6-10-98

DATA EVALUATION RECORD

DIFLUFENZOPYR (SAN 835 H TECHNICAL)

Study Type: 82-1b; 13-Week Feeding Study in Dogs

Work Assignment No. 3-50B (MRID 44194105)

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Diflufenzopyr (SAN 835 H technical) 3-Month Subchronic (§82-1b)

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DATA EVALUATION RECORD

STUDY TYPE: 13-Week subchronic toxicity [feeding]- dog
OPPTS Number: 870.3150 OPP Guideline Number: §82-1b

DP BARCODE: D238413 SUBMISSION CODE: S527347
P.C. CODE: 005107 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): SAN 835 H technical (98% a.i.)

SYNONYMS: Diflufenzopyr; 2-[1-[[[(3,5-Difluorophenyl)amino]-carbonyl]hydrazono]ethyl]-3-pyridinecarboxylic acid (CA)

CITATION: Carpy, S.A. (1996) SAN 835 H technical. 13-Week feeding study in dogs. Sandoz Agro Ltd., Department of Toxicology, B.881, CH-4132 Muttenz 1/Switzerland. Laboratory Study Number 554D. October 8, 1996. MRID 44194105. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., Department of Toxicology, B.881, CH-4002 Basel/Switzerland.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44194105), diflufenzopyr (98% a.i.) was administered to beagle dogs (4/sex/dose) by feeding at dose levels of 0, 1500, 10000, or 30000 ppm (0, 58, 403, or 1131 mg/kg/day for males; 0, 59, 424, or 1172 mg/kg/day for females) for 13 weeks.

Diflufenzopyr affected the bone marrow and liver of dogs in the 30000 and 10000 ppm treatment groups. All dogs in the 30000 ppm treatment group exhibited erythroid hyperplasia in the bone marrow and most (4/4 males and 3/4 females) had an absence of fatty bone marrow. Two males and one female were anemic, with associated reticulocytosis, anisocytosis, polychromasia. The

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ratio of myeloid/erythroid cells in the bone marrow was depressed in 2/4 males and 1/4 females. In addition, all dogs had extramedullary hemopoiesis of the liver. Hemosiderin deposits in Kupffer cells and/or macrophages were observed in 1/4 males and 4/4 females. One female also exhibited centrilobular atrophy, degeneration, and fibrosis of the liver. Final mean body weight gains of males and females were 14 and 83% lower, respectively, than the controls, and mean food consumption was reduced 13 and 16% in males and females, respectively throughout the study. Dogs in the 10000 ppm treatment group exhibited erythroid hyperplasia in the bone marrow (2/4 males, 1/4 females), extramedullary hemopoiesis in the liver (2/4 males, 1/4 females), and hemosiderin deposits in Kupffer cells (1/4 females). Dogs in the 1500 ppm treatment group exhibited no treatment-related responses. No dogs died during the study. There was no observed effect on ophthalmology, clinical blood chemistry or urine. The LOAEL for this study is 10000 ppm (403 mg/kg/day in males and 424 mg/kg/day in females), based on the occurrence of erythroid hyperplasia in the bone marrow, extramedullary hemopoiesis in the liver, and hemosiderin deposits in Kupffer cells. The NOAEL is 1500 ppm (58 mg/kg/day in males and 59 mg/kg/day in females).

