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CHEMICAL	Aluminum tris (O-ethyl phosphonate) Trade Name: Fosety-Al
FORMULATION	MonoSodium Phosphite (metabolite of Fosetyl-Al)
CITATION	Spicer, E. J. F.. 1981. Lifetime Chronic Toxicity and Carcinogenicity in Rats
CONTRACTING LAB	International Research and Development Corporation (IRDC), Mattawan, MI
SPONSOR	Rhone-Poulenc Agrochimie, Lyon, France
REPORT N°	IRDC No. 347-022 of August 21, 1981
	EPA Reg. No. Acc. No. 247176-247182
REVIEWED BY	A. F. Pelfrene, MD, Ph.D, ATS Director of Toxicology Rhone-Poulenc Inc. Pelfrene 1-/20/92 C. Gregorio
REVIEWED ON	July 23, 1982
TEST TYPE	Chronic Feeding and Oncogenicity
TEST MATERIAL	Monosodium phosphite hydrated Purity: 98% Batch No. DA 117

MATERIAL AND METHODS

Animals and Maintenance

Three hundred and ten male and 323 female weanling Charles River CD rats (approximately 3 weeks old) were supplied by Charles River Breeding Laboratories, Michigan and acclimated to the laboratory conditions for 10 days. During this conditioning period, 26 (13 males and 13 females) were used to obtain base line values for clinical pathology, sacrificed and discarded.

Two hundred and forty males (body weight range 77-113g) and 240 females (body weight range 76-97g) with no physical or ocular abnormalites were randomly selected and assigned to either one of the three treatment group or to the control group which consisted of 60 males and 60 females each.

The animals were housed individually in hanging wire-mesh cages in an environmentally controlled room. They were fed Purina Laboratory Chow No. 5001 or Certified Rodent Chow No. 5002. Water was available ad libitum.

The rats were ear tagged for individual identification at study initiation.

Test Compound Administration

Monosodium phosphite was mixed in the diet at the following concentrations: 2,000 8,000 and 32,000 ppm expressed as the anhydrous salt (equivalent to 2,740 - 10,960 and 43,840 ppm of the hydrated salt, the compound containing 27% water). The controls received basal laboratory rat chow.

Monosodium phosphite was added to the basal diet on a weight to weight basis and mixed in a twin shell blender with an intensifier bar for 10 minutess.

Fresh batches of control and test diets were prepared each week. Homogeneity of the diet mixes was evaluated by periodically collecting 100g samples of each batch at all three concentrations and three sub-samples representing the top, middle and bottom of each batch) during the first 4 weeks of study and then on weeks 8, 12, 26, 39, 52, 65, 78, 91, 104 and 117 of study. The results of this extensive analytical work (16 reports of 12 analyses each appear in the main report) show that the actual concentrations were constantly within close range of the nominal concentrations and that homogeneity of the diet mixes was satisfactory.

GENERAL OBSERVATIONS

General Behavior and Appearance

The rats were observed twice daily, seven days a week for signs of overt mortality and for mortality and details accorded and reported on a weekly basis.

Mortality and Moribundity

The above were recorded on the day noted.

Body Weight

Individual body weights were recorded weekly for the first 13 weeks and once every two weeks thereafter.

Food and Compound Consumption

Individual food consumption was recorded weekly for the first 13 weeks and once every 2 weeks thereafter. Average food and compound consumption and food efficiency values, by sex and group, were calculated.

Opthalmoscopic Examinations

These were performed for all rats during the acclimation period and at 3, 6, 12, 18 and 24 months of study, following pupillary dilation with 1% tropicamide solution using a binocular indirect ophtalmoscope.

