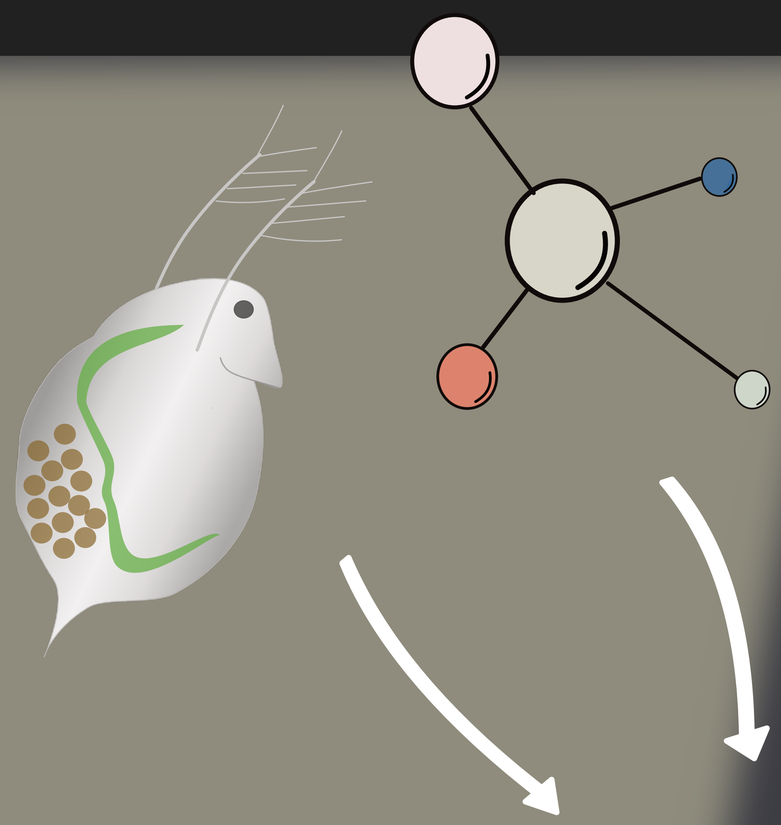
Multiplexed High-Content Screening of Chemicals in *Daphnia magna*

Cedric Abele[1], Amira Perez[1], Paula Pierozan[1], Erik Nylander[1], Magnus Breitholtz[2], Oskar Karlsson[1]

[1]*Science for Life Laboratory, Department of Environmental Sciences, Stockholm University, 114 18 Stockholm, Sweden*
[2]*Department of Environmental Science, Stockholm University, 114 18 Stockholm, Sweden*



1 Introduction

A globally growing economy and developing society demand large amounts of chemicals with specific physico-chemical properties. This requires efficient testing of novel chemicals. High content-screening (HCS) describes automated microscopic image acquisition with subsequent quantitative evaluation of multi-parametric data sets. In this project molecular fluorescence staining applied on aquatic organisms will be used to measure toxicological effects *in vivo*.

Fluorescence Staining

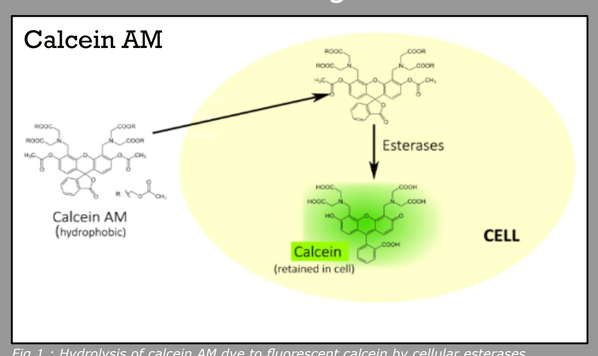


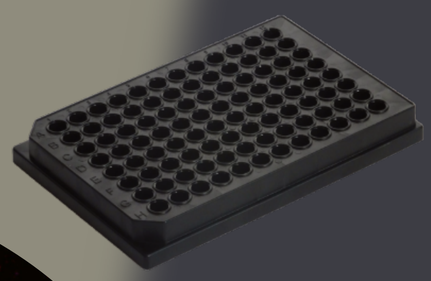
Fig.1.: Hydrolysis of calcein AM dye to fluorescent calcein by cellular esterases (https:/www.gbiosciences.com/)

2 Aims

(i) Develope HCS workflows for toxicological effect screening.

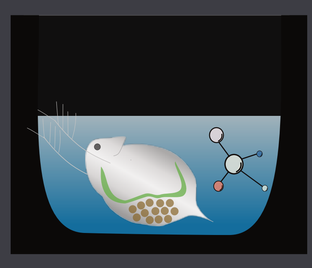
(ii) Asses effects of single, or multiple, chemicals and environmental samples.

(iii) Understand toxic mechanisms in aquatic organisms.

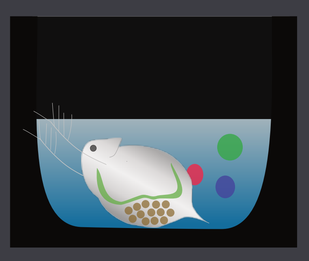


Red fluorescence
Stain: *SYTOX Deep Red*
Localization: *Nucleic Acid*
End point: *Dead cell*

Blue fluorescence
Stain: *DAPI*
Localization: *Nucleic acid*
End point: cell counting



Green fluorescence
Stain: *Calcein AM*
Localization: C*ytoplasm*
End point: *Living cell/ esterase activity/membrane integrity*



3 Method

1. Chemical exposure (dose - response setup)
2. Subsequent staining with molecular dyes
3. Multiplexed image acqusition
4. Analysis of fluorescence intensity with ImageJ

4 Preliminary data

OECD acute immobilisation

concentration (µg/L)	0	5	10	25	50	75	100	200
immobile after 24h	0	0	0	0	0	0	0	0

Calcein Intensity

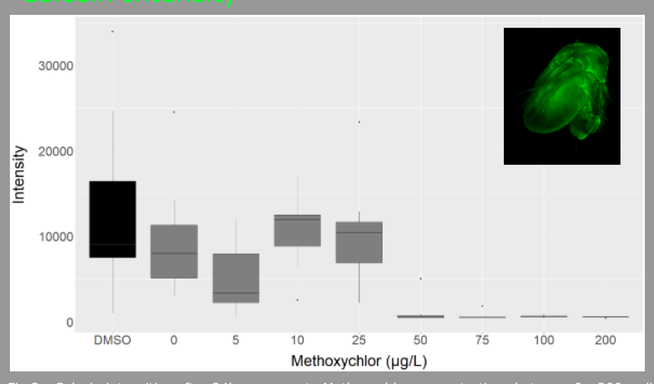


Fig.2.: Calcein intensities after 24h exposure to Methoxychlor concentrations between 0 - 200 µg/L

→ Lower Calcein signal after higher exposure concentrations

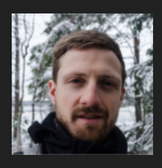
5 Conclusion

HCS allows...

- detection of adverse effects in living individual organisms
- earlier detection of effects (resulting in lethality)
- simultaneous multi-parametric mechanistic characterization

6 Next Steps

- Optimizing the highthrouput workflow
- Finding applicable molecular stains
- Extend to organisms of other trophic levels (i.e. algae, zebrafish embryo)



cedric.abele@aces.su.se
+46 8 16 2911
@CedricAbele

References:

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