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THE EFFECT OF CARBARYL ON REPRODUCTION IN THE RHESUS MONKEY

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DR. FREDERICK COULSTON

DIRECTOR

INSTITUTE OF EXPERIMENTAL PATHOLOGY AND TOXICOLOGY

ALBANY MEDICAL COLLEGE

ALBANY, NEW YORK 12208

INTRODUCTION

The widespread occurrence of pesticides in the environment has necessitated a thorough evaluation of safety of some of these compounds. The effect of certain pesticides on reproduction has become an increasingly important area of evaluation for the toxicologist. For example, Carbaryl (N-methyl-1-naphthyl carbarmate) has been reported to have an effect on reproduction in a variety of species. Marliac (1964), Marliac et al. (1965), Ghadiri et al. (1966) and Khera (1966) demonstrated that Carbaryl was teratogenic in the chick and the duck, when applied directly to or injected into the embryo or yolk sac. Defects of the cartilaginous and osseous skeleton, eye cataracts, ascites, and hepatic degenerative changes were observed. Similarly, Ghadiri et al. (1967) fed Carbaryl to laying hens and they reported that there was a significant decrease in the hatchability of the eggs. In those chicks that hatched, there were marked developmental abnormalities. Carbaryl at a level of 200 ppm (50 mg/kg) in the diet of pregnant mice produced a number of skeletal abnormalities in two litters (FAO/WHO, 1967). Smalley et al. (1968) studied the effects of Carbaryl on reproduction in the beagle dog and they reported a possible "contraceptive effect" at the high dose level of 50 mg/kg. At lower dose levels of 25, 12.5, 6.25 and 3.125 mg/kg, there was resorption of some of the pups. Other pups had developmental abnormalities of the skeletal system and viscera. At parturition, they noticed that the females experienced difficulty in delivery which was attributed to atonia of the uterine musculature and designated as dystocia. Robens (1969) studied the effects of Carbaryl in a number of animals and reported that no terata were produced in hamsters fed 125 mg/kg of Carbaryl, however, a single dose of 250 mg/kg resulted in increased fetal mortality. Similarly, there were no teratogenic effects in rabbits fed 100 mg and 200 mg/kg of Carbaryl. Defects of the skeletal system were seen in guinea pigs fed 300 mg/kg of Carbaryl

from the 11th to the 20th day of gestation. Collins et al. (1970) fed Carbaryl at levels of 10,000 5,000 and 2,000 ppm in the diet to rats and gerbils for three generations and they observed a dose-related decrease in the fertility, viability, survival and lactation indices in these species over three generations.

In view of these observations in lower animals a detailed experiment was undertaken to investigate the effects of Carbaryl on reproduction in the non human primate. Previous work in the Rhesus monkey at the Institute of Experimental Pathology and Toxicology (unpublished results Table I) indicated that Carbaryl may interfere with reproduction in this species. Eleven female monkeys were bred and medicated with 2 mg, 6.3 mg and 2.0 mg/kg of Carbaryl. One of these delivered a baby while four controls conceived and delivered normal babies. The purpose of the present investigation in the monkey was to reexamine these results and evaluate the effects of Carbaryl on reproduction in the Rhesus monkey.

MATERIALS AND METHODS

Twenty-five mature female Rhesus monkeys, five to seven kilograms in weight, with a past history of regular menstrual cycles were chosen for this study. All monkeys were housed individually in air-conditioned quarters and allowed free access to water and food (Monkey Chow, Purina). Each female was mated from day 11 to 16 of the menstrual cycle with the first day of menstrual bleeding being counted as day one of the cycle. Daily vaginal smears, stained by the method of Papanicolaou (1941) were used to detect the onset of menstruation. The initial presence of red blood cells in the smear was indicative of the start of the cycle. The female monkey was put in a cage with a different male Rhesus monkey every 48 hours during the entire 6 day mating period. Vaginal smears were taken each morning during the mating period to determine if copulation had taken place. When sperm were found in the smear, the female was medicated orally by stomach tube with Carbaryl in 1% aqueous gum tragacanth five days a week at either the 2 mg or 20 mg/kg level. The dose range is within the tolerance range of 100 ppm in the diet established for Carbaryl by the FDA (20 mg = 100 ppm, 2 mg = 1 ppm in the diet of a Rhesus monkey). The controls were given 1% aqueous gum tragacanth five days a week by stomach tube. Medication was continued for the entire gestation period in the pregnant females.

