Holistic platform for xenometabolome coverage of zebrafish embryos exposed to triclosan utilizing timsTOFpro and a biotransformation-oriented data processing workflow

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Introduction

Triclosan (TCS) constitutes a common household product ingredient, given its antimicrobial activity, and has been widely used over the past decades. There is clear evidence that TCS persists in aquatic systems. Thus, it is urgent to evaluate its potentially toxic effects to aquatic organisms. Zebrafish has emerged as a powerful model organism to study various aspects of developmental and cell biology, while it provides a promising alternative model for acute toxicological studies. The impact of xenobiotics in the aquatic environment is evaluated in more depth when the whole xenometabolome of aquatic organisms is studied. Biotransformation is known to affect the internal concentration (C_{int}) and the uptake of the parent xenobiotics, while it constitutes a critical factor for the toxic response.

Methods

The zebrafish embryo (ZFE) toxicity assay was used to calculate the LC50 of TCS, as well as for phenotypic evaluation of TCS toxicity. 4 time-interval samples were taken during the exposure experiment. A holistic analytical platform was developed combining orthogonal chromatographic modes (HILIC and RPLC), trapped ion mobility (TIMS), and HRMS for the analysis of TCS and the identification of its bio-TPs.

ZFE were extracted with organic solvent mixtures (Methanol: Water and Dichloromethane :Water). ZFE extracts analysis was conducted by UHPLC (Elute) coupled with a hybrid trapped ion mobility-quadrupole time-of-flight mass spectrometer (timsTOF pro).

A "biotransformation oriented" data treatment workflow, consisted of suspect and non-target screening, was developed utilizing MetaboScape (version 2021)

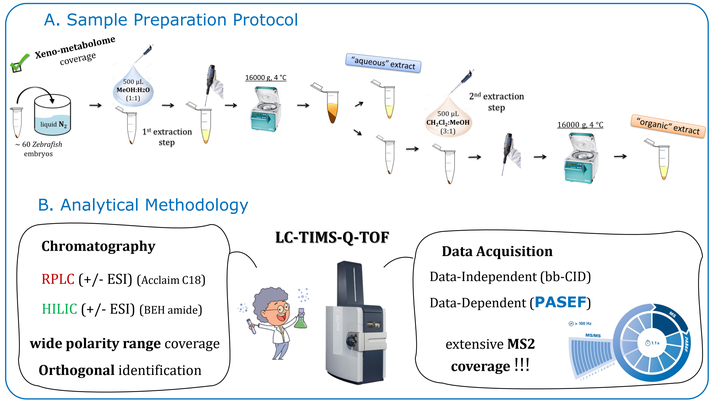

Fig. 1: A. Sample preparation protocol for comprehensive xeno-metabolome coverage of zebrafish, B. Analytical methodology: Extracts were analyzed on a UHPLC-**TIMS**-QTOF.

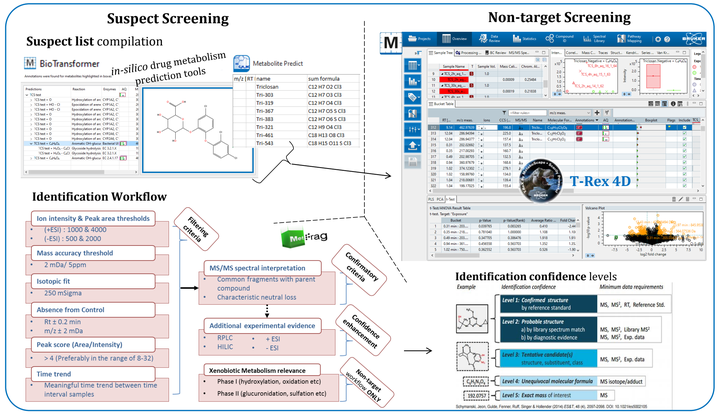

Fig. 2: Bio-transformation oriented data processing workflow (Suspect & Non-target screening) for the identification of tentative bio-TPs.

