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Development of *Daphnia magna* under exposure to the xenobiotic octylphenol

Sự phát triển của Daphnia magna trong phơi nhiễm với hợp chất tổng hợp octylphenol

Research article

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Xenobiotics are of human and environmental concerns due to their potential toxicity. Octylphenol is one of the very common and daily used xenobiotics in door and out door activities of human beings. Toxicity of octylphenol to aquatic organisms, especially to zooplankton (e.g. *Daphnia magna*) was investigated but not fully understood. In this study we evaluated the chronic effects of octylphenol at the concentrations of 5, 50 and 500 µg L^{-1} on *Daphnia magna* over a period of 14 days. The results showed that low concentration of octylphenol (5 µg L^{-1}) stimulated the maturation while high concentrations of the chemical (50 and 500 µg L^{-1}) caused a significant mortality to the *Daphnia*. Besides, all the tested concentrations of octylphenol had serious impacts on fecundity and growth of the animals. Investigations on the presence, distribution, fate and toxicity of xonobiotics including octylphenol in the developing country environment are suggested for human, environmental and ecological health protection.

Những hợp chất tổng hợp đang là mối quan ngại cho con người và môi trường vì khả năng gây độc của chúng. Octylphenol là một trong những hợp chất tổng hợp được sử dụng phổ biến và thường xuyên trong những hoạt động của con người trong nhà và ngoài trời. Độc tính của octylphenol đối với thủy sinh vật, đặc biệt đối với động vật phù du (vd. Daphnia magna) mặc dù đã được nghiên cứu nhưng vẫn chưa được hiểu biết đầy đủ. Trong nghiên cứu này, chúng tôi đánh giá ảnh hưởng mãn tính của octylphenol ở các nồng độ 5, 50 và 500 µg/lít lên Daphnia magna trong thời gian 14 ngày. Kết quả cho thấy ở nồng độ octylphenol thấp (5 µg/lít) kích thích sự thành thục của sinh vật trong khi ở nồng độ cao hơn (50 và 500 µg/lít) gây chết đáng kể Daphnia. Bên cạnh đó, tất cả các nồng độ ocytlphenol dùng trong thí nghiệm gây ảnh hưởng nghiêm trọng lên sức sinh sản và sinh trưởng của sinh vật. Nghiên cứu về sự hiện diện, phân bố, phát tán và độc tính của những chất tổng hợp bao gồm octylphenol ở các nước đang phát triển nên được tiến hành vì mục tiêu bảo vệ sức khỏe con người, môi trường và hệ sinh thái.

Keywords: octylphenol, *Daphnia magna*, chronic effects, reproduction, growth

1. Introduction

Recently, the focus on chemical pollution has been largely directed toward the well-known ‘‘priority’’ pollutants, especially those displaying persistence in the environment. This is likely to be only one piece of a larger puzzle (Daughton and Ternes, 1999). Other surface water contaminants include a variety of metals, carcinogenic organic compounds, xenobiotics, pharmaceutical, veterinary, personal care products, and food supplements among others (Weyer and Riley, 2001; Kolpin et al., 2002). Xenobiotics can act as endocrine disruptors without affecting hormone binding by modulating endogenous hormone levels.

The main sources of endocrine-disrupting compounds in the rivers and lakes of Europe and North America are sewage effluent and agricultural chemicals from runoff. In less developed countries uncontrolled domestic and industrial discharge to waterways contributes to EDCs (Barnhoorn et al., 2004). Many anthropogenic chemicals, as well as naturally occurring estrogens and plant secondary

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metabolites, experimentally have been found to have endocrine-disrupting properties (Naz, 2005). Octylphenol, an alkylphenol, is one of the endorine disrupting compounds widely used in as industrial surfactants, bases for household products, fungicides, textile and leather auxiliaries, veterinary medicine formulations, and thousands of tonnes of octylphenol have been annually used in the world (Brooke et al., 2005).

Researchers were first alerted to aquatic contamination by endocrine-disrupting compounds through observation of a variety of reproductive changes in different aquatic organisms (Folmar et al., 2001; Gagne et al., 2002). Octylphenol could be accumulated in freshwater and marine fish and mussel (Brooke et al., 2005). This chemical caused acute and chronic effects on embryos, larvae and offspring, juvenile aquatic animals, mostly on fish (e.g. fathead minnow, rainbow trout, carp) and invertebrates (freshwater shrimp, nematode worm, mysid shrimp, water flea). Few investigations showed adverse effects of octylphenol on *Daphnia magna* in terms of survival and body length (Brooke et al., 2005). However, other life history traits of *D. magna,* e.g. maturation, dry mass, fecundity, during a long-term exposure to octylphenol are not known, to our best knowledge. Therefore, this study aimed to evaluate the chronic effects of octylphenol at the concentrations of 5–500 µg L^{-1} on life history traits of *D. magna* over a period of 14 days.