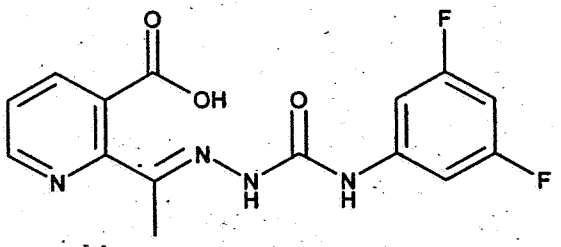
This 90-day subchronic toxicity study is classified acceptable and satisfies the Subdivision F guideline requirement for a subchronic toxicity study in nonrodents (§82-1b). Because the toxic response observed in dogs in the 10000 ppm treatment group does not appear to be life-threatening, it is recommended that a maximum treatment rate of at least 10000 ppm be used in a chronic feeding study.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: SAN 835 H technical
   Description: White powder
   Lot/Batch #: 5904-4
   Purity: 98 ± 0.4% a.i.
   Stability: Expiration date reported to be May 1998
   CAS #: 109 293-97-1
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Dog
   Strain: Beagle
   Age and weight: Approximately 6 months of age; body weight at Week 0, males - 7.13-8.81 kg; females - 6.25-7.90 kg
   Source: BRL Breeding Laboratories, CH-4414 Fullinsdorf, Switzerland
   Housing: Housed in pairs in 2-4 m^2 suspended steel mesh cages. Dogs were exercised for 1 hour each day.
   Diet: KLIBA Powdered Diet No. 24-335-1 (Klingentalmuhle AG Basel, Switzerland, 350-400 g offered each day for a 2-3 hour period
   Water: Municipal tap water, ad libitum
   Environmental conditions:
   Temperature: 21-24°C
   Humidity: 46-80%
   Air Changes: 10-15 per hour
   Photoperiod: 12-Hour light/dark cycle

Acclimation period: 4 Weeks

B. STUDY DESIGN

1. In life dates - Start: 10/27/94 End: 2/3/95

2. Animal assignment

Upon receipt, dogs were allocated to the test groups in Table 1 in chronological order of unpacking. Several days after receipt, the dogs were weighed and, if necessary, exchanged between groups to achieve bodyweight homogeneity between groups. At the start of the study, the weight of each animal was within 15% of the mean value for each sex.

Table 1. Study design.[a]

Test Group	Dose to animal (ppm)	Animals assigned	
		Male	Female
K Control	0	4	4
A Low	1500	4	4
B Mid	10000	4	4
C High	30000	4	4

[a] Dose levels were based upon the results of preliminary studies in which marked toxicity was observed at 30000 ppm. No additional information about the preliminary studies was provided.

3. Treatment preparation

The test diets were prepared fresh each week and stored at room temperature during use. A 3% premix was prepared by adding diflufenzopyr to a portion of the powdered diet and mixing for 1 hour in a rotary mixer. The premix was added to sufficient untreated diet to yield the desired concentrations of diflufenzopyr and mixed for at least

20 minutes using a rotary mixer. Subsamples of the 0-, 2-, 6-, and 12-week feed preparations were collected from the top, middle, and bottom of the mixing container for homogeneity (0 and 2 weeks) and concentration (0, 2, 6, and 12 weeks) analyses.

In addition, the stability of diflufenzopyr in the test diet at room temperature for 15 days was determined in a preliminary study. In the preliminary study, diet was treated with diflufenzopyr at 1000, 5000, or 30000 ppm and mixed as described.

Results:

Homogeneity (Week 0, Week 2):
- 1500 ppm: 90.2-96.7%, 94.2-99.1% of nominal
- 10000 ppm: 85.7-91.4%, 97.8-99.6% of nominal
- 30000 ppm: 76.6-89.7%, 96.7-98.6% of nominal

Concentration analysis:
- 1500 ppm: 92.5-100.9% of nominal
- 10000 ppm: 87.9-101.0% of nominal
- 30000 ppm: 81.4-100.9% of nominal

Stability analysis (stored dry at room temperature):
- 1000 ppm:
  - 0 days: 97.3% of nominal
  - 8 days: 89.8% of nominal
  - 15 days: 84.5% of nominal
- 5000 ppm:
  - 0 days: 98.2% of nominal
  - 8 days: 99.4% of nominal
  - 15 days: 94.7% of nominal
- 30000 ppm:
  - 0 days: 96.5% of nominal
  - 8 days: 98.3% of nominal
  - 15 days: 94.6% of nominal

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

4. Statistics

Analyses of parametric data were performed using standard one-way ANOVA followed by Dunnett's test for equal variances. Analyses of nonparametric data were performed using the Kruskal-Wallis test followed by Mann-Whitney. Count data were analyzed using Chi-square followed by Fisher's exact test.

