Assessing the Ecotoxicological Impact of Plastic-Associated Chemicals from Consumer Products on the Marine Environment: Using Atlantic Halibut (*Hippoglossus hippoglossus*) Hepatocytes

Norwegian Institute for Water Research

Maria T. Hultman[*,1], Prem Chand[1], Cassandra Rauert[2], Pradeep Dewapriya[2], Kevin Thomas[2] & Tânia Gomes[1]

Introduction

❖ The increasing amount of microplastics resulting from fragmentation & degradation of marine litter are well documented, but the environmental risks of plastic-associated chemicals in consumer products are emerging as a much lesser understood threat to the marine environment.

❖ **The aim of this study was to investigate the toxic effects of plastic-associated chemicals originating from consumer products in a marine fish species relevant to the Norwegian environment.** We developed a reproducible, cost-effective New Approach Method (NAM) using primary liver (hepatic) cells from the marine Atlantic Halibut (*Hippoglossus hippoglossus*). Following implementation, the toxicity of leachates from five different consumer products were assessed using the primary hepatocytes.

Leachate preparation Non-target & Suspect chemical analysis

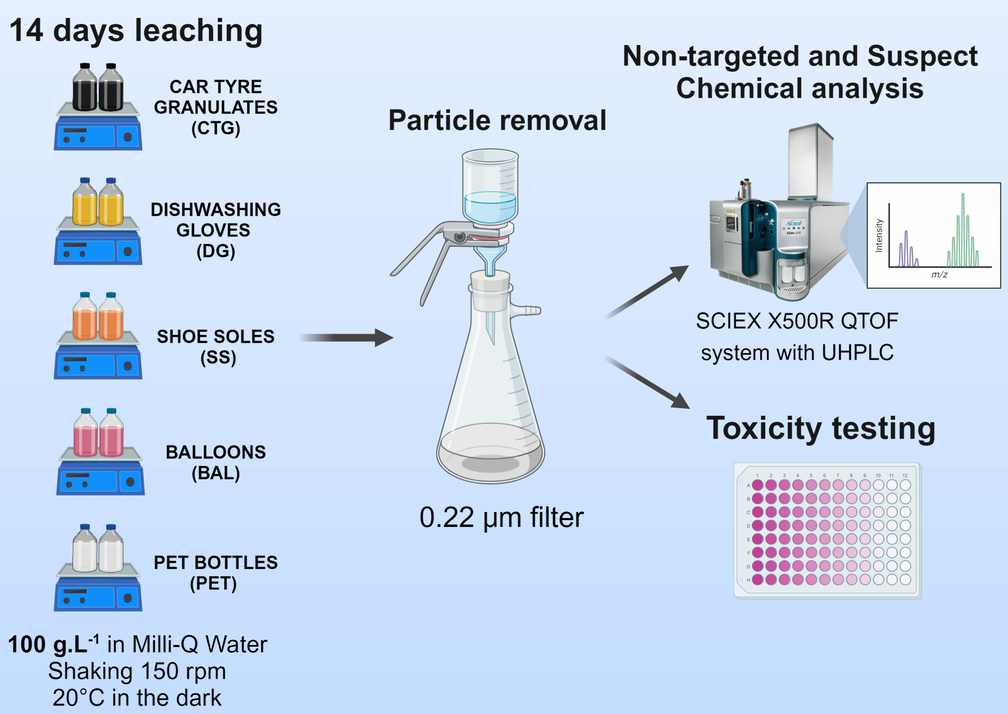


Figure 1. *Five plastic consumer products (CTG, DG, SS, BAL & PET) were cryomilled & sieved in a size range of <500µm. The particles were leached at 150 rpm for 14 days in Milli-Q water, in the dark at 20°C. Following leaching, particles were removed by filtering the solution (0.22µm filter) & aliquotes frozen at -20°C until further analysis. Leachates were subjected to suspect & non-targeted chemical analysis using LCMS (SCIEX X500R QTOF system with UHPLC, LC-QToF). Additional extracts were prepared following the same procedure for further in vitro toxicity assessments.*

In vitro experimental design

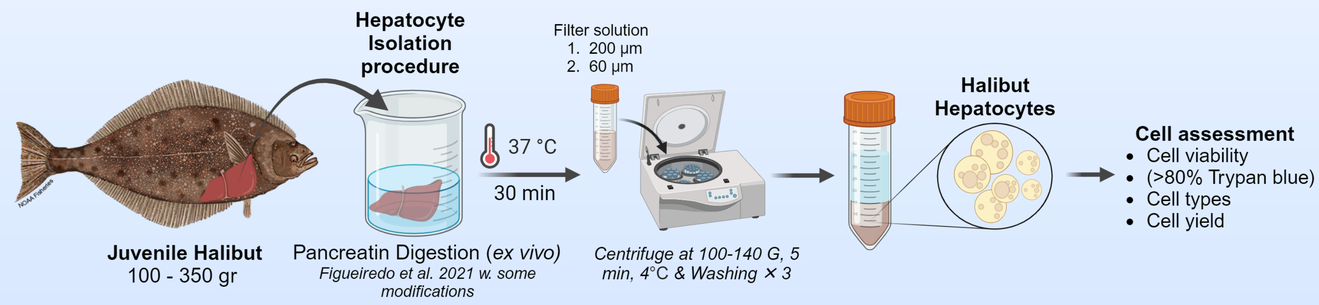


Figure 2. *Hepatocyte isolation procedure using ex vivo pancreatin digestion with modifications* i.e., no histopaque, [1].

