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Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*)

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Abstract

Sexually mature goldfish (*Carassius auratus*) of both sexes were exposed to two doses (100 and 1000 µg/l) of the widely used herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) for a period of 21 days and effects on the concentrations of gonad and plasma sex steroids (testosterone (T), 17β-estradiol (E2) and 11-ketotestosterone (11-KT)), plasma vitellogenin (VTG) and gonad histo-morphology assessed. Atrazine did not show any obvious estrogenic effect in males, as determined by a lack of vitellogenin induction. There were, however, effects of atrazine on plasma androgen concentrations (androgen dynamics) and tissue (plasma and gonad) estrogen concentrations in male goldfish; exposure to 1000 µg/l atrazine induced suppression in both testosterone and 11-ketotestosterone, and resulted in elevated 17β-estradiol, after 21 day of exposure. Further, these suppressive effects on plasma androgens and the induction in estrogen were dose- and time-related. The highest atrazine exposure dose induced structural disruption in the testis and both 100 and 1000 µg/l induced elevated levels of atresia in ovaries.

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1. Introduction

It has now been established that there are a large number of man-made and natural chemicals capable of interfering with the synthesis and action of natu-

ral hormones. Most of these ‘endocrine disrupters’ have been shown to have effects on the reproductive system (Toppari et al., 1995; Tyler et al., 1998; Vos et al., 2000). Products used in agriculture represent a major source of pollutants that are applied to terrestrial and aquatic environments all over the world. In the last 40 years, atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) has been one of the most extensively used herbicides. Atrazine is relatively

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persistent in freshwater, with a half-life between 8 and 350 days, depending on the physio-chemical environment (Diana et al., 2000; Tavera-Mendoza et al., 2002).

The extensive use of atrazine has made this compound a focus for environmental impact studies (Huber, 1993; Solomon et al., 1996; IARC Monographs, 1999). Studies on the toxic effects of atrazine in fish have indicated a great variability in the responses, depending upon the dose and the species, with lethal concentrations in fish ranging between 3 and 45 mg/l (Elia et al., 2002). Effects of sub-lethal concentrations of atrazine in common carp *Cyprinus carpio* include an increase in serum glucose and cortisol, and a decrease in serum protein concentration/cholesterol, and decrease in liver and muscle glycogen (exposure dose 100 µg/l, Gluth and Hanke, 1985); and changes in the activity of alkaline phosphatase in serum, heart, liver and kidney (exposure dose 1.5–6.0 mg/l for 14 days, Neškovic et al., 1993). In rainbow trout *Oncorhynchus mykiss* sub-lethal doses of atrazine (ranging between 10 and 160 µg/l) caused a variety of effects in the renal tubule, including proliferation of smooth endoplasmic reticulum, atypical mitochondria and lysosome, proliferation of peroxisomes and disorganization of Golgi fields (Oulmi et al., 1995). Bioconcentration of atrazine has been shown to occur in banded tilapia *Tilapia sarrmanii* notably in ovaries (50 µg/g, after 72 h at an exposure of 16 mg/l), and in the liver (40 µg/g, after 72 h at the same dose) (Du Preez and van Vuren, 1992).

The possibility of interactions between atrazine and the endocrine system of organisms has been the focus of several studies. In humans, long-term exposure is suspected to increase the risk of breast and ovarian cancers (Donna et al., 1989; Kettles et al., 1997). In rat, some reports have indicated interactions of atrazine with the hypothalamus–pituitary–gonadal axis (Stoker et al., 1999; Cooper et al., 2000). In vitro studies have also shown the induction of aromatase activity in human cell lines (Sanderson et al., 2000, 2001). Induction of aromatase has also been reported to occur in male juvenile alligators when the exposure occurred just after hatching (Crain et al., 1999).

Atrazine has recently been linked with the decline of some amphibian populations in North America (Allran and Karasov, 2000, 2001), although this is

still rather controversial. Studies by Tavera-Mendoza et al. (2002) have shown that *Xenopus laevis* tadpoles exposed to only 21 µg atrazine/l during sexual differentiation, impacts early testicular development. This dose had previously been designated as a no-observed-ecological-effect concentration for atrazine (Solomon et al., 1996). Very recently, Hayes et al. (2002) showed that atrazine caused hermaphroditism and demasculinization in *X. laevis* (exposure dose 0.01–200 ppb). In contrast to this work however, Carr et al. (2002) observed only a weak impact of this compound when tested at similar doses on the same species (*X. laevis*); <5% of the population were found to be hermaphrodites when they were exposed to 25 µg/l atrazine during the critical window of sex differentiation.

