EFFECT(S) OF DIELDRIN ON PLASMA TESTOSTERONE AND PLASMA LUITENIZING HORMONE IN MALE RATS

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SUMMARY

The effect(s) of organochloride pesticides on the reproductive system of adult male vertebrates are inconclusively known. In the present study, we investigated the effect(s) of feeding dieldrin spiked diet to adult male rats on the plasma levels of testosterone and luteinizing hormone. We fed either dieldrin free diet or dieldrin spiked diet (5mg or 10mg/kg body weight) for 20 days *ad libitum*. Dieldrin did not affect body weight gain and plasma testosterone and LH levels (P> 0.05). In summary our results indicate that dieldrin when administered orally at less than a month duration does not decrease the plasma levels of testosterone and LH in male rats.

INTRODUCTION

Effect(s) of environmental and industrial pollutants on the male reproductive system are becoming increasingly important as judged by animal studies. Todate, whether these chemicals have a significant impact on human fertility is still debatable. As it is, the human male is a very subfertile species with an inefficient spermatogenesis compared to animals (rodents in particular) and therefore particularly vulnerable to the adverse effects of chemicals (Traina, *et al*, 1994). Despite the fact that the use of organochlorine pesticides has been either restricted or banned, environmental contamination remains the major source of organochloride pesticides to both animals and man (Raisbeck *et al*, 1989). Organochloride pesticides have oestrogenic activity (Bulger and Kupfer, 1985) and when fed to adult male rats decrease the size of the prostate, seminal vesicles and testes (Tullner and Edgcomb,1962). Further it has been reported that men occupationally exposed to the nematocide dibromochloropropane (DBCP) showed various degrees of testicular damage and even infertility (Whorton *et al*,1977). However, the mechanisms by which organochlorines interfere with reproductive parameters have yet to be elucidated. Wasserman *et al*, (1974) reported that organochlorine pesticides are potent inducers of hepatic microsomal enzymes capable of altering responses to naturally occuring compounds (eg steroid hormones) in the animal body. Also it has been reported that these compounds are capable of interacting with androgen and estrogen receptors (Whilam *et al*,1995; Jonhson *et al*,1988) and depressing the reproductive hormonal levels (Wango *et al*, 1997). Thus, it appears that there are several possible mechanisms by which organochloride pesticides may affect reproductive parameters.

Dieldrin, a chlorinated hydrocarbon, is one of the pesticides still being used especially in developing countries though in restricted manner (i.e. some parts of East Africa) (Baziraki and Kangwaye, 1995) and still is detected in high levels in tissues and blood samples from areas where it was banned long time ago example Australia, Canada and UK (Basri *et al*, 1994; Kannan *et al*, 1994). The present study was undertaken to investigate the effect of this pesticide on rats male reproductive system. We studied the effects of feeding dieldrin on plasma levels of LH and testosterone.

MATERIALS & METHODS

Animals and treatments

Adult male rats *(spraque dawley)* weighing 330-340g (3 months old) were divided into three groups (I, II and III) of 10 rats each according to treatment. They were maintained under natural lighting conditions 12:12 hours light / darkness, and average room temperature of 23^{o}C with a humidity of around 19%. The beddings, which were changed every day, consisted of sawdust. Group I and II were fed *adlibitum* for 20 days with dieldrin spiked feed at two different dosages (i.e 5mg/kg and 10mg/kg body weight of dieldrin for group I and II respectively). Group III was fed with dieldrin free diet and acted as control.

Dieldrin (approximately 90% $C_{12}H_6CL_6O$) was purchased from Sigma Chemicals Co, St Louis, MO. Reagents for radioimmunoassay (RIA) of testosterone and Bioassay of Luitenizing hormone (LH), were provided by the WHO Matched reagent programme. Basal medium eagle (BME) (with earle's and L-glutamine without $NaHCO_3$) was obtained from Kilmarnock UK.

Experimental layout

Groups I, II and III were caged separately. The rats were weighed every other day and were killed by decapitation at the end of the experiment, blood samples were collected in EDTA containing tubes, and plasma separated by centrifugation. Plasma samples were stored at -20^{0}C pending testosterone and LH analysis.

