6-22-98

DATA EVALUATION RECORD

DIFLUFENZOPYR (SAN 835 H)

Study Type: 82-7: Subchronic Neurotoxicity Study in Rats
Work Assignment No. 3-50C (MRID 44329602)

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:
Joan L. Harlin, M.S.

Signature: Joan L Harlin
Date: 6/22/98

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

Signature: Kathleen Ferguson
Date: 6/22/98

Program Manager:
Mary L. Menetrez, Ph.D.

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

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date:

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

Diflufenzopyr (SAN 835 H Technical$ubchronic Neurotoxicity (82-7)

EPA Reviewer: Roger Hawks, Ph.D.
Toxicology Branch II (7509C)

Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T.
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 13-Week subchronic neurotoxicity [feeding] - rats
OPPTS Number: 870.6200 OPP Guideline Number: §87-2

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

TEST MATERIAL (PURITY): Diflufenzopyr (SAN 835 H Technical, 96.4% a.i.)

SYNONYMS: 2-(Methyl(((3,5-difluorophenylamino)-carbonyl)hydrazono)methyl)-3-pyridine carboxylic acid; SAN 835 H

CITATION: Hughes, E.W. and D.P. Meyers (1996) San 835 H Neurotoxicity to rats by dietary administration for 13 weeks. Huntington Life Sciences Ltd., P.O. Box 2, Huntington, Cambridgeshire, PE18 6ES, England. Project Number SNC/187/961253. BASF Reg. Doc. No. 97/5095. November 5, 1996. MRID 44329602. Unpublished.

SPONSOR: Sandoz Agro, Inc., 1300 East Touhy Avenue, Des Plaines, IL 60018.

EXECUTIVE SUMMARY:

In a subchronic neurotoxicity study (MRID 44329602), diflufenzopyr (SAN 835 H technical; 96.4% a.i.) was administered in the diet to Crl:CD BR rats (10/sex/group) at dose levels of 0, 25, 75 or 1000 mg/kg/day for 13 weeks. The rats were evaluated for reactions in functional observations and motor activity testing at 4 hours and during weeks 4, 8 and 13 of treatment.

No treatment-related neurotoxicological effects were observed at any treatment level. Treatment-related toxic effects were

observed at the 1000 mg/kg/day treatment level. Weekly mean body weights were lower in males (8-16%) and females (2-8%) compared to the controls. Mean body weight gains were reduced (20-26%) for both sexes. Weekly food consumption by males was up to 15% lower than the controls. In general, food consumption by females was similar to the controls. All female treatment groups exhibited a concentration-dependent increase in vocalization upon removal from the cage, and the 1000 mg/kg/day group females had lower mean activity and rearing counts (27-31%) than the controls at Week 4 but not at Weeks 8 and 13. There were no treatment-related deaths during the study or treatment-related differences in the general appearance, absolute or relative brain weights, or gross or microscopic histology of the rats. A LOAEL for neurotoxicological effects was not established; the NOEL was 1000 mg/kg/day for both sexes. The toxicological LOAEL for this study is 1000 mg/kg/day, based on decreased body weight gains for both sexes. The toxicological NOAEL is 75 mg/kg/day.

This study is classified Acceptable-Guideline and satisfies the guideline requirement for a subchronic neurotoxicity study in rodents (§82-7).

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

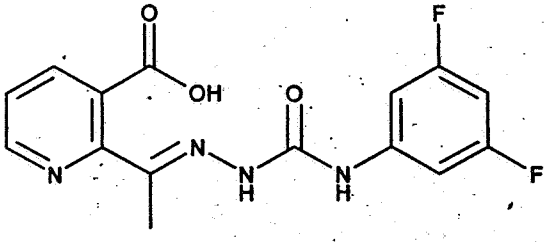
3

Diflufenzopyr (SAN 835 H Technical)Subchronic Neurotoxicity (82-7)

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Diflufenzopyr (SAN 835 H Technical)
   Description: Beige powder
   Lot/Batch #: 6500-19
   Purity: 96.4% a.i.
   Stability of compound: Reported to expire March 1997
   CAS #: 109293-97-2
   Structure:



