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Atrazine Effects on Testosterone Levels and Androgen-Dependent Reproductive Organs in Peripubertal Male Rats

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ABSTRACT: Previous studies have reported that atrazine, a widely used herbicide that selectively inhibits photosynthesis in broadleaf and grassy weeds, has adverse effects on reproductive function in the male, suggesting a direct effect of atrazine on the hypothalamic-pituitary-testicular axis. As yet, however, no studies have critically examined the doses of atrazine that elicit such effects, and few have focused on the mechanism by which atrazine acts. Herein we report a dose-response study of the effects of atrazine ingestion on reproductive function in male Sprague-Dawley rats during a critical developmental period, the peripubertal period. Atrazine was administered by gavage to rats from day 22 to day 47 of age, at doses of 1–200 mg/kg body weight per day. Atrazine administration of up to 50 mg/kg per day had no effect on any of the measured variables. Serum testosterone concentration was reduced by atrazine at doses of 100 and 200 mg/kg per day, as were seminal vesicle and ventral prostate weights. Intratesticular testosterone concentration was reduced in parallel with serum testosterone, suggesting that the reductions in serum testosterone resulted from reduced testosterone production by Leydig cells or from changes in testosterone metabolism within the testis, or both. Serum luteinizing hormone (LH) concentration was reduced despite the reduced serum testosterone, suggesting an effect on the hypothalamus, the pituitary gland, or both. At the termination of the study, the average body weight of rats receiving atrazine at 100 mg/kg per day was found to be reduced by approximately 9%. This suggested the possibility that the effects of atrazine on the reproductive tract may not be direct, but rather, the noted deficits of the male reproductive tract resulted from reduced food intake by the treated rats. We tested this by feeding control (vehicle-gavaged) rats amounts of food equivalent to that consumed by the atrazine-fed rats, and then assessing reproductive tract endpoints. Even mild food restriction resulted in reductions in serum testosterone concentration, in the weights of androgen-dependent organs, and in serum LH concentration; the same deficits that were seen in atrazine-gavaged rats. Indeed, the effects of atrazine on the male reproductive tract seen in rats receiving atrazine at greater than 50 mg/kg per day could not be distinguished from the effects of reduced food consumption. These results suggest that caution must be exercised before concluding that atrazine (or any potentially toxic chemical) has direct, detrimental effects.

Key words: Herbicide, male reproduction, hypothalamic-pituitary-testicular axis, peripubertal period.

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A number of reports have suggested that atrazine, a widely used herbicide that selectively inhibits photosynthesis in broadleaf and grassy weeds, may have adverse effects on reproductive function in both females and males. In female adults, dietary exposure to atrazine has been reported to produce a lengthening of the estrous cycle, reduction of estradiol-induced uterine weight gain, reduced uterine cytosolic progesterone receptor binding, or early onset of mammary and pituitary tumors (Eldridge et al, 1994a, 1994b; Simic et al, 1994; Tennant et al, 1994; Wetzel et al, 1994; Connor et al, 1996; Cooper et al, 1996; Stevens et al, 1999). In males, Kniewald and his colleagues have reported that atrazine exposure may result in suppression of the conversion of testosterone to 5α-dihydrotestosterone (DHT) in the hypothalamus, anterior pituitary, and prostate; reduced pituitary and prostate weights; reduction of DHT binding to the androgen receptor; and reduced number and motility of spermatozoa (Kniewald et al, 1979, 1987, 1995, 2000; Babic-Gojmerac et al, 1989; Simic et al, 1991). As yet, however, few studies have critically examined the doses of atrazine that elicit such effects, or have focused on the mechanism by which atrazine acts.

Herein we report a dose-response study of the effects of atrazine treatment on Sprague-Dawley rats during a critical developmental period of the male, the peripubertal period (postnatal days 22–48). We show that orally administered atrazine at doses of 100 and 200 mg/kg per day produced a delay in preputial separation, significant reductions in serum testosterone and luteinizing hormone (LH) concentrations, and significant reductions in the weights of androgen-dependent organs. However, we not-

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ed that at the termination of the study, the average body weight of rats receiving atrazine at 100 mg/kg per day was reduced by approximately 9%. This suggested the possibility that the effects of atrazine on the reproductive tract may not be direct, but rather that deficits of the male reproductive tract resulted from reduced food intake. We tested this by feeding rats amounts of food that were equivalent to that consumed by the atrazine-fed rats, and then assessing reproductive tract endpoints. Food restriction resulted in reductions in serum and intratesticular testosterone concentrations, androgen-dependent organ weights, and serum LH concentration, the same deficits seen in atrazine-gavaged rats. Indeed, the effects of atrazine on the male reproductive tract that were seen in rats receiving atrazine at greater than 50 mg/kg per day could not be distinguished from the effects of reduced food consumption.

