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DATA EVALUATION RECORD

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

FORMUALTION: Technical

CATATION: Spicer, E.J.F., 1981
Fosetyl-Al
24 month carcinogenicity study in mice

CONTRACTING LAB.: INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION (IRDC) - Mattawan, Michigan

SPONSOR: RHONE-POULENC AGROCHIMIE - LYON - FRANCE

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RHONE-POULENC INC. C. Gregorio

10/20/82 10-20-82

REVIEWED ON: June 14, 1982

TEST TYPE: Chronic feeding study

TEST MATERIAL: FOSETYL-AL technical
purity 96.9%
batch No. DA136

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MATERIAL AND METHODS

ANIMALS AND MAINTENANCE

Three hundred and seven male and three hundred and ten female Charles River CD-1 mice approximately 4 weeks old, were received from Charles River Breeding Laboratories, Wilmington MA. and acclimated for 12 days to the laboratory conditions.

Twice during this period of acclimatation, 10 male and 10 female mice were bled to obtain baseline data for clinical pathology, sacrificed and discarded.

At the end of this 12 day period, 240 males (body weight range 23-28 g) and 240 females (body weight 19-24 g) in good health were randomly selected on the basis of their body weights and assigned to three treatment groups or the control group (60 males and 60 females each). The mice were identified by ear punching and individually housed in suspended wire-mesh cages and maintained in a temperature, humidity and light controlled environment. The mice were fed on control or test diets prepared from Purina Laboratory Chow or certified Purina Rodent Chow. Tap water was freely available.

TEST ARTICLE ADMINISTRATION

Fosetyl-Al was administered to the mice in the diet at dosage levels of 2,500 - 10,000 and 20,000/30,000 ppm*. Control mice received basal laboratory diet only. Fosetyl-Al was added to basal diet on a weight to weight basis and mixed in a twin shell blender with an intensifier bar for 20 minutes with the intensifier bar run for the first and last two minutes of the blending period. Fresh batches of test diet were prepared each week. After each blending procedure duplicate 100-gram samples from the top, middle and bottom of each batch were collected for analysis. Homogenicity and concentration were evaluated on samples collected on days 0 and 7 of each of the following weeks: 1-3-4-9-12-25-38-52-65-78-91-104. The concentrations found at each level of each batch at all sampling intervals were always very close to the nominal concentrations (see table below). A stability blending study was also conducted on a duplicate 100 gram sample allowed to stand for 7 days under the normal laboratory conditions.

x (dosage level increased after 18 weeks of treatment).

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CONCENTRATIONS POUND (in ppm)			
WEEK	LOW CONCENTRATION (2,5000)	MEDIUM CONCENTRATION (10,000)	HIGH CONCENTRATION (20,000/30,000)
1	2,379	10,099	23,772
3	2,193	11,516	20,282
4	2,188	9,794	23,197
9	2,516	10,584	21,982
12	2,320	10,570	20,240
25	2,567	10,694	31,801 *
38	2,384	10,189	30,026
52	2,510	10,143	32,745
65	2,640	10,523	34,410
78	2,740	11,516	38,156
91	NA	10,966	NA
104	2,146	8,702	25,227

*-Concentration increased from 20,000 ppm to 30,000 after 18 weeks of administration.

OBSERVATIONS

APPEARANCE AND BEHAVIOR : the mice wer observed 3 times daily Monday through Friday and twice daily on week-ends and holidays, for signs of overt toxicity and for mortality.

MORTALITY : moribundity and mortality were recorded daily.

BODY WEIGHTS : individual body weights were recorded weekly for the first 13 weeks and once every two weeks after.

FOOD CONSUMPTION AND FOOD INTAKE : Individual food consumtion was recorded weekly for the first 13 weeks and once every two weeks thereafter. Average food and compound consumtpion and food efficiancy values, by sex and group, were calculated during the measures food consumtpion periods.

CLINICAL LABORATORY TESTS : Hematological tests were conducted on 10 mice/sex twice during the pre-test period and on 10 mice/sex/group at 1 year of study.

Hematology, biochemistry and urinalysis evaluations were performed on all animals at 2 years of study. Mice were selected for baseline and 1-year testing using a computer-generated random number sequence. Blood was obtained via puncture of the orbital sinus plexux. Urine was collected overnight (approx. 16 hours) frcm animals housed individually in stainless steel metabolism cages.

Food and water were freely available prior to blood and during urine collections.

HEMATOLOGY : The following parameters were evaluated :

Hemoglobin
Hematocrit (Packed Cell Volume)
RBC count
WBC count (total and differential)
Platelet count
Reticulocyte count
Mean Corpuscular Volume (MCV)
Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin Concentration (MCHC)

BIOCHEMISTRY : The following parameters were evaluated :

Blood Urea Nitrogen (BUN).
Alkaline phosphatases
Serum Glutamic Oxaluacetic Transaminase (SGOT)
Serum Glutanic Pyruvic Transaminase (SGPT)
Total protein
Cholesterol
Glucose

URINALYSIS : included :

Color-appearance
pH
Protein
Ketones
Urobilinogen
Bilirubin
Glucose
Occult blood
Nitrites
Microscopic examination of sediment

PATHOLOGY

MACROSCOPIC : After 24 months of administration of the test compound, all surviving mice were sacrified (CO_2 asphyxiation) and each animal was given a complete post-mortem examination.

Representative sections of tissues and organs were collected and fixed in phosphate-buffered neutral formalin.