2. Vehicle: None

3. Test animals: Species: Rat
   Strain: Crl:CD BR
   Age at study initiation: Approximately 48 days old
   Weight at study initiation: Males, 220-260 g; females, 164-201 g
   Source: Charles River Breeding Laboratories, Margate, Kent, England
   Housing: Individually housed in suspended cages with wire mesh floors
   Diet: Pelleted SDS Rat and Mouse No. 1 modified ground maintenance diet, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 21 ± 2 C
   Humidity: 56 ± 16%
   Air changes: Not reported

1984

Photoperiod: 12-Hour light/dark cycle
Acclimation period: 13 Days

B. STUDY DESIGN

1. In-life dates - 11/14/95 to 2/29/96

2. Animal assignment

Animals were selected based on health and body weights and were randomly assigned to the test groups shown in Table 1. The groups were stratified by bodyweight so that initial group means were approximately equal.

TABLE 1: Study design.[a]

Test Group	Dose (mg/kg)	Males	Females
Control	0	10	10
Low (LDT)	25	10	10
Mid (MDT)	75	10	10
High (HDT)	1000	10	10

[a] The rationale for dose selection was not provided.

3. Treatment preparation

The test diets were prepared fresh weekly. Diflufenzopyr was manually mixed with sieved diet until approximately half of the diet for the pre-mix had been added. The mixture was ground using a coffee grinder and made up to the pre-mix weight using coarse diet from the sieve. The pre-mix was mixed with a mixer for at least 5 minutes. To prepare the test diets, the pre-mix was diluted with plain diet, and mixed with a mixer for at least 5 minutes. Concentrations in the diet were changed as necessary to preserve the required dosage levels for each group and sex.

Homogeneity and stability of treated diet were determined prior to study initiation. Duplicate homogeneity samples of freshly prepared treated diet were randomly collected from the top, middle, and bottom within the mixer drum. Stability samples were stored for 4, 8 or 15 days at room temperature under conditions comparable to those in the actual feeding study. To confirm the concentration of diflufenzopyr in the test diet, duplicate samples of diet freshly prepared during weeks 1 and 13 were analyzed.

Results:

Homogeneity:

100 mg/kg/day: 103.0-108.0% of nominal (mean 105.0%)
400 mg/kg/day: 95.2-100% of nominal (mean 97.5%)
10000 mg/kg/day: 91.0-98.8% of nominal (mean 95.0%)
25000 mg/kg/day: 100.4-102.8% of nominal (mean 101.6%)

Concentration:

Week 1:

245 mg/kg/day: 94.7-109% of nominal (mean 101.8%)
735 mg/kg/day: 99.9-104.9% of nominal (mean 102.4%)
760 mg/kg/day: 98.7-102.6% of nominal (mean 100.6%)
9665 mg/kg/day: 103.5-104.5% of nominal (mean 104%)
10050 mg/kg/day: 102.5-103.5% of nominal (mean 103.0%)

Week 13:

350 mg/kg/day: 98.0-98.6% of nominal (mean 98.3%)
440 mg/kg/day: 98.6-98.9% of nominal (mean 98.8%)
1080 mg/kg/day: 94.4-95.4% of nominal (mean 94.9%)
1330 mg/kg/day: 91.7% of nominal/mean
13495 mg/kg/day: 93.4-94.1% of nominal (mean 93.8%)
16730 mg/kg/day: 91.4% of nominal/mean

Stability (mean of duplicate samples):

100 mg/kg/day:
0 days: 105% of nominal
8 days: 100% of nominal
15 days: 101% of nominal

400 mg/kg/day:
0 days: 97.5% of nominal
4 days: 96.8% of nominal
8 days: 95.2% of nominal
15 days: 91% of nominal

10000 mg/kg/day:
0 days: 95.0% of nominal

6

200

4 days: 93.6% of nominal
8 days: 91.1% of nominal
15 days: 93.2% of nominal
25000 mg/kg/day:
0 days: 101.6% of nominal
8 days: 104.8% of nominal
15 days: 103.2% of nominal

