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KRL 5/10/88

ERB 5/11/88

DATA EVALUATION REPORT

Study Type: Teratology - Rabbit

Doc. No.: 006658
TOX Chem No.: 363A
MRID No.: 144521
Project No.: 8-0126

Test Material: Avenge; Purity: 99.1%

Synonyms: Difenzoquat Methyl Sulfate; AC 84,777

Study Number(s): 6123-114; 981-84-102

Sponsor: American Cyanamid Company

Testing Facility: Hazelton Laboratories America, Inc., Madison, WI

Title of Report: A Teratology Study With AC 84,777 in Rabbits: Final Report

Author(s): Karen MacKenzie

Report Issued: December 14, 1984

Conclusions:

Teratogenic NOEL = > 100 mg/kg (mid-dose)*
Fetotoxic NOEL = > 100 mg/kg*
Maternal NOEL = 100 mg/kg
Maternal LEL = 250 mg/kg (HDT; high mortality and high percentage of animals with resorptions only)

Core Classification: Minimum

Experimental Procedures:

Artificially inseminated New Zealand rabbits (about 4 months old and weighing 3.5 kg), 18 animals/group, were tested with 0, 50, 100, or 250 mg AC 84,777/kg of body weight on gestation days (gd) 7 through 19. The compound was administered by gavage as a solution in distilled water. Immediately prior to insemination,

*Because of very high maternal mortality in the 250 mg/kg group (61%), insufficient pups (only 17) were available for a meaningful evaluation of the teratogenic potential of the test material at that level.

each doe was injected with human chorionic gonadotropin solution (250 IU in 0.25 mL 0.9% saline). Cesarean sections were performed on gd 29 and all dams were examined macroscopically. Gross pathologic lesions were preserved in 10% phosphate-buffered formalin for possible future histopathologic examination. The reproductive tract was evaluated for the following:

- o Number and location of live and dead fetuses;
- o Early and late resorptions;
- o Empty sites and implantation scars;
- o Unusual coloration and variations in amniotic fluid or placentae; and
- o Any other abnormalities.

The ovaries were examined for gross abnormalities and the number of corpora lutea, and the gravid uterus was weighed. Uteri that appeared nongravid were opened and placed in a 10% solution of ammonium sulfide to confirm pregnancy status.

All viable fetuses were individually tagged for identification, weighed, examined externally for gross abnormalities, and then given a thorough examination of the viscera including internal sexing and a heart dissection; the brain was examined by a mid-coronal slice. All fetuses were then eviscerated, skinned, leared, stained, and evaluated for degree of skeletal ossification, variations, and malformations. Intact aborted or premature fetuses and fetuses obtained from dams not surviving until scheduled necropsy were examined externally and placed in 10% phosphate-buffered formalin.

The animals were:

- o Obtained from Hazelton Dutchland, Inc., Denver, PA;
- o Acclimated for at least 19 days;
- o Assigned randomly to test groups;
- o Housed individually;
- o Identified by numbered metal ear tags;
- o Fed unrestricted amounts of food (Purina Certified Rabbit Chow 5322, pelleted) and water;
- o Observed twice daily for toxic signs; and
- o Weighed on gd 0, 7, 12, 15, 18, 24, and 29.

Maternal and litter data were evaluated by appropriate statistical procedures (1, 2, 3, 4, and 5).

Results:

Maternal Data

Survival

Survival was very low in the high-dose group. Of the 18 inseminated females assigned to each group, 17 (94%), 15 (83%), 15

(83%), and 7 (39%) survived until the termination of the study (gd 29) in the control, low-, mid-, and high-dose groups, respectively. One female in each of the first three groups was sacrificed upon evidence of abortion (gd 20-26), and another female in the low-dose group was sacrificed because of a broken back. The remaining animals (1, 2, and 11 in the low-, mid-, and high-dose groups, respectively) died during gd 9 to 18.

Pregnancy Status

The overall pregnancy rates for the study were 94, 83, 89, and 83 percent for the control, low-, mid-, and high-dose groups, respectively. The pregnancy rates for the same groups, for animals surviving until the termination of the study, were 94, 80, 87, and 86 percent, respectively. At the termination of the study, a high percentage of animals with resorptions only, compared with the controls, was observed in the high-dose group. The incidence of these resorptions in the control, low-, mid-, and high-dose groups was 6, 17, 15, and 33 percent, respectively.

Toxic Signs

Possibly treatment-related observations included diarrhea in 7 out of 11 high-dose animals that died during the study, and single incidents of wetness around the muzzle and genital area in the same group.