C. METHODS

1. Observations

Animals were observed twice daily on weekdays and once daily on weekends for mortality and gross ill-health. Each week all animals were given a detailed examination that included palpation.

2. Body weight

Body weights were measured shortly after receipt and weekly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was measured weekly, beginning 4 weeks prior to the initiation of treatment. Food consumption was reported as g food/animal/day. Test article intake was calculated:

[(g food/kg body weight/day) x measured concentration in the feed]

and reported as mg test substance/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were performed prior to treatment (Week -1) and during Week 12 following induction of mydriasis.

5. Blood

Blood was collected from all animals 1 week prior to the initiation of treatment and during Weeks 6 and 12. Animals were fasted for an unspecified period prior to the collection of blood from the vena cephalica without anaesthesia. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential
X	Hemoglobin (HGB)*	X	count*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpusc. HGB
X	Platelet count*	X	conc. (MCHC)
	(thrombocytes)	X	Mean corpusc. volume (MCV)
	Blood clotting	X	Reticulocyte count
X	measurements*	X	Myeloblasts
X	(Partial thromboplastin		Myelocytes
	time)		Metamyelocytes
	(Whole blood clotting		
	time)		
	(Prothrombin time)		

* Required for subchronic toxicity studies.

b. Clinical Chemistry

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

* Required for subchronic toxicity studies.

6. Urinalysis

Urine was collected from all animals 1 week prior to the initiation of treatment and during Weeks 6 and 12. Animals were fasted for an unspecified period prior to the collection of urine by catheterization. The CHECKED (X) parameters were examined in all samples analyzed.

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

* Urinalysis is not required for subchronic toxicity studies.

7. Sacrifice and Pathology

Animals were killed by an overdose injection of Vetanarcol (150 mg/kg) followed by exsanguination. The bodies were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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

* Required for subchronic toxicity studies.

II. RESULTS

A. Observations

1. Mortality - No animals died prematurely.

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2. Clinical Signs - Dogs in the 30000 ppm treatment group exhibited dry skin at the termination of the study. Beginning at week 12, one female in the 30000 ppm treatment group exhibited fur loss and a second female in this group exhibited erythema. No other differences that could be attributed to treatment were observed in the clinical appearance of dogs in the treatment and control groups.

B. Body weight and weight gain

Mean body weight gains of males in the 30000 ppm treatment group were 24-37% lower than other treatment groups and 14% lower than the controls at Week 13 (Table 2). Individual weight gains ranged from 0.76 to 1.12 kg. Mean body weight gains of males in the 10000 and 1500 ppm treatment groups were higher than the controls. The magnitude was due in part to the weight losses of two control males through Week 11, at which time their food rations were increased.

Females in the 30000 ppm treatment group had body weight gains 83% lower than the controls at Week 13. During the study, one female lost 0.77 kg (#293) of body weight, two gained 0.4 kg, and one gained 0.91 kg. Although females in the 1500 and 10000 ppm treatment groups had body weight gains lower, than the controls, the difference was not dose dependent and therefore not considered treatment related.

Table 2. Mean body weights and body weight gains (kg) of dogs before and during treatment with diflufenzopyr.[a]

Treatment rate (ppm)	Mean Body weight (g)			13-Week body weight gain	
	0 Weeks	4 Weeks	13 Weeks	Total (g)	control gain (%)
Males					
0	7.855	8.010	9.000	1.145	---
1500	8.163	8.463	9.448	1.285	+12
10000	7.903	8.472	9.455	1.552	+36
30000	7.875	7.875	8.858	0.983	-14
Females					
0	7.150	7.440	8.520	1.370	---
1500	7.227	7.525	8.370	1.143	-17
10000	7.098	7.455	8.365	1.267	-8
30000	7.068	7.063	7.300	0.232	-83

[a] Body weights obtained from Table 3, pages 34-37, in the study report.
* Significantly different, $p<0.05$.

