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EFFECT OF ESTROGENIC ACTIVITY IN RIVER WATER AND TREATED WASTEWATER ON FEMINIZATION OF JAPANESE MEDAKA

Kiyoaki Kitamura, Koya Komori, Kiyoshi Miyajima, Tadashi Higashitani,
Norihide Nakada and Yutaka Suzuki*

ABSTRACT: The objective of the study is to evaluate the effect of estrogens in river water and treated wastewater on fish feminization. Male Japanese Medaka (*Oryzias latipes*) was used for exposure test of various concentrations of estrone (E1) or 17beta-estradiol (E2), river water and treated wastewater. Generation of vitellogenin in the liver of male Medaka was measured as the index of fish feminization. The effective concentrations of E1 and E2 on feminization of Medaka was evaluated by breeding for 14 days in tanks with the E1 or E2 concentration of 5, 12.6, 31.6, 79.5 and 200 ng/L, respectively. Induced vitellogenin concentration increased along time during the exposure, and the higher estrogen concentration caused the more vitellogenin generation. From the test results, LOEC (Lowest Observed Effect Concentration) of E1 and E2 were estimated as 31.6 and 5.0 ng/L, respectively, and the E2 equivalent of LOEC of E1 was 9.5 ng-E2/L by multiplying the conversion factor 0.3. To investigate the influence of river water or treated wastewater, Medaka was exposed to each water continuously for totally 44 weeks. Recombinant yeast assay was applied to measure the total estrogenic activity of the water. The estrogenic activity, expressed as E2 equivalent concentration, changed widely during the exposure tests, and vitellogenin induction was observed after high level of estrogenic activity in water with an interval of several days. The vitellogenin concentration had the tendency of significant increase if the estrogenic activity exceeded 10ng-E2/L. These results were substantially the same as the results of E1 and E2 exposure tests. Consequently, estrogenic activity over 10 ng-E2/L was revealed to have a potential of causing feminization of male Medaka. According to our nationwide survey of 200 rivers, most of estrogenic activity in river water was ranged from 0.1 to 1 ng-E2/L, however, in some urban rivers receiving treated wastewater, estrogenic activity larger than 10 ng-E2/L was observed. Therefore, in urban rivers, estrogenic activity should be decreased more by wastewater treatment to prevent fish feminization.
KEY TERMS: Japanese Medaka, estrogenic activity, estrone, 17beta-estradiol, feminization, vitellogenin induction

INTRODUCTION

Over the past decade, the effect of endocrine disrupter chemicals (EDCs) on aqueous creatures has raised a number of concerns. For example, feminization of male fish has been observed in various rivers in Japan. In one urban river, approximately 30% of the male carp (*Cyprinus carpio*) generated vitellogenin, one of the specific proteins for female species.

Treated wastewater was considered the cause of feminization since its occurrence was often observed downstream from wastewater treatment plants. Treated wastewater contains various EDCs such as nonylphenol, bisphenol A and dialkylphthalate, and natural estrogens such as 17beta-estradiol (E2) and estrone (E1). However, major contributory chemicals to fish feminization had not yet been clarified.

On the other hand, the LOEC of these chemicals has been determined through numerous studies. Therefore, clarifying the relationship between the concentration of EDCs and estrogens and the feminization effect should provide information about the potential of river water or treated wastewater to promote feminization.

This paper describes the results of exposure tests using river water or treated wastewater and deals with the effect of estrogens in river water and treated wastewater on fish feminization.

Previous PWRI Study

As described above, many chemicals have estrogenic potential. However, measuring the chemical concentration does not provide the total estrogenic potential since each chemical differs in concentration and estrogenic potential. The PWRI uses estrogenic activity as the index of total estrogenic potential of river water and treated wastewater. Estrogenic activity is estimated by recombinant yeast as the E2 equivalent (Routledge et al., 1996). Relative estrogenic activity of estrone (E1) and

*Respectively, Senior Researcher, Senior Researcher, former Senior Researcher, former Researcher, former Researcher, Team Leader, Public Works Research Institute, Water Environment Research Group, Water Quality Team, Minamihara 1-6, Tsukuba, Ibaraki, Japan, Phone: +81-29-879-6777, Fax: +81-29-879-6748, Email: k-kitamu@pwri.go.jp

nonylphenol to that of E2 by recombinant yeast assay is 0.3 and 0.001, respectively (Yakoh et al., 1999).

