Atrazine and the Hypothalamo-Pituitary-Gonadal Axis in Sexually Maturing Precocial Birds

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Summary and Implications

The herbicide atrazine is a putative endocrine disruptor. The present studies investigated the effects of atrazine in male Japanese quail during sexual maturation. Atrazine was administered for 2 weeks in the diet or systemically to birds under long photoperiods. Atrazine had no effect on mortality but depressed both feed intake and growth (average daily gain [ADG] in g/day) at dietary concentrations of 1000 ppm. Atrazine in the diet at 10 ppm, but at no other concentrations, increased testes weight and gonadal-somatic-index and decreased the seminiferous tubule diameter-to-testis weight ratio. Atrazine in the diet at 1000 ppm increased circulating concentrations of testosterone. Dietary atrazine at 10 ppm increased circulating concentrations of estradiol. Atrazine administered systemically exerted no effect on indices of growth or reproduction. Atrazine did not mimic the effects of either estradiol or tamoxifen in male quail; thus, atrazine did not exhibit overt estrogenic or anti-estrogenic activity. It is concluded that atrazine up to 1000 ppm in the diet may exert some effects on reproductive development in sexually maturing male birds, but these are inconsistent and modest.

Introduction

There is increasing concern that chemicals in the environment may be endocrine disruptors. Endocrine disruptors are defined as exogenous chemicals or mixtures that alter the function of the endocrine system, causing adverse effects at the level of the organism, progeny, population or subpopulation. These compounds exhibit estrogenic, anti-estrogenic, androgenic or anti-androgenic activities and/or may affect thyroid, immune and cognitive function. The extensively used herbicide, atrazine, has come under scrutiny as a putative endocrine disruptor.

Low concentrations of atrazine have been reported to feminize amphibians. This feminization is unlikely to be caused by direct activation of the estrogen receptor (ER) but may be attributed to activation of gonadal aromatase. Moreover, plasma concentrations of testosterone are reduced by atrazine in exposed frogs.

Atrazine exhibits low acute toxicity to birds with a dietary LC_{50} of > 5000 ppm. To date, there is little information describing the effect of atrazine on avian reproduction. There are no reports describing the effects of atrazine on reproduction in male birds. In the temperate zone, birds may be exposed to atrazine during the spring field application period. At this time, increases in the natural photoperiod induce sexual maturation of birds. The present studies examine the effects of atrazine on the hypothalamo-pituitary-gonadal (HPG) axis in precocial birds, modeled by the male Japanese quail, during photostimulated gonadal maturation (1).

Materials and Methods

Animals and Procedure

Japanese quail (*Coturnix coturnix Japonica*) were reared from hatch in battery cages under a short daily photoperiod (8L:16D) with free access to feed (Purina Game Bird Chow; Purina Mills LLC, St. Louis, MO) and water. Males were separated from females based on plumage, transferred to individual cages and the treatments initiated. At the start of the treatments, the photoperiod was changed to 16L:8D to induce sexual maturation. All dietary treatments were directly mixed in Purina Game Bird Chow; feed and water were available *ad libitum*.

In each study, male quail were weighed and randomly assigned to treatment groups. The birds were fed dietary treatments or controls *ad libitum* (unless noted) for 2 weeks. They were then re-weighed and blood samples collected after decapitation. Plasma was separated by centrifugation (2500 x *g*, 10 min, 4°C) and stored at -20°C until analysis.

Experimental Treatments

In study 1, 6-week-old male quail (n = 12 per treatment) were administered atrazine at 0, 10, 100 or 1,000 ppm in the diet. In study 2, four-week-old quail were administered 0 or 100 ppm atrazine (n = 11 per treatment) or 1000 ppm atrazine (n = 13) in the diet. In study 1, a positive control of 17β-estradiol (E_2) (Sigma-Aldrich, St. Louis, MO) was administered at 100 ppm (n = 12).

Study 3 investigated the effect of atrazine on growth and sexual maturation in 6-week old birds with an additional group of pair-fed birds (n = 7 per treatment). Birds were assigned to dietary treatment groups of 0 or 1000 ppm atrazine.