Pregnancy was diagnosed by the Tullner-Hentz (1966) modification of the Ascheim-Zondek Pregnancy Test. This test indicates the presence of Monkey Chorionic Gonadotropin (MCG) in the blood through its action on the uterus of immature mice. The hormone is detectable in the blood by the 16th day of pregnancy, peaks at day 21 and is undetectable by day 28. Blood was drawn from the brachial vein of the female monkey 21 days after mating. Serum (0.5 ml) was injected subcutaneously into three 21 day old female mice weighing 10 ± 2 gm. once a day for three days. On the fourth day, the mice

were killed and the uteri removed. The fat was trimmed from the uterine horns and the uterus was weighed on a Roller-Smith torsion balance. Additionally three mice were injected with 3 X 0.5 IU of Human Chorionic Gonadotropin (HCG Ayerst Laboratories, New York, N. Y.) to insure that each group of mice was responding to the hormone. An increase in the mouse uterine weight of approximately 100% as compared to saline controls indicated a pregnancy in the monkey. Positive pregnancy tests were confirmed by bimanual rectal palpation of the uterus according to the procedure of Hartman (1932). The palpations were done weekly for the first two months of pregnancy. Early abortion in the Rhesus monkey is difficult to detect and may be confused with placental bleeding. Weekly measurement of the increase in uterine size by this method indicated that the pregnancy was proceeding normally.

Following delivery, medication of the mother was discontinued. Immediately after birth the baby and mother were separated and examined. The babies were weighed, measured and returned to the mother. This examination of the infant was performed on the 7th and 15th day after birth and then once every month. The babies were weaned at various intervals after birth and the mother returned to the breeding colony.

RESULTS

A. Reproductive Performance of Females in the Control Group

The results of the control group consisting of 7 monkeys are presented in Table II. One of these monkeys (#693) was used as a control in the previous study dealing with Carbaryl and had delivered a normal baby. Five of these seven monkeys had a positive pregnancy test (Table IV). The other two monkeys were mated seven or more times and failed to conceive. Four of the five monkeys (#'s 706, 693, 814, 825) delivered normal babies, two males and two females. The average length of gestation was 164 ± 2.4 days. This agrees with the findings of Valerio et al. (1969) and Van Wagenen (1966). The fifth monkey aborted within the first 60 days of pregnancy and no fetus was recovered. All the monkeys delivered in the late evening or early morning. One monkey, #706, had a prolapse of the uterus after delivery. This condition persisted for about a week after which the uterus returned to its normal position.

B. Reproductive Performance of Females in the Medicated Groups

The results of the groups on medication are presented in Table III. There were four females in the 2 mg/kg group. Two of these females (#621 and #684) had positive pregnancy tests. The results of the pregnancy tests in the medicated groups are presented in Table V. Neither of these monkeys delivered a baby, due to an abortion early in pregnancy. The remaining monkeys in this group were mated 3 or more times and failed to conceive although their menstrual cycles were regular.

A total of 10 female monkeys received 20 mg/kg of Carbaryl. Six of these ten monkeys (#'s 700, 698, 712, 812, 816 and 823) had positive pregnancy tests (Table IV) by the third mating. Three of the six pregnant females delivered normal babies. The average length of gestation for these three births was 166 ± 3.5 days. The remaining three monkeys failed to deliver viable babies. One monkey (#812) delivered a male fetus at 116 days

of gestation. The dead baby was found when the female was medicated. Gross examination of the baby revealed no developmental abnormalities. Subsequent examination of the other two females within sixty days of conception revealed that these monkeys were no longer pregnant and had aborted early in pregnancy.

The dystocia characterized as a delay in delivery accompanied by restlessness and vaginal discharge observed in dogs treated with Carbaryl (Smalley et al. 1968) was not observed in any of the female monkeys that conceived and delivered in the course of this study. There was no indication of any difficulty in the delivery of these babies. A slight amount of bleeding accompanied the delivery and this persisted in all females medicated and control for about a week following birth.

Preliminary observations of the babies is presented in Table VI. The average weight recorded within a week of delivery was 445 gms for the control monkeys. The babies in the medicated group were slightly higher at 500 gms. The size of all the monkeys in terms of length was approximately the same. The age of the infants at weaning is presented in Table VI. The babies had no difficulty adjusting after being weaned and immediately began eating and drinking by themselves. All of the babies from the medicated mothers appear normal as compared to controls.

