Fish cell lines: a screening tool for multiple stress scenarios

Interaction between **fatty acid** profile and **metals** in rainbow trout hepatocytes (RTL-W1)

C . Bonnineau, A. Ferain, I. Neefs, A. Zuyderhoff, C. McGahan, M. Pierloot, J.F. Rees, Y. Larondelle, C. Debier

Institut des Sciences de la Vie, UCLouvain, Louvain-la-Neuve, Belgium

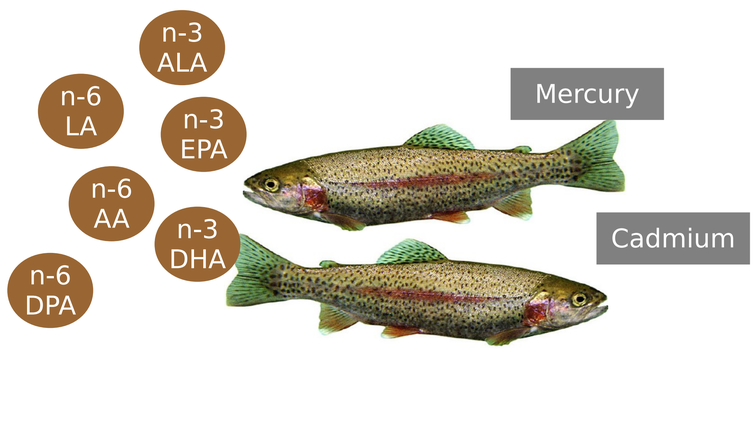
chloe.bonnineau@uclouvain.be

MULTIPLE STRESS SCENARIO

In aquatic ecosystems, organisms undergo multiple stress due to environmental changes (temperature, food quality, water quality...) and contamination. Though classical toxicological tests usually investigate the effect of one pollutant under optimal and stable environmental conditions, the environmental parameters are likely to influence the response of organisms to contamination.

Food quality is expected to influence fish health and so its capacity to cope with stress. In particular, fish fatty acid (FA) profile is strongly dependent on fatty acid composition of its food. **Since fatty acids play an important role in fish cell membrane, and in oxidative stress response, fatty acid composition may influence contaminant uptake and/or sensitivity to oxidative components such as metals.**

=> Is fatty acid composition of food influencing metals toxicity in fish ?



Omega-3 (n-3) FA: ALA: alpha-linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid
Omega-6 (n-6) FA: LA: linoleic acid, AA: arachidonic acid, DPA: docosapentaenoic acid

Complexity
Animal testing

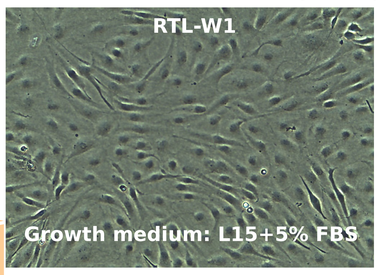
Cause-effect
determination

IN VITRO SCREENING

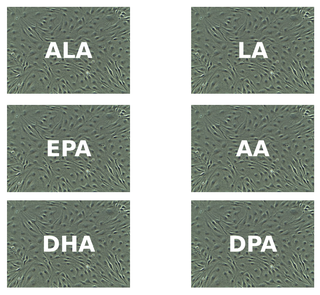
To be able to test the influence of various fatty acids on different metallic compounds, an *in vitro* study was conducted allowing to reduce complexity and focus on cause-effect determination.

=> Is fatty acid composition of fish cells influencing cells sensitivity to metals ?

