Original Article

Effects of Cypermethrin on Male Reproductive System in Adult Rats*

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Abstract

Objective To evaluate effects of cypermethrin on the testis histology and testosterone, LH and FSH in adult male Sprague-Dawley rats.

Methods The intact adult male rats were randomly divided into five groups and were treated with cypermethrin at doses of 0, 7.5, 15, 30, or 60 mg/kg per day by oral gavage for 15-days. After the treatments, serum was collected for hormone assays. The testes, epididymides, seminal vesicles, and prostates were excised and weighed. The right testis was frozen for daily sperm production and the left one was processed for histopathology.

Results Daily sperm production decreased significantly in 30 and 60 mg/(kg·day) groups. Testicular structure abnormalities included atrophic and distorted seminiferous tubules, deformed and disordered arrangement of germ cells, reduced germ cells, Sertoli cells and Leydig cells, vacuolization and multinucleated formations of spermatids in the cypermethrin-treated rats. Vacuolization was found in Sertoli cells and the deformed nucleus was noted in Leydig cells. Serum testosterone reduced significantly in 30 and 60 mg/(kg·day) groups. Serum FSH increased significantly in 60 mg/(kg·day) group.

Conclusion Cypermethrin induces impairments of the seminiferous tubules structure and spermatogenesis in the rats. The damages of the male reproductive system may be attributed to the imbalance of circulating testosterone.

Key words: Cypermethrin; Testosterone; Seminiferous tubule; Spermatogenesis

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INTRODUCTION

Pesticides are widely used in agriculture and public health to control insects, weeds, animals, and vectors of disease. Although the use of pesticides is of benefit in general, abuse of the pesticides a is harmful due tothier their potential toxicity to humans and animals[1-2]. Pyrethroid pesticides are a group of man-made products which are used widely in and around households as well as in agriculture. The use of pyrethroids has been increasing during the past decade with the declining use of organophosphate pesticides, which are more acutely toxic to birds and mammals than pyrethroids[3-4]. However, although pyrethroids are less acutely toxic, some studies have demonstrated that synthetic pyrethroids possess hormonal activities and have been classified as endocrine-disrupting compounds (EDCs), which potentially pose a threat to human and wildlife[5-6]. Research on the endocrine disrupting effects of pyrethroids should be therefore given more concern.

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Over the past years, estrogenic activities of pyrethroids have been the focus of research[7-8]. However, much attention has been recently paid to anti-androgenic effects of the pesticides for the observed degeneration in male reproductive health. It has been shown that some pyrethroids pesticides are associated with certain male reproductive damages including reduced sperm count, testicular lesions, sperm motility changes, sperm morphologic abnormality and genotoxic effects[9-12]. The reproductive impairments caused by the pyrethroids may be related to anti-androgenic activities of the pesticides.

Recently, we have shown that some pyrethroid pesticides including cypermethrin, permethrin, and fenvalerate act as anti-androgens in the *in vitro* study[13-14]. In the studies, we have developed a human androgen receptor (hAR) reporter gene assay to measure anti-androgenic activity of the chemicals[15]. The *in vitro* assay can provide valuable insights into mechanisms of test chemicals involved in AR, while it is not adequate to entirely replace *in vivo* assay for it is unable to evaluate metabolism and distribution in the animal. The anti-androgenic effects involved in testosterone synthesis can neither be clarified by the hAR reporter gene assay.

The pyrethroids may act as an anti-androgen via various pathways. AR mediated signaling has been shown to be a major pathway. However, there is evidence that some pyrethroids do not act via AR, and the alteration of the synthesis, secretion and distribution of testosterone may also play a partial role in the mechanisms of action[16]. Because of the central role of androgens in the male reproductive system, low androgens levels must contribute to decreased male fertility[17]. The pyrethroids may exert anti-androgenic effect by interfering the synthesis of androgens in the testes, the normal secretion and function of endogenous androgen hormones.

