THE ACUTE EFFECTS OF PERFLUOROOCTANE SULFONATE ON MICROSTRUCTURE OF THE GONAD OF ZEBRAFISH (*DANIO RERIO*)

Hu Q[1,2], Zhang J[2], Zhou Q F[1], Liang Y[1,3]

[1] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China; [2] Key lab of subtropical agriculture & environment, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, 430070, China; [3] Faculty of Medicine, Jianghan University, Wuhan, 430056, China

Abstract

The objective of this study was to determine concentration dependent effects of PFOS on gonad morphology. After adult zebrafish were exposed to 0, 0.5, 1 and 2mg/L PFOS for 7 days, results of paraffin section and scanning electron telescope revealed that PFOS could induce obvious histopathological changes of sex gonads, which included reduced number of spermatogonia or spermatophore sacs, appearance of hollow spermatophores together with congestion in spermary of male fish and relaxed follicle, less and unclear yolk granules, large vacuoles, and irregularity in the shape of the oocytes in ovary of female fish, and degrees of these lesions were all dose-dependent. Considering the persistent nature of PFOS, more research is required to determine potential consequences of long-term exposure in aquatic ecosystems.

Introduction

Perfluorinated surfactants have emerged as priority environmental contaminants due to recent reports of their detection in environmental and biological matrices. Perfluorooctane sulfonate (PFOS), found widely in wildlife and humans, is environmentally and metabolically stable. Environmental PFOS may be from its use as a surfactant, hydrolysis of perfluorooctanesulfonyl fluoride, and degradation of perfluorooctanesulfonamide compounds formerly used in numerous applications. In numerous independent studies, the greatest concentration of PFOS in liver of mink from the United States was 5140 ng/g, wet weight, and PFOS was detected in livers of all river otters collected from Washington and Oregon at concentrations ranging from 25 to 994 ng/g, wet weight [1].The potential toxicity of PFOS, such as hepatotoxicity, interference with mitochondrial bioenergetics, impeding intercellular communication through gap junctions, endocrine dysfunction, effects on development and reproduction, carcinogenicity and induction of the yolk precursor protein, vitellogenin (VTG), has been reported recently [2,3,4,5]. However, the consequences of acute PFOS exposure on gonads of adult individual were still limited. Zebrafish (*Danio rerio*) was chosen as the test species for this study because of its small size, ease of maintenance and short reproductive cycle. The objectives of the present study were to determine concentration dependent effects of acute PFOS exposure on gonad morphology of zebrafish by paraffin section and scanning electron microscopy (SEM).

Materials and Methods

Chemicals

Perfluorooctane sulfonate (PFOS, potassium salt; ≥98% pure; FW 538.22) was purchased from Sigma-Aldrich.

Stock solutions of PFOS were prepared in DMSO (dimethyl sulfoxide, Amresco, USA). Solvent concentration was kept at 0.01% (v/v) throughout the experiment.

Exposure assays

Adult zebrafish (wild-type, AB strain) were obtained from Institute of Hydrobiology, Chinese Academy of Sciences. Forty of each sex were chose randomly and divided evenly into four groups exposed to nominal concentrations of PFOS (0.5, 1 or 2 mg/L) or 0.01%DMSO (control) for 7 days, and each group was individually heated using a aquarium heater to maintain a temperature of 26-28 ℃. Aeration and filtration were provided using sponge filters. Fish were maintained on a photoperiod of 14 h light: 10 h dark with pH ranging from 7.0 to 7.6 throughout the duration of the experiment, and fed freshly hatched brine shrimp (*Artemia saline*) twice daily. Fish were allowed to acclimate to laboratory conditions for 7 days prior to the experiment.

Histological examinations

At the end of exposure, 6 fish from each treatment were euthanized using an overdose of MS-222 (3-aminobenzoic acid ethyl ester, methanesulfonate salt). Gonads were removed from individual fish and part of these tissues were fixed in polyoxymethylene fixative for 24 h then transferred to serial ethanol (75%, 85%, 95% and 100%) to dehydrate and embedded in paraffin wax. Longitudinal 5 mm sections were taken by ultramicrotome (Leica RM2145, Germany) and stained using hematoxylin and eosin, and cover slips were mounted with Permount. Sections were examined under a light microscope (Olympus BX51, Japan) to assess the lesions of gonad tissues of both sexes.

