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DATA EVALUATION REPORT

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

FORMULATION : Technical

CITATION : PASQUET,J. and LEBAIL,R. 1976 -
Compound LS 74 783 -
Oral teratogenicity in the rabbit.

CONTRACTING LAB : CENTRE DE RECHERCHES RHONE-POULENC
VITRY sur SEINE - FRANCE

SPONSOR : RHONE-POULENC AGROCHIMIE - FRANCE

REPORT N° : RP/PD/CNG N° 18.917-E of 12/06/1976

EPA Reg. N° Acc. N° 247174-A

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REVIEWED ON : MAY 27, 1982

TEST TYPE : Teratogenicity

TEST MATERIAL : FOSETYL-Al technical
Purity 98 %
Batch : N° FT 795

18/10

MATERIAL AND METHODS

ANIMALS

Eighty-eight NZW derived Ju/RP strain virgin female rabbits, supplied by Elevage Junod, 10160 AIX-en-OTHE - France, and 11 males from the same strain and origine were used in this study. The body weight range at start of the study was from 2.6 to 3.4 kg.

Upon arrival to the testing facilities, the animals were identified with a numbered metal ring tag fitted around one of the hind legs.

MAINTENANCE

The does were individually caged in an environmentally controlled room (temperature : 22 ± 2°C - relative humidity : 30-50 % - artificial lighting from 7:00 am to 7:00 pm - air changes 14-15 times/hour). Those environmental parameters were not permanently recorded.

The animals were fed on UAR N°111 rabbit diet (200 g/day) and had free access to tap water.

All animals were acclimated to the laboratory condition for at least two weeks prior to mating.

TEST PROCEDURE

- Insemination : the mating was made under direct visual supervision of a trained technician on the basis of on male to one female being kept together for approximately 15 minutes. The breeding period took place between July 9 and September 6, 1976. Altogether 11 males were used to breed 88 females, thus each male was used several times during this period.

The day of successful mating was considered as Day 0 of pregnancy.

- Test groups : the mated females were randomly allocated to the treatment or control groups as the mating proceeded, their body weights on day 0 being taken into consideration for group homogeneity.

From day 6 to day 16 inclusively, the pregnant females were orally (via stomach tube) 5 ml/kg b.w. of Fosetyl-Al as a suspension in a 10% aqueous solution of gum arabic at the following daily dose level : 500 - 250 - 125 mg/kg b.w.

The highest dose level was selected from the results of a preliminary 11 day subacute study in non-pregnant female rabbits from the same origine.

One control group received only 5 ml/kg b.w. of the vehicle only under the same regime as the treated ones.

The number of pregnant females per dose level was as follows :

. Control		:	20
. Low dose	(125 mg/kg b.w. day)	:	23
. Intermediate dose	(250 mg/kg b.w. day)	:	23
. High dose	(500 mg/kg b.w. day)	:	22

TEST ARTICLE PREPARATION

Suspensions of test article in the vehicle at any given concentration were freshly prepared daily and administered to the rabbits within on hour of preparation. Therefore no stability analysis was performed on one hand ; on the other hand it was known from data obtained from other studies that Fosetyl-Al technical is quite stable for long periods of time under normal storage conditions at room temperature. Therefore it is highly unlikely that under the conditions of this test the suspension used were not satisfactory.

OBSERVATIONS

The does were weighed on days 0-6-16 and 28 of pregnancy and their daily food intake was recorded daily from day 0 to day 27.

The pregnant females were observed daily for signs of overt toxicity, mortality, premature abortion - general appearance and behavior.

On day 28 of pregnancy, the does were sacrified by cervical dislocation and immediately autopsied in order to examine the genital tract and to count the number of implantation sites, resorptions, and live and dead foetuses.