C. Food consumption and compound intake

1. Food consumption - Mean food consumption (g/animal/day) was reduced 13 and 16% in males and females, respectively in the 30000 ppm treatment groups compared to the controls. During the 13 weeks of treatment, males in the 30000 ppm treatment group consumed an average 309 g/animal/day, compared to 342-356 g/animal/day for other treatment groups and the control. Females in the 30000 ppm treatment group consumed an average 279 g/animal/day, compared to 304-332 g/animal/day for other treatment groups and the control.

2. Compound intake - Measured compound consumption by male dogs in the 1500, 10000, and 30000 ppm treatment groups averaged 58, 403, and 1131 mg/kg/day, respectively, over the 13-week treatment period. Measured compound

consumption by female dogs in the 1500, 10000, and 30000 ppm treatment groups averaged 59, 424, and 1172 mg/kg/day, respectively.

D. Ophthalmoscopic examination

No treatment-related abnormalities were noted in the appearance or function of the eyes.

E. Blood work

1. Hematology - Two males (#273, 274) and one female (#293) in the 30,000 ppm treatment group were anemic, as reflected in the decreased hematocrit, hemoglobin, and erythrocyte values compared to the controls (Table 3). The anemia was associated with reticulocytosis, anisocytosis, polychromasia, and an elevated number of normoblasts and echinocytes. Poikilocytosis and Howell-Jolly bodies were observed in the female at week 12. Higher MCV values, lower MCHC values, and unaffected MCH values for this group suggested large numbers of immature erythrocytes.

No treatment-related effects were observed in hematology parameters in dogs in the 10000 or 1500 ppm treatment groups. All other hematology parameters for dogs in the treated and control groups remained within the expected ranges, and observed differences did not appear to be either treatment-related or biologically significant.

Table 3. Selected hematology parameters in dogs following 12 weeks of treatment with diflufenzopyr.[a]

Treatment rate (ppm)	Mean Hematocrit (%)	Mean Hemoglobin (g/dL)	Mean RBC (MI/CMM)	Mean MCV (fL)	Mean MCHC (g/dL)
Males					
0	48.05	16.57	7.577	63.45	34.50
1500	46.25	16.18	7.413	62.43	34.98
10000	49.55	16.40	7.665	64.60	33.13*
30000	44.13	14.28	6.270	70.53*	32.30*
Females					
0	48.42	17.08	7.515	64.45	35.28
1500	47.72	16.52	7.550	63.23	34.63
10000	51.33	17.08	8.043	63.85	33.30*
30000	42.95	13.13*	6.032*	72.95	30.20*

[a] Data obtained from Table 6, pages 44-75, in the study report.
* Significantly different, $p<0.05$.

2. Clinical Chemistry - All clinical blood chemistry parameters for dogs in the treated and control groups remained within the expected ranges, and observed differences did not appear to be either treatment-related or biologically significant.

F. Urinalysis

Occult blood and red blood cells were detected in the urine of one female at 30,000 ppm. There were no other treatment-related differences observed between animals in the treatment and control groups.

G. Sacrifice and Pathology

1. Organ weight - In the female 30000 ppm treatment group, the relative liver, spleen, and kidney weights of one female (#293) and the relative liver and spleen weights of a second female (#288) were higher than the control means

and outside the range of normal biological variation. The increase in the relative liver weights was 34-55%, in the relative spleen weights was 72-119%, and in the relative kidney weight was 56% compared to the control means. No treatment-related differences in absolute or relative organ weights were observed in the male treatment groups or in the female 10000 and 1500 ppm treatment groups compared to the controls.

2. Gross pathology - Dogs in the 30000 ppm treatment group had an absence of fatty bone marrow (4/4 males, 3/4 females), nodules in the body cavities (2/4 males, 1/4 female), and enlarged, discolored and/or malformed livers (2/4 females). No other treatment-related gross postmortem differences were observed between dogs in the treated and the control groups.