Body Weights

No statistically significant differences were observed in mean body weights or weight changes, uncorrected and corrected for gravid uteri. On gd 29, the mean body weights (corrected for gravid uteri) were 3.25, 2.57, 3.27, and 3.45 kg for the control, low-, mid-, and high-dose groups, respectively.

Necropsy

There were no treatment-related findings. Ascites was observed in the control, low-, and mid-dose groups, but the incidence was higher in the controls. Fluid-filled lungs, noted in four animals (one in low-, one in mid-, and two in high-dose groups) that died during the study because of intubation errors were not attributed to treatment. Other observations noted at necropsy included a displaced kidney in one animal and a lobulated and fragmented spleen in another animal in the mid-dose group, and three fluid-filled cysts in the oviductal connective tissue of one animal in the high-dose group.

Litter Data

The numbers of females examined at the cesarean section in the control, low-, mid-, and high-dose groups were 16, 12, 13, and 6, respectively.

There were no statistically significant differences in the mean number of corpora lutea or implants, implantation efficiency (number of implants x 100/number of corpora lutea), fetal body weights, sex ratio, or in the number of resorptions (total, early, and late). The mean number of live fetuses/dam in the treated groups was smaller than that of the control group but the difference was statistically significant ($p = < 0.05$) only for the low-dose group. Dead fetuses were not observed in this study. (For details, see Attachment I.)

The numbers of litters examined for visceral and skeletal abnormalities in the control, low-, mid-, and high-dose groups were 15, 10, 11, and 4, respectively. The numbers of fetuses examined in these groups were 119, 52, 70, and 17, respectively. There were no treatment-related visceral abnormalities. There was, however, a slight and possibly treatment-related increase in the number of fetuses with vertebral central abnormalities in the high-dose group. The fetal percent incidence of this skeletal malformation in the control, low-, mid-, and high-dose groups was 0, 0, 1, and 12, respectively. The litter percent incidence of the malformation in the same groups was 0, 0, 9, and 25, respectively. According to the submitted report, the increased incidence of vertebral central abnormalities was statistically significant in the high-dose group, but statistical significance was not designated in the data.

Maternal NOEL = 100 mg/kg
Maternal LEL = 250 mg/kg (HDT; high mortality and high percentage of animals with resorptions only)
Teratogenic NOEL = > 100 mg/kg*
Fetotoxic NOEL = > 100 mg/kg*

Core Classification: Minimum

A Quality Assurance Statement, dated December 14, 1984 was included in the report.

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*According to the author of this report, teratogenic (and presumably fetotoxic) NOEL was 250 mg/kg (HDT). TB/HED disagrees. Maternal mortality of 61 percent in this group resulted in insufficient numbers of pups (only 17) to permit meaningful evaluation of the teratogenic potential of the test material at the 250 mg/kg level.

REFERENCES

1. Sokal, R. R. and F. J. Rohlf, Biometry, W. H. Freeman and Company: San Francisco, Chapter 9, pp. 204-252 (1969).

2. Dunnett, C. W., "A Multiple Comparison Procedure for Comparing Several Treatments with a Control," J. Am. Stat. Assoc. 50, pp. 1096-1121 (1955).

3. Sokal, R. R. and F. J. Rohlf, Biometry, W. H. Freeman and Company: San Francisco, Chapter 13, pp. 367-403 (1969).

4. Dunn, O. J., "Multiple Comparisons Using Rank Sums," Technometrics 6, pp. 241-252 (1964).

5. Sokal, R. R. and F. J. Rohlf, Biometry, W. H. Freeman and Company: San Francisco, Chapter 16, pp. 549-620 (1969).

Attachment I

Study No. 6123-114

Table 5

Summary of Cesarean Section Data

Observation		Group (Dose Level in mg AC 84,777/0.5 mL/kg) 1 (0)	2 (50)	3 (100)	4 (250)
Number of animals on test		18	18	18	18
Number (percent) pregnant		17 (94)	15 (83)	16 (89)	15 (83)
Aborted/sacrificed		1	2	1	0
Died		0	1	2	9
Examined at C-Section		16	12	13	6
Corpora lutea	N[a]	17	13	14	14
	Mean	13	14	13	14
	SD	2.7	3.9	5.0	4.9
Implants	N	17	15	16	15
	Mean	8	6	6	7
	SD	2.5	3.5	3.7	3.5
Implantation efficiency	N	17	13	14	14
	Mean	63.8	51.0	58.8	54.5
	SD	20.99	30.72	28.65	21.41
Live fetuses	N	16	12	13	6
	Mean	7	4*	5	3
	SD	2.6	3.3	3.6	3.1
Sex ratio (M/M + F) x 100	N	15	10	11	4
	Mean	44.0	51.7	54.7	38.1
	SD	18.07	29.16	28.00	17.72
Fetal body weights					
Male	N	15	10	11	4
	Mean	39.0	44.7	43.7	47.2
	SD	5.80	9.17	6.10	11.31
Female	N	15	8	9	4
	Mean	37.8	41.7	41.9	44.8
	SD	5.18	5.42	5.58	4.73

*$p < 0.05$.

