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# Non-destructive biological and chemical traits to track down long term effects of environmentally relevant low concentrations of pharmaceutical mixtures using two freshwater species

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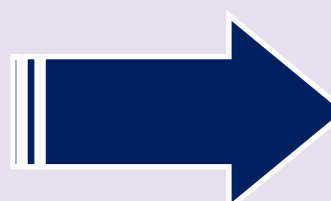
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## Context & Objectives

Currently, there are a lot of normalised **acute bioassays** that evaluate ecotoxicity:

- Of single pollutants
- Under short time exposures (72h or 96h)
- At high concentrations (mg.L<sup>-1</sup>)



There is a **need for new models** to evaluate :

- Pollutant mixtures
- Chronic toxicity
- Low environmental concentrations

## Pollutants & Freshwater species

### Study of 2 pharmaceuticals

not removed in wastewater treatment plants

**Carbamazepine (CBZ), OxCarbazepine (OxCBZ)**

and one of their metabolite **Acridine-9-carboxylic acid (9-CAA)**



### *Lemna minor* L.

- ❖ Cosmopolitan species and fast growth rate
- ❖ Normalised bioassay for acute & chronic toxicity

#### Growth conditions:

Light : 9h/day, Moisture : 60%, Temperature : 15°C, Hoagland media (10%)

- ❖ Optical measurement of early changes in primary and secondary metabolites (anthocyanin, chlorophyll, flavonol, phenol and nitrogen balance indexes)

### Published ecotoxicity values

Only For CBZ<sup>1,2</sup>

*Lemna minor* L., EC<sub>50</sub> : 25.5 mg.L<sup>-1</sup>, growth inhibition, 7d, (chronic toxicity)

*Hydra attenuata* Pall., EC<sub>50</sub> : 15.52 mg.L<sup>-1</sup>, 96h (chronic toxicity)

LC<sub>50</sub> : 29.4 mg.L<sup>-1</sup>, 96h (acute toxicity)

### *Hydra attenuata* Pall.



- ❖ Minimized genetic variability (polyps propagate asexually by budding)
- ❖ Diploblastic organisation

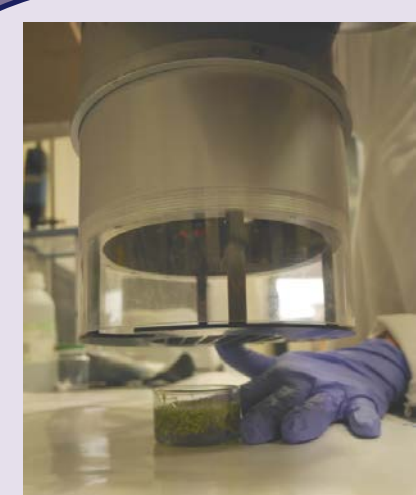
#### Growth conditions:

Single strain, Light : 12h/day, Temperature : 19.5°C, TES\* buffer, EDTA, CaCl<sub>2</sub>

- ❖ Monitoring of morphological alterations, reproductive rates, feeding behaviour

