PESTICIDE BIOCHEMISTRY AND PHYSIOLOGY **62,** 41–50 (1998)
ARTICLE NO. PB982366

Mechanistic Effects of a Triazine Pesticide on Reproductive Endocrine Function in Mature Male Atlantic Salmon (*Salmo salar* L.) Parr

Andrew Moore* and Colin P. Waring†,[1]

**Centre for Environment, Fisheries and Aquaculture Science, Lowestoft Laboratory, Pakefield Road, Lowestoft, Suffolk NR33 0HT, United Kingdom; and †School of Biological Sciences, University of East Anglia, Norwich, Norfolk NR4 7TJ, United Kingdom*

Received March 13, 1998; accepted July 14, 1998

The *s*-triazine pesticide atrazine, a known contaminant of ground and surface water, had a significant sublethal impact upon the pheromonal mediated endocrine system in mature male Atlantic salmon (*Salmo salar* L.) parr. Previous studies have demonstrated that ovulated female salmon release a priming pheromone in their urine (considered to be an F-type prostaglandin) which is subsequently detected by the olfactory system of mature male salmon and results in increased levels of plasma sex steroids and expressible milt. Short-term exposure of the olfactory epithelium of male parr to concentrations of the pesticide ranging from 2.0–20 $\mu g\ L^{-1}$ significantly reduced the olfactory response to prostaglandin $F_{2\alpha}$. In addition, exposure of male parr to atrazine significantly reduced their ability to respond to the priming effect of ovulated female salmon urine. The priming effect on milt and plasma 17,20β-dihydroxy-4-pregnen-3-one levels were reduced at water atrazine concentrations at and above 0.04 $\mu g\ L^{-1}$. The priming effect of urine on plasma testosterone and 11-ketotestosterone concentrations was also affected at pesticide concentrations at and above 3.6 and 6.0 $\mu g\ L^{-1}$, respectively. Atrazine also affected the accumulation of steroids in bile and directly impacted upon the testes, modifying 17,20β-dihydroxy-4-pregnen-3-one and androgen secretion. The results of the study are discussed in relation to the possible sublethal impacts of atrazine on the biology of Atlantic salmon with particular emphasis on olfaction and reproduction

INTRODUCTION

An increasing number of aquatic contaminants have been shown to influence the reproductive endocrine function of fish (30). These endocrine-disrupting chemicals (EDCs) (6) encompass a broad suite of compounds and include a number of pesticides (38). Recent studies (24,43) have demonstrated that exposure to low levels of certain water borne pesticides have a significant impact upon pheromonal mediated endocrine function in mature male Atlantic salmon parr. Exposure of male parr to both an organophosphate (diazinon) and a carbamate (carbofuran) pesticide inhibited the olfactory detection of the female priming pheromone (22, 25,41), which is considered to be involved with the synchronization of spawning between the sexes. This inhibition in the olfactory detection of female pheromones resulted in the subsequent reduction in the levels of plasma sex hormone concentrations and expressible milt in these males.

This present study examined the impact of sublethal levels of a third common pesticide (the *s*-triazine, atrazine) on pheromonal mediated endocrine function in mature male salmon parr. Atrazine (2-chloro-4-ethylamino-6-isopropoylamino-*s*-triazine) is a pre- and postemergence herbicide for the control of annual and perennial grass and annual broad-leaved weeds. Atrazine is known to have high mobility through soil and is a known contaminant of aquatic ecosystems in England and Wales. In 1992 and 1993, atrazine was one of the five pesticides most frequently present in both ground and surface water at levels in excess of the maximum admissible concentration (MAC) 0.1 $\mu g\ L^{-1}$ imposed by the Water Act 1991 (27). In addition, some analyses of UK surface waters demonstrated levels approaching, and in a few cases exceeding, the

[1] Present address: School of Biological Sciences, University of Portsmouth, King Henry I Street, Portsmouth, Hampshire PO1 2DY, UK.

0048-3575/98 $25.00

proposed Environmental Quality Standard (EQS) of 2.0 $\mu g\ L^{-1}$ based on the annual combined average of atrazine and simazine. Since 1993 there has been a general decline in the detection of atrazine in United Kingdom surface waters as a result of a ban on use on noncropped land. However, its main use is now in the production of maize particularly in the south west of England where it is still detected in surface waters exceeding the 0.1 $\mu g\ L^{-1}$ MAC (8). Concentrations of atrazine up to 275 $\mu g\ L^{-1}$ have been detected in runoff water from agricultural land (35), but at these concentrations it is considered not to be a risk to aquatic life.

The study examined three different aspects relating to the effects of atrazine exposure on pheromonal priming and the endocrine response of male salmon. First, an electrophysiological study was carried out to investigate the effects of the pesticide on the olfactory ability of mature male Atlantic salmon parr to detect prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Second, an endocrinological study was carried out to investigate the effects of atrazine exposure on the levels of plasma sex steroids and expressible milt in mature male parr after exposure to ovulated female salmon urine. Female urine has been demonstrated to have a significant priming effect on male salmon (41, 42) and to contain a priming pheromone, $PGF_{2\alpha}$ (25). Third, in order to investigate possible direct effects of the pesticide on the ability of the testes to respond to pituitary stimulation, testes were removed from atrazine-exposed males and incubated *in vitro* with a pituitary extract from mature salmonids. In addition, free and glucuronidated steroid levels in the bile were measured to ascertain whether the pesticide altered aspects of the metabolism and excretion of steroids in mature male salmon parr.

