

Changes in serum concentration of 17 β -estradiol in female rats during estrous cycle after treatment with atrazine and zearalenone

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ABSTRACT: A daily dose of 14 mg atrazine and 2.5 mg zearalenone, given *p.o.* during 5 days of estrous cycle to female rats, changed their estrous cycle in comparison with control animals. On day –1 of expected estrus, a significantly lower ($p < 0.05$) concentration of 17 β -estradiol compared with the control group was recorded in all experimental groups of animals. In the group of animals administered zearalenone, the concentration of 17 β -estradiol on the day of expected estrus was significantly higher ($p < 0.05$). In the group administered a combination of atrazine and zearalenone, the concentration of 17 β -estradiol on the day after expected estrus was significantly higher ($p < 0.05$) than in the control group. In the group of animals receiving atrazine, complete absence of the onset of estrous cycle was recorded, whereas in the group given zearalenone the onset of estrous cycle was delayed by 24 hours. The combination of atrazine and zearalenone induced similar effects as atrazine, however, with the onset of estrous cycle being delayed by 48 hours. Neither of these two groups of animals reached the level of 17 β -estradiol recorded in the control group. *For free full paper in pdf format see*
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Keywords: atrazine; zearalenone; estrous cycle; 17 β -estradiol; serum; rat

INTRODUCTION

Atrazine is an s-triazine herbicide that is mostly used as a weed-killer in corn fields. After several-year use, atrazine residues were found in the soil (Goh *et al.*, 1993), surface waters (Hrlec, 1990), ground water (Graham, 1991) and drinking water (Gojmerac *et al.*, 1994, 1996). Besides the soil and waters, atrazine residues were also detected in feed, mostly corn (Norris and Fong, 1983).

Zearalenone is a mycotoxin produced by molds of the genus *Fusarium* during the plant growth in the field. Contamination of cereals with *Fusarium* spp. is quite common in Croatia, ranging from 20% (Munk and Topolko, 1978) to as much as 82% (Kralj *et al.*, 1988), depending on weather conditions. Atrazine and zearalenone enter the animal body with feed and water, and usually cause reproductive disorders in female animals.

Swine is the animal species most susceptible to the toxic effects of zearalenone and atrazine, in which these agents cause vulvovaginitis with persistent estrus, possible vaginal and rectal prolapse, and abortion in pregnant animals (Mirocha *et al.*, 1971; Chang *et al.*, 1979; Blaney *et al.*, 1984). Atrazine, on the other hand, induces anestrus, as it was found to significantly increase the concentration of progesterone and decrease the concentration of 17 β -estradiol in serum when given in feed in a

dose of 2 mg/kg body weight (b.w.) for 19 days (Gojmerac *et al.*, 1996). Similar disorders were also observed in female rats (Becci *et al.*, 1982), however, with higher active concentrations than in swine (Kuiper-Goodman *et al.*, 1987). Atrazine in uterine cytosol of female rats was found to prolong the estrous cycle of animals by protracting the period of diestrus (Šimić *et al.*, 1994). Zearalenone induces characteristic changes with persistent estrus (Kumagai and Simizu, 1982), has gonadotoxic and teratogenic effects (Barna and Gyürü, 1990), and affects fertility in female rats due to reproductive disorders (Ruzsas *et al.*, 1979).

MATERIAL AND METHODS

Animals

One hundred and twenty Sprague-Dawley sexually mature female rats aged 90 days, body weight ~200 g, were used in the study. During the study, the animals were appropriately housed at a temperature of 21°C, relative humidity of 56%, and 12-hour light/dark cycles. The animals were fed commercial chow for laboratory rats (4 RF 21, Muscedola s.r.l., Settimo Milanese, Italy), and had access to water *ad libitum*.

The animals were divided into four groups of 30 rats. Group 1 animals were administered atrazine, group 2 zearalenone, group 3 atrazine and zearalenone, and group 4 served as a control group.

Chemicals

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s triazine) with 97.5% atrazine (Herbos, Sisak, Croatia) was admixed to liquid paraffin (Kemika, Zagreb, Croatia) used as a vehicle. There were 27.8 mg/ml of working substance. Animals received 0.5 ml of active liquid paraffin suspension or 13.9 mg atrazine/animal/day *p.o.*, i.e. 6.95 mg atrazine/100 g b.w. per day.

Zearalenone [6-(10-hydroxy-6-oxo-trans-1-undecenyl) β -resorcylic acid lactone] is a desiccant (Sigma, St. Louis, USA) in crystalline form. Total amount of the toxic substance was dissolved in 1.0 ml 96% ethyl alcohol and admixed to liquid paraffin used as a vehicle. Working suspension contained 5 mg zearalenone per milliliter. Animals were administered 0.5 ml of working suspension *p.o.* with a probe, i.e. 2.5 mg zearalenone per day or 1.25 mg/100 g b.w.

