Histological alternation and vitellogenin induction in adult rare minnow (*Gobiocypris rarus*) after exposure to ethynylestradiol and nonylphenol

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Abstract

Adult rare minnow (*Gobiocypris rarus*) were exposed to 0, 1, 5, and 25 ng/l (nominal concentrations) of 17 α -ethynylestradiol (EE₂) and 3, 10, and 30 μ g/l (nominal concentrations) of 4-nonylphenol (NP) under flow-through conditions for a period of 28 d. Low mortality was observed at 5 and 25 ng/l EE₂ and the growth of fish reduced significantly at 25 ng/l EE₂ compared to controls. However, the gonadosomatic indices (GSI) of male fish were significantly higher in 1 ng/l EE₂ treatments and in 10 and 30 μ g/l NP treatments ($p < 0.05$). Renal somatic indices (RSI) of male fish in EE₂ treatments were significantly higher than those in controls ($p < 0.05$). In contrast, significantly decreased GSI and RSI of female fish could only be observed in 5 and 25 ng/l EE₂ treatments ($p < 0.05$). Hepatosomatic indices (HSI) of male fish were significantly higher in 25 ng/l EE₂ treatments. However, significantly increased of HSI of female fish could only be observed in 1 ng/l EE₂ treatments. Plasma vitellogenin (VTG) induction could be observed in males after exposed to different concentrations of EE₂ and NP, and plasma VTG concentrations in females exposed to 5 and 25 ng/l EE₂ were also significantly higher than in controls ($p < 0.05$). At level higher than 5 ng/l EE₂ or 30 μ g/l NP, hepatic tissue and renal tissue impairment of males could be observed. The pathological male liver was associated with a hypertrophy of hepatocytes and damages to cellular structure and accumulated eosinophilic material. Renal tissue showed different pathological effects which was reflected by accumulated eosinophilic material, hemorrhages within the kidney tubules and hypertrophy of the tubular epithelia. Also at these levels of exposure, feminization of male fish could be noticed and parts of males manifested the testis-ova phenomenon. Ovaries of female rare minnow in 25 ng/l EE₂ treatment group were degenerated. Therefore when exposed to EE₂ and NP even at environmental observed concentrations, adverse effects could occur in the reproductive system of adult fishes. The observed hepatic tissue and renal tissue impairment should be due to the induction and accumulation of VTG in organs, especially in males.

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1. Introduction

There has been a growing concern on possible harmful consequences of exposure to xenobiotic compounds that

are capable of modulating or disrupting the endocrine system of wildlife species (Ankley et al., 1998). The group of compounds, both natural and man-made, have the capacity to mimic the actions of the female steroids, and is very diverse and includes compounds belonging to the group of organochlorine pesticides, polychlorinated biphenyls, dioxins, alkylphenols, phthalates as well as natural or synthetic female steroids (Jobling et al., 1995).

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4-Nonylphenol (NP) reaches the aquatic environment as a degradation product of nonylphenol ethoxylates (NPE). The concentrations of NP within the aquatic environment vary. They have been found to range from <0.2 to 12 µg/l in polluted British river waters (Blackburn and Waldock, 1995), and <0.3–45 µg/l in the Swiss river Glatt (Ahel et al., 1994). Several studies have been performed to evaluate the potential risk of NP exposure on the endocrine system, which include induction of the female specific egg yolk precursor vitellogenin in male rainbow trout (*Oncorhynchus mykiss*) (Jobling et al., 1996), development of testis-ova in medaka (*Oryzias latipes*), increasing of the ovosomatic index in medaka (Gray and Metcalfe, 1997).

The synthetic estrogen 17 α -ethynylestradiol (EE₂) is a chemical known for inducing vitellogenin in male individuals (Kime and Nash, 1999) and reduction of yolk filled oocytes of mature females (Van den Belt et al., 2001). However, EE₂ was observed extensive distribution in aquatic environment. The reported median concentration of EE₂ ranged from 1 to 17 ng/l in sewage treatment effluents and ranged from below the detection limit to 15 ng/l in surface waters (Stumpf et al., 1996; Ternes et al., 1999).