MATERIALS AND METHODS

In November 1996, mature male Atlantic salmon parr (14–24 g weight range; 110–155 mm length range; 4.7–9.0 % GSI range) were obtained from the Marine Harvest-MacConnell, Loch Shiel hatchery, Scotland, and transported to the CEFAS, Lowestoft Laboratory. The fish were kept in 1000-L tanks, under natural light conditions, with a constant flow of aerated dechlorinated water (flow rate of 85 L min^{-1}). Water temperature ranged from 7.5 to 10°C and the physicochemical characteristics of the water have been reported elsewhere (24). The fish were fed to satiation twice a day with commercial salmon pellets (BP Mainstream).

Effect of Atrazine on the Olfactory Detection of $PGF_{2\alpha}$

The study used the same electrophysiological technique (electroolfactogram, EOG) as that in previous studies on mature male Atlantic salmon parr (21–25,43). EOG recording measures transepithelial voltage gradients from the surface of the olfactory epithelium and is considered to reflect multiunit cell activity (9).

Testing Procedure

The testing procedure was the same as that used previously in the studies on diazinon and carbofuran (24,43). The responses of the olfactory epithelium of mature male salmon parr to a 10^{-9} M concentration of $PGF_{2\alpha}$ were recorded after perfusion of the olfactory rosette with different concentrations of atrazine (0.1, 1.0, 2.0, 5.0, 10.0, and 20.0 $\mu g\ L^{-1}$). The olfactory epithelium was perfused with each concentration of the pesticide for a period of 30 min.

The amplitude of each EOG response was measured from the baseline to the peak of each phasic displacement and expressed in millivolts (mV). All recordings in response to the dechlorinated water controls were subtracted from the EOG responses. In all experiments the EOG responses recorded at each concentration of atrazine were compared to the responses recorded to the relevant control water taken from the inlet of the salmon tank, using a paired two sample for means *t* test.

Effect of Atrazine on the Priming Response of Males to $PGF_{2\alpha}$

In November 1996, spermiating male parr were transferred to glass tanks each of 63-L

volume. Each tank had a constant flow of dechlorinated tap water (1 L min^{-1}) with no recirculation. A natural photoperiod was followed and the water temperature was 11 ± 1°C. Males were gently stripped of milt and groups of five were placed into each tank and left to recover for 96 h without feeding.

Stock solutions of atrazine (Greyhound Chromatography and Allied Chemicals, Birkenhead, Merseyside, UK) were produced by dissolving in water and were stored in amber glass bottles. Atrazine was added to the tanks via a multichannel peristaltic pump (Watson–Marlow) and silicon tubing (Altec) and vigorously mixed by aeration. Stock solutions were renewed every 12 h. At the end of the experiment water samples were taken and stored in the dark at 4°C in amber glass bottles before analysis of actual atrazine contents. Water atrazine concentrations were measured at the CEFAS Burnham-on-Crouch Laboratory using gas chromatography/mass spectrometry (1).

Males were exposed to various concentrations of atrazine (nominally 0–20.0 μg L^{-1}) for 5 days. At the end of this period groups of males were then either given a 5-h exposure to ovulated female salmon urine or to a water control. The urine had been collected from five urinary bladder-catheterized ovulated female salmon as described previously (25) and pooled. One-milliliter aliquots of this pooled urine were mixed with an equivalent volume of dechlorinated tap water of tank water and then added to the relevant tanks. Rapid aeration ensured that the urine was thoroughly mixed with the tank water and that each fish was exposed to similar concentrations of the urine.

At the end of the urine exposure period, males were anesthetised in 0.4 ml L^{-1} 2-phenoxyethanol and milt and blood were sampled (24). Gallbladders were removed from each fish and the contents were left to drain into preweighed 1.5-ml microcentrifuge tubes. Bile was then diluted 1:10 (w/v) with distilled water and stored at −20°C.

Testes were removed from some of the males and were then washed, chopped into 50-mg fragments, and incubated *in vitro* using the procedures outlined in detail previously (26,43).

Free steroids were extracted from plasma (50 μl), bile (50 μl), and testes incubation medium (100 μl) with 3 ml of diethyl ether. Plasma levels of testosterone (T), 11-ketotestosterone (11-KT) and 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) were measured using radioimmunoassay (32,33). Levels of glucuronidated and sulfated T, 11-KT, and 17,20βP were also measured in bile and incubated medium using the methods described previously (31,42,43). The data have been expressed as nanograms of free steroid equivalents and have not been adjusted to take into account the mass of the conjugated moiety.

The milt, bile, and plasma steroid data were analyzed using a one-way ANOVA followed, if significance was indicated, by Student–Neuman–Keuls (SNK) tests as the multiple range test. The testes incubation data were analysed using a two-way ANOVA with the presence or absence of PE and pesticide treatment as factors. When significance was indicated, SNK was used as the multiple range test.

RESULTS

Water Atrazine Concentrations

Samples from the electrophysiological study although stored in the dark and at low temperature suffered rapid degradation as the result of an unavoidable delay in being analyzed. The measured water atrazine concentrations from the endocrinological study were in the range 8–72% of the nominal concentrations (Table 1). The

TABLE 1
Nominal and Measured Concentrations of Atrazine in the Endocrinological Experiment

Nominal (μg L^{-1})	Measured (μg L^{-1})	% of nominal
Control (0)	0	0
0.5	0.04	8
5.0	3.6	72
10.0	6.0	60
20.0	14.0	70

measured water concentrations are referred to in the rest of the text and figures. However, these values were terminal measurements and we have no data regarding water concentrations earlier in the exposure period.

Effect of Atrazine on the Olfactory Detection of $PGF_{2\alpha}$

Electrophysiological responses recorded from the olfactory epithelium of mature male Atlantic salmon parr to 10^{-9} M $PGF_{2\alpha}$ were significantly reduced ($P < 0.01$) after perfusion of the epithelium with nominal concentrations of atrazine ranging from 2.0 to 20 $\mu g\ L^{-1}$ (Fig. 1). Although at the highest concentration tested (20.0 $\mu g\ L^{-1}$), responses were still recorded from the epithelium, the amplitudes were only 56 ± 3.2% compared to the control.

