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DATA EVALUATION RECORD

CHEMICAL:	Aluminum tris (O.ethyl phosphonate) Trade name: Foseytl-Al
FORMULATION:	Technical
CITATION:	Palmer, A.K., Bottomley, A.M., Barton, S.J., Clark, R., Offer, J.M., 1981 Effect of LS 74-783 on reproductive function of multiple generations in the rat
CONTRACTING LAB.:	HUNTINGDON RESEARCH CENTRE
SPONSOR:	RHONE-POULENC AGROCHIMIE, LYON, FRANCE
REPORT NO.:	HUNTINGDON RESEARCH RNP/89/80745 of January 22, 1981
	EPA Reg. No. Acc. No. 247174 - C
REVIEWED BY:	A. F. PELFRENE 09/13/1982 C. Coelho 9-13-82
REVIEWED ON:	August 2, 1982
TEST TYPE:	MULTIGENERATION FEEDING STUDY
TEST MATERIAL:	FOSETYL-AL technical Purity 97.3% Batch No. DA 73

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MATERIALS AND METHODS

ANIMALS AND MAINTENANCE

Specific pathogen free rats of the CFY strain, supplied by Anglia Laboratories, Alconbury Huntingdon, England, with body weight range of 60 to 70 g for both sexes were used to start in the first generation Fo. After an acclimatization period of 7 days, they were weighed and allocated to 4 groups. The animals were maintained in a temperature and humidity controlled room (21±4°C and 50±5% relative humidity) with 12 to 14 air changes per hour. Natural light in the room was supplemented by artificial light between 8 a.m. and 8 p.m.

During the premating periods the rats were housed 5 to a cage in suspended galvanized metal cages. Cages of males were interspersed among those holding females to promote the development of oestrous cycles.

During the mating periods the rats were housed in plastic breeding cages. At the end of the mating periods the males were re-housed to their original cages. Females were retained in the plastic breeding cages until sacrifice of the litter (first mating) or termination (second mating).

TEST MATERIAL ADMINISTRATION

Treatment groups (FO generation) were as follows:

Treatment (dietary concentration)	No. of Rats (FO gen.) Male	Female
Control	25	25
6000 ppm	25	25
12000 ppm	25	25
24000 ppm	25	25

Test diets were prepared freshly each week. Homogeneity and concentrations of test material in feed verified in the 6 samples taken from the 6,000 ppm batch were found to be in the range of 4,170 - 5,870 with a mean of 5120 ppm and a standard deviation of 559 ppm. The 6 samples taken from the 24, 000 ppm batch were in the range of 20,6000 - 23,000 ppm with a mean of 22,300 ppm and a standard deviation of 875.

Stability of Foseytl-Al in the feed was verified from day 1 through day 7 on samples taken from 6,000 ppm batch. The concentrations found were in the range of 4,950 - 5,640 ppm with a mean of 5,180. Concentrations of Fosetyl-Al in day 1 through day 7 samples taken from the 24,000 ppm batch

were found to be in the range of 21,000 - 25,800 ppm with a mean of 23,200 ppm. It is concluded that Fosetyl-Al is stable in the feed for one-week periods when stored at room temperature.

Concentration analysis in feed: The Fosetyl-Al concentrations in the feed averaged 5,481 - 11,140 and 23,942 ppm and were in the ranges of 4,690 - 6,300, 9,680 - 11,900 and 21,900 - 26,000 ppm corresponding to respective nominal concentrations of 6,000 ppm - 12,000 ppm and 24,000 ppm.

Animals of the F0 generation were maintained on their respective diet for 90 days prior to mating. The animals were then mated on a one male to one female basis for a period of 20 days. Resulting litter (F1A) were reared to 21 days post partum. The F1A pups were then sacrificed and subjected to post mortem examination for detection of macroscopic changes.

Approximately 10 days after the weaning of the F1A litters, the F0 generation was remated for a period of 20 days employing different male and female parings. Five pregnant females in each group were sacrificed on day 20 of gestation for teratological examination, (day 0 being the day of appearance of sperm in the vaginal smear).

The remaining dames were allowed to rear their young to 21 days post partum when 25 males and 25 females were selected from each group to form the basis of the F1B generation. Parent (F0) animals and surplus of F1B pups were killed and examined macroscopically.

The F1B generation were reared on their respective diets to an age of at least 90 days and then mated for a period of 20 days. The resulting F2A generation was reared 21 days post partum, sacrificed and examined macroscopically.

