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DATA EVALUATION REPORT

8/14/2001

PYRACLOSTROBIN (BAS 500F)

Study Type: §82-2a; 28-Day Dermal Toxicity Study - Rat

Work Assignment No. 3-01-113C (MRID 45118324)

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PYRACLOSTROBIN (BAS 500F)

28-Day Dermal Toxicity (§82-2[a])

6/14/01

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DATA EVALUATION RECORD

STUDY TYPE: 28-Day Repeated Dose Dermal Toxicity Study - Rat
OPPTS Number: 870.3200 OPP Guideline Number: §82-2[a]

DP BARCODE: D269669
P.C. CODE: 099100

SUBMISSION CODE: S583112
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Pyraclostrobin (99% a.i.)

SYNONYMS: BAS 500 F; 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 - Repeated Dose Dermal Toxicity Study in Wistar Rats Administration for 4 Weeks. BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, Germany. Laboratory Project Id.: 33S0494/96179, October 15, 1999. MRID 45118324. Unpublished.

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

EXECUTIVE SUMMARY: In this repeated-dose dermal toxicity study (MRID 45118324), pyraclostrobin (99% a.i.) was administered to10 Wistar (Chbb:THOM (SPF)) rats/sex/group at doses of 0, 40, 100, or 250 mg/kg in 0.5% aqueous carboxymethyl cellulose. The test agents and the vehicle controls were applied to 10 percent of the clipped dorsal skin of the animals, under semipermeable occlusive dressings, 6 hours a day, 5 days per week for 28 days. The animals were observed daily for mortality/morbidity and weekly for food consumption and body weights. Routine hematological, clinical chemistry and urinalyses were carried out at the end of the study. Ophthalmological examinations were carried out at the beginning of the study and at the end of the study. A number of open field behavioral observations were made during days 1, 5. 12 and 26 days of the study. At the end of the 28 days, the animals were sacrificed, examined grossly, and selected organs were weighed. Organs from the controls and high dose groups and all gross pathological organs were examined histopathologically. The skin application sites examined daily for signs of irritation sections of treated and untreated skin sites were examined

Histopathologically at the end of the study.

No animals died during the study. Body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic observations, open field observations, hematology, clinical chemistry, and urinalysis parameters, and organ weights were unaffected by treatment.

At 40 mg/kg, the only finding noted on the skin was minimal to slight epidermal thickening (8/20 vs. 2/20 controls).Gross dermatological assessments were negative in most animals throughout the experimental period. Late in the study, the high dose males and the mid- and high-dose females showed mild/minimal scale formations on the treated sites. At the end of the 28 day period, the 100 and 250 mg/kg animals showed minimal to slight histological changes which included: slight hyperkeratosis at 100 mg/kg (5/10 males and 4/10 females vs. 0/20 controls) and 250 mg/kg (9/10 males and 10/10 females vs. 0/20 controls); and minimal to slight epidermal thickening at 40 mg/kg (3/10 males and 5/10 females vs. 2/20 controls), 100 mg/kg (9/10 males and 10/10 females vs. 2/20 controls), and 250 mg/kg (10/10 males and 10/10 females vs. 2/20 controls). Slight to moderate dilation of the uterine lumen was observed in the 250 ppm females (6/10 treated vs. 0/10 controls).

The dermal LOAEL was 100 mg/kg based on scale formation, hyperkeratosis, and epidermal thickening at the site of application. The dermal NOAEL was 40 mg/kg. The systemic LOAEL was not observed. The systemic NOAEL was 250 mg/kg.

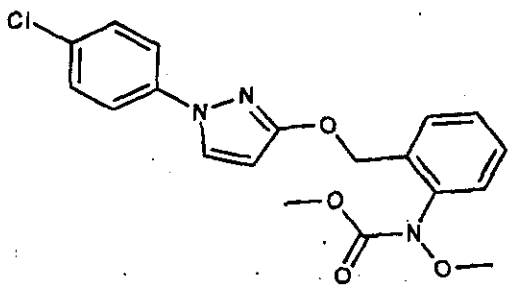
This study is classified by the EPA reviewer as **UNACCEPTABLE (§82-2[a])** because of the lack of systemic effects as the high dose (250 mg/kg/day) which is 1/4th the limit dose of 1000 mg/kg/day. OPPTS GL #870.3200 requires that highest dose tested should result in definite systemic toxicity unless the limit dose is used or there is severe irritation to the skin. The skin effects in this study very minimal.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin
   Description: Yellowish solid, crystalline
   Lot/Batch #: LJ.-No. 28632/147FS, CP 029053
   Purity: 98.9% a.i.
   Stability of compound: The test substance was stable in the vehicle for 96 hours at room temperature.
   CAS #: 175013-18-0



