**IDENTIFICATION OF POTENTIAL EXPOSURE / PATHOLOGICAL METABOLOMIC AND LIPIDOMIC SIGNATURES OF OBESITY AND LIVER PATHOLOGIES USING ZEBRAFISH AS A MODEL**

**N. Papaioannou1,2, C. Gabriel1,2, T. Papageorgiou1,2,** **H. Le Mentec4,5,** **D. Lagadic-Gossmann6, S. Karakitsios1,2,3,** **N. Podechard6, D. Sarigiannis1,2,3\***

1 HERACLES Research Center – CIRI, Aristotle University of Thessaloniki, Greece

2 Environmental Engineering Laboratory, Department of Chemical Engineering, Aristotle University of Thessaloniki, Greece

3 Environmental Health Engineering, School for Advanced Study IUSS, Pavia, Italy

4 Institut National de la Santé et de la Recherche Médicale (INSERM), Institut de Recherche en Santé, Environnement et Travail (IRSET-INSERM UMR 1085), 35000 Rennes, France,

5 Université de Rennes 1, Faculté des Sciences Pharmaceutiques et Biologiques, Structure Fédérative de Recherche Biosit UMS CNRS 3480/US INSERM 018, 35043 Rennes, France

6 Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail)-UMR\_S 1085, University of Rennes, F-35000 Rennes, France.

*\**  denis@eng.auth.gr

**ABSTRACT**

The aim of this study was to assess metabolomic and lipidomic responses of zebrafish (D. rerio) embryos exposed to Amiodarone and DEHP. Amiodarone is an effective and widely used anti-arrhythmic drug with known hepatotoxicity used as positive control in this study [1]. Di-2-ethylhexyl phthalate (DEHP) is a colourless, viscous, and lipophilic plasticiser belonging to the high molecular weight HMW phthalates [2]. We performed global untargeted metabolomics analysis using an Agilent 6540 Ultra High Definition Accurate-Mass QTOF instrument, two different analytical columns (C18 - RP and HILIC), and two different ionisation modes (positive and negative) to increase the coverage of the detected metabolites to the maximum. Data collected with the Agilent MassHunter Workstation Data Acquisition Software v.B.06.01 were translated into the .mzML open format using the tool msConvert included in the ProteoWizard toolkit. Data pre-processing and processing, which included data cleaning, log transformation, normalisation, and batch effects correction, was performed using the Bioconductor R - based package XCMS v.3.10.1. An R package, called xMSannotator, was used for Network-Based annotation retrieving information from HMDB, Metlin, and Lipid Maps. The significantly differential metabolites were determined by fold-change analysis and then mapped to metabolic pathways using Fisher’s method. The databases used were the following: KEGG, WikiPathways, Reactome, HumanCyc, EHMN, PharmGKB, SMPDB, BioCart, INOH, and PID. In total, 202 unique annotated features were found to have significantly different peak areas after exposure to amiodarone and DEHP. Most of the annotated statistically significant belong to the lipids, and more precisely, to the class of glycerophospholipids, followed by the sterol lipids and fatty acyls. Sixty-one biomarkers, including amino acids (e.g. L-Serine) and lipids (e.g. Prostaglandin E2) were common for all three treatments, suggesting a general effect of amiodarone and DEHP in amino acid and lipid metabolism. In addition, DEHP 10 μM and DEHP 25 nM presented 28 metabolites affected by both exposures indicating a similar mode of action. The most significantly perturbated pathway is the one of Sphingolipid metabolism. In conclusion, the untargeted UPLC-HRMS metabolomic approach allowed the identification of potential biomarkers related to toxicity mechanisms of investigated pollutants in these aquatic organisms.

**KEYWORDS:** D. rerio embryos, untargeted metabolomics, amiodarone, DEHP, metabolic disorders

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