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DATA EVALUATION RECORD

FLUROXYPUR

Chronic Toxicity Study in Dogs

APPROVED BY

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Date: 4-4-88

Accession # 40241507

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DATA EVALUATION RECORD

FLUROXYPUR

Chronic Toxicity Study in Dogs

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DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic feeding study in dogs.

ACCESSION NUMBER: 402445-07

TEST MATERIAL: Dowco 433.

SYNONYM: 4 amino 3,5 dichloro 6 fluoro 2 (pyridinyl oxyacetic acid)

STUDY NUMBER: V 65 541

SPONSOR: DOW Chemical Europe, Horgen, Switzerland

TESTING FACILITY: Battelle Institute, Frankfurt, Federal Republic of Germany

TITLE OF REPORT: 12-Month toxicity study in Beagle dogs by dietary administration of DOWCO 433.

AUTHOR(S): Kinkel, H. J., Ehard, H., and Raasch, E.

REPORT ISSUED: December 12, 1984.

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CONCLUSIONS:

Under the conditions of the study, there were no effects of toxicologic importance when Dowco 433 was fed to dogs at 20, 50, or 150 mg/kg/day for one year. Effect levels could not be established. The dogs could have tolerated a higher dose.

Core classification: Supplementary since the dosing was not adequate.

A. MATERIALS:

1. Test Compound: Dowco 433, description: powder; batch no. 433t-0283-10; purity 98%.

2. Test Animals: Species: Dog, strain: Beagle, age: 7 to 8 months, weight: Males: 13.9 kg, Females: 12.9 kg, source: Erkrath, K. G., Rodenbach, Federal Republic of Germany.

B. STUDY DESIGN:

1. Animal assignment. Animals were assigned randomly to the following test groups:

Test Group	Dosage (mg/kg/day)	Main study (12 months) Males	Females
1 Control	0	4	4
2 Low (LDT)	20	4	4
3 Mid (MDT)	50	4	4
4 High (HDT)	→ 150	4	4

The dogs were innoculated against leptospirosis, hepatitis, distemper, and rabies and were treated with an anthelmintic. They were quarantined for 5 weeks prior to study initiation.

2. Diet Preparation: Test substance incorporated in standard diet at either 0.6, 1.5, or 4.5 g/kg diet and 33 g/kg body weight of the respective diets was offered for one hour/day to the dogs. Samples of diets were analyzed at the start of the study and at 3-month intervals. Stability was measured after 3 months (maximal storage).

Results: Analytical data indicated that Dowco 433 was stable in the diets after 3 months storage (96-101% recovery) and levels of Dowco 433 were within 10% of the target level. The coefficient of variation for the low dose was reported to be 3.8%.

3. Food and Water Consumption: Dogs were offered 33 g/kg of food per body weight for 1 hour each day for 52 weeks, and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: the Kruskall and Wallis test was used for analyses of liver-to-body weight ratios. No other details of statistical procedures were presented.

5. Quality Assurance: A quality assurance statement (undated) was present.

METHODS AND RESULTS:

1 Observations: Animals were inspected daily for signs of toxicity and mortality.

Results: One male dog receiving 20 mg/kg/day died in week 12 from uremia induced by stones in the bladder and urethra. No signs of toxicity were seen in other dogs whether dosed or not.

2 Body weight: Dogs were weighed weekly during the entire study.

Results: There were no effects of dosing on mean body weights or individual body weights when dosed males or females were compared with controls. Mean body weights at selected intervals are presented in Table 1.

3 Food Consumption and Compound Intake: Food intake was recorded daily for the first 2 weeks and weekly thereafter. Water intake was determined over 2 consecutive days monthly.

Results: Food consumption was similar for all animals on study (Table 2). The compound intake was appropriate, based on food consumption and analyzed dietary levels.

4 Ophthalmological Examinations: These examinations included the use of a direct ophthalmoscope and were performed prior to study initiation and at weeks 26 and 51 on all animals.

