



*In vivo* information...  
...with *in vitro* tools



1st Prize "Creation", Ile de France 2004  
National Competition in Support of the Creation  
of Innovative Technology Businesses





WatchFrog technology

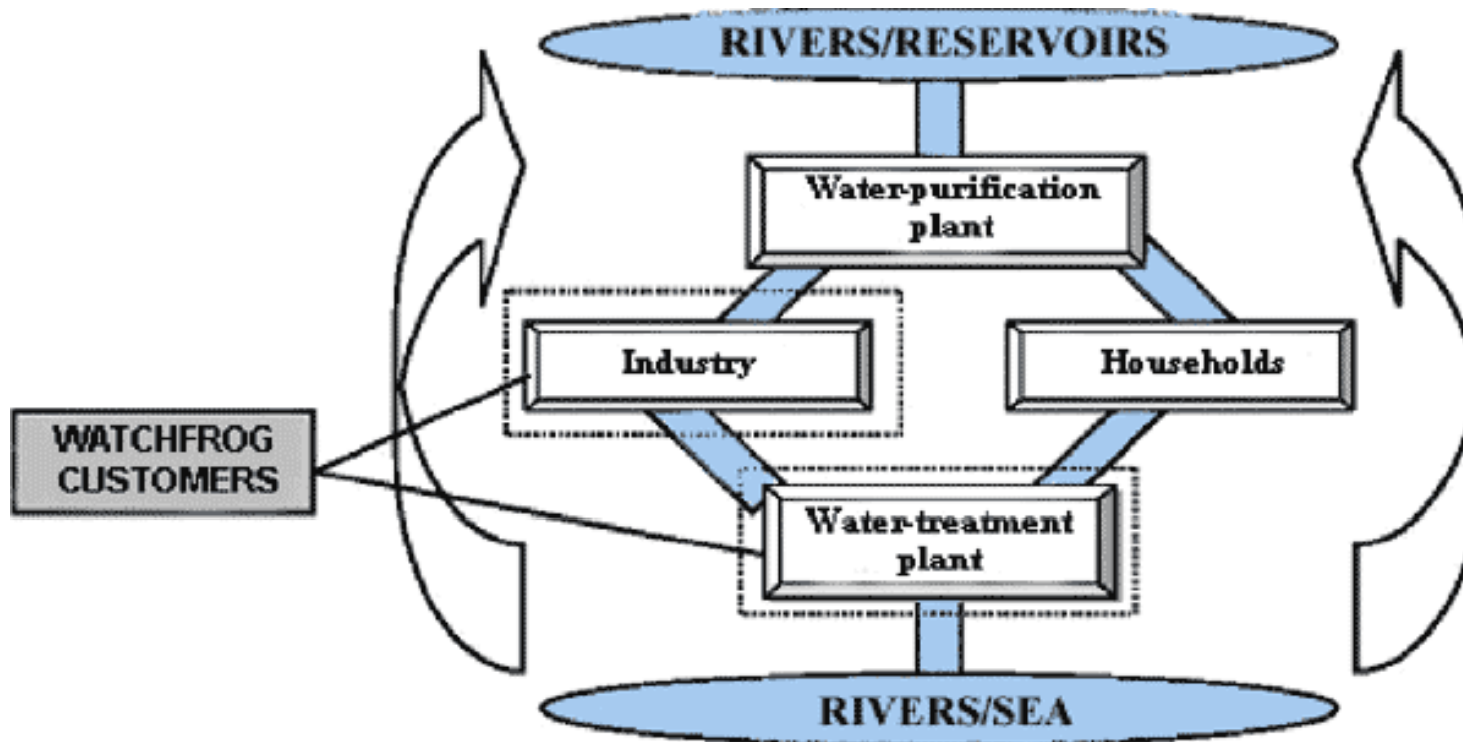
In Vivo risk assessment

Case study: endocrine chemicals

# Monitoring water, detecting pollutants

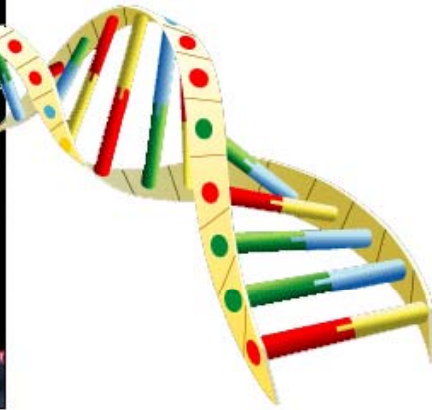
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- Currently implementing effluent management at industrial plants. **heavy metals and endocrine disruptive chemicals detection**
- Looking ahead, WatchFrog will provide water monitoring solutions involving minimal handling on the part of users, **based on models tailored to their needs.**



# Genetic detection *in vivo*

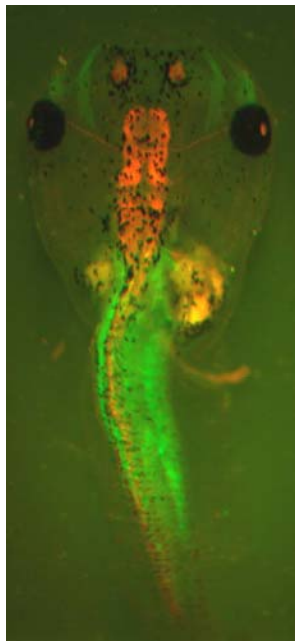
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Fluorescent protein **red**, **green**, **etc.**



**Germinal transgenesis**



Biological system: expression of a finely modulated fluorescent protein by the presence of the molecule to be detected or screened

# Advantages of the **amphibian** model

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- thousands of transparent larvae
- easily placed in contact with the sample
- polydetection
- rapid tests and dose-response
- small size of larvae
- low cost
- tailor-made tests



The only small organism model sufficiently **close to us** to predict the effect on human health

# WatchFrog's amphibians: model of vertebrate development

The rapid amphibian development allows to anticipate physiopathological phenomenon that intervenes at long term in human

Similar specific responses between amphibian vertebrate and human

48 hours of development:

Genes and proteins homologies

Complex nervous system  
(central / peripheral)