MICROSCOPIC : Hematoxylin and eosin paraffin sections of the following tissues and organs were prepared and examined by an outside consultant (Dr L.W. NELSON) :

Abdominal aorta	Lymph nodes (mediastinal mesenteric)
Adrenals	Mammary gland
Brain	Mandibular salivary gland
Eyes and Harderian glands	Sciatic nerve
Gall bladder	Pancreas
Gonads	Pituitary
Heart	Skin
Esophagus	Spinal cord
Stomach	Spleen
Cecum	Thymus
Colon	Trachea
Duodenum	Thyroid/parathyroid
Ileum	Urinary bladder
Kidneys	Prostate
Liver	Cervix uteri
Lung and mainstrem bronchi	Sternum (bone marrow)

as well as any other abnormal tissues.

In addition, 3 coronal sections through the head to include basal cavity, paranasal sinuses, oral cavity, nasopharynx and middle ear and one section of the tongue were prepared and examined from 10 mice/sex/group.

STATISTICS

All statistical analyses compared the treatment groups with the control group, by sex.

Numerical data were compared by analysis of variance, Bartlett's test for homogeneity of variance and the appropriate t-test (for unequal and equal variances) as described by STEELE and TORRIE[1], and OSTLE[2]-DUNNETT's multiple comparison tables were used to judge significance of differences.

The tumor incidences of individual types were compared using the Chi-square criterion with Yates'correction on 2 x 2 contingency tables.

(1) STEELE,R.G.D. and TORRIE,J.H., 1960 - Principles and procedures of statistics - McGraw-Hill book company inc - New York N.Y.

(2) OSTLE,B., 1954 - Statistics in research - Iowa State College Press.

RESULTS

GENERAL OBSERVATIONS

APPEARANCE AND BEHAVIOR

No obvious trends in physical appearance or behavior suggestive of a compound-related effect were observed. Incidental findings such as hair loss, scabbing, abrasions, labored breathing, tremors, palpable masses, eye lids red and swollen, swelling of neck, head or thorax region, abdominal swelling or firmness (more frequent in females) were noted for both the control and treated animals.

MORTALITY

There were no remarkable differences noted in survival for any of the treated groups when compared with the control after 24 months of study as shown in the following table:

SURVIVAL AT 104 WEEKS

Group ppm	Number of surviving/Number initiated Males	Females
0 (Control)	28/60	25/60
2,5000	34/60	27/60
10,000	29/60	25/60
30,000 (a)	29/60	34/60

(a) dosage level increased from 20,000 ppm at study week 19.

BODY WEIGHTS

Through 104 weeks of study, there were no remarkable changes in group mean body weights from treated animals when compared with the controls. A few of the intervals analyzed showed a statistically significant ($p<0.05$) decrease for the 30,000 ppm dose level, but no compound-related trend could be established.

FOOD CONSUMPTION

There were no remarkable change in group mean food consumption for the treated male groups when compared with the controls.

An occasional statistically significant ($p<0.05$ or $p<0.01$) decrease was seen for treated female group at a few intervals of analysis, but no dose related trends could be established.

There were no meaningfull differences in feed efficiency. The individual mean compound intake throughout the study was as follows (mg/kg/day):

	Males	Females
2,500 ppm	352	409
10,000 ppm	1408	1672
30,000 ppm	3,956	4,549

CLINICAL LABORATORY TESTS

HEMATOLOGY

A statistically significant ($p<0.05$) decrease in reticulocyte count was seen in the high dose male group (20,000/30/000 ppm) at 12 months when compared to control males. However this effect was not seen at 24 months

A statistically significant ($p<0.05$) decrease in segmented neutrophile count was observed in the 10,000 ppm and 20,000/30,000 ppm females at 12 months when compared to the control females; this effect was not seen at 24 months. In addition, a statistically significant ($p<0.05$) increase in lymphocytes was seen in the 10,000 and 20,000/30,000 ppm females at 12 months when compared to control females, again this effect was not seen at 24 months.

BIOCHEMISTRY

No statistically or biologically significant differences were seen in any of the parameters examined.

URINALYSIS

No statistically or biologically significant variations were observed in any of the parameters examined.

PATHOLOGY

MACROSCOPY

There were no macroscopic lesions which could be attributed to an eventual effect of the test compound. The changes reported were considered non specific, agonal or representative of the usual spontaneous alterations of this species and strain.

HISTOPATHOLOGY

No distinct or consistent compound related changes were observed in any tissues or organs examined miscroscopically from male and female mice at any dose level of Fosetyl-Al. The types or incidences of neoplasms identified in this study did not indicate any tumorigenic effects related to compound administration.

There was no signs of any earlier onset of any type of neoplasia in any treatment group when compared with controls.

DISCUSSIONS

The observations worthy to describe in this study are quite scarce. Despite this increase of concentration from 20,000 ppm to 30,000 at week 19 of study decided because of the absence of any effect in the early part of the study, there were no apparent effect on the anormals' behavior or appearance and no effect on food consumption or body weight. There were no meaningful changes in the clinical studies.

CONCLUSION

Fosetyl-Al when administered in the diet to mice of both sexes at concentrations of 2,500 - 10,000 and 30,000 ppm for 104 consecutive weeks did not induce any toxicological effects. No oncogenic effect was induced in mice at any dose level under the conditions of this test.

The dose level of 20,000/30,000 ppm may be considered as the maximum tolerated dose.

REVIEWER'S CONCLUSION

This study has been conducted and reported according to the current guidelines.

It is reasonable to consider that in spite of a lack of effect at the highest tested dose level, it was unnecessary to increase it above the selected level of 30,000 ppm.

CLASSIFICATION : Minimum.

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