

Development of the Endocrine Disruptor Experiment in Teaching Program for Teenagers

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Abstract

We investigated the education process of an environmental experiment in middle and high school. The major objective was that students understand about biological toxicity of endocrine disruptor. We used bisphenol A in environmental experiment to educate them about the effects of endocrine disruptor. The test animal was zebrafish (*Danio rerio*, wild type) which is very easy to use in the school experiment class. We educated the high school students (n=25) in Suwon through this teaching program. Finally we analyzed the effect of research education between two groups, education students (test) and non-education students (control). This experiment showed there were some positive effects on environmental reorganization of students. They understood the affect of endocrine disruptors in fish and man. Specifically, understanding the importance of preservation of ecosystem increased in the test-education group.

Key Words : Education for teenager, Endocrine disruptor, Zebrafish, Bisphenol A

1. Introduction

The importance of environmental education in school and public education has increased. However, most environmental education programs in school just stop after elementary school in Korea, because environmental class isn't necessary to enter university. However, after the 1990s, there were numerous environmental agendas and conferences worldwide. Korea must come together with the world environmental flow. For example, the damage from industry chemicals is a major environmental problem¹⁾. Every person has to use a lot of chemical materials. Some of these chemicals may produce several problems of endocrine disruptors. Therefore chemicals problem becomes controlled by the policy of government or international organ. REACH is one of the control regulations.

REACH means Registration, Evaluation and Authorization of CHemicals (REACH), and was issued by EC in 2003²⁾. Chemicals safeties include endocrine disruptors become international important issue like this.

Furthermore, it is very important to educate the public about the harmful effects of endocrine disruptor that are widely used in chemicals. Experimental education to understand the biological harm is better than teaching theory³⁾. However, experimental education is very difficult in Korea's middle and high schools. There are several reasons, 1) public school teacher's experimental skill or training is low; 2) insufficient laboratory spaces; 3) preparation of experiment materials.

Many teachers want training and experiment teaching program should be developed to application easily at middle and high schools⁴⁾.

Furthermore, it is indispensable to experiment education in school, suitable tools, experiment materials include experiment animals to prepare without difficulty.

There are several kinds of experiment animals; rat, rabbit et al., however that are difficult breeding, and

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treatment for teenagers.

Zebrafish (*Danio rerio*, wild type) is very easy to use in the school experiment class. It sells at an aquarium and the space for test animal is just small glass aquarium. The space isn't large; 40 cm×40 cm is enough.

Furthermore, zebrafish is vertebrata animal and their embryogenesis period is just 48 hours under 28.5°C⁵⁾. The development stage of embryo very clearly proves the damage to living organisms under environmental chemicals including endocrine disruptors. Therefore, students can easily observe the abnormal embryo in the development stages using the light microscope.

Bisphenol A (BPA) is one of endocrine disruptors, it is useful in environmental experiment to educate teenagers about the harmful effects of endocrine disruptor.

BPA is widely used in our daily life. BPA has been reported to leach from polycarbonate baby bottles⁶⁾, reusable containers⁷⁾, and drinking water tanks⁸⁾.

BPA has also been detected in plastic waste⁹⁾, in plasma stored in polycarbonate tubes¹⁰⁾, and in aquatic environments¹¹⁾. Furthermore, BPA shows estrogenic activity and is considered an environmental endocrine disrupter by many scientists¹²⁻¹⁵⁾. However there are

many chances to ingest the exposed bisphenol A.

In this study, we developed the endocrine disruptor experiment teaching program for teenagers. That is to educate the relationship with endocrine disruptor and living organism at ecosystem by the observation the effect of bisphenol A on the zebrafish development stage (Fig. 1).

The major objective was to expand the students' understanding of biological toxicity of endocrine disruptor.

We taught the high school students (n=25) in Suwon using this experimental program. Finally we analyzed the effect of education by the research between non-education student's group and education student's group.

2. Development and application of environmental experiment

2.1. Materials

2.1.1. Experimental animals

We purchased zebrafish at an aquarium in Suwon. The lengths of zebrafish were 3~3.5 cm and the average age was 5-6 months old. Before the fishes were exposed, we had been breed during 2 weeks in our

