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Tebuconazole Disrupted Aromatase Activity and Reduced 17β-Estradiol Concentration in Pubertal Developmental Rats

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Abstract

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Around the world, triazoles are used as fungicides for fruit, grain, vegetable, and flower production and as pharmaceuticals for the treatment of human diseases. One type of triazole, tebuconazole, has been reported to combine with other endocrine disrupting pesticides, such as expoxiconazole, mancozeb, prochloraz, and procymidone, leading to dose-additive effects that impair parturition, cause pup mortality, affect sexual differentiation, and disrupt kisspeptin neurons in rats. However, the mechanisms by which tebuconazole effects thyroid function as well as the phase of pubertal development that this compound acts upon remain unclear; therefore, this study investigated pubertal developmental and thyroid function in rats. Our experiments were in accordance with US EPA OCSPP Harmonized Test Guidelines Series Number 890. For female rats, treatments (control; 5 mg/kg/day 17α-ethinyl estradiol [EE]; or 15, 50, or 150 mg/kg/day tebuconazole) were administered daily by oral gavage from postnatal day (PND) 22 to 42. For male rats, treatments (control; 0.4 mg/kg/day testosterone propionate (TP); 3 mg/kg/day flutamide; or 15, 50, or 150 mg/kg/day tebuconazole) were administered daily by oral gavage from PND 23 to 53. Our results showed that tebuconazole did not affect absolute organ weight nor the concentrations of serum blood urea nitrogen (BUN) or creatinine in either male or female rats. In contrast, high doses of tebuconazole decreased the uterus and ovary weights of female pubertal rats, whereas in male pubertal rats, high doses of tebuconazole increased relative testis weight and penis

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width but decreased the relative weights of the prostate, seminal vesicle (both with and without fluid), bladder, and levator ani plus bulbocavernosus muscles (LABC). In female rats, low doses of tebuconazole also delayed the age of vaginal opening (VO) and increased rat body weight at the time that VO was first observed. Furthermore, tebuconazole significantly decreased serum 17β-estradiol (E2) but did not affect the concentrations of serum testosterone, luteinizing hormone (LH), or follicular stimulating hormone (FSH). It did, however, decrease serum aromatase activity in male or female rats. Finally, tebuconazole did not affect the concentrations of serum thyroxine (T4), thyroid stimulating hormone (TSH), or triiodothyronine (T3) at any dosage level. However, dosage levels of 15 and 150 mg/kg/day tebuconazole increased the concentration of serum T3 in male rats. Taken together, our results suggest that tebuconazole primarily inhibits aromatase (CYP19) activity in pubertal male and female rats. However, the underlying mechanism of this inhibition needs to be more comprehensively elucidated.

Key words: thyroid hormone, tebuconazole, vaginal opening, preputial separation, pubertal rats

Introduction

Around the world, imidazoles and triazoles are employed as fungicides for fruit, grain, vegetable, and flower production and as pharmaceuticals for the treatment of human diseases [24]. Triazole antifungal agents inhibit fungal lanosterol-14α-demethylase (CYP51) activity and are effective in preventing the spread of many fungal species [16, 18]. Furthermore, some azole fungicides have been reported to exhibit cytochrome P450 enzyme activities, and the ability of these fungicides to inhibit aromatase (CYP19), which converts androgens to estrogens, is well known [34, 44, 52, 54, 59]. Cytochrome P450 monooxygenases (CYPs) are a large group of enzymes that are found in most organisms, from bacteria to mammals [51]. Moreover, several highly substrate-selective cytochrome P450 enzymes are responsible for regulating steroid hormone synthesis [41]. In mammals, sexual development can be disrupted by genetic and/or environmental factors [26]. This is because perinatal hormones have permanent imprinting effects on the hypothalamus. These effects are expressed early on as morphological sex differences in the brain, which later lead to physiological and behavioral differences between the sexes [20, 55].

Most previous studies which investigated the influence of exogenous chemicals on the

gonadal actions of male rats focused on behavioral and anatomical effects. Specifically, these effects result from endogenous hormonal effects that occur when testosterone levels surge [43], especially during the neonatal period [45, 54]. It is well known that azole fungicides increase gestation length, lead to virilization in female pups, and alter steroid hormone levels in rat fetuses and/or dams [44]. For example, the azo chemical prochloraz was found to induce reproductive and developmental malformations in androgen-dependent tissues of male rat offspring [34]. Tebuconazole was reported to demasculinize male offspring and was found to cause the same endocrine effects as prochloraz [44]. Perinatal exposure to tebuconazole was further found to cause neurobehavioral deficits and neuropathologies in rats, but the compound did not alter immunological or reproductive functions in rats [33].

Tebuconazole has also been found to suppress the invasion and migration of human trophoblasts by affecting the expression of proteases, hormones, angiogenic factors, growth factors, and cytokines [60]. Tebuconazole does not inhibit the aromatase activity of CYP19; rather, this compound exhibits anti-androgenic activities and shows potent CYP3A4 inhibition [28, 58]. In fact, we daily faced a mixture of chemicals; some previous researchers have investigated persistent developmental toxicity in rat offspring exposed to a low dose of an endocrine disrupting pesticide mixture. In those studies, tebuconazole was found to combine with other endocrine disrupting pesticides, such as expoxiconazole, mancozeb, prochloraz, and procymidone, leading to dose-additive effects that impaired parturition, increased pup mortality, affected sexual differentiation, and disrupted kisspeptin neurons in rats [21, 22, 23, 35].

Though tebuconazole was reported to induce endocrine disrupting activities at different endpoints of an important phase of pubertal development, its effects on thyroid function remain unclear. The Endocrine Disrupter Screening and Testing Advisory Committee of the USEPA recommended a screening strategy to investigate endocrine-disrupting compounds that inhibit steroid biosynthesis, alter thyroid hormone function, or act as agonists/antagonists to estrogen and/or androgen receptors [10]. Pubertal female and male rat models have been designed to investigate the effects of fungicides on pubertal development, thyroid function, and hypothalamic-pituitary-thyroid (HPT) function in both male and female rats. In these models, 22- or 23-day-old weanling female and male rats were exposed to the test substance for 20 or 30 days during the pubertal development period [11, 12].

HPT function has been studied extensively in vertebrates, teleosts, amphibians [1, 2], and mammals [56, 57]. When the thyroid gland is functioning normally, thyrotropin-releasing

hormone (TRH) is secreted from the hypothalamus. TRH then travels to the anterior pituitary and triggers the release of thyroid-stimulating hormone (TSH), or thyrotropin, from thyrotrophic cells in the pars distalis of the adenohypophysis. An increase in TSH production in turn leads to an increase in thyroid hormone (TH) synthesis. TSH binds to receptors on the membrane of thyroid follicle cells, thereby stimulating the biosynthesis of the iodine-containing THs, thyroxine (T4), and triiodothyronine (T3). T4 is the primary TH secreted by the thyroid gland; however, it is quickly metabolized into T3, the more potent form of TH. Both T3 and T4 can exert negative feedback control over the anterior pituitary gland to inhibit further TSH release [30, 36].

Based on previous reports which investigated the inhibition of aromatase (CYP19) activity, we hypothesized that tebuconazole may disturb thyroid-related functions in pubertal male and female rats. Therefore, in this research, we sought to link the potential effects of tebuconazole on thyroid function and on HPT function through a study on pubertal development and thyroid function in male and female Wistar rats.

Materials and methods

1. Chemicals

The following materials were obtained from Sinon Co. (Taichung, Taiwan, ROC): testosterone propionate (TP, purity ≥ 97%), flutamide (purity ≥ 97%), corn oil (0.9 g/ml), 17α-ethinyl estradiol (EE, purity ≥ 98%) (Sigma-Aldrich Co., St. Louis, MO, USA), and tebuconazole (purity ≥ 97%).

