005444

STUDY TYPE	2 Year feeding/ONCO - Rats
CHEMICAL	Aluminum tris (0-ethyl phosphonate) Trade Name: Fosetyl-Al.
ACCESSION NUMBER	247162-166
MRID NUMBER	Not assigned.
CITATION	Spicer, E.J.F. 1981 - Fosetyl-Al (LS 74-783) Chronic toxicity (2-year) and carcinogenicity study in rats
CONTRACTING LAB	International Research and Development Corporation. (Report No. 347-016.
SPONSOR	Rhone-Poulenc Agrochimie, Lyon, France
DATE	March 27, 1981
TEST MATERIAL	Fosetyl-Al Technical Batch No. DA 67 purity 99.7 ± 0.3% DA 68 purity 99.5 ± 0.3%

Material and Methods

Three hundred and twenty male and 320 female Charles River CD rats supplied by Charles River Breeding Laboratories, Inc., Wilmington, MA were housed individually in hanging wire-mesh cages and maintained in temperature (65-78°F), humidity and light (12 hour light - 12 hour dark) controlled rooms. Air renewal in the study rooms ranged from 6 to 10 changes/hour. Water and diet were freely available.

The rats were ear tagged for individual identification. The body weight range upon receipt of the animals was 68-140 grams for males and 50-123 grams for females.

Test Compound Administration

The rats (80/sex/dose) were distributed by use of a computer-generated table of random numbers at doses of 0, 2,000, 8,000 and 40,000 ppm Fosetyl-Al for 2 years. The high dose level was reduced to 30,000 ppm after 2 weeks following observation of staining of the abdominal fur and red coloration of the urine. Reduction to 30,000 ppm resulted

in reversal of the above mentioned adverse effects. The control group received only the standard basal diet (Ground Purina Chow No. 5001, Ralston Purina Co.).

The treated diet was prepared by grinding technical Fosetyl Al using a mortar and pestle and mixing with a small amount of basal diet in a Hobart blender. The premix was then mixed with the appropriate amount of basal diet in a twin-shell blender for 5 minutes with an intensifier bar and an additional 10 minutes without the bar. Diet mixes were routinely analyzed for concentration and homogeneity on weeks 1, 13, 26, 39, 52, 65, 78, 91 and 104 of the study. (See table on page 3).

General Observations

Animals were observed twice daily, 7 days a week for signs of overt toxicity, moribundity and mortality. Signs were recorded on the day noted and detailed observations were recorded weekly. Individual body weights, food and compound consumption were also recorded weekly.

Opthalmoscopy

Opthalmoscopic examinations of all rats were conducted in the pre-test period and at 3, 12 and 24 months of study.

Concentration and Homogeneity of Fosetyl-Al

CONCENTRATIONS FOUND (in ppm)			
WEEK	LOW CONCENTRATION	MEDIUM CONCENTRATION	HIGH CONCENTRATION
1	1,812	9,679	47,281
13	2,036	9,530	38,806[a/]
26	1,933	9,145	45,775[a/]
39	1,847	8,298	29,874*
52	1,814	8,404	29,797
65	2,027	8,959	Not analyzed
78	2,121	7,919	32,825
91	1,943	8,488	35,290
104	2,142	8,389	34,500

* Dose level decreased from 40,000 ppm to 30,000 ppm on week 2.

a/ Note the discrepancy in diet concentration levels at weeks 13 and 26. The report indicates that high dose diet was changed at week 2 of the study.

Clinical Laboratory Tests

At 3, 6, 12, 18, and 24 months, blood and urine samples were obtained from 20 male and 20 female rats from each group. Blood was obtained via puncture of the orbital sinus plexus, the rats were housed overnight in stainless steel metabolism cages for urine collection. Food and water were withheld overnight prior to sample collection.

Hematology

Hematological parameters included: hemoglobin, hematocrit, erythrocyte count, reticulocyte count, Heinz bodies, methemoglobin, total and differential leucocyte counts, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and prothrombin time.

Biochemistry

Biochemical studies included: glucose, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase, serum electrophoresis, sodium, potassium, cholesterol, bilirubin and total protein.

Urinalysis

Urinalysis included: volume, pH, specific gravity, color and appearance, albumin, glucose, occult blood, bilirubin, urobilinogen and ketones, and microscopic examination of the sediment.

Pathology

After two years of compound administration, all surviving rats were sacrificed by carbon dioxide asphyxiation, weighed and necropsied. After a thorough external examination, each animal was sacrificed and viscera from all body cavities were examined in situ and after dissection. All abnormalities observed were described. The following organs were trimmed and weighed intact: spleen, liver, kidneys, testes, heart, brain, adrenals. Thyroid and ovaries were weighed after fixation. Bone marrow smears were prepared from a femur.