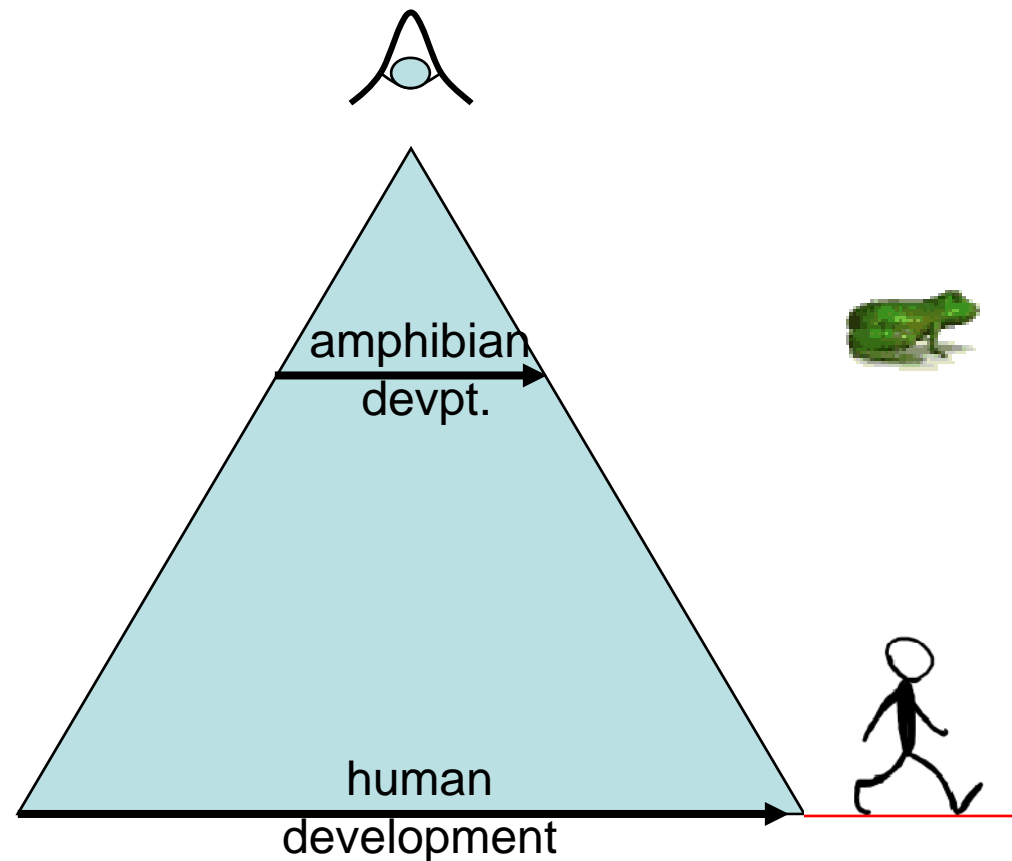
Cartilage (>bones)

Skin (Derma>Epiderma)

Eye

Cardiovascular

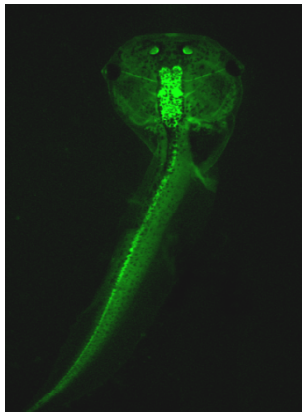
Immune system



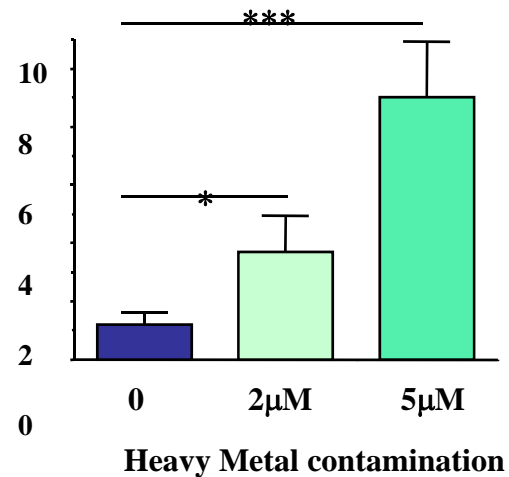
# WatchFrog tailor-made tests: *in vivo* is as simple as *in vitro*

- Absorption/Distribution/Metabolism/Excretion/Tox (ADMET)
- Specific/global responses
- Scalable
- Sensitivity/Specificity
- Reproducible
- Cost effective
- Potential automatization
- Medium rate screening

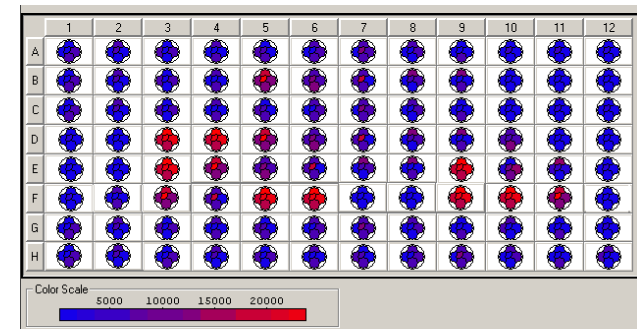
Results:



Light!



Quantifiable!

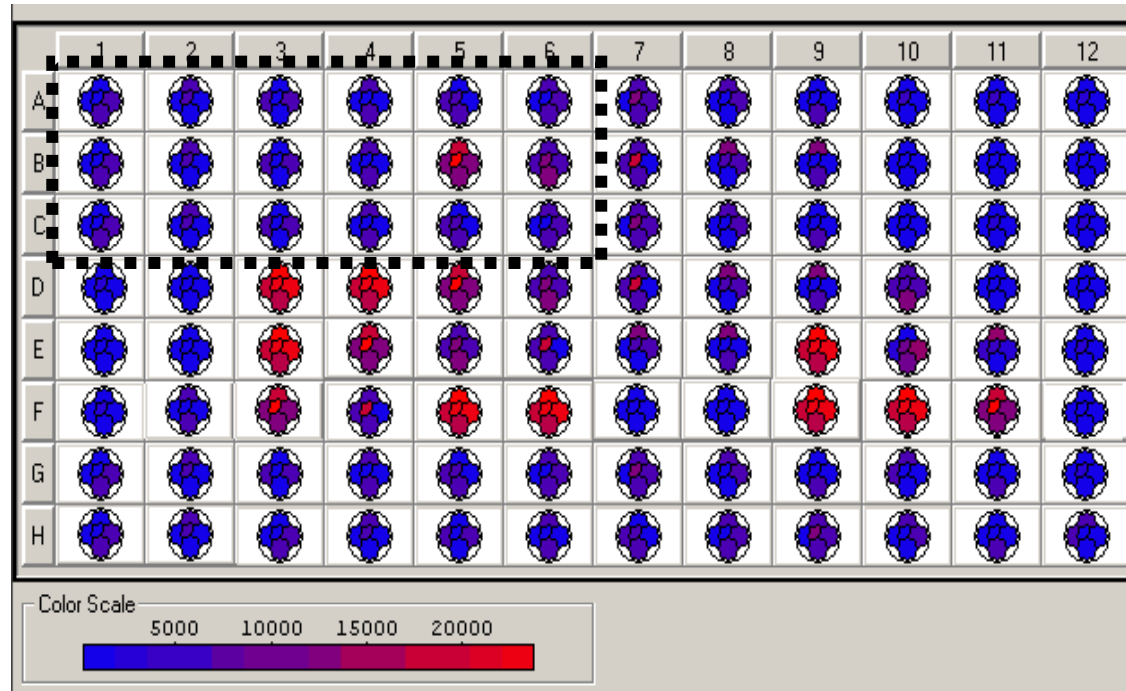
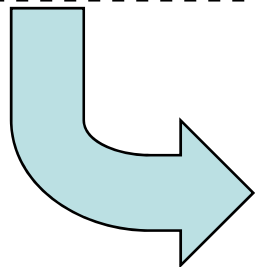


Can be automatized!





In vivo information...  
...with in vitro tools.







