6-10-98

DATA EVALUATION RECORD

DIFLUFENZOPYR (SAN 835 H TECHNICAL)

Study Type: 83-1b; 52-Week Feeding Study in Dogs

Work Assignment No. 3-50D (MRID 44307405)

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

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

Primary Reviewer:
Kathleen P. Ferguson, Ph.D.

Signature: Kathleen Ferguson
Date: 6/6/98

Secondary Reviewer
Joan L. Harlin, M.S.

Signature: Joan L Harlin
Date: 6/6/98

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/10/98

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date:

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Diflufenzopyr (SAN 835 H technical) 52-Week Chronic (§83-1b)

EPA Reviewer: Deborah Smegal, M.P.H.
Toxicology Branch II (7509C)

EPA Secondary Reviewer: Stephen Dapson, Ph.D.
Toxicology Branch II(7509C)

DATA EVALUATION RECORD

STUDY TYPE: 52-Week chronic toxicity [feeding]- dog
OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

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

TEST MATERIAL (PURITY): SAN 835 H technical (98.1% a.i.)

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

CITATION: Carpy, S.A. (1997) SAN 835 H technical. 52-Week feeding study in dogs. Novartis Crop Protection Inc., Department of Toxicology, B.881, CH-4132 Muttenz 1/Switzerland. Laboratory Study Number 555D. January 31, 1997. MRID 44307405. Unpublished.

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

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRID 44307405), diflufenzopyr (98.1% a.i.) was administered to beagle dogs (4/sex/dose) by feeding at dose levels of 0, 750, 7500, or 15000 ppm (0, 26, 299, or 529 mg/kg/day for males; 0, 28, 301, or 538 mg/kg/day for females) for 52 weeks.

All dogs in the 15000 ppm treatment group exhibited slight to marked erythroid hyperplasia in the femoral and sternal bone marrow. Several dogs (3/4 males, 1/4 females) exhibited an accompanying reddish discoloration of the diaphysis of the femur. Bone marrow smears were unremarkable. Hemosiderin deposits were observed in the kidneys and liver of several dogs. All dogs

exhibited mild to moderate reticulocytosis, but there were no signs of anemia and no changes in RBC morphology. Mean body weight gains of males were unaffected, but of females were 44% lower than the controls at Week 52. All dogs in the 7500 ppm treatment group exhibited slight to moderate erythroid hyperplasia in the bone marrow, and two males exhibited a reddish discoloration of the diaphysis of the femur. Moderate hemosiderin deposits were noted in the kidneys of one male. Most dogs (4/4 males, 2/4 females) exhibited mild to moderate reticulocytosis. Females had body weight gains 41% lower than the controls at Week 13 and 12% lower at Week 52. Dogs in the 750 ppm treatment group exhibited no treatment-related responses. The bone marrow from bone sections of all dogs of both sexes of all treated groups and controls were examined. No dogs died during the study. There was no observed effect on clinical signs, food consumption, ophthalmology, clinical blood chemistry, urine, or absolute or relative organ weights. The aspirated bone marrow smears [not bone sections] of dogs in the 7500 and 750 ppm treatment groups was not examined, since there were comparable results between controls and high dose dogs of both sexes. The LOAEL for this study is 7500 ppm (299 mg/kg/day for males and 301 mg/kg/day for females), based on erythroid hyperplasia in the bone marrow in bone sections, reticulocytis, and increased hemosiderin deposits in the liver, kidneys, and spleen. The NOAEL is 750 ppm (26 mg/kg/day for males and 28 mg/kg/day for females).

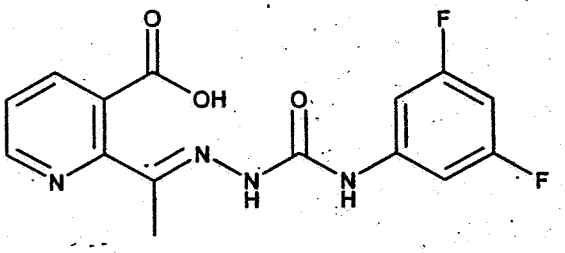
This 52-week chronic toxicity study is classified acceptable and satisfies the Subdivision F guideline requirement for a chronic toxicity study in non-rodents (§83-1b).

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: 98.1% 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 - 7.21-8.99 kg; females - 6.14-8.01 kg
   Source: BRL Breeding Laboratories, CH-4414 Fullinsdorf, Switzerland
   Housing: Housed in pairs in 2 in 2-4 m^2 suspended steel mesh cages. Dogs were exercised for 1 hour each day.
   Diet: KLIBA Powdered Diet No. 24-355-1 (Klingentalmuhle AG Basel, Switzerland), 400 g offered each day for a 2-4 hour period
   Water: Municipal tap water, ad libitum
   Environmental conditions:
   Temperature: 19-23°C
   Humidity: 40-80%
   Air Changes: 6-15 per hour

