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Serum Testosterone and Sexual Behavior in Rats After Prenatal Exposure to Lindane

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A large number of chemicals occurring in our environment may have the potential to interfere with the endocrine system of animals and humans. Several pesticides have been reported to produce gonadal toxicity, among these are the persistent and bioaccumulative organochloride pesticides. Increasing interest has been observed among environmental and health institutions regarding the potential reproductive effects due to exposure to occupational and environmental chemicals. Lindane, γ-1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH) is an organochloride compound that has been used since the early 1950s as a broad-spectrum pesticide for both agricultural and non-agricultural purposes in many parts of the world (WHO 1991). Neurological and behavioral alterations are the principal toxic effects of lindane in animals (Hulth et al. 1976; Joy 1982). Lindane also possesses an endocrine effect (Raizada et al. 1980; Uphouse 1987; Cooper et al. 1989), and has been reported to disturb male reproductive function (Dalsenter et al. 1996).

The purpose of this study was to ascertain the effects of a single dose of lindane on reproduction in adult male rats after prenatal exposure. The effects on testicular weight, spermatid and sperm number, testosterone concentration and sexual behavior were investigated. In the present study, pregnant rats were treated on day 15 of gestation which corresponds to the early fetal phase of testosterone production.

MATERIALS AND METHODS

Pregnant Wistar rats (Bor: spf, TNO; Fa. Winkelmann, Borchen, FRG) were housed singly in standard plastic cages (Macrolon® Type III) with stainless steel covers and wood shavings as bedding. Animals were treated orally by gavage with a single dose of 30 mg lindane/kg b.w. on day 15 post conception (day 0 was considered the day that sperm was detected in vaginal smears). The control pregnant rats (n = 20) received peanut oil on the same day. The rats were kept under a constant day/night cycle (light from 9:00 to 21:00), at a room temperature of 21° ± 1° C and 50 ± 5% relative humidity. They received a standard pellet feed (Altromin® 1324, Lage, FRG) and tap water ad libitum.

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Parameters including litter size and the number of viable and dead offspring were assessed. The body weights of newborn rats were determined on postnatal days 1, 7, 14, and 21. Forty-five male offspring from each group were randomly selected for the assessment of reproductive effects.

Fifteen male offspring from each group were sacrificed on days 100 and 130 postnatally and the testes and epididymides were removed and weighed. The testes were minced and homogenized in 10 ml 0.9% NaCl containing 0.5% triton X-100 at medium speed in an IKA-RW 15 tissuemizer® (Janke und Kunkel, Staufen in Breisgau, FRG) for 1 minute after removal of the tunica albuginea. Resistant spermatids were counted in a hemacytometer (Buerker, Germany). Likewise, the cauda epididymis was minced, homogenized, and spermatozoa counted as described above.

Seven-month-old male offspring (n = 15) from each group were mated with untreated females to determine the effect of lindane on sexual behavior. The sexual cycle of untreated females was predetermined by examining the vaginal smears (Chahoud and Kwasigroch 1977). Only cycling females were utilized. The prenatally exposed male offspring were mated (1:1) with estrus females and the sexual behavior of each male was filmed for 15 minutes using a video compact camera recorder (Hi8 Handycam CCD-V800E, SONY®), under blue light illumination (black light, 75W; 220-230V; Osram, Germany). Video was used subsequently to evaluate the following parameters:

Mount latency: the time (in seconds) which elapsed between the introduction of the female into the same cage with the male rat and the first mounting trial without intromission of the penis into the vagina,

intromission latency: the time (in seconds) which elapsed between the introduction of the female into the same cage with the male rat up to the first penetration of the penis into the vagina,

ejaculatory latency: the time (in minutes) which elapsed between the first penetration of the penis into the vagina and ejaculation,

number of intromissions: number of intromissions up to ejaculation, and

intromission frequency: number of intromissions per minute within a 15 minute period.

