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DATA EVALUATION RECORD

CHEMICAL : Aluminum tris (o-ethyl phosphonate)
Trade Name : Fosetyl-Al

FORMULATION : Technical

CITATION : SPICER, E.J.F., 1981
Fosetyl-Al - Two year dietary Toxicity in dogs

CONTRACTING LAB. : International Research and Development Corporation (IRDC) - Mattawan, Michigan

SPONSOR : RHONE-POULENC AGROCHIMIE - LYON, FRANCE

REPORT NO. : 347-023 of 10/19/1981

EPA Reg. No. Acc. No. 247167

REVIEWED BY : A. F. PELFRENE, MD, PhD, ATS
Director of Toxicology
RHONE-POULENC INC.
Pelfrene C. Gubois
10/20/82 10-20-82

REVIEWED ON : June 10, 1982

TEST TYPE : Chronic feeding study

TEST MATERIAL : FOSETYL-AL technical
Purity : 96.9%
Batch No. DA136

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MATERIALS AND METHODS

ANIMALS

Thirty two male and thirty one female pure bred Beagle dogs, approximately 3-4 months old, were supplied by Ridglan Research Farms, Mt. Horab, Wisconsin. They were quarantined for approximately 4 weeks prior to initiation of the treatment. During this period they all were observed for physical condition and general health, de-wormed and immunized against distemper, hepatitis and leptospirosis and vaccinated against rabies.

Immediately prior to initiation of the study, 24 males (body weight range 5.2 to 9.7 kg) and 24 females (body weight range 4.9 to 8.6 kg.) were selected and randomly (computerized pseudo-random number generator method) assigned to one of the three treatment groups or to the control group (6 animals/sex/dose).

They were given a unique identification number (ear tattoo).

HOUSING AND MAINTENANCE

The dogs were individually housed in suspended cages and maintained in a temperature, humidity and light controlled environment.

Control and test diets were prepared from Purina Canine Diet. Tap water was freely available.

Test Article Administration

Fosetyl-Al was administered to the dogs at the following concentrations in the diet : 10,000 - 20,000 and 40,000 ppm. Control dogs received the basal diet only.

For each group, Fosetyl-Al was added in appropriate amounts to the basal diet and mixed in a twin-shell blender for 20 minutes. Fresh batches of control and test diet were prepared each week. Representative control and test diet samples from top, middle and bottom of each batch (weeks 1 to 4) and single samples thereafter were collected on days 0 and 7 of each of the following weeks 1,2,3,4,8,12,27,40,53,66,79,93,105, and analyzed for homogeneity and concentration. Concentrations found were always very close to the nominal concentrations for each batch at each sampling interval, as shown in the following table.

Week	CONCENTRATIONS FOUND (in ppm)		
	Low concentration (10,000 ppm)	Intermediate concent. (20,000 ppm)	High concantration (40,000 ppm)
1	10,116	19,733	43,749
2	10,565	24,095	42,319
3	9,296	21,090	45,094
4	10,245	19,638	41,625
8	8,325	25,530	40,043
12	10,117	20,735	44,672
27	9,729	18,637	37,686
40	9,013	21,434	43,675
53	9,825	21,013	41,958
66	10,112	23,532	42,124
79	9,574	20,812	44,801
93	9,224	18,448	NA
105	10,723	22,489	46,367

GENERAL OBSERVATIONS

Appearance and behavior:

The dogs were observed daily for signs of overt toxicity, morbidity, moribundity and mortality.

Body weights:

Individual body weights were recorded weekly, beginning with the pre-test period.

Food and compound consumption:

Individual food consumption was measured and recorded weekly beginning with the pre-test period. From these values, mean compound intake and food efficiency values were calculated.

Ophthalmoscopy:

Eye examinations were performed on all dogs once during the pre-test period and at 1-2-3-6-9-12-15-18-21 and 24 months of study.

Clinical laboratory tests: Were performed twice on all dogs, during the pre-test period and then again at 1-2-3-4-5-6-12-18 and 24 months of the study.

The dogs were fasted overnight prior to blood sampling via puncture of the juglar vein.

Urine was collected during the 16 hour fasting period from animals individually housed in metabolism cages.

