Electrochemical Simulation of Triclosan Metabolism and Toxicological Evaluation

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Introduction
Triclosan (TCS), an antimicrobial agent, is considered as an emerging pollutant due to its wide dispersive use in personal care products and high aquatic toxicity. In the present study, phase I metabolism of Triclosan was investigated using electrochemistry.

The products formed in the electrochemical (EC) cell were identified by on-line and off-line MS. The sequential formation and disappearance of each product, with the continuous increase of voltage from 0 to 3500 mV, was observed to reveal the transformation pathways of TCS. Eight metabolites (transformation products = TPs) were found using the EC approach.

In the present study, phase I metabolism of Triclosan was investigated using the ReactorCell. 5

Results
1.2. Electrochemical synthesis of metabolites (TPs)
For online EC-MS experiments, the QTRAP® linear ion-trap equipped with the ReactorCell® (Antec Scientific, Boston, USA) and a boron-doped diamond (BDD) working electrode was used. MS experiments were carried out on QTRAP 2000 (SCIEX, USA) and high-resolution FT-ICR MS (LIT ThermalIonization Mass Spectrometry, USA). Off-line electrochemical analyses were conducted in a bulk reactor, i.e., SynthesisCell® (Antec Scientific, Zoeterwoude, Netherlands; Schematics instrumental setup: Figure 1 B). Off-line MS experiments, the ROXY™ Potentiostat equipped with the ReactorCell™ (Antec Scientific, Zoeterwoude, Netherlands) was used. MS experiments were carried out on QTRAP 2000 ESI MS/MS (SCIEX, USA) and high-resolution FT-ICR MS LIT (ThermoFinnigan, USA). Off-line electrochemical analyses were conducted in a bulk reactor, i.e., SynthesisCell® (Antec Scientific, Zoeterwoude, Netherlands).

The solution was seeded in the SynthesisCell® for approximately 2 h. The quantification of TCS in the reaction solution was performed with a QTRAP ESI-MS instrument (SCIEX) coupled with an Agilent 1200 HPLC (Agilent Technologies, Germany) equipped with a Zorbax SB-C18 column.

Table 1: Predicted structures of metabolites/tranformation products (TPs) of Triclosan. P7 and P8 were not isolated.

The Phase I metabolism of Triclosan was successfully simulated by electrochemistry mass spectrometry.

1.3. Toxicological evaluation of Triclosan and its metabolites (TPs)

The acute toxicity through the transfection pathway after cleavage (Route A), hydroxylation (Route B), and cyclization (Route C) are shown in Figure 4 based on the predicted LC50 values of TCS and its metabolites. The three products formed in the Route A were one or two level less toxic than TCS, which indicates the toxicity decreased through cleavage of the ether bond. The three products (P4, P7 and P8) formed through Route B showed similar toxicity on fish as the parent compound. The results indicate that P6, P7 and P8, which have similar structure as TCS, are less toxic than TCS and the acute toxicity of TCS apparently increased with the formation of highly toxic like products.

Conclusions
The Phase I metabolism of Triclosan was successfully simulated by electrochemistry mass spectrometry.

• All major metabolites/ transformation products (TPs) could be generated within a few minutes in full agreement with literature.
• Two new toxic-dioxygen like metabolites could be predicted for the first time.
• Ether cleavage, hydroxylation and cyclization are the main reaction mechanisms.
• Electrochemical synthesis allowed for rapid synthesis of mg quantities of TPs used in the toxicology and environmental tests.
• Triclosan and the reaction mixture after electrochemical reactions showed high toxicity on zebrafish embryos.

This study highlights that Triclosan and its metabolites may cause serious adverse effects in aquatic species if TCS is continuously used and released into the environment. Therefore, the chemical should be considered on the priority list of emerging contaminants and its utilization in all products should be regulated.

Reference