2. Animals

All experimental animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Taiwan Agricultural Chemicals and Toxic Substances Research Institute. Five-week-old male and female Wistar rats were purchased from Bio-LASCO (Taipei, Taiwan, ROC). The rats were acclimated to the laboratory environment for 7 days in which conditions were as follows: temperature (21 ± 2°C), humidity (40 ~ 70%), frequency of ventilation (at least 10/h), and lighting (alternating 12-h cycles of light and darkness). Rats were provided with a rodent pellet diet and water *ad libitum* until they were sacrificed. At 12 weeks of age, the 18 male and 18 female rats within each treatment group were allowed to mate over a 14-day period. Gestation day (GD) 0 was defined as the day that sperm was observed in the vagina of the female following mating. Dams were allowed to deliver their pups naturally. Any litters with fewer than eight pups (including both males and

females) or that had not been delivered by GD 23 were excluded from the study. The eight-pup threshold was adopted to ensure that at least 7-10 female pups and 10 male pups were included in each treatment group and to avoid the need to place pups from the same litter in the same treatment group. We also ensured that the population of female and male pups was as homogeneous as possible by excluding both the heaviest and lightest pups from the study. Therefore, mean body weight and body weight variance of rats in all groups were similar. Nonetheless, animals were allocated to treatment groups on the basis of body weight, randomization to ensure unbiased weight distribution across all treatment groups.

3. Chemical Treatments

Prior to treatment, male rat pups were weighed and clinically observed daily. Treatments (control; 0.4 mg/kg/day TP; 3 mg/kg/day flutamide; or tebuconazole administered at a dose of 15, 50, or 150 mg/kg/day) were delivered daily by oral gavage from postnatal day (PND) 23 to 53. Beginning on PND 30 or earlier, male rat pups were examined daily for preputial separation (PPS). On PND 53, male rat pups received a final dose of the treatment and were then sacrificed two hours later. Female rat pups were also weighed and clinically observed daily prior to treatment. Treatments (control; 5 mg/kg/day 17α-ethinyl estradiol [EE]; or tebuconazole administered at a dose of 15, 50, or 150 mg/kg/day) were delivered daily by oral gavage from PND 22 to 42. Beginning on PND 30, female rat pups were examined daily for vaginal opening (VO). On PND 42, or earlier females received a final dose of the treatment and were then sacrificed two hours later.

4. Monitoring of clinical signs and body weights

Throughout the study period, we examined each female and male rat at least once per day to determine whether clinical signs of toxicity related to chemical treatment were present. On working days, all cages were checked in the mornings and afternoons for dead or moribund animals. Finally, the body weight of each rat was recorded (to the nearest 0.1 g) daily prior to treatment.

5. Determination of organ weights

All rats (both females and males) were sacrificed 24 hours after the final treatment via 3 mg/kg/day Zoletil in the same sequence that the test substance was administered. Uteri and ovaries were dissected and carefully trimmed of fat to avoid loss of luminal content. The body of each uterus was cut just above its junction with the cervix as well as at the junction where

the uterine horns meet the ovaries. Uteri were then weighed with and without luminal content. Thyroids, livers, kidneys, pituitary glands, adrenal glands, ovaries, seminal vesicles, coagulating glands (with and without fluid), prostates, levator ani plus bulbocavernosus muscles (LABC), epididymides, testes, and penises were also carefully dissected and weighed.

6. Monitoring of vaginal openings (VO)

Each female rat was examined daily for VO beginning at PND 21. On the day that VO was first observed, the age and body weight of the rats were recorded. Vaginal lavage was collected daily from the day after VO was observed until the end of the study. This was achieved by repeatedly pipetting 0.9% saline into the rat vagina. The lavage fluid was then applied to a clean glass slide, and the smear was immediately observed under low magnification (×100) with a microscope. Lavage cytology was evaluated, and the stage of the estrous cycle was determined using the method described by Everett et al. (1989) [13]. In addition to recording the day that complete VO was observed, we also recorded the days that a small "pinhole" and/or vaginal threads were observed. The day of complete VO was the endpoint in the analysis which was used to elucidate the average age at which VO occurred; however, note that pinholes and threads did not represent complete VO. If any animal within any treatment group showed an incomplete opening (indicated by persistent threads or a pinhole) for more than 3 days, they were included in a separate analysis which was conducted using the ages at which incomplete openings were first observed. Even if VO otherwise appeared complete, documentation of vaginal threads was crucial. It was also crucial for us to record the "initiation" of VO. Note that, as much as possible, we recorded VO observations after dosing. However, VO observations were always recorded at approximately the same time each day, regardless of whether this was before or after dosing.

7. Monitoring of estrous cyclicity

From the day of VO up to and including the day of necropsy, daily vaginal smears were collected and evaluated for leukocytes, nucleated epithelial cells, and cornified epithelial cells under a low-power light microscope. The stages of vaginal smears were classified and recorded daily. Specifically, these stages included the diestrus stage (predominance of leukocytes mixed with some cornified epithelial cells), the proestrus stage (predominance of round, nucleated clumps of epithelial cells), and the estrus stage (predominance of cornified epithelial cells). Note that metestrus was classified as an early part of the diestrus stage rather than as a late part of the estrus stage. The ages at which

vaginal estrus were first observed were also recorded.

At the end of the study period, the overall estrus pattern of each female rat was characterized as being regular (recurring 4- to 5-day cycles), irregular (cycles with periods of diestrus longer than 3 days or with periods of cornification longer than 2 days), or as non-existent (prolonged periods of vaginal cornification or leukocytic smears). In cases where there were too few days between VO and the end of the study period for more than one cycle to be observed, estrus patterns were classified using available data. In these cases, if the partial data indicated that rats had a regular estrus cycle, we classified the cycle as regular. If the partial data did not indicate whether the estrus cycle was irregular or non-existent, the estrus pattern was classified as irregular.

8. Monitoring of preputial separation (PPS)

Preputial separation, the separation of the foreskin of the penis from the glans, is a reliable, early marker of the pubertal progression that normally occurs between 40 and 50 days of age. For many rat species, the average age at which PPS occurs is 43 days [26]. In the present study, PPS was monitored from PND 22 to 53. All males were examined at approximately the same time each day. Although partial separations, in which the foreskin and glans are mostly separated but threads of cartilage remain between the glans and the prepuce, were also recorded, only the day of complete separation was used in data analysis. Partial and complete PPS as well as persistent threads of cartilage were all recorded on the days that they were observed. The day of complete PPS was adopted as the endpoint in the analysis, which was conducted to determine the age at which PPS occurred. However, if any animal in any treatment group showed incomplete separation (including persistent threads) for more than three days, they were included in a separate analysis that was conducted using the ages at which partial separation was first observed. Even if PPS otherwise appeared complete, documentation of a thread was crucial. It was also crucial for us to record the "initiation" of PPS. Note that, as much as possible, we recorded PPS observations after dosing, which were always recorded at approximately the same time each day, regardless of whether this was before or after dosing.

9. Hematochemistry

At the end of the study period, blood samples were collected and allowed to coagulate for 30 min in an SST II tube (#367953, BD Co., Plymouth, UK) placed in an ice bath. After coagulation was complete, the blood was centrifuged at 3,000 × g for 15 min. The blood serum was

then transferred into siliconized microcentrifuge tubes and stored at -80°C until use. Serum creatinine and blood urea nitrogen (BUN) levels were detected using an automated clinical chemistry analyzer (DRI-CHEM 4000i, Fujifilm Co., Tokyo, Japan).

10. Determination of hormone levels

Levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), T4, T3, and TSH in serum were determined using a magnetic bead panel (#RPTMAG-86K, #PTHYMAG-30K, Millipore Co., St. Charles, MI, USA). Levels of testosterone (T) and estradiol (E2) in serum were determined using an EIA assay kit (#582701, #582251, Cayman Co.). Levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH) in serum were determined using a magnetic bead panel (#RPTMAG-86K, #PTHYMAG-30K, Millipore Co.). A cytochrome P450 19A1 ELISA kit (CSB-EL006394RA, Cusabio Co.) was used to determine aromatase levels in serum.

11. Statistical analysis

In this study, data are expressed as the mean ± standard deviation (SD). Data pertaining to mean initial weight, body weight at the time of necropsy, mean age, body weight upon VO or PPS, organ weight, and hormone levels were analyzed for homogeneity of variance using Bartlett's test. When samples were found to be homogeneous, nonparametric analysis of variance was applied. Analysis of covariance (in which body weight at the time of necropsy was used as a covariate) was performed to determine absolute organ weight. When a significant treatment effect was observed, Dunnett's test (control vs. treatment groups) was performed. The level of statistical significance was set a priori at $\alpha = 0.05$.

Results

1. Effects on body and organ weights

During the study period, there were no clinical signs of toxicity in any group of male or female rats. However, EE significantly decreased the body weights of the female rats at PND 24 to 42 (Fig. 1A). EE also significantly reduced weight gain in female rats. Conversely, tebuconazole did not significantly affect the body weight of either female (Fig. 1A) or male rats (Fig. 1B). In male rats, neither TP, flutamide, nor tebuconazole had any significant effect on body weight or weight gain (Table 1).