Testosterone and LH Analysis. For the analysis of plasma testosterone the radioimmunoassay (RIA) was used, which depends on the competition for limited number of binding sites on the testosterone specific antibody by hormone in the sample and radioactive hydrogen (H^{+3}) labeled testosterone.

For the LH assay the Bioassay method which utilizes the mice interstitial testicular cells was used. Briefly, the mice testes were decapsulated and minced in BME media (PH 7.4) at 4^{0}C. The minced testes were stirred with a magnetic stirrer for 10 minutes to disperse the tissues and Leydig cells obtained after further purification. Viability of the Leydig cells was determined by dye exclusion method using Trypan blue, and cell suspensions diluted to achieve a working concentration of 2.5 x10^5 viable cells per ml. Replicates of Leydig cells preparation were prepared in triplicate in BME medium and incubated with either 100µl of test samples or standard LH solution for 3hrs in a shaking water bath at 34^{0}C . The tubes were gassed with 95%O_2, 5%CO_2 and kept open in this atmosphere in a hood during incubation. The incubations were stopped at the end of 3hrs by immersing the incubation tubes in an ice bath at 0^{0}C. One ml aliquots of steroid assay buffer were added to each tube, the mixture vortexed and centrifuged at 500g at 4^{o}C for 10min. The supernatant was decanted into separate vials and used for testosterone assay by using RIA. The amount of LH in the samples were calculated from the LH standard curve according to Van Damme *et al*, (1974).

Statistical Analysis
All data are reported as mean ± SEM and statistical differences for all the parameters were determined by an unpaired *t*-test.

RESULTS

There were no adverse clinical signs during the course of treatment and no clear change in body weight (Fig. 1). In this study no significant difference (P>0.05) in plasma testosterone concentrations were observed between rats fed dieldrin free diet and dieldrin spiked diet. The plasma testosterone concentrations for the control group were 3.29 ± 0.74 nmol/L and that for 5mg/kg and 10mg/kg body weight were 2.72 ± 0.68 nmol/L and 4.66 ± 1.19 nmol/L respectively. Furthermore, there were no significant difference (P<0.05) in plasma LH concentrations