EE₂ and NP caused organ toxicity in fish, particularly in the liver and kidney, at relatively lower concentrations. Kidney impairment in fish based on the presence of eosinophilic material within renal tubule and tubule necrosis (Metcalf et al., 2001; Lynn et al., 2003) and accumulated eosinophilic material and enlarged and changes in hepatocyte vacuolization in the liver have been reported recently (Metcalf et al., 2001; Lynn et al., 2003). However, most of the work has been carried out with relatively high exposure concentration. In addition, there is a notable lack of studies that include histopathological examination of the gonads in adult, sexually mature fish after short-term exposure to endocrine disruptors, especially EE₂ or synthetic analogues.

Rare minnow is a fish species of the largest family Cyprinidae. The fishes distribute mostly in upstream waters in the Yangtze River, Sichuan Province, China. The species has been used for aquatic toxicity testing in China (Zhou et al., 1995). The fish are small (30–80 mm in total length) and easy to culture in the laboratory. They have a relatively short life cycle, spawning hundreds of eggs with high fertilization and hatching rates. For adult, sex could be easily determined by secondary characteristics (Wang, 1992). Ma et al. (2004) have reported endocrine disrupting effects on Chinese rare minnow (*Gobiocypris rarus*) fed with field collected *Limnodrilus* sp.

The objectives of the present study were (1) to observe histopathological changes in adult rare minnow exposed to EE₂ and NP at concentrations similar to those in the environment, (2) to build up the relationship of vitellogenin inducing and histological alteration, (3) to determine growth, mortality, gonadosomatic index, hepatosomatic index and renal somatic index, and organ impairs after EE₂ or NP exposure.

2. Material and methods

2.1. Chemicals

Regent 17 α -ethynylestradiol (EE₂) (98% purity) was obtained from Sigma (St. Louis, MO, USA). Technical nonylphenol (NP) was obtained from Sigma Aldrich (Deisenhofen, Germany). Technical nonylphenol consisted of 98% NP isomers (90% 4-NP, 10% 2-NP) and 2% dinonylphenol. Stock solution of EE₂ or NP was prepared by diluting the chemical in HPLC-grade acetone and mixing with distilled water, which was demineralized water. The final acetone concentration in the water was less than 0.01%. To obtain the final concentration for exposure, the appropriate amount of the stock solution of EE₂ and 4-NP was added to the aquarium water via a combination of polytetrafluorethylene (PTFE) and isoversinic tubes (Abimed, Langenfeld, Germany).

2.2. Fish

Rare minnow were provided by the Institute of Hydrobiology, the Chinese Academy of Sciences, China. The brood stock was kept in the follow-through de-chlorine tap water at 25 ± 2 °C with a photoperiod of 16:8 h (light: dark). Fish were generally fed with a commercial granule food (Trea, Germany) at a rate of 0.1% body weight per day and dry brine shrimp (*Artemia*) was supplemented for newly hatched fish.

2.3. Exposure and experimental design

Sexually mature rare minnow (about 7 months old) were the offspring from the same pair of brood stock. The body weights and body lengths were 1.4 ± 0.4 g and 47.3 ± 3.6 mm, respectively. One day prior to the experiment, sex of all fish were determined by observing the shape of the abdomen and the distance between the abdomen fin and the stern fin (Wang, 1992). Fifteen males and 15 females were then randomly assigned to each of seven 30-l glass tanks and acclimated for a week.

These fish were kept in the follow-through de-chlorine tap water 25 ± 2 °C with a photoperiod of 16:8 h (light: dark). Fish were generally fed with a commercial granule food (Trea, Germany) at a rate of 0.1% body weight per day and a dry diet of brine shrimp was supplemented. Waste and uneaten food were removed daily. After one week acclimation, fish were exposed to NP concentrations of 3, 10, or 30 µg/l and to EE₂ concentrations of 0, 1, 5, or 25 ng/l under flow-through conditions (flow rate of test solution was 10 l/h) for 28 d. Rare minnow exposed to 0.01% acetone for 28 d and all endpoints in 0.01% acetone treatment group have no significant difference with that of the control (de-chlorine water). Therefore, de-chlorine tap water was used for the controls in the results. There were no replicate chambers for each concentration.

2.4. Tissue sampling and analyses after 4 weeks exposure to NP and EE₂

2.4.1. Measurement of plasma vitellogenin level

Plasma vitellogenin level was measured using the carp vitellogenin ELISA kit (Bioscience, German). To avoid confounding results with sampling time, individual fish was taken sequentially from each treatment group. Fish were anesthetized in 200 ml of de-chlorine tap water containing 200 mg/l MS-222 (3-aminobenzoic acid ethyl ester methane sulfonate salt; Sigma, St. Louis, MO, USA, A-5040). Body length and wet body weight were determined. The root of the caudal fin of each fish was cut to collect blood with a heparinized microcapillary tube, and the blood volume was measured. The 250 µl blood was transferred to the sampling buffer attached to the ELISA kit. Then the diluted blood specimen was immediately centrifuged (8000g, 10 min, 4 °C) and frozen at –80 °C until use.

