic effects that might shed some light on these results.

Mild hemolytic anemia was evidenced by increased ($p \leq 0.02$ or 0.002) reticulocytes in the 1000 ppm males (↑41%) and in the 1500 ppm animals (↑64-94%), decreased ($p \leq 0.002$) erythrocytes in the 1000 (↓7%) and 1500 (↓11%) ppm females, and increased ($p \leq 0.02$ or 0.002) total bilirubin in the 1000 ppm males (↑58%) and in the 1500 ppm animals (↑35-

95%). Consistent with mild hemolytic anemia, there were small changes in other parameters including increased MCV and decreased MCHC in both sexes, and decreased HCT and HGB in females. In addition, the spleen was increased in weight and had gross and microscopic changes that are indicative of hematological effects (see below).

Other effects on the hematopoeitic system were also evident as, for instance, increased (56-84%) leukocytes, neutrophils, and lymphocytes in the 1000 and 1500 ppm female groups and increased (89-120%) neutrophils in the 1000 and 1500 ppm males.

Gross and microscopic pathology revealed the liver, spleen, and duodenum as the target organs of toxicity. Absolute liver weight was increased ($p \leq 0.01$) in the 1500 ppm females (↑22%). Relative (to body) liver weights were increased ($p \leq 0.05$ or 0.01) in the 500 (↑5%), 1000 (↑14%), and 1500 (↑34%) ppm females. The liver weight increase was associated with minimal to slight hepatocyte hypertrophy (predominantly in zone 3) in the 500 (3/10 treated vs 0/10 controls) and 1000 (6/10 treated) ppm males and in the 1500 ppm animals (14/20 treated).

In addition to increased absolute and relative spleen weights in animals at doses of 500 ppm or higher, there were gross and microscopic spleen changes that are consistent with the hematological effects. These included spleen discoloration, sinus distension, extramedullary hematopoiesis, and histiocytosis.

Thickening of the duodenal wall was observed macroscopically in the 1000 ppm females (2/10 vs 0 controls) and in the 1500 ppm animals (20/20 treated). Microscopically, minimal to slight hyperplasia of the duodenal mucosa was observed in the 500 (4/10 treated vs 2/10 controls) and 1000 (5/10 treated) ppm males and in the 1500 ppm animals (20/20 treated vs 4/20 controls).

The LOAEL is 500 ppm (equivalent to 34.7/40.8 mg/kg/day for males/females) based on decreased body weights/body weight gains (males), decreased food consumption, increased relative liver weight and absolute and relative spleen weight in females, and histopathology of the duodenum, spleen and liver. The NOAEL for this study is 150 ppm (equivalent to 10.7/12.6 mg/kg/day for males/females).

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

C. Study deficiencies - No deficiencies were noted.

APPENDIX

Repeated Dose-Range Finding Study
(MRID 45118322)

Rat 28 Day Dietary Repeat Dose Study (used as dose-rationale for 90 day rat study)

This study is an early range-finding study performed to determine adequate dose levels used in other studies; therefore, only a summary of this study is provided in order to confirm the adequacy of the dose selection rationale used in later studies.

In this non-guideline oral toxicity study (MRID 45118322), pyraclostrobin (BAS 500F; 94-99% a.i.; Batches 27 882/37/a and 27 655/160) was administered in the diet to 5 Wistar rats/sex/group at nominal doses of 0, 20, 100, 500, or 1500 ppm (equivalent to [M/F] 0/0, 1.8/2.0, 9.0/9.6, 42.3/46.6, and 120.2/126.3 mg/kg/day) for 28 days. Dietary analyses indicated that the mixing procedure was adequate and nominal dosages were achieved.

No mortalities occurred during the study. Clinical signs, water consumption, and gross pathology were unaffected by the test substance. No treatment-related findings were observed in the 100 or 20 ppm dose groups.

Throughout the study, food consumption (↓16-44%), body weights (↓14-16%), and body weight gains (↓32-67%) were decreased ($p \leq 0.05$ or 0.01) in the 1500 ppm males. Food consumption was decreased ($p \leq 0.01$) in the 1500 ppm females on days 7, 14, and 28 (↓16-42%); however, body weights were only decreased on day 7 (↓10%, $p \leq 0.01$) and body weight gains were comparable to concurrent controls throughout the study in these animals. Food efficiency was decreased ($p \leq 0.01$) in the 1500 ppm males relative to controls on day 7 (16.2% treated vs. 28.4% controls), but was similar to controls for the remainder of the study; no differences in food efficiency were observed in the females.

Erythrocytes and hemoglobin were decreased ($p \leq 0.01$) in the 500 (↓7% each) and 1500 (↓7-9%) ppm females. Prothrombin time was increased ($p \leq 0.05$ or 0.01) in the 500 and 1500 ppm males (↑3-8%) and the 1500 ppm females (↑12%). Mean corpuscular hemoglobin concentration was slightly decreased in both sexes at 1500 ppm (↓4%, $p \leq 0.05$ or 0.01) and mean corpuscular volume was decreased in the 1500 ppm females (↓6%, $p \leq 0.01$). Additionally, serum cholinesterase activity was decreased in the 1500 ppm females (↓56%, $p \leq 0.01$) while brain and erythrocyte cholineesterase were normal in all dosed groups. At 1500 ppm, inorganic phosphate was decreased in the males (↓17%, $p \leq 0.05$), total bilirubin was increased in the males (↑104%, $p \leq 0.01$), and glucose was decreased in the females (↓22%, $p \leq 0.01$). In the urine, specific gravity was decreased at 1500 ppm in both sexes.

Increased absolute and relative (to body) spleen weights were observed in both sexes at 500 and 1500 ppm. In addition, increased extramedullary hematopoiesis in the spleen was noted in these animals. Additionally at 1500 ppm, increased absolute liver weights were noted in the females and increased relative liver weights were noted in both sexes. In the 500 and 1500 ppm animals, diminishing hepatocellular fat storage was noted; in the 1500 ppm animals, hepatocellular hypertrophy was noted in nearly all treated males and in a single female. Finally, mucosal hyperplasia in the duodenum was observed at 500 and 1500 ppm in both sexes.

The LOAEL for this study is 500 ppm (equivalent to 42.3 mg/kg/day in the males and 46.6 mg/kg/day in the females) based on changes in hematology parameters, organ weights, and histopathology of the spleen, liver, and duodenum.

The NOAEL for this study is 100 ppm (equivalent to 9.0 mg/kg/day in the males and 9.6 mg/kg/day in the females).

Based upon the results of this 28-day range-finding study, the doses of 0, 50, 150, 500, 1000, and 1500 ppm were selected for the 90-day study (MRID 45118321).

The submitted study is classified as **acceptable/non-guideline** and satisfies the purpose for which it was intended as a dose range-finding study.

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

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