In female rats, treatment with 150 mg/kg/day tebuconazole significantly increased absolute liver weight by 11%, decreased absolute ovary weight by 34%, and decreased uterus blotting

Table 1. General growth in the female and male rats

Treatments[3)]	Vehicle Control	EE[1)] 5	TP[2)] 0.4	Flutamide 3	Tebuconazole 15	Tebuconazole 50	Tebuconazole 150
Female							
Sample size (n)	10	10			10	10	10
Initial body weight (g)	54 ± 3	56 ± 6			56 ± 5	56 ± 4	54 ± 2
Final body weight (g)	164 ± 11	116 ± 8***			170 ± 12	164 ± 11	160 ± 9
Final body weight (%)	100	71			104	100	97
Body weight gain (g)	110 ± 12	60 ± 8***			113 ± 13	108 ±10	105 ± 8
Male							
Sample size (n)	10		10	10	10	10	10
Initial body weight (g)	64 ± 2		65 ± 4	66 ± 4	60 ± 6	65 ± 4	63 ± 4
Final body weight (g)	298 ± 18		290 ± 26	304 ± 16	300 ± 33	297 ± 17	277 ± 23
Final body weight (%)	100		97	102	101	100	93
Body weight gain (g)	234 ± 18		225 ± 24	238 ± 14	239 ± 33	232 ± 17	213 ± 21

1) EE: 17α-ethinyl estradiol; p-value = * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.005
2) TP: Testosterone Propionate
3) Treatment dose: mg/kg/day

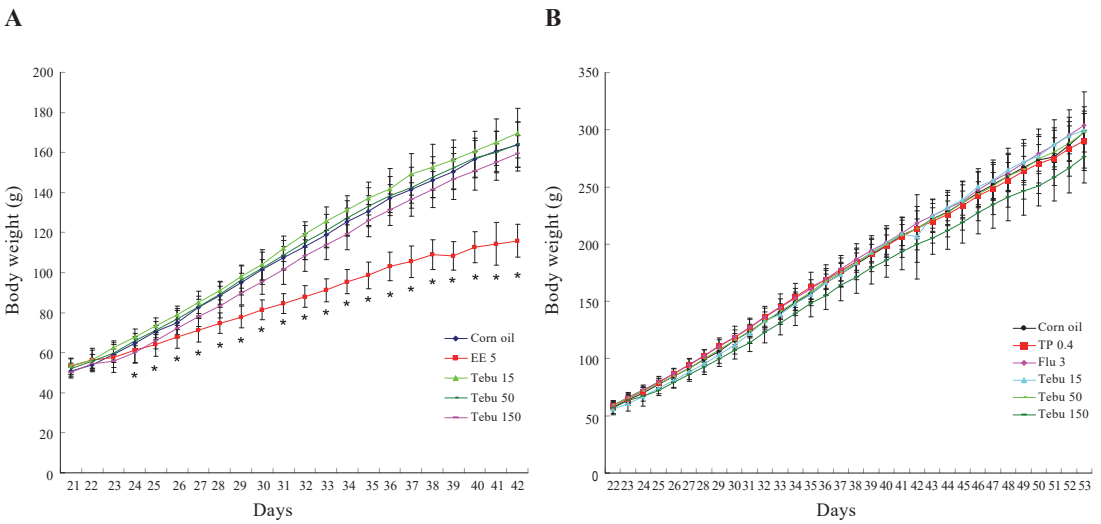

Fig. 1. Body weight changes in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5) and male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4) and 3 mg/kg/day flutamide (Flu 3) and both female and male Wistar rats treated with 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Female rats were dosed daily from 21 days of age until necropsy was performed on day 42 (A). Male rats were dosed daily from 22 days of age until necropsy was performed on day 53 (B). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that the mean body weight was significantly different from that of the control, $P < 0.05$.

by 26% (note that none of these changes were dose-dependent). Treatment with 15 mg/kg/day tebuconazole also significantly increased 13% of thyroid w/o trachea in female rats (Table 2). EE significantly increased the relative weight of the uterus (both with and without fluid), while tebuconazole significantly decreased the relative weight of the uterus without fluid when administered at a dose of 150 mg/kg/day (Fig. 2) but did not affect relative uterus weight in any other cases. EE did not affect the relative weight of the ovaries whereas tebuconazole significantly decreased the relative weight of the ovaries when administered at a dose of 150 mg/kg/day but did not affect relative ovary weight when administered at other dosage levels (Fig. 3). Finally, in female rats, EE significantly increased the relative weights of the thyroid (Fig. 4A) and pituitary (Fig. 5A) glands, but tebuconazole did not.

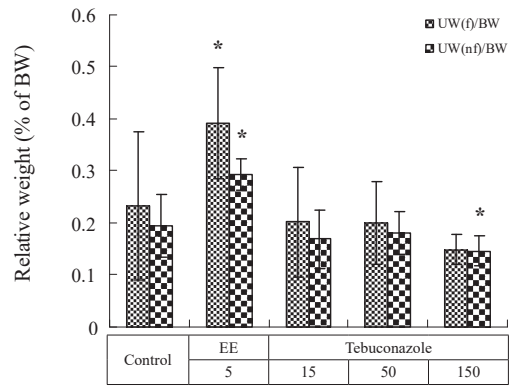

Fig. 2. Relative weights of uterii with and without fluid in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that the mean relative uterus weight was significantly different than that of the control, $P < 0.05$. UW(f): uterus weight with fluid; UW(nf): uterus weight without fluid; BW: body weight.

Table 2. Organ weight at necropsy in the female rats

Treatments[2)]	Vehicle control	EE[1)]	Tebuconazole		
		5	15	50	150
Sample size (n)	10	10	10	10	10
Liver (g)	8.1 ± 0.9	6.2 ± 0.7***	8.3 ± 0.9	8.5 ± 0.7	9.0 ± 0.8*
Kidney (g)	1.6 ± 0.2	1.2 ± 0.1***	1.6 ± 1.2	1.6 ± 0.2	1.5 ± 0.1
Pituitary (mg)	9.1 ± 1.3	11.0 ± 2.3*	8.8 ± 0.9	9.5 ± 1.5	7.9 ± 1.7
Adrenals (mg)	52.3 ± 5.5	37.0 ± 6.3***	53.0 ± 5.8	50.4 ± 5.7	50.2 ± 6.8
Ovaries (mg)	98.4 ± 35.4	64.3 ± 33.5*	84.8 ± 6.2	87.8 ± 12.9	64.7 ± 19.0*
Uterus, wet (mg)	373.0 ± 196	449.0 ± 101	342.0 ± 181	327.0 ± 127	239.0 ± 54
Uterus, blotted (mg)	316.0 ± 87	338.0 ± 25	286.0 ± 95	294.0 ± 68	235.0 ± 54*
Thyroid w/trachea (mg)	136.4 ± 10.3	110.5 ± 7.7***	138.3 ± 7.8	137.8 ± 12.9	128.0 ± 8.0
Thyroid w/o trachea (mg)	15.6 ± 3.2	14.7 ± 2.1	17.7 ± 3.3*	16.4 ± 2.5	17.1 ± 3.9

1) EE: 17α-ethinyl estradiol; p-value = * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.005

2) Treatment dose: mg/kg/day.

In the male rats, TP significantly increased the absolute weights of the following: seminal vesicles plus coagulating glands (both with and without fluid), prostates, LABCs, left and right epididymides, and left and right testes. Flutamide significantly decreased the absolute weights of seminal vesicles plus coagulating glands, LABCs, epididymides, and penises but significantly increased the absolute weight of the testes. Treatment also significantly changed the absolute weight of the penis when administered at a dose of 150 mg/kg/day but did not affect penis weight when administered at any other dosage level (Table 3).

TP, flutamide, and tebuconazole did not significantly change the relative weights of the thyroid (Fig. 4B) nor pituitary (Fig. 5B) glands

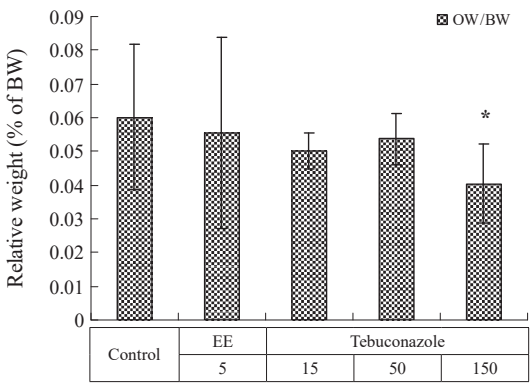

Fig. 3. Relative ovary weights of female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that mean relative ovary weight was significantly different from that of the control, $P < 0.05$. OW: ovary weight; BW: body weight.