Materials and Methods

Chemicals

Atrazine technical, 96.1% purity, was supplied by Novartis, Inc (Greensboro, NC). Atrazine suspensions were prepared in 0.5% carboxymethylcellulose (medium viscosity) by Covance Laboratories (Vienna, Va). The suspensions were stored at 4°C. Testosterone antibody was from ICN Biomedicals, Inc (Costa Mesa, Calif), and tritiated testosterone was from NEN Products (Boston, Mass).

Experimental Design

Dose Response Study—Sprague-Dawley rats of 17–20 days of age were purchased from Harlan (Indianapolis, Ind). After weaning (day 21), the rats were individually caged and received food and water ad libitum on a schedule of 12 hours of light and 12 hours of dark. Rats received atrazine by daily gavage from day 22 to day 47 at doses of 1, 2.5, 5, 10, 25, 50, 100, or 200 mg/kg body weight per day (n = 9–12 rats per dose). Control animals (n = 11) received the same weight-based volume of vehicle (0.5% carboxymethylcellulose). The day of preputial separation was determined for control rats and for rats receiving atrazine at 100 and 200 mg/kg per day. Rats were killed by decapitation 24 hours after the last gavage, at age 48 days. Trunk blood was collected, centrifuged at 3000 × *g* at 4°C for 10 minutes, and then stored at −80°C for subsequent assay. To collect fluid from the interstitial compartment of the testis, the tunica albuginea was perforated at the distal pole of the testis, and fluid was drained by placing the testis in a holder and centrifuging at 54 × *g* at 0°C for 15 minutes (Turner et al, 1984). The collected fluid was stored at −80°C for subsequent assay. Body weight and the weights of the testes, epididymides, ventral prostate, and seminal vesicles (dry weight, with the coagulating gland removed) were recorded for each rat.

Food Restriction Study—As in the dose response study, Sprague-Dawley rats, after weaning, were individually caged. One group of 22-day-old rats (n = 13) received 100 mg/kg atrazine per day, and the amount of food that each rat consumed on a daily basis was measured. A second group of rats (n = 14) received the vehicle by gavage. On each day of the study, each of these rats was fed the mean daily intake of food consumed by the atrazine-administered group on the previous day. Rats in a third group (n = 16) received the vehicle by gavage and were fed ad libitum. Rats were killed by decapitation 24 hours after the last gavage, at age 48 days, and evaluated as described earlier.

All animal protocols were approved by The Johns Hopkins University Institutional Animal Care and Use Committee.

Assays

Testosterone concentration in the serum and interstitial fluid was determined by radioimmunoassay (RIA), as previously reported (Turner et al, 1984). The serum concentration of LH was determined by RIA using a Biotrak assay system (Amersham, Arlington Heights, Ill).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze group means. In cases in which significant differences were found among groups ($P < .05$), individual groups were compared with each other by Fisher's least significant difference test. Means were considered different at $P < .05$.

Results

As seen in Figure 1, serum testosterone concentrations were not significantly different from the control value at atrazine doses of 1–50 mg/kg per day, but were reduced from the control at the 100 and 200 mg/kg per day doses by 34% and 32%, respectively. Reductions in interstitial fluid testosterone concentration (Figure 1) paralleled reductions in serum testosterone concentration; at 100 and 200 mg/kg per day, when serum testosterone concentrations were reduced from the control value, interstitial fluid testosterone concentrations also were significantly reduced, by 41% and 45%, respectively. Interstitial fluid testosterone concentration was not altered at atrazine doses of less than 100 mg/kg per day, as also was the case for serum testosterone.

Reductions in the weights of the ventral prostate and seminal vesicles (Figure 2), both of which are androgen-dependent organs, paralleled reductions in serum testosterone. Thus, at the 100 and 200 mg/kg per day doses, significant reductions in ventral prostate weight were observed, of 26% and 48%, respectively. At these same atrazine doses, significant reductions in seminal vesicle weight, of 32% and 55%, were seen. At lower atrazine doses (1–50 mg/kg per day), the weights of the ventral prostate and seminal vesicles were not different from their respective control values. Preputial separation was delayed by about 3 days in rats receiving atrazine at 100