Live foetuses were weighed and given a careful external examination ; they were then sacrified and autopsied. Autopsy consisted of a detailed examination of the thoracic organs (after sectionning the left ribs and lifting the sternum and ribs sideways) and of the abdominal organs (including sex determination), examination of the eye (eyeball and lens enucleated) and of the brain (after cutting round and lifting backwards the skull cap). Lastly, all skeletons were stained with alizarin red S for examination of the bone segments.

STATISTICS

The results from each treated group were compared statistically with those from the control group, using the Chi-2-test in the case of percentages. In the case of means, either Student's t test or the analysis of covariance was used for roughly normal distributions and the Mann, Whitney and Wilcoxon test was used for asymmetrical distributions.

RESULTS

MATERNAL DATA

- Mortality :

From the 88 does which were mated, 15 were excluded from the study for incidental reasons (accidental death during compound administration or intercurrent infection). See annex

In the remaining females, pregnancy occurred in 12 control rabbits, 14 high dose (500 mg/kg b.w.), 15 intermediate dose level (250 mg/kg b.w.) and in 15 low dose (125 mg/kg b.w.) rabbits.

- Mean body weights of pregnant dams :

A significantly ($p = 0.05$) lower weight gain was observed in the high and intermediate dose levels when compared to the controls. During the treatment period (day 6 to day 16 of pregnancy), the weight gains being + 0.28 kg / + 0.21 / + 0.12 / + 0.15 for the control, low, intermediate and high dose groups respectively.

- Mean daily food consumption :

was comparable to that of the controls for the low and intermediate groups and slightly lower for the high dose group. This decrease was statistically significant ($p = 0.05$) from day 9 to day 16 of the treatment period.

- Pregnancy rate :

was comparable between all three treated groups as well as with that of the control.

Pregnancy occured in 12 out of 18 females (66 %), in the control group, in 14/18 (77 %) in the low dose group, in 15/18 (83 %) in the intermediate dose group and in 15/19 (79 %) in the high dose group.

No case of total loss of litter or total resorption were observed.

LITTER DATA

- Mean number of implantation sites :

was comparable in all groups : 8.3 in control / 8.6 in high dose / 9.3 in intermediate and 7.4 in low dose.

- The mean number of live foetuses :

was comparable in all treated and control group (7.7 / 7.6 / 8 / 6.9 from control to high dose groups respectively).

There was no evidence of an adverse effect on fetal growth in utero as shown by the mean weight of live foetuses (33.8 ± 0.9 g in control, 34.1 ± 2.5 g in the high dose group, 33.2 ± 1.2 g in the intermediate dose group and 36.6 ± 1.3 g in the low dose group).

- Embryotoxicity :

there was no evidence of an embryotoxic effect in any treatment group. The mean number of fetal losses (resorptions & dead foetuses) was not significantly different from one treatment group to the other and when treatment groups were compared to the control.

Also skeletal ossification was comparable.

- Teratogenicity :

no evidence of a teratogenic effect was observed. No malformed pup was found in any of the high or low dose groups and there were 3 malformed young in the control group and 2 in the intermediate dose group. These major malformations in the latter group (caridovascular, vertebral and brain malformation) are known to occur in untreated NZW rabbits. See annex.

All pregnant females carried to term and delivered normally ; therefore the above observations are drawn from respectively 12-14-15 and 15 litters for the control, low, intermediate and high dose groups.

All live foetuses were weighed and autopsied and a detailed gross examination of the thoracic and abdominal cavities, as well as of the brain and eyes was performed. All skeletons were stained and examined. The total numbers examined of foetuses per group were 92 in the control - 107 in the high dose group - 120 in the intermediate group and 103 in the low dose group.

Description of the few major abnormalities seen (3 in the control and 2 in the intermediate group) is given in the annex, along with the incidences reported in the literature for similar malformations in the same strain of rabbits.

CONCLUSION

Fosetyl-Al did not induce any embryotoxic, fetotoxic or teratogenic effects when orally administered to pregnant NZW rabbits at dose levels of 500 - 250 and 125 mg/kg b.w. daily from day 6 to day 16 of pregnancy.