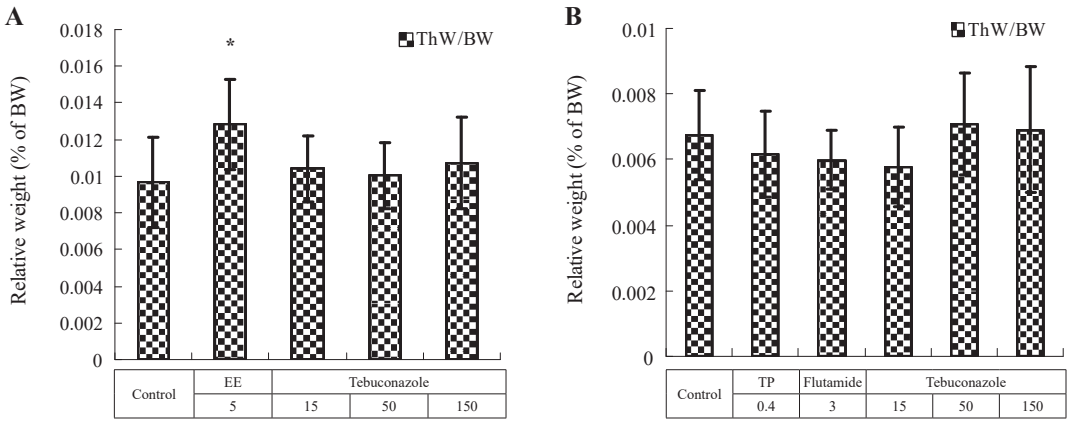

Fig. 4. Relative thyroid weight in female Wistar (A) and male Wistar (B) rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5) (females); 0.4 mg/kg/day testosterone propionate (TP 0.4) (males); 3 mg/kg/day flutamide (Flu 3) (males); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (both females and males). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that mean relative thyroid weight was significantly different than that of the control, $P < 0.05$. ThW: thyroid weight; BW: body weight.

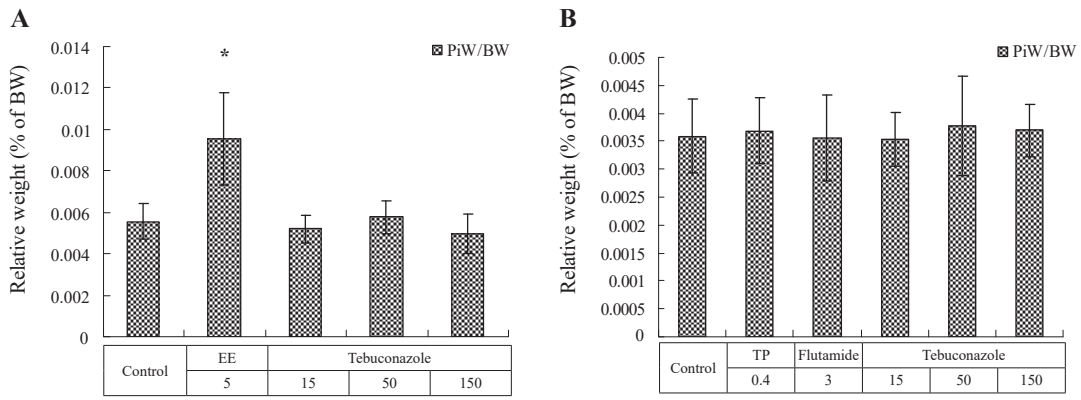

Fig. 5. Relative pituitary weight in female Wistar (A) and male Wistar (B) rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5) (females); 0.4 mg/kg/day testosterone propionate (TP 0.4) (males); 3 mg/kg/day flutamide (Flu 3) (males); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (females and males). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that mean relative pituitary weight was significantly different than that of the control, $P < 0.05$. PiW: pituitary weight; BW: body weight.

Table 3. Organ weight at necropsy in the male rats

Treatments[3)]	Vehicle control	TP[1)] 0.4	Flutamide 3	Tebuconazole 15	Tebuconazole 50	Tebuconazole 150
Sample size (n)	10	10	10	10	10	10
Liver (g)	14.0 ± 1.3	13.3 ± 1.7	14.7 ± 1.6	15.2 ± 1.8	14.7 ± 1.3	15.7 ± 1.5
Kidney (g)	2.5 ± 0.3	2.4 ± 0.2	2.5 ± 0.1	2.5 ± 0.3	2.4 ± 0.2	2.4 ± 0.3
Pituitary (mg)	10.6 ± 1.7	10.7 ± 1.7	10.9 ± 0.0	10.8 ± 1.6	11.2 ± 2.3	10.2 ± 1.2
Adrenals (mg)	58.6 ± 9.7	63.1 ± 10.0	64.1 ± 13.1	60.2 ± 8.8	56.1 ± 8.0	57.8 ± 9.2
Seminal vesicle + coagulating gland, with fluid (mg)	712 ± 186	961 ± 108*	441 ± 82*	695 ± 173	630 ± 102	485 ± 64
Seminal vesicle + coagulating gland, without fluid (mg)	488 ± 112	593 ± 53*	356 ± 61*	490 ± 85	445 ± 54	363 ± 38
Prostate (mg)	185 ± 39	218 ± 30*	124 ± 32	188 ± 49	178 ± 23	138 ± 28
LABC (mg)[2)]	611 ± 97	750 ± 53*	478 ± 85*	640 ± 104	560 ± 154	450 ± 79
Left epididymis (mg)	204 ± 23	155 ± 26*	167 ± 18*	205 ± 27	206 ± 17	177 ± 11
Right epididymis (mg)	205 ± 24	155 ± 21*	163 ± 18*	204 ± 30	204 ± 17	178 ± 14
Left testis (mg)	1267 ± 108	744 ± 36*	1383 ± 64*	1221 ± 312	1342 ± 102	1312 ± 138
Right testis (mg)	1282 ± 112	757 ± 359*	1390 ± 73*	1216 ± 40	1340 ± 94	1316 ± 116
Thyroid with trachea (mg)	179 ± 13	180 ± 14	195 ± 16*	179 ± 12	183 ± 10	172 ± 16
Thyroid without trachea (mg)	20 ± 4.5	17.8 ± 3.1	18.2 ± 3.0	17.6 ± 38	21.0 ± 4.6	18.9 ± 4.4
Penis (mg)	230 ± 18	244 ± 17	205 ± 19**	231 ± 25	222 ± 16	202 ± 14**

1) TP: Testosterone Propionate; p-value = * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.005.
2) LABC: levator ani plus bulbocavernosus muscles.
3) Treatment dose: mg/kg/day.

in male rats. TP and flutamide significantly increased and decreased the relative weight of LABC, respectively. Tebuconazole significantly decreased the relative weights of the bladder and LABC when administered at a dose of 150 mg/kg/day (Fig. 6) but did not affect the relative weights of these organs when administered at any other dosage level. TP significantly decreased the relative weights of the testis and epididymis. Flutamide increased the relative weight of the left testis but decreased the relative weight of the epididymis. Tebuconazole significantly increased the relative weight of the testis when administered at a dose of 150 mg/kg/day (Fig. 7) but did not affect relative testis weight when administered at any other dosage level. TP significantly increased the relative weight of the prostate and seminal vesicle (both with

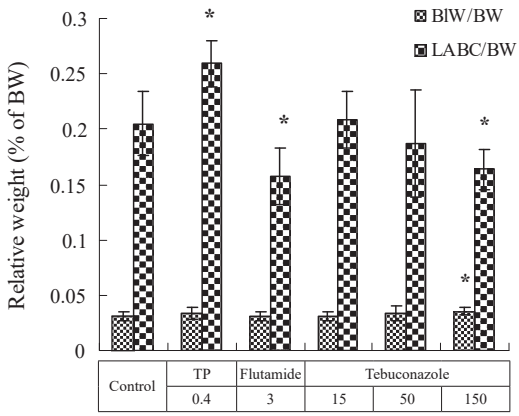

Fig. 6. Relative bladder and levator ani plus bulbocavernosus muscle weights in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that the mean relative organ weight was significantly different than that of the control, $P < 0.05$. BlW: bladder weight; LABC: levator ani plus bulbocavernosus muscles; BW: body weight.

