009610

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 5/21/92
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel
Tox. Branch II, Head Section II (H7509C)

MAY 21 1992

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Feeding - mice TOX. CHEM. NO.: 463-O

MRID NO.: 421373-37

TEST MATERIAL: Fluroxypyr

SYNONYMS: 4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid

STUDY NUMBER: 87-0083

SPONSOR: Dow Chemical Japan, Ltd.

TESTING FACILITY: Mitsukaido Laboratories/The Institute of Environmental Toxicology, Japan

TITLE OF REPORT: Fluroxypyr: 13-Week Oral Subchronic Toxicity Study in Mice.

AUTHORS: Y Shirasu, A Yoshido, K Ebino, S Tsuda, K Inui, S Goto

REPORT ISSUED: October, 1988

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, dietary exposure to fluroxypyr for 13 weeks at dose levels of 200, 500, 2500, and 10000 ppm produced no significant effects in mice. It is concluded that the dose levels used are adequate since the highest dose in either sex is greater than one gram/kg/day. The NOEL can be set at 10000 ppm (1342 mg/kg ♂♂/1748 mg/kg ♀♀), the highest dose tested (HDT). This study satisfies the guideline requirement (82-1) for a subchronic feeding study in the rodent.

Classification: Core Minimum. This study satisfies the guideline requirement (82-1) for a subchronic feeding study in rodents.

A. MATERIALS:

1. Test Compound: Fluroxypyr; Description: white crystalline solid; Batch #: Lot # HM 196; Purity: 99.3%.

2. Test Animals: Species: mouse; Strain: SPF ICR (Crj:CD-1); Age: 6 weeks old at study start; Weight: males ≈30 g, females ≈23 g; Source: Charles River Japan, Inc..

3. Statistics: Body weight, food consumption, hematology, blood biochemistry, and organ weight - Multiple Comparison test (Dunnett's or Scheffé method). Urinary data - Mann-Whitney's U test. Survival data, incidences of clinical signs, and pathological lesions - Fisher's Exact Probability test. Significance of differences was estimated at 5% and 1% levels of probability.

B. STUDY DESIGN

1. Methodology: Sixty males and 60 females were randomly assigned (randomization procedure by computer; body weight distribution among groups ≈ equal) to one of five groups [0, 200, 500, 2500, or 10000 ppm] of 12 mice/sex/group. The dose levels were chosen, based on the results of a 4-week range-finding study (87-0081) in which one of six males died and another showed poor condition with renal atrophy at 20000 ppm; decreased specific gravity of the urine at 10000 and 20000 ppm (males); tubular epithelial degeneration and/or papillary degeneration in the male kidney at dose levels of ≥1000 ppm and in females at 20000 ppm. There were no effects on body weight, food consumption, food efficiency, hematology, blood chemistry, gross lesions, or organ weights. The mice were housed 3/sex/cage; 14 hours of light, 10 hours of dark. The animals were fed a standard laboratory pelleted chow Oriental MF (Oriental Yeast Co., Ltd./Tokyo), which was pulverized and the powdered diet M was used as the basal diet. Both diet and water were available ad libitum. Test diets were prepared once every 3 weeks and stored at room temperature, apparently. Basal diet and test material were mixed together to obtain the desired concentrations. The stability of the test material in the diet was determined in the 4-week range-finding study, and the concentration of the test material in the diet was analyzed for each preparation. Additionally, homogeneity of mixing was determined once prior to study initiation.

RESULTS

The test material was found to be homogeneously mixed in the feed. The pretest stability study indicated that the test material was stable in the diet for up to 28 days at room temperature. Analyses of the test diets indicated that the diets were within 5% of the target concentration.

2. Clinical Observations: The animals were observed daily for clinical signs, mortality/moribundity, with the time of onset, nature/severity/duration of signs being recorded. A weekly careful examination of each animal was conducted, and individual body weights and food consumption (per cage) were recorded weekly. Group mean test material intake was calculated from the data on food consumption/body weight/and dose level. Food efficiency was calculated from the ratio of the mean body-weight gain to mean food consumption.

