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# Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod *Eurytemora affinis* (Poppe)

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**Abstract**

Given recent reports suggesting that certain contaminants may be present in sewage effluents at levels, which may exert a deleterious impact on fish, it seems pertinent to extend ecological hazard evaluation for such substances to aquatic invertebrates. For this reason, we sought to determine whether 17$\beta$-estradiol (E2), benzo(*a*)pyrene (BaP), 4-nonylphenol (NP), di(ethyl-hexyl)-phthalate (DEHP), and atrazine (A), individually or in binary mixture, can inhibit the survival, development, or reproductive output of the estuarine copepod *Eurytemora affinis*. In the first experiment nauplii were exposed to graded concentrations of individual contaminants to determine the 96-h $LC_{50}$, 10-day no observed effect concentration (NOEC) and 10-day lowest observed effect concentration of each compound. In the second experiment newly released (<24-h-old) nauplii were exposed either to an individual contaminant at the NOEC or to binary mixtures, where each compound was used at half NOEC. The effects were monitored daily for development and sex ratio. After 10 days of exposure, adult males and females were paired and exposures continued to investigate effects on reproductive output (maximum 28 day total exposure). Based on these life cycle parameters the lowest 10-day NOECs were $6 \pm 4\,\mu g\,L^{-1}$ for E2, $7 \pm 3\,\mu g\,L^{-1}$ for NP, $12 \pm 3\,\mu g\,L^{-1}$ for BaP, $25 \pm 3\,\mu g\,L^{-1}$ for A, and $109 \pm 29\,\mu g\,L^{-1}$ for DEHP. BaP, NP, and DEHP inhibited naupliar development, but in binary mixture with E2 these compounds did not inhibit larval development. The results suggest that endocrine disruption could occur in copepods following exposure to estrogenic compounds, especially if they are exposed starting from embryonic development.


*Keywords:* Endocrine disrupters; Aquatic invertebrates; Copepod; Development; NOEC

## 1. Introduction

Anthropogenic chemicals that have potential endocrine disruptor activity enter aquatic ecosystems in Western Europe, Japan, and North America. In France, recent studies of the Seine river water quality show that effluents from municipal sewage treatment works contain the natural estrogens estrone and 17$\beta$-estradiol (E2) at levels as great as 25 and $15\,ng\,L^{-1}$, respectively (Mouatassim et al., 2002). Further, alkylphenols, atrazine (A), and benzo(*a*)pyrene (BaP) were detectable in the Seine river at levels from 10 to $300\,ng\,L^{-1}$ (Abarnou et al., 2000; Budzinki, personal communication). Survey of rivers in the United Kingdom found nonylphenol (NP) concentrations up to $180\,g\,L^{-1}$ (Blackburn and Waldock, 1995). Physiological effects of these endocrine-disrupting chemicals (EDCs) have been well described for vertebrates and are distinctly different from one to another. For instance, A inhibits ligand binding to both androgen and estrogen receptors (Danzo, 1997), BaP acts as an antiandrogenic compound (Thomas, 1990), di(ethyl-hexy)-pathalate (DEHP) inhibits binding to the estrogen receptor and is antiandrogenic (Gray et al., 1999; Harris et al., 1997; Jobling et al., 1995; Moore et al., 2001), and NP is an

