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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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DATA EVALUATION REPORT

Study Type: Reproduction (3-gen.) -- Rat

Tox. Chem. No.: 363A
TXR Doc. No.: 006658
MRID No.: 37924
Project No.: 8-0126

Test Material: AC 84,777;
Purity not specified.

Synonyms: Difenzoquat, Avenge

Study Number(s): 362-147

Sponsor: American Cyanamid Company

Testing Facility: Hazelton Laboratories, Inc.,
Vienna, VA

Title of Report: Final Report: Three-Generation Reproduction Study in Rats

Author(s): Reno, F. E.

Report Issued: October 14, 1974

Conclusions:

Parental NOEL = > 2500 ppm (125 mg/kg; HDT)*

Reproductive/Developmental NOEL = 500 ppm (25 mg/kg)

Reproductive/Developmental LEL = 2500 ppm (Decreased body weights of male and female pups at weaning in all generations; decreased body weights of male and female pups at birth in the second and third generation, respectively)

Core classification: Minimum

*Although, compared with the controls, there were statistically significant ($p < 0.05$) decreases in maternal body weights at this dose level during the premating period (the only time when body weights were recorded in this study), these were either small (5 or 6 %) and probably

biologically insignificant or occurred in animals which were smaller (20 %) than controls at the start of the treatment, due to reduced weight at weaning.

Experimental Procedures:

Charles River rats, 10 males and 20 females/ dose level, were fed diets containing 0, 500 or 2500 ppm of AC 84,777 for three successive generations. The feeding of the test material was started 14 weeks (P_1 parents) or 9 weeks (P_2 and P_3 parents) before mating and was continued throughout the mating, gestation and lactation periods. The test material was incorporated in Purina Laboratory Chow and the food (and water) were allowed in unrestricted amounts. The animals weighed 125-149 g (males) and 94-125 g (females) at the start of treatment, were assigned to groups randomly and housed individually. The parents were observed for toxic signs and mortality throughout the study. Individual body weights were recorded on treatment weeks 0, 4, and 9 (P_1, P_2 and P_3 animals) and also on treatment week 14 for the P_1 animals. Food intake was recorded during the same weeks, except that no record was made at the start of the treatment (week 0).

Twenty-four hours after parturition, the litters in each generation were randomly reduced to a maximum of eight pups (4 males and 4 females, when possible). A record was kept of the number of pregnancies, litters born, live and still births, deaths during lactation, and of litter size and weight by sex at 24 hours and at weaning. All pups were examined for external abnormalities. At the weaning of each generation, 10 males and 20 females from each dose level were saved for continuation of the study; about one-third of the pups were subjected to gross necropsy; and the remaining pups and all parents were discarded (without necropsy).

From 20 male and 20 female weanling pups of the third generation (F_{3A}), the following tissues were preserved in formalin for possible future histopathological examination:

brain, pituitary, eye, thyroid, lung, liver, spleen kidney, adrenal, stomach, pancreas, small intestine, urinary bladder, gonad, bone, bone marrow, heart, large intestine, and any unusual lesions.

The following reproductive indices were calculated for each generation: Fertility Index (number of pregnancies x 100 / number of females mated) and Gestation Index (number of litters born x 100 / number of pregnancies observed).

Statistical analysis of parental group mean body weight and food consumption data and of the reproduction data (mean number of pups born, mean number of live pups, mean number of dead pups, and mean weight of live pups at 24 hours and weaning) was

performed by the t-test at the 5.0% probability level[1].

Results:

Parental Data

Males -- The high-dose animals in all generations gained slightly less weight (4-7 %) than did the controls during the premating period, but these differences in weight gains were small and lacked statistical and, probably, biological significance. Also, compared with the controls, the P_2 and P_3 high-dose males weighed less (10-12 %) at the initiation of treatment, due to reduced weights at weaning.