Clinical Laboratory Tests

These were performed for 10 rats/sex/group at various intervals throughout the study. Hematological tests were conducted at 4, 8, 12, 16, 20, 24 and 27 months of study, including the following parameters: Hematocrit, hemoglobin, erythrocyte count, total and differential leucocyte counts, reticulocyte and platelet counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Biochemical tests and urinalysis were conducted at 6, 12, 18, 24 and 27 months of study, including the following parameters: chloride, potassium, sodium, calcium, cholesterol, blood urea nitrogen (BUN), alkaline phosphatase, serum glutamic oxals acetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), albumin, glucose, direct and total bilirubin, lactic dehydrogenase (LDH), total protein, globulin.

Urinalysis includes measurement of volume, specific gravity, pH, description of color and appearance, microscopic examination of the sediment, bilirubin, glucose, ketones, occult blood protein, urobilinogen and nitrites.

Blood was obtained via puncture of the orbital sinus plexus from rats fasted overnight.

Pretest values for the clinical laboratory tests were obtained from 13 male and 13 female weanling rats sacrificed for this purpose prior to commencement of dosing.

Pathology

. Macroscopic

After 12 months of oral compound administration 10 rats/sex/group and after 27 months of treatment all surviving rats were sacrificed by carbon dioxide asphyxiation. Body weights were measured. Each animal received a complete post-mortem examination. The following organs were weighed: heart, kidneys, liver, brain, testes.

Representative sections of the following tissues were checked and fixed in phosphate-buffered neutral formalin for fixation:

adrenals
aorta
eyes (+ Harderian glands)
esophagus
stomach
duodenum
jejunum
ileum
cecum
colon
rectum
liver
kidneys
trachea
spleen
pancreas
urinary bladder
prostate
corpus and cervix uteri
testes
ovaries
sciatic nerve
brain
heart
lungs (+mainstem bronchi)
pituitary
thyroid and parathyroid
lymph nodes (mesenteric-mandibular)
sternum (bone marrow)
spinal cord
salivary gland
skeletal muscle
skin
mammary gland
thymus

also any other tissues with gross lesions. Animals dying or sacrificed during the course of the study were examined by the same procedure except body and organ weights, were not measured.

• Microscopic

Sections of the above listed organs and tissues were prepared for all animals and microscopically examined by Dr. C. E. Gilmore (Experimental Pathology Laboratories, Inc., Herndon, VA).

• Statistics

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

The following tests were used whenever appropriate: analysis of variance (one-way classification) - Bartlett's test for equal and unequal variances as described by Steel and Torrie* using Dunnett's** multiple comparison tables to judge significance of differences.

*Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York.

**Dunnett, C.W. 1964. New Tables for Multiple Comparisons with a Control- Biometrics pp. 482-491.

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RESULTS

General Observations

• Appearance and Behavior

Throughout 117 weeks of study, a higher evidence of soft stool was observed for the 32,000 ppm male group when compared to the controls. No other trends in physical appearance or behaviour suggestive of a compound-related effect were observed.

Incidental findings noted for both control and treated rats included staining of anogenital region, hair loss, red/black material around the eyes, lacrimation, area around eyes red and/or swollen and corneal opacities. Palpable masses, primarily found in the ventral abdominal, anogenital and thoracic regions were observed with greater for females than males.

• Mortality

Through 12 months of study, no remarkable differences or effects were noted for survival. At 27 months of study, male survival for all the treated group was lower than the respective control group due to a greater number of death in the 12-19 month interval. The deaths appeared to occur over a relatively short time (different for each group) and then followed the control group pattern.

Female survival, for all the treated groups, was higher than the respective controls. Survival at 117 weeks of study was as follows:

Dosage Level ppm	Number of survivors/number initiated (less interim sacrifice) male	female
Control	21/50	16/50
2,000	15/50	21/50
8,000	14/50	20/50
32,000	[illegible]	20/50

• Body Weights

Throughout the 117 weeks of study, a statistically significant decrease ($p < 0.05$ or $p < 0.01$) in group mean body weight was seen for both the male and female high concentration group (32,000 ppm) at all intervals analysed except at week 117. The decrease is considered compound-related. No remarkable differences in group mean body weights were seen at the 2,000 and 8,000 ppm dose levels except for the 8,000 ppm male group showing a statistically significant decrease ($p < 0.005$) at week 117.