In fish, increasingly the monitoring of sex steroid hormone concentrations and profiles and vitellogenin (VTG) are proving to be useful tools to study the effects of endocrine disrupting chemicals in wild populations (Goodbred et al., 1997; Lee et al., 2000; Hecker et al., 2002). In fish, testosterone (T) is the precursor of the two hormones 11-ketotestosterone (11-KT), produced predominantly in testis of males, and 17β-estradiol (E2), synthesised predominantly by the ovaries in females. Endogenous E2, in reproductively active female, stimulates the liver to produce vitellogenin, a yolk precursor protein, that is sequestered by developing oocytes (Tyler et al., 1991). The liver of both males and juvenile females contain the necessary E2 receptors and genetic machinery for the production of VTG and in fish VTG induction in males and immature females has been used for detecting the estrogenic activity of chemicals suspected as being endocrine active (Sumpter and Jobling, 1995).

The aim of this study was to investigate the effect of atrazine on steroidogenesis, vitellogenin induction and gonad development in adult goldfish. Reproductively active fish were exposed to atrazine (at nominal concentrations of 0, 100 and 1000 µg/l) for a 3 week period and concentrations of plasma and gonad sex steroids T, 11-KT and E2 and plasma concentration of VTG were measured as biomarkers for possible effects of atrazine on their reproductive biochemistry. Histological examination of the gonads were also undertaken to assess any effects of the atrazine treatments on sex cell development and/or gonad organisation.

2. Materials and methods

2.1. Fish culture and maintenance

Adult goldfish (initial body weight = 150.1 ± 53.2 g) were acclimated to laboratory conditions for 4 weeks before the initiation of the experiment. Fish were maintained on a 16L:8D photoperiod throughout the acclimation period and during the atrazine exposures. Eighty-two-year-old fish of both sexes were divided between three recirculating systems (each with 6, 1201 tanks and a 6001 biofilter). Weekly, pH, temperature, oxygen, nitrate, nitrite and ammonia concentrations were measured in each system. Temperature was maintained at 20 ± 1 °C. Fish were fed ad libitum twice a day (9.00 and 16.00) with pellets (Trouvit, Trow & Co., Belgium) during the acclimation and experimental periods.

2.2. Atrazine exposure and analytical procedures

In goldfish the LC50 (48 h) value for atrazine is 10,000 µg/l (Solomon et al., 1996) and the reported no-observed-effect-concentration (NOEC) value is 1000 µg/l (Huber, 1993). In each of the three recirculating systems, fish were exposed via the water to either one of two different concentrations of atrazine (Atrazine PESTENAL[®], Riedel-de Haën) (nominal concentrations 100 and 1000 µg/l) or to dilution water/solvent (controls) for 3 weeks. For the chemical exposures, atrazine was first diluted in acetone (stock solution concentration 20 µg/µl), and this solution was then dissolved in 1 l distilled water at 90 °C under constant stirring, cooled to 20 °C, and then mixed in the 1500 l test aquaria. For the control tank, acetone only was dissolved in 1 l distilled water. The amount of acetone in the exposure (control) tanks was 5.3 µl/l. Concentrations of atrazine in the exposure tanks were measured 12 h after dosing (designated day 0) and then weekly (days 7, 14, 21) by HPLC (extraction: Oasis HLB SPE, Waters; determination: Symmetry[®] column, Waters, in a Millipore Waters HPLC).

2.3. Fish sampling

Fish were sampled on days 0, 7, 14 and 21, in the morning before noon. At each sampling 8 fish were blood sampled for the analysis of plasma sex

steroids (T, E2 and 11-KT) and VTG. Blood samples were taken from the caudal vessel into heparinized syringes, centrifuged for 20 min at 4500 rpm and the plasma was collected and stored at -20 ° until assayed for steroids (radioimmunoassay) and -80 ° for VTG (enzyme-linked immunosorbent assay). Body and gonad weights were recorded and the gonadosomatic index [GSI = (gonad weight/body weight) \times 100] was determined as a measure of gonad development. Gonad tissue was either stored at -80 ° until used for steroid extraction, or fixed in Bouin's solution for histological examination.

2.4. Histological analysis

Tissues embedded in paraffin were cut at 4 µm sections for ovary and at 3 µm sections for testis. Ovary sections were stained with trichrome with hemalun, phloxine and light green and testis sections with Regaud's haematoxyline, phloxine and light green (Langeron, 1942; Romeis, 1948).

