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Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Effects of dietary microplastic exposure on the organ toxicity of a mixture of chemical contaminants in zebrafish

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OVERVIEW

- Effects of dietary exposure to microplastic and chemical contaminants on the organ toxicity of an aquatic animal model.
- Biological system used: zebrafish (*Danio rerio*) adult fish.
- Exposure protocol based on evaluation of: stability, solubility and uptake of the compounds.
- Biological effects detected by microscopic observation, histopathology and evaluation of gene expression in different organs.
- Major effects were detected on the liver.

INTRODUCTION

Microplastic contamination of the aquatic environment is considered a growing problem. The ingestion of microplastic has been documented for a variety of aquatic animals. Studies have shown the potential of microplastic to affect the bioavailability and uptake route of sorbed co-contaminants such as persistent organic pollutants and metals. The effect of the dietary uptake of microplastic and sorbed co-contaminants in aquatic organisms still needs to be properly understood.

OBJECTIVE

To evaluate the biological effect at organ level of the dietary uptake of microplastic and sorbed co-contaminants in an aquatic model organism.

METHODOLOGY

EXPERIMENTAL DESIGN

- 15 Fish fed daily with 0,2 g contaminated feed
- Duration: 3 weeks

Samples

- Sampling at end of experiment
- Organs collected in pools of 4 in triplicate and homogenised

Extracted organs:

- **Liver**
- **Brain**
- **Intestine**
- **Muscular tissue**

Experimental design approved by the competent Ethical Committee

Table 1. Types of feed used in this work

FEED	CONTENT	DETAILS OF CONTENT
A	Not contaminated feed	Normal feed
B	Feed with microplastic	2% LPDE 125-250 µm diameter
C	Feed with microplastic and contaminants	As B with contamiant of Table 2
D	Feed with chemical contaminants	Contaminants of Table 2 (x 2)

Table 2. Chemical contaminants added to the feed

Compuond	Concentration in feed (ng/g)
HCBD	11.6
2,4,6 TBP	8.7
PFOS	19.9
PFOA	9.8
PFNA	10.9
PFOSA	10.1
MeHg	50.0
DE71 (PBDE)	83.6
PCB 28	10.3
PCB 52	10.5
PCB 101	21.6
PCB 118	20.4
PCB 153	20.6
PCB 138	40.1
PCB 180	5.2

Detected effects

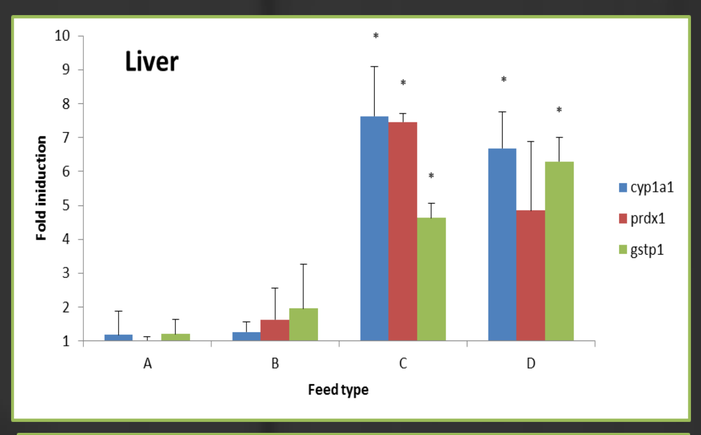
- Organ shape and appearance by visual inspection (stereomicroscope Leica MZ FLIII).
- Histopathology by standard techniques.
- Differential expression of some selected genes by qRT-PCR.