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Group mean body weights at termination were as follows:

Dose Level ppm	Group mean body weights (in grams) (% Difference from Control) male	female
Control	748	469
2,000	677 (-9.5)	506 (+7.9)
8,000	633 (-15.4)	494 (+5.3)
32,000	645 (-13.8	425 (-9.4)

• Food and Compound Consumption

Throughout 27 months of study, the average food consumption values for the treated groups (males and females) were similar to the respective control groups.

The efficiency of food utilization showed no effects for the females whereas a decrease was noted for mid and high-dose males. This decrease appears to be related to the reduced body weights observed.

The average calculated compound consumption from week 1 through week 117 was as follows:

Dosage Level ppm	Average Compound Consumption (hydrated - mg/kg/day) male	female
Control	-	-
2,000	83.9	104.2
8,000	347.6	434.1
32,000	1481.5	1820.1

• Opthalmoscopic Examination

The observations noted throughout the study, during the various opthalmoscopic examinations, were representative of pathology that would be expected for this group of animals considering age, sex and strain. No obvious trends in pathology suggestive of test material related reactions were observed.

• Clinical Laboratory Tests

Hematology. Slight but significant reductions in erythrocyte count, hemoglobin and hematocrit were seen at the 12 month but not at other interval in the mid- and high-dose males.

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Group Mean Erythrocyte Count at the 12-Month Interval
(10 animals/sex/group)

	Control	2,000 ppm	8,000 ppm	32,000 ppm
males	7.61±0.59	7.22±0.69	6.80±0.35*	6.90±0.33*
females	6.81±0.34	6.69±0.33	6.50±0.43	6.65±0.34

*Statistically significant for $p < 0.01$.

Group Mean Hemoglobin Level at the 12-Month Interval (g/100 ml)
(10 animals/sex/group)

	Control	2,000 ppm	8,000 ppm	32,000 ppm
males	16.8±1.14	16.2±1.29	15.3±0.71*	15.3±0.80*
females	16.3±1.05	15.8±0.84	15.5±1.11	15.7±0.78

*Statistically significant for $p < 0.05$.

In view of the lack of consistency or progression in these variations they were not considered to be of toxicological significance.

. Blood Chemistry

There were a number of values in the treated group which were significantly different from control values but which were sporadic and inconsistent in nature. In males these were occasional results for glucose, alkaline phosphatase, SGPT, LDH, albumin, globulin and total protein.

In females, they were for glucose, BUN, SGPT, LDH, cholesterol, albumin, globulin and total protein. These variations were not dose related nor consistent from one examination interval to the next and were therefore not considered as representative of toxicological effect of the compound administration.

The reductions seen in calcium and potassium values at some intervals only are probably sporadic in nature but could also be secondary to the considerable quantities of sodium and phosphorus present in the test article. The increases in sodium values in the high dose level at 27 months is probably due to the same cause.

Urinalysis

There was a tendency to a reduced pH (acidification) in males. At the 32000 p.p.m. dose level compared to control males (Control pH values: 6-8; 32000 ppm male pH values: 5-7).

PATHOLOGY

Macroscopic

There was no compound-related macroscopic lesions present in males or females which died on study, were sacrificed when moribund, were sacrificed at the 12-month interim or were sacrified at termination of the study.

Organ Weights

There was increase in relative weight of the liver, kidney and heart for high-dose males and kidney and heart for high-dose females at terminal sacrifice. The increase in relative kidney weight is probably related to the increased incidence in chronic nephritis in high-dose animals. However, this incidence of chronic nephritis was within the expected range for this strain and species. Thus the increased kidney weight was not of toxicological significance. There was no morphological explanation for the increased relative liver and heart weights and these observations are regarded as toxicologically significant.