Carassius auratus is a group-synchronous multi-spawning fish, therefore the ovaries in mature females contain oocyte at various developmental stages concomitantly. Oocyte development was staged in this study according to the presence of the most advanced oocytes present (Kestemont, 1987; Rinchar and Kestemont, 1996). For each section analysed, 200 oocytes were scored and the proportion of oocytes at each stage of development (pre-vitellogenic, cortical alveolus, early and late vitellogenic) calculated. Only oocytes cut through the nucleus were considered for determining sizes of different oocyte stages. Atretic oocytes were also considered in the ovary analyses. In males, for each histological section three different areas of 1 mm² were scored for sexual status using a 10 \times 10 (0.1 mm) grid. In this analysis, the proportion of the section occupied by spermatogonia (including both spermatogonia a and b), spermatocytes, spermatids and spermatozoa was calculated.

2.5. Measurement of plasma and gonad steroids

2.5.1. Plasma

Plasma concentrations of T, E2 and 11-KT were determined by RIA analysis according to Fostier and Jalabert (1986). Samples of 50 µl of plasma for each steroid were extracted twice with cyclohexane/ethyl

acetate (v/v). All samples were assayed in duplicate and standards in triplicate (anti-T and anti-E2, Laboratoire d'Hormonologie de Marloie, anti-11-KT was a gift from Prof. Fostier, INRA, Rennes, France; all the radioactive hormones were purchased from Amersham Pharmacia).

For estimating intra-assay coefficient of variation, 14 samples at various dilutions were run in each assay and were used as internal standards. For the 3 steroid hormone assays, the sensitivity were 5 pg/ml throughout, and the intra-assay coefficients of variation were 5.79, 7.38 and 6.83% for T, E2 and 11-KT, respectively.

2.5.2. Gonads

The procedure for steroid extraction in gonads was modified from D'Cotta et al. (2001). Briefly, 1 g sections of frozen gonads were homogenized twice in 4 ml of cold 95° ethanol and centrifuged for 20 min at 4500rpm at 10 °C. The steroids in the aqueous phase were extracted by dichloromethane and, after removing the aqueous phase, the extract in the dichloromethane was evaporated to dryness. Steroid concentrations were determined by RIA as described for the plasma analyses.

2.6. Measurement of plasma VTG

Plasma concentrations of VTG were quantified by ELISA, using a carp VTG ELISA according to Tyler et al. (1999).

2.7. Data analysis

Homogeneity of data were tested by Bartlett's test. For GSI, T, E2 and VTG, if necessary data were log-transformed for normalisation; for 11-KT data were root arc-sin transformed, before statistical analyses. All data are expressed as the mean \pm S.E.M. Data for GSI, VTG and steroid concentrations were subjected to a two-way analysis of variance (ANOVA) followed by Scheffé's test ($P < 0.05$). As E2/T data and differences in the proportion of all sex cell types in the testes and ovaries were not normally distributed, they were subjected to nonparametric analyses using a Kruskal-Wallis one-way ANOVA on ranks followed by Mann-Whitney U test ($P < 0.05$). All tests were performed using Statistics 5.5 for Windows.

3. Results

3.1. Measured exposure concentration of atrazine and water physico-chemistry

Mean measured concentrations of atrazine were 102.8 ± 25.3 and 859 ± 142.5 $\mu\text{g/l}$ for nominal concentrations of 100 and 1000 $\mu\text{g/l}$, respectively (see Table 1 for atrazine concentrations at each sampling interval). In the control, there was no trace of atrazine. In the 1000 μg atrazine/l treatment group, the measured concentrations of atrazine were between 75 and 79% of nominals on days 7 and 14, and therefore further atrazine was added to this treatment group produce a measured concentration closer to the nominal 1000 $\mu\text{g/l}$. Neither deisopropylatrazine, nor desethylatrazine, two atrazine degradation products, were found in water of the recirculating systems.

