The Non-Teratogenicity of 2,4,5-Trichlorophenoxyacetic acid in the Rhesus Monkey (Macaca mulatta)[1]

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INTRODUCTION

There have been numerous reports concerning the effect of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on fetal development. These reports indicate that administration of the herbicide produces fetal abnormalities, resorptions, reduced litter sizes and reduced fetal weights (COURTNEY et al. 1970, 1971; SPARSCHU et al. 1970; EMERSON et al. 1970; KHERA and MCKINLEY 1972; HART and VALERIO 1972; COLLINS and WILLIAMS 1971; NEUBERT and DILLMAN 1972; WILSON 1971). These results were obtained using different animal species, dose levels, routes of administration, and impure 2,4,5-T containing varying concentrations of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDBD). The administration of this particular contaminant, TCDBD, to pregnant rats has resulted in fetuses with intestinal hemorrhages, subcutaneous edema, as well as reduced litter sizes, an increase in fetal resorptions and fetal deaths. Therefore, it has been suggested that a high concentration of TCDBD in 2,4,5-T preparations could be responsible for the fetal effects observed when this herbicide is administered to pregnant animals (SPARSCHU et al. 1970). The purpose of this investigation was to determine if 2,4,5-T containing less than 0.05 ppm of TCDBD is teratogenic in the rhesus monkey when administered daily from Day 22 through Day 38 of gestation, at doses of 2,4,5-T which were reasonable potential levels of exposure to man. In addition, the experiment was designed to determine any gross toxicity to the pregnant female monkey and to observe the development of the offspring up to 1 yr of age for signs of delayed toxicity.

MATERIALS AND METHODS

The 40 pregnant rhesus monkeys used in this study were selected from the International Center of Environmental Safety's breeding colony at Holloman AFB, New Mexico. All females were bred for 48 hr beginning on the 11th day of the menstrual cycle. Pregnancy was

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diagnosed by the modification of the Ascheim-Zondek pregnancy test (TULLNER and HERTZ 1966). Blood was drawn from the brachial vein of the female on Day 19 after mating. Immature mice, 21 days old, were injected with 0.5 ml of monkey serum for 3 consecutive days. On the 4th day, the mice were killed, the uteri removed and weighed. A 100% increase in mouse uterine weight as compared to control mice was considered indicative of a pregnancy in the monkey.

Using this procedure, pregnancy was diagnosed by Day 22 of gestation. Vaginal smears were obtained up to Day 100 of gestation. This was done to record the length of implantation bleeding and combined with rectal palpation of the uterus, was used to diagnose abortions. All pregnant animals were housed individually and allowed free access to food and water.

The 2,4,5-T [2] used for this study contained 0.05 ppm of TCDBD. It was administered orally in No. 5 gelatin capsules using a stomach tube. The 40 pregnant monkeys were assigned to four groups of ten monkeys each and received either the empty gelatin capsule or the gelatin capsule containing either 0.05, 1.0, or 10.0 mg/kg doses of 2,4,5-T. All animals were medicated once daily from Day 22 through 38 of pregnancy. All pregnancies were allowed to go to term. The mother and offspring were separated within 24 hr after birth; the infant was given a physical examination, weighed and returned to its mother. The mean birth weights and gestation lengths were statistically analyzed for significance of difference by Student's "t" test (SNEDECOR 1956). Babies were weaned at 4 months of age. In addition to the daily observation of the offspring, monthly body weights were obtained and six offspring from each group were scheduled for necropsy, two shortly after birth, two at 6 months, and two at 1 year. A careful dissection of major organ systems was done on each infant necropsied. Also, a skeletal x-ray examination, clinical chemistry and hematology were done on each animal killed. In addition to the scheduled necropsies, all infant deaths during the study were similarly examined.

RESULTS

A preliminary dose ranging study indicated that 2,4,5-T containing 0.05 ppm of TCDBD at dose levels of 12 mg/kg or higher when administered for 18 consecutive days, produced signs of toxicity in the adult male and female rhesus monkey. This was evidenced by loss of appetite, vomiting, and loss of body weight. Thus, 10 mg/kg was chosen as the highest dose to be used in the experiment with pregnant monkeys. During the course of this study, none of the pregnant monkeys receiving 2,4,5-T showed any of the signs of toxicity. The outcome of the pregnancies for the four groups are presented in Table I. The mean gestation time and mean birth weight of each infant was within normal limits for our colony. Nine of the ten control monkeys delivered live infants. The remaining monkey aborted on Day 68 of

[2] Supplied by C. H. Boehringer Sohn, Ingelheim/Rh, Germany.

TABLE I. The Outcome of Pregnancies of Rhesus Monkeys Dosed with 2,4,5-T Containing Less than 0.05 ppm TCDBD.

Group	No. of Monkeys	No. of Livebirths	No. of Stillbirths	No. of Abortions	GESTATION LENGTH (days) Mean ± SD	t	INITIAL BODY WEIGHT OF INFANT (gms) Mean ± SD	t
Control	10	9	-	1	163.0 ± 10.3		410.3 ± 66.2	
0.05	10	10	-	-	162.9 ± 7.8	.02 [a]	455.7 ± 44.0	1.67 [a]
1.0	10	8	1	1	161.6 ± 16.3	.28 [a]	413.1 ± 63.7	.08 [a]
10.0	10	8	-	2	164.3 ± 8.2	.26 [a]	447.2 ± 21.9	1.49 [a]

[a] Not statistically different from control at $P \leq 0.05$.

gestation. This was accompanied by heavy vaginal bleeding which lasted for 5 days. An offspring born on Day 143 of gestation was considered premature according to the criteria of VAN WAGENEN (1966), i.e. a pregnancy terminating beyond $\pm$ 2 SD. This infant died 21 days after birth. Gross autopsy revealed no developmental abnormalities.