Approximately 10 days after weaning of the F2A litters, the F1B generation were remated for 20 days. Ten pregnant females in each group were sacrificed for teratological examination. The remaining F1B dames were allowed to rear their litters to day 21 post partum when 27 males and approximately 39 females from each group were selected, of these 15 males and 15 females were reared on their respective diets for at least 91 days after which time they were subjected to detailed macroscopic examination and representative tissues were retained for microscopic examination.

The remaining 12 males and 24 females were reared on their respective diets for 91 days (housed 6 to a cage) after which two matings with rearing to day 21 post partum were permitted. Mating was on the basis of 1 male to 2 females, employing different partners at the second pairing. All F3A pups were sacrificed and discarded following macroscopic examinations. Ten male and ten female F3B pups from all groups were subjected to organ weight measurements with histopathological examination initially restricted as per protocol to control and high dosage groups.

INTERIM SACRIFICE (for teratology purposes)

On day 20 of pregnancy selected F0 and F1B generation dames were killed by CO_2 asphyxiation, dissected, and examined for abnormalities, and macroscopical changes in internal organs. The ovaries and uteri were immediately examined for:

Number of corpora lutea
Number and distribution of live young
Number and distribution of embryonic/fetal deaths
Litter weight from which the mean pup weight was calculated
Fetal abnormalities

Embryonic and fetal deaths were classified as: Early (only placenta visible) and late (both placenta and embryonic remnants visible).

Live young were examined externally and weighed. Half the pups in each litter were preserved in Bouin's solution for subsequent researching for visceral abnormalities (Wilson's technique). The remainder were fixed for skeletal examination. Structural deviations were classified as major malformations, minor malformations, and variants.

GROSS AND HISTOPATHOLOGY OF F3B AND NON-MATED F2B GENERATIONS

Animals of the non-mated F2B generation and selected pups of the F3B generation were killed by CO_2 asphyxiation and subjected to detailed post mortem examination for macroscopic pathological changes.

For selected pups of the F3B generation only, the following organs were weighed: BRAIN, HEART, LIVER, KIDNEY, LUNGS, SPLEEN, THYMUS.

The following tissues were preserved and subsequently examined:

Routinely examined microscopically		Preserved but not examined in the first instance
brain	salivary gland	aorta
eye	pancreas	trachea
heart	lymph nodes	oesophagus
lung	(cervical & Mesenteric)	jejunum
liver	thymus	mid-colon
spleen	thyroid	mammary gland
kidney	pituitary	skin
urinary bladder	adrenals	prostate
stomach (gladular & non-glandular)	testes	tongue
	seminal vesicle	second eye
duodenum	ovaries	sciatic nerve
ileum	uterus	
caecum	any other tissue macroscopically abnormal	

The following observations and tests were performed throughout the study:

PARENT ANIMALS

General observation for signs of overt toxicity, mortality was performed daily.

URINALYSIS:

Urinalysis was undertaken following the death of 5 animals from group 4 (high dose level, 24,000 ppm) when autopsy revealed kidney damage. Urinalysis was performed during week 7 of the F1B generations, on individual urine samples collected overnight from 10 males each from group 1 (control) and 4 (high dose - 24,000 ppm).

FOOD AND WATER CONSUMPTION:

Food intake was recorded weekly during the first pre-mating phase of each generation. Water intake was measured during the first and penultimate weeks of the same premating phase.

BODY WEIGHTS:

Body weight changes of each rat of the F0 generation were taken initially and at intervals of 1 week thereafter Animals of all subsequent generations were weighed at birth, 4, 8, 12 and 21 days and at weekly intervals thereafter. Mating performance, pregnancy rate, gestation period (time between the day of successful mating as indicated by presence of spermatoza in vaginal smear and parturition) were recorded.

TERMINAL AUTOPSY:

After the second litters had been weaned, parent animals were sacrificed and macroscopically examined.

LITTER DATA

As soon as possible after birth, all young were counted, identified by toe amputation and examined for external abnormality. All litters were examined daily up to day 21 post partum for dead and malformed young. Pups were weighed individually at 1, 4, 8, 12 and 21 days post partum. Surplus pups were sacrificed at day 21 and examined externally and internally for abnormalities. Sex was determined by gonadal inspection.

Other litter data were: Litter size, litter and mean pup weight, pup mortality, abnormalities.

Tissues were generally preserved in 10% buffered formalin, except eyes which were preserved in Davidson's fluid. For microscopic examination tissues were processed, embedded in paraffin wax, sections cut a 5 µ and stained with haematoxylin and eosin. Frozen cryostat sections of liver and kidney, previously fixed in 10% buffered formalin, were sectioned at 12 µ and stained for fat with Oil Red O.