Mean EOG recordings (in mV) from the olfactory epithelia of the parr in response to $PGF_{2\alpha}$ at each concentration of atrazine were control, 1.7 ± 0.13 mV; 0.1 $\mu g\ L^{-1}$, 1.7 ± 0.12 mV; 1.0 $\mu g\ L^{-1}$, 1.7 ± 0.11 mV; 2.0 $\mu g\ L^{-1}$, 1.54 ± 0.10 mV; 5.0 $\mu g\ L^{-1}$, 1.34 ± 0.11 mV; 10.0 $\mu g\ L^{-1}$, 1.13 ± 0.09 mV; 20.0 $\mu g\ L^{-1}$, 0.99 ± 0.09 mV. There was also a significant reduction in the recorded response to 10^{-5} M L-serine ($P < 0.0001$). The mean EOG recording prior to atrazine exposure was 0.75 ± 0.08 mV. After exposure to the highest concentration of the pesticide (20.0 $\mu g\ L^{-1}$), the EOG recording was reduced to 0.38 ± 0.03 mV, which was 50% of the initial response. Atrazine itself did not elicit a recordable response from the olfactory epithelium at any of the concentrations used in these experiments.

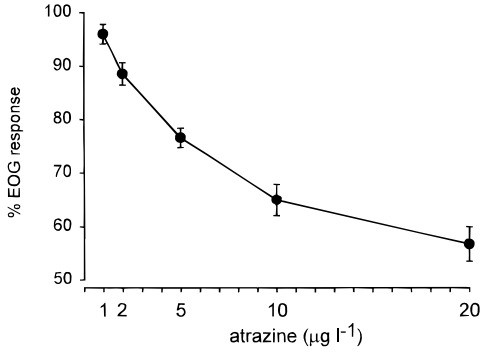

FIG. 1. EOG recordings from the olfactory epithelium of mature male Atlantic salmon parr to $PGF_{2\alpha}$ (concentrations 10^{-9} M; $n = 6$), after perfusion of the olfactory epithelium with various concentrations of atrazine (0.1, 1.0, 2.0, 5.0, 10.0, and 20.0 $\mu g\ L^{-1}$). The responses to $PGF_{2\alpha}$ after exposure to 0.1 $\mu g\ L^{-1}$ atrazine were similar to those after exposure to 1.0 $\mu g\ L^{-1}$ and have not been included. The amplitude of each EOG response was measured from the baseline to the peak of each phasic displacement and expressed as millivolts. The negative value of the EOG response has been changed and each recording expressed as positive millivolts. Vertical bars represent SEM.

Effect of Atrazine on the Priming Response of Males to Ovulated Female Urine

Exposure to ovulated female salmon urine for 5 h significantly increased levels of expressible milt and plasma 17,20βP, T, and 11-KT concentrations (Fig. 2) in male parr compared to the control group. However, when the male parr were exposed to water atrazine concentrations at and above 0.04 $\mu g\ L^{-1}$ significant increases in milt and plasma levels of 17,20βP were not apparent when compared to control males. Exposure of male parr to atrazine in the water also impacted on the plasma androgen response which was affected at water concentrations at and above 3.6 $\mu g\ L^{-1}$ (T) and 6.0 $\mu g\ L^{-1}$ (11-KT).

Bile-free and glucuronidated 17,20βP levels were significantly elevated in the pheromonally primed males compared to controls (Fig. 3). However, this response was not apparent when water atrazine concentrations were 14.0 $\mu g\ L^{-1}$ (bile free) and at and above 0.04 $\mu g\ L^{-1}$ (bile glucuronides). Similar increases in bile free and glucuronidated T concentrations were also evident in males exposed to female urine, but in the presence of atrazine both these responses were attenuated when the pesticide was present at and above 0.04 $\mu g\ L^{-1}$. The exposure to ovulated urine resulted only in an increase in the bile-free and not glucuronidated levels of 11-KT. However, the presence of atrazine at concentrations at 0.04 $\mu g\ L^{-1}$ and above significantly reduced this response in male parr.