Representative sections of the following tissues were collected and preserved in phosphate-buffered neutral 10% formalin:

aorta
nasal cavity
brain
femur-tibia
eye + optic nerve
heart
esophagus
stomach
duodenum
jejunum
ileum
cecum
colon
rectum
kidneys (both)
liver
larynx
trachea
lung and bronchi
mandibular lymph nodes
spleen
pancreas
pituitary (intact)
prostate/uterus
seminal vesicles
sternum (bone marrow)
skin
mammary gland
spinal cord (cervical)
testis + epididymis/ovaries
thymus
thyroid and parathyroid
urinary bladder
thigh muscle
sciatic nerve
salivary gland
tongue

Any other tissue with abnormalities.

Animals found dead or sacrificed moribund were necropsied as above with the following exceptions: when possible a cause of death was established for animals found dead, no body or organ weights were taken, bone marrow smears were not prepared.

Histopathology

All wet tissues were shipped from IRDC to American Histolab, Rockville, MD for histological processing. The slides prepared by American Histolab were sent to Tracor-Jitco Inc., Rockville, MD for microscopical evaluation. Slides were prepared from all tissues for all animals.

Statistics

Statistical analyses compared the treatment groups with the control groups, by sex. Body weights, food consumption, hematological, biochemical and urinalysis parameters and absolute and relative organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal and unequal variances). Dunnett's multiple comparison tables were used to judge significance of differences.

Statistics on tumor incidence were performed by Tracor-Jitco, Inc. The incidences of individual tumor types in high-dose and control groups were compared using Fisher's

exact test, except where the number of tumors in the high-dose group was too small to be of probable statistical significance.

RESULTS

Appearance and Behavior

During the first two weeks of study about 27% of the rats of the high-dose level (40,000 ppm) showed urine staining of the abdomen and red coloration of the urine; because of the poor conditions of the affected animals, the dose level was reduced to 30,000 ppm. These signs were not observed after reducing the dosage.

No changes considered to be compound-related were noted after the high-dose level was adjusted. Incidental findings were seen in similar numbers for treated and control animals.

Masses were seen more frequently in female rats (both treated and controls) than in male rats.

Mortality: Survival through 104 weeks of study was slightly lower for the treated rats (male and female) when compared to the controls, as seen in the following table:

MORTALITY

Dose	Number Surviving/Number Initiated			
ppm	Males		Females	
0	39/80	(48.75%)	43/80	(53.75%)
2000	36/80	(45.00%)	37/80	(46.25%)
8000	29/80	(35.25%)	40/80	(50.00%)
30000	35/80	(43.75%)	40/80	(50.00%)

Body Weights: During the first two weeks of study, rats at the high-dose level (40,000 ppm) showed lower weights (12% in male and 9% in female) when compared to the control rats. After reduction of the concentration level the rats gained slightly more body weight.

At 104 weeks of study, differences in group mean body weights for treated animals when compared to the controls were not indicative of a compound-related effect, with the possible exception of the high-dose females (-8.8% of controls).

Food and Test Article Consumption: In the first week of study, food and test article consumption were lower for the high-dose rats when compared to control rats. Thereafter, through 104 weeks of study, these parameters were similar for the high-dose males and all treated females when compared to the controls. After 25 weeks of study a slight but statistically significant decrease in average food consumption was noted for the mid- and low-dose males at almost all time intervals. This was not considered to be treatmentrelated, and the food efficiency values were similar for control and treated rats.

Opthalmoscopic Examinations: No changes considered to be related to compound administration were seen during the 3 and 12 month opthalmoscopic examinations. At the 24-month examination, the observations noted were representative of pathology that would be expected considering age, sex and strain. No obvious trends in pathology suggestive of test material related reactions were observed.

Hematology: No changes considered to be related to compound were seen in the hematological parameters.

Biochemistry: No changes considered to be related to compound administration were seen in the biochemical parameters.

Urinalysis: At 6 months of study, albumin protein in the urine was noted more frequently for treated male rats when compared with control male rats. At 12, 18 and 24 months, the number of male rats with albumin protein present in the urine sample was increased for all groups, including the control group. Presence of albumin in male rat urine is common and usually not regarded as being of any pathological significance. However, in this case, it seems that the amount of protein was progressively increasing both with dose level and time on study.

Gross Pathology: Gross lesions were of the type commonly encountered in aged, laboratory-housed Sprague-Dawley rats, except for urinary bladder lesions, (including calculi and mineralization), and pheochromocytomas which were seen in treated males only. (Description in Microscopic Pathology section).