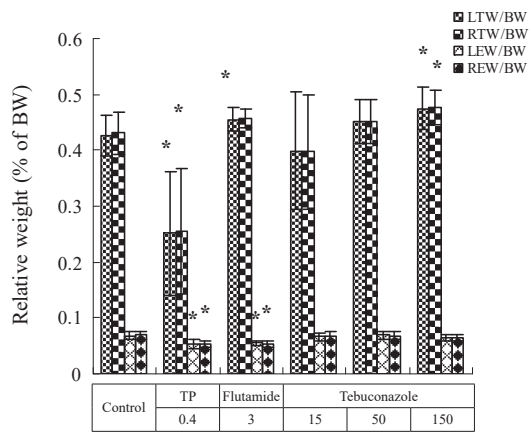

Fig. 7. Relative weights of testis and epididymis in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that mean relative organ weight was significantly different than that of the control, $P < 0.05$. LTW: left testis weight; RTW: right testis weight; LEW: left epididymis weight; REW: right epididymis weight; BW: body weight.

and without fluid) but decreased the relative weight of the scrotum. Flutamide significantly decreased the relative weight of the prostate and seminal vesicle (both with and without fluid). Tebuconazole significantly decreased the relative weights of the prostate and seminal vesicle (both with and without fluid) when administered at a dose of 150 mg/kg/day (Fig. 8) but did not affect the relative weights of these organs when administered at any other dosage level. TP significantly increased the relative weight and width of the penis. Conversely, flutamide significantly decreased the relative weight of the penis. Tebuconazole increased the relative width of the penis when administered at a dose of 150 mg/kg/day (Fig. 9) but did not affect relative penis weight when administered at any other dosage level.

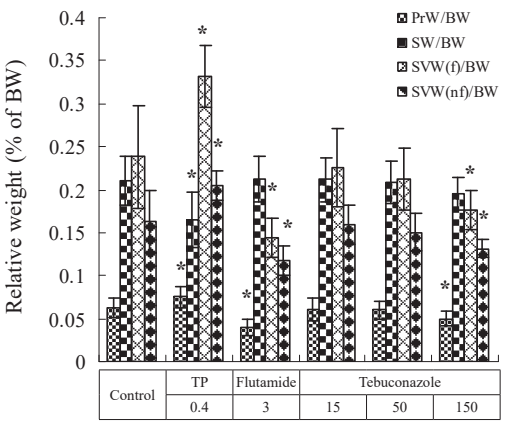

Fig. 8. Relative weights of prostate, scrotum, and seminal vesicles with and without fluid in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that relative organ weight was significantly different than that of the control, $P < 0.05$. PrW: prostate weight; SW: scrotum weight; SVW(f): seminal vesicle with fluid; SVW(nf): seminal vesicle without fluid; BW: body weight.

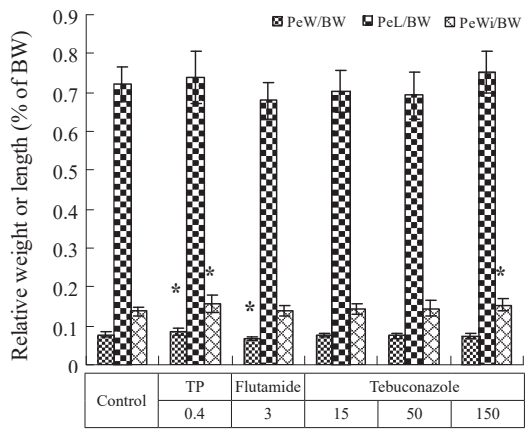

Fig. 9. Relative weights, lengths, and widths of penises in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). Data are expressed as the mean ± SD of 10 animals per treatment group. The * symbol indicates that the mean weight, length, or width value was significantly different than that of the control, $P < 0.05$. PeW: penis weight; PeL: penis length; PeWi: penis width; BW: body weight.

2. Effects on blood urea nitrogen (BUN) and creatinine in serum

In investigating the effects of tebuconazole on blood chemistry, blood urea nitrogen (BUN) and creatinine were used as indicators. In female rats, tebuconazole significantly decreased BUN by 17% and 15% when administered at dosage levels of 15 and 150 mg/kg/day, respectively, compared to the controls. In male rats, tebuconazole decreased BUN by 25% and 29% when administered at dosage levels of 50 and 150 mg/kg/day, respectively. Tebuconazole did not affect creatinine in either female or male rats; however, EE, TP, and flutamide did affect both BUN and creatinine in female and male rats (Table 4).

3. Effects on VO and estrous cyclicity in female rats

For female rats in the control group, mean age and body weight at the time when VO was first observed were 29.0 days and 95.0 g, respectively. Treatment with EE significantly advanced VO to 26.2 days of age and significantly reduced mean body weight at the time of VO to 69.0 g, and VO was first detected at 24 days of age in most EE-treated rats. Treatment with 15 mg/kg/day tebuconazole significantly delayed VO (to 32.6 days of age) and significantly increased mean body weight at the time of VO to 121.2 g. For female rats treated with 50 and 150 mg/kg/day tebuconazole, the mean age and body weight at the time when VO was first observed were comparable to those of controls (Fig. 10A).

Table 4. BUN, creatinine in the female and male rats

Treatments[4)]	Vehicle control	EE[1)] 5	TP[2)] 0.4	Flutamide 3	Tebuconazole 15	Tebuconazole 50	Tebuconazole 150
Female							
Sample size (n)	10	10			10	10	10
BUN (mg/dL)[3)]	22.5 ± 3.0	23.5 ± 3.5			18.6 ± 1.9*	21.7 ± 4.7	19.1 ± 3.4*
Creatinine (mg/dL)	0.3 ± 0.2	0.5 ± 0.9			0.28 ± 0.0	0.28 ± 0.0	0.28 ± 0.0
Male							
Sample size (n)	10		10	10	10	10	10
BUN (mg/dL)[3)]	19.9 ± 2.9		19.3 ± 2.2	17.6 ± 3.5	17.1 ± 2.0	15.0 ± 1.6*	14.1 ± 4.6**
Creatinine (mg/dL)	0.3 ± 0.1		0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1

1) EE: 17α-ethinyl estradiol
2) TP: Testosterone Propionate
3) BUN: blood urea nitrogen; p-value = * ≤ 0.05, ** ≤ 0.01.
4) Treatment dose: mg/kg/day.

The estrous cycles of individual female rats were evaluated from the day after VO until the end of the study period. Most control rats exhibited regular cycling.

4. Effects on PPS in male rats

For male rats in the control group, mean age and body weight at the time when PPS was first observed were 31.0 days and 123.9 g, respectively. TP significantly advanced the mean age of PPS to 29.0 days and significantly reduced mean body weight at the time of PPS to 110.8 g. Conversely, flutamide significantly delayed the mean age of PPS to 33.1 days and significantly increased mean body weight at the time of PPS to 145.3 g. The mean ages as well as body weights at the time when PPS was first

