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DATA EVALUATION RECORD

CHEMICAL:	Aluminum tris (O-ethyl phosphonate) Trade name: Fosetyl-Al
FORMULATION:	Technical
CITATION:	PALMER, A.K. and JAMES, R.W., 1977 Effect of LS74 783 on pregnancy of the rat
CONTRACTING LAB.:	HUNTINGDON RESEARCH CENTRE HUNTINGDON - ENGLAND
SPONSOR:	RHONE-POULENC AGROCHIMIE, LYON, FRANCE
REPORT NO.:	RNP/33/76939 of 06/23/1977
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REVIEWED BY:	A.F. PELFRENE, MD, PhD, ATS, Director of Toxicology RHONE-POULENC INC.
	09/13/1982
	C. Caccavo 9-13-82
REVIEWED ON:	JUNE 9, 1982
TEST TYPE:	TERATOGENICITY
TEST MATERIAL:	FOSETYL-AL technical Purity 99.8% Batch No. FR 794/795

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MATERIAL AND METHODS

ANIMALS AND MAINTENANCE

Eight sexually mature CFY strain (Anglia laboratory animals, Huntingdon, England) SPF female rats weighing between 180 and 250 g were used in this study. They were supplied timed mated by the breeding laboratory.

The day of mating as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug was considered day 0 of pregnancy.

On arrival, the animals were arbitrarly assigned to 4 groups, 5 per cage and ear marked.

They were given free access to Spratt's laboratory diet n° 1 and to tap water.

TEST PROCEDURE

The following treatment groups were set up:

Group	Treatment Fosetyl-Al	Concentration of solution % w/v	Dosage volume (ml/100g)	Number of Females
Gr. 1	Control	-	2.0	20
Gr. 2	500 mg/kg	2.5	2.0	20
Gr. 3	1000 mg/kg	5.0	2.0	20
Gr. 4	4000 mg/kg	20.0	2.0	20

The pregnant females were treated according to the above regimen from day 6 to day 15 of pregnancy inclusively by oral gavage of 2.0 ml/100g of the appropriate concentration.

The test solution was prepared fresh on a daily basis.

OBSERVATION

The animals were observed daily for signs of overt systemic toxicity or reaction to treatment and weighed on days 1-3-6-10-14-17 and 20 of the study.

On day 20 of pregnancy, all animals were sacrificed by CO_2 asphyxiation, dissected and observed for macroscopic pathological changes in maternal organs.

The ovaries and uteri were examined immediately to determine:

- number of corpora lutea
- number and distribution of live youngs
- number and distribution of embryo/fetal deaths
- litter wieght from which the mean pup weight was calculated
- fetal abnormalities.

Uteri or individual uterine horns without visible implatations were stained with a 10% solution of ammonium sulphide to reveal evidence of embryo resorption at very early stages of implantation.

Live young were examined externally and weighed.
Half the pups in each litter were preserved in Bovin's solution for subsequent free-hand sectioning (Wilson's technique) in order to evaluate any eventual visceral malformations. The remaining half of the litter was fixed in methylated spirit for subsequent macroscopic examination, evisceration and determination of sex prior to clearing and alizarin red S staining (modified Dawson's technique) for skeletal examination.

Structural deviation were classified as: major malformations, minor anomalies and variants.

STATISTICS

All numerical data were evaluated on a litter basis using non-parametric methods (Kruskal and Wallis (1) - Jonckheere (2))

RESULTS

PARENT ANIMALS

*General Behavior and Mortality:

Deaths were recorded in the 500 and 1,000 mg/kg/day dose groups (1 and 2 respectively). These deaths were not related ~~with an effect of the~~ compound since they resulted from "dosing errors" (i.e. mis administration). At the high dose level (4,000 mg/kg/day), 5/20 mothers died or had to be sacrificed at days 9,10 and 11. Post mortem examination of each animal showed marked gastric dilation and fluid retention. Prior to death, animals lost weight and 3 animals showed chromodacryorhea.