Mean water temperatures during the experimental period were 20 ± 1 °C (control), 19.3 ± 1.1 °C (100 $\mu\text{g/l}$ atrazine) and 19 ± 1.1 °C (1000 $\mu\text{g/l}$ atrazine), respectively. In the control tanks and in the lowest dose of atrazine pH was 8.3 ± 0.2 , and 8.4 ± 1.2 in the higher dose of atrazine. Dissolved oxygen concentrations were 7.6 ± 0.4 mg/l in the control, 7.8 ± 0.5 mg/l in the 100 $\mu\text{g/l}$ atrazine and 7.7 ± 0.5 mg/l in the 1000 μg atrazine/l treatment tank. Nitrate and nitrite measures were 15.1 ± 2.9 and 0.14 ± 0.1 mg/l, respectively in the control tank, 12.8 ± 4.2 and 0.4 ± 0.1 mg/l in 100 $\mu\text{g/l}$ atrazine and 11 ± 1.1 and 0.3 ± 0.1 mg/l in the 1000 $\mu\text{g/l}$ atrazine dose. Ammonia concentrations were: 0.1 ± 0.08 mg/l in the control tanks, and 0.2 ± 0.07 and 0.3 ± 0.04 mg/l in the lowest and in the higher dose, respectively.

3.2. Gonadosomatic index

No fish died during the experiment. Gonadosomatic indices ranged between 5.7 ± 0.2 and $7.7 \pm 0.4\%$ in

Table 1
Atrazine concentrations at each sampling point during the exposure

Time point	Nominal concentrations of atrazine ($\mu\text{g/l}$)	
	100	1000
Day 0	105.3 ± 1.2	1021 ± 3.5
Day 7	116.8 ± 2.3	754.8 ± 7.7
Day 14	122.4 ± 0.4	775.7 ± 4.5
Day 21	69.4 ± 0.4	1049.2 ± 1.8

Values are given as means \pm S.E.M.

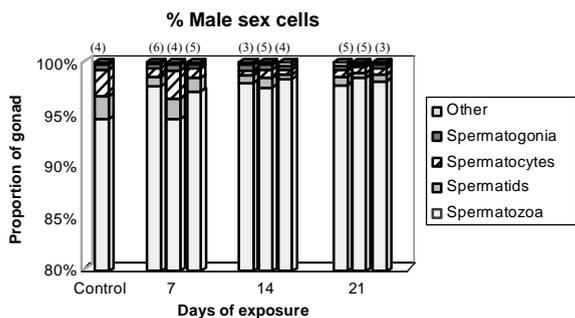


Fig. 1. Proportion of different sex cell types in the testis of *C. auratus* exposed to 100 and 1000 µg/l atrazine at 0, 7, 14 and 21 days. Data are given as means ± S.E.M. Sample numbers are shown in brackets above the columns.

males and between 14.2 ± 4.7 and 21.4 ± 4.5% in females over the experimental period. There was a large variation in GSI between individuals in some of the groups; in the control females the GSI ranged

between 10.1% to 28.4%. No time- or dose-related effects of atrazine were found on GSI for the 3 week exposure.

