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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

30 OCT 1980

MEMORANDUM

SUBJECT: EPA Reg.#707-145; Goal 2E Herbicide; PP#8F2058, 9F2197;
oxyfluorfen; 104-week Toxicity Study in Dogs with RH-2915; Rough
Draft of Final Report. CASWELL#188AAA

FROM: William Dykstra
Toxicology Branch, HED (TS-769)

WJD JDC
9/30/80

TO: Richard Mountfort (23)
Registration Division (Ts-767)
and
Residue Chemistry Branch (TS-769)

Recommendations:

- 1) The study is acceptable as core-minimum data. No NOEL was established in this study. The study needs to be repeated at lower doses.

Review:

- 1) 104-Week Toxicity Study in Dogs with RH-2915 Hazelton Project No. 417-367; Rough Draft of Final Report; July 23, 1980

Test Material

Five samples of RH-2915 were used for this study and are identified as follows:

<u>Lot No.</u>	<u>Receipt Date</u>	<u>Purity(%)</u>	<u>Used Study Weeks</u>
PL76/8046 (TRD 77-118)	4/29/77	73.2	1-3
2-8208	7/5/77	71.4	4-81
PL76/8046 (TD 78-206)	8/29/78	73.2	82-85
PL76/8046	12/26/78	73.2	86-104 for groups 6&7 and 86-108 for group 8
Ref#8/8208	6/20/79	73.8	104 for group 8

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The first two samples received were described as orange-brown and solid and the last three samples were described as light brown and solid.

One hundred ten, young (18-22 weeks old), purebred beagles were received from Hazelton Research Animals, Inc., Cumberland, Virginia, on May 3 and 4, 1977. The dogs were individually housed in elevated steel cages and fed Wayne Dog Meal. Water was available ad libitum. Each dog was uniquely identified by a plastic ear tag. All of the dogs were quarantined for at least two weeks following receipt and all individual animal records were checked to assure that the dogs had received DHL (distemper, hepatitis, and leptospirosis) and rabies vaccine.

Fecal flotations were performed on all dogs to check for the presence of intestinal parasites.

The one hundred four most clinically acceptable dogs (fifty-two males and fifty-two females) were stratified by weight and assigned to groups using a table of random permutations of nine. The dogs assigned to the portion of the study receiving RH-2915 were grouped as follows:

Group	No. of Animals		Dietary Level ppm	Days
	Males	Females		
1 (control)	10	10	0	1-term.
6	6	6	100	1-term.
7	6	6	600	1-term.
8	6	6	3600	1-8
			0	9-14
			2800	15-28
			2000	29-term.

At initiation of treatment, the body weights ranged from 6.4 to 11.4 kg for males and from 6.5 to 10.9 kg for the females. The required amount of test material for each group was weighed and added to a small amount of ground Wayne Dog Meal and blended in a war.ing blender. This premix was then added to the amount of feed required for the group and mixed at a rate of one minute/kg in a twin-shell Patterson-Kelly blender fitted with an intensifier bar. Three hundred grams (pretreatment) or 400 grams (week 1-term.) of the appropriate diet was presented to each dog once daily.

All of the dogs were observed daily for mortality, appearance, behavior, appetite, elimination, and signs of toxic and pharmacologic effect.

These observations were recorded daily for the first six weeks of treatment and at least once weekly for Weeks 7 and through termination unless adverse clinical signs were observed. In these instances signs were recorded on a daily basis. Individual body weights were recorded twice prior to treatment (week 1 and week 0, respectively), weekly for weeks 1-8, biweekly for weeks 20-26, every four weeks for weeks 30-102 and at termination (week 104). Individual food consumptions were recorded daily beginning one week prior to the initiation of treatment.

Clinical laboratory studies were performed on all dogs twice prior to treatment (weeks -3 and -1) and at weeks 4, 13, 26, 52, 82 and 104.

Hematology: hematocrit, hemoglobin, RBC count, RBC morphology, total and differential WBC counts, and reticulocyte counts.

Clinical Chemistry: Alkaline phosphatase, BUN, fasting blood sugar, SGPT, SGOT, total protein, total cholesterol, creatinine, and protein electrophoresis.

Urinalysis: appearance, pH, specific gravity, glucose, ketones, total protein, bilirubin, occult blood, volume, reducing substances, and microscopic examination of the sediment.