Photoperiod: 12-Hour light/dark cycle
Acclimation period: 4 Weeks

B. STUDY DESIGN

1. In life dates - Start: 7/20/95 End: 7/26/96

2. Animal assignment

Upon receipt, dogs were allocated to the test groups in Table 1 in chronological order of unpacking. One week prior to the start of the study, the dogs were weighed and, if necessary, exchanged between groups to achieve bodyweight homogeneity between groups. At the start of the study, the weights of each animal were 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	750	4	4
B Mid	7500	4	4
C High	15000	4	4

[a] Dose levels were based upon the results of the subchronic toxicity study (MRID 44194105) in which diflufenzopyr was administered to beagle dogs (4/sex/dose) by feeding at dose levels of 0, 1500, 10000, or 30000 ppm for 13 weeks. At 30000 ppm (1131 mg/kg/day), effects included anemia, an erythropoietic response in bone marrow, liver, and spleen, and reduced body weights. At 10000 ppm (403 mg/kg/day), effects were limited to erythropoietic responses in the bone marrow and liver. The NOEL was 1500 ppm (58 mg/kg/day).

3. Treatment preparation

The test diets were prepared fresh each week and stored at room temperature during use. A premix was prepared by adding diflufenzopyr to a portion of the powered 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-week feed preparations were collected from the top, middle, and bottom of the mixing container for homogeneity and stability analysis. Subsamples of the feed preparations were collected at 0 and 30 days, and 12, 24, 39, and 52 weeks for concentration analyses.

Results:

Homogeneity:
- 750 ppm: 98.7 ± 1.4% of nominal
- 7500 ppm: 104.8 ± 1.0% of nominal
- 15000 ppm: 100.2 ± 2.8% of nominal

Stability analysis (stored dry at room temperature):
- 750 ppm:
  - 0 days: 92.1% of nominal
  - 8 days: 85.6% of nominal
  - 15 days: 81.7% of nominal
  - 30 days: 74.7% of nominal
- 7500 ppm:
  - 0 days: 97.8% of nominal
  - 8 days: 95.1% of nominal
  - 15 days: 97.1% of nominal
  - 30 days: 96.0% of nominal
- 15000 ppm:
  - 0 days: 100.2% of nominal
  - 8 days: 98.9% of nominal
  - 15 days: 95.2% of nominal
  - 30 days: 96.5% of nominal

Concentration analysis:
- 750 ppm: 93 ± 4% of nominal
- 7500 ppm: 99 ± 3% of nominal
- 15000 ppm: 100 ± 3% 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-U. 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 and holidays 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 51 following induction of mydriasis. The examinations were performed using an indirect ophthalmoscope and a slit-lamp.

5. Blood

Blood was collected from all animals 1 week prior to the initiation of treatment and during Weeks 13, 26, and 51. Animals were fasted for approximately 20 hours 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		Reticulocyte count
X	measurements*		RBC morphology
X	(Partial thromboplastin time)		
	(Whole blood clotting time)		
	(Prothrombin time)		

* Required for chronic 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 chronic toxicity studies.

6. Urinalysis

Urine was collected from all animals 1 week prior to the initiation of treatment and during Weeks 13, 26, and 51. Animals were fasted for approximately 20 hours 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

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	(femur,	XX	Pituitary*
X	Stomach*	XX	sternum)	X	Eyes (plus optic
X	Duodenum*	X	Lymph nodes*		nerve)*
X	Jejunum*		Spleen*		
X	Ileum*		Thymus*		
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
X	Liver*	XX	Kidneys*	XX	Adrenal gland*
X	Pancreas*	X	Urinary		Lacrimal gland
	Gall bladder	XX	bladder*	X	Mammary gland
		X	Testes*	XX	Thyroids*
		X	Epididymides	XX	Parathyroids*
			Prostate		
		XX	Seminal vesicle		
	RESPIRATORY	X	Ovaries*		
		X	Uterus* plus		
X	Trachea*		cervix		
X	Lungs*		Vagina		
	Muzzle				
	Pharynx				
	Larynx				OTHER
X	Diaphragm				
				X	Bone*
					(femur, sternum)
				X	Muscle*
					(skeletal)
				X	Skin*
				X	All gross lesions
					and masses*
				X	Tonsils

* Required for chronic toxicity studies.

II. RESULTS

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A. Observations

1. Mortality - No animals died prematurely.

2. Clinical Signs - No differences that could be attributed to treatment were observed in the clinical appearance of dogs in the treatment and control groups. Skin erythema was observed in male and female dogs in the control group and male and female dogs in all treatment groups during the treatment period; the frequency of the condition was greater in the high dose males from weeks 31 to 52.

B. Body weight and weight gain

Mean body weight gains of males in the treatment and control groups during the 0-13 and 0-52 week periods were similar (Table 2). One male dog in the 7500 ppm treatment group lost weight between Weeks 18 and 52, with on apparent change in food consumption. The reason for this weight loss is unclear.

Females in the 15000 ppm treatment group had body weight gains 47% lower than the controls by Week 13; body weight gains were 44% lower at Week 52. Females in the 7500 ppm treatment group had body weight gains 41% lower than the controls by Week 13 and 12% lower by Week 52. Females in the 750 ppm treatment group had body weight gains similar to the controls throughout the study.