Microscopic examination was restricted to Groups 1 and 4 except for urinary bladder for which all groups were examined.

The tissues listed above were also preserved for the non-mated $F2^{a}$ animals but were not processed.

ASSESSMENT AND ANALYSIS OF RESULTS

In respect of litter data, group mean values generally were calculated in two ways viz:

Mean A: Generally includes all animals showing evidence of pregnancy either at day 20 of pregnancy (sacrificed dams) or at birth (dams rearing young).

Mean B: Generally only includes dams with viable young either at day of pregnancy (sacrificed dams) or at day 21 post partum (dams rearing young).

Mean B has more meaning when group size is low, in which case mean values would be unduly influenced by the presence of a single animal with total litter loss. Mean A is a more accurate index with large group sizes or when several litter are totally lost.

Litter weights, mean pup weights and incidences of abnormality are only calculated as Mean B values. For all values expressed as a ratio e.g. pre- and post-implantation losses, group mean values are derived as the mean of percentages within individual litters.

As litter values do not follow a "normal" distribution, intergroup differences are analysed by non-parametric statistical methods using the litter as the basic sample unit.

Organ weights of the F3B generation were analysed by analysis of variance adjusting for bodyweight at sacrifice as covarite, provided there was found to be a significant relationship (F-test; P 0.1). Treatment means were compared with control values by the method of L.S.D.'s in conjunction with Williams' test.

RESULTS

INTAKE OF TEST COMPOUND (Remark)

Estimation of dosage in terms of mg/kg bodyweight/day in studies employing dietary incorporation as a means of administration to individual animals, particularly in multigeneration studies where animals progress through a marked range of physical and physiological development. Comparisons, therefore, may only be made when appreciable inter-group or in this case intergeneration differences occur.

Allowing for inaccuracies it was evident, nevertheless, that for each dietary concentration employed, the dosage of LS 74-783 in terms of mg/kg bodyweight/day was considerably greater for the F1B and F2B generations than for the F0 generation. It was considered that these differences were largely responsible for the greater responses, particularly in respect of urinary tract changes and bodyweight gains seen in the second and third generations and described in the following sections.

GENERAL OBSERVATIONS:

The general appearance and condition of the animals were unaffected by the test compound.

The following deaths were recorded:

GROUP	MORTALITIES BY SEX AND GENERATION					
	MALES			FEMALES		
	F0	F1B	F2B	F0	F1B	F2B
Control	1	-	-	-	-	-
6000 ppm	-	-	-	2	1	-
12,000 ppm	1	1	1	-	-	2
24,000 ppm	-	7	3	2	1	-

URINALYSIS:

A significant ($p<0.01$) lower specific gravity in the 24,000 ppm male group was observed. In addition, an increase in epithelial or polymorphonuclear cells was observed in the urine of 4/10 males at the 24,000 ppm dose level as compared to 0/10 in the control males.

No other treatment related urinary effect was reported.

WATER CONSUMPTION:

For all generations, water consumption for the 24,000 ppm males and females was greater than that of the controls as seen in the table below:

GROUP ppm	GROUP MEAN WATER CONSUMPTION (G/RAT/DAY)					
	F0	F1B	F2B	F0	F1B	F2B
	Week 1	Week 1	Week 1	Week 12	Week 9	Week 12
Males						
Control	24.3	20.3	19.4	38.9	36.5	43.2
6000	26.8	23.1	17.3	42.5	42.7	46.8
12000	24.3	23.0	16.5	42.7	39.6	39.6
24000	27.5	25.0	19.6	40.0	40.5	48.5
Females						
Control	23.5	20.8	16.8	31.2	32.3	31.0
6000	24.2	22.1	16.5	31.7	35.2	35.9
12000	23.2	20.5	14.8	29.9	31.1	30.5
24000	24.9	26.8	17.8	31.8	36.8	32.7

FOOD CONSUMPTION:

No changes in food consumption were evident when treated groups were compared to the control groups of males and females.