2. Materials and methods

2.1 Materials

The 4-n-Octylphelol (hereafter we call it octylphenol) was obtained from Merck (Dr. Ehrenstorfer GmbH). The chemical was dissolved in MeOH (Merck) at the concentration of 1 mg mL^{-1}. The stock solution was kept at -70ºC prior to the experiment. The purification of the commercial chemical is 99.5%.

2.2 Experimental organisms and experiment set up

The test organism was *Daphnia magna*, obtained from MicroBioTest Inc. (Belgium) and has been maintained in the laboratory of the Institute for Environment and Resources for many generations. The *Daphnia* medium consisted of $CaCl_2$, KCl, $NaHCO_3$ and $MgSO_2$. *Daphnia* were fed with alga *Scenedesmus* sp. three times a week. The alga was cultivated in Z8 medium (Kotai, 1972) with continuous aeration. Both culturing of *Scenedesmus* and *Daphnia* and the exposures were conducted at 20 ± 1°C and a photoperiod of 14h light: 10h dark at a light intensity of around 1000 Lux.

Prior to the experiments, fifteen female *D. magna* were incubated in a 500 mL beaker and fed with *Scenedesmus* sp. for 2-3 weeks. Offspring from the second to fifth clutch of these *D. magna* were used for experiments. *Daphnia* was exposed to octylphenol at the concentrations of 0, 5, 50 and 500 µg L^{-1}. In each treatment, thirty neonates less than 24 h old were randomly selected for each chronic exposure (Adema, 1978) and ten organisms were incubated in 200 mL plastic beakers containing 100 mL of medium. Three replicates were prepared for each treatment.

The animals were fed with *Scenedesmus* sp. at the concentration of 2 mg C L^{-1} per day. The medium were totally renewed 3 times every week. During incubations, animals were observed daily for their survival, maturation and reproduction. Death of the animal was defined as the stop of heartbeat confirmed by microscopic observation (Olympus BX51, coupled with a digital camera). Maturation of *Daphnia* was defined as time point of first egg occurrence in the brood chamber (could be seen with naked eyes). Fecundity of the animals was defined as the accumulative neonates from an exposure (Dao et al., 2010). The experiments lasted for 2 weeks. By the end of the tests, the alive *Daphnia* was dried at 50ºC for 4 hours then weighed for dry mass determination.

2.3 Data analysis

Shapiro Wilk test (Sigma Plot, version 12.0) was applied to determine the significant difference of *Daphnia'*s maturation and dried mass from control and octylphenol exposures.

3. Results and discussion

3.1 Survival of *Daphnia magna*

During two weeks of incubation all *D. magna* in the control were alive. The low concentration of octylphenol (5µg L^{-1}) slightly impacted on the survival of *Daphnia*. However, the higher concentrations of octylphenol, 50 and 500 µg L^{-1}, caused a significant reduction of the organisms' survivorship to 73% and 53%, respectively (Fig. 1). A previous investigation showed that all *D. magna* died after 9 day exposure to the 4-tert-octylphenol at the concentration of 510 µg L^{-1}(Brooke et al., 2005), similar to the highest concentration in our study, 500 µg L^{-1}. The higher survival percentage in the current study could explained as (1) the chemical used in our study (4-n-octylphenol) may be less to toxic to Daphnia than the chemical used in the previous study (4-tert-octylphenol) and (2) different clones of *Daphnia* could have different tolerance to the same toxin concentration (Hietala et al., 1997). On the other hand, the effects of octylphenol on survival of *Daphnia* in this study is in line with a recent publication of Vo et al (2014) in which the animals were exposed to atrazine.

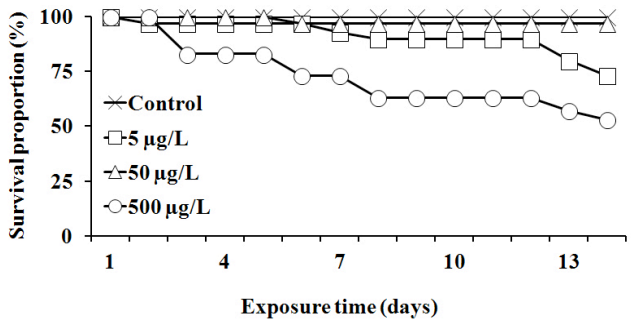

Figure 1. Survival of *Daphnia magna* (n = 30 at the start) during 2 weeks of exposure to 0, 5, 50 and 500 µg/L of octylphenol

3.2 Maturation of *Daphnia magna*

Octylphenol has been linked with estrogenic effects in fish (Jobling et al., 1996). Octylphenol at low concentration, 5µg L^{-1}, made the *Daphnia* reach their maturity age earlier compared to the control. However, at higher octylphenol concentrations, 50 and 500 µg L^{-1}, the exposed animals had a similar age of maturation (Fig. 2). Maybe the maturation of the organisms was stimulated at low chemical concentration but inhibited at higher ones which needs further investigation. To our best knowledge, this is the first information on the maturation of *D. magna* exposed to octylphenol.