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 (kg)				Mean 52-Week body weight gain	
	0 Weeks	13 Weeks	26 Weeks	52 Weeks	Total (kg)	control gain (%)
Males						
0	7.98	9.66	10.12	10.57	2.59	---
750	8.18	10.23	10.78	11.74	3.56	+37
7500	8.08	9.92	10.11	10.52	2.44	-6
15000	8.18	10.03	10.20	10.53	2.35	-9
Females						
0	6.96	8.24	8.24	8.72	1.76	---
750	7.24	8.40	8.51	9.15	1.91	+8
7500	7.01	7.77	8.13	8.56	1.55	-12
15000	7.05	7.73	7.79	8.04	0.99	-44

[a] Body weights obtained from pages 45-56 in the study report.

C. Food consumption and compound intake

1. Food consumption - Overall mean food consumption (g/animal/day) was not significantly affected by treatment. However, statistically significant increases in mean consumption were occasionally noted in 750 and 7,500 ppm males. During the 52 weeks of the study, males in the control group consumed an average 331 g/animal/day, compared to 363, 387, and 348 mg/kg/day for the 750, 7500, and 15000 ppm treatment groups, respectively. Females in the control group consumed an average 292 g/animal/day; females in the 750, 7500, and 15000 ppm treatment groups consumed an average 310, 318, and 276 g/animal/day, respectively.

2. Compound intake - Measured mean compound consumption by male dogs in the 750, 7500, and 15000 ppm treatment groups averaged 26, 299, and 529 mg/kg/day, respectively, over the 52-week treatment period. Measured compound consumption by female dogs in the 750, 7500, and 15000 ppm treatment groups averaged 28, 301, and 538 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 - All dogs in the 15000 ppm treatment group (4/4 males, 4/4 females) and most dogs in the 7500 ppm treatment group (4/4 males, 2/4 females) exhibited mild to moderate reticulocytosis. Animals in the 15000 ppm treatment groups also had slightly higher MCV (statistically significant at week 13) and lower MCHC (Statistically significant at weeks 13 and 51) values compared to the controls. MCH values were not affected, which suggested the presence of large numbers of immature erythrocytes. There were no associated signs of anemia, and no changes in RBC morphology.

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.

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

No treatment-related differences were observed between animals in the treatment and control groups.

G. Sacrifice and Pathology

1. Organ weight - No significant differences in absolute or relative organ weights were observed between the treatment and control groups.

2. Gross pathology - Several dogs (3/4 males, 1/4 females) in the 15000 ppm treatment group and 2/4 males in the 7500 ppm treatment group exhibited reddish discoloration of the diaphysis of the femur. Since the same dogs exhibited microscopic erythroid hyperplasia of the femoral marrow, the discoloration was considered treatment related. 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 15000 and 7500 ppm treatment group exhibited erythroid hyperplasia in the femoral and sternal bone marrow. The severity of the hyperplasia was moderate in males in the 15000 ppm treatment group, slight to marked in females in the 15000 ppm treatment group, and slight to moderate in dogs in the 7500 ppm treatment group.

Hemosiderin deposits were observed in the kidneys of 2/4 females in the 15000 ppm treatment group and 1/4 males in

the 7500 ppm treatment group, and in the livers of 2/4 males and 3/4 females in the 15000 ppm treatment group. Hemosiderin deposits were observed in the spleens of dogs in all study groups (including controls); the severity of the deposits in males increased with increasing treatment rate. In males, hemosiderin deposits were described as moderate in the 7500 ppm treatment group and moderate to marked in the 15000 ppm treatment group.

Bone marrow smears of dogs in the 15000 ppm treatment groups and controls were similar. The bone marrow of dogs in the 7500 and 750 ppm treatment groups was not examined.

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 15000 and 7500 ppm treatment groups. Effects included reticulocytosis, erythroid hyperplasia in bone marrow, and increased hemosiderin deposits in the liver, kidneys, and spleen. The study author identified the LOAEL as 7500 ppm and the NOAEL as 750 ppm.

B. Reviewer's Discussion

We agree with the study author that the LOAEL and NOAEL for this study are 7500 and 750 ppm, respectively. All dogs in the 7500 ppm treatment group exhibited erythroid hyperplasia of slight to moderate severity in the femoral and sternal bone marrow. Most dogs (4/4 males, 2/4 females) exhibited mild to moderate reticulocytosis.

Females had body weight gains 41% lower than the controls at Week 13 and 12% lower at Week 52.

Dogs in the 750 ppm treatment group exhibited no treatment-related responses.

At 15000 ppm, it was apparent that diflufenzopyr had a significant toxic effect on bone marrow. All dogs in this treatment group had bone marrow characterized by erythroid hyperplasia and mild to moderate reticulocytosis. Increased incidence of hemosiderin deposits was noted in the kidneys of 2/4 females and in the livers of 2/4 males and 3/4 females. Females had body weight gains 44% lower than the controls at Week 52.

IV. STUDY DEFICIENCIES

No scientific deficiencies were noted in this study.

DER #4

Chemical Name: 2-Generation Reproduction Study in Rats
BASF. 1996. MRID No. 44170148
HED Doc. No. None