RESULTS

Survival and Clinical Observations

All animals survived until study termination. The only effect noted was a statistically significant increase in the incidence of loss of tactile hair in females at the 2500 ppm dose level. There were no other treatment-related clinical findings seen during the study.

Body Weight and Food Consumption

Body weight was comparable among the groups throughout the study for both sexes, although there was a significant increase in the 200 ppm females compared to the control value at week 4. The authors did not discuss or provide any analysis of body-weight gains. The overall body-weight change as a % and in grams (calculated by TB II; no statistical analysis) are shown below.

Body-Weight Change (%)

Week/Group	0 ppm	200 ppm	500 ppm	2500 ppm	10000 ppm
MALES 0-13	47.4	51.0	45.5	52.7	52.4
FEMALES 0-13	42.2	47.6	47.5	56.4	41.8

Mean Body-Weight Change (g)

Interval/Group	0 ppm	200 ppm	500 ppm	2500 ppm	10000 ppm
MALES 0-13	14.1	15.2	13.5	15.7	15.6
FEMALES 0-13	9.9	12.1	10.9	13.0	9.6

Food consumption and food efficiency were comparable among the groups for both sexes throughout the study. Test material intake is listed below for each sex and dose level.

Dose Levels of Test Material

Dose Level (ppm)	Test Material Intake (mg/kg/day)	
	MALES	FEMALES
200	26.7	32.5
500	67.7	81.7
2500	330	418
10000	1342	1748

3. Blood Analyses

Hematology: Blood samples were obtained from all animals after 13 weeks of treatment. It was not stated whether food was withheld prior sacrifice and sample collection (through the posterior vena cava). The CHECKED (X) parameters were 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		Reticulocyte count
	Blood clotting measurements		Red cell morphology
	(Thromboplastin time)		
	(Activated partial thromboplastin time)		
	Nucleated erythrocytes normoblasts		

RESULTS

There were no treatment-related effects observed on any of the measured parameters in either sex, although males at the 500 ppm dose level displayed a statistically significant increase in hematocrit, hemoglobin, and erythrocyte count compared to the control values (7-8% > control values).

Clinical Chemistry: Blood samples were obtained as stated above. The CHECKED (X) parameters were examined.

	Volume		[illegible]
X	Specific gravity		Bilirubin
X	pH	X	Blood
	Sediment (microscopic)		Nitrate
	Protein	X	Urobilinogen
	Osmolality		[illegible]

X	Electrolytes:
X	Calcium
	Chloride
	Magnesium
	Phosphorous
	Potassium
	Sodium
	Iron
	Enzymes
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
	Creatine kinase (CK)
	Lactate dehydrogenase (LAD)
X	Serum alanine aminotransferase
X	Serum aspartate aminotransferase
	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase (GLDH)
	Ornithine carbamyltransferase (OCT)
	Serum protein electrophoresis*
	Thyroxine, total T4

X	Other:
	Albumin
	Blood creatinine
X	Blood urea nitrogen
X	Cholesterol
	Globulins
X	Glucose
	Phospholipids
	Total bilirubin
X	Total serum Protein (TP)
	Triglycerides
	Lipids, total
	Triiodothyronine, total T3

RESULTS

Although differences were observed in several of the parameters measured (males only), none is considered to be treatment-related.

MALE CLINICAL BIOCHEMISTRY FINDINGS♥

Parameter/Dose	0 ppm	200 ppm	500 ppm	2500 ppm	10000 ppm
GOT	35	29	31	29	27**
GPT	19	13*	14*	15	14*
Proteins TP	4.60	4.83*	4.56	4.85	4.60

♥ GOT/GPT [U/L]; TP [g/dL]

4. Urinalysis: Urine samples were collected from each mouse at 13 weeks by pressing the lower abdomen. The CHECKED (X) parameters were examined.

