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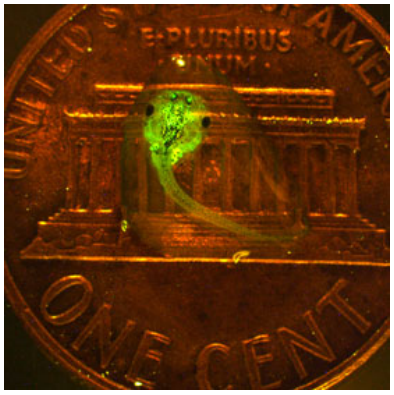
Unhealthy Glow: Fluorescent Tadpoles Expose Chemical Contamination

Transgenic fish fry and larval frogs light up when exposed to hormone-disrupting compounds in water

By Erica Gies | Thursday, February 7, 2013 | 1 comments

In cartoons glowing goo signals that there is bad stuff in the water. Now life imitates art: A French biotechnology company has created a transgenic tadpole that fluoresces when it encounters chemical contaminants in water that disrupt thyroid functioning. The test promises to shine a light on a class of endocrine-disrupting pollutants, which pollution regulators have in their crosshairs.

This month France-based WatchFrog begins its first high-profile trial to test effluent from a hospital near Paris under the auspices of the ministries of environment and industry. The Organization for Economic Cooperation and Development (OECD) is also evaluating the thyroid test, and the U.S. Environmental Protection Agency (EPA) is already using it.



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GLOWING TADPOLE: This tiny tadpole serves as a chemical sentinel, fluorescing when in contact with contaminants and more brightly with increasing exposure. Thanks to its small size, which can be used in an automated read-out system, the tadpoles can be used to test large number of water samples.
Image: © Anthony Sébillot

Tests on living organisms—in vivo—are more revealing than traditional chemical analyses. Instead of targeting specific compounds, they can highlight any toxic chemical or mixture that disrupts a key organ or life system. Until recently, however, in vivo testing for endocrine disruptors has been conducted on full-grown animals, which means the process is slow, taking years to develop and weeks to execute. It is also expensive—each run costs 60,000 to 100,000 euros.

These impediments have let WatchFrog hop ahead. The company's innovation is to combine the best of two worlds: "in vivo testing at the scale of in vitro," says CEO Gregory Lemkine. WatchFrog's three-millimeter-long *Xenopus* tadpoles, a common species in physiology labs, are small enough to fit in standard lab equipment. "Our tests cost between 10 and 20 times less," Lemkine says, and they offer results in 24 to 48 hours.

Regulators are concerned because growing evidence shows that some synthetic chemicals—including those found in in insecticides, herbicides, fumigants, fungicides, detergents, resins and plasticizers—may disrupt the body's endocrine system, which controls many important functions by emitting hormones, or natural chemical messengers. Chemicals can scramble the signal by mimicking hormones, blocking their receptors or altering hormonal levels.

Thyroid hormones, for example, affect brain development, metabolism and aging. They are also critical in amphibian metamorphosis.* Without thyroid hormones, a tadpole will not turn into a frog.

WatchFrog co-founder Barbara Demeneix turned this science into a toxicology test.* She combined a marker—a gene for a jellyfish protein that fluoresces green—with a DNA sequence that turns on the green fluorescent protein in the presence of thyroid hormones. The system shows where and when thyroid hormones (or contaminants mimicking them) induce metamorphosis. It also reveals the

absence of those processes when hormone function is disrupted.

WatchFrog's competitors are pushing ahead with analogous tests using fluorescing fish to detect estrogen disruptors.* Estrogen influences development of female reproductive behaviors, including egg production.*

Many fish can naturally change sex during their lifetimes, an adaptation to maximize sexual reproduction. Individual fish have a genetic predisposition to be male or female, but that can be overridden by hormone signaling. Unlike other fish models, medaka show genetic determination of sex clearly; males have a Y chromosome. Researchers can thereby measure estrogenic or androgenic effect against a fish's genetic predisposition.

When transgenic fish encounter a chemical that prevents them from producing eggs or from changing sex, they light up.

Hong Kong-based Vitargent has commercialized a test using embryos of *Oryzias melastigma*, an Asian fish better known as medaka. Co-founder and chief technology officer Xueping Chen says the product is now in widespread use. "Our tests have been used extensively to ... measure the estrogenic activity of a wide range of consumer products, including cosmetics and skin care products, drugs and health supplementary products, milk powders, meats, seafood and so on," she says. Vitargent has also developed a dioxin test on zebrafish (*Danio rerio*).

It's a market that WatchFrog is targeting, too. The company collaborated with Taisen Iguchi, a professor of environmental endocrinology at the National Institute for Basic Biology in Okazaki, Japan, and Masato Kinoshita, assistant professor of applied biosciences at Kyoto University, who had created a adult medaka laboratory test. WatchFrog adapted the model to a sturdier variety of medaka that would better hold up to commercial testing and uses fish fry to speed the process. WatchFrog also has a test for androgen disruptors, which affect male sexual differentiation.

The market for these tests is growing. The European Commission has identified endocrine disruptors as serious contaminants to be monitored and restricted under its Registration, Evaluation, Authorization and Restriction of Chemical (REACH) legislation and its Water Framework Directive. But both laws are short on key details. "They don't say what an endocrine disruptor is or how to test it," Lemkine says. Because of this lapse, WatchFrog is targeting its tests to OECD guidelines, which define hormone disruptors as chemicals that can disrupt the ability of a fish to produce eggs or change sex or prevent a tadpole from metamorphosing.

The U.S. Environmental Protection Agency (EPA) is already using WatchFrog's tadpole model. To prioritize which of thousands of chemicals need toxicity testing, the agency's ToxCast program uses computational tools and high-throughput testing to identify a signature for the types of chemicals that might be dangerous. The EPA then uses WatchFrog's tadpole test as a middle step to discover which suspicious chemicals require expensive, time-consuming toxicology tests, says Kevin Crofton, acting deputy director of the EPA's National Center for Computational Toxicology.

Another EPA program, Pathfinder, is helping WatchFrog automate its tests for on-site use. Pathfinder funds developing technologies that target environmental and human health challenges. It helped WatchFrog develop the FrogBox, a device the size of a record turntable that uses cartridges containing tadpoles for continuous flow-through monitoring.

One target application is the outflow from water treatment centers. Treatment eradicates targeted chemicals but can generate harmful by-products. FrogBox's ability to survey an ongoing stream will allow it to document hourly, daily, weekly or seasonal changes in pollutant levels.

The hospital outside of Paris is a typical target client. The 1.4-million-euro project, which will run for two to three years, will identify which effluent streams could be better treated. The idea is to develop a mobile lab at the hospital.

As in France, so it goes in the United States. The tadpoles and fish fry are "little sentinel animals," Crofton says. "If you see a signal, it means, gee we'd better take a second look here."

** Editor's Note (2/12/13): These sentences were edited after posting either to correct or clarify the original content.*