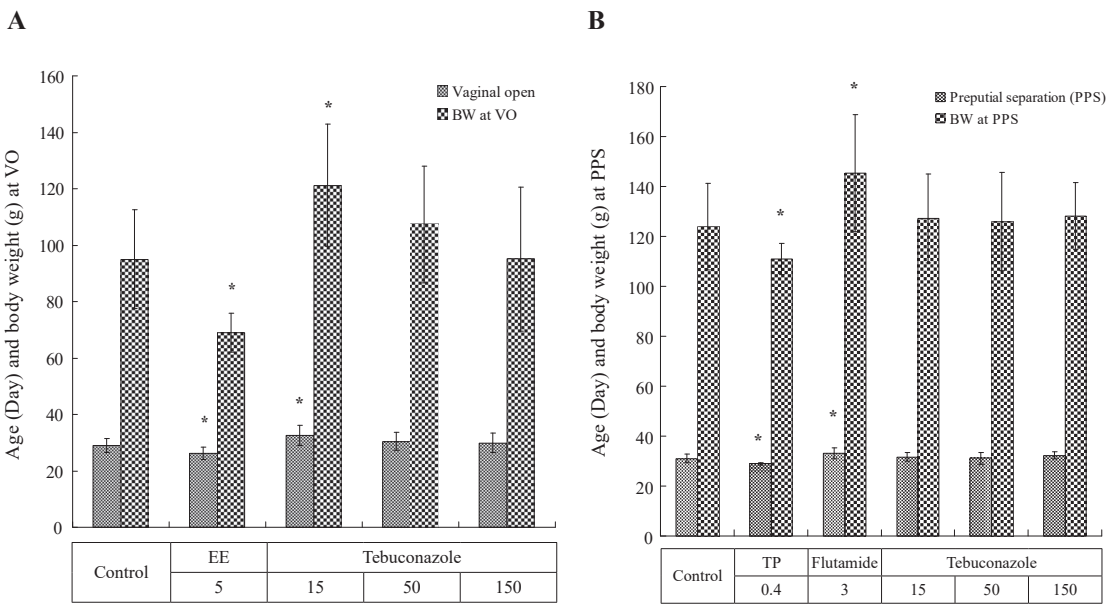

Fig. 10. Mean age and body weight at the time when vaginal opening (VO) was first observed in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A). Mean age and body weight at the time when preputial separation (PPS) was first observed in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B). Female rats were dosed daily from 21 days of age. The body weight of female rats was recorded for 21 days, starting from the day when VO was first observed. Male rats were dosed daily from 22 days of age. The body weight of male rats was recorded for 31 days, starting from the day when PPS was first observed. The * symbol indicates that mean age or mean weight was significantly different from that of the control, $P < 0.05$.

observed in male rats treated with 15, 50, and 150 mg/kg/day tebuconazole were 31.6, 31.2, and 32.3 days as well as 127.3, 125.9, and 128.1 g, respectively, and these values were comparable to those of the controls (Fig. 10B).

5. Effects on serum hormone concentrations in female and male rats

In female rats, EE significantly increased the concentration of serum 17ß-estradiol (E2) by 113%. Tebuconazole significantly decreased the concentration of this hormone by 55%, 45%, and 74% when administered at dosages of 15, 50, and 150 mg/kg/day (Fig. 11), respectively. EE significantly decreased serum luteinizing hormone (LH) (Fig. 13A) and follicular stimulating hormone (FSH) (F. 14A) while tebuconazole did not. EE, however, did not significantly affect serum aromatase activity, while tebuconazole did significantly decrease the activity of this enzyme in a dose-dependent manner (Fig. 15A). Neither EE nor tebuconazole significantly changed the concentrations of serum thyroxine (T4) (Fig. 16A) or triiodothyronine (T3) (Fig. 17A). EE significantly increased the concentration of serum thyroid stimulating hormone (TSH), but tebuconazole did not (Fig. 18A).

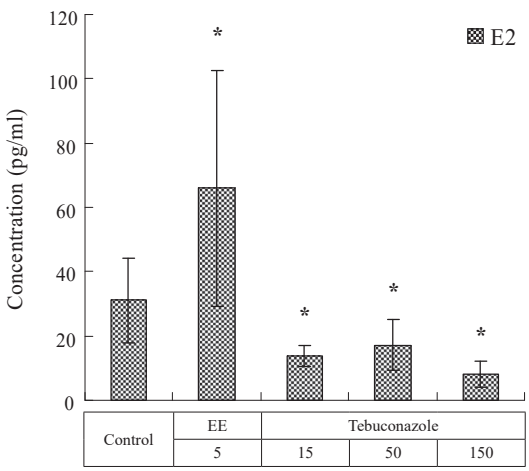

Fig. 11. Concentrations of serum 17β-estradiol (E2) in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). The * symbol indicates that the mean concentration of E2 was significantly different than that of the control, $P < 0.05$.

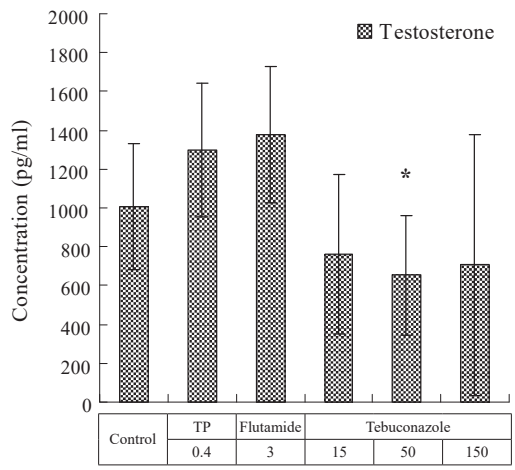

Fig. 12. Concentration of serum testosterone in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150). The * symbol indicates that the mean concentration of testosterone was significantly different than that of the control, $P < 0.05$.

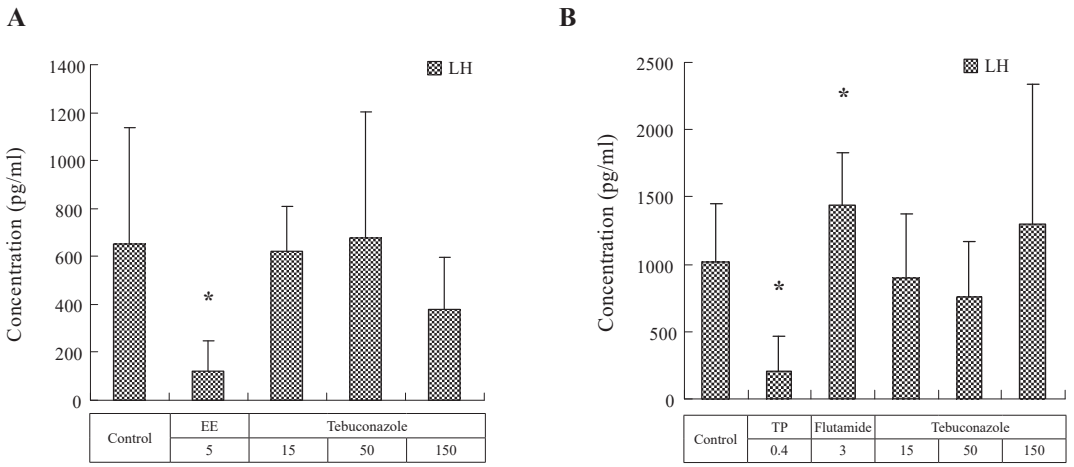

Fig. 13. Concentration of serum luteinizing hormone (LH) in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A). Concentration of LH in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B). The * symbol indicates that the mean concentration of LH was significantly different than that of the control, $P < 0.05$.

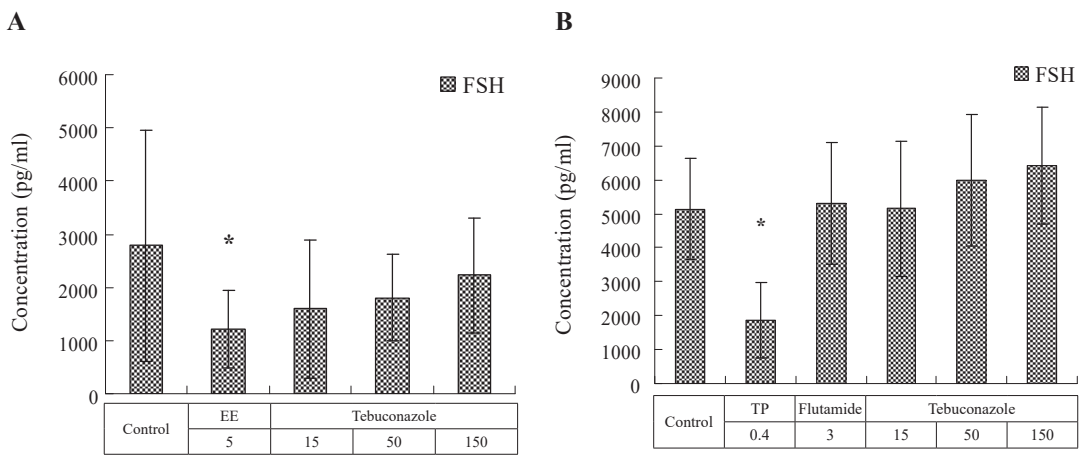

Fig. 14. Concentration of serum follicular stimulating hormone (FSH) in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50 or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A). Concentration of FSH in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B). The * symbol indicates that mean FSH concentration was significantly different than that of the control, $P < 0.05$.