DISCUSSION

Survey of the literature indicated that Carbaryl has an effect on reproduction or embryonic development in every animal tested with the exception of the rabbit. The widespread use of this pesticide has warranted its evaluation in a non-human primate, such as the Rhesus monkey, whose reproductive system is similar to the human (Heuser et al. 1941) and which has a low level of spontaneous teratology (Wilson, J. G. et al. 1970).

The assay for monkey Chorionic Gonadotropin has been reported by Tullner et al. (1968) to be a reliable method for the detection of pregnancy in the Rhesus monkey. This assay combined with the rectal palpation of the uterus indicated that the Carbaryl monkeys in this study had conceived and subsequently aborted. Unpublished results of an earlier study conducted at the Institute of Experimental Pathology and Toxicology indicated that Carbaryl interfered with reproduction in the Rhesus monkey. The results of the present investigation confirmed the previous results and further indicated that administration of this pesticide throughout gestation was associated with a higher abortion rate when compared to control monkeys. The abortion rate in the control group was 20%. The abortion rate in the medicated groups was 100% for those monkeys receiving 2 mg/kg of Carbaryl and 50% in the 20 mg/kg group.

All previous investigations (Marliac 1964, Marliac et al. 1965, Khera, 1966, Smalley et al. 1968 and Robens, 1969) reported that Carbaryl had a teratogenic effect on the developing embryo. The medication schedule in this study insured that the pregnant female received Carbaryl during the period of gestation when the embryo was most susceptible to a teratogen. The results indicated that all the live babies as well as the one aborted fetus did not reveal any abnormal development. The 1:1 ratio of male to female babies in the control and medicated group indicated that Carbaryl did not effect the sex ratio. Similarly, all pregnancies in the Control

and medicated groups were obtained by the third mating. In all groups control and medicated, the conception rate decreased after the third mating period. This indicates that at these dose levels Carbaryl does not have a contraceptive effect in the Rhesus monkey. This schedule of administration in the monkey did not have any effect on embryonic development or conception, but it did affect those factors responsible for the maintenance of pregnancy.

SUMMARY

Mature, cycling female Rhesus monkeys were medicated orally with 2 mg/kg or 20 mg/kg of Carbaryl five days a week throughout gestation. Of the four monkeys medicated with 2 mg/kg of Carbaryl, two conceived but did not deliver a baby. Ten monkeys received 20 mg/kg of Carbaryl. Three out of the six that conceived delivered live babies. In the control group four out of the five monkeys that conceived delivered live babies. The administration of this pesticide during pregnancy in the Rhesus monkey increases the rate of abortion as compared to control monkeys. There was no evidence of abnormal development in the live babies delivered by the medicated mothers.

Table VII is a summary of all the work done on the effect of Carbaryl on reproduction in the Rhesus monkey at I E P &T. A total of eleven control females were mated thirty-two times. Eight of these delivered normal babies, four males and four females. One aborted and two failed to conceive. Seven females were medicated with Carbaryl at the two mg/kg level. These monkeys were mated seventeen times. Two of these conceived and both aborted early in pregnancy. Three females received 6.3 mg/kg of Carbaryl and each was mated once. One of these delivered a normal male baby. The two remaining monkeys failed to conceive. Fourteen females received Carbaryl at the 20 mg/kg level. These monkeys were mated a total of 34 times. Six of these monkeys conceived. Three delivered normal babies and the remaining three females aborted. The results of these two experiments indicate that Carbaryl does have an effect on reproduction in the Rhesus monkey.

TABLE I

CARBARYL REPRODUCTIVE STUDY

Monkey No.	Date Bred	Dose mg/kg	Date Delivered	Gestation Period (days)	Sex of Infant
693	11/18/66	*Control	5/1/67	164	female
698	11/20/66	Control	5/3/67	164	male
694	12/1/66	Control	5/15/67	166	male
684	5/20/67	Control	10/30/67	163	female
619	10/5/67	+2.0	-	-	-
704	10/26/67	2.0	-	-	-
689	11/28/67	2.0	-	-	-
463	10/13/67	6.3	-	-	-
564	10/13/67	6.3	3/17/68	157	male
613	10/13/67	6.3	-	-	-
614	11/15/67	6.3	-	-	-
717	10/30/67	20.0	-	-	-
703	11/13/67	20.0	-	-	-
714	11/13/67	20.0	-	-	-
706	11/15/67	20.0	-	-	-

* 1% aqueous gum tragacanth administered daily by stomach tube, 6 days a week, throughout gestation.