2.4.2. Gonadosomatic, hepatosomatic and renal somatic indices

Gonads, liver and kidney of each fish were removed and weighed after the blood was collected. Gonadosomatic index (GSI), hepatosomatic index (HSI) and renal somatic index (RSI) were calculated as following: $GSI = \text{gonad weights (g)} / \text{body weights (g)} \times 100$; $HSI = \text{liver weights (g)} / \text{body weights (g)} \times 100$; $RSI = \text{kidney weights (g)} / \text{body weights (g)} \times 100$.

2.4.3. Histological analysis

After complete necropsy of all fish from all treatment and control groups, tissues of gonad, liver and kidney were removed, gonads (including testis and ovaries), livers and kidneys of male rare minnows were fixed in Bouin's Solution and buffered formalin (4%), respectively, dehydrated and processed for paraffin wax embedding. The sections were cut at 3–4 µm and stained with hematoxylin and eosin (H&E). The sections of the paraffin blocks were monitored microscopically.

2.5. Statistics

All statistical analyses were performed by using Statistica (version 99) (Sattsoft. Inc., USA). The experimental data were checked for homogeneity of variance across treatments by using Levene's test. When the assumptions were met, the data were subjected to one-way analysis of variance followed by Dunnett's multiple comparison tests. When no homogeneity was observed, the nonparametric Kruskal–Wallis test was used, followed by the Mann–Whitney *U* test with Bonferroni's adjustment. Statistical significance was set at $p \leq 0.05$.

3. Results and discussion

3.1. Mortality and growth

After 28 d of exposure, the body weights and body lengths of rare minnow at 25 ng/l EE₂ were significantly lower than those in control (Table 1). One and three fish died during the 28 d exposure period at 5 ng/l EE₂ and 25 ng/l EE₂, respectively (Table 2). In previous studies, the decreasing of total body length and weight of zebrafish (*Danio rerio*) was observed after 24 d exposure to EE₂ at 10 ng/l (Van den Belt et al., 2002). In addition, Nimrod and Benson (1998) reported that newly hatched medaka (*Oryzias latipes*) fry exposed at 0.01 µg/l to 1.66 µg/l EE₂ for 28 d followed by a post-dosing 28 d recover period significantly reduced their total body lengths and weights.

3.2. Tissue somatic indices

Tissue somatic indices, such as the gonadosomatic index (GSI), hepatosomatic index (HSI) and renal somatic index (RSI) are general measurements of the overall condition of fish or the growth status of a specific tissue (West, 1990). Tissue somatic indices are also commonly reported in fisheries studies because the indices can be easily determined and some indices such as the HSI can be an excellent predictor of adverse health in fish (Adams and McLean, 1985). In the present experiments, GSI of males exposed

Table 1
Growth, gonadosomatic index (GSI), hepatosomatic somatic index (HSI) and renal somatic index (RSI) of adult rare minnow at the end of NP and EE₂ exposure period

Exposure concentrations	Body length (mm)		Body weight (g)		GSI (%)		HSI (%)		RSI (%)	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Control	49.4 ± 3.7 ^a	51.2 ± 4.1	1.4 ± 0.3	2.3 ± 0.8	3.4 ± 2.1	14.0 ± 5.4	2.3 ± 0.8	2.3 ± 0.2	0.5 ± 0.5	0.5 ± 0.3
3 µg/l NP	49.2 ± 4.2	51.6 ± 3.8	1.6 ± 0.6	2.4 ± 0.6	3.3 ± 2.2	12.4 ± 4.3	2.3 ± 0.6	2.7 ± 0.2	0.6 ± 0.3	0.6 ± 0.2
10 µg/l NP	49.7 ± 3.4	51.9 ± 4.3	1.5 ± 0.5	2.2 ± 0.7	4.0 ± 1.5*	12.3 ± 4.4	2.7 ± 0.5	2.6 ± 0.2	0.7 ± 0.4	0.5 ± 0.5
30 µg/l NP	49.1 ± 3.8	51.0 ± 3.5	1.4 ± 0.4	2.1 ± 0.5	4.9 ± 1.8*	13.4 ± 5.2	2.8 ± 0.6	2.1 ± 0.4	0.6 ± 0.4	0.6 ± 0.4
1 ng/l EE ₂	50.0 ± 4.2	52.2 ± 4.1	1.5 ± 0.5	2.2 ± 0.7	5.6 ± 0.8*	13.5 ± 5.8	2.8 ± 0.7	3.0 ± 0.2*	1.0 ± 0.4*	0.7 ± 0.4
5 ng/l EE ₂	49.2 ± 3.8	51.1 ± 3.2	1.5 ± 0.4	2.1 ± 0.8	3.9 ± 1.1	9.1 ± 5.0*	2.8 ± 0.9	2.3 ± 0.3	1.4 ± 1.3*	1.3 ± 0.4*
25 ng/l EE ₂	47.4 ± 3.2*	49.1 ± 3.1*	0.9 ± 0.6*	1.5 ± 0.5*	3.8 ± 1.2	4.4 ± 1.6*	3.5 ± 0.7*	2.5 ± 1.1	2.6 ± 1.4*	2.7 ± 0.9*