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estrogen receptor agonist which reduces estradiol binding to the estrogen receptor (Danzo, 1997; Lascombe et al., 2000; Rajapakse et al., 2001; Soto et al., 1991, 1995). In the past few years, numerous effects of EDCs on wildlife have emerged including changes in the sex of riverine or estuarine fish (Harries et al., 1997; Hashimoto et al., 2000; Minier et al., 2000). Much less is known with regard to endocrine disruption in marine or estuarine invertebrates, the key structural and functional components of aquatic ecosystems. However, ecological studies have reported abnormal sexual development in copepods (Moore and Stevenson, 1991) and amphipods (Gross et al., 2001) sampled from areas receiving sewage sludge discharges. More recently, Andersen et al. (2001) and Marcial et al. (2003) identified inhibition of larval development of *Acartia tonsa* and *Tigriopus japonicus*, respectively, by several EDCs that presumably differ with respect to the mechanism of action and Yamashita et al. (2001) observed intersexuality in *Acanthomysis mitsukurii* in Sendai Bay (Japan). Considering these observations, when conducting ecological risk assessments for natural and environmental estrogens present in the aquatic environment, it looks clearly important to know, the potential for effects on crustacea and other invertebrates. The early life stages of many species are often more sensitive to toxicants than later developmental stages (Rand et al., 1995). The metamorphosis from nauplius to copepodid is a major developmental event in the copepod lifecycle. Thus, the toxicity tests starting from the nauplius stage were designed specifically to incorporate this particularly sensitive period. Moreover, invertebrates, such as copepods with short generation times (3 weeks) offer the possibility of examining transgenerational and population effects of contaminants (Bechmann, 1999). The copepod 21-day life-cycle assay has been developed and used successfully in several studies (Breitholtz and Bengtsson, 2001; Hutchinson et al., 1999a, b; Marcial et al., 2003; Pounds et al., 2002). Both molting and metamorphosis, are regulated by ecdysteroids, and metamorphosis is presumably controlled by compounds similar to the juvenile hormones that are known for insects. Therefore, a test based on the time period required for nauplii to develop into copepodids is suitable for screening chemicals for chronic toxicity and for detecting disruption of processes controlled by ecdysteroids or juvenile hormones.

The aim of this study was to examine the potential lethal and sublethal effects of EDCs on a sexually reproducing species, the calanoid copepod *Eurytemora affinis*. This species was chosen since calanoid copepods have a widespread biogeographic distribution and are important food items for macroinvertebrates and fish. *E. affinis* is also particularly amenable to laboratory testing. The 50% lethality concentration ($LC_{50}$), lowest observed effect concentration (LOEC), no observed effect concentration (NOEC) and life cycle studies were completed for BaP, E2, NP, DEHP, and A. These substances are also being used for the evaluation of new endocrine disrupter screens as recommended by the European Union (96/22/CE directive).

## 2. Materials and methods

### 2.1. Test organisms

Copepods *E. affinis* used for this study were collected by planktonic netting (200 μm) in the oligohaline zone of the Seine river estuary near the Tancarville bridge (France). They were conveyed to the laboratory in containers and then placed in "clean" water, consisting of Volvic natural water salted at 2‰sea salt (Sera premium, w/v), for 5 days before use for exposure experiments. The animals were kept at $18 \pm 1\,^{\circ}C$ (field temperature) under static-renewal conditions (every 3 days) and a 12-h light:12-h dark photoperiod.

*E. affinis* undergoes six naupliar stages and five copepodid stages before the animals reach adulthood and sexual maturity (Fig. 1). The maturation typically required 15–18 days under the previously described conditions (Katona, 1970a, b). Individual *E. affinis* can be sexed using light stereomicroscopy from stage IV copepodid; then, males can be distinguished from females on the basis of body length and other morphological features (fifth leg and antennules) (Fig. 1).

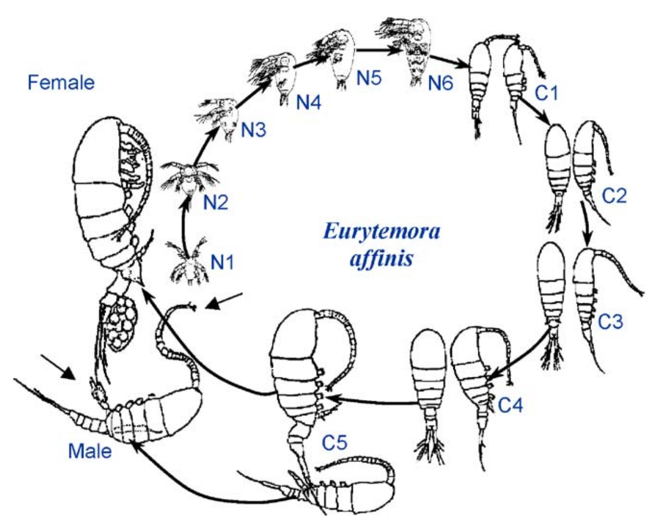


Fig. 1. Summary of the development stages of *E. affinis* (Crustacea: Copepoda). Adapted from Katona (1970a, b) by Souissi (with permission). N, nauplius stage, the difference among nauplli stages is caudal ends; C: copepodid stages, the difference between copepodid stages is the number of swimming legs, female and male, female body slightly longer than male (1.42 mm for 1.15 mm), male fifth legs larger, with a protuberance on inner side of right second basipode segment, Male antennule with spine-like projections.