The US Environmental Protection Agency (EPA) recommended the 15-day intact adult male assay as an *in vivo* alternative for assessing potential androgenic and antiandrogenic effects. The assay has been shown to be a viable means for assessing the endocrine potential of chemicals[18-19]. The endpoints of this assay consist of serum concentrations of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The alterations of hormones are critical to the regulation of the hypothalamic-pituitary-testicular (HPT) axis. Then the assay is capable of identifying an anti-androgen that interferes testosterone and acts at the HPT axis.

In this study, the 15-day intact adult male assay was used to evaluate the anti-androgenic effect of one of pyrethroid pesticides, cypermethrin. We investigated the contributions of reproductive hormones to cypermethrin induced anti-androgenic effects. Cypermethrin was selected because the chemical is widely used to kill insects on cotton and lettuce, and to kill cockroaches, fleas, and termites in houses and other buildings in China.

MATERIALS AND METHODS

Chemicals and Reagents

Cypermethrin was bought from ChangZhou Pesticide Factory with the purity of 98% (Jiangsu, China). Citrate Buffer Solution (CBS) and Phosphate Buffered Saline (PBS) were purchased from Beijing *Zhongshan Golden Bridge Biotechnology Co., Ltd* (Beijing, China). *3% Hydrogen Peroxide was supplied by Jiangmen Hengjian Pharmaceutical Co., Ltd* (Jiangsu, China). Testosterone, FSH and LH radioimmunoassay *(*RIA) kits were purchased from Beijing North institute of Biological Technology (Beijing, China).

Animals and Housing

The male *Sprague-Dawley* rats, approximately 9 weeks of age and 160-190 g in weight, were obtained from Shanghai Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). The rats were allowed to acclimate in the animal care facility for 7 days before the start of treatment. The animals were maintained in plastic cages with a controlled temperature of 25±2 °C. A 12-h light-dark cycle was maintained. The rats were ad libitum fed with water and food supplied by Laboratory Animal Center of Xuzhou Medical College (Jiang Su, China). The animal studies were approved by Ethical Committee for Animal Research of Xuzhou Medical College. The animal care and the experimental procedures were conducted following the national law on animal use.

Experimental Design

The sixty animals were randomly assigned to 5 equal groups. Cypermethrin was dissolved in corn oil and the rats were administered by oral gavage for 15

consecutive days. The rats in test groups was administered with different doses of cypermethrin of 7.5, 15, 30, and 60 mg/(kg·day) respectively. The control group received the same volume of corn oil without cypermethrin. All animals were weighed and were observed daily for signs of treatment-related effects during the 15-day treatments.

Blood and Tissue Collection

On the day 15 after treatment, rats were sacrificed by decapitation 2 h following the last dose. The decapitation procedure was completed within 5 to 10 sec to avoid stress and pain. Following decapitation, the blood was placed in a centrifuge tube until the serum was prepared. The blood was allowed to clot and to be centrifuged under approximately 1500 ×g for 20 min. Serum was stored at -80 °C until the hormones were measured. The livers, kidneys, testes, epididymides, seminal vesicles and prostates were excised quickly and weighed. The relative weights were calculated. After weighing, the right testes were frozen (-80 °C) for use of testicular sperm head counts and daily sperm production. Some sections of the left testes were processed for investigation of histopathological change .

Evaluation of Daily Sperm Production

Daily sperm production in the rat testis was measured as described previously[11,20]. Briefly, the rat testis stored at -80 °C was thawed and decapsulated before being weighed and placed in 50 mL of ice-cold 0.9% sodium chloride solution containing 0.01% Triton X-100 to be homogenized for spermatid count. The homogenization tissue was mixed using a vortex mixer and was then allowed to stand for one minute. Afterwards, the sample spermatozoa were counted using a hemocytometer. The number of spermatozoa produced per gram of testicular tissue per day was calculated according to the following formula: A×B×C×D/E/F. A=average count of sperm heads from chambers, B=square factor (5.0), C=hemocytometer factor (10^4), D=dilution factor (50), E=test weight (g), and F=the time (days) during spermatogenesis that these cells are resistant to homogenization (6.1 days).