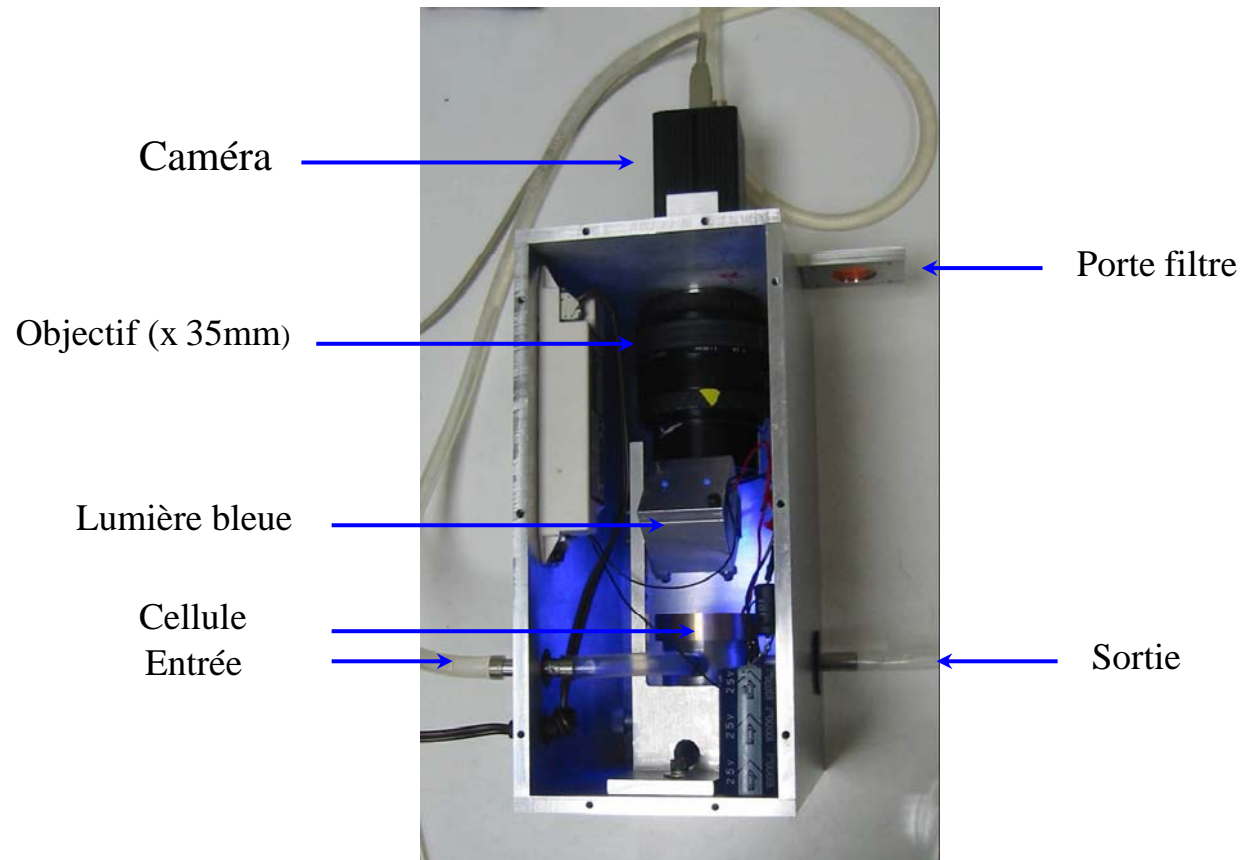
WatchFrog technology

In Vivo risk assessment

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# Monitoring water quality

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# On line monitoring

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Image de la cellule en flux au passage d'une larve fluorescente d'intensité moyenne)