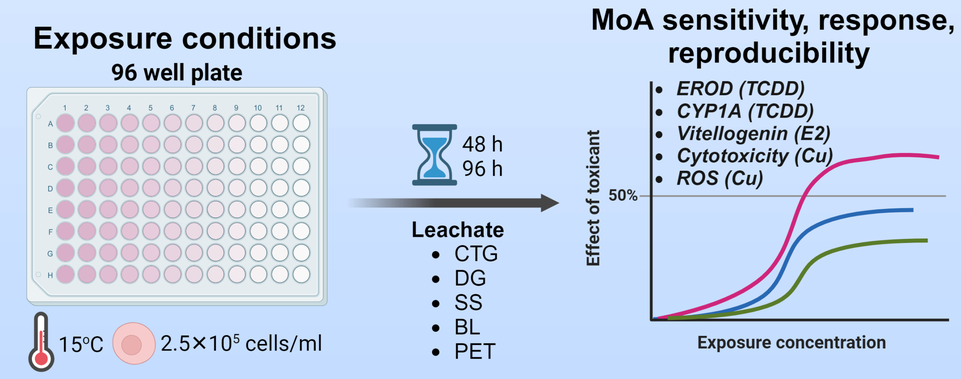


Figure 3. *Isolated primary hepatocytes were plated (96 well plate) & acclimatized 24h prior to assessing the assay's sensitivity, responsiveness, reproducibility & endpoint suitability, when exposed to a solvent control (DMSO) & positive controls for the following endpoints: Cytotoxicity: Cell membrane integrity & Metabolic activity (Copper sulphate, Cu), Cytosolic Reactive Oxygen Species, ROS (Cu/H_2O_2), Vitellogenin, Vtg (β-Estradiol, E2), Ethoxyresorufin-O-deethylase, EROD & Cytochrome P450 1A, CYP1A (2,3,7,8-Tetrachlorodibenzodioxin, TCDD) for 48 & 96 hours* [2]*. Following its assessed suitability, the assay was utilized to assess toxicity of a leachate control (only Milli-Q water) & five consumer product leachates: CTG, DG, SS, BAL & PET (0.0033 – 33.3 g/L) for 48 & 96 hours.*

Results

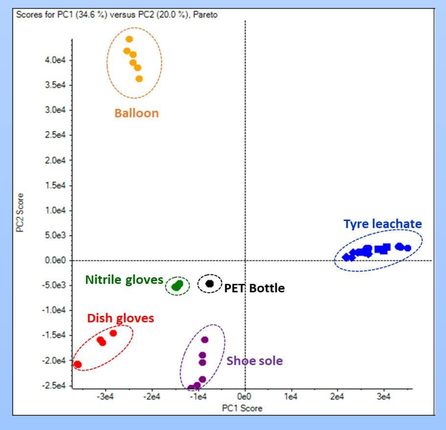
Chemical feature extraction

A.

		Aligned features	Car Tyre Granulates	Balloons	Dishwash gloves	Nitrile Gloves	PET bottle	Shoe Sole
SCIEX OS	Feature finding	17094						
In all replicates	Noise Filter	13159						
Intensity > $10e^4$	Intensity filter	13101						
	RSD filter	6743						
	# of true features*	6220	3830	3962	4004	1607	813	2456
In one sample	Unique features	1298	668	116	374	97	2	41

**Some features present in multiple products*

B.



C.

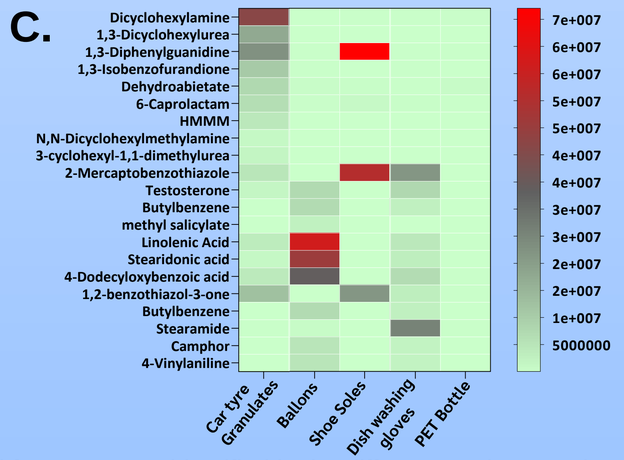


Figure 4: *Non-targeted chemical analysis & suspect screening of leachates originating from five different plastic consumer products (A). A total 1298 unique chemical features were identified in the different products & plotted in a principal component analysis (PCA) (B). The PCA displayed the chemicals to be clearly grouped in their individual leachates. Chemicals found in different leachates & intensity across the leachates (obtained by LC-QToF) displayed that several were present in more than one leachate (C).*

