Application Note
Environmental Degradation

Electrochemical Reactions upfront MS – EC/MS

Proteomics & Protein Chemistry
S-S bond reduction
HDX
Peptide bond cleavage
Na+, K+ removal
Drug-protein binding

Lipidomics & Fatty Acids
Cholesterol
Oxysterol
FAME Biodiesel

Drug Metabolism
Mimicking CYP 450
Phase I & II
Biotransformation

Synthesis (mg)
Metabolites & Degradants

Pharmaceutical Stability
Purposeful degradation
API testing
Antioxidants

Environmental
Degradation & persistence
Transformation products
Surface & drinking water

Food & Beverages
Oxidative stability
Antioxidants

Forensic Toxicology
Designer drugs
Illicit drugs

Healthcare & Cosmetics
Skin sensitizers

Genomics
DNA Damage
Adduct formation
Nucleic acid oxidation

Electrochemical Simulation of Triclosan Metabolism and Toxicological Evaluation

- **All major transformation products generated by EC**
- **Fast prediction and identification by on-line EC-MS**
- **Rapid electrochemical synthesis of metabolites for toxicological studies**

Introduction

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

This application note is by courtesy of Dr. Stephan Küppers, Research Center Jülich, Department of Analytics, Jülich, Germany. For more info see reference [1]

Instrumentation

In Figure 1 the schematics of the instrumental setup for on-line EC-MS and for the electrochemical synthesis is shown.

Figure 1: Schematics instrumental setup: (A) On-Line EC-MS for metabolite (transformation product, TP) generation using the ReactorCell. (B) Off-line EC-MS for metabolite (TP) synthesis using the SynthesisCell

Method

For online EC-MS experiments, the ROXY™ Potentiostat equipped with the ReactorCell™ (Antec Scientific, Boston, USA) and a boron doped diamond (Magic Diamond™) working electrode was used. MS experiments were carried out on QTRAP 2000 ESI-MS/MS (ABSciex, USA) and high-resolution ESI-FTICR-MS Ultra (ThermoFisher Scientific, USA). Offline electrochemical reactions were conducted in a bulk reactor, i.e., SynthesisCell (Antec Scientific). 5 mg of TCS with 10 mM ammonium acetate was dissolved in a 50 mL mixture of methanol and water (1:4 v/v). The solution was oxidized in the SynthesisCell for approximately 2 h. The quantification of TCS in the reaction solution was performed with a QTRAP 6500 instrument (ABSciex) coupled with an Agilent 1260 HPLC (Agilent Technologies, Germany) equipped with a Zorbax C18 column.

Results

Electrochemically induced metabolism

In Figure 2 the 2D mass voltammogram of selected masses of the metabolites, i.e.,transformation products (TPs) of Triclosan are shown. While the reaction voltage increased from 0 to 3500mV with 10 mV/s, the formation of the metabolites (TPs} can be directly monitored. Due to multiple peaks of each TP, only one mass of each TP was recorded. We found that P1 (m/z 143), P2 (m/z 161), P3 (m/z 179), P4 (m/z 267), P6 (m/z 303) and P7 (m/z 317) were almost formed at the beginning of the reaction (at approximately 1400 mV). The intensities of P1, P2, P3 and P7 continuously increased until the reaction ended. The intensity of P4 slightly declined at voltages higher than 2500mV, indicating that P4 can be oxidized to other product at higher voltages. We found P5 formed at the voltage of 2000 mV and increased until the end of the reaction, which indicate P5 may be transformed from other products. P8 is excluded in the figure due to the low intensity comparing with the other products.

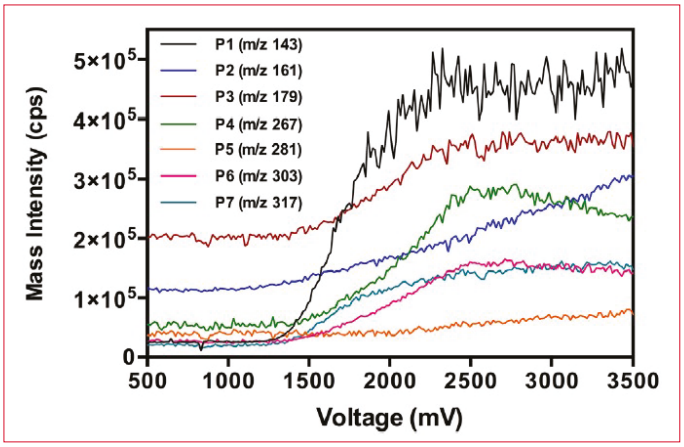


Figure 2: 2D mass voltammogram of selected masses of the metabolites, i.e., TPs formed during the flow of the Triclosan solution (50 µM of TCS in 5 mM ammonium acetate buffer, in a mixture of methanol and water (2:3 v/v)) through the ReactorCell (Antec Scientific) equipped with a Magic Diamond electrode. Voltage ramp 0 to 3500 mV.