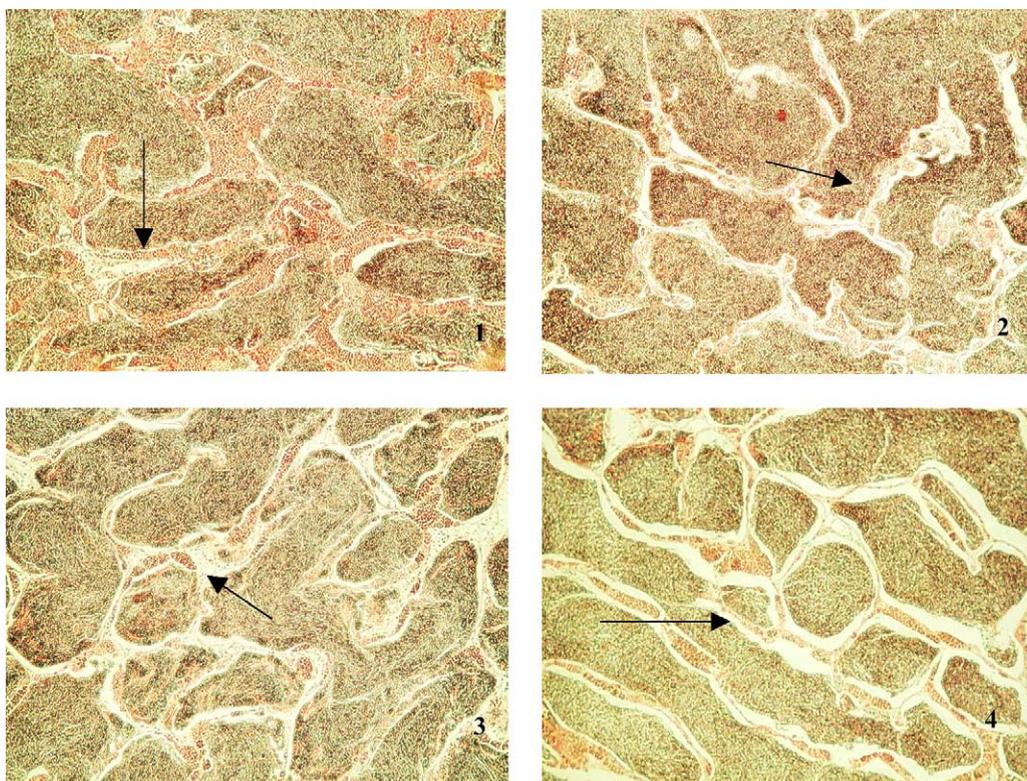
3.3. Histo-morphological analysis

3.3.1. Males

Spermatozoa were abundant in all males throughout the experimental period, representing between 95 and 98% of the testicular sex cells present. Atrazine exposure did not affect the percentage of spermatozoa or any of the other male sex cells (spermatogonia, spermatocytes and spermatids: see Fig. 1). At the highest dose of atrazine, there was a progressive increase in gaps in the interstitium between lobules, with longevity of exposure (Schemes 1–4).

3.3.2. Females

At the onset of the experiment all females were in late exogenous vitellogenesis characterised by the



Schemes 1–4. Histological sections of testis of *C. auratus* after exposure to atrazine. Scheme 1: Control on day 0; fish exposed to 1000 µg/l atrazine on day 7 (Scheme 2), day 14 (Scheme 3) and day 21 (Scheme 4). Arrows indicate the progressive increase in gaps in the interstitium between lobules (magnification 10×).

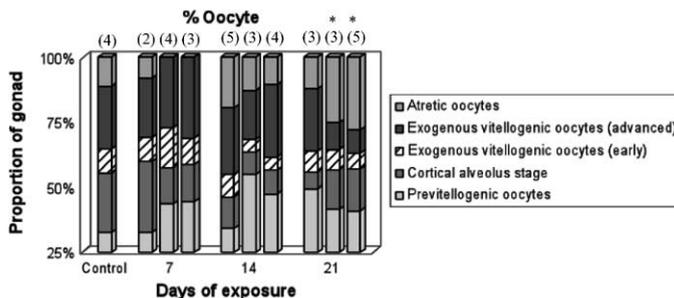


Fig. 2. Proportion of different sex cell types in the ovaries of *C. auratus* exposed to 100 and 1000 µg/l atrazine at 0, 7, 14 and 21 days. Asterisks designate significant differences ($*P < 0.05$) between treatments and control. Sample numbers are shown in brackets above the columns. At day 7, as the control had only two females, no statistical analysis could be applied.

simultaneous presence in the ovary of pre-vitellogenic oocytes and oocytes at different stages of cortical alveolus and exogenous vitellogenesis (Fig. 2; Scheme 5). Some atretic oocytes were also present (Scheme 6). On day 21, the proportion of oocyte undergoing atresia was significantly ($*P < 0.05$) higher in females exposed to atrazine (at both doses), compared to control; atretic oocytes constituted 20 and 25% of the oocytes in the ovary for the 100 and 1000 µg/l treated fish, respectively (Fig. 2).

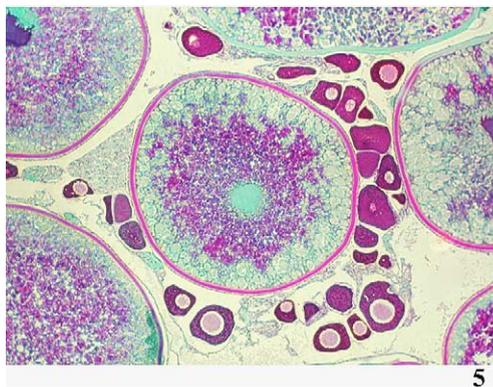
3.4. Sex steroids

3.4.1. Gonad and plasma concentrations of T

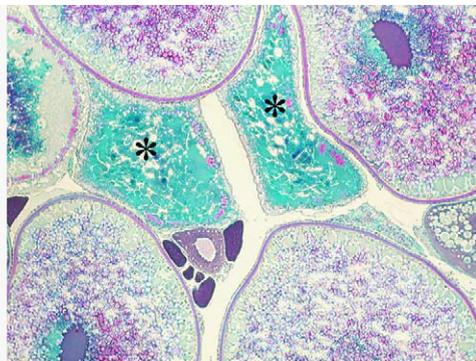
Testosterone concentrations of testis in control fish ranged between 39.4 and 395.3 ng/g tissue. There was

no significant dose- or time-related effects of atrazine on the testis T level. Plasma concentrations of T in control males ranged between 2.3 and 16.0 ng/ml over the study period. At the highest exposure dose of atrazine there was a reduced plasma content of T at all sampling points ($*P < 0.05$, $F = 6.41$) (Fig. 3). Furthermore a time-related effect occurred ($*P < 0.05$; $F = 3.46$).