Blood samples for the hematology determinations were collected by jugular puncture from dogs which had been food and water fasted overnight prior to collection. The urine samples were collected from cage floor run-off. Approximately sixteen hours prior to urine collection, all of the dogs were water loaded by oral intubation at a level of 25 ml/kg. Following water loading, the dogs were returned to their cages and water fasted overnight. On the following morning the collection interval began and the first voided sample was collected and submitted for analysis.

Ophthalmologic examinations were performed on all dogs initially (week -3) and at weeks 13, 26, 52, 82 and 104 using a hand held slit lamp, a binocular indirect ophthalmoscope, and tropicamide ophthalmic solution as a mydriatic.

Neurological and physical examinations were performed on all dogs pretreatment (week -3), at weekly intervals during the first thirteen weeks of study, biweekly for weeks 15-25, monthly for weeks 30-52 and on eight occasions between weeks 68 and 104. The neurological examinations consisted of evaluating pupillary response to light; pain perception; gait; and the extensor thrust, placement, righting, and patellar (knee) jerk reflexes. The physical examinations consisted of a visual evaluation of the condition of the dogs and heart and lung auscultations.

With the exception of one mid-dose female, two males and two females from the control and each of the compound-treated groups were sacrificed after fifty-two weeks of study (week 53 interim sacrifice). The remaining dogs, were sacrificed after 104 weeks of study (week 105 terminal sacrifice). Sacrifice was by exsanguination while under the influence of Surital anesthesia (Sodium Thiomybal for injection, N.F., Parke, Davis and Company, Detroit, Michigan). Necropsies were performed under the supervision of a staff pathologist on all dogs which died or were sacrificed.

The following organs from each sacrificed dog were weighed and the organ/body weight ratios were determined: brain, pituitary, thyroids, heart, liver, spleen, kidneys, adrenals, testes (males) or ovaries (females) and uterus (females).

The following tissues from all sacrificed dogs were preserved in 10% neutral buffered formalin: brain, pituitary, spinal cord, eyes, salivary gland, thyroids esophagus, lungs, heart, liver, gall bladder, spleen, kidneys, adrenals, stomach, pancreas, small intestines (duodenum, jejunum, ileum), large intestines (cecum, colon, and rectum), lymph nodes (mandibular and mesenteric), urinary bladder, gonads, prostate (males), uterus (females), skin, rib junction, femur with marrow, sciatic nerve with adjacent muscle, mammary gland, larynx, and any unusual lesions.

With the exception of the spinal cord, cecum, larynx, rectum, and skin, all of the preserved tissues from the control and high-dose dogs (Groups 1 and 8) were embedded in paraplast, sectioned stained with hematoxylin and eosin and examined microscopically. The following tissues from the low- and mid-dose dogs (groups 6 and 7) were processed in a like manner and examined microscopically: adrenals, brain, gonads, heart, kidneys, liver, lungs, thyroid with parathyroid and any unusual lesions.

Statistical analysis of the data was performed.

Results:

One group 7 (mid-dose) female was found dead during week 35 from unexplained causes and one group 8 (high-dose) male was sacrificed moribund during week 83 following deterioration of its physical condition.

Marked inappetance and resulting losses in body weight were noted during the first week of study for the group 8 dogs. Because of this, the dogs were placed on control diet for days 9-14 and returned to treatment at a level of 2800 ppm on day 15. The level was further reduced to 2000 ppm beginning on day 29 and continuing until termination of treatment.

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(conc. from last para.)

With the exception of the group 6 males for weeks 0-26 and 0-104, a dose-related decrease was noted for the mean body weight changes of the compound-treated dogs at all of the intervals evaluated. Most of the decreases for the high-dose males and females were statistically significant.

A treatment-related thin appearance was noted in two group 8 males by the end of the fourth week of study which persisted through week 17 and again observed at weeks 29 and 30 in one of the dogs. Other scattered incidences of a thin appearance were also noted in group 8 dogs during the study. Additional treatment-related signs observed in the group 8 dogs were an increased incidence of lacrimation and sores on the muzzle.