Scanning electron microscopy

For scanning electron microscope (SEM) observation, remaining part of the gonad tissues from those fish at the end of exposure were fixed in 2.5% glutaraldehyde for 24 h at 4 ℃ then rinsed with 0.1M PBS for three times, and then were fixed in 1% osmium, dehydrated with serial ethanol (30%, 50%, 70%, 85%, 95% and 100%) and dried with liquid CO_2. The tissues membranes were detached and coated with gold/palladium in an argon atmosphere in a vacuum evaporator (Hitachi E-1010, Japan) and then examined with a scanning electron microscope (Hitachi S-3000N, Japan).

Results and Discussion

Histological examination of gonad sections from control zebrafish indicated normal testicular and ovarian (Fig. 1a and e). No obvious histological abnormality was observed in 0.5mg/L PFOS groups. There was a mild reduction in the number of spermatogonia in the spermary from male fish of 0.5mg/L PFOS, accompanied by appearance of small hollow spermatophores (Fig. 1b). In the spermary from male fish of 1mg/L PFOS, there was reduced number of spermatogonia and spermatophore sacs, accompanied by congestion with erythroblasts (Fig. 1c). Gonad histology revealed significant abnormal testicular morphologies in male fish exposed to 2mg/L PFOS, besides reduced number of spermatogonia and spermatophore sacs, hollow spermatophores and congestion were also observed (Fig. 1d). In the ovary from female fish of 0.5mg/L PFOS, previtellogenic follicles were arranged slightly relaxed (Fig. 1f). Other than relaxed previtellogenic follicles, there were less and unclear yolk granules

in mature follicles in the ovary from female fish of 1mg/L PFOS (Fig. 1g). Severe disruptions including relaxed previtellogenic follicles and less mature follicles were observed in the ovary from female fish of 2mg/L PFOS. Furthermore, appearance of large vacuoles, and irregularity in the shape of the oocytes were also observed (Fig. 1h). The relative severity degree of pathological changes of the gonads between male and female fish in paraffin sections were showed in Table 1.

Photos of gonad surface taken by SEM were complement and confirmation to the results of histological examination. However, it was unable to explore the inside of the tissues, only photos taken from sections of the spermary from male fish were showed. From outside of the spermary, numerous spermatophore sacs could be identified, each of which contained abundant sperms (Fig. 2a and b). Exposure to a high concentration of PFOS (2mg/L) appeared less number of spermatophore sacs compared to control (Fig. 2c), and congestion with flat erythroblasts inside spermatophore sacs (Fig. 2d).

PFOS has been suggested to interfere with mitochondrial bioenergetics [6,7], and a previous study has reported an increase in caspase-3 activity and apoptosis in cultured tilapia hepatocytes after PFOS exposure [8]. PFOS in rats and mice showed developmental toxicity and other adverse effects *in vivo*. These effects included reduction of fetal weight, cleft palate, anasarca, delayed ossification of bones, and cardiac abnormalities, as well as, decreased neonatal survival, reduction in mean post natal body weight, and a significant delay in sexual maturation [6,9,10,11]. In this study, the findings clearly indicated that PFOS exposure could impair the reproductive systems of both male and female zebrafish. Numerous studies also have shown that fish exposed to environmental endocrine disrupting chemicals experienced disrupted sex differentiation, and reproductive dysfunction [12]. Such consequences have the potential to impact the stability of wild fish populations, given its high potency and wide distribution. Considering the persistent nature of PFOS, more investigation is required to be conducted to evaluate potential toxicity effects of long-term exposure in aquatic ecosystems.

Table 1. Comparison of pathological severity of the gonad between male and female zebrafish in paraffin sections(n=4)

	Spermary	Ovary
Control	−	−
0.5mg/L PFOS	+	+
1mg/L PFOS	++	++
2mg/L PFOS	+++	++

Relative severity degree of pathological changes within each column indicated by (−): no pathological changes seen; (+): mild; (++): moderate; (+++): heavy.