Asterisks indicate significant difference from the control ($p < 0.05$).

^a Data expressed as mean ± SE.

Table 2

Number of lesion gonads, liver and kidney of males by histological investigations at the end of NP and EE₂ exposure period

Concentrations	No. fish at the end of exposure period		No. lesion gonad		No. males with lesion liver	No. males with lesion kidney
	Males	Females	Testes-ova	Degeneration		
Control	15	15	0 ^a	0	0	0
3 µg/l NP	15	15	0	0	0	0
10 µg/l NP	15	15	0	0	4	0
30 µg/l NP	15	15	3	0	6	0
Control	15	15	0	0	0	0
1 ng/l EE ₂	15	15	1	0	6	2
5 ng/l EE ₂	14	15	6	1	10	7
25 ng/l EE ₂	13	14	9	5	13	12

^a Showed that this characters were not observed.

to 10 and 30 µg/l NP and 1 ng/l EE₂ was significantly greater than that of control, whereas GSI of males exposed to 5 and 25 ng/l EE₂ had no significantly effect compared to that of control. In addition, significantly lower GSI of female was only found at 5 and 25 ng/l EE₂ (Table 1). This indicates that male is more sensitive to EE₂ and NP exposure when GSI is used as endpoint. Increased GSI of male medaka might indicate the endocrine disruption effects while decreased GSI of female might due to ovaries degeneration (Scholz and Gutzeit, 2000). Scholz and Gutzeit (2000) reported GSI of male medaka was decreased at EE₂ exposure levels of 10 and 100 ng/l, respectively. Decreased GSI was also observed when male fathead minnows (*Pimephales promelas*) were exposed at 48 and 58.6 µg NP/l for 21 d (Harries et al., 2000).

Significantly higher HSI of males could only be observed at 25 ng/l EE₂ treatments (Table 1). However, hepatosomatic indices (HSI) of female fish were significantly higher in 1 ng/l EE₂ treatments. HSI of female fish in higher EE₂ treatment group had no significantly effect compared with that of the control. The HSI for females and male in other treatments contained no statistically differences compared to control groups.

RSI of males was significantly higher in treatments at concentrations higher than 1 ng/l EE₂ than that of control. For females, RSI of fish at 5 and 25 ng/l EE₂ were significantly higher than that of control (Table 1). In NP treatments, no statistical significance between treatments and control of both male and female fish were found. The results suggest that renal tissue may be less affected by NP than by EE₂.

In summary, the lowest-observed-effect concentrations (LOECs) calculated based on tissue somatic indices were 10 µg/l for NP and 1 ng/l for EE₂ and the male rare minnow were generally more sensitive to the chemicals compared to female ones.

3.3. Plasma vitellogenin (VTG) concentrations

After 28 d of exposure to NP and EE₂, plasma VTG concentrations of males ranged from 150 ng/ml plasma in the control to 620 000 ng/ml plasma in 25 ng/l EE₂ exposure

group (Fig. 1), while plasma VTG concentrations of females ranged from 300 000 ng/ml plasma in the control to 500 000 ng/ml plasma at 25 ng/l EE₂ (Fig. 1). Plasma VTG concentrations of male from all NP and EE₂ treatments were significantly increased compared to controls. However, the plasma VTG concentrations of females increased significantly only in 5 and 25 ng/l EE₂ treatments (Fig. 1).