NOEL : $\geq$ 500 mg/kg/day.

CLASSIFICATION : minimum

7

ANNEXES

INCIDENTAL DEATHS

Daily dose level (mg/kg po)	Doe N°	Time of death or sacrifice	Observations
Controls	164	Day 28	Bilateral pyometra
	173		Unilateral pyometra
	265-a	Day 16	Accidental death (maladministration of the compound)
500	267	Day 28	Unilateral uterine torsion (of the upper 1/3 of the horn)(a)
	279		Bronchial pneumonia
	361-a	Day 13	
	365	Day 11	Accidental death (maladministration of the compund)
	365-a	Day 13	
	366-a	Day 11	
250	378	Day 13	Found death with advanced autolysis on day 14; had presented the previous evening with almost total anorexia, dyspnoea and a frothy discharge with traces of blood.
	465	Day 11	
	465-a	Day 12	Accidental death (maladministration of the compound)
	466-a	Day 7	
125	467-a	Day 8	
	471-a	Day 21	Bronchial pneumonia

(a) From day 26, this doe showed a reduced food intake, then anorexia and, on day 28, a vaginal discharge with traces of blood. The horn showing the torsion was severly congested and contained 3 dead and 2 live foetuses, the other horn contained 2 dead and 7 live foetuses, all foetuses apparently being normal.

8

SUMMARY OF STUDY

Determinations		Control	Daily dose level (mg/kg p.o.) 500	250	125
Mated rabbits kept throughout the study (a)		18	18	18	19
Pregnant does (b)		12 (67)	14 (78)	15 (83)	15 (80)
Number of implantation sites	Total number	99	120	139	111
	Mean number (c)	8,3 ± 0,4	8,6 ± 0,5	9,3 ± 0,3	7,4 ± 0,4
Foetal losses (resorptions + dead foetuses)	Total number	7 (7 + 0)	13 (12 + 1)	19 (18 + 1)	8 (8 + 0)
	Mean number	0,6	0,9	1,3	0,5
Number of does which carried to term		12	14	15	15
Live foetuses	Total number	92	107	120	103
	Mean number (a)	7,7 ± 0,4	7,6 ± 0,6	8,0 ± 0,5	6,9 ± 0,5
	Male (b)	42 (46)	61 (57)	61 (51)	42 (41)
	Female (b)	50 (54)	46 (43)	59 (49)	61 (59)
	Mean weight (g) (c)	33,8 ± 0,9	34,1 ± 2,5	33,2 ± 1,2	36,6 ± 1,3
Foetuses showing no malformation	Hypotrophic (b)	0	0	2 (1,7)	0
	with one minor abnormality (b)	0	1 (0,9)	0	0
	ossification stage	Normal	Normal	Normal	Normal
Malformed foetuses (b)		3 (3,3)	0	2 (1,7)	0

(a) Not including rabbits excluded from the study
(b) Number (and %).
(c) ± standard error.

9

MALFORMED FETUSES

Daily dose level (mg/kg p.o.)	Litter No.	Number per litter	Findings
Controls	162	2	Foetuses with cardiovascular malformation (dilatation of the aortic arch and pulmonary artery).
	168	1	Talipes, front left paw
500	No malformed foetuses.		
250	364	1	Cardiovascular malformation (dilatation of the aortic arch) and vertebral malformation (dorsal spondylolisthesis + 2 fused ribs).
	372	1	1 foetus with malformed face (cyclocephalus).
125	No malformed foetuses.		

Note: The frequencies of spontaneous cardiovascular and vertebral malformations and cyclocephalus in the New Zealand rabbit are 0.29%, 0.16%, and 0%, respectively for our strain, and 0.06%, 0.10%, and 0.02% according to A.K. Palmer [Lab. Animal, 2, 195-206, 1968: "Spontaneous malformations of the New Zealand white rabbit: the background to safety evaluation tests."]