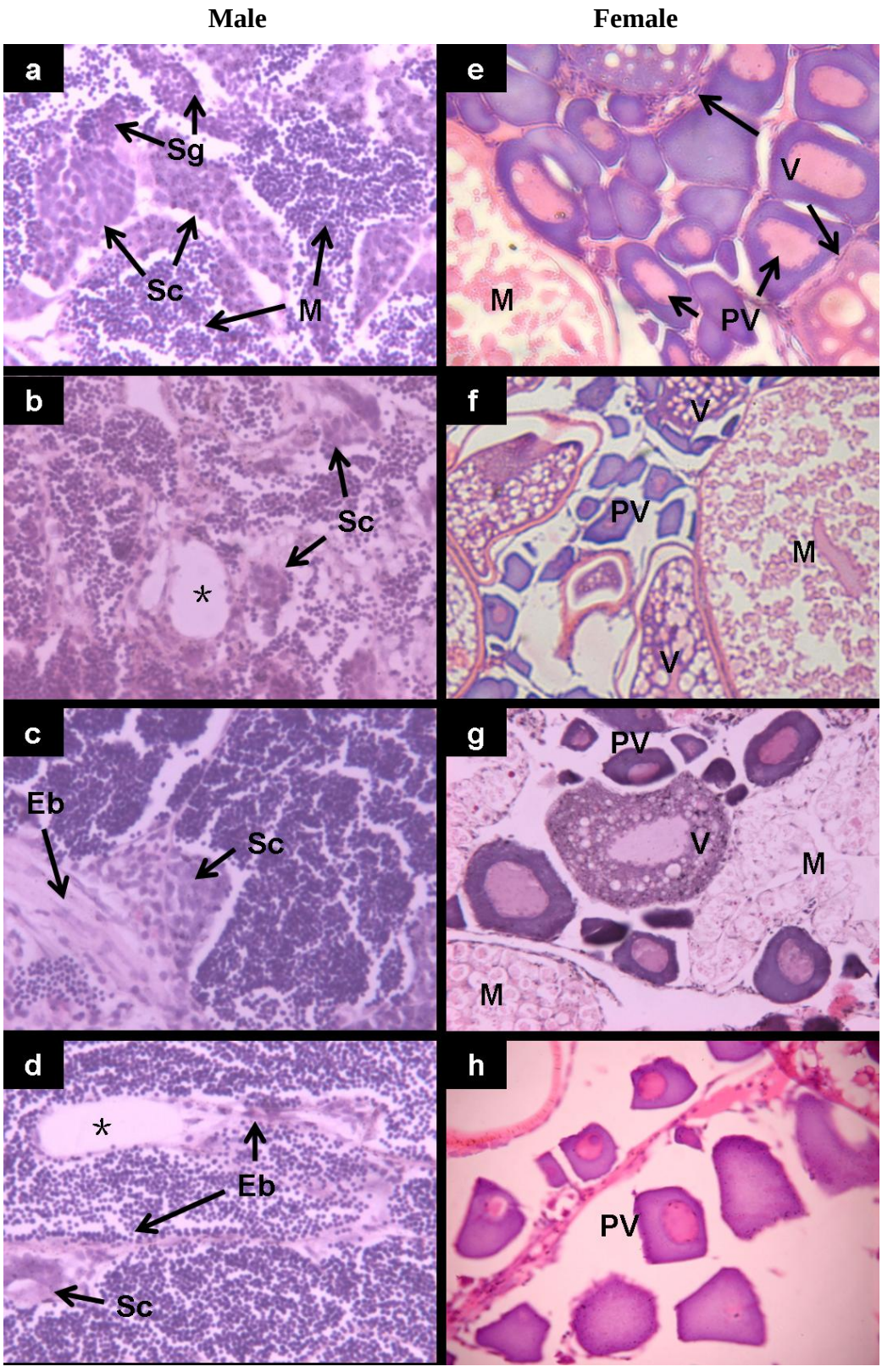
Male
Female
a
Sg
Sc
M
e
V
M
PV
b
Sc
*
f
V
PV
M
V
c
Eb
Sc
g
PV
V
M
M
d
*
Eb
Sc
h
PV

Fig.1 The lesions of gonads of zebrafish exposed to PFOS observed in paraffin sections (objective, 40×). (a) Spermary from male fish of control; (b) Spermary from male fish of 0.5mg/L PFOS; (c) Spermary from male fish of 1mg/L PFOS; (d) Spermary from male fish of 2mg/L PFOS; (e) Ovary from female fish of control; (f) Ovary from female fish of 0.5mg/L PFOS; (g) Ovary from female fish of 1mg/L PFOS; (h) Ovary from female fish of 2mg/L PFOS. Sc, spermatocyte; Sg, spermatogonia; M, mature sperm or follicle; Eb, erythroblast; PV, previtellogenic follicle; V, vitellogenic follicle.