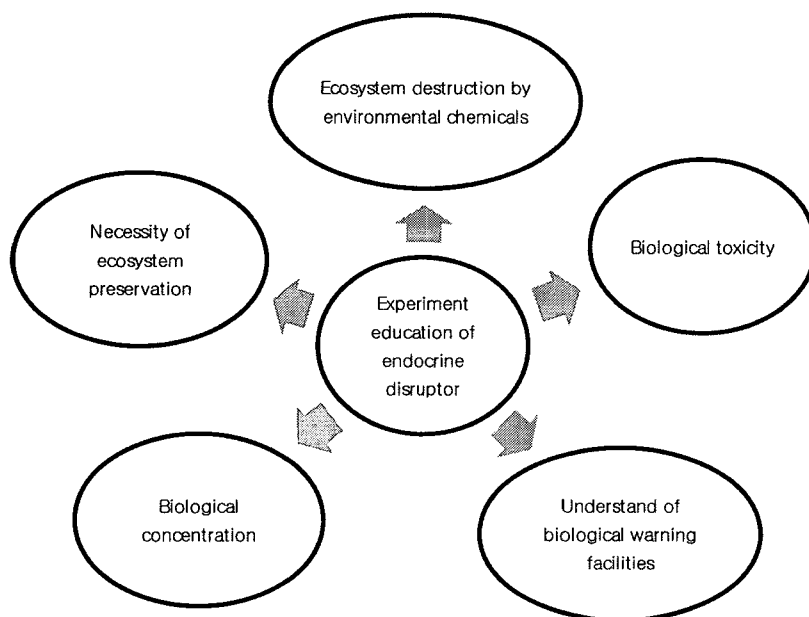


Fig. 1. The relationship with the endocrine disruptor experiment teaching program for teenagers and the aspect of environment is appeared the diagram.

laboratory. Experiment animals need to adapt to new environmental condition. However, the adaption period can be shorter than 2 weeks, because the experimental class in the public school is different from the research laboratory. It is enough to product eggs and finds the properties of embryogenesis. The zebrafish breeding conditions, developmental stage, morphology, and hatching rate were modified based on previous studies¹⁶.

2.1.2. The care condition of zebrafish

A 20-L glass aquarium contained the zebrafish and civil water that stayed overnight to protect chloride effect. The water temperature was maintained at $28 \pm 1^\circ\text{C}$, and the light/dark cycle was 14/10 h. We used the automatic timer switch for the light/dark cycle.

Adult fish were fed blood worms, dry flake food, and brine shrimp.

The blood worms are suitable to provide protein, and brine shrimp is the type of fresh protein food. Instant dry flake food is very easy to use but does not contain enough protein to stimulate breeding.

2.1.3. The tools of laying of eggs

The tools of laying of eggs are shown in Fig. 2. The rate of female and male was 6:4.

Eggs were laid and fertilized within 1 hour(h) of the beginning of the light cycle, which provided large populations of synchronously developing embryos.

The embryos were collected by net, pooled, and rinsed several times with embryo medium. The embryo medium temperature was 20.5°C , to make slow the de-

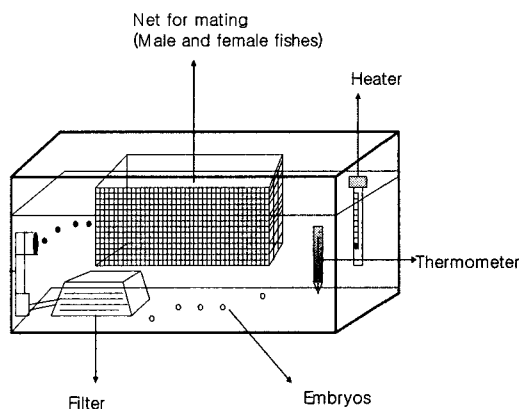


Fig. 2. The tools of laying of eggs.

velopment process. The composition of embryo medium is like Table 1⁵.

Embryonic staging was conducted according to the standardized staging series described by Kimmel et al.¹⁷. The embryos were immersed in BPA ($10 \mu\text{g/l}$) or control solutions beginning at the 64- to 256-cell stage, 2.5 h post-fertilization (hpf). The concentration of BPA is the same level of nature stream. It means this experiment is safe for students.

Dead embryos were removed to avoid contaminating the test solution. Embryos were observed with a microscope (Olympus, SZ61, Japan) to determine morphology and abnormalities.

2.2. Exposed condition

The BPA stock solution was diluted with tap water.