One week after recording sexual behavior, the same male rats were mated with other control female rats (1:1) three hours daily for eight consecutive days. Vaginal smears were collected daily and examined for the presence of sperm. Day 0 was considered the day that sperm was detected in vaginal smears. The dams were killed on day 21 post conception and implantation sites, viable and dead fetuses, resorptions, as well as external malformations were enumerated and recorded.

The serum testosterone concentration was determined in the same male rats used for examination of sexual behavior. After decapitation, blood was collected, centrifuged, serum removed, and stored at -20° C until analysis. Testosterone concentration was determined using a radioimmunoassay (RIA) technique. Testkits

were acquired from Euro-Diagnostica (Giessen, Germany). Fifty µl of standard, control, and rat serum samples were pipetted into labeled coated tubes, immediately 500 µl testosterone [^{125}I] reagent were added to each tube. Racks of tubes were gently shaken by hand for 30 s and placed in a water-bath at 37 ± 1° C for 1 h. After 1 h, all tubes, except total count tubes were decanted, aspirated and then counted for 1 min. in a gamma counter. Duplicate measurements were made on each sample, and the average result is reported in ng/ml.

For the statistical analysis, the variables body weight, testis and epididymis weight, number of sperm, spermatids, as well as testosterone concentration were analyzed using the student's t-test. The overall mortality rate in pregnancy outcome was statistically tested using a chi-square test.

RESULTS AND DISCUSSION

On postnatal day 1, birth weights of rats exposed prenatally to lindane were not significantly different from the control group. The number of stillborn (zero for all groups), the mortality rate and body weights of offspring at day 21 were not affected (Table 1) by prenatal exposure to lindane.

Table 1. The effect of prenatal exposure to lindane on reproductive performance, litter size, and body weight parameters of rats. Data are presented as mean and standard deviation.

Parameter	Control	Lindane
Litters (n)	20	20
Total newborn (n)	195	194
Postnatal day 1		
live births (%)	100	100
litter size	9.8 ± 2.6	9.7 ± 2.5
birth weight (g)	6.2 ± 0.8	5.9 ± 0.7
Postnatal day 21		
mortality rate (%)	3.1	2.6
body weight (g)	31.0 ± 6.5	33.2 ± 6.6

The number of spermatids (control = 254 ± 37, lindane = 196 ± 40) counted on day 100 postnatally was significantly reduced by lindane (Table 2). At the same age

period, the number of sperm was similar between the control and lindane group (control = 326 ± 71, lindane = 294 ± 34). On day 130 postnatally, we observed no differences in numbers of spermatids or sperm and testes weights between control and treatment group.

Table 2. The effect of prenatal exposure to lindane on spermatid and sperm numbers per animal and testicular weight in 100- and 130-day-old rats. Data are presented as mean and standard deviation.

Group	Number (10^6) of spermatids at day		Number(10^6) of sperm at day		Testicular weight (g) at day	
	100	130	100	130	100	130
Control (n)**	254 ± 37	267 ± 22	326 ± 71	334 ± 38	1.62 ± 0.19	1.76 ± 0.26
Lindane (n)**	196 ± 40*	258 ± 38	294 ± 34	329 ± 35	1.69 ± 0.20	1.75 ± 0.17

*t-test = $p < 0.01$, different from control of same age
(n)** = 15 rats/study period

Seven-month-old male offspring rats were used to study sexual behavior and testosterone concentration. In the control group shortly after introducing the female rat into the cage of the male rat, exploratory activities including sniffing, nose-nose contact, genital exploring, and grooming were observed. These activities were followed by mounting and copulation. Exploratory activities which were not quantitatively registered seem to be reduced in the lindane group. Of 15 male offspring rats of dams treated with lindane prenatally, only one rat manifested sexual desire. The other 14 animals showed a minimum interest in their female partners. In contrast only one rat failed to copulate in the control group. Since only 1 male rat exhibited sexual activity in lindane group, the variables of the sexual behavior will not be presented. Serum concentrations of testosterone in male rats from treated mothers were significantly reduced compared to the control rats (Table 3).