There were evidences that NP and EE₂ could induce the plasma VTG of male fish. Harries et al. (2000) reported that male fathead minnows exposed to 48.8–58.6 µg/l NP had plasma VTG levels 4000–45 000 times higher than males in control. Increased VTG mRNA expression was observed in liver of male medaka after 7 d exposure to nominal 4-nonylphenol concentrations of 20 and 50 µg/l (Islinger et al., 2002). In adult male zebrafish, a 21 d exposure to EE₂ at measured concentrations down to 5 ng/l caused significant increases in VTG formation in blood plasma (Van den Belt et al., 2001). A similar result was also obtained in a study which exposed adult zebrafish for 14 d to a nominal EE₂ concentration of 5 ng/l and the level of VTG induction was roughly 3-fold higher in female than in male zebrafish (Kime and Nash, 1999). In a recently study, Pawlowski et al. (2004) reported that the level of VTG of plasma of mature fathead minnow exposed to 1 ng/l EE₂ was significantly elevated. The present study indicates that concentrations of 3 µg/l NP or 1 ng/l EE₂ could induce significant increase of plasma VTG of male rare minnows, suggesting that mature rare minnow was among the most sensitive species to endocrine disruptors.

3.4. Histopathology

3.4.1. Gonads

The histological changes of ovaries of all observed female fish over 28 d exposure to 3, 10, and 30 NP µg/l or 1 and 5 ng/l EE₂ were similar to those in control (Fig. 2A). However, ovaries were severely degenerated when mature female rare minnow were exposed to 25 ng/l EE₂ (Table 2, Fig. 2C). Histological inspection of the ovaries revealed all stages of oocyte development including primary, secondary, and tertiary follicles at all exposure groups (Figs. 2A and C).

Fig. 1. Plasma VTG concentrations in mature male and female rare minnow after 28 d exposure to EE₂ and NP. Asterisks indicate significantly difference from the control ($p < 0.05$). The error bars indicate standard deviation of the mean.

For fish in control, seminiferous tubules of testes were filled with large numbers of fertile sperm (Fig. 2B). In contrast, only primary and secondary spermatogonia could be detected and no more sperm were detectable at 5 and 25 ng/l EE₂ and 30 µg/l NP treatment (Fig. 2D). In addition, oocytes were detected in testes at this level of observation (Table 2, Fig. 2D).

Testes-ova of mature rare minnow were detected at relatively low concentrations of EE₂ and NP. However, some studies reported that other adult fish exposed to endocrine disruptors were not detected with testes-ova. For example, mature fathead minnow exposed to 10 and 100 ng/l EE₂ could not be observed with testes-ova and only showed degeneration of gonads (Pawlowski et al., 2004). In addition, adult zebrafish exposed to 10 ng/l and 25 ng/l EE₂ reduced the number of mature, yolk-filled oocytes (Van den Belt et al., 2001). In contrast, Gray and Metcalfe (1997) reported medaka larvae exposed for three months to NP at concentrations of 50 and 100 µg/l were shown to undergo testis-ova formation in males. Reinhard et al. (2001) also reported that long-term exposures resulted in

formation of ova-testes and the appearance of sex reversals in fathead minnows after 305 d of exposure to EE₂ at 4 ng/l. Gray and Metcalfe (1997) also reported that medaka with long-term exposure to *p*-nonylphenol at 50 µg/l caused the formation of ova-testes. This report showed a difference in adult fish with the formation of ova-testes at relatively low concentrations of endocrine disruptors. So our results showed that rare minnows were fish sensitive enough to screen endocrine disruptors.

3.4.2. Hepatic tissue

In male rare minnows, abundant cytoplasmic lipid in hepatocytes was observed in control and 3, 10 µg/l NP and 1 ng/l EE₂ treatment (Fig. 3A). After 28 d of exposure, hepatic tissue of all male fish at 30 µg/l NP and 5, 25 ng/l EE₂ treatment showed hypertrophy of the hepatocytes and damaged cellular disarray, as well as an enlargement of the cell nuclei accompanied by a distinct reduction of glycogen deposits (Table 2, Fig. 3C). In addition, a lot of eosinophilic material was observed to have accumulated in hepatic tissue (Fig. 3B). An increase in liver cell size detected in estradi-