Structure:

2. Vehicle: 0.5% carboxymethyl cellulose in distilled water.

3. Test animals: Species: Rat
   Strain: Wistar (Chbb:THOM (SPF))
   Age and weight at study initiation: Approximately 9 weeks old; 269.3-333.6 g (males), 168.4-226.1 g (females)
   Source: Boehringer Ingelheim Pharma KG, FRG
   Housing: Individually, in suspended, stainless steel, wire mesh cages
   Diet: Kliba maintenance diet (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 20-24°C
   Humidity: 30-70%
   Air Changes: Not reported
   Photoperiod: 12-hour light/dark cycle
   Acclimation period: 8-9 days

B. STUDY DESIGN

1. In-life dates - Start: 02/24/99 End: 03/26/99

2. Animal assignment - Animals were randomly assigned by computer (stratified by body weight) to treatment groups as indicated in Table 1.

Table 1. Study design [a]

Test Group	Dose (mg/kg/day)	Animals assigned	
		Males	Females
Control	0	10	10
Low	40	10	10
Mid	100	10	10
High	250	10	10

a Adapted from the study report, page 19.

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

4. Preparation and treatment of animal skin - Each day the test substance was weighed, diluted with 0.5% carboxymethyl cellulose in doubly distilled water, applied to the exposed treatment area (clipped dorsal and dorsolateral parts of the trunk; approximately 10% of the total body surface area), and covered with a semiocclusive dressing. The test substance was administered for 6 hours/day, 5 days/week, for 4 weeks. After each exposure period, the dressing was removed and the treated skin was washed with lukewarm water. Control animals received vehicle only.

5. Test substance analysis - Homogeneity (top, middle, and bottom) and concentration were determined on all dose formulations (1, 2.5, and 6.25 g/100 mL) before study initiation, during the first week of dosing, and near study termination. Stability analyses were conducted on two samples of a 50 mg/100 mL dose formulation stored for 96 hours at room temperature.

Results - Homogeneity analysis (range as % of nominal): 95.1-101.6%

Concentration analysis (range as % of nominal): 95.1-101.6%

Stability analysis (% of day 0): 98.7-105.1%

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

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6. Statistical analyses: The following statistical tests were used [a]:

Statistical Test	Parameters Investigated
One-way ANOVA followed by Dunnett's test ($p \leq 0.05$ and 0.01)	Body weight Body weight gains Food consumption Food efficiency
One-way Kruskal-Wallis test followed by Mann-Whitney U test ($p \leq 0.05$, 0.02, and 0.002)	Hematology (except differential blood count) Clinical chemistry
Fisher's exact test ($p \leq 0.05$ and 0.01)	Urinalysis (except volume, color, tubidity, and specific gravity)
One-way Kruskal-Wallis test followed by Wilcoxon test ($p \leq 0.05$ and 0.01)	Organ weight

a Extracted from the study report, pages 26, 34, and 39.

C. METHODS

1. Observations - All animals were observed twice daily (once daily on weekends and holidays) for mortality and morbidity. In addition, the rats were observed at least once a day for clinical signs of toxicity and dermal effects immediately prior to treatment.

2. Body weight - All rats were weighed weekly throughout the study. Body weight gains were calculated at weekly intervals.

3. Food consumption/food efficiency - Food consumption (g/animal/day) was determined weekly. Food efficiency was calculated from individual food consumption and body weight data.

4. Ophthalmoscopic examination - The eyes of all animals were examined prior to the start of treatment using an ophthalmoscope. At the end of the study, the eyes of the control and high-dose animals were examined.

5. Open field examination - All animals were subjected to detailed clinical observations in the open field prior to treatment and on days 5, 12, 19, and 26. The open field assessment included the following parameters:

Behavior during handling	Abnormal movements
Fur	Gait impairment
Skin	Lacrimation
Posture	Palpebral closure
Salivation	Exophthalmus
Respiration	Feces
Activity/arousal	Urine
Tremors	Pupil size
Convulsions	

5. Blood - Upon study termination, blood was collected from the retroorbital venous plexus of all animals following an overnight fast. The CHECKED (X) 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
X	Blood clotting measurements		
	(Partial thromboplastin time)		
	(Capillary clotting time)		
X	(Prothrombin time)		

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
X	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
		X	Total bilirubin
	ENZYMES	X	Total protein
		X	Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophores
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT)		
X	Aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis - Prior to study termination, urine was collected overnight from all animals. The following CHECKED (X) parameters were evaluated.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Nitrite
X	Protein		Urobilinogen