X	
	Appearance
	Volume
X	Specific gravity
X	pH
	Sediment (microscopic)
X	Protein
	Osmolality

X	
X	Glucose
X	Ketones
	Bilirubin
X	Blood
	Nitrate
X	Urobilinogen
	Color

RESULTS

There were no differences observed among the females in any of the measured parameters. The high-dose males displayed a statistically significant reduction in pH and the urobilinogen value at the 500 ppm dose level was significantly elevated compared to the control value. With regard to pH, a similar change occurred in the 4-week study (see the tables below). There are no baseline data with which to compare this apparent effect, and its significance is not known. Additionally, since there was no dose response with regard to urobilinogen, it is not considered treatment-related.

URINALYSIS FINDINGS

Parameter/Dose	0 ppm	200 ppm	500 ppm	2500 ppm	10000 ppm
MALES					
pH					
6	4	6	9	8	10
7	7	6	3	4	2*
8	1	-	-	-	-
FEMALES					
pH					
6	12	10	9	11	10
7	-	2	3	1	2

* $p<0.05$

URINALYSIS FINDINGS (range-finding study)

pH/Dose	0 ppm	100 ppm	1000 ppm	5000 ppm	10000 ppm	20000 ppm
MALES						
pH						
6	2	-	1	2	5	5
7	4	3	3	4	1	-
8	-	3	2	-	-	-
FEMALES						
pH						
6	5	5	6	5	5	6
7	1	1	-	1	-	-
8	-	-	-	-	1	-

5. Gross Pathology: All animals were subjected to a full macroscopic examination at sacrifice (no information on whether animals were fasted overnight was provided). The following organs were weighed: kidneys, liver, testes, brain, and adrenals.

RESULTS

There were no significant differences noted in the incidence of any gross lesions. The only statistically significant effect noted in organ weight was a reduction in relative liver weight in the females at 2500 ppm.

6

6. Histopathology: The following organs/tissues (CHECKED (X)) were preserved from all animals at terminal sacrifice and processed for histological examination.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Periph. nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord□
X	Stomach♣	X	Lymph nodes♦	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes/Harderian gland
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		X	Adrenal gland
X	Cecum	X	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland area ♀
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
X	Gall bladder	X	Prostate	Other	
X	Pancreas	X	Seminal vesicle	X	Bone♥
Respiratory		X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus♠	X	Skin
X	Lung		Vagina	X	All gross lesions
X	Nose	X	Coagulating gland		
X	Pharynx				
X	Larynx				

♥femur & sternum; ♦ cervical & mesenteric; ♣ forestomach & glandular stomach; ♠ cornua, corpus, & cervix; □cervical, thoracic, & lumbar

RESULTS

The only difference noted among the groups was a statistically significant decrease in the incidence of micro-granuloma in the liver in males at the 200, 500, and 10000 ppm dose levels, compared to the control incidence.

DISCUSSION

There were no significant differences observed in either sex following oral administration of fluroxypyr for 13 weeks in any of the parameters measured (mortality, body weight, food consumption/efficiency, clinical observations, hematology, clinical chemistry, urinalysis, necropsy, organ weight, or histopathology). The kidney effects observed in the 4-week range-finding study (tubular epithelial degeneration and/or papillary degeneration) in males at 1000 ppm and above and in females at 20000 ppm were not duplicated in the current study.

CONCLUSION

Under the conditions of the study, exposure to fluroxypyr via the diet for 13 weeks at dose levels of 200, 500, 2500, and 10000 ppm produced no significant effects in mice. It is concluded that the dose levels used are adequate since the

7

highest dose in either sex is greater than one gram/kg/day. The NOEL can be set at 10000 ppm (1342 mg/kg ♂♂/1748 mg/kg ♀♀), the highest dose tested (HDT). This study satisfies the guideline requirement (82-1) for a subchronic feeding study in the rodent.