Single and combined effects of Di-2-ethylhexyl phthalate and Bisphenol A on life traits of a tropical micro-crustacean, *Ceriodaphnia cornuta*

Van-Tai Nguyen[1,2], Manh-Ha Bui [3], Emilie Strady [4,5], Thuy-Chung Kieu-Le [1,2,4], Ba-Trung Bui [6], Thanh-Son Dao[1,2]

[1] Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam
[2] Vietnam National University, Ho Chi Minh City, Vietnam
[3] Saigon University, Ho Chi Minh City, Vietnam
[4] CARE, Ho Chi Minh City University of Technology, VNU-HCM, Viet Nam
[5] Aix-Marseille Univ., Mediterranean Institute of Oceanography (M I O), Marseille, Université de Toulon, CNRS/IRD, France
[6] Institute for Environment and Resources, Ho Chi Minh City, Vietnam

INTRODUCTION

Plastics can contain various harmful chemicals, mainly plastic additives (e.g., phthalate, bisphenol A, etc.). They are added to the plastic polymers to provide their specific characteristics like making them harder, more flexible and/or durable [1]. However, these chemicals can enter the water bodies through different pathways like the discharge through the industrial manufacture or the leach out of plastic materials in the use and disposal processes under natural conditions such as high temperature and radiation [1]. Di-2-ethylhexyl phthalate (DEHP) and bisphenol A (BPA) are widely used in the plastic manufacture and frequently detected in the aquatic environment. However, they are known as endocrine-disrupting compounds (EDCs) that can cause intracellular disruption and interfere with the functions of hormones in the endocrine systems of living organisms [2]. Previous studies have reported the negative impacts of these additives on aquatic organisms such as phytoplankton, zooplankton, fish [2, 3, 4]. Therefore, the presence of these additives have been considered as the potential risk causing biological disorder in animals and ecological imbalance in aquatic environment.

In aquatic ecosystems, zooplankton (e.g. *Daphnia*, *Ceriodaphnia*) play an important role as they are at the central position in the food chain, and among the most vulnerable organisms upon the pollutant occurrence [5]. These organisms have been commonly used in toxicological assessment due to their wide distribution in aquatic ecosystems, high sensitivities to toxins, and easy to culture under the laboratory conditions [6]. Although previous studies have showed negative impacts of phthalates and BPA on various aquatic organisms, the chronic effects of these chemicals to zooplankton from tropical regions have not been fully understood. Therefore, the aim of this study is to assess the chronic impacts of DEHP and BPA on the survival, reproduction, and growth of *Ceriodaphnia cornuta*, a tropical micro-crustacean isolated from Vietnam.

MATERIALS AND METHODS

The test organism and chemical for experiment

- The tropical micro-crustacean, *C. cornuta* (Fig. 1), was isolated from the Mekong River in Vietnam and has been maintained for over one year in the laboratory conditions at the temperate of 25 ± 1 ºC, the light intensity of 600 Lux, and photoperiod of 12h light: 12h dark [7, 8]. The organism was raised in the artificial medium called M4/4 [7] and fed with a mixture of green alga Nannochloropsis sp. and YTC, a rich nutrient mixture [9].
- The plastic additives DEHP and BPA, from Aldrich Sigma, were dissolved in acetone (Merck) at the concentration of 1000 and 5000 mg/L, respectively, and used as mother solutions for the experiments. The mother solutions were stored at the temperature of 4 ºC prior to the experiment.

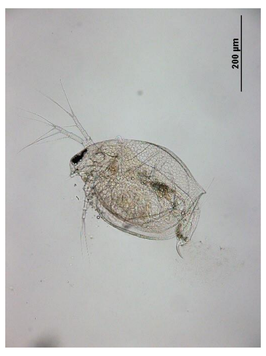

Figure 1 – Adult *C. cornuta*. Scale bars = 200 µm.

Experimental setup

- The chronic experiments were conducted according to APHA (2012) with minor modifications [8].