Table 1. The composition of Hank's solutions

Solution type	Composition
Hank's Stock	Solution 1 (8.0 g NaCl, 0.4 g KCl, in 100 ml dd H ₂ O)
	Solution 2 (0.358 g Na ₂ HPO ₄ Anhydrous, 0.60 g KH ₂ PO ₄ , in 100 ml ddH ₂ O)
	Solution 3 (0.72 g CaCl ₂ , in 50 ml ddH ₂ O)
	Solution 4 (1.23 g MgSO ₄ ·7H ₂ O, in 50 ml dd H ₂ O)
	Solution 5 (0.35 g NaHCO ₃ , 10.0 ml dd H ₂ O)
Hank's Premix	Solution 1 10 ml
	Solution 2 1 ml
	Solution 3 1 ml
	Solution 4 1 ml
	DDW 86 ml
Hank's (Final): Hank's embryo medium	9.9 ml Hank's Premix
	0.1 ml solution 6

Before use, the tap water was allowed to stand for 24 h to evaporate any chlorine. The final concentration was BPA 0.1 µg/l.

Each group of embryos was placed in a 1-L glass beaker and maintained in carbon-filtered water at 28±1°C. Each group contained 300 embryos. Embryos were randomly divided into the following groups: Group 1 was the control group, and Groups 2 was exposed to 0.1 µg/l BPA, respectively. Embryos were observed at 2, 5, 8, 22, 27, 32, 48, 52, and 72 hpf, which are time points based on known developmental stages¹⁷⁾. We calculated the hatching rate for each group at 72 hpf.

3. The experiment education and result analysis

We educated the high school students (n=24) in Suwon using the experiment teaching program, and then analyzed the effect of experiment education with student's research. The composition of groups is shown in Table 2.

4. Results and Discussion

The effect of experiment education about endocrine disruptor had 5 analysis aspects (Fig. 3).

Finally, after the program ended we gave the students an evaluation questionnaire. Analysis should those students who understood the objectives in advance had a higher level of knowledge of endocrine disruptor.

We taught the problem of endocrine disruptor using

Table 2. The constitution of education group and control group

Group	Constitution	Number	%	Total
Non-education group (control)	Male	14	58.33	24
	Female	10	41.66	
Education group (test)	Male	18	75.00	24
	Female	6	25.00	

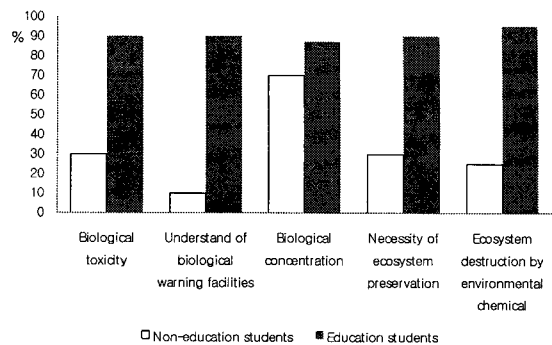


Fig. 3. The analysis of experiment education effects about endocrine disruptor. The percentage appears that the answer of students were "very effective" to understand these environment concepts.

the experiment, and explained the connection with environment concepts. In the analysis of education effects, education group answered that they knew the importance of preservation ecosystem by this experiment education. Both control group and educated group, students answered that environmental technology is necessary. However only the education group learned that the environmental technology must have the basis to understand Korea's ecosystem. Specially, the observation of development stages of zebrafish's organs was effective. For example, students could observe the vertebra, heart, eye, blood tube without dissection because the zebrafish embryo is transparent (Fig. 4).

The development stage of embryo very clearly lead to understanding the damage of living organisms under

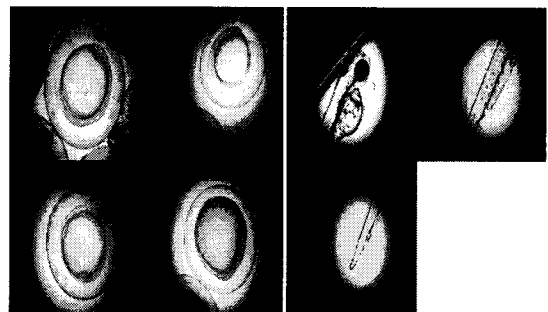


Fig. 4. Students observed zebrafish development stages. It is the student's data in our education program. Left photo is the point after 6 hours from experiment start point, and right photo is after 48 hours.

environmental chemical include endocrine disruptors.

Environment education in middle and high school is controlled by the Korean Ministry of Education and Environment. School teachers want to use the new teaching tools for the environmental education. However experiment education has some problems; materials cost, experiment animals, and the laboratory space. Our experiment with environmental education tools will solve the problems.

5. Conclusion

We used bisphenol A in environmental experiment to educate middle and high school students about the effects of endocrine disruptor. We educated the high school students (n=25) in Suwon through this teaching program. The test animal was zebrafish for experiment. Finally we analyzed the effect of research education between two groups, education students (test) and non-education students (control). They understood the affect of endocrine disruptors in fish and man. In addition, there was a positive effect to understanding the importance of preservation of ecosystem.

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