The following parameters were evaluated:

- HEMATOLOGY:
  - hemoglobin
  - hematocrit (packed cell volume)
  - RBC count
  - WBC count (total and differential)
  - platelet count
  - reticulocyte count
  - mean corpuscular hemoglobin concentration (MCHC)
  - mean corpuscular volume (MCV)
  - mean corpuscular hemoglobin (MCH)
  - heinz bodies
  - erythrocyte sedimentation rate (ESR)
  - methemoglobin (18 month interval only)

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BIOCHEMISTRY :

- sodium
- potassium
- chloride
- calcium
- blood urea nitrogen (BUN)
- alkaline phosphatase
- total and direct bilirubin
- serum glutanic oxaloacetic transaminase (SGOT)
- serum glutanic pyronic transaminase (SGPT)
- lactic dehydrogenase (LDH)
- total protein
- cholesterol
- glucose
- cholinesterase (at termination brain - RBC - plasma)
- protein electrophoresis

URINALYSIS :

- color
- appearance
- volume
- microscopic examination of sediment
- specific gravity
- pH
- protein (albumin)
- glucose
- occult blood
- nitrites
- urobilinogen
- ketones
- bilirubin

PATHOLOGY

After 24 months of administration of test compound, 23 male and 24 female dogs were sacrified and each animal given an external and internal post-morten examination.

The following organs were weighed :

- Adrenals - heart - kidneys - liver - pituitary - testes - ovaries - thyroïd with parathyroïd -.

Bone marrow smears were taken from the rib and fixed in methanol.

Representative section of the following tissues were collected, fixed in phosphate buffered neutral formalin and then processed for histopathological examination :

adrenals	lung (with mainstem bronchi)
aorta	mesenteric lymph nodes
nasal cavity	striated muscle
brain	skin
eye and optic nerve	mammary gland
gall bladder	sciatic nerve
heart	spleen
trachea	pancreas
tongue	pituitary
esophagus	prostate
stomach	corpux and cervix uteri
duodenum	salivary gland
ileum	spinal cord
jejunum	testis
cecum	ovaries
colon	epidioymis

rectum	thymus
kidneys	thyroid with parathyroid
liver	urinary bladder
larynx	bone
	bone marrow smear

Any other tissues which appeared grossly abnormal were also collected.

STATISTICS

All numerical values were statistically evaluated in order to compare the treatment groups with the control groups by sex.

Appropriate methods were used:

- analysis of variance
- Barlett's test for homogeneity of variances
- t-test for equal and unequal variances
- Dunnett's multiple comparison tables.

RESULTS

General observations

Appearance and behavior:

Incidental findings notes for both control and treated dogs included soft stool and diarrhea, infection of sclera, relaxed nictiting membranes, emesis, ptyaliom, dermatitis, alopecia, lacrimation. These observations were randomly distributed among all groups.

Several dogs from all treatment groups (with a higher frequency found in the 40,000 ppm dosage group) showed "parts of stool covered with grey elastic-like material" or "grey material". This observation was first made at approximately one year of study. A fecal sample obtained from a 40,000 ppm male dog was analyzed for test article content. The results from this analysis suggested that the material in question was water insoluble, contained traces of aluminum tris (O-ethylphosphonate) and phosphoric acid equivalents and the white flakes themselves, were constituted of an unknown substance and not the test article.

Mortality :

One animal died (male in the 10,000 ppm dose) at week 81 of the study. No precise cause of death was determined at necropsy.

Body weights:.

Throughout the 104 weeks of study, no statistically significant compound related differences were evident in the group mean body weights for the treated group when compared to the controls.

However, there was a very slight nonsignificant reduction in the percentage change in mean body weights, at week 104 from pre-test, for all treated groups when compared to the controls as shown in the following table:

STUDY WEEK	CONTROL 0 ppm		10,000 ppm		20,000 ppm		40,000 ppm	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
PRE-TEST	7.5 (1.54)	6.4 (0.95)	7.2 (1.42)	6.8 (0.97)	6.7 (0.77)	6.2 (0.62)	7.7 (0.70)	6.5 (1.34)
104	12.7 (3.24)	11.3 (3.61)	12.7 (1.61)	11.4 (2.77)	11.2 (1.80)	10.3 (2.04)	12.7 (2.39)	10.7 (3.45)
WEIGHT GAIN [a]	+69.3	+76.5	+76.3	+67.6	+67.1	+66.1	+64.9	+64.0

(a) percent gain over initial weight

Food and compound consumption:

No dosage - or compound - related trends could be established in mean food controls despite incidental statistically significant increased or decreased values observed.