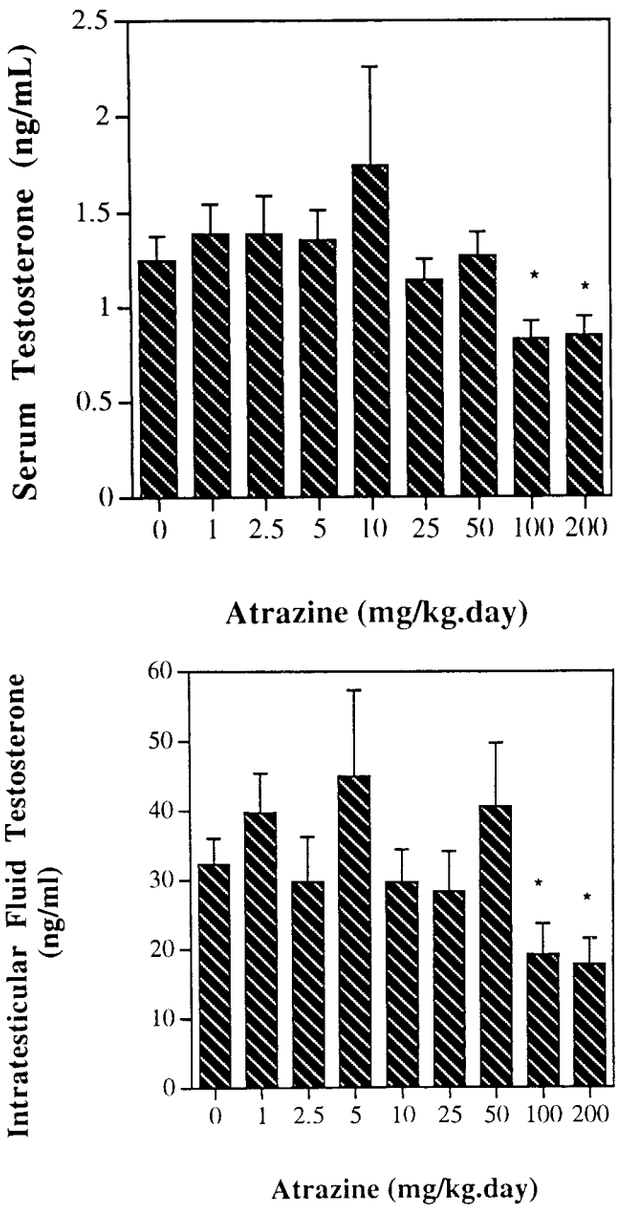

Figure 1. Testosterone concentration in the serum **(above)** and testicular interstitial fluid **(below)** of rats administered atrazine (1–200 mg/kg per day) by gavage. *Significantly different from control (0 mg/kg) values ($P < .05$).

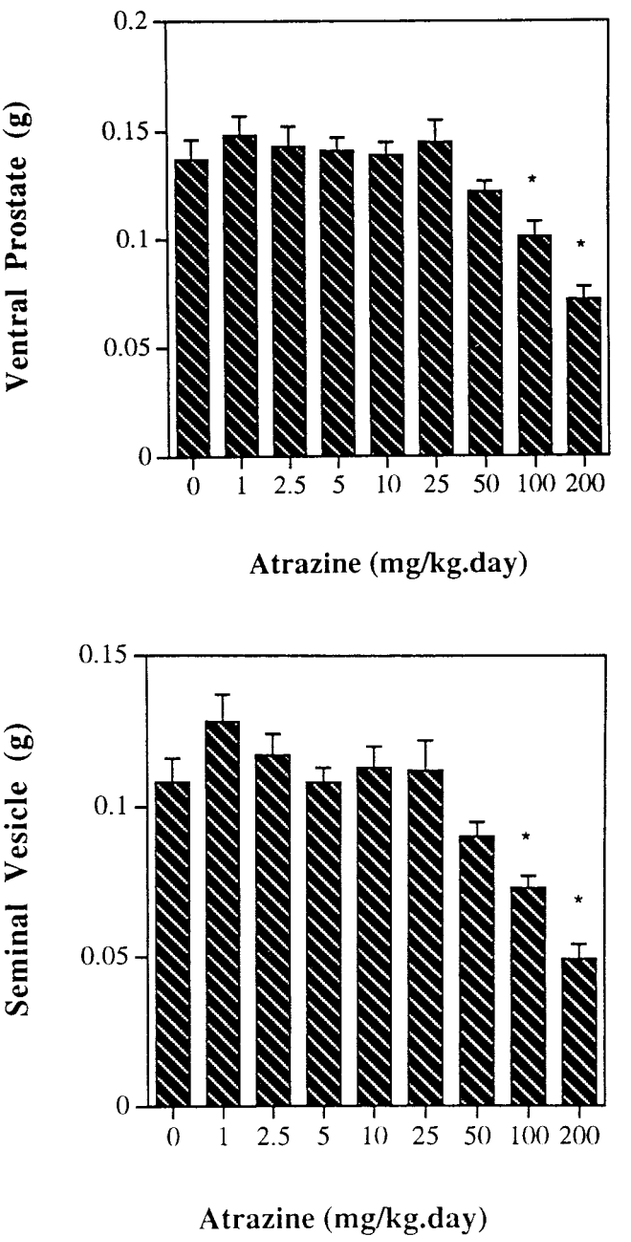

Figure 2. Ventral prostate **(above)** and seminal vesicle **(below)** weights in rats administered atrazine (1–200 mg/kg per day) by gavage. *Significantly different from control (0 mg/kg) values ($P < .05$).

mg/kg per day compared with controls (postnatal day [pnd] 44 vs pnd 41), and by about 4 days in rats receiving 200 mg/kg per day.