Organ Weights: Statistical comparison of the organ weights showed incidental, sporadic differences in absolute weights of some organs without a consistent trend in pattern. The toxicological significance of relative weight increases in kidney weights in females at the two highest dose levels is questionable and this observation was not considered to be due to the treatment.

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However, the same effect occurred in both sexes in 90-day rat study IFREB-R 77-03-5

Microscopic Pathology

a. Male Rats: An increased incidence of transitional cell carcinoma (no metastases observed), and transitional cell papilloma were observed in the urinary bladder of males at the high dose group (40,000/30,000 ppm) when compared in controls. See the following table:

Urinary Bladder (Males)

Lesion	Control	2000 ppm	8000 ppm	40000/30000 ppm
Transitional cell carcinoma	1/78	0/80	0/81	5/79
Transitional cell papilloma	0/78	1/78	1/81	9/79*
Total	1/78	1/78	1/81	14/79*

* P = 0.05 (Fisners Exact Test).

"Tumors judged to be malignant had either a significant degree of anaplastic cells, invasion of basement membranes and underlying tissues, or both. No metastases were observed."

"Hyperplasia and inflammation of the urinary tract...were characterized by an increase in thickness of the transitional elithelium forming folds and small club formed processes on the luminal surfaces. Transitional cells were hyperplastic, sometimes hypertrophied but not generally dysplastic and these proliferations were contained by basement membranes. Acute and/or acute and chronic inflammation frequently accompanied the hyperplastic lesions but in many cases inflammation occurred without appreciable hyperplasia. Calculi, mineralized deposits, and proteinaceous casts were occasionally seen histologically. They were more frequently described grossly and their less frequent presence in histological sections would suggest loss due to handling and/or processing. Cortical cysts and hydronephrosis were also more common in the kidneys of the high treatment animals reflecting obstruction or interference with urinary flow by proliferative processes or calculi."

The registrant has stated that "the treatment-related increase in urinary bladder neoplasia in males at 40,000/30,000 ppm is most likely secondary to the presence of calculi and mineralization in this group. The latter condition is almost certainly related to the considerable intake of phosphorus present in the test compound, resulting in an unbalanced phospho-calcic metabolism as evidenced by the results of a

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side study performed in rats fed for a month at dose levels of 10,000 ppm, 20,000 ppm and 40,000 ppm. The male rats had a marked increased level of urinary calcium excretion and a decreased urinary phosphorus level. Fecal phosphorus excretion was increased. These variations were clearly dose and sex related. This increase in the level of urinary calcium is with no doubt a major factor in the formation of calculi and mineralization which in turn are responsible for chronic irritation of the bladder epithelium resulting in secondary reactional lesions of the bladder wall."

The registrant cites the following literature: "Cheng states that calculi are spontaneously present in the urinary bladder of male rats (50% of germ-free male rats). She also indicates that hypercalciuria is a metabolic contributing factor in the production of urinary stones.

Clayson states that, based on data from the literature, there is a correlation between bladder stone and bladder tumors in rats and mice.

The Registrant further states "Therefore it is strongly felt that the increased incidence in epithelial neoplasia in the urinary bladder of the rats treated at the highest dose level is only secondary to the chronic irritation of the bladder walls induced by the presence of stones and mineral deposits itself resulting from unbalanced calcium excretion secondary to high intake of phosphorus from the test article. A direct relationship between Fosetyl-Al and epithelial lesions of the urinary bladder cannot be made."

An increased incidence of pheochromocytoma was observed in males at the mid dose group (8000 ppm) and the high dose group (40,000/30,000 ppm) when compared to control males. See the following table:

Pheochromocytoma (Males)

Lesion	0	2000 ppm	8000 ppm	40000/30000 ppm
Benign	5/80	7/79	15/81	16/81
Malignant	1/80	0/79	1/81	2/81
Total	6/80	7/79	16/81*	18/81*

* P = 0.0095 (Fishers Exact Test)

b. Female Rats:

No compound related lesions were observed in females.

CONCLUSION: Based on the submitted data, Fosetyl-Al is oncogenic in rats.

Systemic NOEL = 2000 ppm
Systemic LEL = 8000 ppm

Oncogenic NOEL = 2000 ppm
Oncogenic LEL = 8000 ppm

A provisional oncogenic risk assessment has been completed by Anne Barton (Deputy Division Director/HED) and Bert Litt (Toxicology Branch Statistician/HED) for whole pineapples and ornamentals. (See attached memo A. Barton to J. Akerman, dated June 23, 1983).

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