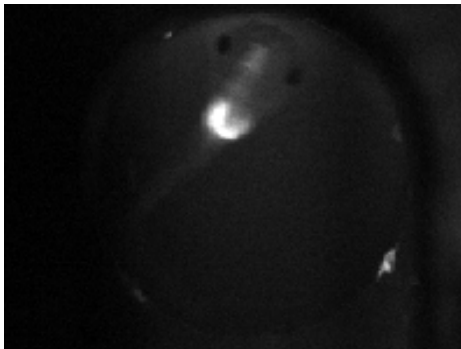


Image de la cellule en flux vide

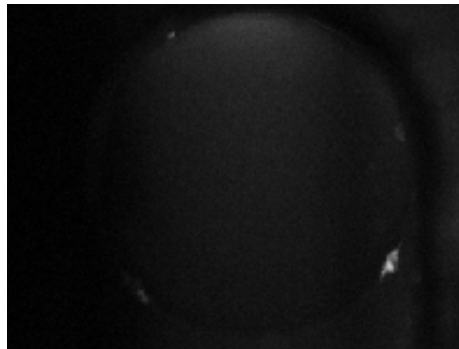
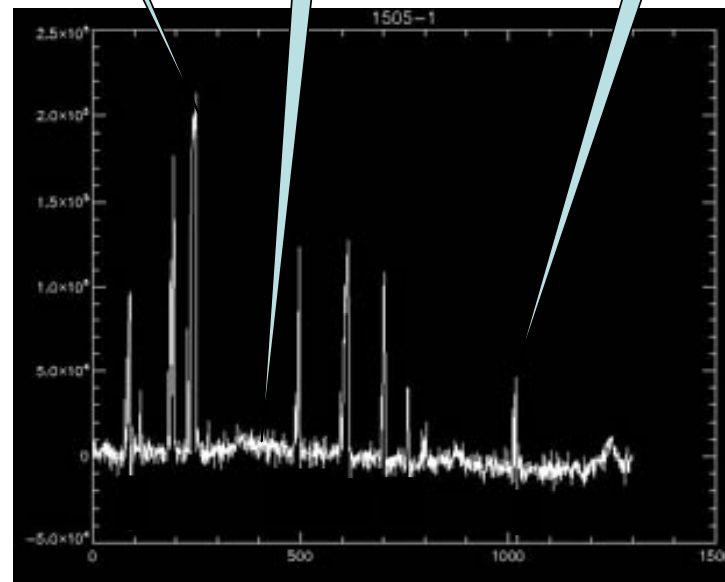
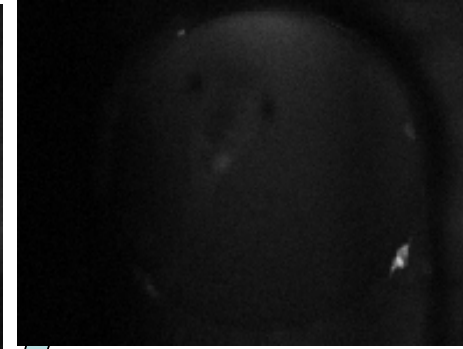
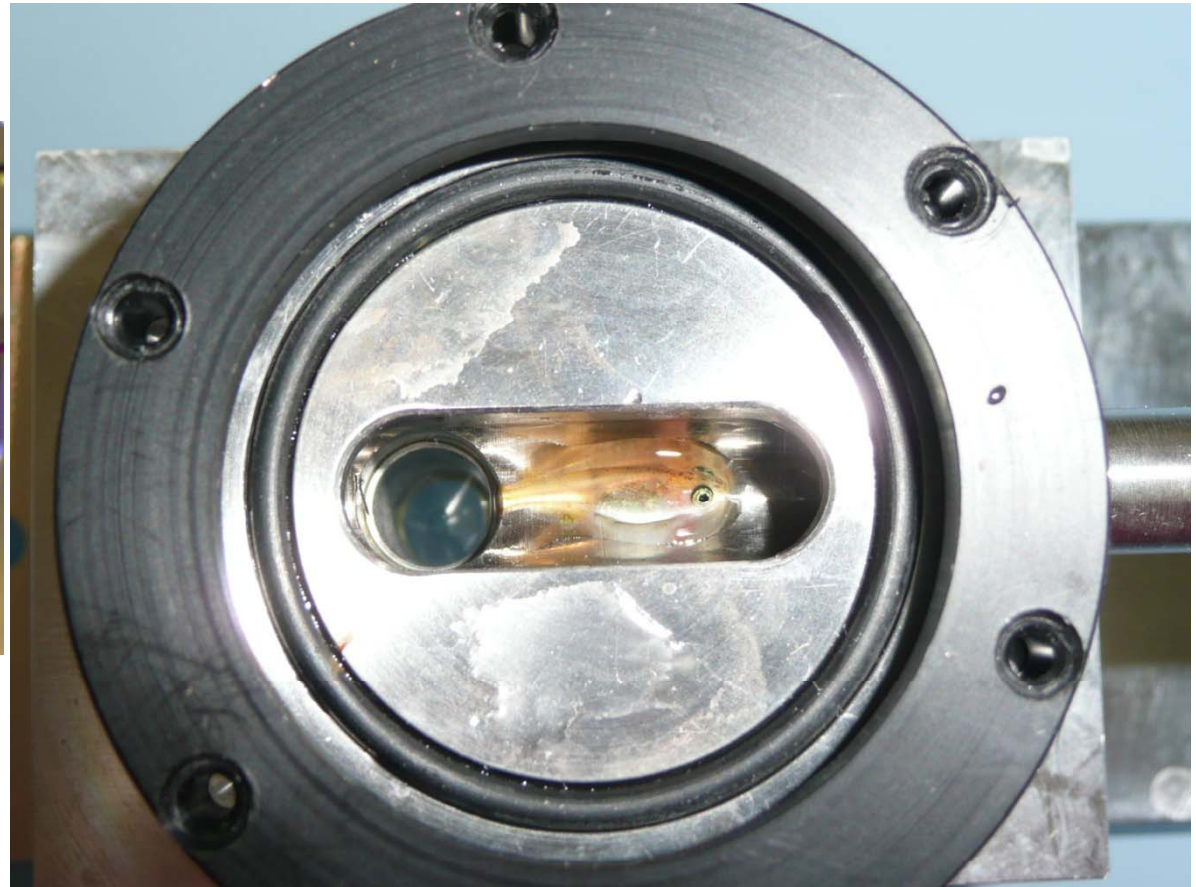
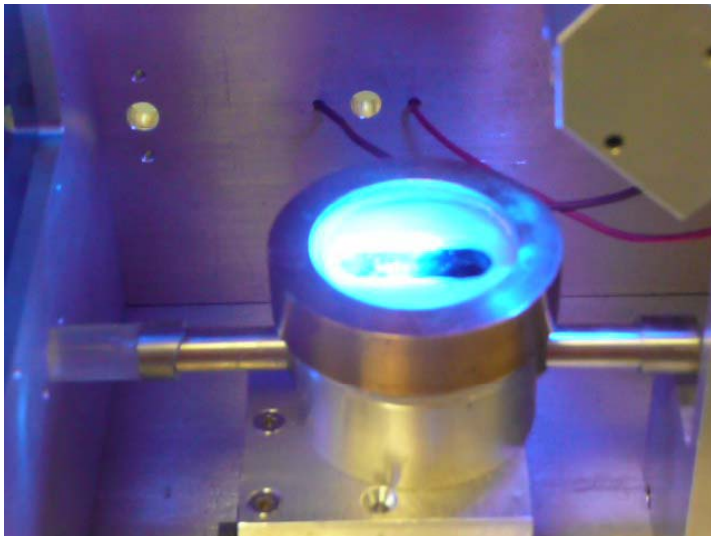


Image de la cellule en flux au passage d'une larve non fluorescente (ou très faible)



Also with japanese medaka!

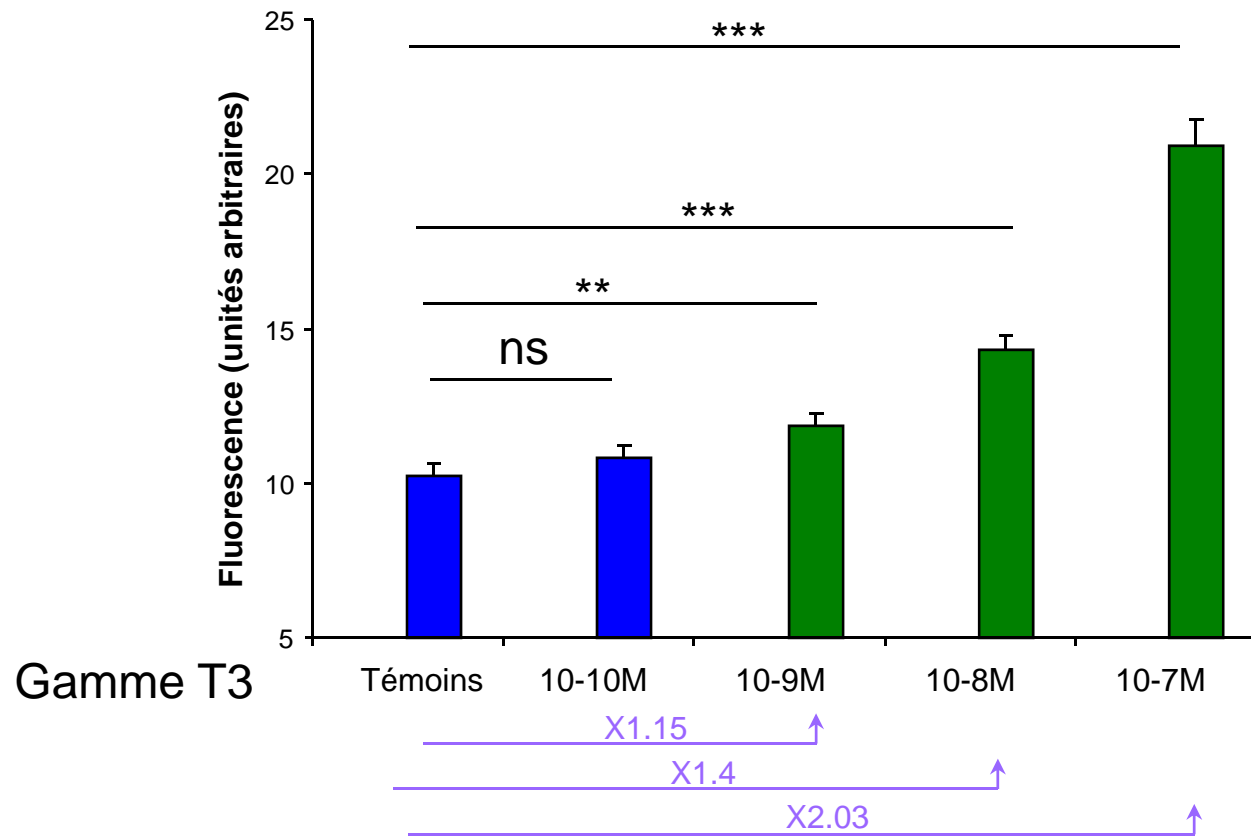
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# Versatile tool adapted to industrial need

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Sensitivity and specificity: benchmark to eliminate the risk of false positive



