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Uterine and Fetal Characteristics in Rats Following a Post-implantational Exposure to Permethrin

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The natural pyrethrins constitute a group of potent contact insecticides which are extracted from dried chrysanthemum flowers (CREMLYN 1978). Permethrin, one of the newly synthesized pyrethroid and an ester of chrysanthemic acid, is presently being widely used as a component in household and farm sprays. This extensive usage is attributable to its high insecticidal activity and low mammalian toxicity (ELLIOTT et al. 1973; VERSCHOYLE & BARNES 1972). From a reproductive standpoint, pyrethrum displayed a strong teratogenicity and produced sterility when administered to chickens (LUTZ-OSTERTAG & LUTZ 1970).

The purpose of the present study was: 1. to determine the effects of the post-implantational exposure to permethrin on mammalian reproduction using certain aspects of placental biochemistry and intra-uterine development as endpoints; and 2. to assess the scope of permethrin's possible reproductive toxicity with rats as the mammalian model.

MATERIALS AND METHODS

Virgin, female Sprague-Dawley rats (Holtzman Company, WI.), weighing 180-200 grams at the start of the test, and those of our stock which were bred from the original supplies, were employed. Rats were caged individually in stainless steel cages in temperature ($20 \pm 2^{0}C$) controlled, windowless quarters. Light from fluorescent banks were automatically regulated under a photoperiodic regimen of 14 hours light: 10 hours darkness (lights on at 0700 h). Water and Purina laboratory chow were provided freely. Upon arrival from the breeder, the animals were allowed a 2-3 days acclimation period. Thereafter, the stages of the estrous cycles were determined microscopically. Only those rats which exhibited two consecutive 4-or 5-days estrous cycle, were used in the investigation. Whereas control rats received untreated water and lab chow during the experimental periods, test animals were dosed with permethrin mixed with the water, plus free access to untreated lab chow.

Permethrin (technical grade) was stored in a refrigerator and protected from possible decomposition due to light exposure. Serial concentrations were prepared from a stock solution. Employable concentrations ranged from 500 to 4000 ppm. These doses were de-

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termined by a pilot tolerance study which utilized the LD_{50} value which was greater than 4000 mg/kg rat for acute oral toxicity with technical grade permethrin (FARM CHEMICALS HANDBOOK 1979), and relied on taste preferances. Doses were administered from Days 6 through 15 of gestation.

Pregnancy was effected by caging the female with a fecund male overnight at proestrus. The first morning following cohabitation, when the vaginal smear was sperm-positive, was designated as Day 1 of pregnancy. Implantation sites were verified on Day 6 of gestation by cesarean section, following a tail vein intravenous injection of Chicago blue dye (DICKMANN et al. 1977). Laparotomy was performed on Day 20 of gestation at which time the live fetuses were counted. Placentae were excised and cleaned of extraneous connective tissue, and assayed for protein and glycogen on Day 16 of pregnancy. The supernatant protein concentrations were determined by the Folin phenol method of LOWRY et al. (1951) using bovine serum albumin as the standard. Glycogen analyses were conducted in accordance with the method of SEIFTER et al. (1950), modified by ZARROW et al. (1964), and employed Type III rabbit liver glycogen as the standard.

Control and experimental groups consisted of 5-8 randomly selected animals. The data were analyzed for statistically significant differences by using one-way analysis of variance and by the Student's two tailed t test. Effects at the 0.05 level or less were considered to be statistically significant. Data are expressed as means $\pm$ standard errors of the means.

RESULTS AND DISCUSSION

Placental analysis of biochemical constituents (Table I) under feeding regimens of 500 to 4000 ppm permethrin, indicates a sensitivity ($p < 0.05$) to the pesticide in protein and glycogen contents only at the highest concentrations (2500-4000 ppm). Generally however, the response in these two parameters to permethrin was not in a step-wise and consistent manner, nor was it overwhelmingly substantial. Whereas an analysis of variance demonstrated no significant differences attributed to the exposure levels with protein ($p > 0.05$), the treatment did influence the glycogen concentration ($F= 2.86$, $p < 0.01$).