In study 4, atrazine was administered in 0.1 ml of propylene glycol daily at 1 and 10 mg/kg through sc injections in the nape of the neck (n = 8 per treatment). In study 5, atrazine was administered through Silastic implants (1.47 mm ID, 1.96 mm OD, 1.5 cm length; Dow-Corning, Midland, MI) (n = 8 per treatment).

Studies 6 and 7 examined the putative anti-estrogenic effects of atrazine on the HPG axis in 4-week old male quail. In study 6, treatments of 100 ppm tamoxifen (an anti-estrogen,

Sigma-Aldrich), 100 ppm E_2, 1000 ppm atrazine and combinations of 100 ppm E_2 + 100 ppm tamoxifen and 100 ppm E_2 + 1000 ppm atrazine were administered in the diet for 2 weeks (n = 8 per treatment group).

Hormone Analysis

Plasma concentrations of estradiol and testosterone were determined in duplicate using an ELISA from DRG International, Inc. (Mountainside, NJ). For estradiol and testosterone, the intra-assay coefficient of variation (CV) was 4.1 and 0.7%, respectively, with all samples within a study analyzed in one assay. Plasma concentrations of LH were determined in duplicate and the intra- and inter-assay CV were 8.3 and 10.9%, respectively.

Histologic and Morphometric Analysis

The left testis was fixed in Bouin's solution (Sigma-Aldrich) and transferred to 70% ethanol for storage. The tissues were embedded in paraffin and sectioned at 4 µm at two different levels within the tissue.

Ten seminiferous tubules representative of the tissue were examined and classified (two sections per sample, 5 tubules per section). Tubule diameters were measured with an ocular micrometer and the qualitative presence of a lumen was evaluated.

Statistics

All data were analyzed by the MIXED procedure using SAS version 9.0 (SAS Institute, Cary, NC). Histological data from studies 1, 2, 3 and 7 (classification, tubule diameter, tubule:testis weight ratio and lumen presence) were averaged across an individual sample, analyzed by two-way ANOVA for differences between studies (treatment and study). Where significance was found, the least-squared means were separated by the Dunnett-Hsu t-test. In all studies, values are reported as least-squared mean ± pooled SEM (calculated from MSE). Significance was determined at $p < 0.05$.

Results and Discussion

General Toxicity

In the seven studies, only four instances of mortality were observed with atrazine or the positive and negative controls. Concentrations of atrazine up to 1000 ppm in the diet had no effect on mortality in sexually maturing male Japanese quail. Atrazine at 1000 ppm decreased growth and feed intake by 32 and 15%, respectively, versus control ($p < 0.001$), but had no effect on liver weights or the liver-somatic index. In contrast, estradiol (100 ppm) reduced growth ($p < 0.05$) and feed intake 21% each, respectively, versus control ($p < 0.001$). Moreover, estradiol increased liver weights and liver-somatic index 50 and 58%, respectively, versus control ($p < 0.001$).

Sexual Development and Circulating Hormones

Atrazine up to 1000 ppm had no effect on testes weight or the ratio of the left-to-right testis weight. However, atrazine at 10 ppm increased testes weight 46% ($p < 0.01$) and gonadal-somatic index 49% versus control ($p < 0.01$). In contrast, estradiol at 100 ppm decreased testes weight and gonadal-somatic index by 92 and 91%, respectively, versus control ($p < 0.001$). Atrazine up to 1000 ppm had no effect on circulating concentrations of LH, but increased circulating concentrations of testosterone 3.0-fold versus control ($p < 0.01$).

Atrazine and Feed Restriction

The effects of atrazine administered in the diet were compared using pair-fed birds. Atrazine at 1000 ppm reduced growth by 63.6% versus control ($p < 0.05$), but it had no effect on liver weights or liver-somatic index versus control.

Systemic Atrazine

Atrazine administered at 1 and 10 mg/kg/day through sc injections had no effect on indices of growth or reproductive development. In contrast, sc injections of estradiol-benzoate decreased both testes weights and gonadal-somatic index by 46.8 and 42.9%, respectively, versus control ($p < 0.05$).