Figure 3 shows the effect of atrazine dose on serum levels of LH. Atrazine doses of 100 and 200 mg/kg per day resulted in reductions in serum LH concentration of 17% and 20%, respectively, from the control value. (The reduction in LH concentration reached significance only at the 200 mg/kg per day dose.)

Examination of the body weights of atrazine-treated rats (Figure 4) revealed that rats administered atrazine at a dose of 100 mg/kg per day gained significantly less weight over the course of the treatment than did the vehicle-gavaged (control) rats. At the termination of the experiment, significant reductions in body weights of 9% and 21% were seen at doses of 100 and 200 mg/kg per day, respectively (Figure 4). The body weight reductions suggested the possibility that the effects of atrazine may

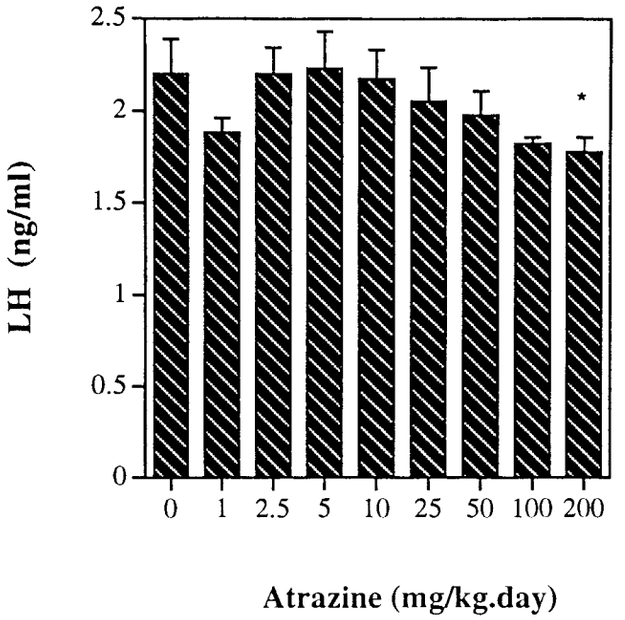

Figure 3. LH concentration in the serum of rats administered atrazine (1–200 mg/kg per day) by gavage. *Significantly different from control (0 mg/kg) value ($P < .05$).