## WatchFrog technology



In Vivo risk assessment

Case study: endocrine chemicals

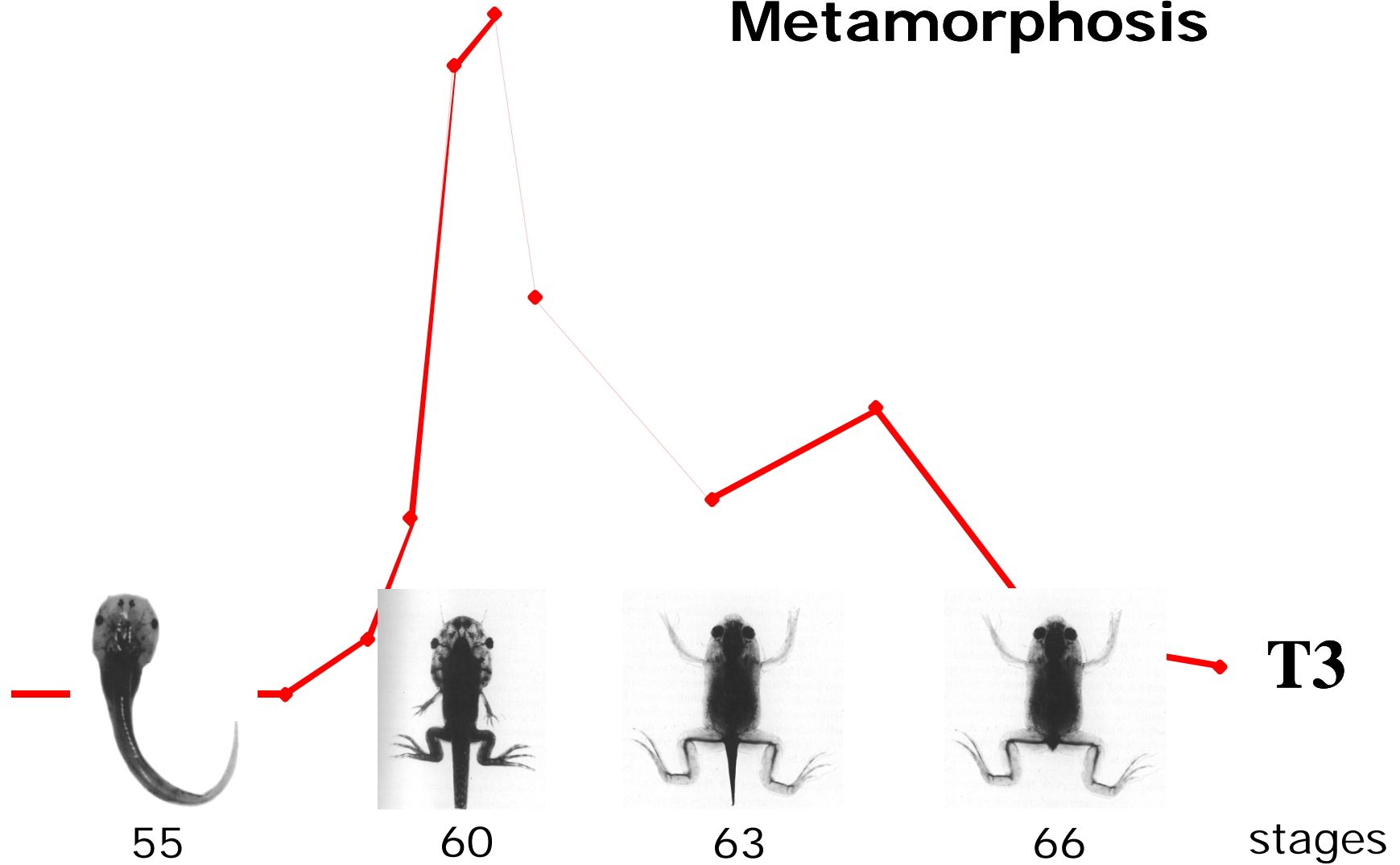


## The problem : detecting endocrine disrupting chemicals in the water/food and the environment.

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- **Need for screening that is rapid, sensitive, physiological ... *in vivo***
- **Why: Because chemistry and *in vitro* methods cannot predict/detect how substances will be distributed, modified and accumulated in organisms**
- **EU and OECD have (re)examined testing and Assessment of Endocrine Disruptors and underlined the importance of rapid and reproducible tests including germinal transgenic approaches.**
- **Reduce, Replace, Refine**
- **Validation of the Amphibian Metamorphosis Assay**

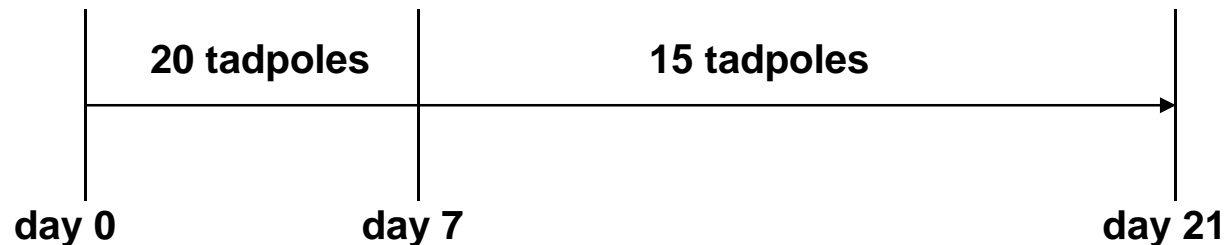
# Metamorphosis



Leloup and Buscaglia, 1977

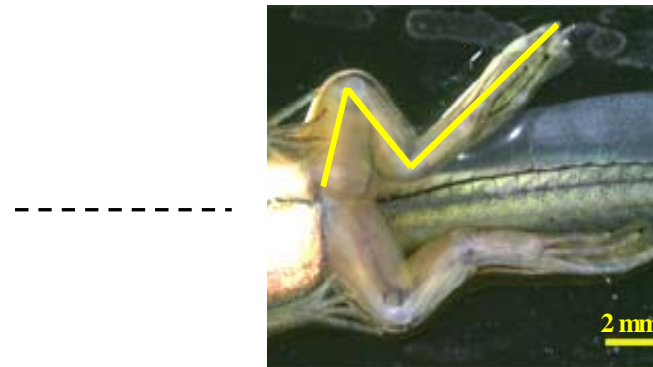
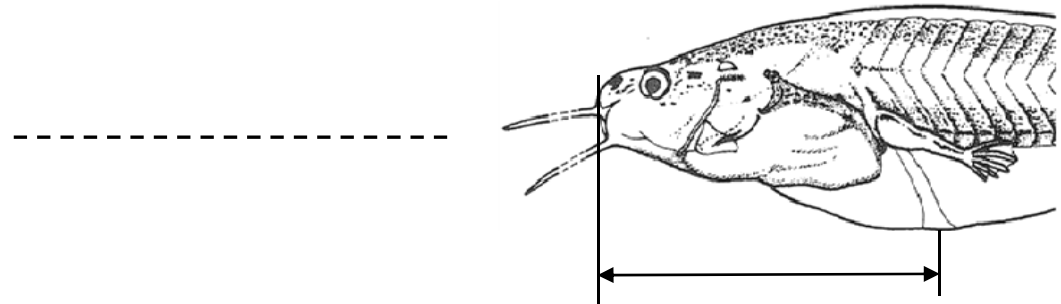
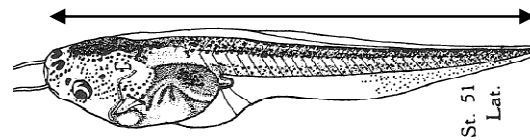
# OCDE Test - Overview