Atrazine administered by Silastic implant (~1.42 mg/kg/day) had no effect on indices of growth and reproductive development. In contrast, the estrogen control delivered by Silastic implant increased liver weights and liver-somatic index 44.4 and 50.0%, respectively, versus control ($p < 0.001$).

Testis Histology and Morphometric Analysis

Atrazine and pair-feeding had no effect on the stage of development of the testes (class), lumen size or seminiferous tubule diameter. However, atrazine at 10 ppm decreased the tubule diameter-to-testis weight ratio 33.3% versus control ($p < 0.05$).

Effects of Atrazine in the Presence of Reproductive Steroids

As expected, atrazine decreased growth and feed intake 31.6 and 14.1%, respectively, versus control ($p < 0.05$). Concurrent administration of estradiol (100 ppm) and atrazine (1000 ppm) in the diet decreased growth and feed intake 63 and 27%, respectively, versus control ($p < 0.05$). Addition of tamoxifen (an anti-estrogen) to the diet marginally inhibited the deleterious effects of estradiol on growth, feed intake and liver-somatic index.

In study 7, the effects of atrazine on growth in the presence of systemically administered testosterone were examined. Addition of atrazine (1000 ppm) to the diet or the presence of a testosterone implant did not influence growth, feed intake or liver weights versus control. However, concurrent administration of atrazine (1000 ppm) and systemic testosterone decreased growth 42.1% versus control ($p < 0.05$).

Atrazine and the Hypothalamo-Pituitary-Gonadal Axis

Administration of estradiol in the diet decreased testes weight 84% versus control ($p < 0.05$). Concurrent administration of atrazine and estradiol in the diet decreased testes weight 96% versus the negative control ($p < 0.05$).

In study 7, atrazine at 1000 ppm in the diet had no effect on testes weight but reduced circulating concentrations of LH 35.7% versus control ($p < 0.05$). Addition of testosterone in the form of subdermal implants decreased testes weight and circulating concentrations of LH 91 and 69%, respectively, versus control.

Atrazine is a triazine herbicide that inhibits photosynthesis in plants. It is widely used in the United States with up to 36,000 metric tons applied per year. It has low acute toxicity to birds and mammals.

Birds in the temperate zone undergo rapid sexual maturation due to photostimulation in the spring. At this time, they may be particularly sensitive to the effects of putative toxicants. The combination of the photoperiodic threshold and these similarities in gonadotropic surge suggest our system is a reasonable model for a springtime effect.

It is well documented that, at the threshold photoperiod, the Japanese quail exhibits a sharp increase in circulating concentrations of gonadotropin. These hormones induce the rapid development of the gonads, testes weight increasing logarithmically for the first half of development. The results presented support the low general toxicity of atrazine. There was no effect of atrazine on mortality.

Atrazine exhibited an additive toxic effect on growth and feed intake with exogenous estradiol. Furthermore in one study, atrazine alone had no effect on growth or feed intake but in the presence of testosterone decreased growth without a change in feed consumption.

In the present studies, atrazine exhibited only modest effects on the HPG axis of the sexually maturing bird. In male quail, circulating concentrations of estradiol are extremely low and as such, the male may be expected to be highly sensitive to changes in concentrations of estrogen.

Male quail receiving atrazine in the diet at 1000 ppm exhibited an increase in circulating concentrations of testosterone. Atrazine increased testes weight and the gonadal-somatic index in quail receiving atrazine at 10 ppm in the diet, and decreased the seminiferous tubule-to-testis weight ratio.

In Japanese quail, as predominantly in other birds, testosterone exerts a negative-feedback effect at the level of the hypothalamus following aromatization to estradiol. The present results demonstrate that atrazine at concentrations at or above 10 ppm in the diet exhibit few and inconsistent effects on the reproductive system in the sexually maturing male Japanese quail. It is unlikely that the changes observed would have a profound effect on sexual development or reproduction of male birds in the wild.