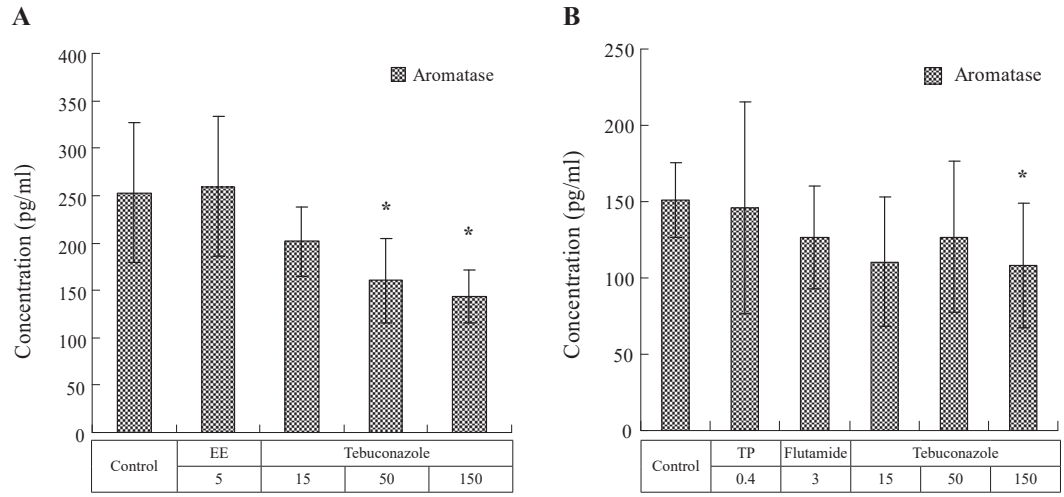

Fig. 15. Serum aromatase activity treated in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A) Serum aromatase activity in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B). The * symbol indicates that serum aromatase activity was significantly different than that of the control, $P < 0.05$.

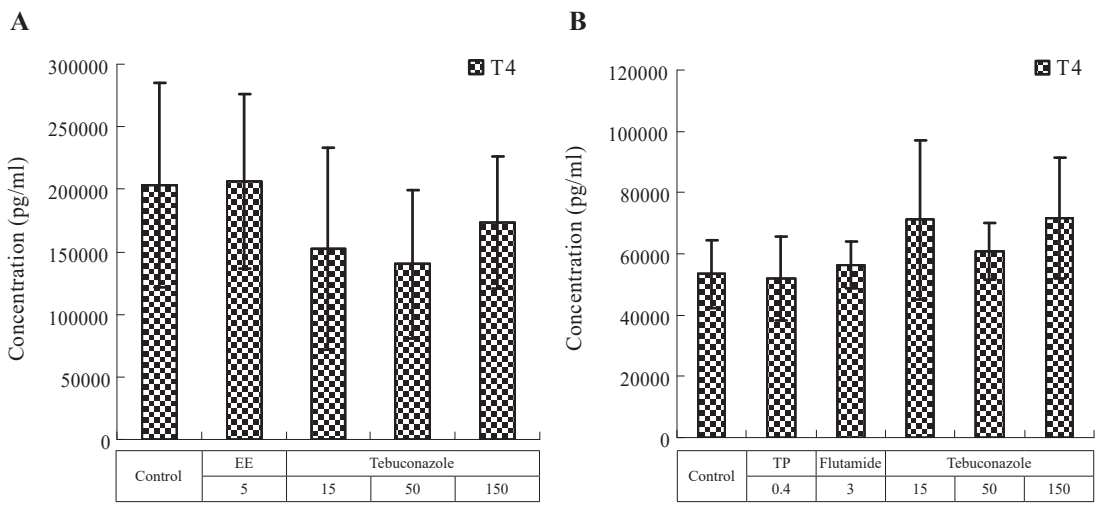

Fig. 16. Concentration of serum thyroxine (T4) in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A). Concentration of T4 in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B).

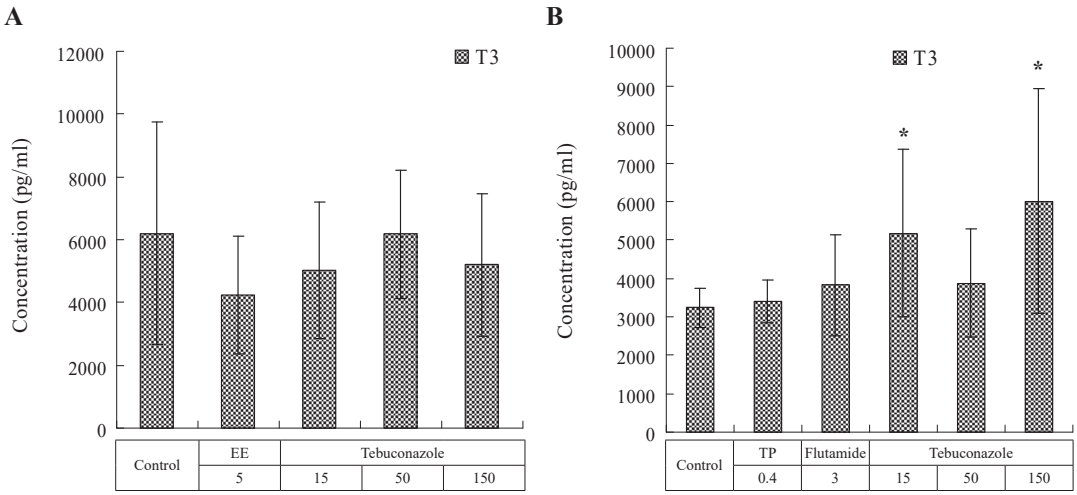

Fig. 17. Concentration of serum triiodothyronine (T3) in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A). Concentration of T3 in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B). The * symbol indicates that the concentration of T3 was significantly different than that of the control, $P < 0.05$.

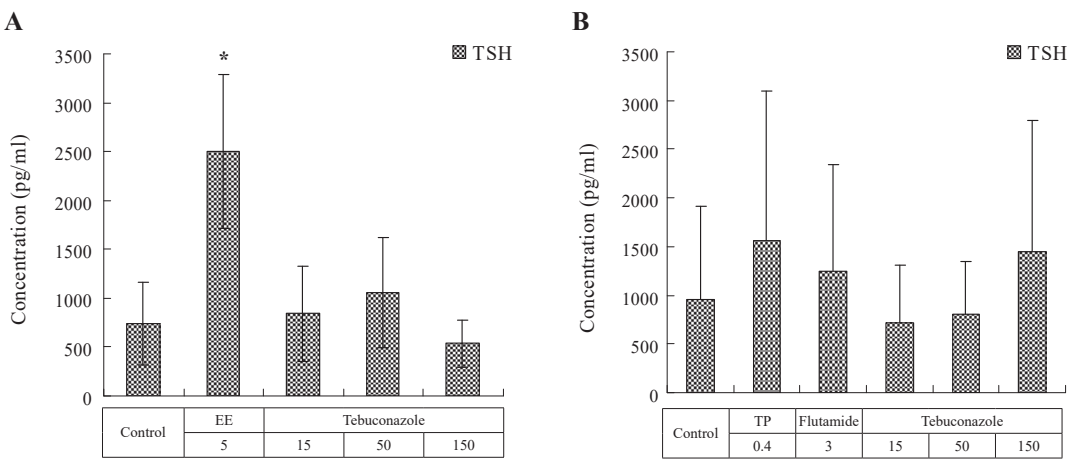

Fig.18. Concentration of serum thyroid stimulating hormone (TSH) in female Wistar rats treated with 5 mg/kg/day 17α-ethinyl estradiol (EE5); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (A). Concentration of TSH in male Wistar rats treated with 0.4 mg/kg/day testosterone propionate (TP 0.4); 3 mg/kg/day flutamide (Flu 3); or 15, 50, or 150 mg/kg/day tebuconazole (Tebu 15, Tebu 50, and Tebu 150) (B). The * symbol indicates that the concentration of TSH was significantly different than that of the control, $P < 0.05$.

For male rats, flutamide significantly increased the concentration of serum testosterone, while tebuconazole decreased it when administered at a dosage level of 50 mg/kg/day (Fig. 12). TP significantly decreased the concentrations of serum LH (Fig. 13B) and serum FSH (Fig. 14B); however, flutamide significantly increased the concentrations of these serums, while tebuconazole did not affect them. Neither TP nor flutamide affected serum aromatase activity, while tebuconazole decreased the activity of this enzyme when administered at a dosage level of 150 mg/kg/day (Fig. 15B). TP, flutamide, and tebuconazole did not affect the concentrations of serum T4 (Fig. 16B), T3 (Fig. 17B), or TSH (Fig. 18B); however, 15 and 150 mg/kg/day tebuconazole increased the concentration of serum T3.