Based on the mass spectrometric analysis of the QTRAP and FTICR-MS, the chemical structures of possible transformation products (TPs) were elucidated. In Table 1 the exact mass, elemental compositions and proposed structures of possible TPs are shown.

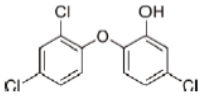
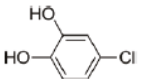
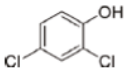
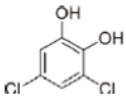
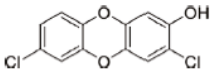
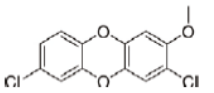
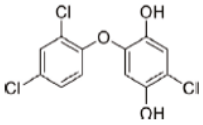
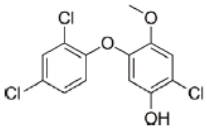
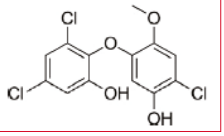
Name	Mass [M-H]-	Elemental composition	Structure
Triclosan	286.94376 288.94082 290.93793 292.93793	$C_{12}H_7O_2Cl_3$	
P1	142.99054 144.98759	$C_6H_5O_2Cl$	
P2	160.95667 162.95371 164.95076	$C_6H_4OCl_2$	
P3	176.95158 178.94863 180.94574	$C_6H_4O_2Cl_2$	
P4	266.96219 268.95928 270.95627	$C_{12}H_6O_3Cl_2$	
P5*	280.9 282.9 284.9	$C_{13}H_8O_3Cl_2$	
P6	302.93889 304.93596 306.93302 308.92997	$C_{12}H_7O_3Cl_3$	
P7	316.95457 318.95160 320.94861 322.94586	$C_{13}H_9O_3Cl_3$	
P8	332.94937 334.94644 336.94353 338.94059	$C_{13}H_9O_4Cl_3$	

Table 1: Proposed structures of metabolites/transformation products (TPs) of Triclosan. P5* not found in FTCIR-MS

Electrochemical simulation showed excellent agreement with the biotransformation reactions in the literature. Monohydroxylated TCS (P6), which has been identified as metabolite in rats (Tulp et al., 1979; Wu et al., 2010) and also found as biotransformation product in biological waste-water treatment (Chen et al., 2015; Lee et al., 2012), was successfully predicted by electrochemical simulation. 4-Chlorocatechol (P1), 2,4-DCP (P2) and 3,5-DCC (P3), known as major metabolites via ether bond cleavage during the biotransformation (Kim et al., 2011; Mulla et al., 2016; Tulp et al., 1979), were also found electrochemically.

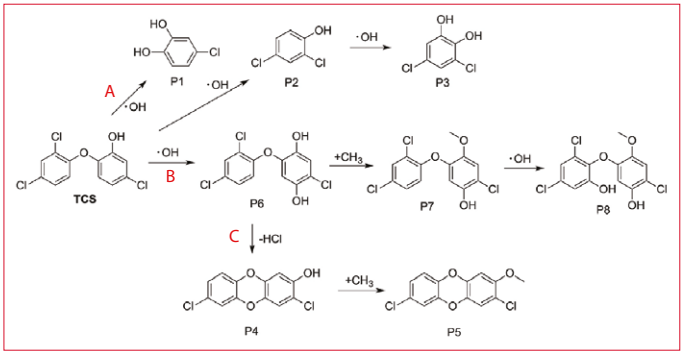


Figure 3: Transformation pathways of TCS during the electrochemically simulated metabolism, with route A, B and C.

Methylation of P6 leads to the formation of P7, which is hydroxylated to P8. Besides successful prediction of the known metabolites, two dioxin derivatives P4 and P5 were predicted as potential metabolites for the first time. The results indicate dioxin-like products are formed, known to be very toxic, highlighting the potential risk of TCS usage in personal care products.

Ether cleavage (Route A), Hydroxylation (Route B), and cyclization (Route C) are the major transformation mechanisms of Triclosan. TCS was attacked by the hydroxyl radicals and formed the metabolites, 4-Chlorocatechol (P1) and 2,4-dichlorophenol (DCP) (P2) via the mechanism of ether bond cleavage. 2,4-DCP (P2) was then oxidized to 3,5-dichlorocatechol (DCC) (P3).