When exposed to PE *in vitro*, there was no

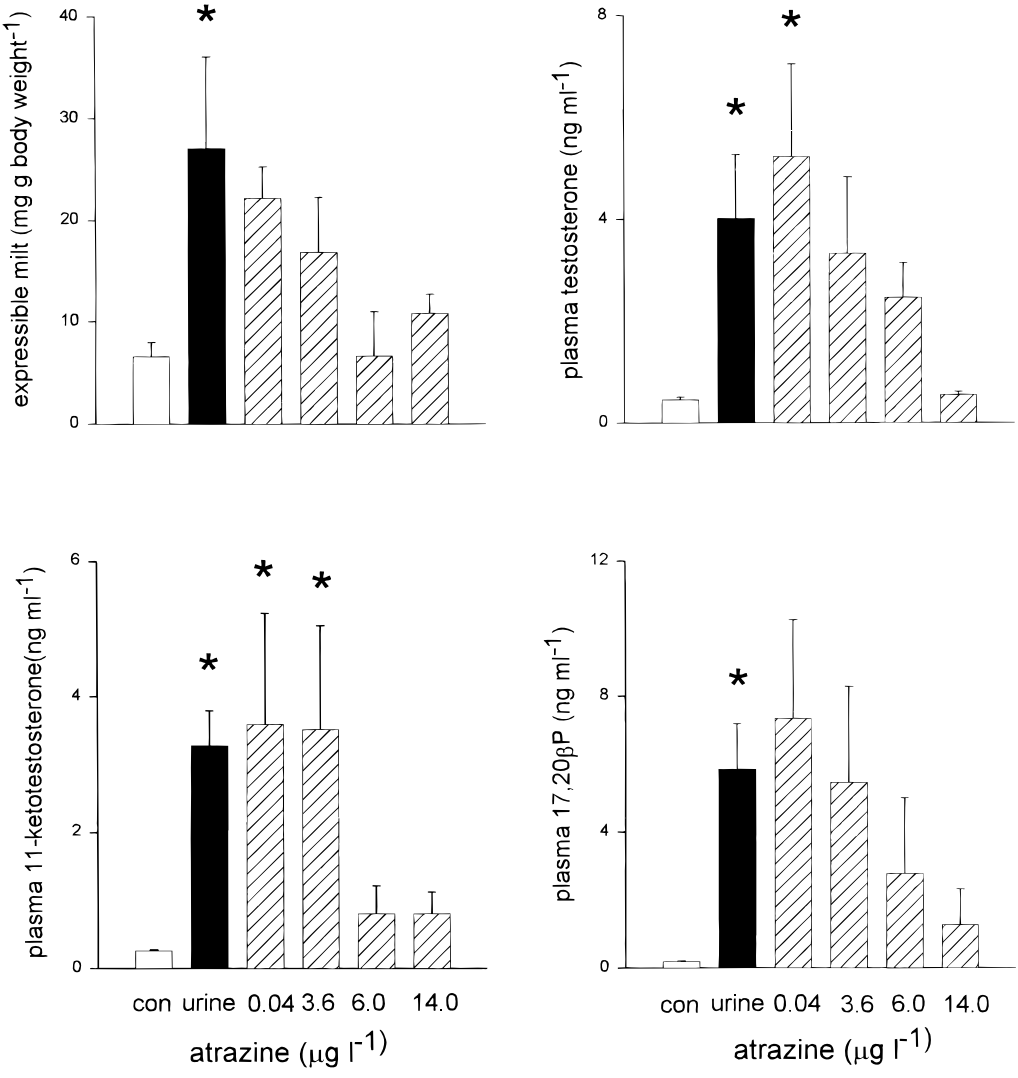

FIG. 2. The effect of atrazine on expressible milt, plasma 17,20βP, testosterone, and 11-ketotestosterone concentrations in various groups of mature male salmon parr. Open column, controls not exposed to ovulated female salmon urine; solid column, controls exposed to ovulated female salmon urine; slashed column, exposed to atrazine and ovulated female salmon urine. Data represent the mean + SEM of five fish per group. $*P < 0.05$ compared to control fish not exposed to ovulated female salmon urine.

significant difference in the release of free, glucuronidated, and sulfated 17,20βP and T from testes of male parr that had been exposed to ovulated female urine and the control group (Table 2). However, in fish exposed to atrazine concentrations of 0.04 μg L^{-1} there was a significant increase in the release of 17,20βP from the testes of nonstimulated fish and at 3.6 μg L^{-1} an increase in release of 17,20βP from both nonstimulated and pituitary stimulated testes.

In fish exposed to the higher pesticide concentrations of 6.0 and 14.0 μg L^{-1} there was a significant decrease in the release of glucuronidated T from both PE-exposed and nonexposed testes (Table 2). However, in the case of T, there was a significant decrease in the release of the

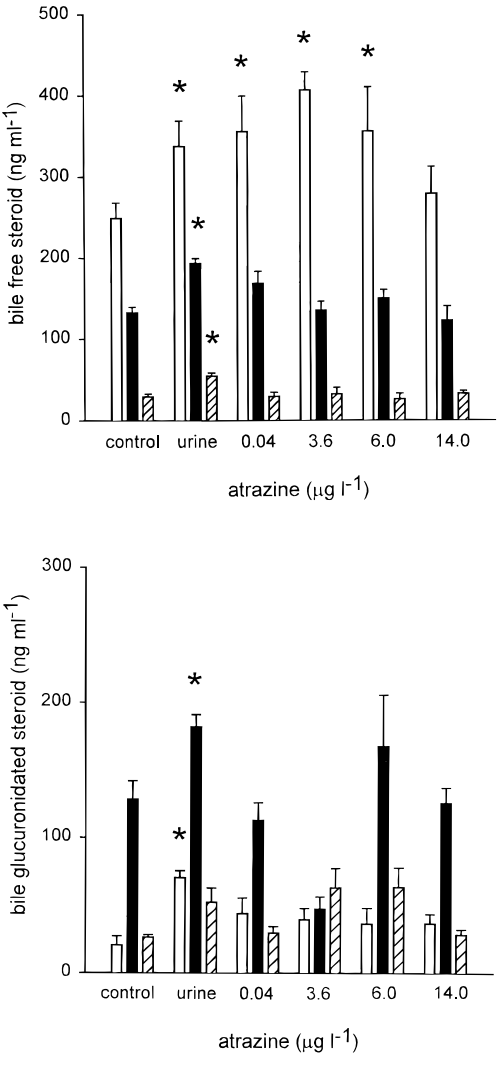

FIG. 3. Bile free and glucuronidated 17,20βP, testosterone, and 11-ketotestosterone in male salmon parr exposed to various concentrations of atrazine. Data represent the mean ± SEM of five fish per group. *$P < 0.05$ compared to control fish.

androgen from nonexposed testes at atrazine concentrations of 0.04 and 14.0 μg L^{-1}, but a significant increase at the intermediate concentration of 3.6 μg L^{-1}.