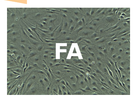
1. Modification of cellular fatty acid profile



1 week incubation
in growth medium
+ 50 µM FA
complexed with BSA



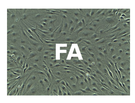
2. Cell characterisation after incubation with each fatty acid



Membrane fluidity by fluorescence anisotropy

Determination of FA profile
- extraction (Bligh & Dyer 1959)
- lipid class separation (SPE)
- methylation & GC analyses

3. For each fatty acid, determination of cells sensitivity to metals



24h **exposure** to:
- **Organic mercury** CH_3HgCl
- **Inorganic mercury** $HgCl_2$
- **Cadmium** $CdCl_2$

Cell viability
- Membrane integrity (CFDA,AM)
- Metabolic activity (Cell Titer Blue)

Medium FA composition influences the cellular FA profile

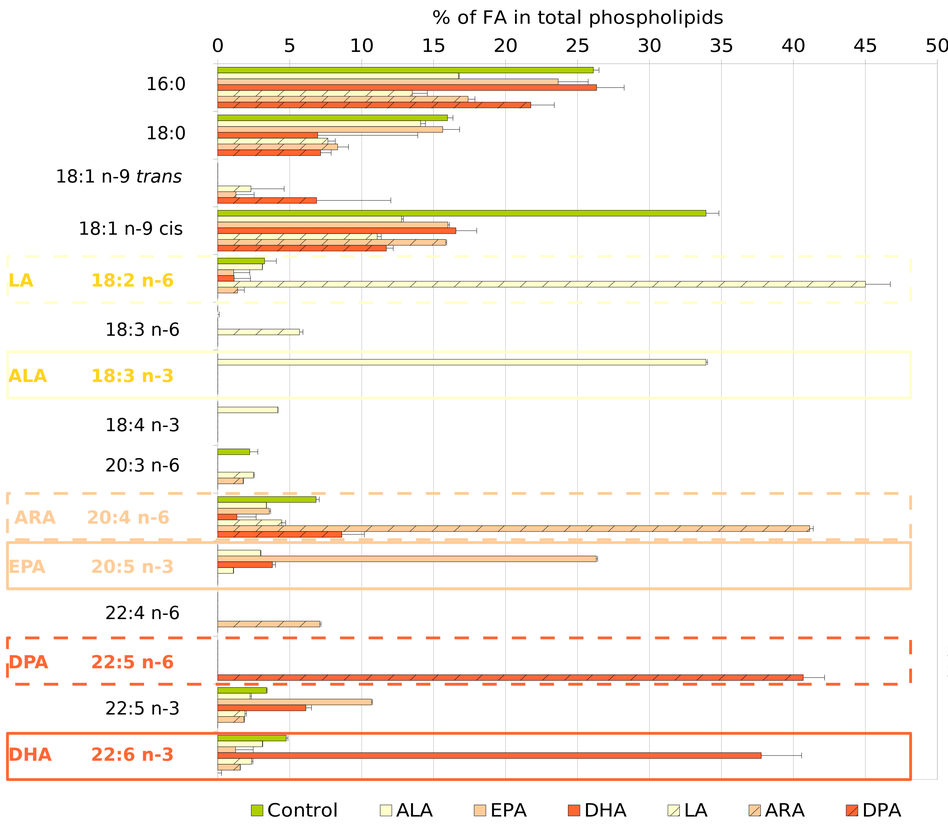
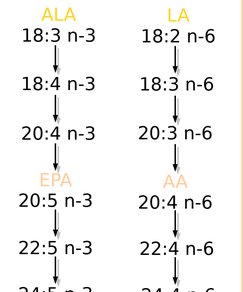


Figure 1: FA composition (%) of phospholipids in control cells or in cells enriched in different FA.



Biosynthesis of n-3/n-6 FA in fish hepatocytes

- **Assimilation** and incorporation of **fatty acids from growth medium into the cells**.
- **Biotransformation** of fatty acids by the cells => accumulation of some FA intermediates of the biosynthesis pathways of long chain FA.

