Influence of pH on the toxicity, uptake and biotransformation potential of citalopram in zebrafish (Danio rerio) embryos

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Abstract
The contamination of the aquatic environment has raised concerns in the scientific community and regulatory authorities. Given the large number of xenobiotics, for most of them there is a striking deficit in the literature concerning their adverse effects on aquatic organisms. Citalopram (CTR) is a worldwide highly consumed antidepressant which has demonstrated incomplete removal by conventional wastewater treatment, hence resulting in the contamination of aquatic ecosystems. Consequently, it is urgent to evaluate its potentially toxic effects on aquatic organisms.

The fish embryo test (FET) with zebrafish (*Danio rerio*) is a well-established and standardised (OECD Guideline 236) *in vivo* toxicity test that is commonly used to evaluate potential adverse effects on early development of fish. Thus, it is a helpful tool for risk assessment in aquatic environments. However, until now, neither pH nor the particular properties of ionisable organic compounds (IOC), like CTR, have sufficiently been considered in risk assessment. Characteristics of IOC vary, depending on their presence either as ions or as neutral species, in particular in respect to the uptake into organisms. Due to their electrical charge, ions pass poorly through biological membranes, whereas neutral species permeate more easily through membranes and are, thus, potentially of higher toxicity. The pH is one major factor influencing the proportion of dissociated and non-dissociated ions. Shifts in ambient pH cause alterations of the ionic proportions and thus, are key to IOC toxicity. Although many IOC are partly or completely ionized under environmental relevant conditions and already slight variances of pH can cause considerable changes in toxicity, little attention has been paid to pH and its consequences in toxicity testing.

The objectives of the current study were (1) to assess to what extent CTR induces toxicity to zebrafish embryos. In addition (2), we evaluated the uptake and biotransformation processes of CTR by zebrafish and examined whether biotransformation data could be used in a complementary way to the concentration of the parent compound to interpret the induced toxicity. The final goal was to evaluate to which extent the pH is influencing CTR's uptake, potential bioaccumulation and biotransformation, as well as toxicity.

More specifically, the zebrafish embryo toxicity assay was used to calculate the LC50 of CTR, as well as to evaluate potential sub-lethal endpoints (e.g. the hatching rate). Exposure experiments were conducted at three different pH values (6, 8 and 9), to assess potential pH-dependent differences in an environmental relevant pH range. Concerning the toxicokinetic part of the study, exposure experiments were conducted at the LC50 value of each pH.

The extraction was carried out with the tissue homogenizer Cryolis Evolution® (Bertin Technologies, France) operating at 8200 rpm for 5 cycles of 15 sec with a 60 s break at 4ºC. Two different organic solvent mixtures (methanol-water and methanol-dichloromethane) were used for the extraction of zebrafish embryos in order to cover a very wide range of physicochemical properties. Exposure water samples and zebrafish extracts were analysed by RPLC and HILIC methods, in both positive and negative ionization mode, to cover the widest possible range of polarities, using LC-QTOF-HR-MS/MS. Detection and identification of tentative CTR biotransformation products (bio-TPs) were performed through in-house developed suspect and non-target screening workflows. Internal concentration of parent CTR and its bio-TPs was determined. Potential pH dependent differences of CTR's uptake and biotransformation were evaluated as well. . Finally, the biotransformation pathway of CTR in zebrafish embryos was proposed.

Keywords: citalopram, zebrafish embryo, pH dependent toxicity, biotransformation

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