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

4. Statistics

Bodyweight, bodyweight gain, and food consumption recorded during the functional observation battery (FOB), grip strength, hindlimb splay, activity and rearing counts, rectal temperature, Colbourn activity measures and brain measurements were analyzed using a one-way analysis of variance. With the exception of pre-dose data, analyses of variance were followed by the Student's t-test and William's t-test for a dose-related response. Kruskal-Wallis analyses were followed by the nonparametric equivalents of the t-test and Shirley's test. For predose data, analyses of variance were followed by the Student's t test. When a difference between the control and treated groups was indicated, the data were analyzed using the Linear by Linear Association test. A one-tailed test was applied for abnormal gait, palpebral closure and tremors. A two-tailed test was applied for all other parameters.

C. METHODS

1. Observations

Animals were observed and palpated at least once daily for signs of behavioral change, reaction to treatment or ill health on weekdays for the first 4 weeks, and once weekly thereafter. Animals were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality.

7

2. Body weight

Animals were weighed 1 week prior to dosing, on the day of dosing, and at weekly intervals following dosing. Animals were also weighed on each occasion that the functional observational battery was performed.

3. Food consumption and compound intake

Food consumption for each animal was measured weekly beginning 1 week prior to dosing. Food intake per rat (g/rat/week) was calculated based on the amount of food remaining in each cage each week. Food efficiency (food consumption/body weight gain) was calculated weekly for each test group during all treatment weeks. Mean compound intake for each group was calculated weekly.

4. Neurobehavioral Studies

Motor Activity - Motor activity of all animals was measured at approximately the same time of day, before initiation of treatment and during weeks 4, 8, and 13. The placement of each animal within a cage was balanced as much as possible across groups. The test session was initiated when all animals were placed in cages, and lasted one hour for each animal. Motor activity was monitored using a Colbourn Infra-Red Activity Monitoring System which uses an infra-red detector. For each animal, the time and number of events spent in no movement, locomotor, and non-locomotor activity were recorded. Data were collected every 2 minutes.

Functional Observational Battery - A functional observational battery (FOB) was performed on all animals at approximately the same time of day, before initiation of treatment and during weeks 4, 8, and 13. The following parameters were evaluated:

	HOME CAGE OBSERVATIONS		OBSERVATIONS IN THE ARENA
X	Posture in cage	X	Convulsions, tremors, twitches
X	Convulsions, tremors, twitches	X	Level of activity in arena
X	Spontaneous vocalizations	X	Level of arousal
X	Palpebral closure	X	Rearing count
		X	Grooming
		X	Assessment of gait
	OBSERVATIONS IN THE HAND	X	Presence of fecal boluses, urine
X	Ease of removal from cage		MANIPULATIONS
X	Ease of handling rat		
X	Salivation/lacrimation	X	Approach response
X	Palpebral closure	X	Touch response
X	Exophthalmus	X	Startle response
X	Piloerection	X	Tail pinch response
		X	Righting reflex
X	Vocalization on handling	X	Pupil response
		X	Grip strength; fore and hindlimb
		X	Landing foot splay
		X	Body temperature

		X	Body weight

5. Sacrifice and Pathology

All animals were sacrificed at the end of the 13-week treatment period by intraperitoneal injection of sodium pentobarbital and perfused in situ with heparinized 0.7% sodium nitrite followed by a 1.5% glutaraldehyde/4% paraformaldehyde solution. Neuropathological examination was conducted only on tissues from five rats/sex from the control and high dose groups. The following tissues were examined:

BRAIN	SPINAL NERVE ROOT FIBER AND GANGLION
Forebrain (3 levels)[a]	Cervical dorsal root ganglia
Midbrain[a]	Lumbar dorsal root ganglia
Cerebellum[a]	Dorsal and ventral fibers (cervical level)[c]
Pons[a]	Dorsal and ventral root fibers (lumbar level)[c]
Medulla oblongata[a]	Gasserian ganglia
SPINAL CORD	PERIPHERAL NERVES
Lumbar spinal cord[b]	Sciatic nerve[b]
Cervical spinal cord[b]	Sural nerve[b]
	Tibial nerve[b]

[a] Cross sections of these tissues were evaluated.
[b] Cross and longitudinal sections of these tissues were evaluated.
[c] Longitudinal sections of these tissues were evaluated.