SELECTED GENES

- ***Cyp1a1:*** cytochrome P450 enzyme. Deotxification phase I enzyme.
- ***Prdx1:*** peroxiredoxin 1 involved in oxidative stress.
- ***Gstp1:*** gluthation S transferase. Detoxification phase II enzyme

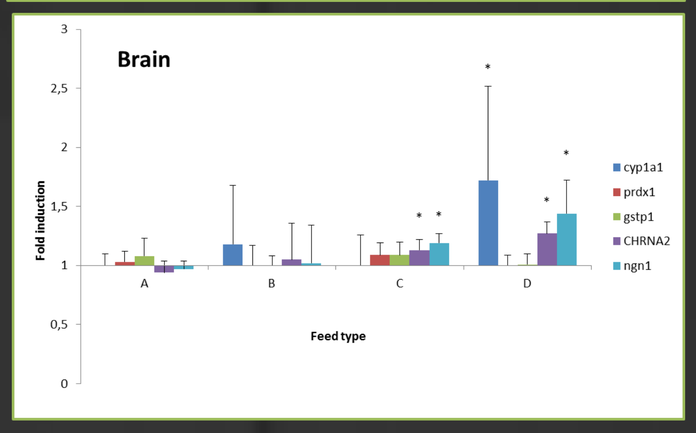
RESULTS

DIFFERENTIAL EXPRESSION OF THE SELECTED GENES IN DIFFERENT ORGANS



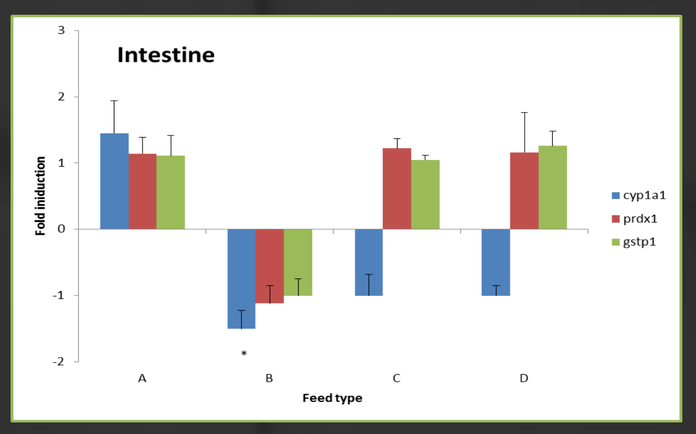
Liver gene expression

- **Feed A:** control
- **Feed B Microplastic alone** = no differential expression
- **Feed C Microplastic + contaminants =** highest levels of induction for all the genes tested.



Brain gene expression

- **Feed A:** control
- **Feed B Microplastic alone** = no differential expression
- **Feed C Microplastic + contaminants =** induction of genes CHRNA2 and ngn1.

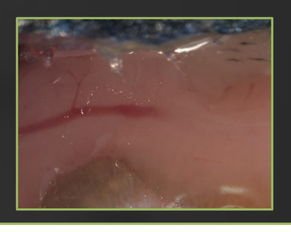


Intestine gene expression

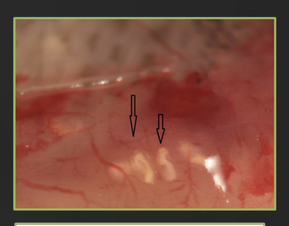
Only cyp1a1 is downregulated in fish fed with feed B.

No other effects were detected.

MICROSCOPY OBSERVATION OF THE LIVER



Liver of fish fed with feed B and D



Liver of fish fed with feed C

60% of the livers of fish fed with C showed some rice shaped formation not observed in other livers.

HISTOLOGICAL SECTION OF LIVER OF FISH FED WITH FEED A AND C

CONCLUSIONS

Microplastic alone showed no effects on the exposed fish.

Effects of microplastic + contaminants were detected in the liver.

Microplastic affects the effect of chemical contaminants.

ACKNOWLEDGEMENTS

We are grateful to Michiel Kotterman (IMARES Wageningen University) for providing microplastic and advice on this work.

Project funded by the European Commission (Project ECsafeSEARFOOD) and the Spanish Minsitry of economy and competitivness (Project SAFEFOOD—CTQ 2014-55711-R)

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 311820.

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