All ten pregnant monkeys that received 0.05 mg/kg doses of 2,4,5-T delivered live babies. One died 37 days after birth. Eight of the ten monkeys assigned to the 1.0 mg/kg group delivered live infants. One monkey aborted, and one delivered a stillborn infant of 119 days gestational age. Of the ten pregnant females receiving 10.0 mg/kg, two aborted and eight delivered live infants. The abortions in both the 1.0 and 10.0 mg/kg groups occurred prior to Day 50 of gestation.

Scheduled necropsies of the offsprings revealed no evidence of teratogenicity. This was also so for the stillborn infants and for those that died subsequent to premature birth. Generally, all infants exhibited a similar growth pattern as compared to the controls. Values for the clinical chemistry and hematology obtained from infant monkeys sacrificed at birth, 6 months, and 1 yr of age were found to be within normal limits for monkeys of this age.

DISCUSSION

The teratogenicity of 2,4,5-T has been the subject of much discussion since the work of COURTNEY et al. (1970). Their investigation indicated that this herbicide was teratogenic and fetocidal in both mice and rats, at dose levels of 4 mg/kg in rats and 46.4 mg/kg in mice. The concentration of the contaminant TCDBD in the 2,4,5-T preparation used in this study was 30 ppm. In view of the study reported by SPARSCHU et al. (1970) which indicates that TCDBD administered orally could produce fetal resorption and death in rats at levels as low as 0.5 ug/kg, it was concluded by these investigators that the fetal effects associated with 2,4,5-T could be the result of the TCDBD contaminant. This conclusion appears to be supported by the results of EMERSON et al. (1970) who demonstrated that 2,4,5-T containing less than 1.0 ppm of TCDBD produced no abnormalities at a high dose of 24 mg/kg. In a later study reported by COURTNEY et al. (1971), it was demonstrated that two preparations of 2,4,5-T containing 0.5 ppm and 0.05 ppm of TCDBD produced cleft palate and kidney malformations in three different strains of mice at levels of 100 mg/kg or higher. In addition, they demonstrated that TCDBD alone produced cleft palate and a marked increase in the incidence of kidney abnormalities at levels of 1 ug/kg and 3 ug/kg. However, when 100 mg/kg of 2,4,5-T was administered to mice with 1 ug/kg of TCDBD there was no increase in the rate of abnormalities. KHERA and MCKINLEY (1972) reported that oral administration of

2,4,5-T containing 0.5 ppm of TCDBD was associated with an increase in fetal deaths, and skeletal abnormalities at dose levels of 100 mg/kg or higher. At lower dose levels of 25 and 50 mg/kg, there appeared to be no effect of 2,4,5-T contaminated with 0.5 ppm of TCDBD. HART and VALERIO (1972) reported that subcutaneous administration of 2,4,5-T stated to be greater than 99% pure produced a marginal increase in cleft palate in CD-1 mice at a dose level of 100 mg/kg. COLLINS and WILLIAMS (1971) demonstrated that 2,4,5-T with no detectable TCDBD produced no malformations in the golden hamster at doses lower than 100 mg/kg. However, at 100 mg/kg there was a number of malformations among the live born. When TCDBD was added to the 2,4,5-T preparations, the abnormalities per litter were related to the levels of the TCDBD contaminant. NEUBERT and DILLMAN (1972) likewise showed that terata increased when levels of TCDBD in 2,4,5-T exceeded 1.5 ppm. The aforementioned studies indicate that levels of nearly pure 2,4,5-T exceeding 100 mg/kg can be teratogenic in a number of animal species, and that in some species, the effect can be additive by increasing concentrations of the TCDBD contaminant.

This rhesus study was designed to evaluate the teratogenicity of nearly pure 2,4,5-T in this primate at dose levels approximating human exposure. The rhesus was chosen because of its similarity in embryonic development to that of man (WILSON 1971). The results of our study indicate that nearly pure 2,4,5-T is not teratogenic in the non-human primate at the dose levels given. This result is in agreement with similar work in the rhesus reported by WILSON (1971). His study indicated that a dose of 40 mg/kg of 2,4,5-T given three times/wk between Day 20 and 48 of pregnancy, produced no abnormalities in four rhesus monkeys delivered by hysterectomy at 100 days gestation. One other female in this group aborted. The level of TCDBD in this 2,4,5-T preparation was not stated. The present study extends Wilson's observations and indicates that there is no effect of 2,4,5-T on the outcome of pregnancies at term. The rate of abortions and stillbirths that occurred during this study were no higher than the normal rate for our colony (DOUGHERTY et al. 1971). Examination of mean gestational weight and mean body weight (Table I) indicate that the differences between the medicated and control groups were not statistically significant ($P \leq 0.05$). Finally, our observation of the infants up to 1 year of age indicates no long term effect due to the administration of 2,4,5-T. It would appear then that 2,4,5-T containing 0.05 ppm of TCDBD produces no evidence of teratogenicity or toxicity in the fetus of the non-human primate at the dose levels administered.

SUMMARY

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) containing 0.05 ppm of tetrachlorodibenzo-p-dioxin, was administered to pregnant rhesus monkeys daily from Day 22 through Day 38 of

gestation. At the dose levels administered, 0.05 mg/kg, 1 mg/kg, and 10 mg/kg, no evidence of toxicity was seen in the mother and no evidence of teratogenicity was seen in any of the offspring. Observations of the infants for 1 year following birth indicated that there was no toxicity due to the 2,4,5-T.

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