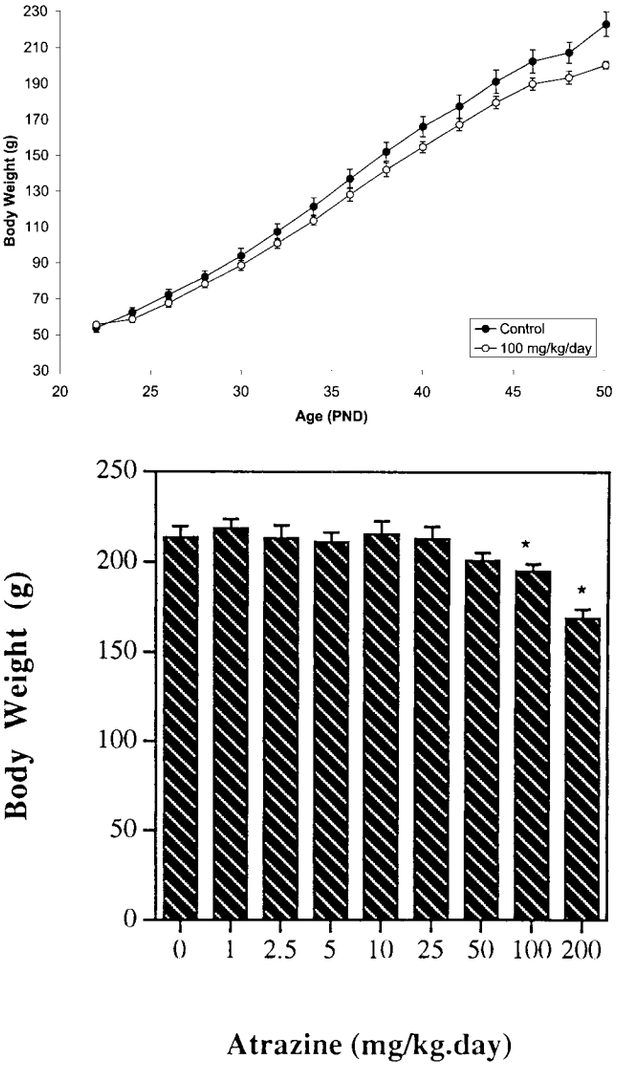

Figure 4. **Above**: Body weights over time of vehicle-treated (control) rats and rats administered atrazine at 100 mg/kg per day by gavage. **Below**: Mean terminal body weights of rats administered atrazine (1–200 mg/kg per day) by gavage. *Significantly different from control (0 mg/kg) value ($P < .05$).

not be direct, but rather result at least in part from food deprivation. To examine this possibility, a second study was designed that involved 3 groups: 1) vehicle-gavaged rats fed ad libitum (controls), 2) rats gavaged with 100 mg/kg atrazine per day, and 3) vehicle-gavaged rats provided each day with the mean daily intake of food consumed by the 100 mg/kg per day group on the previous day. Over the course of the study, rats receiving atrazine at 100 mg/kg per day ate an average of 10% less food per day than the vehicle-gavaged controls (total of 420 g versus 463 g, respectively), and gained less weight (Figure 5). At the termination of the study, the average weight of the vehicle-gavaged rats that had been fed amounts of food comparable to that consumed by the 100 mg/kg per day group also was reduced by about 10% from the ad libitum-fed controls (Figure 5). It is interesting that as in the atrazine-gavaged group, significant reductions in serum concentrations of testosterone and LH (Figure 6), and in the weights of the ventral prostate and seminal vesicles (Figure 7, were seen in the rats in which food had been restricted.

Discussion

We show herein that atrazine, when fed by gavage to peripubertal rats at doses of 100 or 200 mg/kg per day, resulted in reduced serum levels of testosterone in comparison with vehicle-fed controls. The observation of reduced serum testosterone at these doses was consistent with the reduced weight gain of the androgen-responsive seminal vesicles and ventral prostate at the same atrazine doses. The significant reductions in seminal vesicle and ventral prostate weight gain were seen whether the values were expressed as absolute weights of the organs or relative weights (ie, organ weight divided by body weight).

The administration of atrazine at doses that resulted in reduced serum testosterone also produced significant reductions in the weight gain of the rats; rats administered atrazine at 100 mg/kg per day weighed about 9% less than control rats. Although a 9% reduction in weight gain is not dramatic, this, together with the results of previous

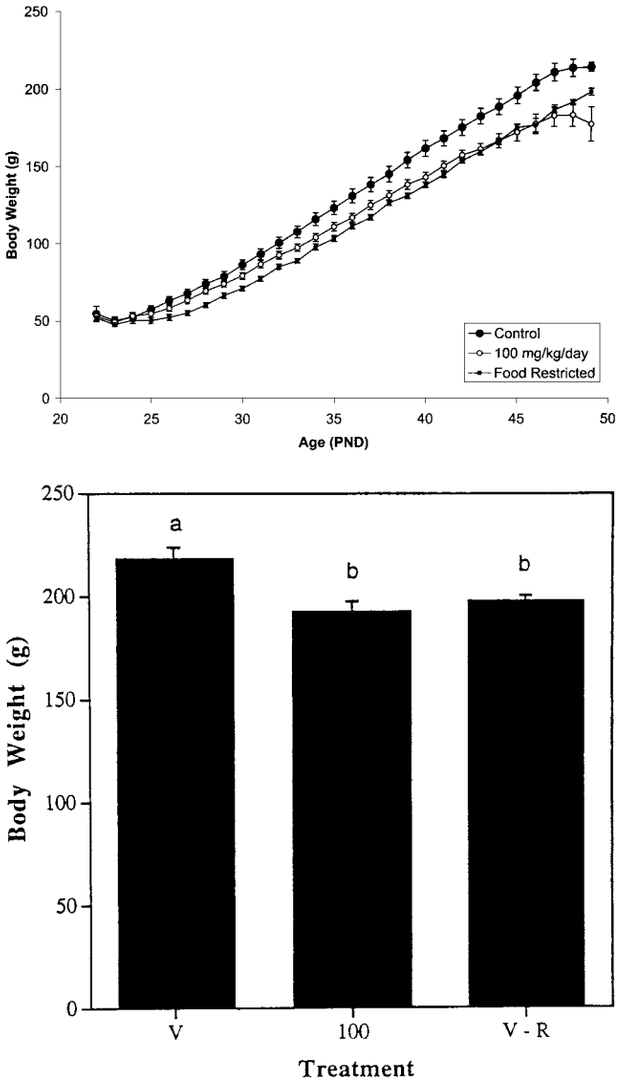

Figure 5. **Above**: Daily body weights of vehicle-treated rats fed ad libitum, rats administered atrazine at 100 kg/mg per day, and vehicle-treated rats restricted to the average daily intake of food consumed by the atrazine-treated group. **Below**: Mean terminal body weights of vehicle-treated rats fed ad libitum (V), rats administered atrazine at 100 mg/kg per day (100), and vehicle-treated rats restricted to the average daily intake of food consumed by the atrazine-treated group (V-R). Different letters over the bars signify significant differences ($P < .05$).

