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Effects of pubertal exposure to thiazole-Zn on thyroid function and development in female rats

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ABSTRACT

Thiazole-Zn is a newly chinese-created systemic fungicide, and belongs to the sort of thiadiazole compounds, some of which have been found to be thyroid disrupters. To determine the probable adverse effects of thiazole-Zn on thyroid gland and development function, the rodent 20-day Pubertal Female Assay in this study was used. Postnatal days (PND) 22-old Sprague–Dawley rats were administered with thiazole-Zn daily by oral gavage at doses 0, 40, 100, 200 mg/kg/day for 20 days. The thyroid endpoints and development endpoints were assessed. The results indicated that serum TT4 and TSH levels were significantly increased at all concentrations, serum TT3 levels were significantly reduced only at 40 mg/kg thiazole-Zn, but had no difference from controls at the other doses. Thyroid histology was significantly altered at all doses with a clear dose-dependent hypertrophy and hyperplasia of thyroid cell. No histological changes were observed in any of the other observed organs. In addition, this study also found that ovarian weights were significantly decreased, but age and weight at vaginal opening (VO), serum E2 levels were unaffected in all treatment groups. These results demonstrate that thiazole-Zn is likely a thyroid disrupter, but did not demonstrate that it has estrogenic/anti-estrogenic activity.

1. Introduction

Thiazole-Zn, [bis (2-amino-5-sulfhydryl-1,3,4-thiadiazole) Zn], which is a type of thiadiazole fungicide with high efficiency and low toxicity, is a Chinese-created systemic fungicide that has been used to control bacterial and fungal diseases. Thiazole-Zn has two active groups that show fungicidal effects. One group is a thiazole group, which has a strong inhibiting effect on bacteria inside plants, but does not cause inhibition outside of plants. The active ingredient of thiazole-Zn taken up by plants causes severe damage to bacteria and induces the cell membrane of bacteria thinning out to die. The other group is Zn^{2+}, which exchanges with positive ions, such as H^+ and K^+ on the cell surface of disease-producing germs. Some zinc ions cause the protein of cell membranes to curdle and kill germs, and other zinc ions penetrate cells of disease-producing germs and result in enginery dysfunction by combining with some types of enzymes of germs. Under the combined action of these two active groups, thiazole-Zn can cause antifungal and antibacterial effects on different targets (Wei et al., 2008).

The acute oral LD50 for rat was greater than 5000 mg/kg/day, and acute dermal LD50 for rat was greater than 2000 mg/kg/day. There were no skin and eye irritability in rabbits. The allergic test on the skin of the guinea pigs showed it has weak allergic reaction. Genotoxicity test of 95% thiazole-Zn indicated that it had no mutagenic and teratogenic action. The data from these tests showed that the toxicity of thiazole-Zn was low. Since thiazole-Zn has a broad spectrum of activity and low toxicity, it is used widely on vegetables, rice and fruit trees for more than 60 types of bacterial and fungal diseases in China. The structure of thiazole-Zn shows a close relationship to N,N-methylene-bis(2-amino-1,3,4-thiadiazole) (Bis-A-TDA) and bismerthiazol [*N,N*-methylene-bis (2-amino-5-sulfhydryl-1,3,4-thiadiazole)], which belong to the thiadiazole derivatives and have been confirmed as thyroid hormone disruptors (Fig 1). Bis-A-TDA has been banned in China because of its strong teratogenicity and thyroid toxicity (Gu and Qian, 1991; Xu and Gao, 1991). Bismerthiazol, which is a Chinese-created systemic fungicide that has been used to control bacterial leaf blight, Cercospora leaf spot on rice and canker on orange, for more than 20 years, can induce thyroid cell hypertrophy and hyperplasia (Xiong et al., 2008; Zhang et al., 2009). Therefore, we speculate that thiazole-Zn could have specific toxic effects on the thyroid gland. Nevertheless, little information on the toxicity of thiazole-Zn is available in the published literature.

In the present study, we used the pubertal female rat assay to determine the effect of thiazole-Zn on pubertal development and

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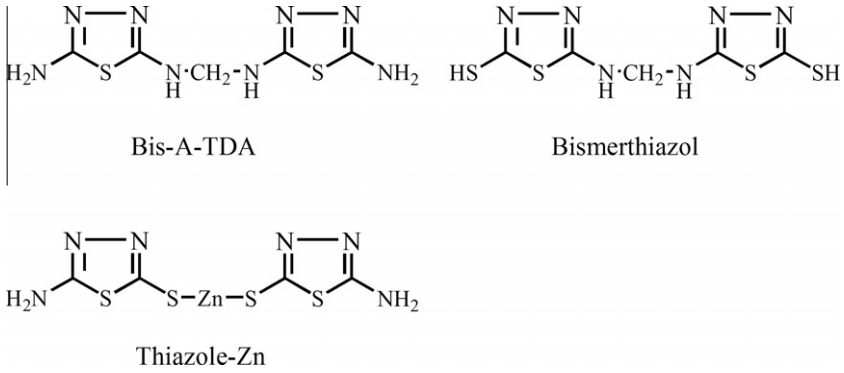

Fig 1. Structures of Bis-A-TDA, bismerthiazol and thiazole-Zn.

thyroid function in the intact juvenile female rat (US EPA 2009b, 2010a). We examined the effect of thiazole-Zn on several thyroid endpoints (serum hormone concentrations, thyroid gland weight and thyroid gland histopathology). Furthermore, the pubertal female assay was also used to examine the endpoints associated with the development of female sex organs and secondary sexual characteristics, and vaginal opening (VO) was used as an indicator of pubertal development.