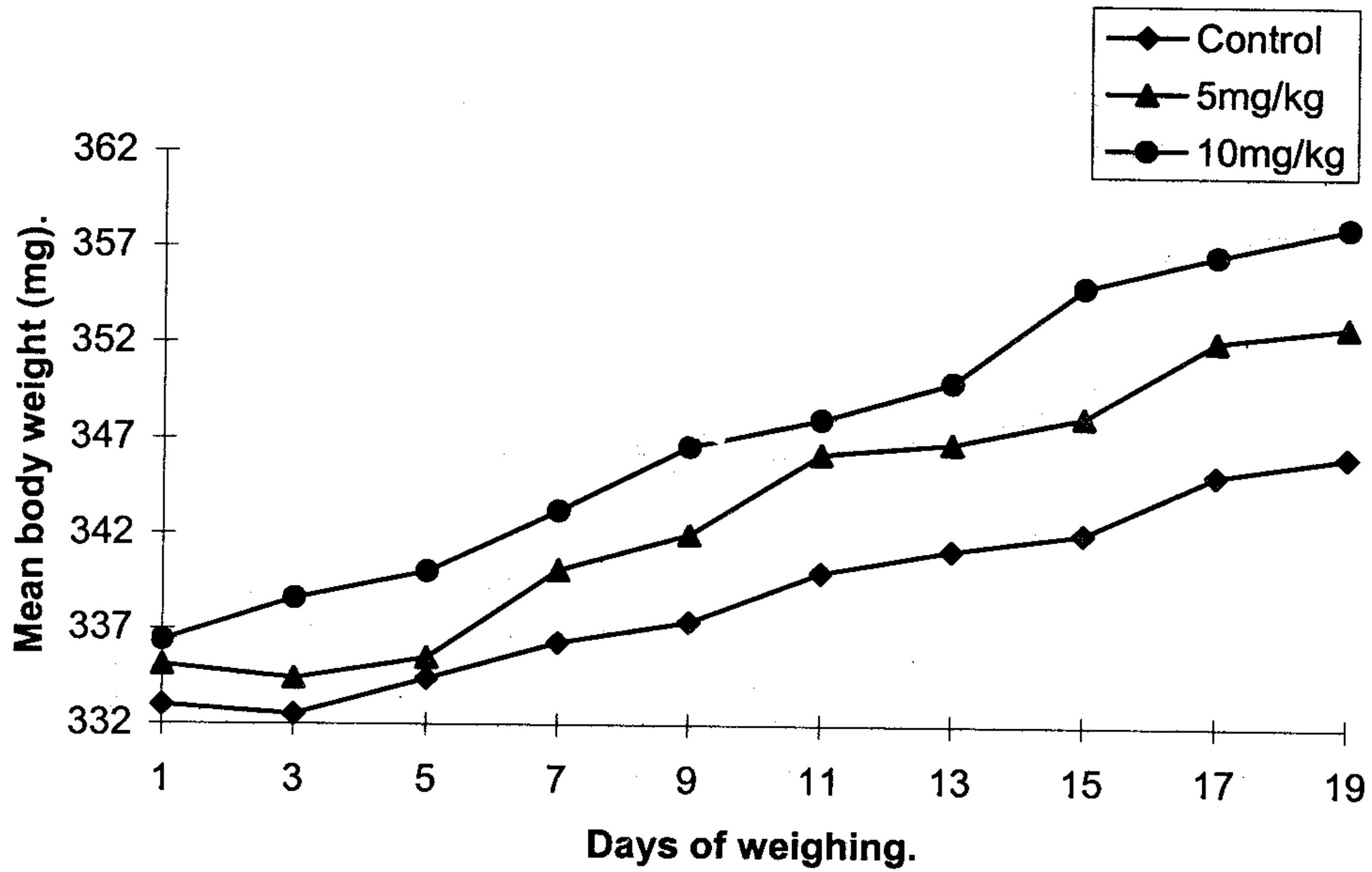

Fig.I. The mean body weight of rats fed on dieldrin free feed (control) and dieldrin spiked feed.