[a] N: number of data points.
SD: standard deviation.

Study No. 6123-114

Table 5 (Continued)

Summary of Cesarean Section Data

Observation		Group (Dose Level in mg AC 84,777/0.5 mL/kg) 1 (0)	2 (50)	3 (100)	4 (250)
Total Resorptions	N	16	12	13	6
	Mean	1	3	1	2
	SD	2.2	3.0	0.8	1.0
Early resorptions	N	16	12	13	6
	Mean	1	2	0	2
	SD	2.2	2.7	0.7	1.2
Late resorptions	N	16	12	13	6
	Mean	0	2	1	0
	SD	0.6	2.5	0.4	0.4

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Table 6

Summary of Fetal Visceral Abnormalities

	Group (Dose Level in mg AC 84,777/0.5 mL/kg)							
	1 (0)		2 (50)		3 (100)		4 (250)	
Number of litters examined	15		10		11		4	
Number of fetuses examined	119		52		70		17	
OBSERVATION		%		%		%		%
Major Vessels								
V Left carotid arising from innominate								
Litter incidence	5	33	2	20	1	9	0	
Fetal incidence	7	6	2	4	1	1	0	
V Accessary left subclavian artery								
Litter incidence	4	27	1	10	3	27	0	
Fetal incidence	6	5	1	2	5	7	0	
Heart								
M Enlarged atrium								
Litter incidence	1		0		0		0	
Fetal incidence	1		0		0		0	
M Septal defect								
Litter incidence	0		0		1		0	
Fetal incidence	0		0		1		0	
Gall Bladder								
M Reduced in size								
Litter incidence	0		2		0		0	
Fetal incidence	0		2		0		0	

V = Variation
M = Malformation

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Table 7

Summary of Fetal Skeletal Abnormalities

	Group (Dose Level in mg AC 84,777/0.5 mL/kg)							
	1 (0)		2 (50)		3 (100)		4 (250)	
Number of litters examined	15		10		11		4	
Number of fetuses examined	119		52		70		17	
OBSERVATION		%		%		%		%
Skull								
M Mandible reduced in ossification								
Litter incidence	0		0		1		0	
Fetal incidence	0		0		1		0	
Vertebrae								
M 27 presacral								
Litter incidence	2	13	0		3	27	0	
Fetal incidence	3	3	0		3	4	0	
M 25 presacral								
Litter incidence	1		0		0		0	
Fetal incidence	1		0		0		0	
M Absent								
Litter incidence	0		0		0		1	
Fetal incidence	0		0		0		1	
M Centra abnormalities								
Litter incidence	0		0		1	9	1	25
Fetal incidence	0		0		1	1	2	12
Ribs								
V Rudimentary - unilateral								
Litter incidence	13		8		5		2	
Fetal incidence	21		15		8		2	
V Rudimentary - bilateral								
Litter incidence	7		2		2		2	
Fetal incidence	10		3		2		4	
V Full unilateral								
Litter incidence	11		4		5		3	
Fetal incidence	13		8		5		3	

M = Malformation
V = Variation

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Study No. 6123-114

Table 7 (Continued)

Summary of Fetal Skeletal Abnormalities

	Group (Dose Level in mg AC 84,777/0.5 mL/kg)			
	1 (0)	2 (50)	3 (100)	4 (250)
Ribs (Continued)				
V 13 full pairs				
Litter incidence	4	4	7	2
Fetal incidence	7	5	9	4
M 11 full pair				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0
M Fused				
Litter incidence	0	0	1	1
Fetal incidence	0	0	1	1
M Interrupted ossification				
Litter incidence	0	0	1	0
Fetal incidence	0	0	1	0
Sternebrae				
V Chain fusion				
Litter incidence	5	1	4	1
Fetal incidence	10	1	5	1
V Unossified (5 and/or 6)				
Litter incidence	6	5	3	0
Fetal incidence	18	8	3	0
M Bipartite				
Litter incidence	0	0	1	0
Fetal incidence	0	0	1	0
Pectoral Girdle				
M Bent scapular spine				
Litter incidence	0	0	1	0
Fetal incidence	0	0	1	0
Pelvic Girdle				
M Unossified pubis				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0

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