PARENTAL BODY WEIGHTS:

Mean body weights (in grams) was similar in the 6,000 and 12,000 ppm treatment groups when compared to the control group. The 24,000 ppm group (males and females) also gained weight. The F_0 generation 24,000 ppm dose animals showed that female weight gain to be slightly superior to that of the respective control dams and male weight gains slightly lower than respective controls animals as shown in the following table:

F_0 GENERATIONS (25 ANIMALS/SEX/GROUP)
MEAN BODY WEIGHT (GRAMS)

	CONTROL		24,000 ppm	
Study week	Males	Females	Males	Females
0	111	103	110	102
13	552	307	537	339
22	663	355	653	368
30	724	384	715	390

The F1b generation 24,000 ppm dosed females and males weight gains were approximately 9% lower than that of their respective controls as seen in the following table:

F1b GENERATION (25 males/sex/group)
MEAN BODY WEIGHT (GRAMS)

	CONTROL		24,000 ppm	
Study week	Males	Females	Males	Females
0	79	77	47	48
11	499	307	427	280
20	609	365	539	334
26	644	415	578	355

The F2b generation 24,000 ppm dosed females and males weight gains were approximately 8.5% lower than that of their respective controls as seen in the following table:

F_2b GENERATION (25 ANIMALS/SEX/GROUP)
MEAN BODY WEIGHT (GRAMS)

	CONTROL		24,000 ppm	
Study week	Males	Females	Males	Females
0	68	64	51	48
14	542	308	498	313
22	686	360	601	333
30	746	445	653	371

MATING PERFORMANCES AND PREGNANCY RATES:

As can be seen in the following tables, from the most part mating performance assessed by the number of females becoming pregnant, was comparable for all groups at both matings of each generation. One exception was the lower pregnancy rate recorded at 6000 ppm for both matings of the F_2b generation. This is considered as coincidental in the absence of a similar reduction at higher concentrations.

MATING PERFORMANCE AND PREGNANCY RATE

F_2 GENERATION
FIRST AND SECOND MATINGS

Group (ppm)	Paired	Mated	Pregnant	Mating index	Pregnancy index
Control	25	21	21	0.84	1.00
6000	25	24	24	0.96	1.00
12000	25	24	24	0.96	1.00
24000	25	22	22	0.88	1.00
Control	25	20	15	0.80	0.75
6000	25	22	16	0.88	0.72
12000	25	25	20	1.00	0.80
24000	23	18	13	0.78	0.72

F_1b GENERATION
FIRST AND SECOND MATING

Group	Paired	Mated	Pregnant	Mating Index	Pregnancy Index
Control	25	19	19	0.76	1.00
6000	25	18	18	0.72	1.00
12000	25	21	21	0.84	1.00
24000	25	20	20	0.80	1.00
Control	25	21	11	0.84	0.52
6000	24	16	6	0.66	0.37
12000	25	20	11	0.80	0.55
24000	25	22	12	0.88	0.54

F_2b GENERATION
FIRST AND SECOND MATING

Group	Paired	Mated	Pregnant	Mating Index	Pregnancy Index
Control	24	18	18	0.75	1.00
6000	24	13	13	0.54	1.00
12000	24	20	20	0.83	1.00
24000	22	19	19	0.86	1.00
Control	24	18	18	0.75	1.00
6000	24	14	14	0.58	1.00
12000	22	16	16	0.72	1.00
24000	24	20	20	0.83	1.00

GESTATION PERIOD:

The gestation time was comparable for all groups.

LITTER LOSS:

The incidence of litter loss was scattered and limited and therefore not treatment related.

LITTER SIZE AND PUP MORTALITY:

Mean litter size and viability of the pups were comparable for all groups.

LITTER AND MEAN PUP WEIGHTS:

For both matings of each generation, values for litter and mean pup weights were essentially comparable for all groups at birth and day 4 post partum. Subsequently however, pup weight gain at 24,000 ppm was consistently retarded, leading to lower values for litter and mean pup weights during the latter part of lactation. Divergence from control values was most marked at 21 days but was also frequently discernible at 12 days and sometimes as early as 8 days post-partum. Differences frequently attained statistical significance.

A similar but less marked and consistent effect on litter and mean pup weights was also seen at 12,000 ppm.

There was no conclusive evidence of a similar effect at 6000 ppm.

The effects on litter and mean pup weights were considered to be related to lower maternal weights gain during lactation.

PATHOLOGY

FO GENERATION:

Gross necropsy and histopathology of the F0 males and females showed no treatment related effects.

F1 GENERATION:

Gross necropsy and histopathology of the F1 animals demonstarated urinary bladder changes described as "hemorrage of the bladder wall, increased pelvic dilation, interstitial nephritis and papillary necrosis" in males at the following incidences: 0/25, 2/25, 0/25 and 8/25 in males and in females: 0/25, 3/25, 3/25 and 10/25 for the controls - 5,000, 12,000 and 24,000 ppm dose levels respectively. No other pathological changes were seen.

F_2 GENERATION:

Gross necropsy and histopathology of the F_2 generation animals demonstrated similar urinary bladder and kidney changes (described above) in males 1/25, 0/25, 1/25 and 5/25 and in females 0/25, 0/25, 1/25 and 5/25 for the controls, 6,000-12,000 and 24,000 ppm dose levels respectively. No other pathological treatment related effects were seen.