No deaths were reported in the control group.

(1) KRUSKAL, W.H. and WALLIS, W.A., 1952 - J.AMER. STAT. ASSOC. 47: 583-621

(2) JONCKHEERE, A.R., 1954 - BIOMETRIKA 41: 133-145

*Body Weight of Dams

A dose related retardation of weight gain was observed during the first four days of dosing in all the treatment groups when compared to the controls. The weight gain retardation was only significant at the high dose level (4,000 mg/kg/day) as seen in the following table:

Group mean body weight of dams

Dose (mg/kg/day	Body Weight (gram) at day 1	6	10[a]	17	20
0 Control	212	246	269	322	371
500	219	257	278	331	375
1,000	220	259	277	333	384
4,000	218	257	260	310	365

(a) 4 days of dosing.

As also observed in this table, the high dose dams never regain their body weight momentum which was seen prior to dosing.

*Pregnancy rate

In the controls, 500 and 1,000 mg/kg/day groups, 1/20 mated animals did not become pregnant. All females from the high dose level group were pregnant.

REPORTED LITTER DATA

*Litter size and post implantation sites

All treatment groups were comparable to controls with respect to litter size.

There was a statistically significant ($p<0.05$) increase of total resorptions when the 4,000 mg/kg/day (1.3 total resorptions) and the 500 mg/kg/day (0.9 total resorptions) were compared to controls (0.5 total resorptions).

*Litter and mean fetal weights

Values for litter and mean fetal weights at 500 and 1000 mg/kg/day were essentially comparable with those of controls. At 4,000 mg/kg/day, a combination of slightly lower size and slightly lower fetal weight led to a low litter weight which was significantly ($p<0.05$) different from the control values as seen in the following table:

Group mg/kg/day	Litter weight (grams)	Mean fetal weight (grams)
Control	48.05	3.79
500	42.07	3.74
1,000	48.50	3.72(a)
4,000	39.71(a)	3.46(a)

(a) significant at p 0.05 (KRUSKAL-WALLIS)

*Fetal malformations

Examination for fetal malformations and developmental variations was performed on 19,18,17 and 14 litters for the control,500,1,000, and 4,000 mg/kg/day groups respectively

A total of 4 kinds of malformations, and 11 kinds.of varitions were observed. The numbers of exposed fetuses with soft tissues and skeletal variations were comparable to controls in the 500 and 1,000 mg/kg/day dose groups.

At the 4,000 mg/kg/day there was slight increase in malformations when compared to controls (5/161-3.1%-fetuses examined as compared with 2/242-0.8% - in the controls). These malformations at the 4-000 mg/kg/day level were described as "thoracic asymmetry-caudal displacement of the right kidney, moderate internal hydrocaphaly and transposition of the azygos vein, aortic arch and ductus arteriosus". A non significant number of minor visceral anomalies at the 4,000 mg/kg/day dose level were reported in 8/78-(10.2%) fetuses examined when compared to 8/119 (6.7%) fetuses examined in the control group. These were described as "mild to moderate subcutaneous edema and medial displacement of the left testis". Skeletal variations at the 4,000 mg/kg/day dose level were statistically different ($p<0.05$) from controls (7/78 fetuses examined as compared to 9/121 respectively). The skeletal variations were described as retarded ossification which correspounds to the lower mean fetal weight described in the litter and mean fetal weight section).

CONCLUSION:

Treatment with Fosetyl-Al in rats, caused maternal toxicity (5/20 pregnant dams died, retarded body weight gain) at the highest dose tested (4,000 mg/kg/day), where administered orally by gavage from day 6 to day 15 of pregnancy, inclusively. As a result of the maternally toxic effect at this dose level, litter and mean fetal weights were reduced, total resorptions were increased and delayed ossification of fetuses were observed.

The NOEL is 1,000 mg/kg/day
The LEL is 4,000 mg/kg/day

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