When exposed to PE *in vitro*, there was no significant difference in the release of free and glucuronidated 11-KT from testes of male parr that had been exposed to ovulated female urine

TABLE 2

In Vitro Release of Free, Glucuronidated, and Sulfated 17,20βP, Testosterone, and 11-Ketotestosterone from Testes from Mature Male Parr Exposed to Female Urine in Water with Various Concentrations of Atrazine

Atrazine μg L^{-1}	Steroid (pg mg testes^{-1})																			
	17,20βP		17,20βP-G		17,20βP-S		T		T-G		T-S		11-KT		11-KT-G		11-KT-S	
	Control	PE	Control	PE	Control	PE	Control	PE	Control	PE	Control	PE	Control	PE	Control	PE	Control	PE
0, control	3.6 ± 0.46	164.4 ± 42.7	6.7 ± 0.73	19.6 ± 8.48	17.3 ± 2.59	163.9 ± 95.64	8.0 ± 0.59	65.0 ± 51.92	6.6 ± 1.15	19.2 ± 8.71	6.2 ± 1.35	11.6 ± 3.14	4.1 ± 0.65	14.6 ± 7.15	2.5 ± 0.24	6.7 ± 2.94	4.1 ± 0.66	9.7 ± 3.28
0, urine	2.79 ± 0.56	135.7 ± 6.8	1.8 ± 0.20	4.8 ± 1.11	13.8 ± 2.34	59.4 ± 14.31	4.2 ± 1.00	10.9 ± 7.68	2.8 ± 0.61	4.6 ± 1.62	6.6 ± 0.78	5.7 ± 0.79	2.3 ± 0.42	1.8 ± 0.22	1.9 ± 0.35	3.2 ± 0.73	1.8 ± 0.27	2.0 ± 0.26*
0.04	21.0 ± 5.0*	285.6 ± 86.4	13.5 ± 7.98	11.8 ± 3.83	49.6 ± 20.16	69.8 ± 13.92	3.1 ± 0.94*	6.8 ± 1.44	18.6 ± 9.1	22.2 ± 8.66	7.7 ± 1.37	7.5 ± 1.02	2.0 ± 0.37	10.9 ± 4.32	8.3 ± 2.56	6.9 ± 2.30	2.2 ± 0.49	1.3 ± 0.25*
3.6	34.7 ± 7.93*	563.5 ± 51.92*	17.1 ± 8.90	24.2 ± 6.50	75.3 ± 33.5	99.9 ± 14.28	11.3 ± 2.18*	47.3 ± 21.5	42.7 ± 17.94	75.5 ± 33.42	9.5 ± 1.98	12.5 ± 1.91	9.5 ± 1.98*	13.0 ± 5.42	21.0 ± 8.2*	23.6 ± 8.7	3.8 ± 0.61	6.4 ± 1.41
6	16.32 ± 11.1	171.8 ± 51.69	2.1 ± 0.17	4.7 ± 1.33	11.4 ± 1.13	64.6 ± 14.69	3.9 ± 0.72	4.5 ± 1.62	2.2 ± 0.41*	3.2 ± 0.77*	8.2 ± 1.93	8.6 ± 1.68	1.7 ± 0.16	2.0 ± 0.31	1.4 ± 0.08*	1.8 ± 0.02	1.9 ± 0.19	3.1 ± 0.37
14	3.5 ± 1.25	101.9 ± 44.02	2.4 ± 0.58	8.4 ± 3.34	11.7 ± 3.74	56.4 ± 10.74	1.6 ± 0.20*	5.2 ± 0.55	1.6 ± 0.32*	2.6 ± 0.71*	4.4 ± 1.28	5.0 ± 0.50	2.8 ± 0.84	4.3 ± 1.20	1.2 ± 0.07*	1.7 ± 0.02	1.32 ± 0.12	1.5 ± 0.10*

Note. Data represents SEM of $n = 5$ per group.
* $P<0.05$ compared to the control.

and the control group (Table 2). However, there was a significant decrease in the levels of sulfated 11-KT from testes of male exposed to female urine when compared to the control group. At the pesticide concentration of 3.6 $\mu g\ L^{-1}$ there was a significant increase in the release of glucuronidated 11-KT from the testes of nonstimulated fish, but a decrease in release from nonstimulated testes at the higher concentrations of 6.0 and 14.0 $\mu g\ L^{-1}$. The release of sulfated 11-KT was significantly reduced in PE-exposed testes at atrazine concentrations of 0.04 and 14.0 $\mu g\ L^{-1}$. An atrazine concentration of 3.6 $\mu g\ L^{-1}$ also significantly increased the release of free 11-KT from testes which had not been exposed to PE. The results suggest that atrazine may have a direct effect on the testes modifying the release of 17,20βP and the androgens.

DISCUSSION

Exposure of mature male salmon parr to sublethal levels of water-borne atrazine clearly inhibited their ability to detect and respond to female priming pheromones. Similar results were obtained when mature male parr were exposed to the pesticides diazinon and carbofuran (24,43). However, in the case of atrazine the mode of action on the reproductive system appears to be more complex. The increase in expressible milt and plasma sex hormones was significantly reduced as the result of impaired olfactory detection of the priming pheromones. But unlike carbofuran, there was evidence that exposure to atrazine influenced both the metabolism of steroids and their accumulation within the bile. Males continued to accumulate the steroid 17,20βP in the bile as a result of atrazine-induced secretion from the testes. Atrazine also had a further direct impact upon the testes modifying the release of androgens and suggesting an additional toxic mechanism on reproduction in the male salmon. Previous studies on endocrine-disrupting chemicals in United Kingdom rivers and streams have indicated that many are estrogen mimics and act as xenoestrogens (30). The data from the present study would suggest that atrazine is a modifier of endogenous androgen metabolism.