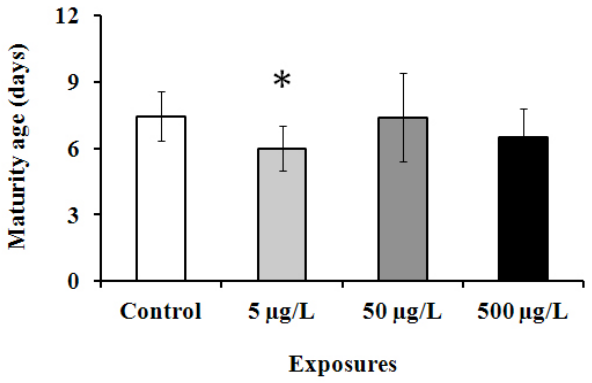

Figure 2. Maturation of *Daphnia magna* during 2 weeks of exposure to 0, 5, 50 and 500 µg/L of octylphenol (*, p < 0.05, Shapiro Wilk test)

3.3 Reproduction of *Daphnia magna*

Out of the 2 week incubation, there were 427 off spring from control experiment. However, the total accumulative neonates in the octylphenol treatments were from 11-55 (Fig. 3). Zhang et al. (2003) indicated that 4-nonylphenol at the concentration from 50 – 1000 µg L^{-1} arrested the *Daphnia*' egg development and caused malformation of *Daphnia* neonates. Because nonylphenol and octylphenol are both endocrine disrupting compounds and may share similar effects on animals, therefore, the strong influence of octylphenol on *Daphnia*' embryo development is the root of the severe effect of the chemical on the reproduction of *Daphnia*.

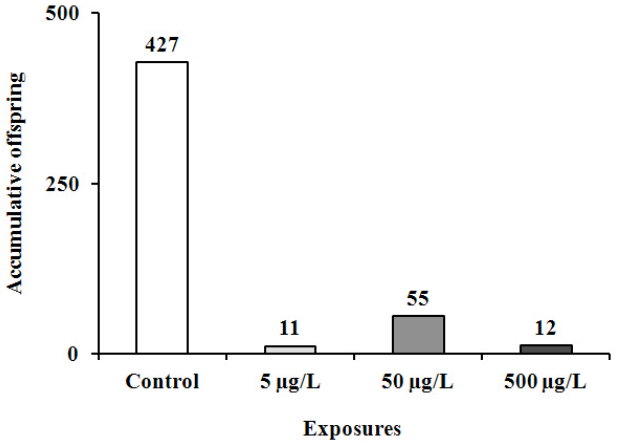

Figure 3. Accumulative offspring produced by mother *Daphnia magna* from control and octylphenol exposures

3.4 Growth of *Daphnia magna*

The average dry mass of one mother *D. magna* in the control was around 0.28 mg. In the exposures to 5, 50, 500 µg octylphenol L^{-1}, the dry mass of one animal was around 0.17, 0.20, 0.09 mg, respectively (Fig. 4). It was reported that octylphenol caused significant decrease of *Daphnia*'s body length when they exposed to 120–230 µg octylphenol L^{-1}, but the effect was not observed when the octylphenol concentration was 62 µg L^{-1}Brooke et al., 2005). Our study proved that the strong impact of the chemical on the growth of *Daphnia* was already observed at the very low concentration of octylphenol, 5 µg L^{-1}.

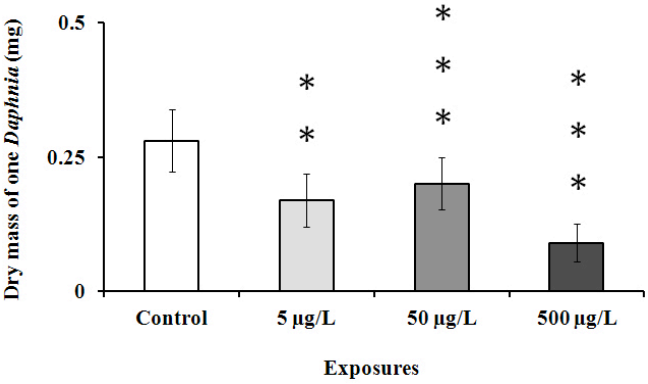

Figure 4. Dry mass of *Daphnia magna* after 2 weeks of exposure to 0, 5, 50 and 500 µg/L of octylphenol (, p < 0.01; ***, p< 0.001; Shapiro Wilk test)**

4. Conclusions

The results of our investigation showed that the commonly and widely used chemical octylphenol had serious effects on the life history traits of *D. magna* including survival, maturation, growth and reproduction. To our knowledge, this is the first report on the chronic and negative effects of octylphenol on maturation and dry mass of *D. magna*. Therefore, more attention should be paid to the presence, distribution, fate in nature and impacts of octylphenol in particular and endocrine disrupting com-

pounds in general on aquatic organisms. Additionally, investigations on the bio-accumulation and bio-transformation of xenobiotics in animals are suggested to get more understanding on the toxicity of the toxins on the aquatic organisms and ecosystem.

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