The investigation of fractionated treated wastewater by HPLC revealed that the major contributing chemicals for estrogenic activity in treated wastewater are E1 and E2, with E1 being dominant. Nonylphenol contributed only slightly (Nakada *et al*., 2006).

PWRI also investigated estrogenic activity in untreated wastewater, treated wastewater and river water at various sites throughout Japan. The wide-ranging occurrence of estrogenic activity in river water and in wastewater can be seen in Fig. 1. Estrogenic activity in river water is about two orders lower than that in treated wastewater, which can be reduced through wastewater treatment.

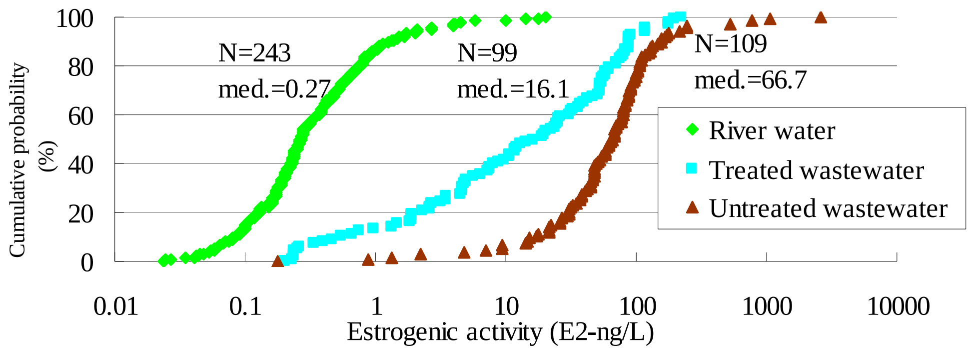

Figure 1. Occurrence of estrogenic activity in river water and in wastewater

Methodology

In order to determine LOEC, Medaka were bred in tanks with E1 or E2 concentration of 5, 12.6, 31.6, 79.5 and 200 ng/L for 14 days. A total of 60 mature male Medaka were treated in each tank. After the exposure tests, vitellogenin concentration in the liver was measured.

The exposure tests using river water or treated wastewater were carried out using specially made equipment. Test water was continuously led into this experimental apparatus for over two weeks. The temperature of the test water was controlled at 25°C and suspended solids were removed by sedimentation and sieves. The concentration of estrogens and EDCs and the estrogenic activity was measured during the exposure period. During the exposure tests, vitellogenin concentration in the liver and estrogenic activity was checked. The exposure tests were carried out for a total of 20 weeks for the river water test and 24 weeks for the treated wastewater test.

E1 and E2 were analyzed by LC/MS/MS. Vitellogenin in the liver of male Medaka was measured by ELISA method after homogenization. Estrogenic activity was measured using recombinant yeast.

RESULTS AND DISCUSSION

LOEC of E1 and E2 to induce vitellogenin

Average vitellogenin concentration after 14 days of exposure to E1 or E2 is shown in Fig. 2. Vitellogenin was induced as early as 2 days after exposure and its concentration increased with time during the exposure period. Vitellogenin concentration after 14 days was considered saturated concentration. Vitellogenin concentration was dependent on the exposure concentration, and a higher estrogen concentration caused greater vitellogenin generation. Using the statistical treatment of the Shirley-Williams method, LOEC after 14 days of exposure was estimated as 31.6 ng/L for E1 and 5.0 ng/L for E2. If the LOEC of E1 is transformed to the E2 equivalent by multiplying by conversion factor 0.3 (relative estrogenic