The gonads are known key target organs for the bioconcentration of lipophilic pollutants such as atrazine (4,15,40), principally due to their high lipid contents during oogenesis and spermatogenisis. In fish there is a rapid uptake of atrazine from the water which occurs via the gills with the other organs contaminated via the blood circulation (17). This uptake often occurs with increasing environmental concentration (16). Other pesticides such as the organochlorines have also been implicated in a number of reproductive abnormalities in salmonid species living in the Laurentian Lakes of North America. In male coho salmon, these abnormalities include decreased fertility, lower plasma concentrations of gonadotropins and steroids (testosterone and 11-ketotestosterone), poor expression of secondary characteristics, and high precocious sexual maturation.

The toxic mechanism by which atrazine inhibits the olfactory response to the priming pheromone in the male parr is not known. Atrazine is a known inhibitor of acetylcholinesterase (AChE) and has been shown to operate in a dose-dependent manner in the freshwater fish species *Oreochromis niloticus* and *Chrysichthyes auratus* (18). AChE is known to be localized within the olfactory epithelium of fish and amphibia (11) and, although not unequivocally demonstrated, it has been proposed that a cholinergic mechanism within the olfactory epithelium of amphibia performs a particular role in creating the receptor (generator) potential (39). However, studies on the effect of the anticholinesterase phosdrin on the EOG and AChE activity in the olfactory epithelium of the goldfish demonstrated that cholinergic transmission does not directly participate in generation of the EOG (12). Studies on the toxicity of heavy metals on the EOG of the Atlantic salmon have suggested that the toxic effect operates on the transduction mechanisms of the olfactory receptor cells (3,44). However, the peripheral sensitivity to sex pheromones in fish is controlled and modified by endogenous androgens (5,29), and therefore atrazine may affect the expression of the PGF receptors within the olfactory epithelium of the

male parr, significantly reducing their ability to respond to the priming pheromones.

It is not evident from the present study what the long-term impact of exposure of Atlantic salmon to atrazine is in relation to reproductive function and population viability. However, the rapid accumulation of atrazine by salmonids, the direct effect of the pesticide on steroid synthesis of the testes, and inhibition of the priming response suggest that exposure of male fish to atrazine should be viewed with concern. Of particular concern is the spring run component of the Atlantic salmon population which are resident within freshwater for longer periods and would be exposed for longer periods. Exposure to atrazine may either enhance the males' reproductive status too early or delay it and this may subsequently have adverse effects on the motivational mechanisms controlling the run timing of the fish and subsequent reproductive success.

The inability of the salmon parr to detect the pesticide would suggest that it has no mechanism by which to avoid contaminated areas which in turn may have a compounding effect on the fish (43). In addition, it is not known what effect atrazine may have on the reproductive biology of mature female salmon. Atrazine is known to have a direct effect on kidney structure and function in freshwater salmonids (10,28). Disturbance to renal function may also inhibit the release of pheromones within the urine of the female (25,42) presuming there had been no initial effect on reproductive status.

Atrazine may also impact upon other critical life history stages of the Atlantic salmon particularly smoltification and migration into the marine environment. The toxicological effect on the olfactory system was not only restricted to priming pheromones and reproduction. The pesticide also significantly reduced the ability of the olfactory epithelium to respond to the amino acid L-serine. The occurrence of atrazine in the aquatic environment may therefore have significant effects on other olfactory-mediated behavior and physiology during the salmon life cycle (e.g., smoltification).

Using standard toxicity tests, atrazine seems to be a relatively harmless contaminant of the aquatic ecosystem with calculated 96-h LC_{50} of >18 and 6.3 mg L^{-1} for rainbow trout and brook trout, respectively (20). However, studies have demonstrated a range of significant sublethal effects by the pesticide on both the physiology and the behavior of other freshwater species. Reduced reproductive success has been demonstrated in bluegill sunfish (*Lepomis macrochirus*) due to an indirect effect of atrazine on diet and prey availability rather than direct toxicity (19). Early life history stages of the zebrafish (*Brachydanio rerio*) were also shown to be sensitive to atrazine. Exposure resulted in deformities of the embryos but did not influence hatching rate or growth (13). Short-term exposure of carp to atrazine resulted in an increase in muscle glycogen levels but a significant reduction after longer exposure (14). Exposure of *Galaxias maculatus* to the pesticide resulted in a reduction in levels of muscle DNA levels and hepatic GSH, (7). Atrazine concentrations as low as 5 $\mu g\ L^{-2}$ were shown to have an effect on the swimming behavior of zebrafish (37). The persistence of atrazine and its widespread temporal and spatial occurrence in both ground and surface waters may be a hazard to other indigenous fish species. The dual hormone/pheromone reproductive system in cyprinids has been well described (2,34,36), and both the priming and the releasing pheromones involved in spawning may be the target for similar toxic mechanisms to those described in the Atlantic salmon. A study on the effects of pesticides on the pheromonal control of reproduction in cyprinids could provide a suitable model for examining the impact of persistent aquatic contaminants at the population level.

ACKNOWLEDGMENTS

We thank Dr. Sandy Scott for the use of steroid antisera and radiolabels and for his advice on *in vitro* teste incubations. We also thank Steve Morris (CEFAS, Burnham-on-Crouch Laboratory) for the analyses of water atrazine concentrations. This work was funded by the UK Ministry of Agriculture, Fisheries and Food.