One week after recording the sexual behavior, above rats were mated with female rats for 8 consecutive days and all impregnated female rats which yielded viable fetuses. The pregnancy and resorption rate, number of implantations, as well as the number of viable and dead fetuses were similar among groups (Table 4).

Mechanisms underlying the effects of substances on reproductive components and functions are variable and extremely complex.

Table 3. The effect of prenatal lindane exposure on serum testosterone concentration (ng/ml) in seven-month-old male rats. Data are presented as mean and standard deviation.

	Control	Lindane
Number of animals	15	15
Serum testosterone (ng/ml)	1.65 ± 0.16	0.94 ± 0.08*

*t-test = $p < 0.01$

Table 4. The effect of prenatal lindane exposure on the future reproductive performance of adult male rats

	Control	Lindane
Pregnancy rate (%)	15/15[a] (100)	15/15 [a](100)
Number of implantations[b]	10.7 ±1.6	10.4 ± 2.9
Number of viable fetuses[b]	10.3 ± 1.7	10.4 ± 2.9
Resorption rate (%)[b]	3 ± 0.5	2 ± 0.4
Number of dead fetuses	1	0

a) Number of mated females/number of pregnant females x 100
b) The data are given as mean and standard deviation

Effects of chemicals on reproduction may be induced directly by interference of the substance with reproductive components or indirectly by influencing hormonal regulations. In recent years, more interest has been focused on the possibility of reproductive disturbances induced during late pregnancy and the early postnatal period. Generally, prenatal exposure of dams to toxic substances may induce either delay or persistent impairment of organ function of the offspring. Though the pregnant rats in the present study had been treated prenatally, the period of exposure to lindane of the offspring could occur even after birth because lindane accumulates in fat tissues, including mammary glands, and can slowly be eliminated in milk during the lactation period (Pompa et al. 1994, Dalsenter et al. 1997). Substances with endocrine disrupting effects may induce alterations on prenatal development of sexual organs and function when they are exposed prenatally (Wilson et al. 1981; Maclusky and Naftolin 1981; Colborn et al. 1993). Examples of such effects are reduction of testicular weight, reduced sperm count and disturbances in adult sexual behavior (Mably et al. 1992a; 1992b; 1992c).

After prenatal exposure to lindane, no clear-cut effect was observed on spermatogenesis parameters. The male offspring prenatally exposed to lindane showed on day 100 a significantly reduced number of spermatids (approximately 80% of the control). On day 130, no effect on either sperm or spermatid number was reported. It is difficult to explain the transitory depression of sperm obtained in the study. However, the most interesting results were the effect of lindane on testosterone and on sexual behavior of the male rats. The testosterone concentration in the prenatally-treated adult rats was markedly reduced compared to the control male offspring (approximately 50% of the control). Previously, histological investigations on the testes of rats exposed to lindane during lactation revealed a decreased number of Leydig cells which may explain the reduction of testosterone production after lindane exposure (Dalsenter et al. 1997).

For the assessment of the sexual behavior the mating activities were filmed for 15 minutes. The results indicated that lindane induces disruption of libido in adult male rats. The behavioral alteration probably results from the reduction in testosterone concentration. This could be the reason why only one male offspring from the treated group showed sexual interest and was able to copulate during the 15 minutes of recording. However, impaired reproductive performance was not detected after mating the same lindane exposed males with control females for 3 h per day for 8 consecutive days. All males were able to impregnate the females which yielded a normal pregnancy outcome; indicating only an apparent transitory decrease in libido and not a total inhibition of adult mating behavior. A decrease in sexual stimulation and testosterone concentration at adulthood was reported after exposure to lindane during lactation (Dalsenter et al. 1997).

Our data indicated that treatment of female rats on day 15 of pregnancy with a single dose of 30 mg lindane/kg b.w. affects the sexual behavior of adult male offspring by changing the state of libido and by reducing the testosterone concentration without compromising fertility.

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