Histological Evaluation of Testes

The left testes slices were fixed in modified Davidson's fluid and embedded in paraffin. Serial sections of 4 μm were cut with a microtome and stained by standard hematoxylin-eosin (HE) double staining procedure and observed under a light microscope. The stained sections were subjected to routine morphometric analysis using the eye piece scale and the stage micrometer.

Hormone Measurements

The serum stored at -80 °C was thawed. The circulating levels of testosterone, FSH and LH in serum were measured by ^{125}I-based radioimmunoassay (RIA) according to the protocol the kits. All samples were measured in the same assay to decrease the variation. The intra-assay and inter-assay variations were found to be less than 10% and 15%, respectively.

Statistical Analysis

Statistical analyses were performed with SPSS16.0 for Windows. The results are expressed as the mean±standard deviation (SD). Multigroup comparisons of the data were carried out by one-way analysis of variance (ANOVA) followed by the Dunnett's test, as appropriate. The results were considered statistically significant if the *P* values were less than 0.05.

RESULTS

General Condition and Body Weight

The food consumptions in any treatment groups were not significantly different from those in the control group throughout the treatment period. There was no abnormality in behavior and appearance in any groups of rats receiving cypermethrin as well as in the control group. Cypermethrin-treated rats did not show any adverse signs and mortality throughout the experiment. No statistically significant differences in the final body weights were observed in the rats treated with cypermethrin (Table 1). As shown in Table 1, no significant differences were noted for weights of liver and kidney between the treatment and control groups.

Effect of Cypermethrin on Reproductive Organ Weights

No significant differences were noted for testis, epididymis, seminal vesicle, and prostate weights in any of the treatment groups compared to those in the control group (Table 2).

Table 1. Effect of Cypermethrin on Body Weight and Absolute Weights of Liver and Kidney

Treatment [mg/(kg·day)]	**Initial Body Weight** (g)	**Final Body Weight** (g)	**Liver** (g)	**Kidney** (g)
0	169.80±9.11	200.03±19.94	7.62±1.18 (3.79±0.30)	1.53±0.16 (0.77±0.09)
7.5	172.00±11.73	211.49±9.64	8.33±0.56 (3.81±0.25)	1.69±0.15 (0.77±0.02)
15	171.70±8.26	208.30±15.92	8.33±0.56 (3.81±0.25)	1.59±0.12 (0.76±0.03)
30	171.70±9.39	211.49±6.93	7.24±0.57 (3.38±0.23)	1.59±0.11 (0.74±0.05)
60	168.20±5.51	197.52±8.71	7.52±0.52 (3.91±0.23)	1.47±0.12 (0.76±0.04)

Note. Values are expressed as means±SD of twelve animals.

Table 2. Effect of Cypermethrin on Absolute Weight of Reproduction Organs

Treatment [mg/(kg·day)]	**Testes** (g)	**Epididymides** (g)	**Seminal Vesicles** (g)	**Prostates**(g)
0	2.19±0.25 (1.10±0.14)	0.56±0.09 (0.28±0.05)	0.52±0.19 (0.26±0.08)	0.246±0.042 (0.12±0.03)
7.5	2.27±0.18 (1.04±0.07)	0.58±0.09 (0.27±0.04)	0.61±0.21 (0.28±0.09)	0.247±0.068 (0.11±0.02)
15	2.13±0.48 (1.04±0.26)	0.53±0.11 (0.26±0.06)	0.67±0.18 (0.32±0.08)	0.237±0.076 (0.11±0.03)
30	2.32±0.21 (1.09±0.10)	0.49±0.04 (0.23±0.02)	0.65±0.13 (0.30±0.06)	0.231±0.054 (0.10±0.02)
60	2.23±0.17 (1.09±0.15)	0.49±0.08 (0.26±0.03)	0.62±0.15 (0.32±0.08)	0.210±0.064 (0.09±0.03)

Note. Values are expressed as means±SD of twelve animals.