REFERENCES

1. M. Ahel, K. M. Evans, T. W. Fileman, and R. F. C. Mantoura, Determination of atrazine and simazine in

estuarine samples by high-solution gas chromatography and nitrogen selective detection, *Anal. Chim. Acta* **268,** 195–204 (1992).
2. R. Bjerselius and K. H. Olsen, A study of the olfactory sensitivity of crucian carp and goldfish to 17α, 20β-dihydroxy-4-pregnen-3-one: 17α20βP and prostaglandin $F_{2\alpha}$, *Chem. Senses* **18,** 427–436 (1992).
3. R. Bjerselius, S. Winberg, Y. Winberg, and K. Zeipel, Ca^{2+} protects olfactory receptor function against acute Cu (II) toxicity in Atlantic salmon, *Aquat. Toxicol.* **25,** 125–138 (1993).
4. D. A. Brown, R. W. Gosset, and K. D. Jenkins, Contaminants in white croakers *Genyonemus lineatus* from the Southern California Bight II: Chlorinated hydrocarbon detoxification/toxification, *in* "Physiological Mechanisms of Marine Pollution Toxicity" (W. B Vernberg, A. Calabrese, F. P. Thurberg, and F.J. Vernberg, Eds.), pp. 197–213, Academic Press, New York, (1982).
5. J. R. Cardwell, N. E. Stacey, E. S. P. Tan, D. S. O. McAdam, and S.L.C. Lang, Androgen increases olfactory receptor response to a vertebrate sex pheromone, *J. Comp. Physiol. A* **176,** 55–61 (1995).
6. T. Colborn, F. S. vom Saal, and A. M. Soto, Developmental effects of endocrine-disrupting chemicals in wildlife and humans, *Environ. Health Perspect.* **101,** 378–384 (1993).
7. P. E. Davies, L. S. J. Cook, and D. Goenarso, Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout, *Environ. Toxicol. Chem.* **13,** 1341–1354 (1994).
8. Environment Agency, "Pesticides in the Aquatic Environment," Update of the Report of the National Rivers Authority, Water Quality Series No. 26, National Centre for Toxic and Persistent Substances, (1997).
9. R. E. Evans and T. J. Hara, The characteristics of the electro-olfactogram (EOG): Its loss and recovery following olfactory nerve section in the rainbow rout (*Oncorhynchus mykiss*), *Brain Res.* **330,** 65–75 (1985).
10. T. Fischer-Scherl, A. Veeser, R. W. Hoffman, C. Kuhnhauser, R.-D. Negele, and T. Ewringmann, Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchus mykiss*), *Arch. Environ. Contam. Toxicol.* **20,** 454–461 (1991).
11. P. A. Gdovskiy and N. N. Ruzhinskaya, Assessment of functional development of the olfactory and visual systems of fishes by acetylcholinesterase activity, *J. Ichthy.* **30,** 47–59 (1990).
12. P. A. Gdovskiy, N. N. Ruzhinskaya, S. A. Menzikov, and O.V. Menzikova, Action of phosdrin on the olfactory system of the goldfish, *in* "5th All-Union Conference on Aquatic Toxicology," Abstracts of Reports, Odessa, (1988).
13. G. Gorge and R. Nagel, Toxicity of lindane, atrazine and deltamethrin to early life history stages of zebrafish (*Brachydanio rerio*), *Ecotoxic. Environ. Safety* **20,** 246–255 (1990).
14. G. Gluth and W. Hanke, A comparison of physiological changes in carp, *Cyprinus carpio*, induced by severall pollutants at sublethal concentrations. I the dependency on exposure time, *Ecotoxic. Environ. Safety* **9,** 179–188 (1985).
15. G. Gluth, D. Freitag, W. Hanke, and F. Kortes, Accumulation of pollutants in fish, *Comp. Biochem. Physiol. C* **81,** 273–277 (1985).
16. E. Grobler-van Heerden, J. H. J. van Vuren, and H. H. du Preez, Bioconcentration of atrazine, zinc and iron in the blood of *Tilapia spaamanii* (Cichlidae), *Comp. Biochem. Physiol. C* **100,** 629–633 (1991).
17. G. Gunkel and B. Streit, Mechanisms of bioaccumulation of a herbicide (Atrazine, s-Triazine in a freshwater mollusc (*Ancylus fluviatilis*) and a fish (*Coregonus fera*), *Water Res.* **14,** 1573–1584 (1980).
18. S. Y. Hussein, M. A. El-Nasser, and S. M. Ahmed, Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt, *Bull. Environ. Contam. Toxicol.* **57,** 503–510 (1996).
19. W. D. Kettle, F. deNoyelles Jr., H. D. Bradley, and A. M. Kadoum, Diet and reproductive success of bluegill recovered from experimental ponds treated with atrazine *Bull. Environ. Contam. Toxicol.* **38,** 47–52 (1987).
20. K. J. Macek, K. S. Buxton, S. Sauter, S. Gnilka, and J. W. Dean, "Chronic Toxicity of Atrazine to Selected Aquatic Invertebrates and Fish." U.S. EPA Report No. EPA-600/3-76-047, 1976.
21. A. Moore, An electrophysiological study on the effects of pH on olfaction in mature male Atlantic salmon (*Salmo salar*) parr, *J. Fish Biol.* **45,** 493–502 (1994).
22. A. Moore and A. P. Scott, 17α,20β-Dihydroxy-4-pregnen-3-one 20-sulphate is a potent odorant in precocious male Atlantic salmon (*Salmo salar* L.) parr which have been pre-exposed to the urine of ovulated females, *Proc. R. Soc. Lond. B* **249,** 205–209 (1992).
23. A. Moore and C. P. Waring, Seasonal changes in olfactory sensitivity of mature male Atlantic salmon (*Salmo salar* L.) parr to prostaglandins, *in* "Proceedings of the Fifth International Symposium on Reproductive Physiology of Fish" (F. W. Goetz and P. Thomas, Eds.), p. 273, Univ. of Texas Press, Austin, TX, 1995.
24. A. Moore and C. P. Waring, Sublethal effects of the pesticide Diazinon on olfactory function in mature male Atlantic salmon (*Salmo salar* L.) parr, *J. Fish Biol.* **48,** 758–775 (1996).
25. A. Moore and C. P. Waring, Electrophysiological and endocrinological evidence that F-series prostaglandins function as priming pheromones in mature male Atlantic salmon (*Salmo salar* L.) parr, *J. Exp. Biol.* **199,** 2307–2316 (1996).
26. J. J. Nagler, A. P. Scott, C. R. Tyler, and J. P. Sumpter, Gonadotropins I and II do not stimulate the *in vitro* secretion of 17α,20β-dihydroxy-4-pregnen-3-one 20-sulphate by rainbow trout gonads during final sexual maturation, *Fish Physiol. Biochem.* **15,** 149–156 (1996).
27. NRA, "Pesticides in the Aquatic Environment," Report