No significant changes in cellular membrane fluidity

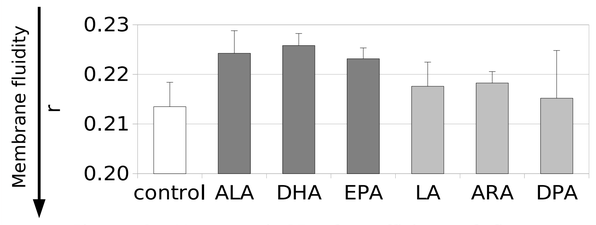


Figure 2: Mean and SE of coefficient of fluorescence anisotropy (r) after 1 week incubation with one of the different FA tested or with no addition of FA (control). (n=4, except for DPA: n=2)

Membrane fluidity tends to be lower in cells enriched in omega-3 fatty acids than in cells enriched in omega-6 fatty acids and in control cells. Nevertheless, these results need further confirmation.

Some FA influence cell response to metals

METHYLMERCURY

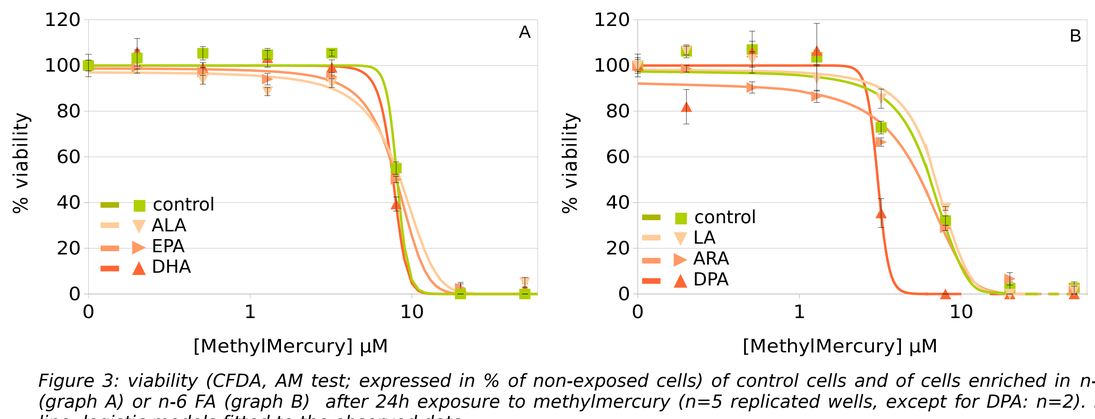


Figure 3: viability (CFDA, AM test; expressed in % of non-exposed cells) of control cells and of cells enriched in n-3 FA (graph A) or n-6 FA (graph B) after 24h exposure to methylmercury (n=5 replicated wells, except for DPA: n=2). Plain line: logistic models fitted to the observed data.

MERCURY

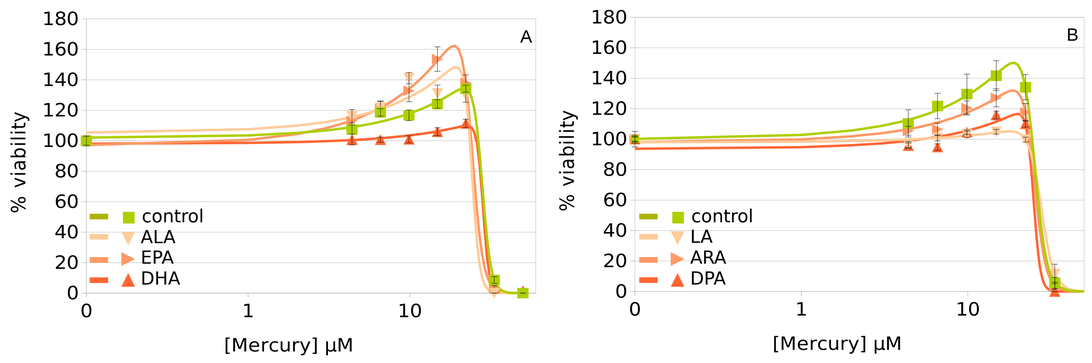


Figure 4: viability (CFDA, AM test; expressed in % of non-exposed cells) of control cells and of cells enriched in n-3 FA (graph A) or n-6 FA (graph B) after 24h exposure to mercury (n=5 replicated wells). Plain line: Brain-Cousens model fitted to the observed data.

