(8-3-99) 17

[Trifloxystrobin] (TXR 013599) Subchronic Oral Study 870.3151; 82-1 b

EPA Reviewer: Anna P. Bearden _____, Date ___
EPA Secondary Reviewer: Stan Gross _____, Date ___

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Rangefinding Oral Toxicity Dog; OPPTS 870.3151 §82-1(b)

DP BARCODE: D244009 SUBMISSION CODE:S-53879
P.C. CODE:129112 TOX. CHEM. NO.:N/A

TEST MATERIAL: CGA 279202 (Trifloxystrobin; 96.2%)

SYNONYMS: Not given

CITATION: Altman, B. (1994) 28-Day range finding toxicity study in beagle dogs. Short/Long-term Toxicology; Novartis Crop Protection, AG (Formerly Ciba-Geigy Limited) Stein, Switzerland. Laboratory test number 933163, September 28, 1994. MRID [44496642]. Unpublished.

SPONSOR: Novartis Crop Protection, Inc.

EXECUTIVE SUMMARY:

In a subchronic toxicity study trifloxystrobin (MRID no. 444966-42; purity = 96.2%) was administered to 2 beagle dogs/sex/dose by capsule at dose levels of 0 (empty capsule), 20 , 50, and 150 mg/kg/day for 29 days. At day 29, dogs in control, 20 and 50 mg/kg/day groups were sacrificed. Due to a lack of toxic effects the dosage level for dogs in the 150 mg/kg/day group was increased to 500 mg/kg/day for an additional 21 days. All dogs were dosed 7 days a week. Dogs were observed daily in addition to weekly weighing and urine and blood analysis at pretest, week 4 and at termination. At termination gross and microscopic pathology were observed.

Doses were selected based on an exploratory test where dogs were exposed to 280 mg/kg/day in the diet. Due to poor palatability, route of administration for the exploratory test was changed to capsule at 300, 150, and 50 mg/kg/day for 16, 8, and 7 days.

No animals died or had to be euthanized during the range-finding study. There were "moderate" diarrhea and vomiting incidences which occurred at the beginning of the study in both sexes in the high dose group. Moderate congestion of the splenic red pulp was observed in 3/4 animals in the high dose group. Treatment for with trifloxystrobin did not effect hematology, clinical chemistry, and urinalysis in all treatment groups. In the high dose group, body weights of male dogs decreased 0.55 kg (-6% of pretest body weight). Organ weights of liver (+29% males, +34% females), kidney (+34% male), and spleen (+73% female) increased in the high dose group.

1/11

The LOAEL is 150 mg/kg/day based on clinical signs (diarrhea and vomiting), body weight reduction, liver, spleen, and kidney weight measures. The NOAEL is 50 mg/kg/day.

Due to the number of dogs (2/sex/dose) used and duration (28-49 days) of the study, this range finding subchronic toxicity study is classified as **guideline** but **not acceptable**. It does **not** satisfy the guideline requirement for a subchronic oral study (82-1) in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: CGA 279202 tech. (Trifloxystrobin)
   Description: powder
   Lot/Batch #: KGL4617/
   Purity: 96.2%.
   Stability of compound: Not given
   CAS #: not given

2. Vehicle control: Empty gelatin capsule; supplied by Torpac Limited, Canada

3. Test animals: Species: Dog
   Strain: pedigree beagle
   Age and weight at study initiation: Age ranged from 31 to 34 weeks old; Weight averaged 10.54 kg for males (range 9.10 to 13.20) and 8.73kg for females (range 7.40 to 10.0).
   Source: Animal production CIBA-GEIGY Limited, 4332 Stein/Switzerland
   Housing: Two/kennel. Fastened with chain during feeding.
   Diet: Certified pelleted standard diet NAFAG 9405 Tox) 350g/animal/day
   Water: Tap water was given ad libitum
   Environmental conditions: Temperature: Not controlled; Minimum temperature of 15°C.
   Humidity: Monitored, not given
   Air changes: not given
   Photoperiod: 12hr light/12hr dark
   Acclimation period: 25 days between delivery and treatment

B. STUDY DESIGN:

1. In life dates - start: not given end: March 21, 1994 for control, low, and medium

concentration group. April 10, 1994 for high concentration group.

2. Animal assignment

Animals were assigned by a randomized complete block design using the Statistical Analysis System to the test groups in table 1.

TABLE 1: STUDY DESIGN

Test Group	Dose to Animal (mg/kg)	Male	Female
Control	0	2	2
Low	20	2	2
Mid	50	2	2
High	150/500	2	2

3. Dosing rationale:

Doses were selected during an exploratory test where dogs were exposed to 280 mg/kg/day in the diet. Due to poor palatability, route of administration was changed to capsule. Additional testing was done at 300, 150, and 50 mg/kg/day for 16, 8, and 7 days.