Results: There were no compound related effects.

5 Clinical Studies: Blood was collected prior to study initiation and at 4, 13, 26, and 52 weeks for hematology and clinical chemistry from all animals. The checked (X) parameters were examined.

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TABLE 1. Representative Body Weight Data from Dogs Fed Dowco 433 for 12 Months

Dosage Group (mg/kg/day)	Mean Body Weights (kg ± SD) at Week 1	13	26	52
Males				
0	12.4 ± 1.0	14.0 ± 0.9	14.6 ± 1.2	14.3 ± 1.1
20	12.0 ± 1.8 97	14.3 ± 2.6 102	13.0 ± 3.8[a] 89	13.5 ± 3.3 94
50	12.6 ± 3.1 102	14.1 ± 2.8 101	14.8 ± 2.6 101	14.8 ± 2.6 103
150	11.8 ± 1.1 95	13.2 ± 1.5 94	13.8 ± 1.3 95	13.2 ± 1.4 92
Females				
0	10.7 ± 2.4	12.2 ± 2.6	13.3 ± 3.1	12.8 ± 3.0
20	10.6 ± 1.7 99	11.4 ± 2.1 93	12.0 ± 2.0 90	11.6 ± 2.1 91
50	10.7 ± 1.7 100	12.0 ± 1.5 98	12.6 ± 1.3 95	12.4 ± 1.6 97
150	10.5 ± 2.8 98	11.7 ± 2.9 96	12.7 ± 3.0 95	11.8 ± 2.6 92

[a]One animal died

15.3%
11-8%
19.6%
↑12% Compared to week 1.

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TABLE 2. Representative Food Consumption Data from Dogs fed Dowco 433 for 12 Months

Dosage Group (mg/kg/day)	Mean Food Consumption (g ± SD) at Weeks 1	13	26	52
		Males		
0	408.8 ± 33.5	475.0 ± 31.4	500.0 ± 40.4	467.5 ± 37.5
20	391.3 ± 55.9	465.0 ± 100.0	431.7 ± 115.5[a]	443.3 ± 106.8
50	408.0 ± 100.4	466.3 ± 96.8	491.3 ± 88.3	482.5 ± 88.8
150	383.8 ± 43.3	423.8 ± 49.7	458.8 ± 30.9	433.8 ± 47.1
		Females		
0	347.0 ± 78.2	408.8 ± 86.3	457.5 ± 107.5	417.5 ± 95.3
20	345.8 ± 60.5	383.8 ± 80.9	400.0 ± 71.8	382.5 ± 68.9
50	339.5 ± 65.2	400.0 ± 53.1	416.3 ± 43.3	407.5 ± 50.6
150	326.3 ± 59.8	392.5 ± 103.6	425.0 ± 102.3	392.5 ± 77.9

[a]One animal died.

a. Hematology

- X Hematocrit (HCT)†
- X Hemoglobin (HGB)†
- X Leukocyte count (WBC)†
- X Erythrocyte count (RBC)†
- Platelet count†
- X Erythrocyte Sedimentation Rate (ESR)
- X Leukocyte differential count
- X Mean corpustular HGB (MCH)
- X Mean corpustular HGB concentration (MCHC)
- X Mean corpuscular volume (MCV)
- X Calcium-Thromboplastin Time (PT)
- X Partial Thromboplastin Time (PTT)

Results: Hematology values for all animals were in the normal range; no compound-related findings were noted. Clotting parameters were similar in dosed and control groups with the exception that there was a decrease in Ca/Thromboplastin time in all dosed males when compared to controls. The decrease was reported not to be statistically significant or of toxicologic importance.

b. Clinical Chemistry

Electrolytes
- X Calcium†
- X Chloride†
- Magnesium†
- X Phosphorus†
- X Potassium†
- X Sodium†