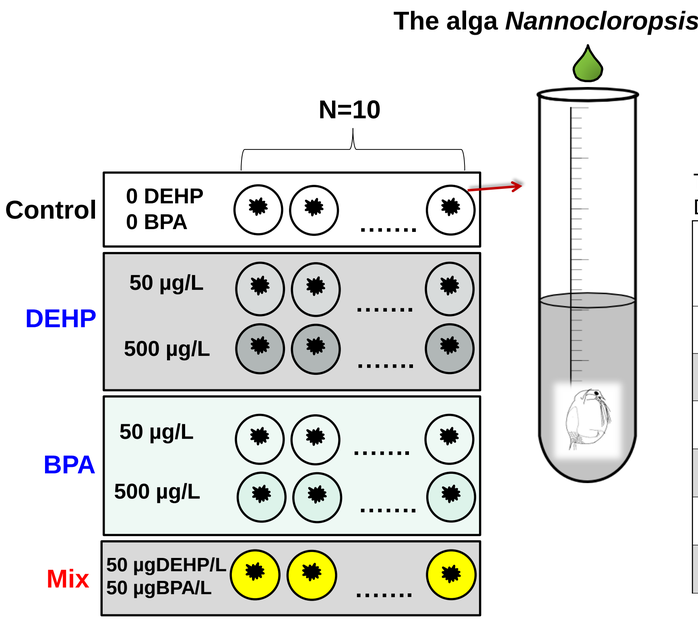

Table 1. Summary of the test concentrations of DEHP and BPA.

No.	Abbreviations of the exposures	DEHP (µg/L)	BPA (µg/L)
1	**Control**	**0**	**0**
2	**D50**	**50**	**-**
3	**D500**	**500**	**-**
4	**B50**	**-**	**50**
5	**B500**	**-**	**500**
6	**Mix**	**50**	**50**

In each concentration:

- The organism was individually incubated in a 15 mL glass tube containing 10 mL M4/4 medium at the test concentration of chemicals (one organism/ tube).
- There were 10 replicates (N = 10) in each treatment.
- The organisms were fed daily with a mixture of green alga *Nannochloropsis* sp. and YTC (a rich nutrient mixture) [9].
- The medium in each incubation was totally renewed three times per week.
- The test lasted 10 days under the laboratory conditions as mentioned above.
- The life-history traits including survivorship and reproduction of *C. cornuta* were daily recorded.
- By the end of the test, the body length of living organisms in each treatment was measured by using the microscope (Olympus BX 51) coupled with a digital camera (DP71) [10].

Data treatment

- Sigma Plot version 12.0 was used for data analyses. The ANOVA test was applied for calculating the statistically significant difference in the body length of *C. cornuta* between the control and exposures.
- A gap of more than 20% in the survival proportion of *C. cornuta* in treatments was considered as significant difference [8].

RESULTS AND DISCUSSION

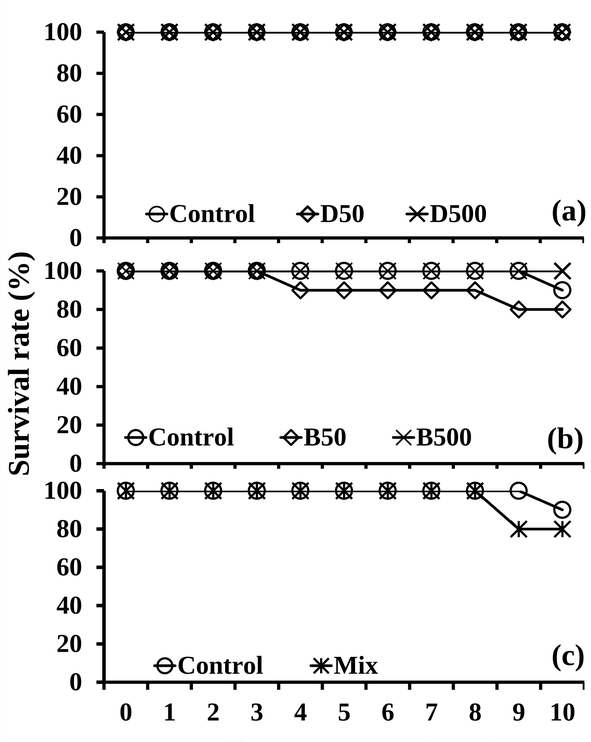

Figure 2 – The survival rate of *Ceriodaphnia cornuta* exposed to DEHP (a), BPA (b), and a mixture of DEHP and BPA (c). Abbreviations as in Table 1.