Brains, spinal cords, ganglia, and dorsal and ventral root fibers were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Peripheral nerves from the right side were embedded in epon, sectioned, and stained with toluidine blue.

III. RESULTS

A. Observations

1. Mortality - No rats died during the study.

2. Clinical signs - No differences in clinical signs in any of the test groups were considered to be treatment-related.

B. Body weight and body weight gain

Weekly mean body weights of the 1000 mg/kg/day group males were 8-16% lower (p 0.05 or p 0.01) than the controls during weeks 1-13. Weekly mean body weights of the 1000 mg/kg/day group females were 3-8% lower (p 0.05 or p 0.01) than the controls at weeks 3-13 except at week 5 (2% lower). Mean body weight gains for the 1000 mg/kg/day group males and females were 26 and 20% lower than the respective control groups. No differences were observed in body weights or body weight gains between the 75 and 25 mg/kg/day treatment groups and corresponding control groups. At the end of the study, mean body weights for males were 463 g for the 1000 mg/kg/day treatment group compared to 523-552 g for the other test groups, and for females, were 267 g for the 1000 mg/kg/day treatment group compared to 287-298 g for the other test groups.

C. Food consumption and compound intake

1. Food consumption - The 1000 mg/kg/day group males consumed 13-15% less food (p 0.01) than the controls during weeks 1 and 13; food consumption during other study weeks was 1-10% lower than the controls and not statistically significant. Feed efficiency values for males were higher than control values during most weekly intervals. The 1000 mg/kg/day group females consumed 7% less food (p 0.05) than the controls during weeks 9 and 12; during other study weeks, food consumption was similar or slightly lower than the control values. Mean feed efficiency values for females were highly variable throughout the study, and indicated an overall impairment. No treatment-related differences in food consumption or food efficiency were observed between the 75 and 25 mg/kg/day treatment groups and corresponding control groups.

2. Compound intake - Calculated compound intake by male rats in the 25, 75, and 1000 mg/kg/day treatment groups averaged 25.2, 75.8, and 998 mg/kg/day, respectively, over the 13-week period. Calculated compound intake by female rats in the 25, 75, and 1000 mg/kg/day treatment groups averaged 24.5, 74.7, and 987 mg/kg/day, respectively.

D. Functional Observational Battery

The female treatment groups exhibited a dose-related increase in vocalization upon removal from the cage at 4 weeks compared to the controls. Vocalization was noted in 2/10 females in the 25 mg/kg/day group, 4/10 females in the 75 mg/kg/day group, and 5/20 females in the 1000 mg/kg/day group compared to 1/10 control females; the increased incidence was significant ($p=0.05940$) at the high dose level. In the 1000 mg/kg/day group females at Week 4, mean activity and rearing counts were lower than the control activity. Mean activity counts were 27% lower than the controls (11 versus 15), and mean rearing counts were 31% lower than the controls (11 versus 16); the decrease was significant only for the rearing counts (p 0.05) compared to the controls. No differences in activity or rearing counts were noted for the 1000 mg/kg/day group females during Weeks 8 and 13, or for any other treatment group. No other differences in FOB parameters for any of the treatment groups were considered to be treatment-related.

E. Motor Activity Measurements

No differences in motor activity measurements in any treatment group were considered to be treatment-related. Although females treated at 75 mg/kg/day exhibited increased motor activity during Weeks 4 and 8 compared to the controls, similar increases were not observed during Week 13 or in the 1000 mg/kg/day group females during any observation week. Therefore, the increased motor activity was not considered to be treatment-related.

