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Impairment of Testicular Endocrine and Exocrine Functions after Dieldrin Exposure in Adult Rats

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Abstract

In this study we examined the mechanism of action of Dieldrin on the reproductive function in adult male rats. Experimental data suggest that exposure to pesticide at 3 and 6mg/kg/day for 10 consecutive days, induced a dose-dependant decrease of the number and the mobility of epididymal spermatozoa. Testicle histological analysis shows alteration of spermatogenesis with marked reduce of gametes production combined with reduction in the seminiferous tubule diameters in treated relative to control. Concentration of plasma testosterone dropped significantly in a dose-dependent manner after exposure to Dieldrin, while circulating levels of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) and weight of accessory sex glands were not affected significantly. Thus, it may be concluded that Dieldrin exposure can impair both testicle gametogenesis and steroidogenesis in adult rats.

Keywords: dieldrin, rat, fertility, testosterone, LH, FSH, testis, spermatozoa

Introduction

During the past 50 years, increases of testicular anomalies including testicular cancer [1] and a decline in human semen quality have received considerable attention [2, 3]. A number of possible causes, including exposure to environmental pollutants, have been suggested. Among the environmental compounds incriminated, pesticides such as Dieldrin are obvious because of their impact on and diffusion in the environment and their adverse effects on the genital system [4].

Dieldrin is a highly effective insecticide for soil-dwelling pests and for the protection of wooden structures against termites and woodborers [5].

Although the use of dieldrin has been severely restricted or banned in many parts of the world since the early 1970s, it is still used in termite control in some countries [6]. It remains at low levels in the environment because of its high persistence, its lipophilicity and its continued use in developing countries, where environment contamination is still important.

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Animals studies demonstrate that Dieldrin causes a wide variety of health problems such a neurological defects [7] and immune system dysfunctions, multiplying the risk of carcinogenic processes [8]. Potential toxic effects on the genital system have also been reported after Dieldrin and numerous pesticides exposure [4]. However, the mechanism of action of Dieldrin on the reproductive function remains unclear.

The objective of the present study is to evaluate the effects of Dieldrin administered for 10 consecutive days on the adult male rat reproductive function by determining epididymal sperm number and mobility, testicle histology as well as the serum concentrations of testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH).

Materials and Methods

Animals and Reagents

2- to 3-month-old male Wistar rats (200–250g) were used in this study. Rats were housed under controlled

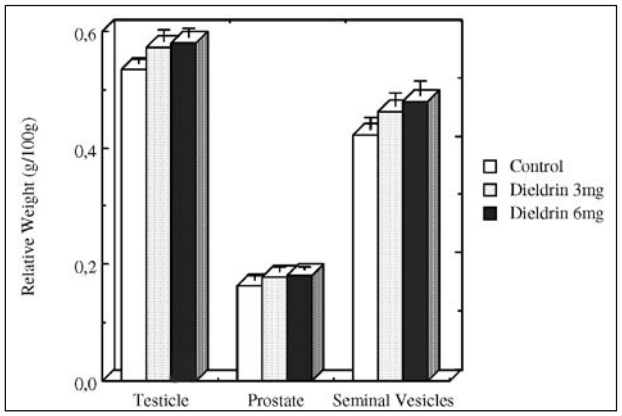


Fig.1. Effect of Dieldrin treatment on testes, prostate and seminal vesicles relative weight in rat.
Dieldrin (3 or 6mg/kg body weight day^{-1}) is administered i.p in corn oil during 10 consecutive days, control animals received the vehicle. Each value is the mean ± SE of 11 determinations. *p < 0.05 compared to control (Student's t-test).

conditions of temperature (22 ± 1°C), with 16h light/dark cycle. Food and water were provided *ad libitum*. Dieldrin (hexachloro–6,7-epoxy–octahydroendo1,4,5,8 dimethanophtalen) was purchased from Sigma chemical Co. (St. Louis, Mo, USA), Earles' medium was obtained from Gibco BRL (Paisley, Scotland). Before initiation of experiments, adult male rats were randomized into 3 experimental groups (n=6–7) of approximately similar weight as follows:

1. animals received an intraperitoneally injection (i.p) [9, 10] of Dieldrin diluted with corn oil at a dose of 3mg/kg body weight (b.wt),
2. animals were administered 6mg of pesticide/kg b.wt,
3. control group received equal volumes of vehicle.