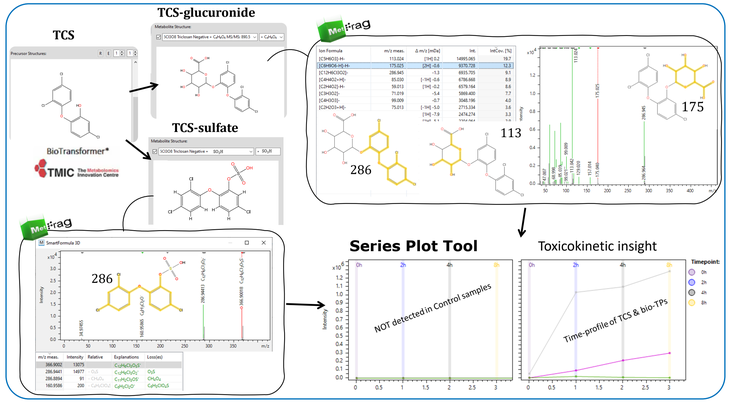

Fig. 3: Identification of TCS-glucuronide and TCS-sulfate. Structures of bio-TPs were predicted using BioTransformer. The *in-silico* fragmentation prediction tool MetFrag was utilized for MS/MS interpretation. Time profile provided additional insights.

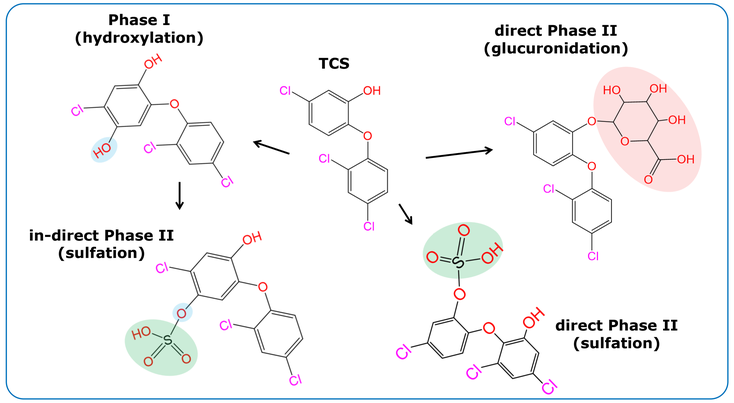

Fig. 4: Proposed biotransformation pathway of TCS by zebrafish embryos, based on the main bio-TPs detected. TCS-sulfate, TCS-glucuronide, TCS-hydroxyl and TCS-hydroxyl-sulfate were observed by means of the applied analytical procedure.

Results

An efficient biotransformation-oriented data processing workflow (Fig. 2) was developed to facilitate the identification of bio-TPs. Suspect screening was based on predicting potential bio-TPs (phase I and II), utilizing **BioTransformer** (inside MetaboScape user interface). *In-silico* fragmentation (**MetFrag**) proved particularly helpful in suspect and non-target screening workflows (Fig. 3) to compare different annotated structure candidates to peaks in the measured MS/MS spectrum. Evaluation of the time profile of the potential bio-TPs was used as a filtering step.

Bio-TPs arising from both phase I and conjugative (phase II) metabolic reactions were identified. Nevertheless, it should be noted that Phase II (conjugative) reactions prevailed in the biotransformation process. Overall, 7 bio-TPs were identified through suspect and non-target screening workflows, while 3 are reported for the first time. The main categories are hydroxylated bio-TPs, sulfate conjugated and glucuronic acid conjugated bio-TPs. In addition, retention time information in two orthogonal modes and collision cross-section values provided additional evidence to the accurate mass measurements and isotopic profiles and enhanced the overall identification confidence.

Conclusions

- The power of combining different orthogonal separation modes (RPLC, HILIC, TIMS) with HRMS for the identification of un-known bio-TPs had been highlighted.
- A powerful bio-transformation oriented data processing workflow was developed.
- MetaboScape proved to be a very promising tool for the identification of bio-TPs.
- A comprehensive xeno-metabolome coverage of aquatic organisms requires the use of holistic approaches that provide extensive analytical evidence and high-throughput identification.

timsTOF / Environmental