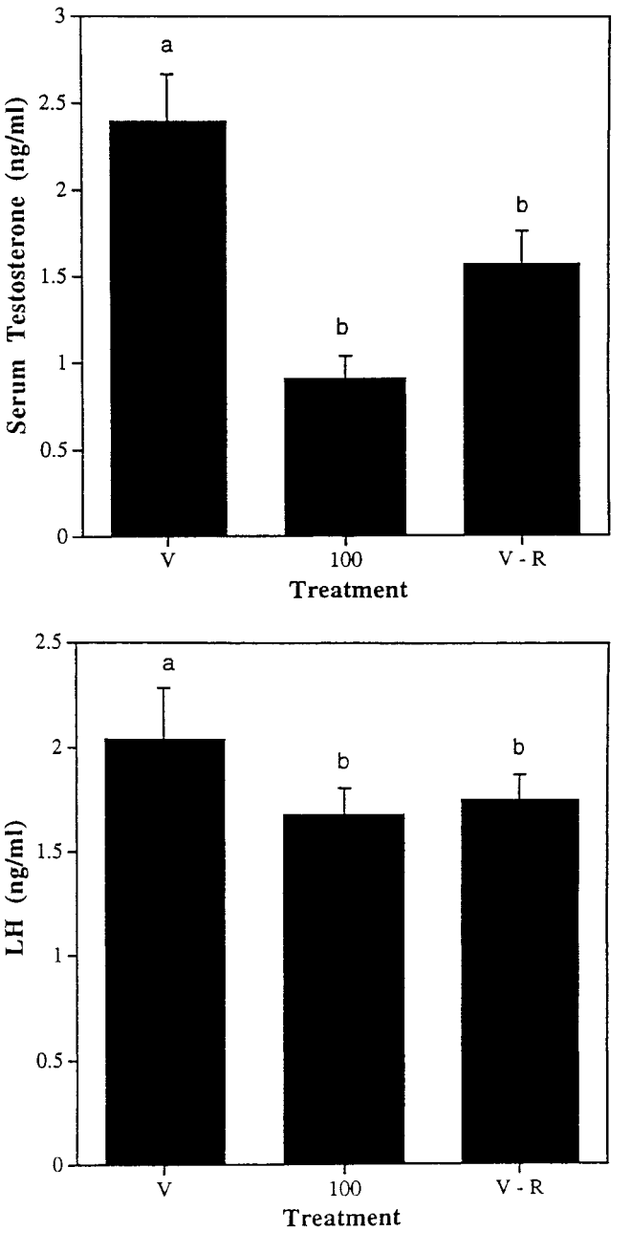

Figure 6. Testosterone **(above)** and LH **(below)** concentrations in the serum of vehicle-treated rats fed ad libitum (V), rats administered atrazine at 100 mg/kg per day (100), and vehicle-treated rats restricted to the average daily intake of food consumed by the atrazine-treated group (V-R). Different letters over the bars signify significant differences ($P < .05$).

studies showing that food deprivation may result in decreased circulating concentrations of testosterone (Chik et al, 1987; Bergendahl et al, 1991; Grizard et al, 1997) led us to examine the possibility that the reduced serum testosterone levels of atrazine-fed rats may result at least in part as a sequelae of reduced food intake. Indeed, in a study designed to compare the effects of food deprivation with the effects of atrazine, we found that the effects seen after atrazine administration could not be distinguished from the effects of reduced food consumption. The observation that there also was a delay in preputial separation suggests that both atrazine and food deprivation may cause delays in pubertal progression, and perhaps, as a consequence, delays in reproductive tract development.