7. Sacrifice and Pathology - Upon study termination, all rats were fasted, anesthetized with carbon dioxide, sacrificed by decapitation, and subjected to gross pathological examination. The following CHECKED (X) tissues were collected from all animals; the tissues from the control and high-dose animals were examined microscopically. Furthermore, all gross lesions and masses, the vagina, epididymides, and treated skin from the animals in the remaining groups were examined microscopically. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	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	XX	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	Thyroids
	Gall bladder	XX	Epididymides	X	Parathyroids
X	Pancreas	X	Prostate		
		X	Seminal vesicle		OTHER
	RESPIRATORY	XX	Ovaries	X	Bone
X	Trachea	XX	Uterus	X	Skeletal muscle
X	Lung	X	Oviducts	X	Skin (treated and untreated)
X	Nose	X	Vagina	X	All gross lesions and masses
X	Pharynx				
X	Larynx				

II. RESULTS

A. Observations

1. Toxicity - The most frequently noted clinical signs were scale formation and erythema

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(Table 2). In the 250 mg/kg males, focal, multifocal, and diffuse scale formation was observed (1/10, 2/10, and 1/10, respectively vs. 0/10 controls). These findings were also noted in the 250 mg/kg females (6/10, 9/10, and 3/10, respectively vs. 0/10 controls). In addition, focal and multifocal scale formation was observed in the 100 mg/kg females (1/10 and 2/10, respectively vs. 0/10 controls). Slight erythema was noted in the 250 mg/kg females (7/10 treated vs. 0/10 controls).

Table 2. Clinical observations noted in rats treated with pyraclostrobin for 28 days [a]

Clinical Observation	Dose (mg/kg)							
	Males				Females			
	0	40	100	250	0	40	100	250
Erythema, slight	0	0	0	0	0	0	0	7
Scale formation								
focal	0	0	0	1	0	0	1	6
multifocal	0	0	0	2	0	0	2	9
diffuse	0	0	0	1	0	0	0	3

a Data obtained from the study report pages 56-57; n=10.

2. Mortality - No animals died during the study.

B. Body weight and weight gain - Body weights and body weight gains were comparable between treated animals and controls throughout the study.

C. Food consumption/food efficiency - Food consumption and efficiency were comparable between treated animals and controls throughout the study.

D. Ophthalmoscopic examination - No treatment-related effects were observed.

E. Open field examination - All parameters were comparable between treated animals and controls during the open field examinations.

F. Blood work

1. Hematology - All hematological parameters were comparable between treated animals and controls.

2. Clinical Chemistry - No treatment-related differences in clinical chemistry parameters were observed in any treated group. Decreased ($p \leq 0.05$) alanine aminotransferase was noted in the 100 mg/kg males (↓15%); however, this decrease was not dose-dependent and was considered unrelated to treatment.

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G. Urinalysis - All urinalysis parameters were comparable between treated animals and controls.

H. Sacrifice and Pathology

1. Organ weights - No treatment-related differences in organ weights were observed. Decreased ($p \leq 0.05$) absolute thymus weight was observed in the 40 mg/kg males (↓19%); however, this decrease was not dose-dependent and was considered unrelated to treatment. In the 250 mg/kg females, absolute and relative (to body) uterus weight was increased (130 and 27%, respectively; $p \leq 0.05$).

2. Gross pathology - Scale formation on the treated skin (Table 3) occurred in 1/10 100 mg/kg females and 3/10 250 mg/kg females. This lesion was not observed in any treated male or in the controls of either sex. Dilation of the uterus was observed in a single 250 ppm female; however, this finding was considered incidental and unrelated to treatment.

Table 3. Gross pathology observations (# affected animals) noted on the treated skin of rats treated with pyraclostrobin for 28 days. [a]

Observation	Dose (mg/kg)							
	Males				Females			
	0	40	100	250	0	40	100	250
Scale formation	0	0	0	0	0	0	1	3

a Data obtained from the study report page 99; n=10.

3. Microscopic pathology - Treatment-related microscopic skin lesions at the application site (Table 4) included slight hyperkeratosis at 100 mg/kg (5/10 males and 4/10 females vs. 0/20 controls) and 250 mg/kg (9/10 males and 10/10 females vs. 0/20 controls); and minimal to slight epidermal thickening at 40 mg/kg (3/10 males and 5/10 females vs. 2/20 controls), 100 mg/kg (9/10 males and 10/10 females vs. 2/20 controls), and 250 mg/kg (10/10 males and 10/10 females vs. 2/20 controls). Slight to moderate dilation of the uterine lumen was observed in the 250 ppm females (6/10 treated vs. 0/10 controls).