CADMIUM

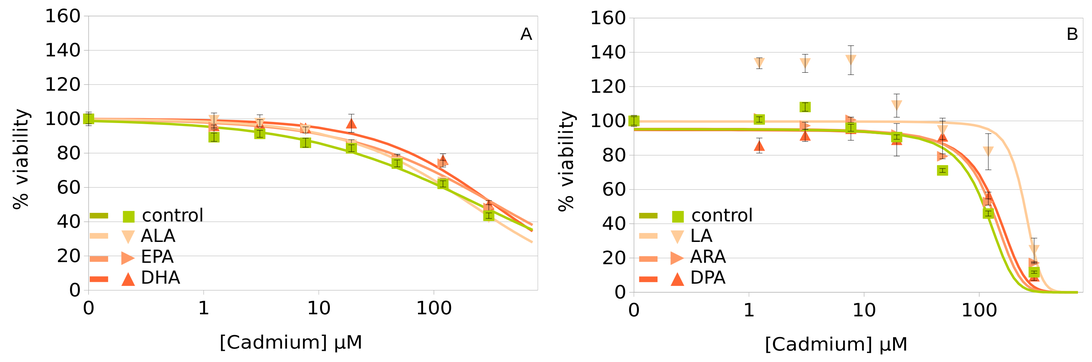


Figure 5: viability (CFDA, AM test; expressed in % of non-exposed cells) of control cells and of cells enriched in n-3 FA (graph A) or n-6 FA (graph B) after 24h exposure to cadmium (n=5 replicated wells). Plain line: logistic model fitted to the observed data.

- Cells enriched in **DPA** are **more sensitive to methylmercury**: EC_{50} of cells enriched in DPA = 3.0 [1.5-4.6] µM
  EC_{50} of control cells = 6.5 [5.8-7.1] µM
- **Hormesis** effect due to **mercury** exposure in control cells (0.016±0.002 to 0.029±0.007)
  Reduction of hormesis effect in cells enriched in **LA** (0.004±0.002), **DPA** (0.012±0.002) or **DHA** (0.005±0.001)
  Increase of hormesis effect in cells enriched in **EPA** (0.038±0.004)
- **Protective** effect of **LA** against **cadmium** exposure: EC_{50} of cells enriched in LA = 248.2 [175.7-320.7] µM
  EC_{50} of control cells = 110.7 [99.4-122.1] µM

Few interactions between cell FA composition and response to metal exposure

- The **fatty acid composition** of the **cell line RTL-W1** was **successfully manipulated *in vitro*** by modifying growth medium composition. Therefore, the cell model was particularly useful to screen different conditions and thus to investigate the interaction between nutritional stress and chemical contamination.
- The influence of **6 fatty acids** on cell response to 24h exposure to **3 different metallic compounds** was investigated. Methylmercury was found to be the most toxic compound, followed by mercury and cadmium. As shown by these first results, some of the **fatty acids tested influenced cell viability**, (estimated as membrane integrity), in response to metals contamination. In particular, the omega-6 fatty acid **DPA** may interact with **mercury toxicity** since DPA enriched cells were more sensitive to methylmercury and presented a lower hormesis effect in response to inorganic mercury. Moreover, long chain highly unsaturated fatty acids in general may interact with inorganic mercury toxicity since a similar **reduction of mercury hormesis** was also observed in cells enriched in **omega-3 fatty acid DHA.** Finally, further investigation should be conducted on the potential protective effect of the **omega-6 fatty acid LA** against **cadmium** toxicity.

The research leading to these results has received funding from BELSPO for the IAP AQUASTRESS (P7/31), from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement PIEF-GA-2012-332049 for FISHSTRESS project and from FNRS (A.Ferain PhD grant: F3/5/5–MCF/ROI/BC -19727).

Pdf available