Establishing a method for measuring Cyp1a activity using precision-cut liver slices from Atlantic cod (*Gadus morhua*)

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Introduction

- Cyp1a is a protein that plays a key role in the metabolism of drugs and other xenobiotics.
- Measurement of Cyp1a, or ethoxyresorufin-O-deethylase (EROD) activity, in fish is a common biomarker of exposure to environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) and structurally related compounds[1].
- The transcriptional activation of the *cyp1a* gene is mediated through a cytosolic receptor called aryl hydrocarbon receptor (AhR).
- Cyp1a catalyses the conversion of 7-ethoxyresorufin (7-ER) to resorfin (R), a fluorescent molecule that can be quantified (Figure 1).
- Traditionally, EROD activity is measured in liver S12 or microsomal fractions (Figure 2a). Previously, EROD activity has been measured directly in salmon liver slices[3].
- The **aim of this study** was to establish a method of measuring EROD activity in precision-cut liver slices (PCLS) from Atlantic cod (*Gadus morhua*) (Figure 2b).

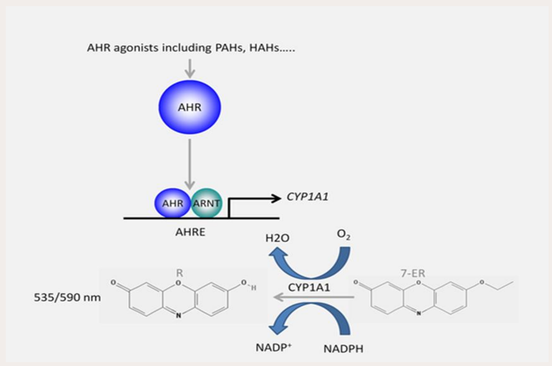


Figure 1 - Illustration of AhR activation and the conversion of 7-ethoxyresorufin to resorufin Cyp1a[2]

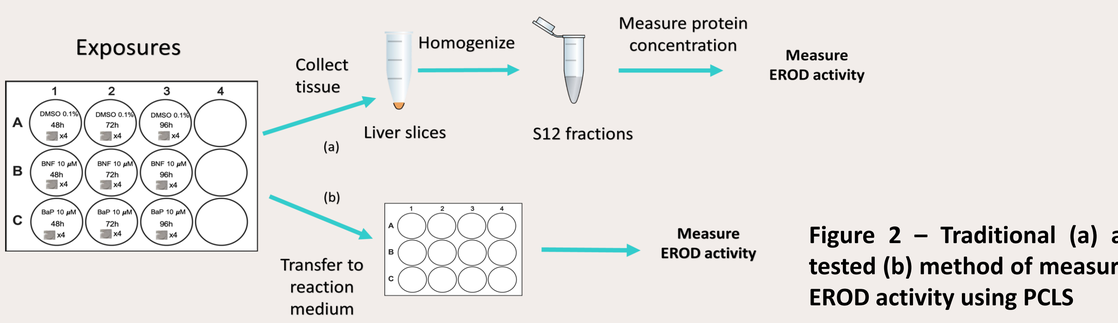


Figure 2 – Traditional (a) and tested (b) method of measuring EROD activity using PCLS

Methods

Precision-cut liver slices (PCLS)

- PCLS (250 µm thickness) from Atlantic cod were prepared using a vibratome. Liver slices were exposed to solvent control (DMSO 0.1%) and the Cyp1a-inducers beta-naphtoflavone (BNF, 10 µm) and benzo(a)pyrene (BaP, 10 µm) for 48, 72 and 96 hours (Figure 3).

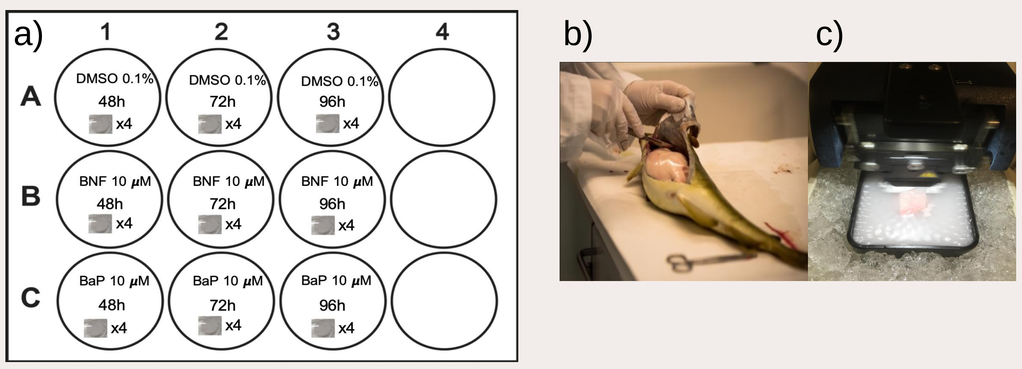


Figure 3 – PCLS exposures setup. a) shows exposure setup in a 12-well plate, b) shows excision of the cod liver and c) shows liver slicing in the vibratome

