"From toxicity assay to metabolomics analysis" An integrated approach to assess the toxicity of three Benzotriazoles in zebrafish (Danio rerio) embryos

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EXTENDED ABSTRACT

The ever increasing contamination of the aquatic environment from xenobiotics has raised concerns in the scientific community and the regulatory authorities. Given the large number of xenobiotics, there is an important gap in the literature concerning their adverse effects on aquatic organisms (Farré, Pérez, Kantiani, and Barceló (2008)). Benzotriazoles (BTs), a high production volume chemical class,(Weiss, Jakobs, & Reemtsma, 2006) pose low biodegradability and sorption tendency, consequently they are partially or not at all removed from WWTPs (Giger, Schaffner, & Kohler, 2006). Thus, there is clear evidence that BTs persist in aquatic systems, as they are measured in almost every surface water sample (Loos, Gawlik, & Locoro, 2008; Reemtsma, Miehe, Duennbier, & Jekel, 2010). Consequently, it is urgent to evaluate their potentially toxic effects to aquatic organisms. The zebrafish has emerged as a powerful model organism to study various aspects of developmental and cell biology (Beis & Stainier, 2006) as well as physiology. In addition, it provides an alternative model for toxicological studies, since mammalian and zebrafish toxicity profiles are strikingly similar (McGrath, 2011).

The objectives of this study were to assess to what extent 1-H-benzotriazole (BT), 4-methyl-1-H-benzotriazole (4-MeBT) and 5-methyl-1-H-benzotriazole (5-MeBT) induce toxicity in zebrafish embryos. In addition, we evaluated the uptake and biotransformation of BTs by zebrafish and examined whether biotransformation data could be used complementary to the concentration of the parent compounds to interpret the induced toxicity. The final goal was to establish a wide-scope targeted metabolomics screening method to investigate the induced toxicity in a biochemical perspective and associate the observed toxicity/phenotype with changes in molecular level.

More specifically, the zebrafish embryo toxicity assay was used to calculate the LC50 values of BTs as well as to perform the morphological phenotyping. In brief, newly fertilized eggs were exposed to the tested chemicals and their development was recorded up to 96-hours post fertilization (hpf). Concerning the biotransformation and the metabolomics experiment, 96 hpf zebrafish were used. Samples were collected at 5 different time intervals, from 30 s up to 24 hours post exposure (hpe), to examine the time profiles of the parent BTs, their biotransformation products (bio-TPs) and the endogenous metabolites of zebrafish. Extracts were analyzed by RPLC and HILIC methods, in both positive and negative ionization mode, to cover the widest possible range of polarities, using a LC-QTOF-HR-MS/MS instrument.

For the detection and identification of tentative bio-TPs, both suspect and non-target screening workflows have been applied. Both oxidative (hydroxylation) and conjugative (sulfation, glucuronidation) bio-TPs were identified. Moreover, the biotransformation rate proved to be informative and correlated well with the observed toxicity. As regards the metabolomics part of the study, a database of over 600 endogenous metabolites (carboxylic acids, amines, nucleotides, mono- and disaccharides etc.) was established, covering a broad range of primary metabolism pathways. The wide-scope targeted metabolomics method proposed in this study constitutes an alternative to the classic targeted methods, as it did not focus at a predefined set of metabolic pathways. The approach to cover a broad range of primary metabolism pathways is hypothesis-generating rather than hypothesis-driven, as it enables to unravel the involvement of unexpected metabolic pathways.

The combination of morphological phenotyping information from acute toxicity test with internal concentration and biotransformation data from toxicokinetic experiment, in addition to the biochemical information from the wide-scope targeted metabolomics analysis, constitutes a high-throughput and integrated approach for the toxicity assessment.

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