After 10 days of treatment, all animals were killed by decapitation; the left testes and the seminal vesicles were dissected and weighed.

The choice of the dose was made according to work of Vanio et al. [11] and those of Graham et al. [9]. In rats, acute i.p LD50 was of 35mg/kg of body weight [5].

Sperm Preparation

Epididymal sperm suspensions were prepared by mincing and homogening two excised cauda epididymides of rat in watch–glass containing 1ml of Earle's medium with 0.2% bovine serum albumin, penicillin (100units/ml) and streptomycin (1µg/ml), pH 7.2. The sperm suspensions were incubated for 10min at 37°C [12]. The numeration and straight mobility of sperm were determined by hemacytometer (Buerker, Germany).

Enzyme Linked Immuno Sorbent Assay (ELISA) and Radio-immunoassay (RIA)

The serum FSH, LH and testosterone were determined in the same male rats used for examination of relative weight of testicle and seminal vesicles. After decapitation, trunk blood was collected, centrifuged (500g for 10min at 4°C), serum was removed and stored at –20°C until analysis. Plasma FSH and LH concentrations were determined in duplicate by enzyme-immunoassay using reagents from commercial kits (Biotrak^{Tm} from Amersham, Greece). Testosterone levels were measured using radio-immunoassay kit (Immunotech, France).

Table 1. Effect of Dieldrin on epididymal sperm numbers and mobility in control and Dieldrin treated rat.

		Number x10^6/ml	Mobility (%)
Control		18.21 ± 0.37	53.69 ± 2.89
Dieldrin	3mg/kg	14.21 ± 0.19* *(21.9)*	33.09 ± 2.13* *(38.3)*
	6mg/kg	11.62 ± 0.21* *(36.1)*	23.70 ± 1.71* *(55.8)*

Each value is the mean of 10-15 determinations with standard error (SE). Rats received daily i.p injection of Dieldrin (3 and 6mg/kg body weight), or an equivalent volume of vehicle during 10 days.
p < 0.05 compared to control (Student's t-test t).
Percentage of decrease in parentheses.

Histological Analysis

Testicle was fixed overnight at room temperature by direct immersion in a 4% paraformaldelyde in 0.1M phosphate buffer, PH 7.4. The samples were dehydrated with ethanol and toluene series and embedded in paraffin. Serial sections (5µm) were mounted on gelatin-coated glass slides cut and stained with hematoxylin and eosin.

Statistical Analysis

Data were analyzed using stat view 512$^+$ software (Abacus Concept, Inc.). The results were expressed as means ± SE and a comparison of two means was made using Student's t- test.

Results

As shown in Figure 1, a moderate and not significant effect in testes relative weight was observed in rats injected with Dieldrin for 10 consecutive days. The increase of testes relative weight reached 7.5% and 9% of controls for 3 and 6mg Dieldrin/kg b.wt day^{-1} respectively. Similarly, the relative weight of prostate and seminal vesicles raised by the same amount after exposure to the two doses of the pesticide.

Semen characteristics, evaluated by microscopic observations, are reported in Table 1. The number of epididymal spermatozoa was significantly decreased in treated rats compared to control animals (14.21 ± 0.19x10^6/ml and 11.62 ± 0.21x10^6/ml respectively with 3 and 6mg

Table 2. Effect of Dieldrin on serum testosterone levels in control and Dieldrin-treated rats.

		Testosterone (ng/ml)
Control		2.35 ± 0.05
Dieldrin	3mg/kg	0.98 ± 0.06*
	6mg/kg	0.92 ± 0.02*

Each value is the mean of 6 determinations in duplicate with standard error (SE). Rats received daily i.p injection of Dieldrin (3 and 6mg/kg body weight), or an equivalent volume of vehicle during 10 days. Testosterone concentrations were determinated using a radioimmunoassay technique.
* $p < 0.05$ compared to control (Student's t-test t).

