# EFFECTS OF 17α-ETHINYLESTRADIOL AND TRENBOLONE ON GROWTH AND REPRODUCTION OF *Caenorhabditis elegans* (NEMATODA)

Sebastian Höss[1], Gabriele F. Severin[2], Wolfgang Jaser[2], Karl-Werner Schramm[2]

[1]ECOSSA (Ecological Sediment & Soil Assessment), Thierschstr. 43, 80538 München, Germany
[2]Institute of Ecological Chemistry, GSF National Research Center of Environment and Health, Ingolstaedter Landstr. 1, D-85764 Neuherberg, Germany

## Introduction

Synthetic hormones and veterinary pharmaceuticals enter the environment through waste water effluents and accumulate in the sediment. Therefore they cause potential danger on benthic habitats. Nematodes are usually the most abundant and species-rich metazoan organisms in sediments and soils and represent an important component of benthic food webs[1,2,3,4]. The nematode *Caenorhabditis elegans* has been studied intensively and proved to be a suitable test organism for ecotoxicological assessment of liquid and solid substrates, using lethal and sublethal toxicity endpoints[5,6]. We conducted a bioassay with the nematode *Caenorhabditis elegans* with Trenbolone (17β-Hydroxyestra-4,9,11-trien-3-on), a synthetic anabolic steroid used as veterinary pharmaceutical and the synthetic hormone 17α-ethinylestradiol (EE), a compound of the contraceptive pill. Aim of the study was to investigate the effect of chemicals with estrogenic and androgenic properties on the growth and reproduction of the worm.

## Methods and Material

Test solutions were prepared by mixing the respective stock solutions of 17α-ethinylestradiol (Sigma Aldrich) and Trenbolone (Sigma Aldrich) with a suspension of *Escherichia coli* OP50 (as food supply; approximately 1010 cells/mL) in M9 medium[1], yielding in final nominal test concentrations of 0.025, 0.5, 25, and 250 µg/L for EE and 0.05, 0.5, 5, and 50 µg/L for Trenbolone. *Caenorhabditis elegans* var. Bristol, strain N2, was maintained in stocks of dauer larvae (an alternative juvenile stadium, that occurs at lack of food) on NG agar[7] according to standard procedures[8,9]. Bioassays with *C. elegans* were carried out according to Traunspurger et al.[6]. Briefly, five juvenile worms of the first stage (J1; mean body length: 270 ± 16 µm standard deviation; n = 50) were transferred to each test vial (12 well polystyrene plate) containing 1 mL of the test medium. Five replicates were set up for each concentration. The samples were incubated on a shaker at 21 °C in the dark and after 72 h the juvenile worms in the controls developed to reproducing hermaphrodites. After that time, the test was stopped by heat-killing the worms at approximately 50 °C. The number of offspring per worm was determined as an indicator of nematode reproduction, using a dissecting microscope (25 ×). Worms were transferred onto a slide and body length and number of eggs inside the body were determined under a microscope at 100 and 400 × magnification, respectively. One-way ANOVAs[10] with post-hoc tests (Bonferroni) were carried out using SPSS® microcomputer software[11].

**Results and Discussion**

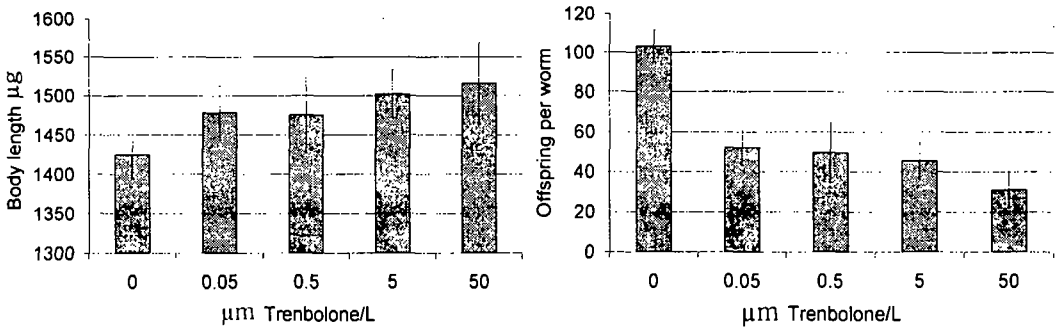


Fig. 1: Body length (in µm; left) and number of offspring (right) of *C. elegans* after 72 h of exposure to various concentrations of Trenbolone; bars = mean; error bars = standard deviation (n = 5).