- Exposure of *X. laevis* tadpoles (stage 51) to 0, 65, 125, 250 and 500 µg perchlorate/L for total 21 days
- Sub-sampling of 5 tadpoles after 7 days; comparison of different endpoints
- Flow-through system (22°C, pH 6.5 - 8.0, photoperiod 12h:12h)
- 6 replicates per test group (4 rep. required) – statistical power



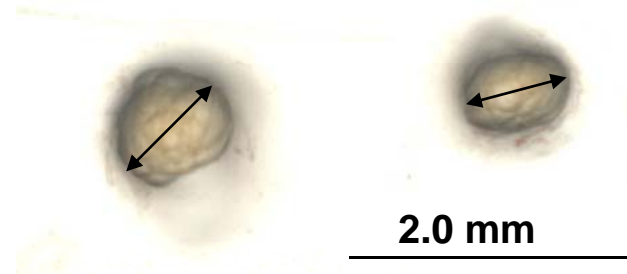
# Endpoints – Apical Endpoints

- Weight
- Whole Body Length
- Snout-Vent Length
- Developmental Stage  
(Nieuwkoop and Faber 1967)
- Hind Limb Length

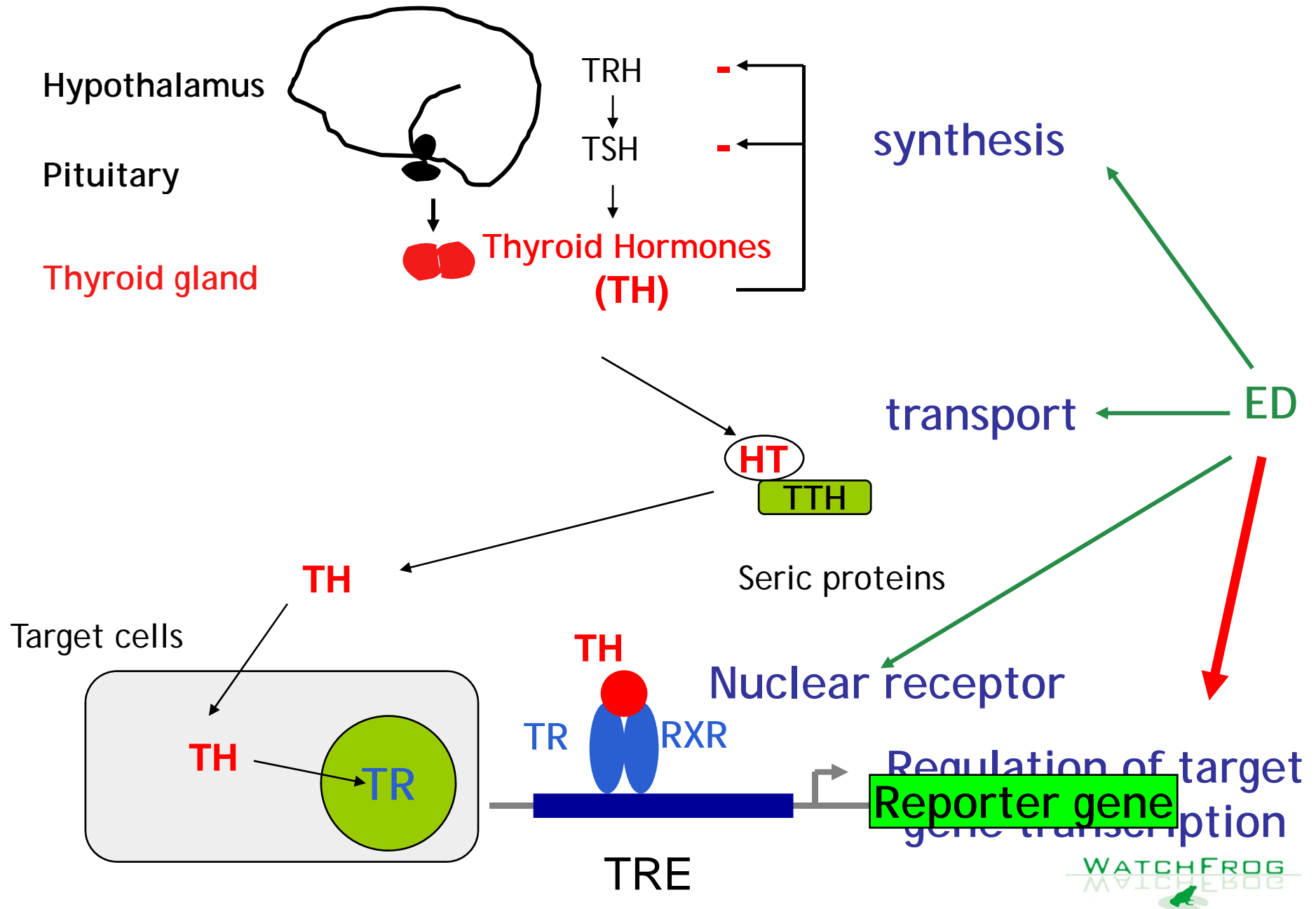


# Endpoints – Thyroid Glands

- Thyroid Gland Diameter
- Thyroid Gland Histology



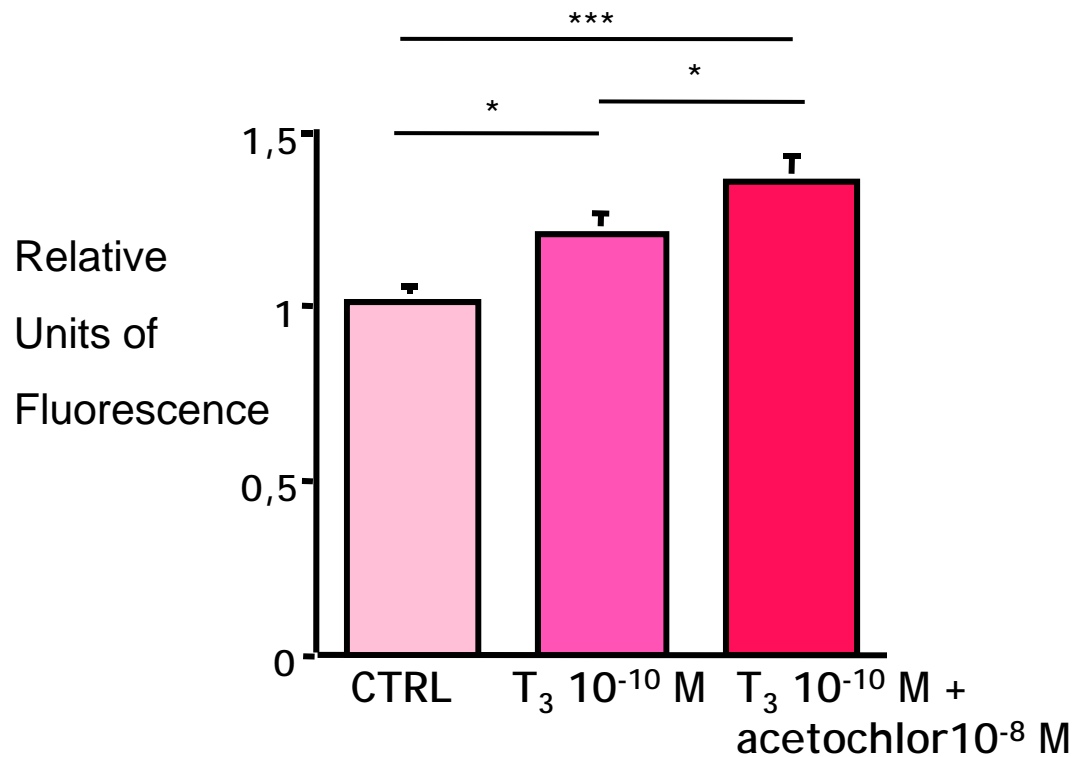
# In vivo Transcriptional assay: thyroid example