Fig. 2. Light micrographs of gonad tissue in adult rare minnow, stained with H&E: (A) normal mature ovary from control ($\times 100$); (B) normal mature testis from control ($\times 400$); (C) degeneration ovary from 25 ng/l EE2 exposure group ($\times 100$); (D) ovary-testis from high dose EE2 and NP groups; arrow shows perinucleolar oocytes ($\times 400$).

diol-treated flounder was suggested to be the consequence of enhanced metabolic activity (Zaroogian et al., 2001) or the consequence of the stimulation of the vitellogenin synthesis when exposed to EE2 (Purdum et al., 1994). An estrogen-dependent depression in gluconeogenesis had been demonstrated in rainbow trout (Cevdet et al., 2003). A reduction of hepatic glycogen deposits observed in the present work has also been observed in estrogen-treated carp (Schwaiger et al., 2000) and flounder (Zaroogian et al., 2001). The results showed that endocrine disruptors caused hepatic tissue impairment of rare minnows due to inducing and accumulating VTG. We thought that inducing and accumulating VTG was a main reason for the impairing of hepatic tissue. However, the chronic toxicity of endocrine disruptors in relative environmental concentrations was only a secondary reason.

3.4.3. Renal tissue

In male rare minnows, normal renal tissues are composed of numerous renal corpuscles with well-developed glomeruli and system of tubules. The segment is lined with large, clear columnar epithelial cells with central nuclei and the brush border is reduced or non-existent (Table 3, Fig. 3D). Renal tissues of fish from all NP treatment and 1 ng/l EE₂ treatment were shown to be normal. After

exposed at 30 $\mu\text{g/l}$ NP and 5, 25 ng/l EE₂, lesions of renal tissue were characterized by severe hemorrhaging within the kidney tubules, the Bowman's space and the renal interstitium (Table 3, Fig. 3E). Distinct changes of the tubular epithelia could be observed, including hypertrophy, degeneration and necrotic of epithelia (Fig. 3F). In addition, we observed a lot of esinophilic material accumulated in the sinusoids of the head kidney (Fig. 3E). Accumulation of esinophilic material was reported in flounder injected with 10 mg/kg E₂ (Zaroogian et al., 2001). Esinophilic material was most probably VTG (Herman and Kincaid, 1988), synthesized under the stimulation of estrogenic chemicals such as EE₂, and accumulated in various tissues. The hypertrophy and degenerative and necrotic of the tubular epithelia were observed in estrogen treated carp (Schwaiger et al., 2000) and flounder (Zaroogian et al., 2001). The results showed that renal tissue impairment of rare minnow was caused not only by chronic toxicity of endocrine disruptors but also by VTG accumulation. Therefore we concluded that renal impairment of fish exposed to relative environmental concentrations endocrine disruptors were mainly due to VTG accumulation.

In summary, synthetic steroids and synthetic analogues resulted in plasma vitellogenin concentrations increasing and gonadal sex reversal in adult rare minnows. Synthetic

Fig. 3. Light micrograph investigation of hepatic and renal tissue of male adult rare minnow (HE, 400 $\times$): (A) normal hepatic tissue from the control; (B) hepatic tissue from 5 ng/l EE₂ treatment group, showing accumulated eosinophilic material; (C) hepatic tissue from 25 ng/l EE₂ treated group showing swelling of hepatocytes and damaging with cell structure and enlargement of cell nuclei (arrow); (D) normal renal tissue; (E) renal tissue from 5 ng/l treatment group (arrow and arrowhead shows hemorrhages within the lumen of kidney tubules and esinophilic material accumulated, respectively); (F) renal tissue from 25 ng/l EE₂ treated group (arrow shows necrotic of epithelia).

steroids (1, 5, and 25 ng/l EE₂) and synthetic analogues (3, 10, and 30 μ g/l NP) induced significant increase of plasma VTG concentrations in male adult rare minnow. However, only relatively high exposure concentrations of synthetic steroids (5, 25 ng/l EE₂) and synthetic analogues (30 μ g/l NP) induced testes-ova and caused severe lesions of liver with a hypertrophy of hepatocytes and damages to cellular structure and accumulated eosinophilic material and kidney by accumulated eosinophilic material, hemorrhages

within the kidney tubules and hypertrophy of the tubular epithelia. Growth and tissue somatic indices were also sensitive endpoints. Since plasma VTG concentration changed and testes-ova were induced during the NP or EE₂ exposure, hepatic and renal impairment might be due to the induction and accumulation of VTG in organs, especially in males. The finding would be useful for detecting endocrine disrupting activity with the rare minnow in laboratory studies as well as in the field.

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