



Overall Diagnosis of Water

WatchFrog

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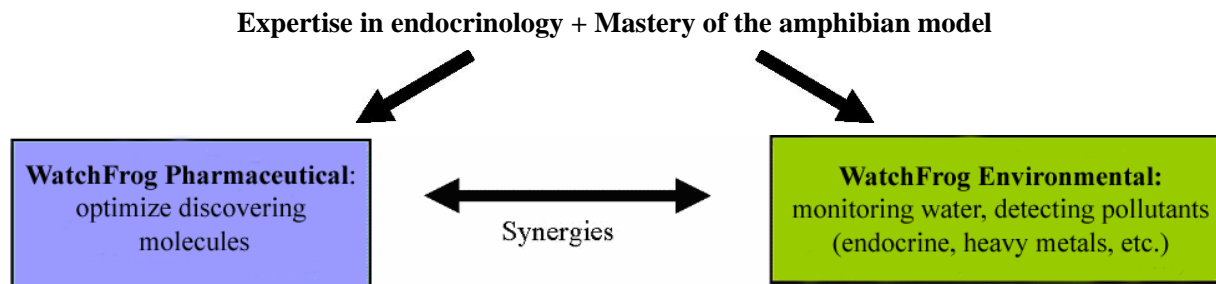
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WatchFrog business profile

WatchFrog creates and markets innovative solutions for the detection of a wide range of chemical, pharmaceutical and cosmetic substances. We provide low-cost diagnosis of the interaction of a chemical compound or an industrial effluent with a complete complex organism. Our genome technology combines maximum relevance of results with high biological significance while keeping detection costs to a minimum.

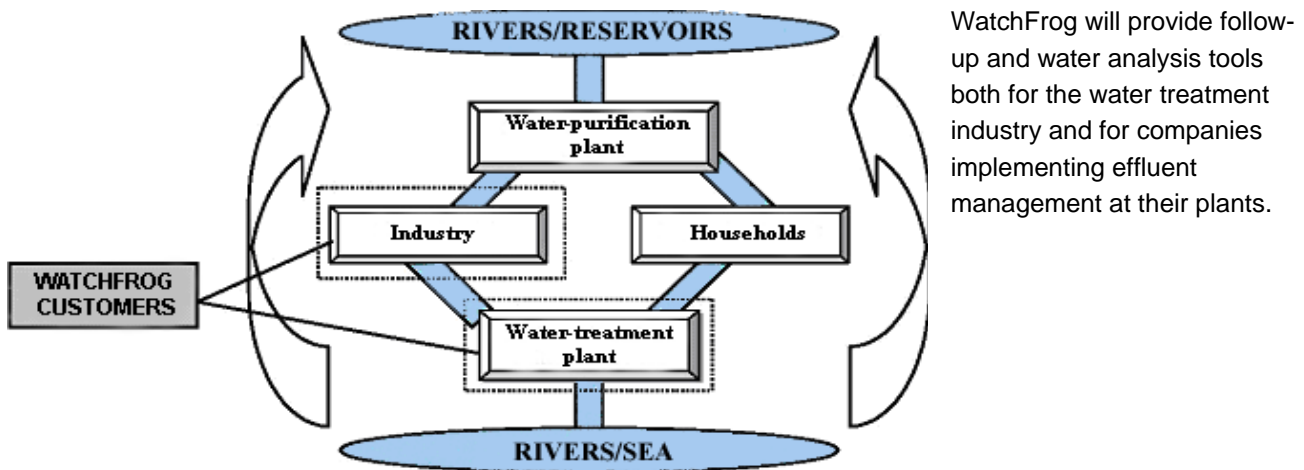
WatchFrog's versatile technology derives from a fundamental research program carried out within an internationally recognized research laboratory affiliated to the CNRS (National Research Council) and located within the French Natural History Museum.

WatchFrog technology is applicable to two main areas: the environmental market and the pharmaceutical market, as schematized below.



WatchFrog Environmental

The physico-chemical tests currently used do not respond to biological pollutants and hence are no longer adequate for ensuring water quality.

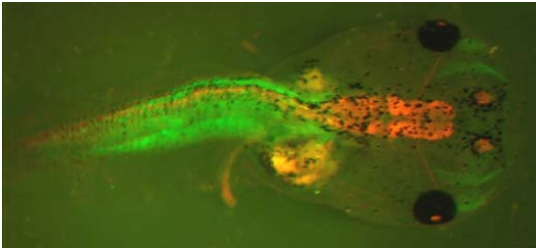


We already have available models that can detect hormonal disruptors of the thyroid function and heavy metals such as zinc and copper. These tools are able to fully meet the regulatory requirements specified in the European Union directive 2000/60/CE.

Looking ahead, WatchFrog will provide water monitoring solutions involving minimal handling on the part of users, based on models tailored to their needs.