\* 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid

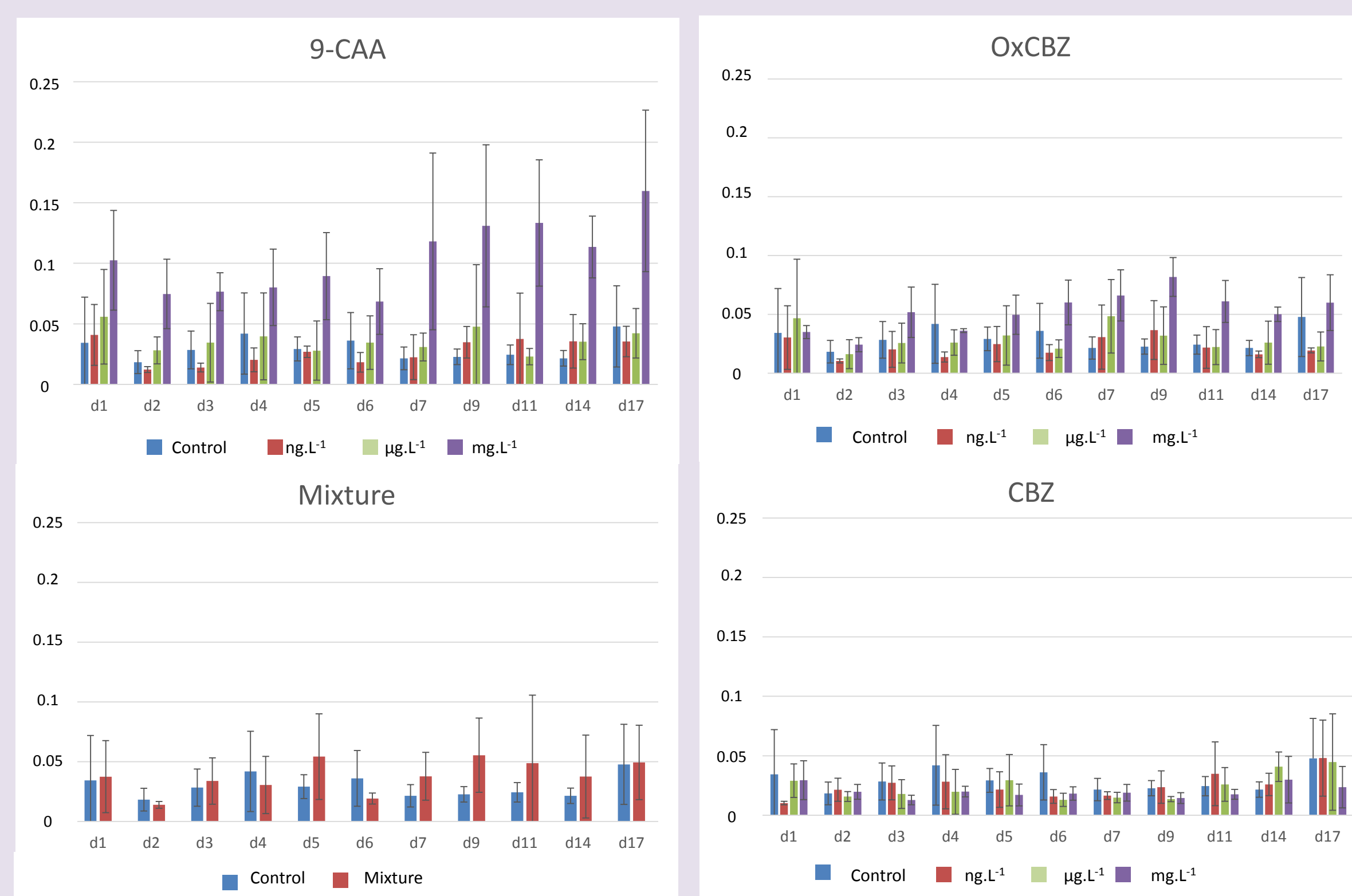
## Results



Compounds	Concentration range		
	600 ng.L <sup>-1</sup>	600 µg.L <sup>-1</sup>	60 mg.L <sup>-1</sup>
CBZ	27 ng.L <sup>-1</sup>	270 µg.L <sup>-1</sup>	24 mg.L <sup>-1</sup>
OxCBZ	900 ng.L <sup>-1</sup>	900 µg.L <sup>-1</sup>	15 mg.L <sup>-1</sup>
Mixture	Σ <sub>CBZ+OxCBZ+9CAA</sub>		



Compounds	Effluent realistic concentrations
CBZ	605 ng.L <sup>-1</sup>
OxCBZ	27 ng.L <sup>-1</sup>
9-CAA	900 ng.L <sup>-1</sup>
Mixture	Σ <sub>CBZ+OxCBZ+9CAA</sub>



Phenolic index of compounds and control during a 17 day-long exposure

### ➤ Phenolic index:

The highest concentration of 9-CAA induced an early metabolic **stress** in *Lemna minor* L. as shown by the increase of the phenolic index probably due to a **defence mechanism**.

For OxCBZ, a slight metabolic stress was observed latter whereas neither CBZ nor the mixture at low concentrations impacted this index.

### ➤ Nitrogen balance index:

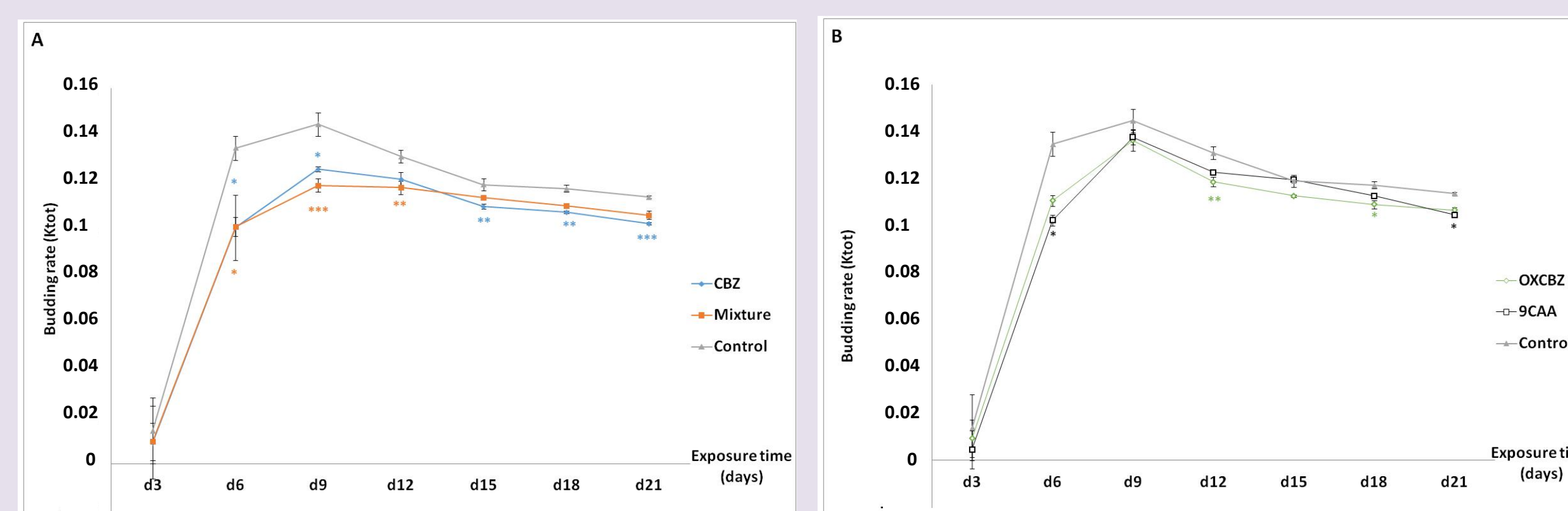
OxCBZ and CBZ at the highest concentrations increased the nitrogen balance index after 11 days. It shows a modification of **allocation of nitrogen and carbon** that may **impact photosynthesis** in of duckweed.