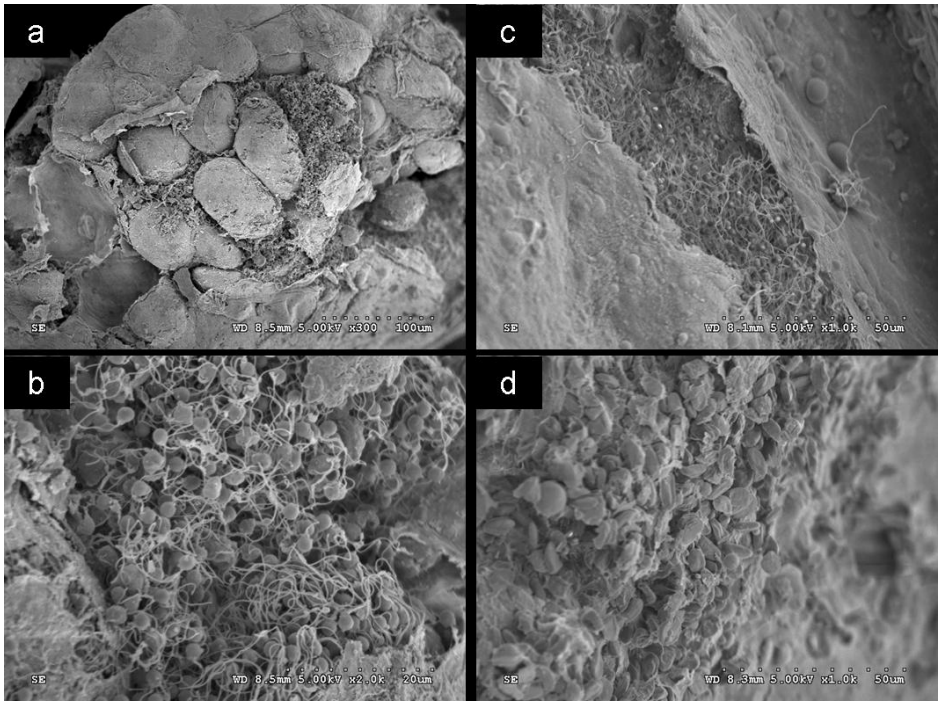

Fig.2 The lesions of spermaries of zebrafish exposed to PFOS observed under scanning electron telescope. (a) Outside spermatophore sacs from male fish of control; (b) Inside spermatophore sacs from male fish of control; (c) Outside spermatophore sacs from male fish of 2mg/L PFOS; (d) Inside spermatophore sacs from male fish of 2mg/L PFOS.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (No. 20890112), and National Key Technology R&D Programe (No. 2007BAC27B01).

References

1. Kannan K., Newsted J., Halbrook R. S.and Giesy J. P. *Environ. Sci. Technol.* 2002; 36(12):2566-2571.
2. Lau C., Butenhoff J. L.and Rogers J. M. *Toxicol. Appl. Pharmacol.* 2004; 198: 231-241.
3. Austin M. E., Kasturi B. S., Barber M., Kannan K., MohanKumar P. S.and MohanKumar S. M. J. *Environ. Health Perspect.* 2003; 111: 1485-1489.
4. Hu W., Jones P. D., Upham B. L., Trosko J. E., Lau C.and Giesy J. P. *Toxicol. Sci.* 2002; 68: 429-436.

5. Du Y. B., Shi X. J., Liu C. S., Yu K.and Zhou B. S. *Chemosphere*.2009; 74: 723-729.
6. Starkov A. A.and Wallace K. B. *Soc Toxicol.* 2002; 66(2): 244-252.
7. O'Brien T. M.and Wallace K. B. *Toxicol Sci.* 2004; 82(1): 333-340.
8. Liu C.S., Yu K, Shi X. J, Wang J. X., Lam P. K. S., Wu R. S. S.and Zhou B. S. Aquat Toxicol. 2007; 82(2): 135-143.
9. Case M. T., York R. G.and Christian M. S. *Int. J. Toxicol.* 2001; 20(2): 101-109.
10. Lau C., Thibodeanx J. R.and Hanson R. G. *Toxicol. Sci.* 2003; 74(2): 382-392.
11. Thibodeaux J. R., Hanson R. G.and Rogers J. M. *Toxicol. Sci.* 2003; 74(2): 369-381.
12. Weber L. P., Hill Jr. R. L.and Janz D. M. *Aquat. Toxicol.* 2003; 63(4): 431-446.