PERMANENT STERILITY IN THE MALE RAT AFTER A SINGLE ORAL DOSE OF A PIPECOLINOMETHYL-HYDROXYINDANE

A. BORIS, L. DEMARTINO AND THELMA TRMAL

Research Division, Hoffmann-La Roche Inc., Nutley, New Jersey 07110, U.S.A.

(Received 12th November 1973)

Summary. A single oral dose of DL-6-(N- α -pipecolinomethyl)-5-hydroxy-indane maleate caused subnormal fertility in the male rat during the 3rd and 4th week after administration. Sterility ensued from the 5th week and persisted to the termination of the experiment 20 weeks after dosing.

The antitesticular activity of DL-6-(N- α -pipecolinomethyl)-5-hydroxyindane maleate (PMHI) in the rat and other species has been described (Boris, Ng & Hurley, 1974; Boris, DeMartino & Cox, 1974). These studies also indicated that treatment with PMHI for 21 days caused sterility in male rats, and that the reversibility of the antifertility effect depended on the dose which had been given. The present study was carried out to determine: (a) whether a single oral dose of PMHI could cause permanent sterility in the male rat, and (b) how soon after dosing sterility would occur.

The rats used were Sprague-Dawley descendants obtained from a local supplier. All animals were housed in temperature-controlled, air-conditioned quarters and were permitted free access to food and water. An artificial lighting system alternated 12-hr periods of light and darkness. The results obtained in preliminary dose-finding experiments suggested that the sterilant dose was between 64 and 270 mg/kg and probably close to 130 mg/kg. Based on these data, PMHI was dissolved in distilled water and administered by gavage to male rats at a dose of 150 mg/kg. From the day of treatment, each male rat was permitted to cohabit with two untreated female rats. Fresh females were supplied at weekly intervals. Rats which did not litter during the interim period were examined for pregnancy at autopsy 2 weeks after removal from the male rats. The incidence of pregnancy was determined for 20 weeks after the single oral dose of PMHI. The male rats were then killed and their sex organ weights were determined. Testes were also examined histologically.

The effect of a single oral dose of PMHI on the subsequent fertility of male rats is shown in Table 1. These data indicate that subnormal fertility was detectable during the 3rd and 4th week after dosing and that sterility began during the 5th week and persisted to the termination of the experiment 20 weeks after dosing. Serial mating studies after administration of antispermatogenic agents can provide information as to the particular spermatogenic cell types affected and indicate whether epididymal spermatozoa are influenced (Jackson, 1966). The apparent normal fertility of rats treated with PMHI during the

first 2 weeks after dosing suggests that PMHI does not affect epididymal spermatozoa. The significant reduction in the number of pregnant rats during the 3rd week and sterility from the 5th week onwards indicates that PMHI affects spermatids, spermatocytes and spermatogonia. It is evident from the data shown in Table 2 that the testicular weight was significantly reduced in rats which had received a single oral dose of PMHI 140 days previously.

Table 1. Effect of PMHI on the subsequent fertility of male rats

Week	Controls		Treated		Significance
	No. pregnant/total no.	%	No. pregnant/total no.	%	
1	23/38	61	22/37	59	N.S.
2	24/40	60	23/37	62	N.S.
3	29/40	73	15/35	43	*
4	26/38	68	4/33	12	***
5	28/38	74	0/35	0	***
6	31/37	84	0/34	0	***
7	29/33	88	0/36	0	***
8	26/35	74	0/37	0	***
9	20/34	59	0/33	0	***
10	19/34	56	0/30	0	***
11	20/30	67	0/34	0	***
12	23/31	74	0/31	0	***

In rats which received a single oral dose of 150 mg PMHI/kg body wt. sterility continued from Week 13 to Week 20 when the experiment was terminated. During this period, the pregnancy incidence in the control group ranged from 64 to 77%.

N.S.=not significant, * $P<0.05$, *** $P<0.001$ (Fisher, 1958).

Table 2. Effect of PMHI on sex organ weights of adult male rats determined 140 days after treatment

Treatment	No. of animals	Mean body weight (g)		Mean organ weight (mg) $\pm$ S.E.		
		Initial	Final	Testes	Seminal vesicles	Ventral prostate
Controls	13	394 $\pm$ 9	549 $\pm$ 12	3687 $\pm$ 44	1206 $\pm$ 44	560 $\pm$ 28
PMHI	13	392 $\pm$ 10	530 $\pm$ 25	1293 $\pm$ 94***	1248 $\pm$ 72	566 $\pm$ 50

Rats received a single oral dose of 150 mg PMHI/kg body wt.

***Significantly different from the control value ($P<0.001$).

Histological examination of these testes revealed tubules completely devoid of spermatogenic cells. A comparison of treated rats with control rats revealed no significant differences in body, seminal vesicle and ventral prostate weights 140 days after dosing. The prolonged duration of sterility and the absence of histological evidence of regeneration of the spermatogenic epithelium implies that PMHI causes permanent destruction of spermatogenic capability and irreversible sterility.

REFERENCES

- BORIS, A., NG, C. & HURLEY, J. F. (1974) Antitesticular and antifertility activity of a pipercolino-methylhydroxyindane in rats. *J. Reprod. Fert.* (in press).
- BORIS, A., DeMARTINO, L. & COX, D. C. (1974) Further studies in rats on the antitesticular activity of a pipercolinomethylhydroxyindane. *J. Reprod. Fert.* (in press).
- FISHER, R. A. (1958) *Statistical Methods for Research Workers*, 13th edn. Hafner, New York.
- JACKSON, H. (1966) *Antifertility Compounds in the Male and Female*. Charles C. Thomas, Springfield.