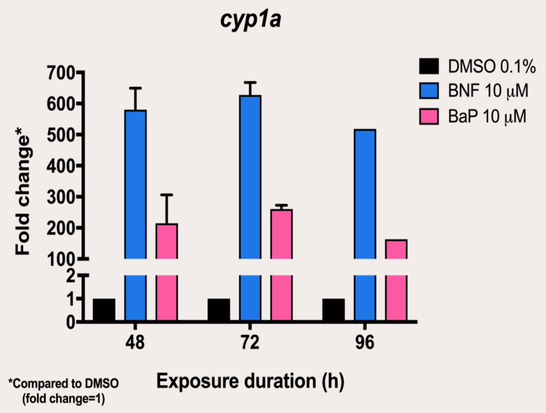
Proof of induction: qPCR and Western blot

- qPCR and Western blot were performed to verify the induction of the *cyp1a* gene and Cyp1a protein expression, respectively.

EROD assay

- PCLS were subjected to EROD analyses in two ways: 1) Using the traditional method by homogenization of PCLS tissue (S12 fractions) and determining protein concentration prior to EROD assay and 2) Direct incubation of PCLS in reaction medium containing substrate (7-ER) prior to EROD measurements.

Results



Proof of gene and protein induction

BNF and BaP cause induction of the *cyp1a* gene (Figure 4), but a weaker induction of Cyp1a protein expression (Figure 5), compared to DMSO.

Figure 4 – Expression of *cyp1a mRNA* in precision-cut liver slices exposed to BNF and BaP.

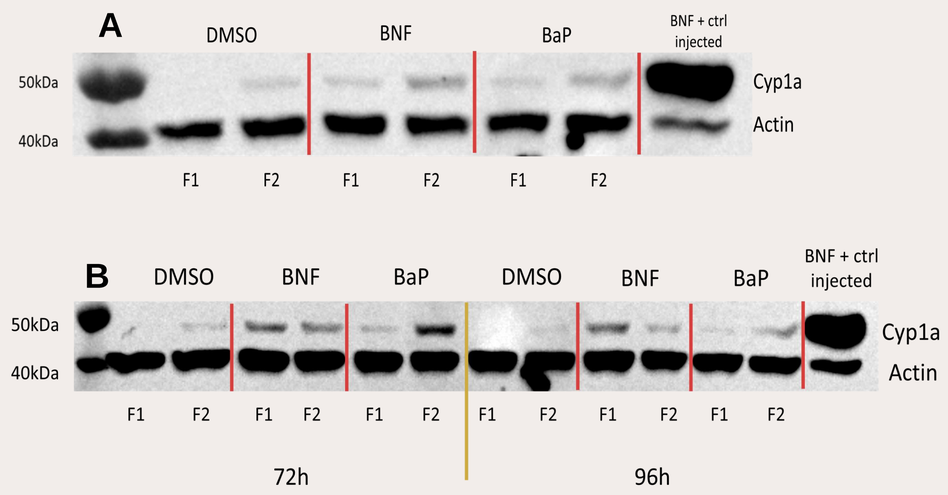


Figure 5 – Expression of Cyp1a protein in PCLS exposed to DMSO, BNF and BaP for 48 hours (A) and 72 and 96 hours (B) determined by Western blot.

EROD activity measurements

Table 1: EROD activities measured with PCLS

		Liver S12 fractions from PCLS	Incubation with PCLS (n.d = not detected)
Exposure (PCLS)	**Exposure duration (h)**	**pmol/min/mg protein**	**pmol/min/mg slice**
DMSO	48	3.41	0.067
BNF	48	1.37	n.d
BaP	48	2.82	0.144
DMSO	72	1.90	n.d
BNF	72	1.15	n.d
BaP	72	3.21	0.162
DMSO	96	1.24	n.d
BNF	96	1.26	n.d
BaP	96	1.41	0.120
S12 fraction controls from *in vivo* exposed fish:			
S12 + CTRL injected		196	
S12 – CTRL injected		31.4	

Discussion

- There was a strong induction of the *cyp1a* gene for both BNF (~600 fold) and BaP (~200 fold).
- The Cyp1a protein synthesis is less induced compared to the strong *cyp1a* gene expression.
- Compared to samples from *in vivo* exposed fish, EROD activity in S12 fractions was very low, and the activities measured directly in PCLS were near or below the limit of detection.

References

1. Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin- *O* -deethylase (EROD) Activity in Fish as a Biomarker of Chemical Exposure. Crit. Rev. Toxicol. 30, 347–570.
2. Mohammadi-Bardbori, A. (2014). Assay for quantitative determination of CYP1A1 enzyme activity using 7-Ethoxyresorufin as standard substrate (EROD assay). Protocol Exchange. https://doi.org/10.1038/protex.2014.043
3. Lemaire, B., Beck, M., Jaspart, M., Debier, C., Calderon, P. B., Thomé, J.-P., and Rees, J.-F. (2011). Precision-Cut Liver Slices of Salmo salar as a tool to investigate the oxidative impact of CYP1A-mediated PCB 126 and 3-methylcholanthrene metabolism. Toxicology in Vitro, 25(1), 335–342. https://doi.org/10.1016/J.TIV.2010.10.002

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