The mean hematocrit and hemoglobin values of the group 8 males and females were consistently, and frequently significantly, lower than the respective control values at all treatment intervals as were the mean erythrocyte count values of the group 8 females at week 13 and males and females at weeks 26, 52, 82, and 104. In most incidences through week 52, these changes occurred because of low individual values for one male and one female and a general trend toward anemia was not indicated. The individual hematocrit, hemoglobin and erythrocyte count values of the three surviving group 8 males at week 104 were all lower than any of the individual control or groups 6 and 7 male values and the mean values were statistically significantly lowered. These findings must be considered a result of treatment with RH-2915.

Evaluation of the clinical chemistry results revealed a dose-related increase in the mean alkaline phosphatase values which, in most instances for the group 8 females, was statistically significant.

Additional treatment-related changes in the clinical chemistry studies included the total cholesterol and total protein values. The group 8 mean total cholesterol values for the females at week 13 and for the males and females at weeks 26, 52, 82 and 104 were slightly but consistently lower. Protein values of the group 8 males and females at all treatment intervals were consistently lower than the control total protein values with many of the findings through week 82 being statistically significant.

During the week 104 ophthalmologic examinations, epiphora was noted in many of the group 8 dogs. During the physical and neurological examinations, thinness and an unthrifty appearance were noted in five of the group 8 dogs at week 3 which persisted through week 6 and three of the surviving group 8 dogs appeared thin at week 91 with the finding persisting through to termination of treatment. All of these observations substantiate the clinically observed findings of lacrimation and thinness.

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Treatment-related increases occurred for the absolute and relative liver weights of the PH-2915 treated dogs. These changes were increased absolute liver weights for the groups 6, 7 and 8 males and the group 8 females sacrificed after fifty-two weeks of treatment and the groups 7 and 8 males and the group 8 females sacrificed after 104 weeks of treatment. The relative liver weights of the groups 6, 7 and 8 males and females showed a dose-related increase at both the 52 and 104 week sacrifices. The absolute and relative liver weights of the groups 7 and 8 males, the absolute liver weights of the group 8 females, and the relative liver weight of the groups 7 and 8 females were statistically significantly increased over the respective control values at the terminal sacrifice interval.

No distinct treatment-related changes were noted at gross pathology. No treatment-related histopathological findings were observed in the dogs sacrificed after fifty-two weeks of treatment.

At the terminal sacrifice, compound-related histopathologic changes included minimal to moderate bile pigmented hepatocytes in the liver of four group 8 males, four group 7 females, three group 6 males, four group 8 females, four group 7 females and one group 6 female. The relationship of test compound exposure to renal tubular epithelial vacuolization in three group 8 males, three group 7 males, and two group 6 males; and lymphocytic thyroiditis, frequently with follicular atrophy and/or diffuse "C" cell hyperplasia in the thyroids of two group 8 males, one group 7 male, two group 8 females, one group 7 female, and three group 6 females is not clear. Minimal to moderate bile pigmented hepatocytes were also seen in the livers of two group 1 males and one group 1 female.

Minimal tubular epithelial vacuolization was observed in the kidneys of three group 1 males.

Various spontaneous disease lesions and incidental findings were of a similar frequency and severity in control and treated males and females with a few sporadic exceptions.

The pituitary of one group 8 male, one group 6 male, and two group 8 females contained cysts/microcysts. Focal/multifocal thyroid "C" cell hyperplasia was frequently noted in control and treated males and females. Focal nodular thyroid "C" cell hyperplasia was noted in a group 6 male. The thymus of three group 1 males, two group 8 males, three group 1 females and four group 8 females contained cysts/microcysts.

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The lungs of treated and control males and females contained various spontaneous lesions, the most common of which was chronic peribronchiolitis, and/or alveolitis and/or pleuritis.

Splenic siderotic nodules were seen in one group 8 male, one group 6 male, one group 1 female and one group 6 female. Splenic nodular lymphoid hyperplasia was noted in a group 8 female.

Additional spontaneous disease lesions and incidental findings were reported.

Conclusion: No NOEL was established in this study. The LEL is 100 ppm and the effects consisted of bile pigmented hepatocytes, dose-related liver weight increase, dose-related alkaline phosphatase, renal tubular epithelial vacuolization and lymphocytic thyroiditis frequently with follicular atrophy and/or diffuse "C" cell hyperplasia.

Classification: Core-Minimum Data

TS-769:TOX/HED:th:CM#2:Rm. 816:X73710:9-24-80

WMB/nalb