Dissertation - Investigations into the toxic potential of the two selective serotonin reuptake inhibitors fluoxetine and citalopram in the zebrafish (Danio rerio) embryo

The potential risk posed by fluoxetine and citalopram in aquatic ecosystems is still unclear, and there is the need for studies investigating the toxic potential and toxicokinetics of these compounds in embryonic life-stages of fish. Therefore, this thesis aimed to investigate potential effects of fluoxetine and citalopram on zebrafish (*Danio rerio*) embryos. To this end, (1) light was shed on the accumulation of fluoxetine and citalopram as well as on the biotransformation of fluoxetine in the zebrafish embryo, (2) a new test protocol was developed to analyze effects of neurotoxic compounds on tail movements of zebrafish embryos, (3) effects of fluoxetine and citalopram as well as three neurotoxic model substances (i.e., ethanol, cadmium and dichlorvos) on coiling activity and embryogenesis (fish embryo toxicity test; FET) of zebrafish embryos were investigated, (4) and effects of fluoxetine, a metabolite of fluoxetine (norfluoxetine), and citalopram on stress-related swimming activity of zebrafish embryos were analyzed in the visual motor response (VMR) test with regard to exposure scenarios in the environment.

Accumulation experiments documented that zebrafish embryos exposed to different concentrations of fluoxetine and citalopram from 48 to 120 h post-fertilization (hpf) accumulated fluoxetine more strongly than citalopram. Exposure of embryos to 5 mg/L of fluoxetine resulted in increased phase I biotransformation activity and continuous accumulation of fluoxetine and eleven fluoxetine metabolites including norfluoxetine. Norfluoxetine accumulated to higher levels than fluoxetine and the remaining metabolites, while total concentration levels (summed up) of fluoxetine and norfluoxetine increased over the entire test period. Embryos exposed to fluoxetine at concentrations < 50 µg/L reduced fluoxetine levels over time, while norfluoxetine was the only metabolite that accumulated to detectable levels in these embryos. It was concluded that norfluoxetine is the only metabolite of fluoxetine that accumulates in zebrafish embryos at environmentally relevant exposure scenarios.

Development of the coiling assay revealed that ambient illumination modulates coiling activity from onset of first coiling movements. This observation enables new test strategies by integrating later coiling movements controlled by a more developed nervous system into coiling analyses which allow for an improved interpretation of chemical-induced effects on tail movements of zebrafish embryos. Fluoxetine, citalopram, ethanol, cadmium, and dichlorvos altered coiling activity in different ways, while chemical-induced effects on coiling activity overlapped with effects on embryogenesis (FET). Teratogenic concentrations of fluoxetine and citalopram caused absence of spontaneous tail movements at 24 hpf, while exposure to a non-teratogenic concentration of citalopram resulted in decreased coiling frequency at multiple time points. Based on these experiments, it was not possible to distinguish between chemical-induced effects resulting from unspecific developmental toxicity and effects from specific neurotoxic mechanisms.

In the VMR test, it was shown that both fluoxetine and citalopram reduce stress-related swimming activity of zebrafish embryo after sudden change from light to darkness. Since results demonstrated that environmentally relevant concentrations of fluoxetine impair stress-related swimming activity of zebrafish embryos, a series of further VMR tests was conducted to improve the understanding of fluoxetine-induced effects on the embryonic VMR with regard to more complex exposure scenarios. In this context, it was shown that fluoxetine-induced effects on the embryonic VMR depend on the pH of the test medium and to a large extent can be attributed to effects of norfluoxetine. Moreover, when zebrafish embryos were exposed to equimolar mixtures of fluoxetine, norfluoxetine and citalopram, the test substances were shown to have an additive effect on the embryonic VMR.

Taken together, zebrafish embryos exposed to fluoxetine and citalopram displayed very similar effects in the FET, coiling assay and VMR test, albeit effects by fluoxetine generally occurred at lower concentration levels than effects by citalopram. The different effective concentrations of fluoxetine and citalopram may in part be explained by different toxicokinetics of both compounds. The high accumulation of fluoxetine and its metabolite norfluoxetine, as well as the neuroactive potential of norfluoxetine, contribute significantly to the effects of environmentally relevant concentrations of fluoxetine on stress-related behavior in zebrafish.