3. Microscopic pathology

a) Non-neoplastic - All dogs in the 30000 ppm treatment group exhibited erythroid hyperplasia in the bone marrow and extramedullary hemopoiesis of the liver. Hemosiderin deposits in Kupffer cells and/or macrophages were observed in 1/4 males and 4/4 females. One female (#293) also exhibited extramedullary hemopoiesis in the kidneys, liver and lungs; and centrilobular atrophy, degeneration, and fibrosis of the liver which were attributed to severe anemia. Abnormalities attributed to stress included myeloid hyperplasia in the bone marrow (2/4 females); sinusoidal granulocytosis in the liver (2/4 males and 3/4 females); and thymic involution (4/4 females). Nonspecific skin lesions, urothelial hyperplasia, and cystitis of the urinary bladder were also noted in the 30,000 ppm dose group.

Dogs in the 10000 ppm treatment group exhibited erythroid hyperplasia in the bone marrow (2/4 males, 1/4 females), extramedullary hemopoiesis in the liver (2/4 males, 1/4 females), and hemosiderin deposits in Kupffer cells and macrophages (1/4 females).

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In bone marrow smears, it was determined that the ratio of myeloid/erythroid cells was lower in 2/4 males and 1/4 females (#293) in the 30000 ppm treatment groups and 1/4 males in the 10000 ppm treatment group. No effects were observed on the composition of the erythroid cells; however, the proportion of polymorphs in the myeloid cells was lower in 4/4 females in the 30000 ppm treatment group compared to the controls.

No other treatment-related microscopic postmortem differences were observed between dogs in the treated and the control groups. All other abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in dogs in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that treatment-related effects were observed in dogs in the 30000 and 10000 ppm treatment groups. At 30000 ppm, effects included anemia, an erythropoietic response in bone marrow, liver, and spleen, degenerative changes in the liver, and reduced body weights. At 10000 ppm, effects were limited to erythropoietic responses in the bone marrow and liver. The study author identified the LOAEL as 10000 ppm and the NOAEL as 1500 ppm.

B. Reviewer's Discussion

We agree with the study author that the LOAEL and NOAEL for this study are 10000 and 1500 ppm, respectively. Three dogs (2/4 males, 1/4 females) in the 10000 ppm treatment group exhibited erythroid hyperplasia in the bone marrow; however, unlike the 30000 ppm group, no abnormalities were apparent in hematological parameters. Extramedullary hemopoiesis in the liver was also observed in this group.

Dogs in the 1500 ppm treatment group exhibited no treatment-related responses.

At 30000 ppm, it was apparent that diflufenzopyr had a significant toxic effect on bone marrow. All dogs in the 30000 ppm treatment group exhibited erythroid hyperplasia in the bone marrow and, with the exception of one female, an absence of fatty bone marrow. Two males and one female exhibited anemia, with associated reticulocytosis, anisocytosis, polychromasia, and an elevated number of normoblasts and echinocytes. The ratio of myeloid/erythroid cells in the bone marrow was depressed in 2/4 males and 1/4 females. In addition, all dogs had extramedullary hemopoiesis of the liver. Hemosiderin deposits in Kupffer cells and/or macrophages were observed in 1/4 males and 4/4 females. One female exhibited centrilobular atrophy, degeneration, and fibrosis of the liver. Abnormalities attributed to stress associated with the anemia included myeloid hyperplasia in the bone marrow (2/4 females); sinusoidal granulocytosis in the liver (2/4 males and 3/4 females); and thymic involution (4/4 females). Dry skin and other nonspecific skin lesions, urothelial hyperplasia, and cystitis of the urinary bladder were also observed. Mean body weight gains of males in the 30000 ppm treatment group were 24-37% lower than other treatment groups and 14% lower than the controls at Week 13. Final mean body weight gains of females were 83% lower. Mean food consumption for both groups was reduced 13-16%.

Because the responses at 10000 ppm are not life-threatening and the responses at 30000 ppm were sufficiently severe to be life-threatening in a longer-term study, it is recommended that the chronic study be done at a maximum rate at or slightly higher than 10000 ppm.

IV. STUDY DEFICIENCIES

No scientific deficiencies were noted in this study.