Enzymes
- X Alkaline phosphatase (ALP)
- Cholinesterase
- Creatinine phosphokinase†
- X Lactic acid dehydrogenase
- X Serum alanine aminotransferase (SGPT)†
- X Serum aspartate aminotransferase (SGOT)†
- X Gamma-glutamyl transpeptidase (GGT)

Other
- X Albumin†
- X Blood creatinine†
- X Blood urea nitrogen†
- X Cholesterol†
- Globulins
- X Glucose†
- X Total bilirubin†
- X Total protein†
- X Triglycerides
- X Iron
- X Uric Acid

Results: No compound-related findings were noted.

6 Urinalysis: Urine was collected from fasted animals prior to start of study and at 4, 13, 26, 36 and 52 weeks. The checked (X) parameters were examined:

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

†Recommended by Subdivision F (October 1982) Guidelines

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Results: No compound-related findings were observed.

7. Sacrifice and Pathology: All animals that were sacrificed on schedule were subject to gross pathological examination, and the checked (X) tissues were collected; a complete histological examination was performed on control and high-dose groups only. The (XX) organs from all animals were also weighed:

	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain† (3 levels)
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†		Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	XX	Thymus†		
X	Ileum†				Glandular
X	Cecum†		Urogenital	XX	Adrenals†
X	Colon†	XX	Kidneys†		Lacrimal gland
	Rectum†	X	Urinary bladder†	X	Mammary gland†
XX	Liver†	XX	Testes†	X	Parathyroids†
X	Gallbladder†	X	Epididymides	XX	Thyroids†
X	Pancreas†	XX	Prostate	X	Harderian glands
			Seminal vesicle		
	Respiratory	XX	Ovaries		Other
X	Trachea†	XX	Uterus†		Bone (sternum)†
X	Lung†	X	Ureter	X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses
				X	Sternum
				X	Femur

Heart, lung, liver, stomach, and duodenum were examined histologically for dogs in the low- and mid-dose groups. Kidney sections were stained with PAS, Oil Red-O, Prussian blue, and Turnbull's blue in addition to hematoxylin-eosin.

Results:

a. Organ Weights: It was reported that there was no effect of dosing on organ weight data. There was a non-significant increase in the liver weight and liver-to-body weight ratio

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†Recommended by Subdivision F (October 1982) Guidelines.

in dosed female groups when compared to controls (Table 3); however, there was no effect in males. Both absolute and relative ovary and uterus weights were increased in females receiving 150 mg/kg/day. This correlated with estrus or postestral changes and was not related to dosing. All four high-dose females had mature Graafian follicles or postestral corpora lutea on histologic examination of ovaries and epithelial and stromal cell activation in the uterus. Similar findings were seen in one control female and only one female receiving 50 mg/kg/day. There were no significant findings in other organs weighed nor any apparent dose trends.

b. Gross Pathology: There were no striking gross findings and no compound-related change.

c. Histopathology: There were no histologic findings that were considered related to compound administration. The kidneys of many dogs had unilateral or bilateral granulomas. These were related to parasitic infection which occurred before study initiation. No larvae or live parasites were found. The granulomas were circumscribed and contained histocytes, macrophages and fibrotic tissue surrounded by lymphocytes. Non-degenerative lipid storage (Red Oil-O positive) in the proximal tubule was found in most dogs and was more marked in females than in males. Hemosiderin deposits (positive staining pigment with Prussian blue and Turnbull's blue) was found in histiocytic but not parenchymal cells. The findings in the kidneys were not increased in incidence in dosed dogs (Table 4). They were all considered the result of parasitic infection prior to study initiation. Livers showed minimum to slight non-degenerative lipid storage in Kupffer cells or hepatocytes as well as mononuclear cell infiltration in the parechyma or periportal space. These findings were not increased in dosed dogs (Table 4). Histologic findings in ovaries and uterus were related to estrus or metestrus. Other lesions found were considered spontaneous.