of the National Rivers Authority, Water Quality Series No. 26, HMSO p. 88, 1995.
28. Y. Oulmi, R.-D., Negele and T. Braunbeck, Segment specificity of the cytological response in rainbow trout (*Oncorhynchus mykiss*) renal tubes following prolonged exposure to sublethal concentrations of atrazine, *Ecotoxicol. Environ. Safety* **32,** 39–50 (1995).
29. T. G. Pottinger and A. Moore, Characterisation of putative receptors in the membrane, cytosol and nuclear fractions from the olfactory tissue of brown and rainbow trout, *Fish Physiol. Biochem.* **16,** 45–63 (1997).
30. C. E. Purdom, P. A. Hardiman, V. J. Bye, C. R. Eno, C. R. Tyler, and J. P. Sumpter, Estrogenic effects of effluents from sewage treatment works, *Chem. Ecol.* **8,** 275–285 (1994).
31. A. P. Scott and N. R. Liley, Dynamics of excretion of 17α,20β-dihydroxy-4-pregnen-3-one 20-sulphate, and the glucuronides of testosterone and 17β-oestradiol, by urine of reproductively mature male and female rainbow trout (*Oncorhynchus mykiss*), *J. Fish Biol.* **44,** 117–129 (1994).
32. A. P. Scott, E. L. Sheldrick, and A. P. F. Flint, Measurement of 17α,20β-dihydroxy-4-pregnen-3-one in plasma of trout (*Salmo gairdneri* Richardson): Seasonal changes and response to salmon pituitary extract, *Gen. Comp. Endocrinol.* **46,** 444–451 (1982).
33. A. P. Scott, D. S. MacKenzie, and N. E. Stacey, Endocrine changes during natural spawning in the white sucker, *Catostomus commersonii*. II. Steroid hormones, *Gen. Comp. Endocrinol.* **56,** 349–359 (1984).
34. P. W. Sorensen, Hormones, pheromones and chemoreception, *in* "Fish Chemoreception" (T. J. Hara, Ed.), pp. 199–221, Chapman & Hall, London (1992).
35. L. M. Southwick, G. H. Willis, R. L. Bengtson, and T. J. Lormand, Effect of subsurface drainage on run-off losses of atrazine and metalochlor in Southern Louisiana, *Bull. Environ. Contam. Toxicol.* **45,** 113–119 (1990).
36. N. E. Stacey, J. R. Cardwell, N. R. Liley, A. P. Scott, and P. W. Sorensen, Hormonal sex pheromones in fish, *in* "Perspectives in Endocrinology" (K. G. Davey, R. E. Peter, and S. S. Tobe, Eds.), pp. 438–448, National Research Council, Ottawa, (1994).
37. C. E. W. Steinberg, R. Lorenz, and O. H. Speiser, Effects of atrazine on swimming behaviour of zebrafish, *Brachydanio rerio, Water Res.* **29,** 981–985 (1995).
38. J. Toppari, J. C. Larsen, P. Christiansen, A. Giwercman, P. Grandjean, L. J. Guillette, B. Jegou, T. K. Jensen, P. Jouannet, N. Keiing, H. Leffers, J. A. McLachlan, O. Meyer, J. Muller, E. Rajpert-De Meyts, T. Scheike, R. Sharpe, J. Sumpter, and N. E. Skakkebaek, Male reproductive health and environmental xenoestrogens, *Environ. Health Perspect.* **104,** 741–803 (1996).
39. Ye. M. Tyrryshkina and Ya. D. Finkinshteyn, Activity of olfactory nerves and bulbs of frogs when acetylcholine synthesis is blocked in the olfactory epithelium, *Fiziol. zhurn SSSR*, **60,** 1043–1048 (1974).
40. H. Von Westerhagen, V. Dethlefsen, P. Cameron, and D. Janseen, Chlorintaed hydrocarbons residues in gonads of marine fish and effects on reproduction, *Sarsia* **72,** 419–422 (1978).
41. C. P. Waring and A. Moore, F-series prostaglandins have a pheromonal priming effect on mature male Atlantic salmon (*Salmo salar*) parr, *in* "Proceedings of the Fifth International Symposium on Reproductive Physiology of Fish" (F. W. Goetz and P. Thomas, Eds.), pp. 255–257, University of Texas Press, Austin, TX (1995).
42. C. P. Waring, A. Moore, and A. P. Scott, Milt and endocrine responses of mature male Atlantic salmon (*Salmo salar* L.) parr to water-borne testosterone, 17,20β-dihydroxy-4-pregnen-3-one 20-sulphate, and the urines from adult female and male salmon, *Gen. Comp. Endocrinol.* **103,** 142–149 (1996).
43. C. P. Waring and A. Moore, Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar L.*) parr, *Fish Physiol. Biochem.* **17,** 203–211 (1997).
44. S. Winberg, R. Bjerselius, E. Baatrup, and K. B. Døving, The effect of Cu (II) on the electro-olfactogram (EOG) of the Atlantic salmon (*Salmo salar* L.) in artificial freshwater of varying inorganic carbon concentrations, *Ecotoxicol. Environ. Safety* **24,** 167–178 (1992).