Fig. 1 shows that growth and reproduction of *C. elegans* were influenced by Trenbolone. Body length of the nematodes was found to be significantly increased from 1425 µm in the controls to 1502 and 1516 µm at 5 and 50 µg/L, respectively ($p<0.001$). In contrast to growth, nematode reproduction was reduced to less than 50 % in the presence of Trenbolone, with first significant effects at 0.05 µg/L ($p<0.001$). Moreover, effects on growth and reproduction seemed to be dependent on the concentration of Trenbolone.

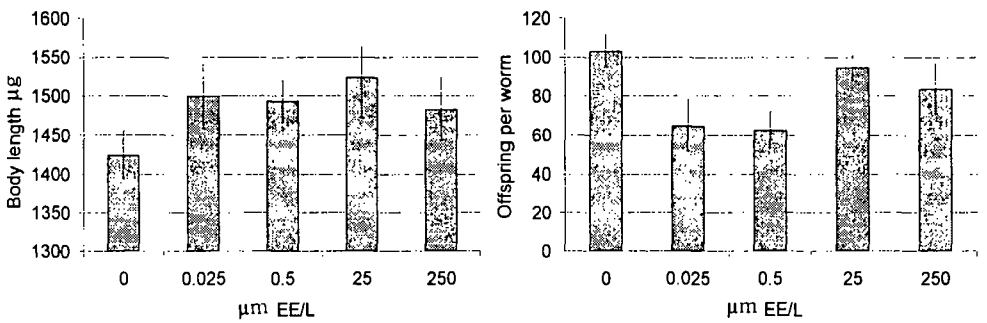


Fig. 2: Body length (in µm; left) and number of offspring (right) of *C. elegans* after 72 h of exposure to various concentrations of 17α-ethinylestradiol (EE); bars = mean; error bars = standard deviation (n = 5).

EE showed a positive effect on nematode growth, with first effects occurring at 0.025 µg/L ($p<0.001$), while body length at 250 µg was not significantly different to the control ($p>0.05$; Fig. 2). Reproduction of *C. elegans* was significantly reduced at low concentrations of EE (0.025 and 0.5 µg/L; $p<0.001$), while this effect did not occur at higher concentrations (25 and 250 µg/L; $p>0.05$; Fig. 2).

These preliminary results show that endocrine active substances, such as the synthetic hormones EE and Trenbolone can influence growth and reproduction of the nematode *C. elegans* at ecologically relevant concentrations. While Trenbolone showed a dose dependent effect on nematode growth and reproduction, it was not possible to unequivocally interprete the dose-response curves of EE. Further investigations are necessary to confirm the U-shape of this curve (hormesis effect?)[12]. However, it is indicated that also in natural benthic habitats, nematodes might be negatively influenced by substances with endocrine activity. These substances are often released into aquatic systems via waste water effluents and subsequently accumulate in sediments, thus posing a potential threat to benthic organisms.

**References**

1. Yeates, G.W., 1981. Nematode populations in relation to soil environmental factors: a review. Pedobiologia 22, 312-338.
2. Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanography and Marine Biology Annual Reviews 23, 399-489.
3. Traunspurger, W., 1996. Distribution of benthic nematodes in the littoral of an oligotrophic lake (Königssee, Nationalpark Berchtesgaden, FRG). Archiv für Hydrobiologie 135, 393-412.
4. Traunspurger, W., Bergtold, M., Goedkoop, W., 1997a. The effect of nematodes on bacterial activity and abundance in a freshwater sediment. Oecologia 112, 118-122.
5. Williams, P.L., Dusenbery, D.B., 1990. Aquatic toxicity testing using the nematode *Caenorhabditis elegans*. Environmental Toxicology and Chemistry 9, 1285-1290.
6. Traunspurger, W., Haitzer, M., Höss, S., Beier, S., Ahlf, W., Steinberg, C., 1997b. Ecotoxicological assessment of aquatic sediments with *Caenorhabditis elegans* (nematoda) – A method for testing in liquid medium and whole sediment samples. Environmental Toxicology and Chemistry 16, 245-250.
7. Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. Genetics 77, 71-94.
8. Sulston, J., Hodgkin, J., 1988. Methods. In: Wood, W.B. (Ed.), The Nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp.587-606.
9. Lewis, J.A., Fleming, J.T., 1995. Basic culture methods. In: Epstein, H.F., Shakes, D.C. (Eds.), *Caenorhabditis elegans*: Modern Biological Analysis of an Organism. Academic Press, San Diego, CA, pp.4-29.
10. Zöfel, P., 1988. Statistik in der Praxis. Gustav Fischer Verlag, Stuttgart, Gemany.
11. SPSS. SPSS for Windows. (7.5). 1996. Munich, Germany.
12. Calabrese E.J., Baldwin L.A., 1998. Chemical Hormesis: Scientific Foundations. Final Report. School of Public Health, Environmental Health Sciences, University of Massachusetts, Amherst, MA 01003.