Summary

The aquatic environment is exposed continuously and increasingly to chemical substances such as pharmaceuticals. These medical compounds are released into the environment after having being consumed and body-excreted by patients. Pharmaceutical residues are synthetic molecules that are not always removed by traditional sewage treatment processes and thus escape degradation. Among pharmaceuticals that escape sewage treatment plants (STPs), the anticancer drugs were measured in STP effluents and natural waters. In the aquatic environment, their long-term effects at low concentrations are sparsely known on non-target species.

Tamoxifen is an anticancer drug that is widely prescribed worldwide for the prevention and treatment of hormone receptor-positive breast cancers. Two of its metabolites, i.e., endoxifen and 4-hydroxy-tamoxifen (4OHTam), have high pharmacological potency *in vivo* and such as tamoxifen, they are excreted via faeces by patients. Tamoxifen was measured in STP effluents and natural waters but, to the best of our knowledge, its metabolites concentrations in waters have never been reported. Imatinib is another and recent anticancer compound that targets specific tumour cells. This pharmaceutical is also body excreted and because of its increasing use in cancer treatment, imatinib may reach the natural water. The effects of tamoxifen and imatinib are unknown upon more than one generation of aquatic species. And the effects of 4OHTam, endoxifen have never been studied in ecotoxicology so far.

The aims of this thesis were threefold. First, the sensitivity of *D. pulex* exposed to tamoxifen, 4OHTam, endoxifen or imatinib was assessed using ecotoxicological experiments. Ecotoxicology is the science that considers the toxic effects of natural or synthetic substances, such as pharmaceuticals, on organisms, populations, community and ecosystem. Acute and multigenerational (2-4 generations) tests were performed on daphnids considering several studied endpoints, such as immobilisation, size, reproduction, viability and intrinsic rate of natural increase. Additional prospective assays were designed to evaluate whether 1) low concentrations of tamoxifen and 4OHTam were able to induce toxic effects when used in combination, and 2) daphnids were able to recover when offspring were withdrawn from solutions carrying the pharmaceutical. Second, the stability of tamoxifen, 4OHTam and endoxifen in incubation medium was evaluated in solution exempted from daphnids. Because the nominal concentrations of tamoxifen, 4OHTam and endoxifen did not correspond to the measured, we provide a predictive method to estimate the concentrations of these chemicals during long-term ecotoxicological tests. Finally, changes in protein expressions were analysed in *D. pulex* exposed 2 or 7 seven days to tamoxifen using ecotoxicoproteomic experiments with a shot-gun approach inducing a peptide fractionation step.

Our results show that tamoxifen, 4OHTam and endoxifen induced adverse effects in *D. pulex* at environmentally relevant concentrations. At very low concentrations, these molecules displayed

unusual and teratogenic effects because morphological abnormalities were observed in offspring, such as thick and short antennas, curved spines, premature neonates and aborted eggs. Tamoxifen was the most toxic compound among the test chemicals, followed by 4OHTam, endoxifen and imatinib. Tamoxifen no-observed effect concentrations (NOECs) that were calculated for size, reproduction and intrinsic rate were below or in the range of the concentrations measured in natural waters, i.e., between 0.12 µg/L and 0.67 µg/L. For instance, the tamoxifen NOECs that were calculated for reproduction were between 0.67 and 0.72 µg/L, whereas the NOEC was < 0.15 µg/L when based on morphological abnormalities. The NOECs of 4OHTam were higher but still in the same order of magnitude as tamoxifen environmental concentrations, with a value of 1.48 µg/L. Endoxifen NOEC for the intrinsic rate of natural increase (r) and the reproduction were 0.4 and 4.3 µg/L, respectively. Daphnids that were withdrawn from tamoxifen and 4OHTam were not able to recover. Also, the reproduction of *D. pulex* was reduced when the treated animals were exposed to the combination of tamoxifen and 4OHTam while no effects were observed when these chemicals were tested individually at the same concentration. Among the anticancer drugs that were tested during this thesis, imatinib was the less toxic molecule towards *D. pulex*. No effects on size and reproduction were observed within two generations, except for the first whose reproduction decreased at the highest test concentration, i.e., 626 µg/L.

Our results also underline the need to use measured or predicted concentrations instead of the nominal during aquatic experiments, particularly when lipophilic molecules are tested. Indeed, notable differences between nominal (i.e., theoretical) and measured concentrations were found with tamoxifen, 4OHTam and endoxifen at all test concentrations. A cost and time sustainable method was proposed to predict the test exposure levels of these chemicals during long-term experiments. This predictive method was efficient particularly for low concentrations, which corresponded to the test concentrations in multigenerational tests.

In the ecotoxicoproteomic experiments a total of 3940 proteins were identified and quantified in *D. pulex* exposed to tamoxifen. These results are currently the largest dataset from *D. pulex* that is published and the results of proteomic analyses are available for the scientific community. Among these 3940 proteins, 189 were significantly different from controls. After protein annotation, we assumed that treated daphnids with tamoxifen had shifted cost-energy functions, such as reproduction, to maintain their basic metabolism necessary to survive. This metabolic cost hypothesis was supported by the presence of proteins involved in oxidative stress. Biomarkers for early detection of tamoxifen harmful effects on *D. pulex* were not discovered but the proteins of the vitellogenin-2 family (E9H8K5) and the ryanodine receptor (E9FTU9) are promising potential biomarkers because their expression was already modified after 2 days of treatment.

In this thesis, the effects of tamoxifen, 4OHTam and endoxifen on daphnids raise questions about the potential impact of tamoxifen and 4OHTam in other aquatic ecosystems, and therefore, about

metabolites in ecotoxicology. Because the NOECs were environmentally relevant, these results suggest that tamoxifen and 4OHTam may be interesting pharmaceuticals to consider in risk assessment. Our findings also emphasize the importance of performing long-term experiments and of considering multi-endpoints instead of the standard reproductive endpoint. Finally, we open the discussion about the importance to measure test exposures or not, during ecotoxicological studies.