Tests

During the first four days of the 10-day study period, estrus synchronization was performed, i.e. the stage of estrus was determined by vaginal swab microscopy in each animal (Weihe, 1987), so that animals in the same estrous stage were allocated to the same group. The experiment started on day 0, i.e. on the day the animals were in estrus, the next estrus being expected on day 5 of the study. Ten animals from each group were sacrificed at the end of day 4, 5 and 6 of the study (at 9.00–10.00 a.m.), and blood was collected for hormone analysis. Blood sampling was performed by exsanguination upon decapitation in ether anesthesia. Blood samples were refrigerated overnight, then they were centrifuged at $3\,000\times g$ for 30 min, and serum was stored at -25°C until analysis. The level of 17β -estradiol was determined in blood serum pool by time-resolved fluoroimmunoassay using Delfia[®] commercial sets (Wallac Oy, Turku, Finland).

Statistical data processing was performed by use of the STATISTICA for Windows software, Release 4.3 (Stat-Soft Inc., 1993).

RESULTS

Results of the hormone tests performed in pooled serum of the studied animals are presented in Table 1 and Figure 1.

DISCUSSION

Hormone concentrations measured in rat serum on days 4, 5 and 6 of intoxication were compared with the control group values which, according to the results of previous studies, were within the expected limits (Yoshinaga *et al.*, 1969; Brown-Grant *et al.*, 1970). Pronounced differences were observed for 17β -estradiol on day 4, when the concentrations of 46.65, 23.63, 21.22 and 21.77 pg/ml were recorded in the control, atrazine, zearalenone and atrazine + zearalenone group of animals, respectively. In all experimental groups, the difference from the control group was statistically significant at a level of $p < 0.05$. Thus, the onset of estrus was not recorded in any of the groups on day 4. The animals administered atrazine had even lower levels of 17β -estradiol on days 5 and 6. The level of 17β -estradiol increased on day 5 in the group of animals given zearalenone, and on day 6 in the animals administered a combination of atrazine and zearalenone.

The onset of the next estrus in the atrazine treated animals could not be determined due to the short study period. The concentration of 17β -estradiol failed to reach maximal values in the animals administered zearalenone as well as in those administered atrazine and zearalenone, however, an increase did occur during continuation of the study.

The animals administered atrazine exhibited complete absence of proestrus and estrus, with a prolonged period of diestrus. The mean serum level of 17β -estradiol showed a steady decrease, while the ovaries failed to show the characteristic hormonal activity after only 4 days of atrazine intoxication.

Table 1. 17β -estradiol levels on days 4, 5 and 6 according to the groups of animals

17β -estradiol (pg/ml)	No. of animals (n/group)	A (mean \pm SE)	Z (mean \pm SE)	A + Z (mean \pm SE)	Control group (mean \pm SE)
Day 4	10	23.63 \pm 3.92	21.22 \pm 3.36	21.77 \pm 4.02	46.65 \pm 3.31
Day 5	10	18.99 \pm 1.89	34.41 \pm 5.03	22.77 \pm 0.61	21.79 \pm 0.77
Day 6	10	13.17 \pm 1.28	16.13 \pm 1.37	30.22 \pm 3.29	23.37 \pm 1.19

A = atrazine; Z = zearalenone; A + Z = atrazine + zearalenone

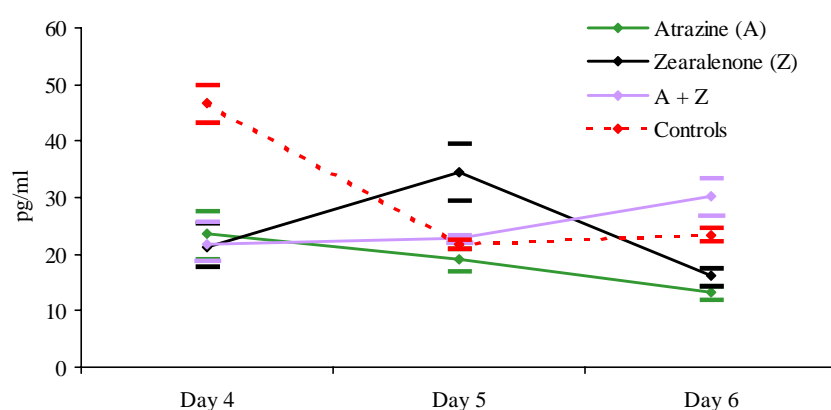


Figure 1. Graphic presentation of 17 β -estradiol levels on days 4, 5 and 6 according to groups of animals

The animals administered zearalenone exhibited the signs of estrus (as assessed by vaginal swab microscopy), however, the corresponding pattern of 17 β -estradiol concentration in the circulation was absent on day –1 of expected estrus, or an elevated concentration was recorded 24 h later, yet not reaching the control group level. The shape of the curve of 17 β -estradiol concentration shows the peak increase to have occurred with a 24-h delay, i.e. the 17 β -estradiol secretion to be prolonged by one day, however, the 17 β -estradiol level declined on day 6 of intoxication again.