Effect of Cypermethrin on Daily Sperm Production

Daily sperm production decreased significantly of the rats in the 30 mg/(kg·day) group and the 60 mg/(kg·day) group when compared to that in the control group (P<0.05) (Figure1).

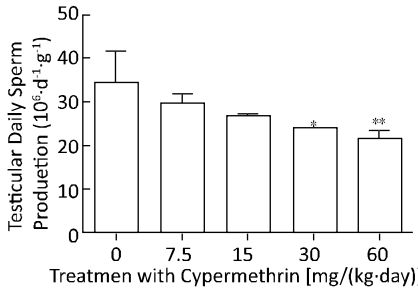

Figure 1. Effect of cypermethrin on testicular daily sperm production in adult male rats. Values are expressed as means±SD of twelve animals. * denotes P<0.05 statistically different from the control rats, ** denotes P<0.01 statistically different from the control rats.

Effect of Cypermethrin on Testis Histopathological Structure

In the control group, the arrangement of the seminiferous tubule was regular, and most of tubal walls were smooth. Slight distortion of seminiferous tubules in the 7.5 mg/(kg·day) group and the 15 mg/(kg·day) group was observed. Atrophic and distorted seminiferous tubules, deformed and disordered arrangement of germ cells were observed in the 30 mg/(kg·day) group and 60 mg/(kg·day) group. The numbers of germ cells, Sertoli cells and Leydig cells reduced, and the number of cell layers of the seminiferous tubules decreased as well. By using high-magnification dark-field microscopy (100×), we fund that the arrangement of germ cells was in disorder. The intraepithelial vacuolization and multinucleated formation of spermatocyte were observed in the treatment groups. The impairments of Sertoli cells and Leydig cells were viewed in the cypermethrin-treated rats. Vacuolization was found in Sertoli cells and the deformed nucleus was noted in Leydig cells (Figure 2). The perimeters of the seminiferous tubule decreased in the treatment groups (P<0.05). The diameters of seminiferous lumen shrank in the 7.5, 15, and 30 mg/(kg·day) groups (P<0.05). The numbers of cell layers of seminiferous tubules decreased in the treatment groups (P<0.05) (Figure 3).

Effect of Cypermethrin on Serum Hormone Concentrations

The effects of cypermethrin treatment on testosterone, FSH and LH in serum were analyzed. The concentrations of testosterone in serum reduced significantly in the 30 mg/(kg·day) group and the 60 mg/(kg·day) group (P<0.05). A significant increase of serum FSH was observed in the 60 mg/(kg·day) group when compared to that in the control group (P<0.05). An upward trend of concentrations of LH in serum was observed, while no significant differences were noted between the treatment groups and the control group (Figure 4).