F. Sacrifice and Pathology

Absolute brain weights for the 1000 mg/kg/day male and female treatment groups and the 75 mg/kg/day male treatment group were each 7-8% lower (p 0.05 or p 0.01) lower than the respective control weights (Table 3). No intergroup differences in mean brain length and width were observed. No macroscopic or microscopic findings were observed in any of the nervous system tissues in the treated rats, based on histological examination of the central nervous tissues, spinal cords with ganglia, and the peripheral nerves of the control and 1000 mg/kg/day groups (5 rats/sex/group examined). An increased incidence of trace axonal degeneration in the tibial nerve in the 1000 mg/kg/day treatment groups (3 rats/sex) was considered a normal background change.

Table 3. Mean absolute brain weights for control and treatment groups.[a]

Dose Group (mg/kg)	Brain Weight (g)	
	Males	Females
0	1.82	1.63
25	1.74	1.65
75	1.69**	1.59
1000	1.68**	1.52*

[a] Data obtained from Table 15, page 47, of the study report.
* Significantly different from the control, $p \leq 0.05$.
** Significantly different from the control, $p \leq 0.01$.

III. DISCUSSION

A. Investigator's Conclusions

The study authors concluded that rats administered diflufenzopyr at 25, 75 or 1000 mg/kg in the diet did not exhibit any behavioral or neuropathological changes that were due to treatment. Non-neurological treatment-related effects observed at the high-dose level were impaired body weight gain and impaired efficiency of food utilization.

Based on these findings, the NOAEL was established at 75 mg/kg/day

B. Reviewer's Discussion

We agree with the study authors that no neurotoxicological effects were observed in any treatment group. Several changes in FOB parameters in treated females at Week 4 were not clearly attributable to treatment since they lacked associated neurotoxicological changes, and were transient, as they were not observed at Weeks 8 and 13. These findings were a dose-related increase in vocalization in all females treatment groups upon removal from the cage, and decreased mean activity counts (27% lower) and rearing counts (31% lower) in the 1000 mg/kg/day group females. Statistically, these differences were only borderline significant or were not significant compared to the controls.

Treatment-related differences limited to the 1000 mg/kg/day treatment groups were decreased body weights, food consumption, and food utilization by both sexes compared to the controls. Mean body weights for the males were 9-15% lower during Weeks 4, 8, and 13, and for the females were 7% lower during weeks 9 and 12 compared to the controls. Males consumed 13-15% less food (p 0.01) than the controls during weeks 1 and 13 and 1-10% less for during the other study weeks compared to the controls. Females consumed 7% less food than the controls during week 9 and 12; otherwise, there were no differences. Overall food utilization by both sexes was impaired compared to the controls throughout the study.

Decreased absolute brain weights (7-8%) for the 75 mg/kg/day group males and the 1000 mg/kg/day treatment groups appeared to be due, in part, to the depressed body weights for these groups. Relative brain weights for all male and female treatment groups were similar to the respective control group relative brain weight.

Based on these findings, a LOAEL for neurotoxicological effects was not established. The NOAEL was 75 mg/kg/day for both sexes. The toxicological LOAEL for this study is

1000 mg/kg/day, based on decreased body weight gains for both sexes. The toxicological NOAEL is 75 mg/kg/day.

IV. STUDY DEFICIENCIES

No scientific or guideline deficiencies were noted. Brief summaries of four neurotoxicity positive control (validation) studies (MRIDs 44447801, -02, -03, -04) are included in a memorandum from Dr. Marion Copley [Fipronil ID#000264-LTT: Evaluation of Neurotoxicity Positive Control (Validation) Studies in Support of a Rat Neurotoxicity Screening Study on MB46513), a Photodegradate of Fipronil (81-8ss)]. The memorandum is included with this review. These positive control studies demonstrate the capability of the testing laboratory, Huntington Life Sciences Ltd., P.O. Box 2, Huntington, Cambridgeshire, PE18 6ES, England, to conduct adequate functional operational battery, grip strength, motor activity testing and neuropathology evaluations.