Food consumption was occasionally reduced among the high-dose animals, but decreases were small (3-7 %), inconsistent, and, in most instances, statistically insignificant.

Females -- Compared with controls, decreases in body weights were observed in the high-dose animals (5 to 6 in P_1 and P_3 generations, and 11 to 15 % in the P_2 generation). These decreases generally persisted throughout the premating period (the only time when weight was recorded) and were statistically significant consistently for the P_1 and P_2 animals. Body weights among the low-dose animals were also occasionally slightly (6 to 8 %) but significantly ($p < 0.05$) lower than those of the controls. However, due to reduced weight at weaning, the P_2 animals weighed 20% less at the initiation of treatment than did the controls. This lower initial weight did not worsen as treatment with AC 84,777 was continued.

There were no consistent differences in food intake among the control and the treated groups in all generations.

Reproductive/Developmental Data

Male and female pups born in the high-dose group weighed less at weaning in each generation than did those in the control group. The mean weight decreases ranged from 13.9 to 15.5 % and were statistically significant. In the low-dose group, the mean weight decreases ranged from 2.1 to 8.7 % and all were statistically insignificant.

The test material had no effect on the fertility and gestation indices, number of pups born alive and dead, weight of live pups at birth (F_{1A} generation only) and pup mortality during the lactation period (F_{1A} and F_{3A} generations only).

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[1]Wilfred J. Dixon and Frank J. Massey, Jr., Introduction to Statistical Analysis, pp. 123-124, p. 232 - Section 13-6, McGraw-Hill, 1957.

Statistically significant decreases in the weight of the male pups (7 %) and female pups (8.2 %) at birth were observed in the F_{2A} and F_{3A} generations, respectively. These weight decreases were attributed to smaller size of the parents, compared with their respective controls. There was also an increase in the number of the high-dose male pups dying during the lactation period, but only int the F_{2A} generation. (For details on the above findings, see Attachment I).

Gross necropsy of randomly selected pups did not reveal treatment-related abnormalities. Incidental observations included a roughened spleen in one F_{1A} control female and one F_{3A} low-dose female; thickened stomach walls of one F_{3A} female (dose level not specified); and thinness of two F_{3A} high-dose males.

Comments:

This study was conducted some 14 years ago and some of the data required in the guidelines are missing, such as: 1) Parents were not necropsied (and neither was one low-dose female which was found dead during the premating period); 2) Animals were weighed only during the premating period, a total of four times during 14 weeks (P_1 group) and a total of three times during 9 weeks (P_2 and P_3 groups); and 3) Litters were weighed only at birth and at weaning. However, enough of the essential data were reported to accept this study as an adequate reproduction study. Although two levels, rather than at least three, of the test material were used, the highest level caused developmental effects.

Parental NOEL = > 2500 ppm (125 mg/kg; HDT. See page 1 for comments)

Reproductive/Developmental NOEL = 500 ppm (25 mg/kg)

Reproductive/Developmental LEL = 2500 ppm (See page 1 for toxic signs)

Core Classification: Minimum

Quality Assurance Statement was not included in the report.

Attachment I

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Figure No. 3 - A summary of reproduction and weaning survival data

$P_1 \rightarrow F_{1A}$

	GROUP NUMBER 1 CONTROL	2 500 PPM	3 2500 PPM	
No. of Matings	20	19	20	
No. of Pregnancies	18	18	19	
Fertility Index	90.0%	94.7%	95.0%	
No. of Litters Born	18	18	18	
Gestation Index	100.0%	100.0%	94.7	
Mean:				
No. of Pups Born	10.4	11.1	9.3	10.6% ↓
No. of Pups Born Alive -				
Males	5.4	5.6	4.7	13% ↓
Females	5.0	5.5	4.6	8% ↓
No. of Pups Born Dead -				
Males	0.0	0.6	0.0	
Females	0.0	0.0	0.0	
Weight of Live Pups at Birth -				
Males	6.8	6.6	6.5	
Females	6.6	6.2	6.2	
No. of Pups Left to Nurse -				
Males	3.5	4.2	3.8	
Females	3.6	3.8	3.3	
No. Died During Lactation -				
Males	0.1	0.2	0.2	
Females	0.1	0.2	0.0	
No. of Pups Weaned -				
Males	3.4	4.0	3.6	
Females	3.4	3.6	3.3	
Weight at Weaning -				
Males	49.3	47.2	4.3% ↓ 42.4 S-	14.0% ↓
Females	47.5	46.5	2.1% ↓ 40.9 S-	13.9% ↓