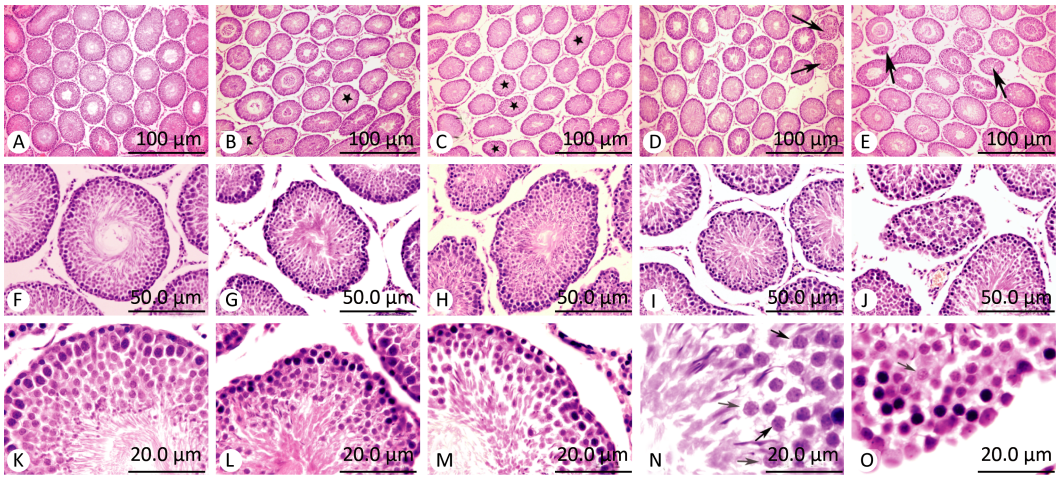

Figure 2. Histopathological changes in testes after cypermethrin treatment (HE). (A) Control group, the arrangement of the seminiferous tubule is regular, and most of the tubal walls were smooth . (B, C, D and E) Treatment groups, the arrangement of the seminiferous tubule is distorted (star), atrophy as well as necrosis (black arrows). The interspaces between seminiferous tubules enlarged. (F) Control group, normal male rat seminiferous tubule. (G, H, I, and J) Treatment groups, the arrangement of germ cells was disordered. The number of cell layers of the seminiferous tubules was significantly reduced and necrosis was present. (K) Control group, normal male rat seminiferous tubule. (L, M, N, and O) Treatment groups, the arrangement of germ cells was in disorder. The intraepithelial vacuolization (grey arrows) and multinucleated formation (black arrows) of spermatocyte were observed .

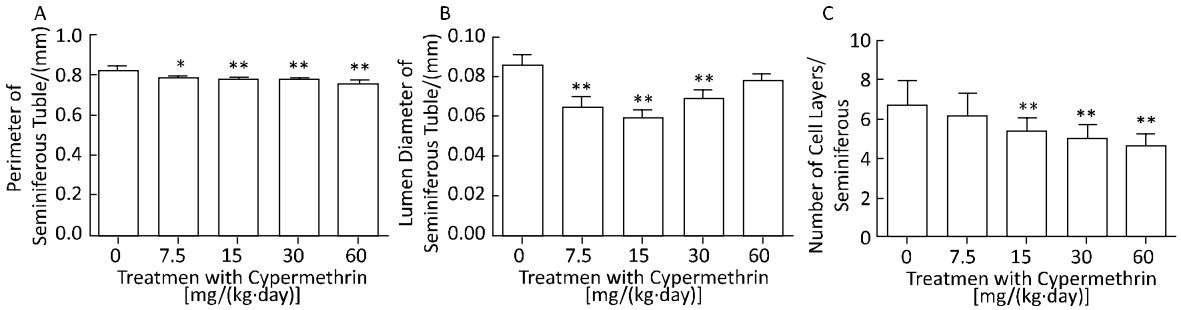

Figure 3. Perimeter, lumen diameter and number of cell layers of seminiferous tubule changes after cypermethrin treatment. *denotes $P<0.05$ statistically different from the control rats, **denotes $P<0.01$ statistically different from the control rats.