2. Materials and methods

2.1. Chemicals

Thiazole-Zn (purity of 95%) was provided by Dr. Li Lin, the pharmaceutical college of Hebei Medical University. It was well-mixed and well-distributed in corn oil prior to and throughout dosing.

2.2. Animals

Sprague–Dawley female rats were obtained from Weitong Lihua Experimental Animal Central (Beijing, China) Laboratory Animal Ltd. under specific pathogen-free conditions. Dams with litters of 20-day-old female rats were received and housed in clear polycarbonate cages for 2 days prior to the start of dosing. Prior to the experiment, all animals were checked for overt signs of illness, and only healthy animals were selected for the study. All animals were maintained under a 14-h light, 10-h dark cycle. Ambient air temperature was controlled at 23 ± 2 °C, and relative humidity was maintained at 50 ± 10%. Food and tap water were provided ad libitum. On PND 21, litters that were as homogeneous as possible were selected for the study by eliminating the largest and smallest of the pups with a range of 8 g above or below the mean. The pups were then randomized to different treatment groups in accordance with their body weight. All animals, including the control group used in this experiment, were handled in an accredited Association for Assessment and Accreditation of Laboratory Animal Care facility.

2.3. Experimental protocol

The study design consisted of 15 animals per group. In the present study, the highest dose level was chosen as the dose to reduce the terminal body weight no more than 10% of the mean for the controls. No clinical signs of toxicity associated with the dose level were observed in a preliminary pilot study. The following treatment groups were administered thiazole-Zn daily by oral gavage from PND 22 for 20 days: vehicle control, and 40, 100 and 200 mg/kg body weight. The test compound was prepared once 3 days before treatment. Test chemicals were administered in 5.0 ml corn oil/body weight at 9.00–11.00 daily for each treatment.

2.4. Clinical signs of toxicity and body weight

Throughout the study period, each animal was observed at least once daily for clinical signs of toxicity related to chemical treatment. The body weight of each rat was recorded daily to the nearest 0.1 g and was measured just daily prior to treatment.

2.5. Vaginal opening

Each animal was examined daily for VO, beginning on PND 22. On the day that VO was first detected, age and body weight were recorded.

2.6. Hormonal measurements

Blood samples were collected from the abdominal vein approximately 24 h after the last treatment of test compounds. Serum was prepared immediately and stored at −20 °C until later analysis for serum hormone levels. Serum Thyroid Stimulating Hormone (TSH) concentrations were assayed by commercial kit employing the quantitative sandwich enzyme immunoassay technique (Beijing Biotopped Science&Technology CO., Ltd., China). This assay kit was specific to assay the level of rat TSH in the sample of rat's serum, blood plasma. Primary antibody specific for rat TSH provided by Santa Cruz biotechnology compony (Santa Cruz, USA) has been pre-coated onto a microplate. Standards were serially diluted with standard diluent for the standard curves. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for TSH. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TSH bound in the initial step. The color development is stopped and the intensity of the color is measured under 450 nm wavelength within 10 min. Serum total T4, T3 and E2 concentrations were measured according to the ELISA kits specific for rat (Beijing Biotopped Science&Technology CO., Ltd, China). TT4, TT3, TSH and E2 assay sensitivity were of 0.4 ng/ml, 30 pg/ml, 0.08 mIU/L and 1 ng/ml, respectively. The inter- and intra assay coefficients of variation were less than 8% and 6% for all hormones measured.

2.7. Measurement of organ weights and histopathological examination

Twenty-four hours after the last treatment, rats were anesthetized with CO_2. The thyroid gland, liver, kidney, pituitary, adrenals, uterus and ovaries were dissected and weighed from rats immediately. The thyroid gland, kidney, uterus and ovaries were then fixed in 10% buffered formalin for at least 5 days. Each tissue was processed in an automatic issue processor and embedded in paraffin. Thin sections were cut at a thickness of 4–5 mm, and stained with hematoxylin and eosin for pathological evaluation under a microscope.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation (SD). All statistical analyses were performed using SPSS 15.0 for Windows. Data for mean initial or necropsy body weights, organ weights and hormone levels were statistically analyzed for homogeneity of variance using Levene's test. If homogeneous, the data were analyzed by one-way analysis of variance (ANOVA). When samples were proven to be heterogeneous, nonparametric ANOVA was applied. When a significant treatment effect was present, Dunnett's test was used to compare treatment groups. The level of statistical significance was set a priori at $\alpha = 0.05$.

3. Results

3.1. Clinical signs and body weight

During the study period, there were no clinical signs of toxicity in any treatment group. Significant decreases in mean body weight gains were observed in rats treated with 100 and 200 mg/kg of thiazole-Zn at the interval of 36–42 days of age (93.6% and 90.3% of controls, respectively) (Fig 2), but these reductions in necropsy body weight were not greater than approximately 10% of the mean for controls (Table 2).