Efficacy of feed conversion:

Showed no differences between groups.

Opthalmoscopic examination:

The observations, noted during the 24 months of the study were only representative of the pathology that would be expected for these groups of dogs considering age, sex and strain.

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No trends in pathology suggestive of test compound related effects could be established.

Clinical laboratory test

Hematology:

A number of statistically significant differences from control values were seen sporadically throughout the study in different dose groups and at different intervals (RBC count - hemoglobin - PCV - platelet count - etc...). However, the lack of consistent trends in these variations deprived them from any toxicological significance.

Blood chemistry:

Statistically significant variations from control values were seen for sporadic values at various intervals for glucose - SGPT - SGOT - LDH and potassium in males and for BUN - SGOT - total and direct bilirubin in females.

There was a slight but consistent reduction in total protein in males treated with 40,000 ppm for the 12-18 and 24 month intervals. Slight but significant reductions were seen in BUN in females on several occasions in the high dose group.

The biological significance of BUN reduction is questionable.

Urinalysis:

There were no biologically significant differences between groups.

Pathology

Macroscopic:

There were no macroscopic lesions which could be attributed to an effect of the compound. Various gross variations from normal were observed in each treated and control groups. They were considered as common findings considering this strain of laboratory dogs, age and sex.

They were randomly distributed among groups.

Organ weights:

No statistically significant variations in the weights of any of the organs that were weighed in any group.

Histopathology:

Microscopic evaluation of histology slides was performed by an outside consultant (D.E. Gordon, Highland Park, Illinois).

There was a variety of microscopic observations among all sexes and treatment groups. They were regarded as incidental, spontaneous with regard to strain, age and sex or agonal lesions unrelated to treatment with the test compound. They occured in most instances with a comparable incidence and severity among both control and treated animals.

There were compound related significant changes confined to the testis of animals treated at the intermediate (20,000 ppm) and high (40,000 ppm) concentrations. The incidence was respectively 2/6 and 6/6.

Microscopically the lesions consisted of the presence of spermatocytic and/or spermatidic giant cells with the lumen of seminiferous tubules. Their severity was also dose-related. In group 3 (intermediate), the lesions were focal, involved one or both gonads and were graded as "trace".

In group 4 (high), the lesions were more numerous focal to multifocal, bilateral but similar in severity to those in group 3 ("trace").

In the epididymis of group 4 (high dose animals, the ducts revealed scant amounts of cellular debris from the testis and or absence of sperm.

A slight increase in severity of a naturally occurring vascular tubular variation in the inner renal cortex was seen among females treated at the intermediate and high dose groups. The incidence did not seem to be affected by the dosage since 5 out of 6 controls were affected compared to 4/6 - 6/6 and 6/6 in the low intermediate and high dose groups respectively. The "lesion" was graded as "trace" among control and low dose affected animals and as "trace" to "mild" in all except one female in intermediate and high dose groups. One female in group 4 (high) had a "lesion" which was graded as "moderate". Histologically, it consisted of bilateral, cytoplasmic vacuolation of the distal convoluted tubules in the inner renal cortex. The actual toxicological significance of such a finding is questionable, as it might only reflect a physiological state of the normal kidney function since such "variation" was also seen in 5 out 6 control animals.

DISCUSSION

There were very few biologically meaningfull variations seen in the auto-mortem phase of this study.

The clinical laboratory studies showed no evidence of any treatment related findings of any biological or toxicological significance. The reductions of total proteins in males at 40,000 ppm and in BUN in females at the same dose level were not considered meaningful in the absence of related laboratory or pathology findings.

There were no opthalmoscopic treatment related observations.

Histopathological examinations revealed a slight testicular effect at the intermediate (20,000 ppm) and high (40,000 ppm) levels in 2 of 6 and 6 of 6 males respectively.

CONCLUSION

Feeding of Fosetyl-Al to male and female Beagle dogs at concentrations of 10,000 - 20,000 and 40,000 ppm in the diet for two years did not induce any toxic effect on the biological parameters (clinical, hematological, biochemical parameters) and induced a slight degenerative effect on the testis of intermediate and high dose males.

- Based on histopathological findings the No Observed Effect Level is considered to be 10,000 ppm and the Lowest Effective Level (LEL) is 20,000 ppm.

CLASSIFICATION : Minimum.

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