Table 3. Effect of Dieldrin on serum FSH and LH levels in control and Dieldrin-treated rats.

		FSH (ng/ml)	LH (ng/ml)
Control		25.00 ± 1.01	0.595 ± 0.005
Dieldrin	3mg/kg	26.25 ± 1.75	0.925 ± 0.025
	6mg/kg	23.00 ± 1.45	0.630 ± 0.060

Each value is the mean of 6 determinations in duplicate with standard error (SE). Rats received daily i.p injection of Dieldrin (3 and 6mg/kg body weight), or an equivalent volume of vehicle during 10 days. Gonadotrophin concentrations were determinated using the ELISA method.
$p < 0.05$ compared to control (Student's t-test t).
FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone.

Dieldrin/kg b.wt versus 18.21 ± 0.37x10^6/ml). Epididymal sperm mobility was also significantly reduced in the treated group. The mobility was 33.09 ± 2.13% and 23.70 ± 1.71%, respectively, in rats receiving 3 and 6mg Dieldrin/kg compared to 53.69 ± 2.89% in control group. Light microscopic inspection of testes revealed active spermatogenesis in the seminiferous tubules of untreated rat showing various stages of spermatogenesis from spermatogonia to mature spermatozoa (Figure 2a), whereas in rats exposed to 3mg Dieldrin a pronounced alteration of the architecture of the testis was observed with dramatically reduce of spermatozoa produced in the lumen of 90% of seminiferous tubules sections (Figure 2b) accompanied by a significant reduction of tubular diameters (data not shown).

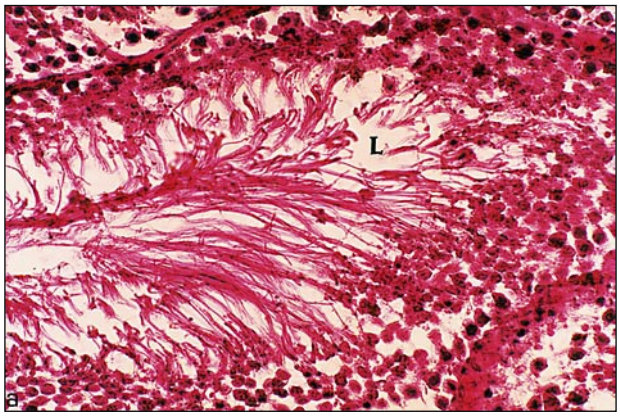
Dieldrin exposure also severely diminished serum testosterone levels from 5.612 ± 0.050ng/ml to 2.108 ± 0.060ng/ml and 2.007 ± 0.020ng/ml respectively for 3 and 6mg/kg (Table 2). In contrast, the pesticide poisoning has no significant effects on both circulating LH and FSH for the two doses of pesticide (Table 3).

Discussion

Various data suggest a deleterious effect of pesticides on the male reproductive function in man and animals [2, 3, 4]. Thus, towards the end of the 1960's, the substantial decline in the population of numerous avian species due to a decrease in successful reproduction, was shown to be consecutive to permanent pollution by organochlorines of continental and marine ecosystems [13]. Animals studies have shown that Dieldrin, an organochlorine pesticide affect male and female fertility [14].

In the present study we demonstrate that the exposure of adult male rats to 3 and 6mg Dieldrin/kg b.wt during 10 consecutive days, induced genital toxicology.