In control fish, mean concentrations of testosterone in the ovary were 4 times lower than in the testis. No dose- and time-related effects were found. In control females, plasma T concentrations were similar to those assayed in males (2.1 to 16.8 ng/ml). Despite an apparent gradual decrease in plasma T from 12.0 ± 3.2 on day 0 to 3.8 ± 1.6 ng/ml on day 21, there were no differences between sampling dates (probably due to the high variation between individuals).



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6

Schemes 5–6. Histological sections of ovaries of *C. auratus* after exposure to atrazine. Scheme 5: control on day 0 (magnification 10×). Scheme 6: ovary of female goldfish after 21 days of exposure to 1000 µg/l atrazine on day 21. Asterisks indicate atretic oocytes (magnification 10×).

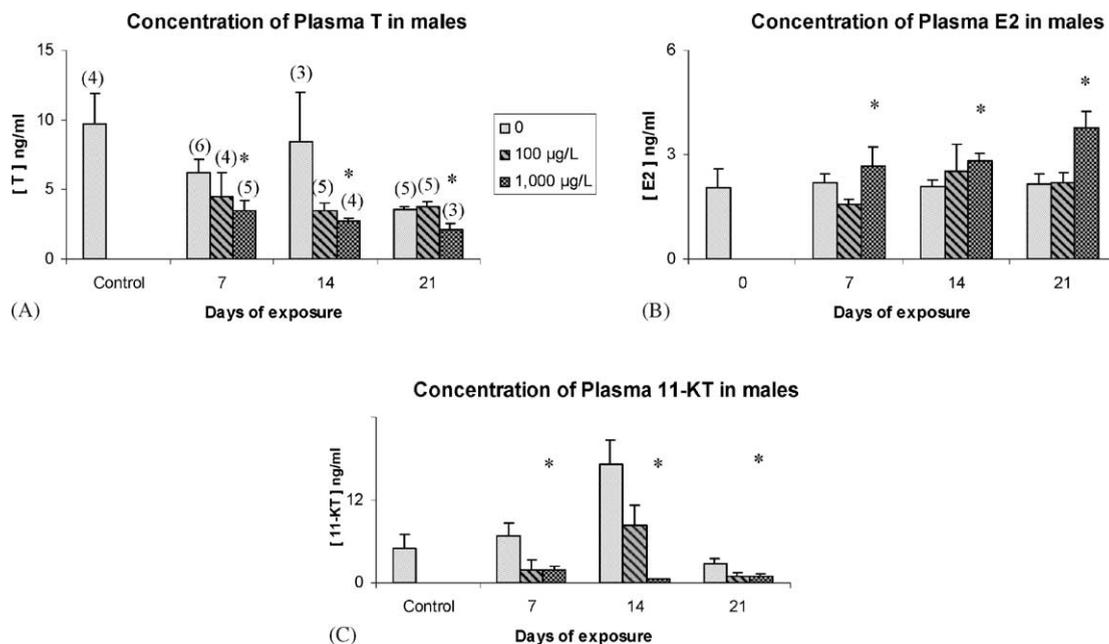


Fig. 3. Concentration of plasma sex steroids in male and female goldfish (mean \pm S.E.M.). Asterisks show significant differences ($*P < 0.05$) between treatments and control at that sampling time point. Sample numbers are shown in brackets above the columns of T concentrations.

3.4.2. Gonad and plasma concentrations of E2 and E2/T ratio

Estradiol concentrations in testis were not affected by the two doses of atrazine. In testis, E2 concentrations ranged between 14.5 ± 5.1 and 36.0 ± 11.0 ng/g tissue. In plasma E2 concentrations ranged between 1.6 ± 0.2 and 3.8 ± 2.0 ng/ml; a significant time- ($*P < 0.05$; $F = 5.26$) and dose-related increase ($*P < 0.05$, $F = 5.74$) was found for the highest atrazine dose (Fig. 3).

In females, E2 levels were not affected by atrazine exposure, either in ovary, or in the plasma. E2 concentrations in the ovary were similar to those measured in testis, ranging between 9.7 ± 1.5 and 25.8 ± 4.8 ng/g tissue while, in plasma, E2 were higher in females than in males, ranging between 2.4 ± 0.4 and 10.8 ± 4.0 ng/ml. No dose- or time-related effects of atrazine occurred.