This range finding study was originally designed to last 28 days. Dogs were dosed once a day using gelatin capsules. Method of capsule administration was not noted in methods. At day 29, dogs in the control, 20 and 50 mg/kg/day groups were sacrificed. Due to the low toxicity of the test chemical, 21 additional days of testing of the 150 mg/kg/day group was done. During this time, dose was increased from 150 mg/kg/day to 500 mg/kg/day. The high dose group was sacrificed at day 50. For the additional 21 days, there is no control data for comparisons of toxic effects.

3. Diet Certified pelleted standard diet NAFAG 9405 Tox 350g/animal/day

4. Statistics -

For each time point and parameter, univariate statistical analysis was performed using nonparametric methods. Medium and high dose groups were tested for increasing or decreasing trends from control group by Jonckheere's test for ordered alternatives. Each point represents only 2 animals, therefore limiting statistical quantitation and calculation of standard deviations.

C. METHODS:

1. Observations:
Animals were inspected twice daily on weekdays and once on the weekend for signs of toxicity and mortality.

2. Body weight
Animals were weighed weekly.

3. Food consumption
350 g of food was offered to each animal daily during a restricted feeding time. Amount consumed by each day on each day was recorded. Food efficiency was not determined.

4. Ophthalmoscopic examination
Eyes were examined at pretest and at day 28. Following external inspection, the lens, iris and fundus were examined with an opthalmoscope. The fundus was photographed. Mydriaticum^{tm} (Dispersa AG) was applied to induce mydriasis. The pupillary reflex was checked in all animals and the third eyelid was examined after local anesthesia using Novesin^{tm} 0.4% (Dispersa AG).

5. Blood collection:
Occurred in the morning. Dogs were fasted for 16 hours prior to collection. Blood was removed from the jugular vein on day 28. The CHECKED (X) parameters were examined.

a. Hematology

Table 2. Hematological parameters examined.

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*	X	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

Table 3. Clinical chemistry parameters examined.

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X			

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from dogs at pretest and at day 28 for all groups and also at day 49 for the high dose group. The CHECKED (X) parameters were examined. It was not stated that the animals were fasted. Methods of urine collection was not mentioned in methods.

Table 4. Urine parameters examined.

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

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals survived the duration of the study. Control, low, and medium dose groups were sacrificed on schedule (i.e., day 29) and were subjected to gross pathological examination. The high dose group was sacrificed at the day 50 following dose increase to 500 mg/kg/day (see above). The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

Table 5. Organs examined.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*Periph.
X	Salivary glands*	XX	Heart*		nerve*
X	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)^{T}
X	Stomach*	X	Lymph nodes* (cervical,	X	Pituitary*
	Duodenum*		mesenteric, popliteal)	X	Eyes (optic n.)^{T}
	Jejunum*	XX	Spleen*		
	Ileum*	XX	Thymus*		GLANDULAR
	Cecum*			XX	Adrenal gland*
	Colon*		UROGENITAL	X	Lacrimal gland^{T}
	Rectum*	XX	Kidneys*+	X	Mammary gland^{T}
XX	Liver*+	X	Urinary bladder*	XX	Parathyroids*++
X	Gall bladder*	XX	Testes*+	XX	Thyroids*++
X	Pancreas*	X	Epididymides		
		X	Prostate		OTHER
	RESPIRATORY		Seminal vesicle	X	Bone (sternum, rib)
X	Trachea*	XX	Ovaries	X	Skeletal muscle
X	Lung*	X	Uterus*	X	Skin
	Nose			X	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

Only the following organs were microscopically examined: brains, heart, liver, kidneys, testes, ovaries, spleen, thymus adrenal gland, thryoid, parathyroid gland, and any tissues with gross lesions.

II. RESULTS

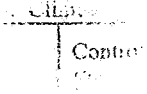
A. Toxicity and Mortality :

1. Toxicity -"Moderate" diarrhea and vomiting were observed in the high dose group. Because of significant vomiting episodes, degree of absorption is unknown in the high dose group.

Table 6. Clinical signs observed. Number of incidences reported.

