

EFFECTS OF 17 α -ETHINYLESTRADIOL ON THE REPRODUCTION OF *Ceriodaphnia reticulata* AND *Sida crystallina* (Cladocera)

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Introduction

Numerous chemical and technical products which mimic the female sex hormone estradiol are released into the environment. Among these so-called endocrine disruptors with estrogenic properties is the synthetic hormone 17 α -ethinylestradiol (EE), a compound of contraceptive pill. Different toxicity tests with EE caused variable results: In a Lumistox test and also in an experiment with *Daphnia magna* EC50 (24h) of 212 mg/L respectively 5.7 mg/L were calculated¹. Effects at considerably lower concentrations were observed in a *Scenedesmus* growth inhibition test and in a yeast assay (2.20 μ g/L)^{1,2}. The effect of EE on the endocrine system of rainbow trout (*Onchorhynchus mykiss*) was shown for concentrations >2 ng/L³. The reproduction of the rotifer species *Brachionus calyciflorus* was affected at EE concentrations >201 μ g/L⁴. In this study single-species-tests were conducted to assess on the one hand acute toxic and on the other hand chronic effects of endocrine disruptors under laboratory conditions. 3-generation-tests with *Ceriodaphnia reticulata* and *Sida crystallina* were carried out to examine the influence of the synthetic hormone 17 α -ethinylestradiol on the reproduction of these cladoceran species.

Methods and Materials

According to a SOP (Standard Operating Procedure) of the ecotoxicological laboratory of the GSF National Research Center the acute toxicity of EE for *Ceriodaphnia reticulata* and *Sida crystallina* was specified. The test organisms were exposed in two replicates with eight concentrations and controls in a climate chamber. Four different concentrations of potassium dichromate served as reference. For 10 juvenile cladocerans per concentration the swim inhibition was observed after 24h. EC10, EC50 and EC90 were calculated with the Probit method.

In the 3-generation-tests for each species, six different concentrations (10–500 μ g/L EE, A-F, eight replicates each) and a control (K, 16 replicates) were tested for a duration of four weeks. The test was initiated by transferring one neonate individual into a test vessel, which was incubated under standardised conditions (52–56% humidity, 16h light (25°C), 8h (22°C) darkness). Every two days, the medium was renewed and life history parameters such as survivorship of the adults and juveniles, clutch size, first appearance and number of produced offspring were investigated. The ANOVA method as well as Kaplan-Meier analyses of survivorship were applied.

Results and Discussion

The juvenile phase of *Sida* was significantly shorter at concentrations above 100 µg/L EE (4 days) (Fig. 1) possibly induced by a stimulating effect of EE on the egg production or a reaction on stress to ensure the survival of the species, as it was shown in a study on the effects of xenobiotica on cladocerans⁵.

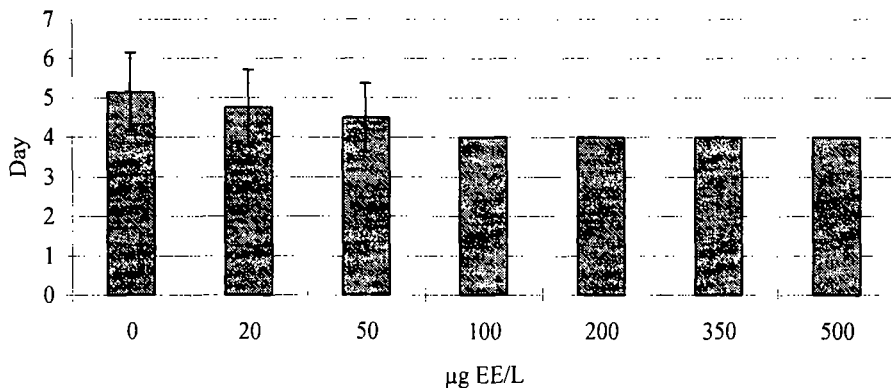


Fig. 1: Length of juvenile phase of *Sida crystallina* at different concentrations of EE

In contrast to this there was no comparable result for *Ceriodaphnia*, but 17 α -ethinylestradiol caused a higher mortality of the newly hatched juveniles of this species for concentrations above 200 µg/L (Fig. 2), acute toxicity tests showed that *Ceriodaphnia reticulata* (EC50 (24h): 1814 µg/L) was generally more sensitive towards the substance compared to *Sida crystallina* (EC50 (24h): >4100 µg/L) (Tab. 1) and *Daphnia magna* (5700 µg/L)¹.

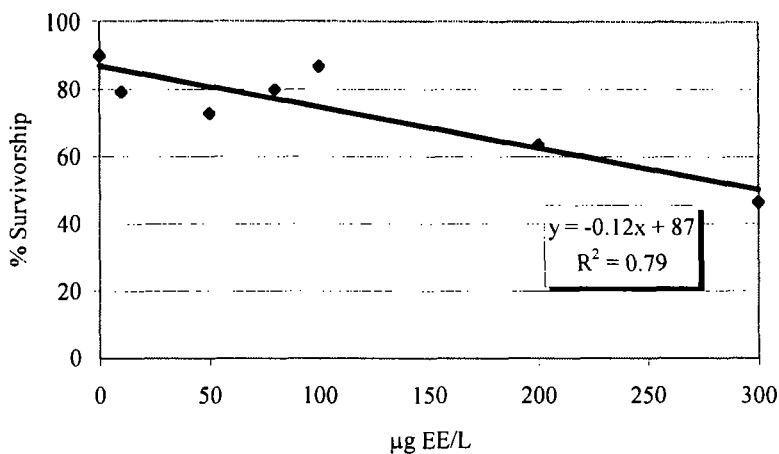


Fig. 2: Survivorship of newly hatched juveniles of *Ceriodaphnia reticulata*

<i>Ceriodaphnia reticulata</i>		
Mean	EE	Potassium dichromat
EC 10 (µg/L)	606	100
EC 50 (µg/L)	1814	300
EC 90 (µg/L)	5473	700

<i>Sida crystallina</i>		
Mittelwert	EE	Potassium dichromat
EC 10 (µg/L)	>4100	112
EC 50 (µg/L)	>4100	485
EC 90 (µg/L)	>4100	2411

Tab. 1: Effective concentrations (24h, swim inhibition) 17 α -ethinylestradiol on *Ceriodaphnia reticulata* and *Sida crystallina*

Conclusions

Acute toxicity test proved that *Ceriodaphnia* was more sensitive towards 17 α -ethinylestradiol in comparison to *Sida* and *Daphnia*.

In 3-generation-tests a higher mortality of juveniles of *Ceriodaphnia* in concentrations was observed. Thus negative consequences for the population dynamic can not be excluded. There was no similar effect for *Sida* but their juvenile phase was one day shorter at EE-concentrations >100 µg/L. No effects were found for mortality of adult animals, birth rate, number of juveniles per female and net reproduction rate of *Sida* and *Ceriodaphnia*.

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