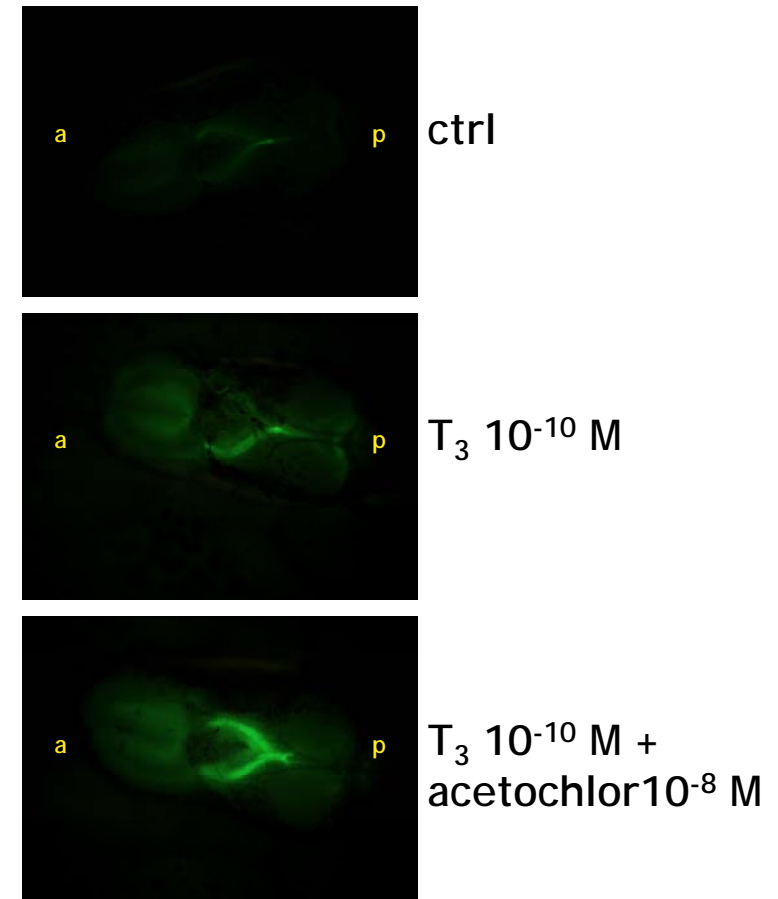
# WatchFrog model lights up to reveal at early stage the physiopathological / therapeutical process

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In 48 hours synergistic effect on thyroid signaling - predicting morphological effect occurring 6 weeks later



Targeted organ: CNS

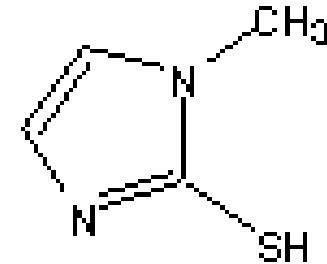




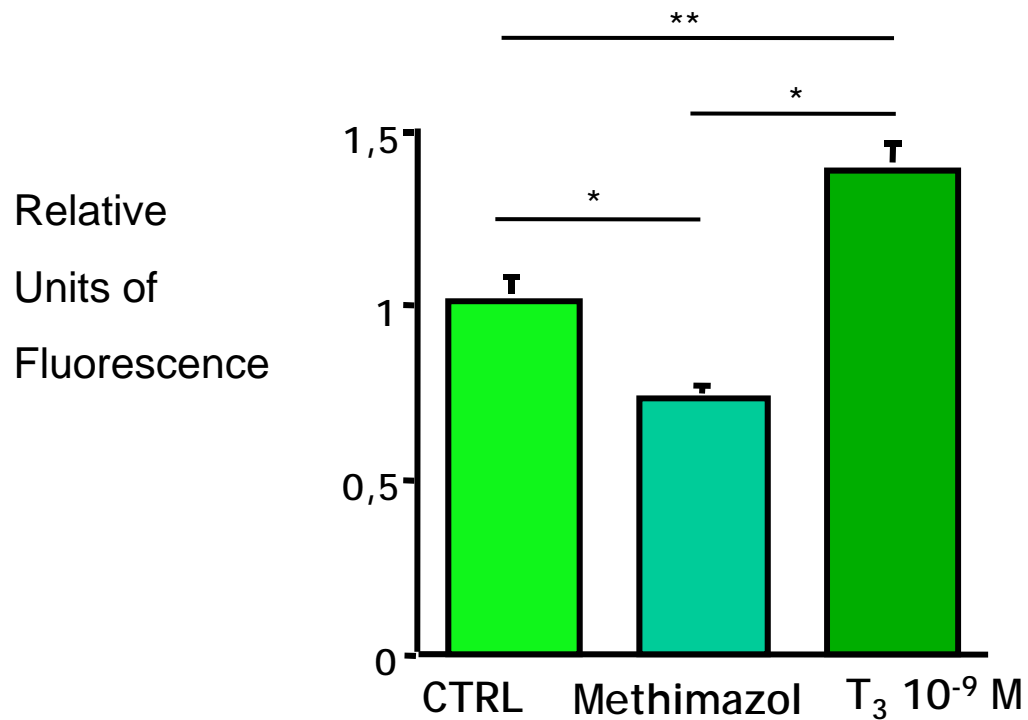
# WatchFrog model to detect anti-thyroid effect : medical drug methimazol

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*Chemical Formula:*  
(METHYL-1 IMIDAZOLE)THIOL-2



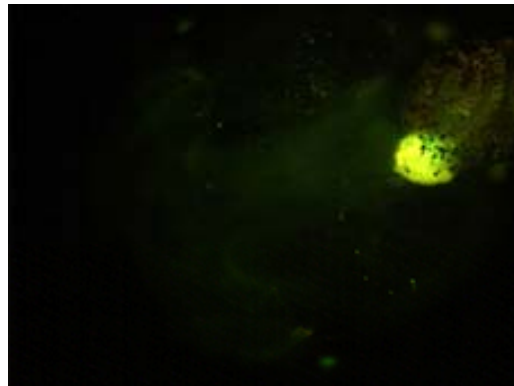
Blocking thyroid hormones synthesis  
Potential inhibitor of thyroid peroxydases