	Control (0 mg/kg/day)	Low (20 mg/kg/day)	Medium (50 mg/kg/day)	High (150/500 mg/kg/day)
Males				
Diarrhea	0	0	0	10 at 500 (day 29-49)
Vomiting	0	1	0	6 at 150 (day 0-28) 8 at 500 (day 29-49)
Females				
Diarrhea	0	0	0	5 at 500 (day 29-49)
Vomiting	1	0	0	22 at 150 (day 0-28) 21 at 500 (day 29-49)

2. Mortality - None of the animals died or had to be sacrificed over the duration of the study.



B. Body weight and weight gain: As shown in table 7: Males in control, low, and medium dose groups gained 0.2 to 0.3 kg over the course of the study. Males in the high dose group lost -0.55 kg. As shown below in Table 8, food consumption of males in the high dose group was lower than other groups. Female dogs in all groups gained approximately 0.2 kg. No changes in body weight or food consumption were statistically significant.

Table 7. Body weight changes in males upon exposure to trifloxystrobin.

Dose Level	Initial body weight	Day 28	Day 49	Body Weight Gain
Control (0 mg/kg/day)	11.20	11.50	N/A	0.30
Low (20 mg/kg/day)	11.65	11.85	N/A	0.35
Medium (50 mg/kg/day)	9.65	9.85	N/A	0.20
High (150/500 mg/kg/day)	9.65	9.35	9.10	-0.20 for 28 days -0.55 for 49 days

Table 8. Food consumption in males.

Dose	Control (0 mg/kg/day)	Low (20 mg/kg/day)	Medium (50 mg/kg/day)	High (150/500 mg/kg/day)
Pretest	350.0	350.0	342.1	350.0
Day 7	350.0	350.0	332.9	268.6
Day 14	350.0	350.0	313.6	259.3
Day 21	350.0	350.0	300.7	260.0
Day 28	350.0	350.0	330.0	288.6
Day 35				253.6
Day 42				281.4
Day 49				320.7

D. Ophthalmoscopic examination - Examination of the conjunctiva, sclera, cornea, lens and fundus did not show any adverse effects caused by the pesticide treatment. All animals showed conjunctivitis follicularis at pretest and at week 4. The intensity of the lesions did not worsen with pesticide treatment. Additional eye exams were not performed on the high dose group at week 7.

E. Blood work:

1. Hematology - Hematological profiles of treatment groups were similar to those of control groups.

2. Clinical Chemistry - There were no treatment related differences in blood chemistry profiles of males and females. Profiles in treatment groups were similar to the control group.

F. Urinalysis - There were no treatment related differences in urinalysis profiles of males and females. Profiles in treatment groups were similar to the control group.

G. Sacrifice and Pathology:

1. Organ weight -In both males and females, liver weights increased significantly in the 150/500 mg/kg/day group. Kidney and spleen weights were increased in males and females, respectively.

Table 10. Organ weights and organ to body weight ratios for males.

Organ Weight Organ to Body Weight Ratio	Control (0 mg/kg/day)	Low (20 mg/kg/day)	Medium (50 mg/kg/day)	High (150/500 mg/kg/day)
Males				
Liver	323.3 29.76	321.2 29.35	317.7 34.05	344.4 38.52*
Kidney	53.80 4.89	50.62 4.53	46.38 5.00	49.89 5.58*
Females				
Liver	261.1 29.19	280.1 33.38	247.1 33.37	345.5 39.11*
Spleen	24.31 2.71	27.87 3.37	25.09 3.38	41.19 4.70*

*Statistical significance using the Jonckheere test ($p < 0.05$).

2. Gross pathology -There were no macroscopical findings resulting from exposure to trifloxystrobin. Gross pathology was typical for dogs.

3. Microscopic pathology -

a) Non-neoplastic - Moderate congestion of the splenic red pulp was seen in 3/4 animals in the high dose group. No other organs showed significant microscopic treatment related effects.

b) Neoplastic - There were no neoplastic lesions found that could be attributed to pesticide treatment.

III. DISCUSSION

A. Discussion: This study is guideline in design but unacceptable for 870.3151. It follows the OECD guideline Nr. 409 as adapted to a range finding toxicity study. The purpose of this study was to determine the appropriate concentrations to be used in a guideline subchronic study. There are obvious deficiencies particularly the number of animals used and time of exposure. This study could be used qualitatively for comparison of toxic effects. The high dose of trifloxystrobin resulted in significant vomiting incidences. Actual concentration absorbed is likely to be reduced due to vomiting.

The LOAEL is 150 mg/kg/day. The NOAEL is 50 mg/kg/day.

B. CalEPA Review:
California EPA also reviewed this study. Although the review by CalEPA was incomplete, the conclusions of this review agree with those of CalEPA.

SignOff Date:	8/3/99
DP Barcode:	D243979
HED DOC Number:	013599
Toxicology Branch:	TOX2