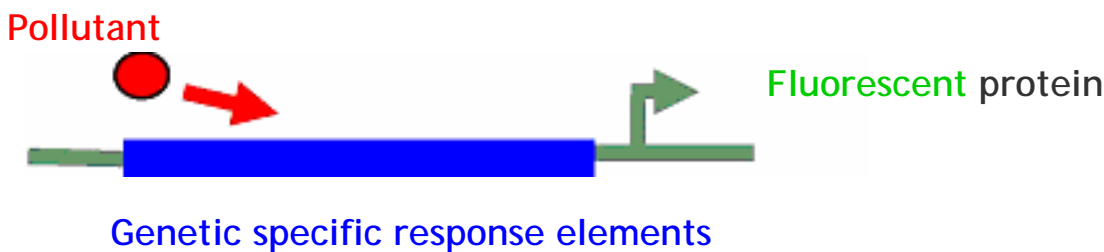
The new generation of tests offered by WatchFrog is allied to the development of amphibian models that "light up" (through emission of fluorescence) when a biological function is activated.

Technology: WatchFrog technology is covered by a number of patents.



The detector-larvae light up in response to a pollutant. Various luminous signals (in the image shown, red and green) can indicate the presence of several pollutants at the same time or a specific bio-availability of an organ (here, nerve tissue red and muscles green).

Our system enables this activation to be located and quantified *in vivo* on the pertinent and easy-to-use xenopus model.



The basic principle involves creating genetic constructions that enable a GFP (Green Fluorescent Protein) to be expressed in response to the physiological action of whatever type of molecules our customers may be interested in. This “molecular dosimeter” is then incorporated a xenopus larva, thereby taking into account all the biochemical regulations that can respond *in vivo* to the sample being tested.

For example, if an endocrine disrupter is present, it will activate the response element of various hormones, such as estrogen or thyroid hormone, triggering the synthesis of fluorescent proteins. The fluorescence is visible through the transparency of the organism, and can therefore be detected and quantified without sacrificing the animal. The larvae simply need to be placed in the liquid sample to implement the test. The genetic constructions can be altered as required to produce a tailor-made range of tests to respond to various disruptive or pharmacological effects.

This test methodology combines the advantages of *in vivo* with the flexibility of *in vitro*. It rapidly and simply furnishes accurate information of high sensitivity and specificity, together with low cost, economic use of material, and the potential for automatization.

WatchFrog technology is **innovative** in several respects:

- Detection at the genetic level or “**geno-detection**” of the *in vivo* distribution of pharmacological molecules. This allows a rapid response, since the expression of a gene in response to a signal is detectable after a few hours, while the assessment of biological consequences can require several days. What’s more, this integrates all the biochemical regulations that precede the activation of the genetic system while remaining specific to a given function.



In cases of contact or absorption, the physiological action of a product/substance on the organism will modify the expression of the genes relating to this effect. The mechanism brings into play genetic elements which are activated in the presence of the substances to be detected.

- The **choice of species** as the basis for the tests. *Xenopus* has complete immune system, as well as a more complex heart and circulatory system. In addition, in terms of endocrine physiology, the conservation of biochemical mechanisms between *xenopus* and humans has been demonstrated and proved. *Xenopus* is an investigated and recognized model in the research world, but its industrial potential has yet to be fully exploited.
- The method of **rapid selection of genetic constructions**, which brings considerable time savings and offers great flexibility for creating a battery of tailor-made tests.
- The **automatization of reading results**, which allows the simplicity and low cost of *in vitro* tests to be combined with the biological pertinence of *in vivo* analysis.
- Mastering **the techniques of breeding amphibians** in large numbers. This variously involves ensuring perfect sanitary conditions, obtaining high reproduction levels.

Competitive advantages

In vitro tests have little predictive power and conventional *in vivo* tests are cumbersome and expensive to implement. WatchFrog's competitors consist mainly of US and European companies which have developed other animal models (such as worms and fish) that are less close to humans than amphibians are (amphibians having the advantage of 150 million years evolution over fish).

WatchFrog amphibian tests have a number of technical advantages similar to those of *in vitro* tests:

- **simplicity of administering** by simply dissolving the substance in water and direct absorption
- **compatibility** with standard equipment through the use of 96-well micro-plates
- **small quantity of active substance** needed (tests in micro-wells)
- **high statistical validity** of results due to the very large number of genetically identical eggs in each laying
- **rapidity** of screening, with fluorescence detectable after a few hours
- **ease of reading** results by measuring fluorescence via transparency
- **sensitivity** from concentrations of 10^{-11} molar upward
- **quantification of the effect** by measuring the dose-response relation on a fluorimeter,
- minimization of **ethical** issues by virtue of using amphibian larvae
- **low cost** of maintenance and production of amphibians in comparison with mammals.

WatchFrog amphibian tests also have various technical advantages similar to those of *in vivo* tests:

- an **integrated approach** (on a vertebrate) that enables the test to have a **biological significance** by taking into account the complete metabolism and the distribution of the molecule in the organism
- the use of the *xenopus* **model** gives results recognized as being similar to those of mammals and humans, in particular for the endocrine, angiogenic and neurogenic systems
- **poly-detection** of a number of metabolic functions and genes simultaneously through the use of multiple markers and fluorochromes
- the possibility of producing ubiquitous and/or tissue-specific constructions

Our location and development within the Natural History Museum/CNRS conforms to the standards for experimental analysis demanded by a leading cosmetics company.