### ➤ Chlorophyll index:

This primary metabolite was **stable** for all the compounds and their mixture.

### ➤ Anthocyanin and flavonol indexes were below detection limits.

➤ Morphological changes, reflecting **progressive intoxication**, were observed after only one day of exposure : 21% of polyps exposed to CBZ, OxCBZ or 9-CAA showed first signs of intoxication and 16,6% of polyps exposed to the mixture showed more pronounced signs of intoxication. Interestingly, after 6 days of exposure morphological signs of intoxication tended to disappear and polyps exhibited normal morphological stage until the end of exposure (21 days). These observations suggest that *H. attenuata* possesses **defence mechanisms** to face intoxication.



A-B : Budding rate (Ktot) of exposed polyps and controls followed during 21 days.

K<sub>tot</sub> values were calculated<sup>3</sup> with the following formula  $K_{tot} = (\ln(n_{tot}) - \ln(n_0)) / d$   
n: number of polyps at the beginning of the experiment (d0); n<sub>tot</sub>: the sum of attached buds plus the number of polyps (i.e. detached buds and initial polyps) after x days of exposure (d3 to d21).  
\*:P< 0,05, \*\*:P< 0,01, \*\*\*:P< 0,001, significantly different from control (n= 24 in triplicate; Kruskal-Wallis followed by Dunn test).

➤ In exposed polyps, **slowing down and decrease of budding rate were observed** (Figs A-B) that could be related to morphological changes. Indeed, as the decrease of budding rate occurred from the d6 after exposure, i.e. the time required to the *Hydra* polyps to restore their normal morphology, trade-offs between the survival of polyps and the reproductive success of the population could be hypothesized. **As polyps exposed to CBZ and mixture never reached the maximal budding rate observed in control**, these compounds have negative effect on the reproductive success.

➤ Whatever the exposure conditions, all *Hydra* polyps were still able to catch and ingest prey : no alteration of feeding behaviour was observed.

## Conclusion

❑ Both models enable to study **non-destructive biological pertinent endpoints**.

- ❑ The macrophyta, *Lemna minor*, showed **deleterious effects only at high concentrations**. This specie does not seem highly sensitive to these compounds under the tested conditions.
- ❑ Bioassays using *Hydra attenuata* showed that the selected **compounds at environmental concentrations induced deleterious effects** at early (morphological changes) and later stages (budding rates). Such negative effects raise the question of the impact on natural populations that are chronically exposed to pollutants.
- ❑ These preliminary results could help to develop **new tools** for water quality monitoring and **for chronic toxicity assessment at realistic environmental concentrations**.

## References

- <sup>1</sup> Cleuvers, M. 2003. Aquatic Ecotoxicity of Pharmaceuticals Including the Assessment of Combination Effects. *Toxicology Letters* 142 (3): 185-94.  
<sup>2</sup> Brian Q., Gagné F., Blaise C. 2008. An Investigation into the Acute and Chronic Toxicity of Eleven Pharmaceuticals (and Their Solvents) Found in Wastewater Effluent on the Cnidarian, *Hydra Attenuata*. *Science of The Total Environment* 389 (2-3): 306-14.  
<sup>3</sup> De Jong L., Pech N., de Aragão Umbuzeiro G., Moreau X. 2016. Multi-scale biomarker evaluation of the toxicity of a commercial azo dye (Disperse Red 1) in an animal model, the freshwater cnidarian *Hydra attenuata*. *Water Research* 96 : 62-73.

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