Discussion

This study investigated whether tebuconazole inhibits aromatase activity, thereby disturbing thyroid-related functions in pubertal male and female rats. We concluded the following. Tebuconazole induced slight changes in the ovaries, testes, epididymides, seminal vesicles, bladders, and LABCs of female and male pubertal rats. Furthermore, tebuconazole slightly delayed VO in female pubertal rats. However, the most notable effects that were associated with tebuconazole were significant decreases in serum aromatase activity and 17ß-estradiol (E2) concentration. Tebuconazole was also found to slightly increase serum T3 concentrations in pubertal male rats.

Reproductive and developmental toxicity are known to disrupt endocrine activity, but do not equal endocrine disrupting activity. Based on the endocrine disrupting activity that we observed for tebuconazole, we can infer that tebuconazole disrupted aromatase and led to a reduction of E2 in female pubertal rats. This finding supports a previous report that investigated neurobehavioral deficits and neuropathologies which were induced in adult rats following perinatal exposure to tebuconazole [33]. Our finding may also support a previous study which reported on (1) virilization in female rat offspring by increasing anogenital distance (AGD) at postnatal day (PND) 0 and (2) feminizing effects in male offspring by increasing nipple retention. The effects observed in that study may have been induced by a reduced testosterone concentration in fetal male rats [44]. That study also reported that tebuconazole increased testicular concentrations of progesterone and 17α-hydroxyprogesterone in male rat fetuses, which suggests that tebuconazole directly impacts the steroid synthesis pathway in Leydig cells [44]. In the current research, we found that the decreases in serum aromatase activity and 17ß-estradiol (E2) concentration induced by tebuconazole were also related to the report of high-frequency of post-implantation loss [45]. In female rats, the delay in VO onset induced by tebuconazole was

comparable to the delay in VO onset induced by epoxiconazole [6] (i.e., the delays induced by both tebuconazole and epoxiconazole were slight).

We did not measure parameters related to androgen receptors (ARs) in this study; however, tissues from the following organs were found to be affected by chemical treatments in pubertal male rats: testis, epididymis, seminal vesicle, bladder, and LABC. Moreover, a previous report showed that certain triazoles, including tebuconazole, exhibited dose-dependent antiandrogenic effects in human ARs [28]. Another report further confirmed that certain triazoles, including econazole, epoxiconazole, propiconazole, tebuconazole, and vinclozolin, were potent antiandrogenic disruptors [3]. There was also an earlier report postulating that the mechanism which underlies the anti-androgenic effects of certain triazoles, such as tebuconazole, involve an inhibition of testosterone-induced AR activation, which in turn leads to decreases in testosterone secretion in murine Leydig cells [40]. Triazoles of different sizes and functional moieties have distinct anti-androgenic activities in human ARs. Tebuconazole, which has shown the greatest potential to disrupt human ARs, has a chemical structure which includes a side chain with a tertiary butyl and hydroxyl group [28].

In the present study, we confirmed that tebuconazole can decrease aromatase activity (CYP19, which is the enzyme responsible for converting testosterone to estrogen) in pubertal male and female rats. Linking to the possible anti-androgenic activity as above the androgenic disrupting effects may be also induced indirectly to due to the interference of enzymatic activities and function of steroidogenic CYP450s is related to the biosynthetic pathways of endogenous steroid hormones or " cross-talk" pathways relevant to AR signaling [30, 41]. Steroidogenic CYP enzymes control steroidogenesis and are able to induce changes in steroid hormone concentrations. Some triazoles possess the ability to inhibit the activity of CYP19, thereby disturbing normal steroid hormone synthesis [51]. Some triazoles have also been reported to disturb the enzymatic activity of CYP19 in human placental microsomes [42] or in rainbow trout ovarian microsomes [32]. Moreover, the inhibition of both testosterone-induced AR activation and testosterone secretion in murine Leydig cells, tebuconazole was reported to disrupt steroidogenesis in Xenopus laevis by suppressing the activity of CYP17, even at low dosage levels [28]. Therefore, there are likely multiple mechanisms which underlie the anti-androgenic effects of triazoles.

In performing a thyroid function and pubertal developmental study to investigate reproductive and developmental toxicity, we did not measure typical parameters. Our finding that tebuconazole induced slight disturbances

in the ovary, testis, epididymis, seminal vesicle, bladder, and LABC in female and male pubertal rats suggests that this chemical is linked to reproductive and developmental toxicity. The aromatase inhibitor letrozole is known to delay sexual maturation in both sexes [37] and is also known to cause a dose-dependent increase in post-implantation loss of embryos [48,50]. Furthermore, some triazoles have been reported to induced reproductive and developmental toxicity. These include 1,2,4-triazol-5-one (NTO) [27], itraconazole [7, 49], fluconazole [15, 29, 47], propiconazole [4, 53], 3-amino-5-mercapto-1,2,4-triazole [23], efinaconazole [17], triadimefon [39], flusilazole [14], and paclobutrazol [5]. Conversely, it also been reported that some triazoles, such as mefentrifluconazole [46] and ultraviolet absorber 2-(3',5'-di-tert-butyl-2'-hydroxyphenyl)-5-chlorobenzotriazole, do not induce developmental toxicity [8, 9]. Findings from the current study have confirmed that tebuconazole can disturb the activity of aromatase, lead to a reduction in serum E2 concentration, and induce slight reproductive and developmental toxicity. Nonetheless, the mechanisms which underlie aromatase disruption as well as reproductive and developmental toxicity need to be more comprehensively elucidated.

Acknowledgements

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得克利干擾青春期大鼠血清中環化酶與降低雌素二醇濃度

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摘要

呂水淵、陳敏貞、廖婧淳、蔡韙任。2019。得克利干擾青春期大鼠血清中環化酶與降低雌素二醇濃度。臺灣農藥科學　7：81-110。

三唑類被全球廣泛應用於水果穀類蔬菜及花等作物，同時也被用於人類生藥治療疾病。得克利曾被報導結合其他內分泌干擾農藥如依普座、鋅錳乃浦、撲克拉及撲滅寧等誘發劑量相加性的傷害，如分娩子代死亡、性別分化異常及干擾 kisspeptin 神經等。得克利對大鼠青春期發育與甲狀腺功能影響仍未知，本研究乃利用美國環保署第一階段檢測疑似內分泌干擾青春期發育與甲狀腺功能影響評估指引進行，使用藥劑與劑量包括炔雌醇 (5 mg/kg/day, 17α-ethinyl estradiol)、得克利 (15, 50 及 150 mg/kg/day) (雌大鼠)及睪固酮 (0.4 mg/kg/day, testosterone)、氟他胺 (3 mg/kg/day, flutamide)、得克利 (15, 50 及 150 mg/kg/day) (雄大鼠)。投予時間分別為大鼠出生後第 22 至 42 天 (雌) 與 23 至 53 天 (雄)。試驗結果顯示，得克利未改變青春期雌、雄大鼠器官絕對重、血清中尿素氮及肌酸肝濃度。相反地，得克利高劑量 150 mg/kg/day 降低青春期雌大鼠子宮與卵巢重。得克利高劑量增加青春期雄大鼠睪丸與陰莖寬度但降低前列腺、儲精囊(含與不含液)、膀胱、球海棉體肌與提睪肌等相對重。得克利在低劑量延遲陰道開啓年齡及增加陰道開啓時體重。在血清中荷爾蒙濃度影響方面，得克利降低雌大鼠血清中雌素二醇濃度但對雌雄大鼠血清中睪固酮、黃體生成素及激濾泡素等無明顯影響，得克利顯著降低雌、雄大鼠血清中環化酶活性。此外，除在 15 與 150 mg/kg/day 劑量增加雄大鼠血清中三碘甲狀腺素濃度外，大致而言，得克利對雌、雄大鼠血清中甲狀腺素、三碘甲狀腺素及激甲狀腺素濃度無明顯影響。綜上結果，本試驗推論得克利主要干擾青春期雌、雄大鼠環化酶活性但

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詳細作用機制仍有待進一步探討。

關鍵詞：甲狀腺素、得克利、陰道開啓、陰莖與包皮分離、發身大鼠