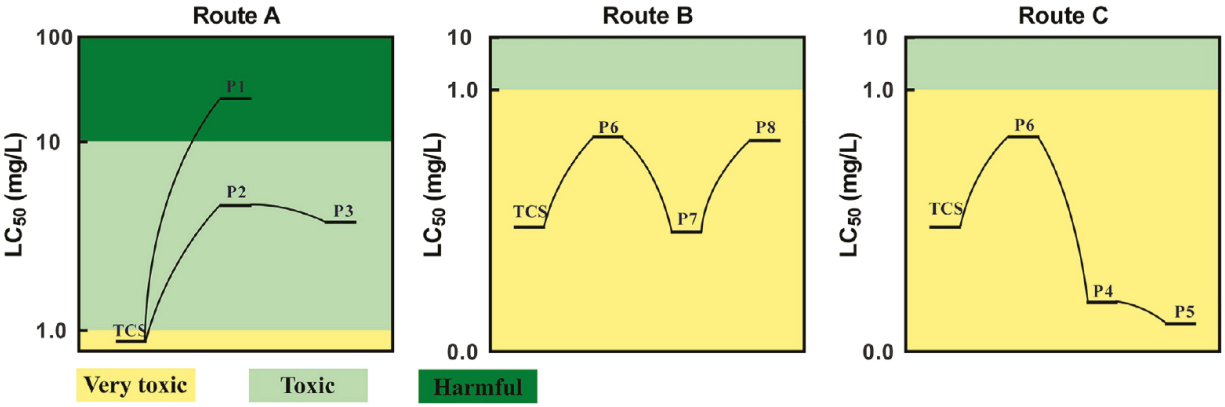


Figure 4: Evolution of acute fish toxicity through the 3 transformation pathways A, B and C based on the predicted LC50 values of TCS and its metabolites.

Meanwhile, hydroxylation of TCS occurred at the less chlorinated benzene ring to form monohydroxyl-TCS (P6). Subsequently, methylation of P6 led to the formation of P7, which later hydroxylated to P8. Monohydroxyl-TCS (P6) was also transformed to hydroxyldichlorodibenzodioxin (DCDD) (P4) via C-Cl bond breaking and hydroxyl radical induced cyclization (Kanetoshi et al., 1987; Nilsson et al., 1974). Methylation of P4 led to the formation of P5.

Electrochemical synthesis of metabolites (TPs)

For the synthesis of the metabolites a bulk reactor (SynthesisCell) controlled by the ROXY Potentiostat (Antec) was used as shown in Figure 1 B. Based on the properties of TCS and its transformation products, a CHROMABOND® HR-X column (3 mL/200 mg, Macherey-Nagel, Düren, Germany) was chosen as the solid phase extraction. The eluate was dried using a SpeedVac concentrator and then reconstituted into stock solution in DMSO, for more details see [1].

Toxicological evaluation of Triclosan and its metabolites (TPs)

The acute toxicity through the 3 transformation pathways ether cleavage (A), hydroxylation (B), and cyclization (C) are shown in Figure 4 based on the predicted LC50 values of TCS and its metabolites. The three transformation 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 (P6, 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, may retain the toxicological properties of TCS.

Through Pathway C, the acute toxicity of TCS apparently increased with the formation of highly toxic dioxin like products P4 (LC50, predicted 0.19 mg/L) and P5 (LC50, predicted 0.11mg/L). The results suggest the high risk of TCS transformed through a cyclization mechanisms. In conclusion, only ether-bond cleavage reactions can detoxify TCS; the other metabolism mechanisms may form similar or higher toxic products.

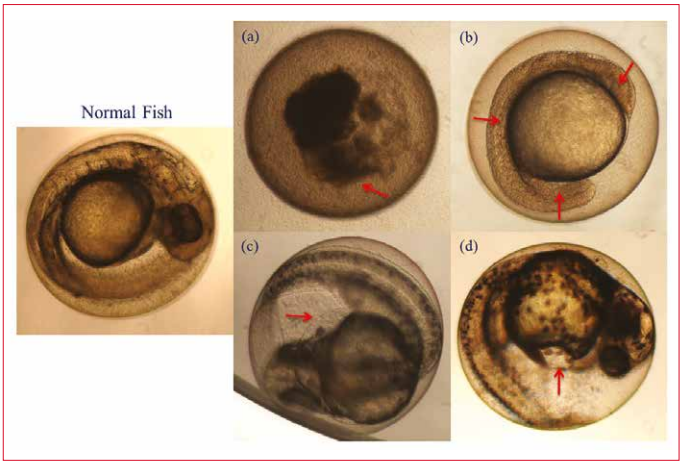


Figure 5: Observed lethal and sub-lethal effects of the reaction mixture on zebrafish embryos (Danio rerio) after 48 h exposure (directed by red arrows): (a) Coagulation (10 mg/L); (b) No pigmentation, undeveloped tail and no eye primordial (2.5 mg/L); (c), (d) Heart edema and slow heartbeats (1.25 and 0.625 mg/L). Solvent control: Normal fish.

References

1. Linyan Zhu et al.; Science of the Total Environment, 622–623 (2018) 1193–1201

Conclusion

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 dioxin-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 studies. 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 system if TCS is continuously used and released into the environment. Therefore, this chemical should be considered on the priority list of emerging contaminants and its utilization in all products should be regulated.

Ordering information

210.0070A	ROXY™ EC system, incl. dual syringe pump, ReactorCell, electrodes, LC connection kit for phase I and II reactions and Dialogue Elite software for system control. All parts included for described application.
206.0037	SynthesisCell with Reticulated Glassy Carbon (RGC) WE - complete
206.0306	Boron Doped Diamond (Magic Diamond) working electrode for SynthesisCell

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333