Primary halibut hepatocytes

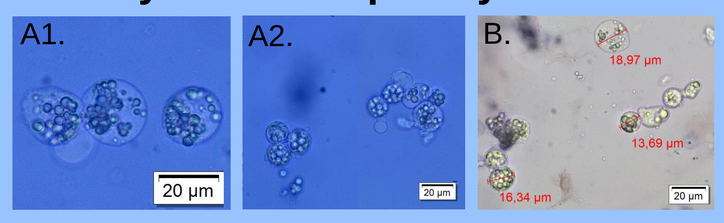


Figure 5. *Primary hepatocytes isolated from juvenile Atlantic Halibut. A light microscope was utlized to assess cell viability using Trypan blue (A) & cell diameter (B). Cell diameter was Ø 15.1 ± 2.4 µm (mean ± SD). Visual inspection of the cells implies minimum two different populations (lipid droplets & complexity). Cell viability was 96.6 ± 5.1 % (mean ± SD) in three independent cell isolations.*

In vitro exposure

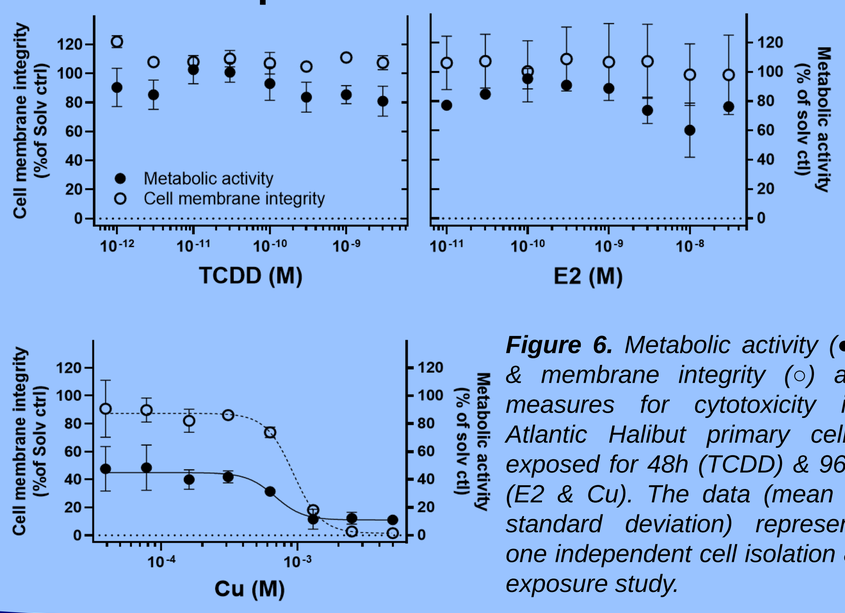


Figure 6. *Metabolic activity (●) & membrane integrity (○) as measures for cytotoxicity in Atlantic Halibut primary cells exposed for 48h (TCDD) & 96h (E2 & Cu). The data (mean ± standard deviation) represent one independent cell isolation & exposure study.*

Conclusion

❖ Chemical suspect screening identified CTG > DG > BAL as containing the highest number of unique chemical features.

❖ Chemicals present in leachates that might be of concern, e.g. Testosterone, 2-Mercapto-benzothiazole, dicyclohexylamine, etc.

❖ *The in vitro* approach was highly suitable for chemical toxicity assessment, as it had sensitive & responsive cytotoxicity to the positive controls at 48 & 96h, in accordance with literature [2]. The assay is highly suitable for further toxicity assessment of the five leachates.

Future work

❖ The *in vitro* toxicity assessment to the five leachates is currently ongoing, focusing on cytotoxicity, EROD, CYP1A, Vtg & ROS.

References: [1] Figueiredo *et al*. Int J of Env Res and Pub Heal. 2021; 18(4), p.1380; [2] Petersen *et al*. Aquat tox. 2017; 187, 141-152.

Contact: mhu@niva.no

Affiliations: [1]Norwegian Institute for Water Research (NIVA), Oslo, Norway; [2] University of Queensland, Queensland Alliance for Environmental Health Sciences Faculty of Health and Behavioural Sciences, Brisbane, Australia.

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