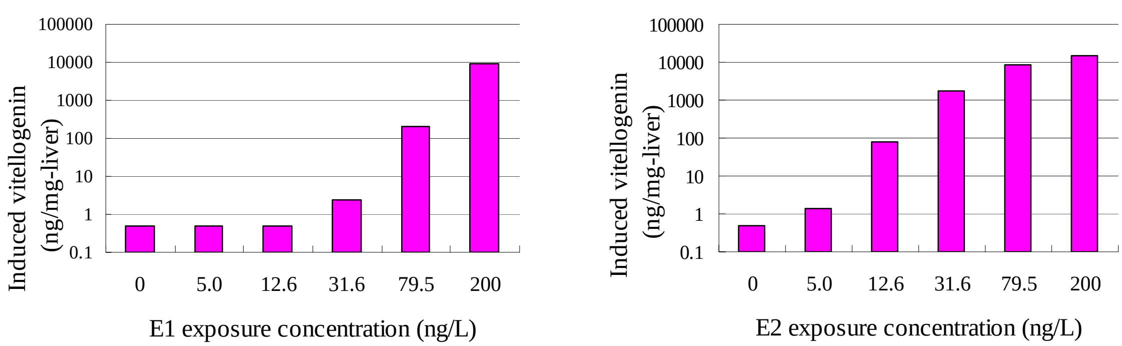

Figure 2. Concentration of induced vitellogenin by E1 or E2 exposure test

activity towards E2 estimated by recombinant yeast),the LOEC of E1 is calculated as 9.5 ng-E2/L of estrogenic activity. Therefore, both exposure tests support the theory that the threshold E2 equivalent estrogenic activity to induce vitellogenin is around 5 or 10 ng-E2/L.

Exposure test using river water

Estrogenic activity in the river water used for the exposure test was relatively high compared to the data shown in Fig. 1 since the source river runs through an urban area and the river water contains a percentage of treated wastewater.

Through the four series of exposure tests using river water, a remarkable concentration of vitellogenin was induced only during Test 3, as shown in Fig. 3. In Test 3, the average estrogenic activity was 14.6 ng-E2/L, which is the largest average concentration among the four tests and slightly larger than the threshold level of 10 ng-E2/L. On the contrary, the average estrogenic activity was less than 10ng-E2/L and vitellogenin was scarcely induced during the other three tests.

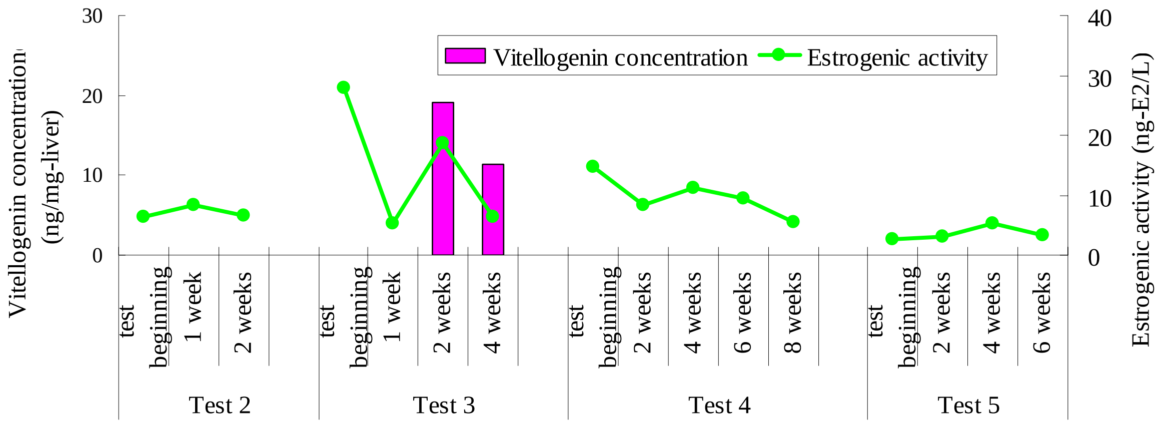

Figure 3. Estrogenic activity and vitellogenin induction during exposure test using river water

Exposure test using treated wastewater

Estrogenic activity in the treated wastewater used for the exposure test moved extensively and rose to almost 100 ng-E2/L in Test B.

Through the four series of exposure tests using treated wastewater, definite vitellogenin induction was observed in Tests B and C, as shown in Fig. 4. In Test B, the average estrogenic activity was 28.2 ng-E2/L, which is considerably larger than the threshold level. In Test C, estrogenic activity at the beginning of the test was 32.3 ng-E2/L, which is also markedly larger than the threshold level, although the average estrogenic activity was slightly less than the threshold level. In this case, vitellogenin might be induced after an interval of several days.

In Tests A and D, only a small concentration of vitellogenin was induced and relatively low estrogenic activity was observed, similar to the results of Tests 2, 4, 5 for the Medaka in river water.