Although the results of the food-deprivation studies suggest that deficits in the male reproductive tract of atrazine-gavaged rats may be partly or even entirely attributable to the effects of atrazine on food consumption, they

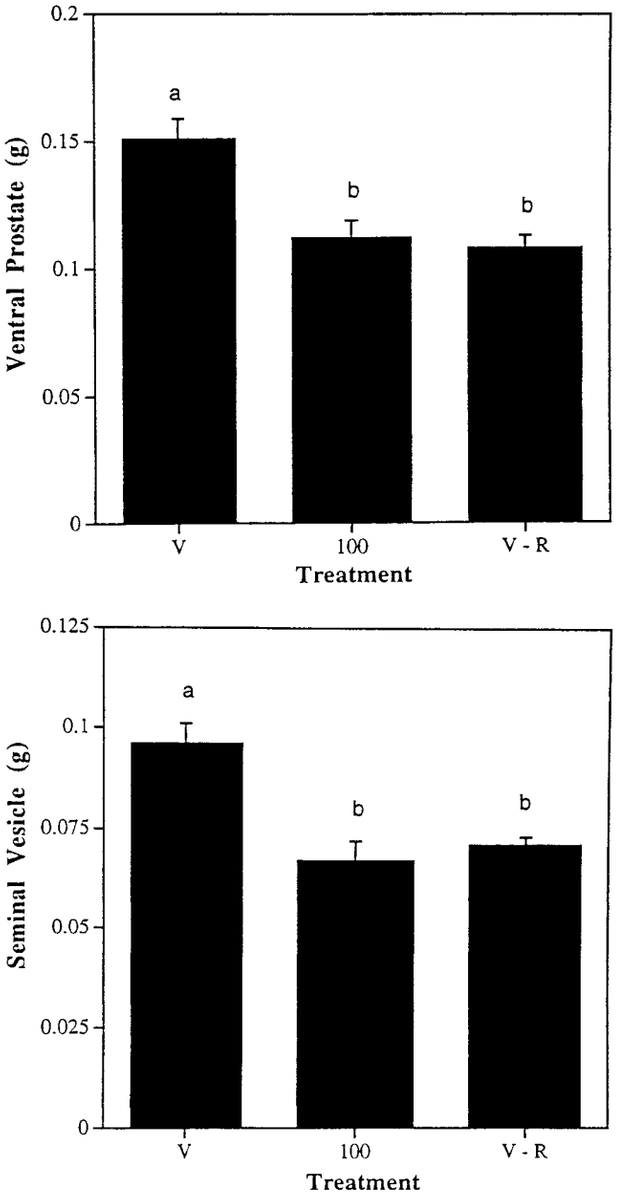

Figure 7. Ventral prostate **(above)** and seminal vesicle **(below)** weights of vehicle-treated rats fed ad libitum (V), rats administered atrazine at 100 mg/kg per day (100), and vehicle-treated rats restricted to the average daily intake of food consumed by the atrazine-treated group. Different letters over the bars signify significant differences ($P < .05$).

do not rule out the possibility that atrazine itself affects the hypothalamic-pituitary-testicular axis of peripubertal male rats. Thus, atrazine and food restriction, although they produce many of the same effects, may do so through different mechanisms. For example, atrazine itself, or a metabolite, may directly affect components of the male reproductive tract, perhaps the Leydig cells, thereby causing diminished levels of serum testosterone.

The doses of atrazine that resulted in diminished serum testosterone concentration also resulted in diminished intratesticular (interstitial fluid) testosterone concentration, suggesting a Leydig cell deficiency of some kind. Two possibilities may pertain: atrazine treatment may result in a reduced number of Leydig cells, or it may result in the reduced ability of individual Leydig cells to produce testosterone. If the latter was the case, one may hypothesize that atrazine produces a deficit at the level of the hypothalamic-pituitary axis. With this in mind, we measured serum LH levels in atrazine-treated and food-restricted rats in comparison with controls. We found that LH levels were reduced at atrazine concentrations of 100–200 mg/kg per day (or after food deprivation), suggesting a primary effect on the hypothalamus, pituitary, or both, with secondary effects on Leydig cells. This conclusion is consistent with the results of previous studies reporting that atrazine affects pituitary function (Chapin et al, 1996; Cooper et al, 1999), and that food deprivation may result in decreased circulating levels of gonadotropins (Campbell et al, 1977). Another possibility is that atrazine treatment may affect testosterone metabolism within the testes, and not testosterone production itself. This is under study at present in our laboratory.

These results taken together indicate that atrazine administration of up to 50 mg/kg per day had no effect on the hypothalamic-pituitary-testicular axis, and that at levels greater than 50 mg/kg per day, the effects seen after atrazine could not be distinguished from the effects of reduced food consumption. It is clear from these studies that caution must be exercised before concluding that atrazine (or any potentially toxic chemical) has direct, detrimental effects. Indeed, the results argue that for any chemical, body weight must be both monitored and accounted for in the assessment of potential toxic effects.

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References

Babic-Gojmerac T, Kniewald Z, Kniewald J. Testosterone metabolism in neuroendocrine organs in male rats under atrazine and deethylatrazine influence. *J Steroid Biochem.* 1989;33:141–146.