It is well established that placental function influences fetal growth. For instance, the number and maintenance of fetuses in rabbits and rats normally depend on the functional integrity of the placenta (BEYDOUN et al. 1972; ZAMBRANA & GREENWALD 1971). Based on these findings, it may be expected that the development of fetal endpoints generally should reflect modifications in placental proteins and glycogen. Results from Table 2 indicate no significant changes regarding the interaction between the treatment levels and implantational sites/intra-uterine fetuses. However, the resorptive rates for concentrations at 500 and 1500-4000 ppm when Day 6-Day 20 comparisons were made, displayed significant differences ($0.05 > p < 0.01$). Albeit, none of these abortifacent manifesta-

TABLE 1. The Effect of Permethrin on Placental Biochemistry in Rats[a].

Dietary Treatment[b] (ppm)	Daily Water Intake (ml/rat/day) $\bar{X} \pm SE$	n	Placental Protein (100 µg/100mg) $\bar{X} \pm SE$	t-test (p)	Placental Glycogen (mg/100g) $\bar{X} \pm SE$	t-test (p)
0	-----	8	61.2 ± 2.5	-----	760 ± 78	----
500	40.6 ± 1.4	8	64.1 ± 4.4	NS	691 ± 46	NS
1000	37.2 ± 1.2	8	59.4 ± 1.5	NS	668 ± 52	NS
1500	34.7 ± 0.5	8	51.7 ± 4.5	NS	614 ± 99	NS
2000	32.7 ± 1.5	8	55.1 ± 2.8	NS	508 ± 61	<0.05
2500	37.8 ± 2.0	8	48.9 ± 2.6	<0.01	534 ± 68	<0.05
3000	34.1 ± 2.1	8	47.9 ± 2.8	<0.01	459 ± 69	<0.05
3500	40.8 ± 1.5	8	53.1 ± 3.6	NS	429 ± 63	<0.01
4000	30.2 ± 1.3	5	51.3 ± 3.4	<0.05	510 ± 77	<0.05
ANOVA	p <0.05	-	NS		p <0.01	

[a]Rats were sacrificed on Day 16 of pregnancy.

[b]Permethrin was administered in the diet from Days 6 through 15 of pregnancy.

TABLE 2. Influence of Permethrin on Intra-uterine Profiles.

Dietary Exposure[a] (ppm)	n	Implanation Sites (Day 6) $\bar{X} \pm SE$	p value[b]	Live Fetuses in Utero (Day 20) $\bar{X} \pm SE$	p value[c]	Resorption Index[d] (%)
0	8	11.0 ± 1.1	NS	10.1 ± 1.2		8
500	8	11.9 ± 0.6	<0.05	9.0 ± 1.1	NS	24
1000	8	11.4 ± 0.5	NS	9.4 ± 1.2	NS	18
1500	8	11.4 ± 0.5	<0.05	9.0 ± 07	NS	21
2000	8	12.4 ± 0.3	<0.05	8.0 ± 1.5	NS	35
2500	8	13.5 ± 0.8	<0.01	7.1 ± 1.4	NS	47
3000	8	13.0 ± 0.6	<0.05	9.3 ± 1.1	NS	28
3500	8	11.8 ± 0.7	<0.05	9.0 ± 0.7	NS	24
4000	8	11.3 ± 1.0	<0.05	7.1 ± 1.1	NS	37

[a]Permethrin was administered in the diet from Days 6 through 15 of pregnancy.

[b]Student's t test between Day 6 and Day 20 readings.

[c]Student's t test when compared with Day 20 control.

[d]$\text{Resorption} = \frac{(\text{IS at Day 6} - \text{Fetuses at Day 20})}{\text{IS at Day 6}} \times 100$

tations was drastic: all were below 50%, especially at the lower applications (600-2000 ppm).

These alterations in fetal physiology generally reflect the overall effects of permethrin on placental biochemistry. Herein, permethrin exerted a somewhat weak to moderate influence on the in utero fetal development, particularly at concentrations of 2000 to 4000 ppm. These results were apparently in agreement with the observation that the pesticide possesses low mammalian toxicity (ELLIOTT et al. 1973) and attest to the minor role of permethrin with regards to mammalian reproduction during pregnancy.

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