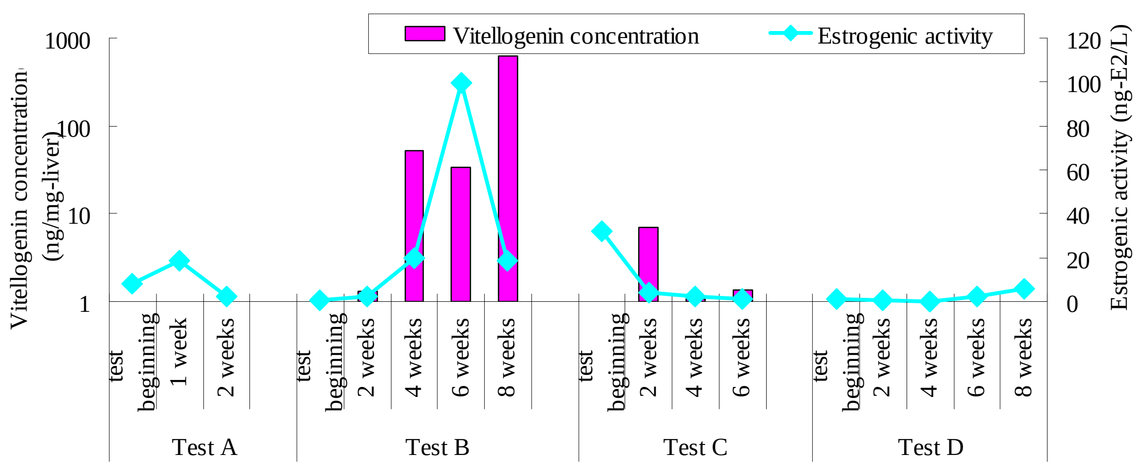

Figure 4. Estrogenic activity and vitellogenin induction during exposure test using treated wastewater

Threshold level to induce vitellogenin

Figure 5 summarizes the relationship between estrogenic activity in the test water and induced vitellogenin concentration in male Medaka. All data from the exposure tests using river water or treated wastewater and converted LOEC of E1 and E2 are indicated together in this figure.

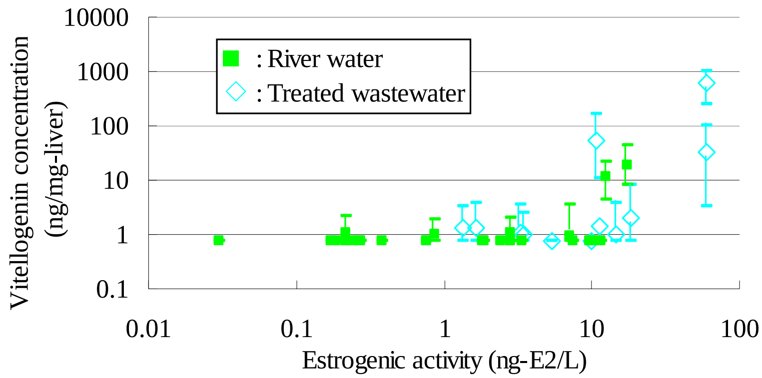

Figure 5. Relationship between estrogenic activity in test water and induced vitellogenin concentration in male Medaka

There is an increasing tendency in vitellogenin induction when estrogenic activity exceeds 10 ng-E2/L in both river water exposure and treated wastewater exposure. Converted LOEC of E1 and E2 is also around 10 ng-E2/L. Consequently, estrogenic activity measured by recombinant yeast can be used as a principal index of environmental water to predict feminization of fish, and approximately 10 ng-E2/L is considered the threshold level to induce vitellogenin in the liver of male Medaka.

Potential of river water and treated wastewater to promote fish feminization

If the threshold level of 10 ng-E2/L as estrogenic activity to induce vitellogenin in the liver of male Medaka is compared to the nationwide data shown in Fig. 1, 3 of 247 samples from river water and approximately 60% of the samples from treated wastewater exceed the threshold level. Therefore, estrogenic activity in treated wastewater should be further reduced through wastewater treatment in view of preventing vitellogenin induction in male fish.

CONCLUSION

The threshold level of estrogenic activity to induce vitellogenin in male Medaka was evaluated as 10 ng-E2/L by estimating the LOEC of E1 and E2 and conducting exposure tests using river water or treated wastewater. On the contrary, although estrogenic activity in river water was detected in the range roughly from 0.1 to 1 ng-E2/L, in some urban rivers, estrogenic activity exceeded the level of 10 ng-E2/L. Consequently, in urban rivers, estrogenic activity should be further reduced through wastewater treatment to prevent vitellogenin induction, one of the indexes of feminization of fish.

Future studies should include clarification of the effect of estrogenic activity on other forms of feminization, such as testis-ova, and effective methods for wastewater treatment to achieve a safe level of estrogenic activity in treated wastewater.

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