Bergendahl M, Perheentupa A, Huhtaniemi I. Starvation-induced suppression of pituitary-testicular function in rats is reversed by pulsatile gonadotropin-releasing hormone substitution. *Biol Reprod.* 1991;44: 413–419.

Campbell GA, Kurcz M, Marshall S, Meites J. Effect of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin: response to LH-releasing

hormone and thyrotropin-releasing hormone. *Endocrinology*. 1977; 100:580–587.

Chapin RE, Stevens JT, Hughes CL, Kelce WR, Hess, RA, Daston GP. Endocrine modulation of reproduction. *Fundam Appl Toxicol.* 1996; 29:1–17.

Chik CL, Ho AK, Brown GM. Effect of food restriction on 24-h serum and pineal melatonin content in male rats. *Acta Endocrinol (Copenh).* 1987;115:507–513.

Connor K, Howell J, Chen I, Liu H, Berhane K, Sciarretta C, Safe S, Zacharewski T. Failure of chloro-s-triazine-derived compounds to induce estrogen receptor-mediated responses in vivo and in vitro. *Fundam Appl Toxicol.* 1996;30:93–101.

Cooper RL, Goldman JM, Stoker TE. Neuroendocrine and reproductive effects of contemporary-use pesticides. *Toxicol Ind Health.* 1999;15: 26–36.

Cooper RL, Stoker T, Goldman J, Parrish M, Tyrey L. Effect of atrazine on ovarian function in the rat. *Reprod Toxicol.* 1996;10:257–264.

Eldridge J, Fleenor-Heyser D, Extrom P, Wetzel L, Breckenridge C, Gillis J, Luempert L, Stevens J. Short-term effects of chloroatrazines on estrus in female Sprague-Dawley and Fischer 344 rats. *J Toxicol Environ Health.* 1994a;43:155–167.

Eldridge J, Tennant M, Wetzel L, Breckenridge C, Stevens J. Factors affecting mammary tumor incidence in chloroatrazine-treated female rats: hormonal properties, dosage, and animal strain. *Environ Health Perspect.* 1994b;102:29–36.

Grizard G, Artonne C, Grizard J, Boucher D. Effect of short-term starvation on Leydig cell function in adult rats. *Arch Androl.* 1997;38: 207–214.

Kniewald J, Jakominic M, Tomljenovic A, Simic B, Romac P, Vranesic D, Kniewald Z. Disorders of male reproductive tract under the influence of atrazine. *J Appl Toxicol.* 2000;20:61–68.

Kniewald J, Mildner P, Kniewald Z. Effects of s-triazine herbicides on hormone-receptor complex formation, 5alpha-reductase and 3alpha-hydroxysteriod dehydrogenase activity at the anterior pituitary level. *J Steroid Biochem.* 1979;11:833–838.

Kniewald J, Osredecki V, Gojermac T, Zechner V, Kniewald Z. Effect of s-triazine compounds on testosterone metabolism in the rat prostate. *J Appl Toxicol.* 1995;15:215–218.

Kniewald J, Peruzovic M, Gojmerac T, Milkovic K, Kniewald Z. Indirect influence of s-triazines on rat gonadotropic mechanism at early postnatal period. *J Steroid Biochem.* 1987;27:1095–1100.

Simic B, Kniewald J, Kniewald Z. Effects of atrazine on reproductive performance in the rat. *J Appl Toxicol.* 1994;14:401–404.

Simic B, Kniewald Z, Davies JE, Kniewald J. Reversibility of the inhibitory effect of atrazine and lindane on cytosol 5alpha-dihydrotestosterone receptor complex formation in rat prostate. *Bull Environ Contam Toxicol.* 1991;46:92–99.

Stevens JT, Breckenridge CB, Wetzel L, Thakur AK, Liu C, Werner C, Luempert LG, Eldridge JC. A risk characterization for atrazine: oncogenicity profile. *J Toxicol Environ Health.* 1999;56:69–109.

Tennant M, Hill D, Eldridge J, Wetzel L, Breckenridge C, Stevens J. Possible antiestrogenic properties of chloro-s-triazines in rat uterus. *J Toxicol Environ Health.* 1994;43:183–196.

Turner TT, Jones CE, Howards SS, Ewing LL, Zegeye B, Gunsalus GL. On the androgen microenvironment of maturing spermatozoa. *Endocrinology.* 1984;115:1925–1932.

Wetzel L, Luempert L, Breckenridge C, Tisdel M, Stevens J, Thakur A, Extrom P, Eldridge J. Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague Dawley and Fischer 344 rats. *J Toxicol Environ Health.* 1994;43:169–182.