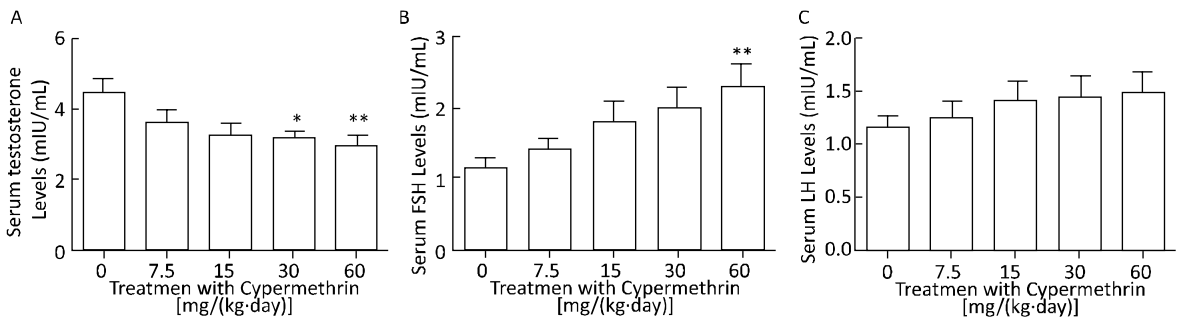

Figure 4. Effect of cypermethrin on serum levels of testosterone, FSH and LH. Values are expressed as means±SD of twelve animals. *denotes $P<0.05$ statistically different from the control rats, **denotes $P<0.01$ statistically different from the control rats.

DISCUSSION

Due to its wide use in many fields, cypermethrin might present a risk to the human health and environment. The concern about its safety has grown[21]. The anti-androgenic activity of the pesticide has been reported by interfering with AR *in vitro*, which involves in AR mediated signaling[13,15,22]. However, the anti-androgenic effects *in vivo* of cypermethrin involved in inhibiting testosterone synthesis are not well established.

In this study, the 15-day intact adult male rat assay was established and was used to evaluate the anti-androgenic effect of cypermethrin involved in the potential to inhibit the production of testosterone. We found that the serum testosterone level decreased significantly in the 30 mg/(kg·day) group and the 60 mg/(kg·day) group. Elbetieha et al. also demonstrated that the serum levels of testosterone were reduced in the adult male Sprague-Dawley rats exposed to tap water containing cypermethrin for 12 weeks[23]. Similar results were reported in male rats exposed to other synthetic pyrethroids such as fenvalerate, permethrin and Beta-cypermethrin[16,24-25]. Leydig cells play a crucial role in testosterone synthesis. Leydig cells synthesize testosterone and secrete it into the bloodstream in males. Some pyrethroids have been reported to cause a decrease in testicular enzymes like 17β-hydroxysteroid dehydrogenase (17β-HSD), and glucose-6-phosphate dehydrogenase and therefore interfere testicular testosterone synthesis[16]. We inferred that cypermethrin acted directly on the testes and affected the androgen biosynthesis in Leydig cells. Abnormal Leydig cells reduced the steroidogenic potential of the testis. Based on histological analysis in this study, structural impairments and number decrease of Leydig cells were observed. The decreased concentrations of serum testosterone could result from decreases in the number of Leydig cells and/or the damage of their structure. Hence, the results may provide clues to reveal the exact molecule mechanisms involved in testosterone biosynthesis pathway, which should be further explored.

Because testosterone plays a crucial role in male reproductive system, disruption of its production may impair male reproductive health[26-28]. Testosterone is needed for the continued production of different generation of germ cells in the seminiferous tubules. Therefore, reduction of testosterone level may lead to the separation of germ cells from the epithelium of the seminiferous tubules[29-30]. The finding of the present study revealed a significant reduction in daily sperm production of the rats treated with 30 mg/(kg·day) and 60 mg/(kg·day) of cypermethrin, which was consist with the reduction in concentrations of serum testosterone. It is suggested that cypermethrin might suppress male spermatogenesis and induce low daily sperm production by disturbing testosterone biosynthesis. Testosterone stimulates sperm production by acting on the seminiferous tubules. Therfore, the reduction in testosterone may also have adverse effects on the seminiferous tubular. In addition to the decreased daily sperm production, the reduced testosterone might also be responsible for morphological abnormality of testis in cypermethrin-treated rats in this study. Similar results have been reported of impairments of cypermethrin on male reproductive system[31-33]. In the current study, testicular structure abnormalities included atrophic and distorted seminiferous tubules, deformed and disordered arrangement of germ cells, reduced germ cells, Sertoli cells and Leydig cells, as well as vacuolization and multinucleated formations of spermatids in the cypermethrin-treated rats. Reduced concentration of testosterone following treatment with cypermethrin could be considered as a cause of the degeneration of germinal epithelium which is needed for normal spermatogenesis. We hypothesized that reduction in serum testosterone level caused by cypermethrin impaired the structure of testis and consequently suppressed spermatogenesis.