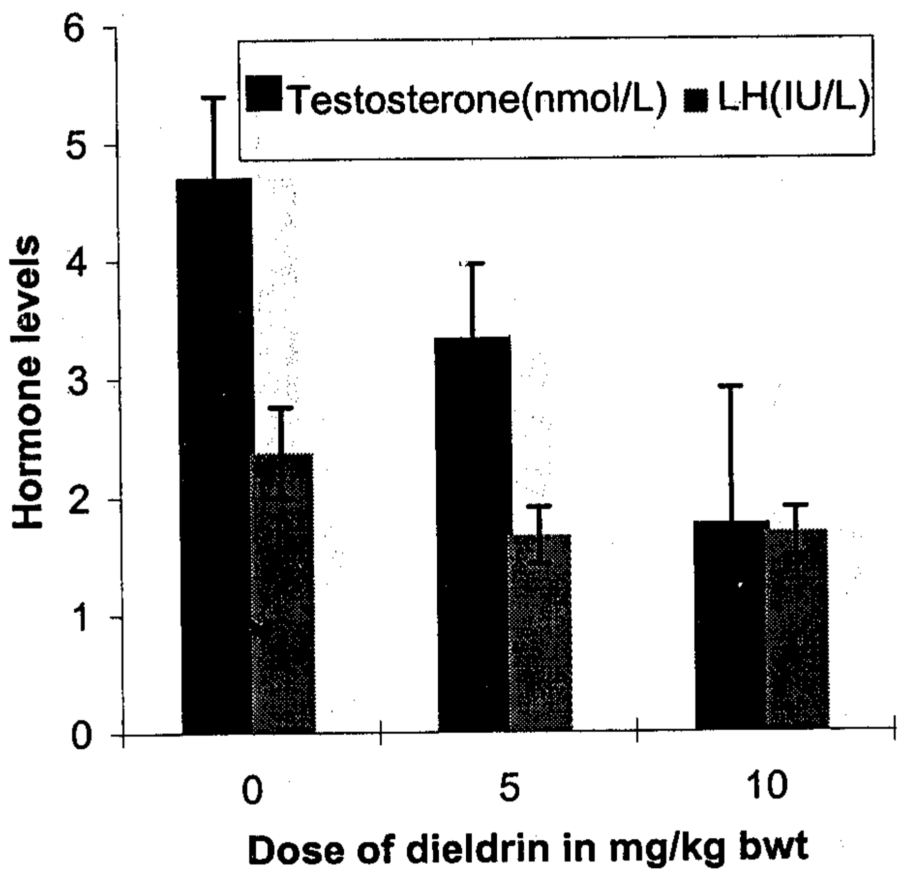

Fig.2. Plasma levels of testosterone and LH in rats fed on either dieldrin free diet or dieldrin spiked diet.

between the control group and the dieldrin fed rats (Fig. 2).

DISCUSSION

These results show that feeding of 5mg and 10mg/kg body weight of dieldrin to male rats over a period of 20 days did not affect their body weight. This observation is in agreement with several other studies which have failed to demonstrate a clear cut effect of organochlorine compounds on body weight of different animal species. For example, dietary heptachlor did not change body weight patterns in beagle dogs (IRDC, 1973). Bruce and Gail, (1977) did not demonstrate body weight change in female rats after feeding with dieldrin for two years at a dose of 20 and 40ppm. Also, Chatterjee *et al*, (1988) observed no change in body weight of male rats after treatment with aldrin for 26 days.

Furthermore the present study showed that there was no statistically significant reduction in plasma LH and testosterone levels at both dosages. These observations differ with those of Fuller and Draper (1975) who reported the inhibition of LH release in rats after treatment with Mirex ^{R} , an organochloride compound for 30 days and that of Chatterjee *at al*,(1988) who also demonstrated that subcuteneous injection of aldrin an isomer to dieldrin for 26 days to male rats decrease plasma testosterone and LH. Furthermore, these results disagree with results from our laboratory (Reproductive Biology Unit) (Unpublished) which demonstrated clearly that subcuteneous injection of dieldrin at a dose of 10mg/kg body weight to mature male rats for 20 days depressed plasma testosterone and LH.

The reason for this discrepancy in observations is not very clear. Nevertheless, the rapid transformation of dieldrin to its metabolites when it passes through the liver after oral administration, may alter its ability to affect reproductive functions (Oduma *et al*, 1994). According to Chasseaud, (1970) and Matsumura, (1982), during hepatic detoxification of apolar and lipophilic compounds (e.g. dieldrin) they are converted to polar compounds which are less toxic and are easily excreted. Also, it has been reported that biliary excretion of dieldrin starts shortly after absorption, mainly in the form of hydrophilic metabolites (Ludwig *et al*, 1964; Hutson and Roberts, 1985) which reduces the concentrations of the chemical reaching the target tissues. It is therefore possible that in this study dieldrin did not affect the plasma levels of testosterone and LH, as a result of having undergone metabolic degradation to less toxic metabolite or/and intestinal absorption which reduced the concentrations of its active ingredients. Furthermore, probably the time for exposure and dose of dieldrin used might not have been enough to interfere with the process of testosterone and LH synthesis and/or clearance.

These results are in agreement with results from study by Rosebella and Jensen,(1993) where dieldrin feeding for 60 days did not alter the plasma levels of progesterone in gilts. However, these results are not explicitly comparable as different species of animals and different exposure time were used.

On the basis of these results, we conclude that, dieldrin when fed to male rats at a dose of 5mg or 10mg/kg body weight for 20 days has no statistically significant effect(s) on plasma testosterone and LH levels. Further, work should be carried out with larger dosage and/or longer duration than the 20 days to evaluate the effect of dieldrin on the levels of reproductive hormones and their clearance rate.

ACKNOWLEDGEMENT

We thank Mr. H Odongo for his technical assistance and Mr M. Okaka and V. Okoth for animal handling. This work was supported by grants from World health Organization's Human Reproduction Programme (WHO/HRP).

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