Table 4. Histopathology (# affected animals) of the treated skin in rats treated with pyraclostrobin for 28 days [a]

Observation	Dose (mg/kg)							
	Males				Females			
	0	40	100	250	0	40	100	250
Hyperkeratosis (Total)	**0**	**0**	**5**	**9**	**0**	**0**	**4**	**10**
Slight	0	0	5	9	0	0	4	10
Epidermal thickening (Total)	**2**	**3**	**9**	**10**	**0**	**5**	**10**	**10**
Minimal	2	3	3	2	0	3	6	3
Slight	0	0	6	8	0	2	4	7

a Data obtained from the study report page 103; n=10.

III. DISCUSSION

A **Investigator's Conclusions** - Dermal administration of pyraclostrobin for 28 days caused local, irritating effects (scale formation, hyperkeratosis, erythema) in a dose dependent way including the low dose (40 mg/kg/day). There was no systemic toxicity observed at any dose. The investigators concluded that the systemic NOEL was 250 mg/kg/day and that the NOEL for dermal effects was 40 mg/kg and the dermal LOAEL was

B. **Reviewer's Discussion/Conclusion** - In this repeated-dose dermal toxicity study, pyraclostrobin was administered for 28 days to 10 Wistar (Chbb:THOM (SPF)) rats/sex/group at doses of 0, 40, 100, or 250 mg/kg in 0.5% carboxymethyl cellulose. The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosages to the study animals was acceptable.

No animals died during the study. Body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic observations, open field observations, hematology, clinical chemistry, and urinalysis parameters, and organ weights were unaffected by treatment. During clinical examinations of the skin, focal, multi focal, and diffuse scale formation was observed in the 250 mg/kg males (1/10, 2/10, and 1/10, respectively vs. 0/10 controls).

These findings were also noted in the 250 mg/kg females (6/10, 9/10, and 3/10, respectively vs. 0/10 controls). In addition, focal and multi focal scale formation was observed in the 100 mg/kg females (1/10 and 2/10, respectively vs. 0/10 controls). Slight erythema was noted in the 250 mg/kg females (7/10 treated vs. 0/10 controls). At necropsy, scale formation on the treated skin occurred in 1/10 100 mg/kg females and 3/10 250 mg/kg females vs. 0/10 controls. Treatment-related microscopic skin lesions at the application site included slight hyperkeratosis at 100 mg/kg (5/10 males and 4/10 females vs. 0/20 controls) and 250 mg/kg

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(9/10 males and 10/10 females vs. 0/20 controls); and minimal to slight epidermal thickening at 40 mg/kg (3/10 males and 5/10 females vs. 2/20 controls), 100 mg/kg (9/10 males and 10/10 females vs. 2/20 controls), and 250 mg/kg (10/10 males and 10/10 females vs. 2/20 controls).

The dermal LOAEL is 100 mg/kg based on scale formation, hyperkeratosis, and epidermal thickening at the site of application. The dermal NOAEL is 40 mg/kg.

Histologically, slight to moderate dilation of the uterine lumen was observed in the 250 ppm females (6/10 treated vs. 0/10 controls), resulting in fluid accumulation. Thus, increased absolute and relative (to body) uterus weights were observed in these animals (130 and 27%, respectively; $p \leq 0.05$). These findings were considered incidental based on the absence of uterine lesions in the subchronic rat study (MRID 45118321).

The systemic LOAEL was not observed. The systemic NOAEL is 250 mg/kg.

This study was carried out using acceptable procedural (guideline) methods, however, the lack of systemic toxicity suggests that the dosing was too low. This study is classified by the EPA reviewer as **UNACCEPTABLE (§82-2[a])** because of the lack of systemic effects as the high dose (250 mg/kg/day) which is 1/4th the limit dose of 1000 mg/kg/day. OPPTS GLN #870.3200 requires that highest dose tested should result in definite systemic toxicity unless the limit dose is used or there is severe irritation to the skin. The skin effects described in this study are so minimal, they do not preclude higher dosing levels.

The lack of more severe adverse effects on the skin in this study is not consistent with skin irritation studies in rabbits (Wieman, C., et al , BASF Doc. # 1999/12021 etc., sited in the BASF toxicological summary dated March 28, 2000), in which a 4 hour dermal exposure to technical BAS 500F produced "moderately severe skin toxicity"in the rabbit. The lack of more significant toxicity (systemically or locally to the skin) in the current 28 day rat dermal study, may be due to the fact the low dosing, that the agent suspended in the carboxyl methyl cellulose was not ground fine enough, was too tightly bound to the vehicle (CMC) or taken up by the 4 layers of gauze thereby preventing more adequate direct contact with the rat skin.

C. **Deficiencies.** None.

\pyraclostrobin\45118324B per Bill Greear June 13, 2001