Regulation of male reproductive system occurs via a negative feedback loop involving the hypothalamus, anterior pituitary and testicles, which is referred to as the HPT axis. The gonadotropin releasing hormone (GnRH) is from the hypothalamus.Gonadotropin including FSH and LH from the pituitary are affected by a negative feedback from testicular hormones including testosterone and other sexual hormones[34]. The mechanism of feedback control of FSH is regulated by a Sertoli cell product called inhibin B[35-36]. Impairments of Sertoli cells are accompanied with decreased production of inhibin B, which is associated with reciprocal elevation of FSH levels by negative feedback[35]. In this study, we found a trend toward increase in the serum FSH levels in cypermethrin-treated rats, and a significant increase was observed in the 60 mg/(kg·day) group. It was shown that treatment with cypermethrin decreased

serum testosterone levels while increasing serum FSH levels. The increase in FSH secretion may be principally due to a feedback signal from the damaged seminiferous tubules. Under such a situation, the Sertoli cells are found to produce less inhibin B, and then FSH released from the pituitary is increased significantly due to a negative feedback action. The impairments of Sertoli cells including vacuolization and number decrease were noted in the current study, which was attributed to elevation of serum FSH level.

Cypermethrin induces impairment of the structure of seminiferous tubules and spermatogenesis in the male adult rats. The said impairment may be attributed to the imbalance of circulating testosterone.

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REFERENCES

1. Eddleston M, Karalliedde L, Buckley N, et al. Pesticide poisoning in the developing world-a minimum pesticides list. Lancet, 2002; 360, 1163-67.
2. Ferrer A. Pesticide poisoning. An Sist Sanit Navar, 2003; 26, 155-71.
3. Bateman DN. Management of pyrethroid exposure. J Toxicol Clin Toxicol, 2000; 38, 107-9.
4. Shafer TJ, Meyer DA. Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs, and relationship to assessment of cumulative neurotoxicity. Toxicol Appl Pharmacol, 2004; 196, 303-18.
5. Kim SS, Lee RD, Lim KJ, et al. Potential estrogenic and antiandrogenic effects of permethrin in rats. J Reprod Dev, 2005; 51, 201-10.
6. Han Y, Xia Y, Han J, et al. The relationship of 3-PBA pyrethroids metabolite and male reproductive hormones among non-occupational exposure males. Chemosphere, 2008; 72, 785-90.
7. Kojima H, Katsura E, Takeuchi S, et al. Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ Health Perspect, 2004; 112, 524-31.
8. Wilson VS, Bobseine K, Gray LE Jr. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. Toxicol Sci, 2004; 81, 69-77.
9. Bian Q, Xu LC, Wang SL, et al. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. Occup Environ Med, 2004; 61, 999-1005.
10. Xia Y, Bian Q, Xu L, et al. Genotoxic effects on human spermatozoa among pesticide factory workers exposed to fenvalerate. Toxicology, 2004; 203, 49-60.
11. Xu LC, Zhan NY, Liu R, et al. Joint action of phoxim and fenvalerate on reproduction in male rats. Asian J Androl, 2004; 6, 337-41.
12. Song L, Wang YB, Sun H, et al. Effects of fenvalerate and cypermethrin on rat sperm motility patterns in vitro as measured by computer-assisted sperm analysis. J Toxicol Environ Health A, 2008; 71, 325-32.
13. Xu LC, Sun H, Chen JF, et al. Androgen receptor activities of p,p'-DDE, fenvalerate and phoxim detected by androgen receptor reporter gene assay. Toxicol Lett, 2006; 160, 151-7.
14. Xu LC, Liu L, Ren XM, et al. Evaluation of androgen receptor transcriptional activities of some pesticides in vitro. Toxicology, 2008; 243, 59-65.
15. Xu LC, Sun H, Chen JF, et al. Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol in vitro. Toxicology, 2005; 216, 197-203.
16. Zhang SY, Ito Y, Yamanoshita O, et al. Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. Endocrinology, 2007; 148, 3941-9.
17. Travison TG, Shackelton R, Araujo AB, et al. The natural history of symptomatic androgen deficiency in men: onset, progression, and spontaneous remission. J Am Geriatr Soc, 2008; 56, 831-39.
18. O'Connor JC, Frame SR, Ladics GS. Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. Toxicol Sci, 2002; 69, 92-108.
19. O'Connor JC, Marty MS, Becker RA, et al. Results of the negative control chemical allyl alcohol in the 15-day intact adult male rat screening assay for endocrine activity. Birth Defects Res B Dev Reprod Toxicol, 2008; 83, 117-22.
20. Xu LC, Wang SY, Yang XF, et al. Effects of cadmium on rat sperm motility evaluated with computer assisted sperm analysis. Biomed Environ Sci, 2001; 14, 312-17.
21. Bradberry SM, Cage SA, Proudfoot AT, Vale JA. Poisoning due to pyrethroids. Toxicol Rev, 2005; 24, 93-106.
22. Sun H, Xu XL, Xu LC, et al. Antiandrogenic activity of pyrethroid pesticides and their metabolite in reporter gene assay. Chemosphere, 2007; 66, 474-79.
23. Elbetieha A, Da'as SI, Khamas W, et al. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol, 2001; 41, 522-8.
24. Mani U, Islam F, Prasad AK, et al. Steroidogenic alterations in testes and sera of rats exposed to formulated Fenvalerate by inhalation. Hum Exp Toxicol, 2002; 21, 593-97.
25. Wang XZ, Liu SS, Sun Y, et al. Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology, 2009; 72, 599-611.
26. McLachlan RI, O'Donnell L, Stanton PG, et al. Effects of testosterone plus medroxyprogesterone acetate on semen quality, reproductive hormones, and germ cell populations in normal young men. J Clin Endocrinol Metab, 2002; 87, 546-56.
27. Holstein AF, Schulze W, Davidoff M. Understanding

spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol, 2003; 1, 107.
28. O'Shaughnessy PJ, Verhoeven G, De Gendt K, et al. Direct action through the sertoli cells is essential for androgen stimulation of spermatogenesis. Endocrinology, 2010; 151, 2343-8.
29. Blanco-Rodriguez J, Martinez-Garcia C. Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormone suppression by short-term oestradiol treatment of rats. Int J Androl, 1998; 21, 109-15.
30. Zitzmann M. Effects of testosterone replacement and its pharmacogenetics on physical performance and metabolism. Asian J Androl, 2008; 10, 364-72.
31. Yousef MI, El-Demerdash FM, Al-Salhen KS. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J Environ Sci Health B, 2003; 38, 463-78.
32. Rodriguez H, Tamayo C, Inostroza J, et al. Cypermethrin effects on the adult mice seminal glands. Ecotoxicol Environ Saf, 2009; 72, 658-62.
33. Wang H, Wang Q, Zhao XF, et al. Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes. Arch Toxicol, 2010; 84, 53-61.
34. Hayes FJ, DeCruz S, Seminara SB, et al. Differential regulation of gonadotropin secretion by testosterone in the human male: absence of a negative feedback effect of testosterone on follicle-stimulating hormone secretion. J Clin Endocrinol Metab, 2001; 86, 53-8.
35. Meachem SJ, Nieschlag E, Simoni M. Inhibin B in male reproduction: pathophysiology and clinical relevance. Eur J Endocrinol, 2001; 145, 561-71.
36. Boepple PA, Hayes FJ, Dwyer AA, et al. Relative roles of inhibin B and sex steroids in the negative feedback regulation of follicle-stimulating hormone in men across the full spectrum of seminiferous epithelium function. J Clin Endocrinol Metab, 2008; 93, 1809-14.