# Estrogens : different lines for various applications

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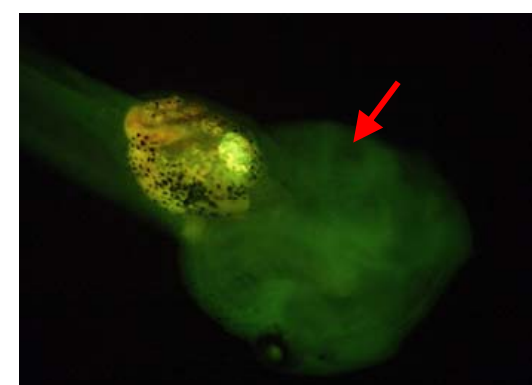
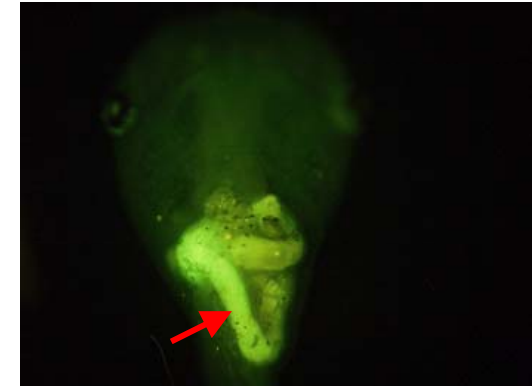
**3ERE-GFP**



**Vtg-GFP**

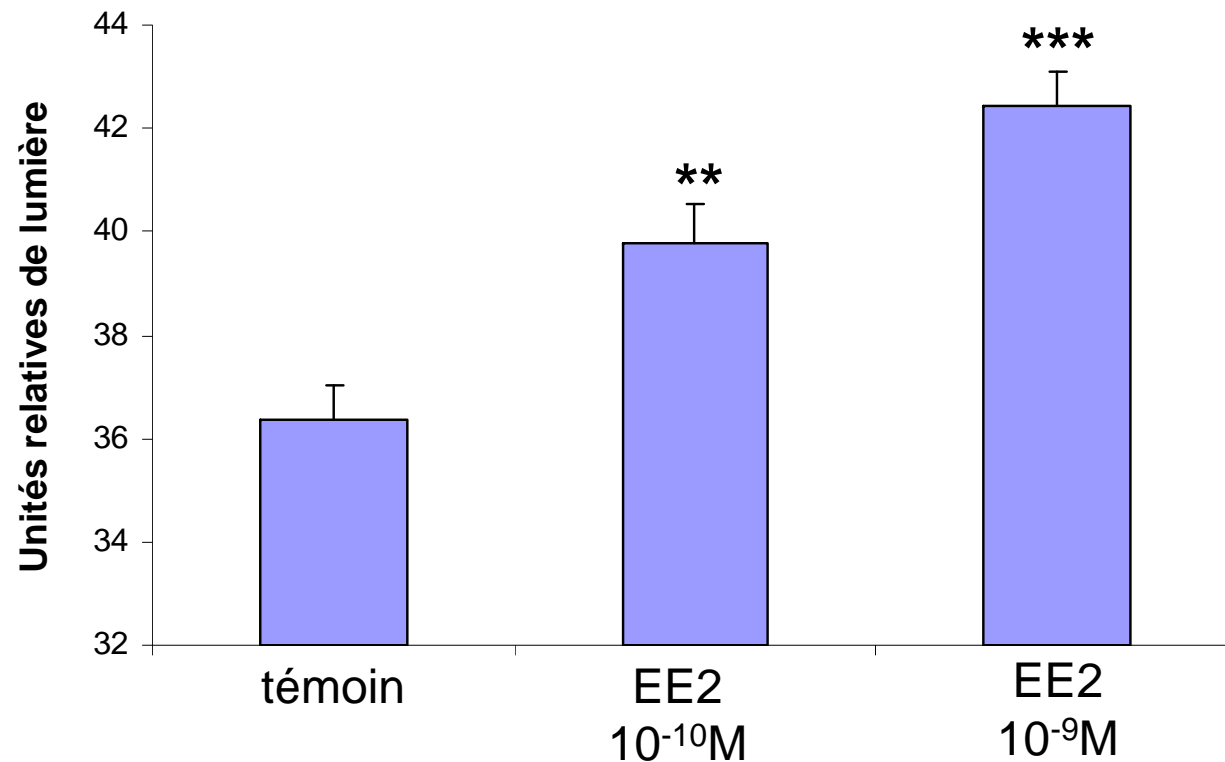


**Arom-GFP**



# Quantification of estrogenic response

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\*\* very significant

\*\*\* extremely significant

**Significant induction significative from 10<sup>-10</sup>M EE2**

## WatchFrog's amphibians (& fish):

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### Existing models :

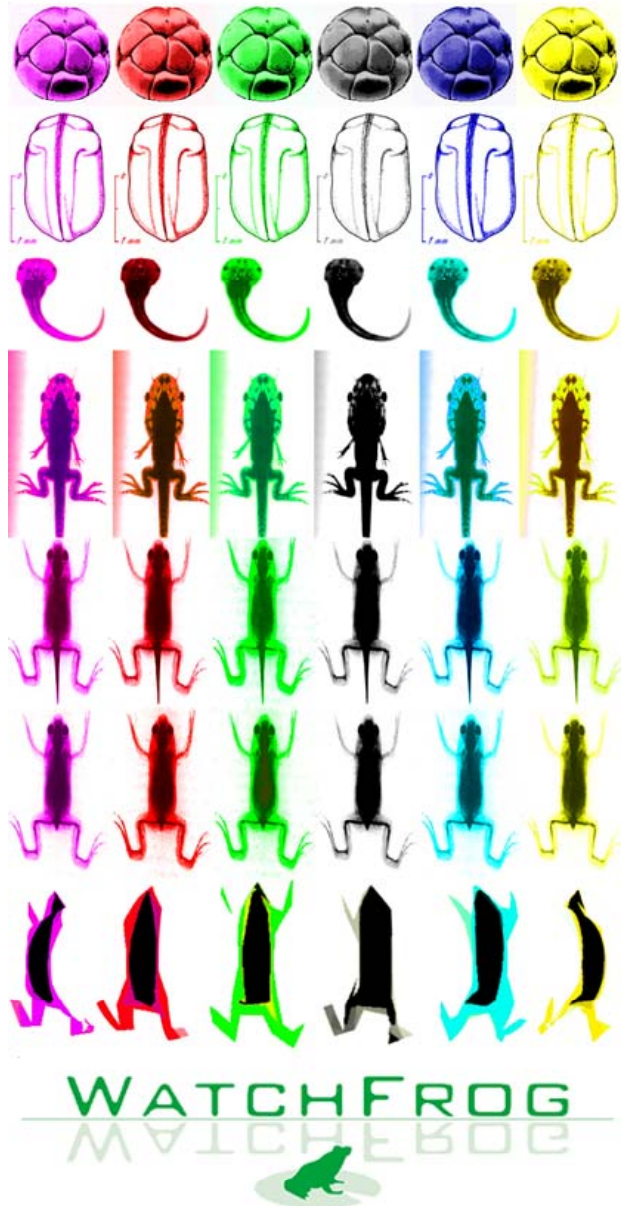
- neuronal labeling
- thyroid functions
- estrogenic functions
- heavy metals
- FETAX

### In progress:

- chronic toxicity
- genotoxicity
- immune system
- ardio-vascular

### Perspectives:

- automatized monitoring on industrial site



**WatchFrog** enables the potential of mixtures to be tested rapidly and *in vivo* creating dedicated models.

Evolutionary technology of **WatchFrog** enhances the ability to take advantage of, cost-effective, predictability for environmental health.

Our partnership with **Veolia – Anjou Recherche** is helping us to industrialize our tools.

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