• One female was found dead during the premating period; was not necropsied.

S- = Significantly lower than control at $p \leq 0.05$.

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Figure No. 3 - Continued

$P_2 + F_{2A}$

	GROUP NUMBER 1 CONTROL	2 500 PPM		3 2500 PPM	
No. of Matings	20	20		20	
No. of Pregnancies	20	19		19	
Fertility Index	100.0%	95.0%		95.0%	
No. of Litters Born	20	19		19	
Gestation Index	100.0%	100.0%		100.0%	
Mean:					
No. of Pups Born	11.3	11.9		10.5	7% ↓
No. of Pups Born Alive -					
Males	5.2	5.7		5.5	
Females	5.9	6.0		5.1	
No. of Pups Born Dead -					
Males	0.5	0.0		0.5	
Females	0.2	0.2		0.5	
Weight of Live Pups at Birth -					
Males	7.1	7.1		6.6	S- 7% ↓
Females	6.6	6.7		6.2	6%
No. of Pups Left to Nurse -					
Males	3.9	3.9		3.6	
Females	3.8	3.9		3.9	
No. Died During Lactation -					
Males	0.2	0.3		0.5	S+ 150% ↑
Females	0.3	0.4		0.3	
No. of Pups Weaned -					
Males	3.7	3.6		3.2	13.5% ↓
Females	3.5	3.5		3.6	
Weight at Weaning -					
Males	49.5	47.6	3.8% ↓	42.0	S- 15.2%
Females	47.7	45.8	4.0% ↓	40.3	S- 15.5%

S+ = Significantly higher than controls at $p \leq 0.05$.

S - = Significantly lower than controls at $p \leq 0.05$.

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Figure No. 3 - Continued

$P_3 \rightarrow F_{3A}$

	GROUP NUMBER 1 CONTROL	2 500 PPM	3 2500 PPM
No. of Matings	20	20	20
No. of Pregnancies	18	20	20
Fertility Index	90.0%	100.0%	100.0%
No. of Litters Born	18	20	20
Gestation Index	100.0%	100.0%	100.0%
Mean:			
No. of Pups Born	9.4	12.2 S+ 29.8% ↑	12.5 S+ 33% ↑
No. of Pups Born Alive -			
Males	4.3	5.7	6.3 ↑
Females	5.1	66.4 ??	6.2 ↑
No. of Pups Born Dead -			
Males	0.5	0.1	0.5
Females	0.0	0.5	0.0
Weight of Live Pups at Birth -			
Males	6.5	6.5	6.1 6.2% ↓
Females	6.1	6.1	5.6 S- 8.2% ↓
No. of Pups Left to Nurse -			
Males	3.4	3.9	3.9
Females	3.8	3.9	4.1
No. Died During Lactation -			
Males	0.1	0.4	0.1
Females	0.2	0.3	0.1
No. of Pups Weaned -			
Males	3.3	3.5	3.9
Females	3.6	3.6	4.0
Weight at Weaning -			
Males	46.4	43.3 6.7% ↓	39.7 S- 14.4% ↓
Females	44.7	40.8 S- 8.7% ↓	38.1 S- 14.8% ↓

S+ = Significantly higher than controls at $p \leq 0.05$.

S- = Significantly lower than controls at $p \leq 0.05$.

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