17β -Estradiol and T contents were also evaluated by the E2/T ratio. Nonparametric statistics showed that the plasma E2/T ratio differed significantly ($*P < 0.05$) in males, between the treatments (Fig. 4) with a dose-related effect for day 21. There were no effects of atrazine on E2/T ratios in the plasma

of females and gonad tissues in both males and females.

3.4.3. Gonad and plasma concentrations of 11-KT

11-KT levels in the testis of male control fish ranged between 11.6 and 62.4 ng/g tissue. There was no dose- or time-related effect of atrazine on the testis content of 11-KT. Plasma 11-KT concentrations in control

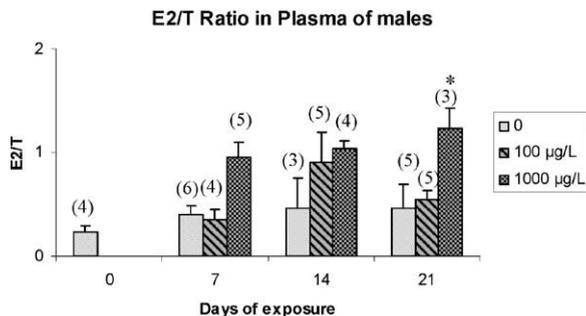


Fig. 4. E2/T ratio in plasma of males of *C. auratus* exposed to atrazine on day 0, 7, 14 and 21 (mean \pm S.E.M.). A dose-related effect ($*P < 0.05$) occurred at day 21. Sample numbers are shown in brackets above the columns.

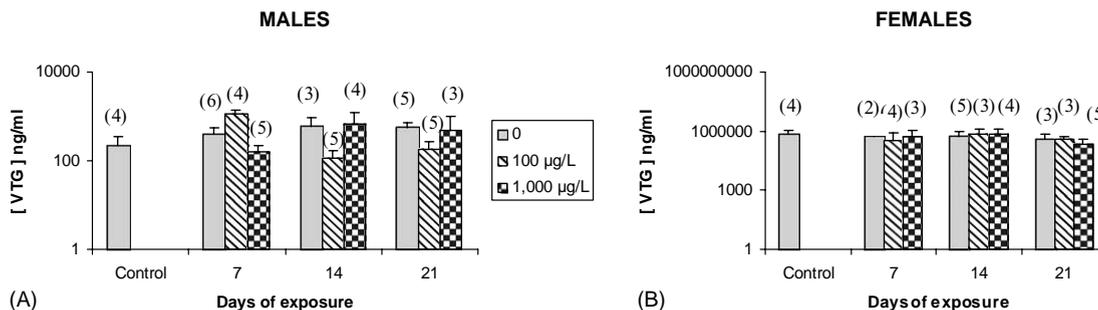


Fig. 5. VTG content in plasma of adult A: male and B: female *C. auratus* exposed to 100 and 1000 µg/l of atrazine sampled at days 0, 7, 14 and 21 (mean \pm S.E.M.). Sample numbers are shown in brackets above the columns. For females, on day 7, there was only two fish in the controls and thus not statistical analysis could be applied.

males ranged between 1.4 and 29.1 ng/ml. There was a dose-related reduction ($*P < 0.05$, $F = 4.37$) on plasma 11-KT levels in males exposed to atrazine at 1000 µg/l for all sampling times (Fig. 3). For plasma 11-KT there was also a time-related reduction in fish treated with 1000 µg/l atrazine ($*P < 0.05$; $F = 5.32$).

In females, 11-KT concentrations in the ovary ranged between 12.0 ± 4.5 and 20.3 ± 7.0 ng/g tissue, and plasma concentrations of 11-KT, that ranged between 0.5 ± 0.1 and 4.5 ± 1.5 ng/ml. No significant dose- or time-related effects of atrazine were observed for ovary or plasma 11-KT in females.

3.5. Vitellogenin

Plasma VTG content in control males ranged between 68.97 and 1263.17 ng/ml across the different sampling points. There were no changes (time- or dose-related effects) in plasma VTG in males across the 21 day study (Fig. 5). Plasma VTG concentrations in control females were about 4000 fold higher than in the males (Fig. 5). There was no effect of atrazine treatment on plasma VTG in females throughout.