Values for the increased relative organ weights are presented in the following table:

Relative (% Body Weight) Organ Weights at Termination

Group Sex	Group Weight (grams)	Liver	Kidneys	Heart
Control				
males	747	3.27±0.39	0.79±0.23	0.31±0.06
females	470	3.62±0.65	0.72±0.12	0.34±0.05
2,000 ppm				
males	685	3.19±0.47	0.80±0.17	0.32±0.06
females	504	3.41±0.75	0.72±0.15	0.34±0.07
8,000 ppm				
males	631	3.88±1.48	1.07±0.46	0.37±0.08*
females	496	3.68±0.61	0.79±0.22	0.34±0.09
32,000 ppm				
males	633	3.88±0.85*	1.15±0.37*	0.43±0.12*
females	424	4.05±0.82	0.9[illegible]*	[illegible]*

*Significantly different from control group mean for p 0.05.

Histopathology

• Twelve-month Sacrifice

There were few neoplastic changes in any of the male or female rats. Those few present were scattered with approximately equal frequency among animals in each of the group and between males and females. Non-neoplastic changes, principallly inflammatory and hyperplastic were also found with similar frequency in males and females throughout the four groups. Most frequent of the inflammatory lesions were intersterestrial pneumonitis of the lungs and chronic nephritis of the kidneys. The lung change consisted of increased thickening and inflammatory cells in the interalveolar septa with varying degrees of congestion. Chronic nephritis (chronic progressive heptopathy) of the kidneys was characterized by a few to several dilated tubules containing proteins varying degrees of intertubular mononuclear inflammatory cells and in some cases face of regenerative tubular epithelium. This lesion was observed more frequently in males than in the females at 12 months and the incidence was also somewhat higher in the test than in the control males.

. Terminal Sacrifice

There was a greater variety of both neoplastic and non-neoplastic changes in rats sacrificed at termination of the study than in those sacrificed at 12 months. The changes were generally those expected in any comparable group of rats.

Among neoplasms, tumors of the pituitary and mammary gland were much more frequent in females than males. Distribution of these tumors was approximately equal between the groups.

Incidence of Pituitary and Mammary Tumors

Tumor Type	Control		Low Dose		Mid Dose		High Dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Pituitary Adeno-carcinoma	5	6	6	3	7	4	4	6
Pituitary Adenoma	24	44	23	44	17	41	21	40
Mammary Fibro-adenoma	-	1	-	1	-	2	-	3
Mammary Carcinoma	-	-	-	-	-	-	-	1

Most of the other tumors were of lower incidence and with about equal frequency in males and females. There were more benign than malignant tumors. The incidence of both was slightly higher in the low and mid-dose male rats and females had more malignant growths in the high dose group than in the other group. None of the tumors appeared to have an increased incidence that could be related to the test compound. The various tumor incidences are summarized in the table below.

Tumor Summary Table

Dose Level	Controls		2,000 ppm		8,000 ppm		32,000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Total number of animals initiated on study	60	60	60	60	60	60	60	60
Total number of animals examined	60	60	60	60	60	60	60	60
Total number of animals with tumors	37	53	40	50	43	52	36	50
Total number of animals with benign tumors	29	50	35	47	35	50	27	45
Total number of animals with malignant tumors	14	15	17	14	19	16	17	22

Chronic nephritis was also a common finding in all four groups (treated and control) of male rats. A comparable number of the high dose group of females were affected but control and other test groups of females had fewer affected animals. This disorder is generally found in comparable group of rats principally in males.

CONCLUSION

When orally administered through the diet to rats of both sex for 117 weeks, at concentrations of 2,000, 8,000 and 32,000 ppm (as anhydrous salt) monosodium phosphite did not induce any clinical signs of toxicity, increased mortality or hematological and biochemical alterations which could be attributed to the compound. No treatment related increased incidence of non-neoplastic or neoplastic lesions was induced.

Monosodium phosphite did not show any carcinogenic potential in rats.

THE SYSTEMIC NOEL is 8,000 ppm; the LEL is 32,000 ppm (soft stools in males, reduced mean body weights in both sexes, reduction in calcium and potassium values, increase in urinary sodium values).

Classification: minimum

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