\+ Administered in 1% aqueous gum tragacanth daily by stomach tube, 6 days a week, after breed[illegible]

TABLE II

REPRODUCTIVE PERFORMANCE OF

CONTROL MONKEYS+

Monkey No.	Times Bred Prior to Conception	Positive Preg. Test	Date Delivery	Gestation Period (Days)
706	3	+	3/28/69	164
628	10	-	--	--
693	3	+	4/30/69	164
814	3	+	5/19/69	167
820	7	-	--	--
825	1	+	4/15/69	161
828	1	+	aborted	--

+ 1% aqueous gum tragacanth administered daily by stomach tube, 5 days a week, throughout gestation.

TABLE III

REPRODUCTIVE PERFORMANCE OF

MEDICATED MONKEYS+

Monkey No.	Dose Level mg/kg	Times Bred	Preg. Test	Delivery	Gestation
621	2	3	+	aborted	-
687	2	7	-	-	-
684	2	3	+	aborted	-
702	2	2	-	-	-
698	20	3	+	8/25/69	164
700	20	2	+	aborted	-
712	20	3	+	3/8/69	168
812	20	1	+	aborted	-
815	20	3	-	-	-
816	20	1	+	4/4/69	161
817	20	5	-	-	-
822	20	5	-	-	-
823	20	1	+	aborted	-
827	20	6	-	-	-

+ Dose administered daily by stomach tube, 5 days a week, throughout gestation.

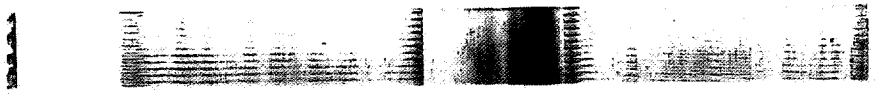

TABLE IV

PREGNANCY TEST

CONTROL MONKEY

Monkey No.	Control Mice Uterine wt. (mg) 3 X .5 ml Saline			Test Mice Uterine wt. (mg) 3 X .5 ml Serum			Delivery Date
706	*83	101	67	140	156	100	3/28/69
693	30	52	29	56	50	59	4/30/69
814	39	34	30	46	52	83	5/19/69
825	28	28	29	67	47	78	4/15/69
828	30	52	29	55	70	96	10/5/69

* Immature rats were used for this test.

TABLE V

PREGNANCY TEST

MEDICATED MONKEY

Monkey No.	Group mg/kg	Control Mice Uterine wt. (mg) 3 X .5 ml Saline			Uterine Wt. (mg) 3 X .5 ml Serum			Delivery Date
621	2	11	12	12	30	37	21	--
684	2	30	25	34	116	154	172	--
698	20	23	21	24	54	45	66	8/25/69
700	20	11	19	25	20	40	45	--
712	20	23	23	31	80	49	61	3/8/69
812	20	30	32	28	78	90	100	--
816	20	24	44	28	105	109	116	4/4/69
823	20	31	17	27	31	31	46	--

TABLE VI

OBSERVATIONS ON THE BABIES

Group	No.	Sex	Wt. gms.	Length in Inches Head to Foot	Length in Inches Head to Tail	Age at Weaning in Days
Control	706	M	490	13	6.5	75
Control	693	F	397	14	8	63
Control	814	M	460	14	8	44
Control	825	F	434	14	7.5	57
Medicated						
20 mg	712	M	542	15	8	45
20 mg	816	M	458	14.5	8	68
20 mg	698	F	425	12	7	--
20 mg	812	F	--	--	--	--

TABLE VII

Summary of Two Carbaryl Experiments as Outlined in Tables I and II

Group	No. of Monkeys	No. of Mating	Gestation and No. of Babies	Sex of Baby M	Sex of Baby F
Control	11	32	8	4	4
2.0 mg/kg	8	18	-	-	-
6.3 mg/kg	3	3	1	1	-
20.0 mg/kg	14	34	4	2*	2

* One of these was an aborted fetus delivered at 116 days of gestation.

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