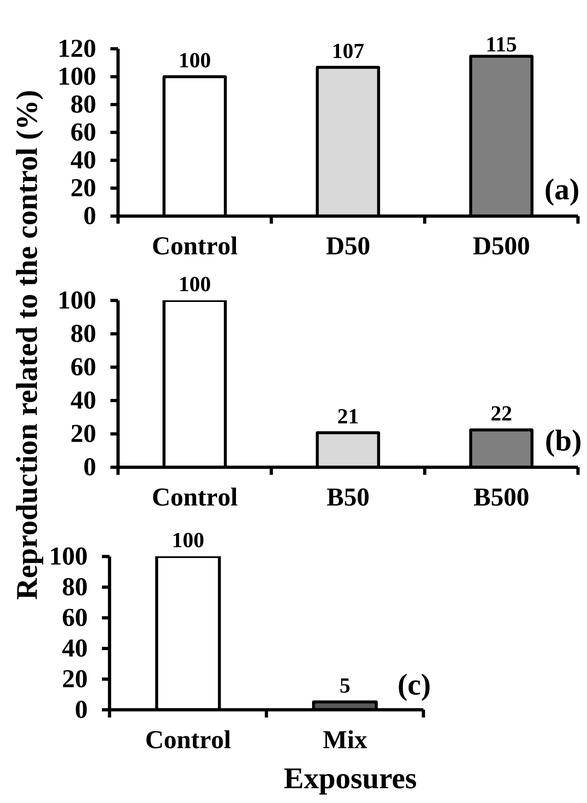

Figure 3 – Total neonates of *Ceriodaphnia cornuta* exposed to (a) DEHP, (b) BPA, and (c) a mixture of DEHP and BPA relative to the control. Abbreviations as in Table 1.

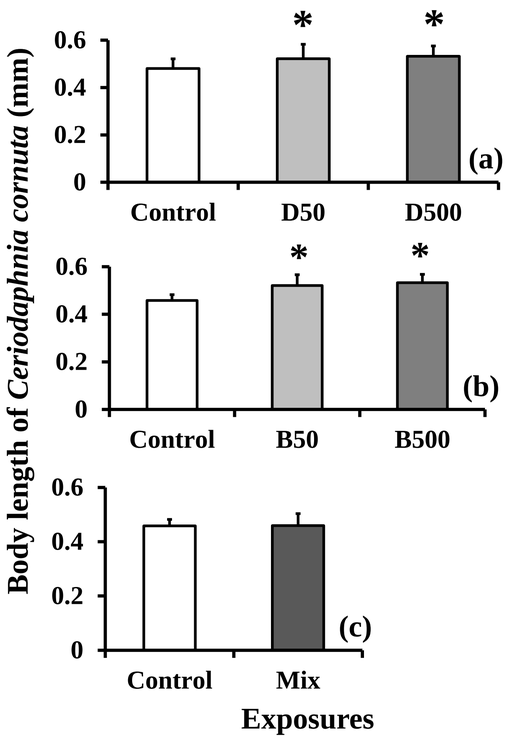

Figure 4 – Body length of *Ceriodaphnia cornuta* exposed to (a) DEHP, (b) BPA, and (c) a mixture of DEHP and BPA. The asterisk indicates a significant difference between the control and exposures ($p<0.05$) by ANOVA followed by Tukey's test.

- The results showed that the exposures to the individual and mixture of DEHP and BPA at the test concentrations during the 10-d period did not negatively influence the survival of the tropical micro-crustacean *C. cornuta*.

- On the other hand, while DEHP marginally enhanced the reproduction of the animals, BPA and the mixture of these additivies strongly inhibited it (Fig. 3a & 3b). Moreover, both DEHP and BPA induced a significantly longer body of *C. cornuta* (Fig. 3c).

- Our results indicated the mixture of DEHP and BPA caused a synergistic effect on reproduction but an antagonistic effect on the growth of C. cornuta.

- The energy cost and biotic ligand competition could be the mechanisms behind the observed impairments on *C. cornuta* exposed to DEHP and BPA in our study.

- More specifically, there have been numerous studies indicated that both DEHP and BPA can strongly alter the antioxidant and biotransformation enzyme activities in micro-crustaceans [22, 23, 35] leading to an energy cost over chronic exposures. This would then imbalance the energy distribution that the animals use to maintain their survival, conduct normal activities such as swimming and feeding, and for their growth and reproduction.

CONCLUSIONS

- Although both DEHP and BPA did not strongly affect the survival rate of the tropical micro-crustacean C. cornuta, BPA and the mixture of these additives could inhibit the reproduction of the organisms. Besides, exposure to DEHP and BPA could influence on the growth of organisms. Therefore, the occurrence of these additives could be considered as potential risks for organisms in aquatic ecosystems.
- Further investigations of the biochemical responses of C. cornuta after exposure to DEHP and BPA are suggested to make clear the mechanisms of the toxicity of these additives on organisms in tropical areas.
- Our findings enrich the knowledge of DEHP and BPA toxicity to tropical micro-crustaceans. Besides, our results are also of significant value to freshwater monitoring and environmental risk assessments of plastic additives.

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Nguyen Van Tai – Email: *tai.nv96@gmail.com*

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