In the male dog receiving 20 mg/kg/day that died during week 12, microscopic examination showed acute tubular nephrosis and tubular lipid accumulation in the kidney. Lungs, liver, and bladder had severe congestion, and there was muscular degeneration in the heart.

D STUDY AUTHOR'S CONCLUSIONS:

The authors concluded that there were no effects of toxicologic importance when Dowco 433 was administered to dogs at doses up to and including 150 mg/kg/day for one year. The only abnormal findings were related to a probable parasitic infection prior to the beginning of the study. This did not compromise the validity of the study.

TABLE 3. Mean Liver Weights (± SD) and Terminal Body Weights of Female Dogs Fed Dowco 433 for 1 Year.

Dosage Group (mg/kg/day)	Body Weight (kg)	Liver	
		grams	percent
0	12.9 ± 2.9	377.2 ± 63.3	2.97 ± 0.34
20	11.8 ± 2.1	388.0 ± 69.5 103	3.30 ± 0.17
50	12.5 ± 1.6	417.3 ± 49.7 111	3.36 ± 0.45
150	12.0 ± 2.5	410.2 ± 50.1 109	3.48 ± 0.34

TABLE 4. Incidence of Lesions in Kidneys and Liver of Dogs Fed 006688
Dowco 433 for 1 Year

Organ/finding	Dosage group (mg/kg/day) Males 0	20	50	150	Females 0	20	50	150
Kidneys								
Granuloma	1	0	1	0	3	2	2	1
Mononuclear cell infiltration	3	1	2	2	0	1	0	0
Pigment deposit (minimal)	3	2	4	4	0	2	4	4
Proximal tubule lipid deposit								
minimum	3	2	3	4	0	0	0	0
moderate/marked	1	2	1	0	4	3	4	4
Liver								
Lipid storage								
minimal	3	2	1	2	3	2	2	1
moderate	0	0	1	0	0	0	0	0
Mononuclear cell infiltration								
minimal	3	1	3	2	2	1	1	1
moderate	0	0	0	1	1	0	0	0

E REVIEWERS' DISCUSSION AND INTERPPETATION OF RESULTS:

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It is our assessment that the dogs could have tolerated a much higher dose than they received. No effects of toxicologic importance were found after dosing dogs for up to one year at levels including 150 mg/kg/day. The conduct and reporting of the study were adequate. There was a fairly large spread in initial body weights. In males receiving 50 mg/kg/day the range was 9.0 to 16.0 kg and in females at the same dose the range was 10.0 to 15.8 kg. Mean initial weights for male and female groups, however, ranged from 11.8 to 12.6 kg (males) and 12.6 to 13.3 kg (females). The variability in the weights combined with the minimum number of dogs per group may have resulted in the relatively large standard deviations noted for absolute and relative organ weights. We assess that, because of the scatter in organ weight data, no evaluation of effect of dosing could be made on these parameters.

There was no summary tabulation of gross or histopathologic findings. Not all tissues in mid- and low-dose groups were examined histologically. In these groups heart, lung, liver, spleen, kidneys, bladder, stomach, small and large intestine were examined. The death of male dog No. 6 (50/mg/kg/day) did not appear to be related to dosing. The effect of prestudy parasitic infection of dogs on the results of the study could not be assessed.

There appeared to be an increase in the number of epithelial cells qualitatively found in urine sediment in some dosed groups of animals at various intervals in the study. We do not assess this to be of biological importance. Epithelial cells were present in all controls at one or more intervals of examination, there was no consistency from interval to interval in the severity of the finding in individual dosed animals and no apparent dose related trends.

At 52 weeks, the LDH levels in all groups including controls was much lower (35-45 U/mL) than expected or found in controls at other intervals (180 U/mL). It could not be determined if this value was within the normal laboratory variability or there was a technical problem.

Under the conditions of the study a LOEL could not be established and the apparent NOEL was the highest dose tested.