4. Discussion

The aim of this work was to investigate the effects of atrazine on the reproductive system of adult goldfish, with a special emphasis on sex steroid concentrations and E2/T ratios (in both gonads and plasma), plasma vitellogenin concentration and gonadal sex cells development.

Atrazine, at doses of 100 and 1000 µg/l, had no dose- or time-related effects on gonad growth (GSI) in either males or females over the 21 days study period.

Atrazine did not affect T concentrations in the testis. Atrazine did, however, affect plasma T concentrations in males (causing a decrease) at the highest dose tested at all exposure times.

Atrazine also affected E2 levels in plasma of males; there was a significant increase at the highest exposure dose. The increase in plasma E2 and concomitant decrease in plasma T in males treated with atrazine indicates that atrazine impacted the sex steroid biosynthesis pathway in male fish; these effects on the sex steroids E2 and T could be explained by a stimulatory effect of atrazine on aromatase activity, increasing the conversion of T to E2 (Simpson et al., 1994). This effect was further confirmed by an increase in the E2/T ratio, linked with the exposure dose. An effect of triazine on aromatase activity (enhancing it) has been shown in a variety of species and has been the subject of several recent in vivo and in vitro studies (Crain et al., 1999; Sanderson et al., 2000; Sanderson et al., 2001; Hayes et al., 2002). In this study, however, there was no appreciable increase in E2 concentration in gonad (in both sexes) or plasma (females), and no induction of VTG, in either male or female goldfish. The lack of induction of VTG in male fish exposed to 1000 µg/l atrazine on day 21 where there was a significant increase of plasma E2 may be due to the fact that the increase in plasma E2 may not have yet attained the critical threshold for inducing VTG concentration above the relatively high background levels in the controls (about 100 ng/ml). The finding that atrazine did not induce VTG in fish, supports a report from a study

on male carp hepatocytes *in vitro* (Sanderson et al., 2001). Ishibashi et al. (2001) have shown that VTG induction in the goldfish is equally responsive to the estrogen mimic, bisphenol A, compared with the fathead minnow (Sohoni et al., 2001), and therefore, we would suggest that the goldfish is not necessarily an insensitive model for studying the induction of VTG.

The more sensitive axis to the disruption by atrazine, was effects on sex steroidogenesis as shown by the changes in 11-KT dynamics. Pronounced differences in plasma 11-KT were observed in males exposed to atrazine and these effects were dose-related. In the plasma, in males there was also a concomitant decrease in T concentration with the decrease in 11-KT concentration. In a study on mature male *Salmo salar* L., Moore and Waring (1998) reported in that species that atrazine concentrations at and above 3.6 and 6.0 $\mu\text{g/l}$ altered plasma testosterone and 11-ketotestosterone concentrations, respectively. This effect of atrazine on androgens in males is also reported in mammals. In Sprague–Dawley rats exposure to atrazine (100 and 200 mg/kg per day) during the peripubertal period, caused a reduced serum and intratesticular T concentration (Trentacoste et al., 2001). Friedmann (2002) demonstrated that this decrease in T concentration was due to the inhibitory effects on the Leydig cell function. Histo-morphological investigations of the goldfish gonads after 21 days of atrazine exposure (at the highest dose tested) induced alterations in testis organisation and induced higher levels of ovarian atresia. The increased level of atretic oocytes in the ovaries of fish exposed to 1000 μg atrazine/l indicates a toxic effect of this herbicide on ovary development. The atresia induced could be due to an apoptotic programming mechanism, as suggested by other researchers (Janz et al., 1997; Janz and Van Der Kraak, 1997). In males too, although no adverse effects were seen on the testis sex cells, the increase in spaces in the interstitium of the testis indicate tissue damage. Together those data strongly support the contention that atrazine affects steroidogenesis in animals generally.

5. Conclusion

These results have shown that overall atrazine has a relatively weak effect on sex steroidogenesis, but it

does impact the E2 and 11-KT biosynthetic pathways in adult male goldfish. The finding of no VTG induction as a consequence of atrazine exposure, albeit for a short term of exposure, suggests that atrazine is not estrogenic in fish, and this supports the studies *in vivo* and *in vitro* by Connor et al. (1996). A toxic impact of atrazine on gonad development occurred at the highest exposure dose adopted (1000 $\mu\text{g/l}$), as shown by histo-morphological changes in testis and by an increase in atretic oocytes in the ovary. These findings have shown that atrazine disrupts steroidogenesis at concentrations below the presently proposed NOEC for fish (goldfish) (1000 $\mu\text{g/l}$, Huber (1993)).

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