The number and the mobility of epididymal spermatozoa are dramatically reduced and profound histological changes are observed in the gonad with a marked loss of mature gametes associated to a reduction of seminiferous tubule diameters. These results indicate that Dieldrin passes through the blood/testis barrier and affects both the



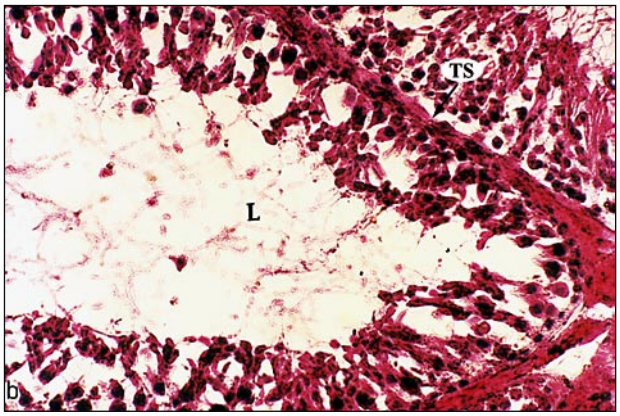


Figure. 2. Photomicrographs of sections of testes from control rats (a) and Dieldrin treated rats (b). (Magnification x1000).
Testicle from control and 6mg Dieldrin-treated rats was fixed by direct immersion in a 4% Paraformaldehyde in 0.1M phosphate buffer, pH 7.4. Serial section (5µm) were mounted on gelatin-coated glass slides cut and stained with Haematoxylin and Eosin.
L: Lumen, TS: Seminiferous Tubule.

production and the maturation of sperm in the epididymis. Similar findings were obtained with Dioxin [15] and other pesticides such as DDT [16, 17] and lindane [18].

The impairment of spermatogenesis appeared to be as a consequence of the decline of testosterone serum in Dieldrin-treated rats since the androgen is clearly essential to the gametogenesis [19]. Leydig cells secrete the male hormone, testosterone, which is a prerequisite for the production of sperm. But, in disagreement with previous findings for other pesticides [20, 21], we have observed no significant differences for circulating LH and FSH following Dieldrin poisoning. However, our result is partially consistent with previous findings showing a significant reduction in plasma gonadotrophins only for LH after 13 days of treatment with aldrin, a Dieldrin metabolite [22].

The fall in plasma testosterone levels in Dieldrin-exposed rats is consistent with previous data showing alteration in Leydig cells after administration of Lindane, another organochlorine compound [23, 24]. Moreover, injection of the Methoxychlore, a pesticide currently used as a substitute for DDT, decreased serum concentrations of testosterone [25] and its metabolite inhibited the steroid production in developing Leydig cells [26]. Therefore, Leydig cells express receptors for glucocorticoid hormone secreted by the adrenal gland, and glucocorticoid action directly suppresses androgen biosynthesis.

Various studies suggest an interaction of pesticides with the hypothalamo-hypophysis axis controlling spermatogenesis. These products may also interact directly with Sertoli or Leydig cells, responsible for testicular production of proteins involved in the transport and the production of testosterone, respectively. The reduction level of the androgen leads normally to the increase of serum FSH and LH as a consequence of the impairment of the negative feedback control on hypothalamic–pituitary axis.

The alteration of gonadotrophin secretions may also be explained by the well-known estrogen-like effect of Dieldrin [27] and the failure in the inhibin production by Sertoli cells, since FSH secretion is modulated by inhibin [28]. However, the fact that in the present experiments circulating levels of LH and FSH were found to be unchanged after exposure to Dieldrin might be attributed to several factors. First, the reduction of serum testosterone concentration is probably not sufficient to produce activation of hypothalamic-pituitary-gonad axis. Second, as described for other xenobiotics [24], Dieldrin treatment may contribute to the establishment of a stress state associated with elevation of plasma corticosterone, which is known to depress testicle testosterone production [29]. Finally, it has been demonstrated for a long time that organochlorine insecticides have some estrogenic and anti-androgenic properties and may impair the negative feedback mechanism of testosterone on the hypothalamic and pituitary levels [25, 10].

Although the growth and maintenance of accessory sex glands are highly dependent on the level of circulating testosterone [30], in the present study no relationship could be made between the unexpected unchanged relative weight of prostate and seminal vesicles in Dieldrin-exposed rats and the decrease of circulating levels of testosterone. This discrepancy may be related to the treatment period and/or the non-androgenic role of testis in the development and maintenance of these organs [31].

In conclusion, it appears that Dieldrin exposure can impairs both testicle gametogenesis and steroidogenesis in adult rats.

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