F_1 GENERATION:

Minimal epithelial hyperplasia and/or hypertrophy of the transitional epithelium, sometimes associated with small papillary projections and/or desquamation cells in the lumen of the urinary tract were observed. These were associated with the presence of crytalline or calcareous deposits usually in the lumen but also in individual cases, in the serosa the mucoid epthelium or tubules of the seminal vesicles. These changes were observed in 8/10 males and 8/10 females at 24,000 ppm and 1/10 males at the 12,000 ppm level. However, young rats are known to be prone to have spontaneous inflammatory lesions of the urinary tract and to easily develop such lesions in presence of crystalline deposits (1-2-3). In the present case, the very high phosphorus intake has been shown to induce increase calciuria (4) in the treated animals and therefore the changes observed in the urinary tract are most likely related to the presence of crystalline calcareous deposits.

PATHOLOGY OF FETUSES

No treatment related effects were observed in fetuses from the F_0 (2nd generation) and F_1b dams sacrificed at day 20 of gestation.

ORGAN WEIGHTS

All organs of both sexes showed a marked correlation with bodyweight.

In case of males significant deviation from control values was recorded with respect to lower liver, spleen and thymus weights of all test groups and higher lung weight of males at 24,000 ppm. For females a similar pattern was evident for thymus, spleen and lung weight but differences from controls attained statistical significance only with respect to thymus weight. The corresponding values are presented in the following table.

The biological reference of the differences was considered uncertain in view of the lack of marked dosage-related trends within test groups, the marked effect at 24,000 ppm and 12,000 ppm on maternal body weight gain and pup weight gain during lactation and most important the absence of significant dosage-related histopathological findings in these organs.

1) CHENG, L.. J. ENVIRON. PATHOL. TOXICOL. 1980; 4:317-349.
2) ROBERTSON, J.L.. TOXICOL. PATHOL. 1980; 8:9-13.
3) CLAYSON, D.B.. J. NAT. CANCER INST. 1974; 52:1685-1689.
4) KALIFAT, R. et al: unpublished report. RHONE-POULENC. C.R. Vitry/C.N.G. No. 20765-E of 1.29.1981.

MEAN GROUP ORGAN WEIGHTS OF F_3B ANIMALS
(grams)

GROUP (ppm)	LIVER		LUNG		SPLEEN		THYMUS	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
CONTROL	3.870	3.303	0.648	0.644	0.338	0.277	0.308	0.322
6000	3.513	3.293	0.725	0.681	0.277	0.255	0.250	0.226
12000	2.963	2.842	0.600	0.576	0.229	0.220	0.199	0.210
24000	2.884	2.460	0.653	0.537	0.184	0.200	0.191	0.210

CONCLUSION

Considering the three generations there was no evidence of an adverse effect on Fertility or Reproduction at any dosage. Similarly there was no indication of an adverse effect on in utero or development of young. Parental animals were adversely affected at the highest concentration (24,000 ppm) and to a lesser extent at 12,000 ppm but not at 6,000 ppm.

At 24,000 ppm the most remarkable effects were: 1) lower body weight gain for males of all generations, and females of the F1B and F2B gerations; the more marked deviations of the F1B and F2B generations being associated with both higher ingestions of material and lower weight at weaning. 2) for all generations a specific effect on the pattern of maternal weight changes during lactation leading to retarded mean pup weight gain and lower litter and mean pup weights in mid- and late lactation. 3) A high incidence of animals showing pathological changes in the urinary tract, particularly for the F1B and F2B generations. This was associated with an increased incidence of male but not female deaths for the F1B and F2B generations. 4) Correlating with the urinary tract changes observed in adults, the more detailed microscopic examination at weaning of 10 male and 10 female per group (F3B generation) revealed crystalline or calcareous deposits in the lumen of the urinary bladder of most animals. The presence of these deposits was frequent

associated wiht minimal hyperplasia/hypertrophy of transitional epithelium and sometimes also with papillary projections and/or desquamation of epithelial cells. These epithelial abnormalities are most likely reactive to the presence of crystalline, calcareous deposits in the bladder.

At 12,000 ppm similar but much less marked effects were evident principally in respect of lower overall weight gains of the F2B generation, lower litter and mean pup weight in late lactation, and the recording of urinary tract changes in occasional adults andl/10 weanling males of the F3B generation.

The No Observable Effect Level (NOEL) in this study is considered to be 6,000 ppm in adult and young rats. (300 mg/kg and 600 mg/kg respectively).

Classification: Minimum