The group of animals administered a combination of atrazine and zearalenone showed a similar pattern of 17 β -estradiol concentration as the atrazine treated group, with an increase in the hormone concentration observed 48 h after its increase in the control group. Thus, the curve of the measured 17 β -estradiol concentrations paralleled the one recorded in the control group with a 48-h delay and lower intensity, being recorded at the control group level on day 4.

REFERENCES

- Becci P.J., Johnson W.D., Hess F.G., Gallo M.A., Parent R.A., Taylor J.M. (1982): Combined two generation reproduction teratogenesis study of zearalenone in the rat. *J. Appl. Toxicol.*, 2, 201–206.
- Blaney B.J., Bloomfield R.C., Moore C.J. (1984): Zearalenone intoxication of pigs. *Aust. Vet. J.*, 62, 24–26.
- Barna J., Gyürü F. (1990): The effect of mouldy corn on the genetic background of reproduction performance and on the feeding parameters in the rats. *Mag. Allatorv. Lapja*, 45, 603–608.
- Brown-Grant K., Exley D., Naftolin F. (1970): Peripheral plasma oestradiol and luteinizing hormone concentrations during the oestrous cycle of the rat. *J. Endocrinol.*, 48, 295–296.
- Chang K., Kurtz H.J., Mirocha C.J. (1979): Effects of the mycotoxin zearalenone on swine reproduction. *Am. J. Vet. Res.*, 40, 1260–1267.
- Goh K.S., Weaver D.J., Hsu J., Richman S.J., Trau D., Barry T.A. (1993): ELISA regulatory application: compliance of simazine and atrazine in California soils. *Bull. Environ. Contam. Toxicol.*, 51, 333–340.
- Gojmerac T., Kartal B., Žurić M., Zidar V. (1994): Primjena imunoenzimne metode (ELISA) u određivanju ostataka s-triazinskog herbicida atrazina u vodi za piće. *Vet. Stanica*, 25, 75–80.
- Gojmerac T., Kartal B., Bilandžić N., Roić B., Rajković-Janje R. (1996): Seasonal atrazine contamination of drinking water in pig-breeding farm surroundings in agricultural and industrial areas of Croatia. *Bull. Environ. Toxicol.*, 56, 225–230.
- Graham J.A. (1991): Monitoring ground water and well water for crop protection chemicals. *Analyt. Chem.*, 63, 613–622.
- Hrlec G. (1990): Ograničenja u primjeni herbicida. *Glasnik Zaštite Bilja*, 13, 252–255.
- Kralj M., Bidjin Z., Nemanich A. (1988): Skupni prikaz sindroma otrovanja mikotoksinima prema podacima iz literature s naročitim osvrtom na pojavu u peradi. *Peradarstvo*, 23, 213–265.
- Kuiper-Goodman T., Scott P.M., Watanabe H. (1987): Risk assessment of the mycotoxin zearalenone. *Reg. Toxicol. Pharmacol.*, 7, 253–306.
- Kumagai S., Shimizu T. (1982): Neonatal exposure to zearalenone causes persistent anovulatory estrus in the rat. *Arch. Toxicol.*, 50, 279–286.
- Mirocha C.J., Christensen C.M., Nelson G.H. (1971): F-2 (Zearalenone) estrogenic mycotoxin from *Fusarium*. *Microbiol. Toxins*, Vol. 7. Academic Press Inc., New York. 17–138.
- Munk M., Topolko S. (1978): Ispitivanja frekvencije mikotoksina u krmivima. *Krmiva*, 20, 95–96.
- Norris R.F., Fong J.L. (1983): Localization of atrazine in corn (*Zea mays*), oat (*Avena sativa*) and kidney beans (*Phaseolus vulgaris*) leaf cells. *Weed Sci.*, 31, 664–671.
- Ruzsas C., Biro-Gosztanyi M., Woller L., Mess B. (1979): Effect of the fungal toxin (zearalenone) on the reproductive system and fertility of male and female rats. *Acta Biol.*, 30, 335–345.

Šimić B., Kniewald J., Kniewald Z. (1994): Effects of atrazine on reproductive performance in the rat. J. Appl. Toxicol., 14, 402–404.

Weihe W.H. (1987): The laboratory rat. In: Poole T.B. (ed.): The UFAW Handbook on the Care and Management of

Laboratory Animals. 6th edition, Longman Scientific & Technical, Harlow.

Yoshinaga K., Hawkins R.A., Stocker J.F. (1969): Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. Endocrinology, 85, 103–112.

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