Linking cell-population to whole-fish growth

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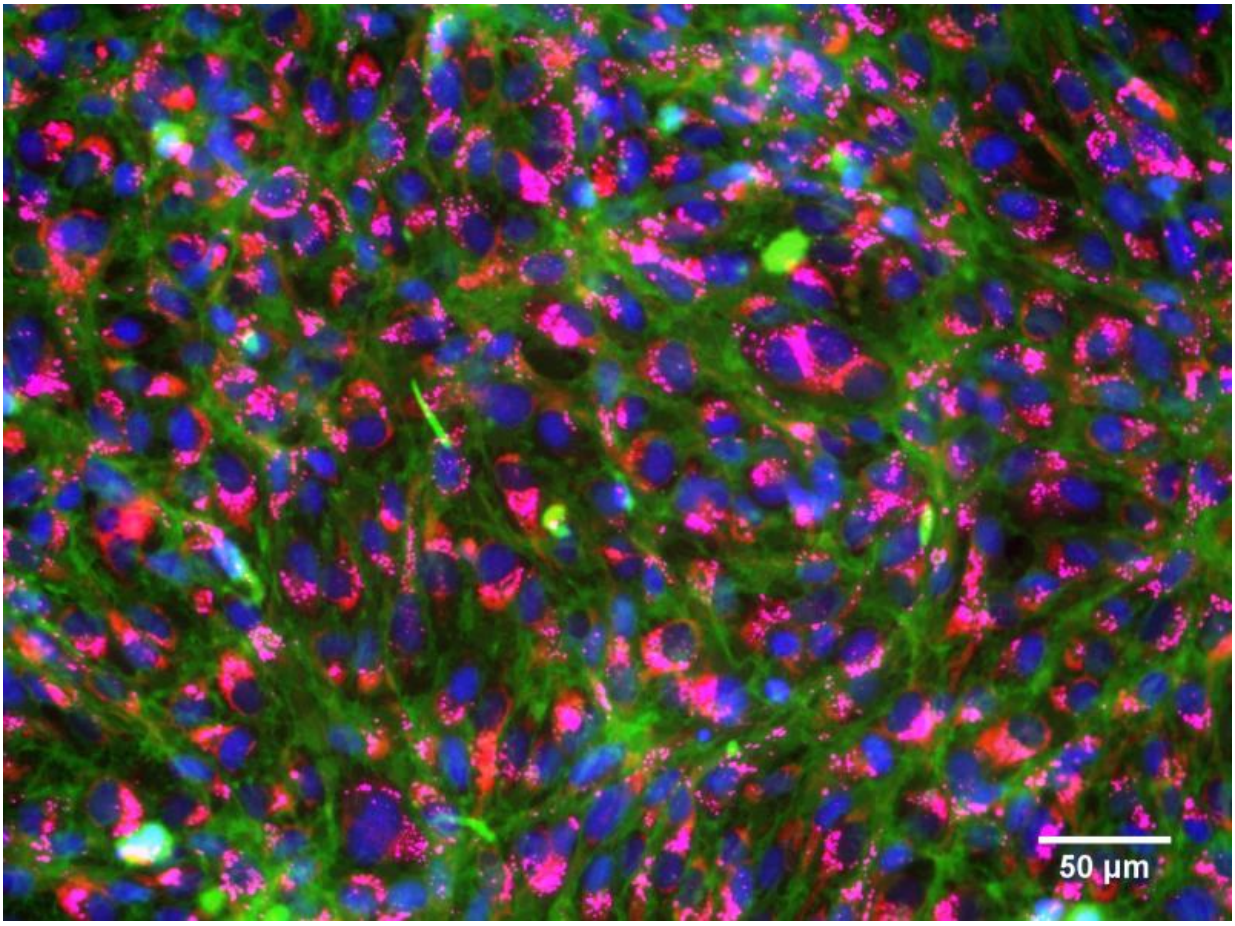


Rainbow trout gill cells were live-stained using molecular probes for nuclei (blue), cell membrane (green), mitochondria (red) and lysosome (magenta). These cells were healthy control cells unexposed to chemical stimuli. Credit: © Vivian Lu Tan, Eawag

Every year, more than a million fish are used for toxicity testing and scientific research in the EU alone, and around 400 fish are needed for a single fish early-life stage test. Such toxicity tests are often required by regulatory authorities for new chemical substances, as fish are particularly sensitive to contaminants in water at early developmental stages. However, the increasing use of experimental animals is ethically questionable. In addition, conventional tests are complex, expensive and take weeks or months to complete. Alternative approaches are therefore being sought by scientists, regulators and industry. A promising new method has now been demonstrated by an Eawag study, conducted in collaboration with the ETH, the EPFL and the University of York (UK).

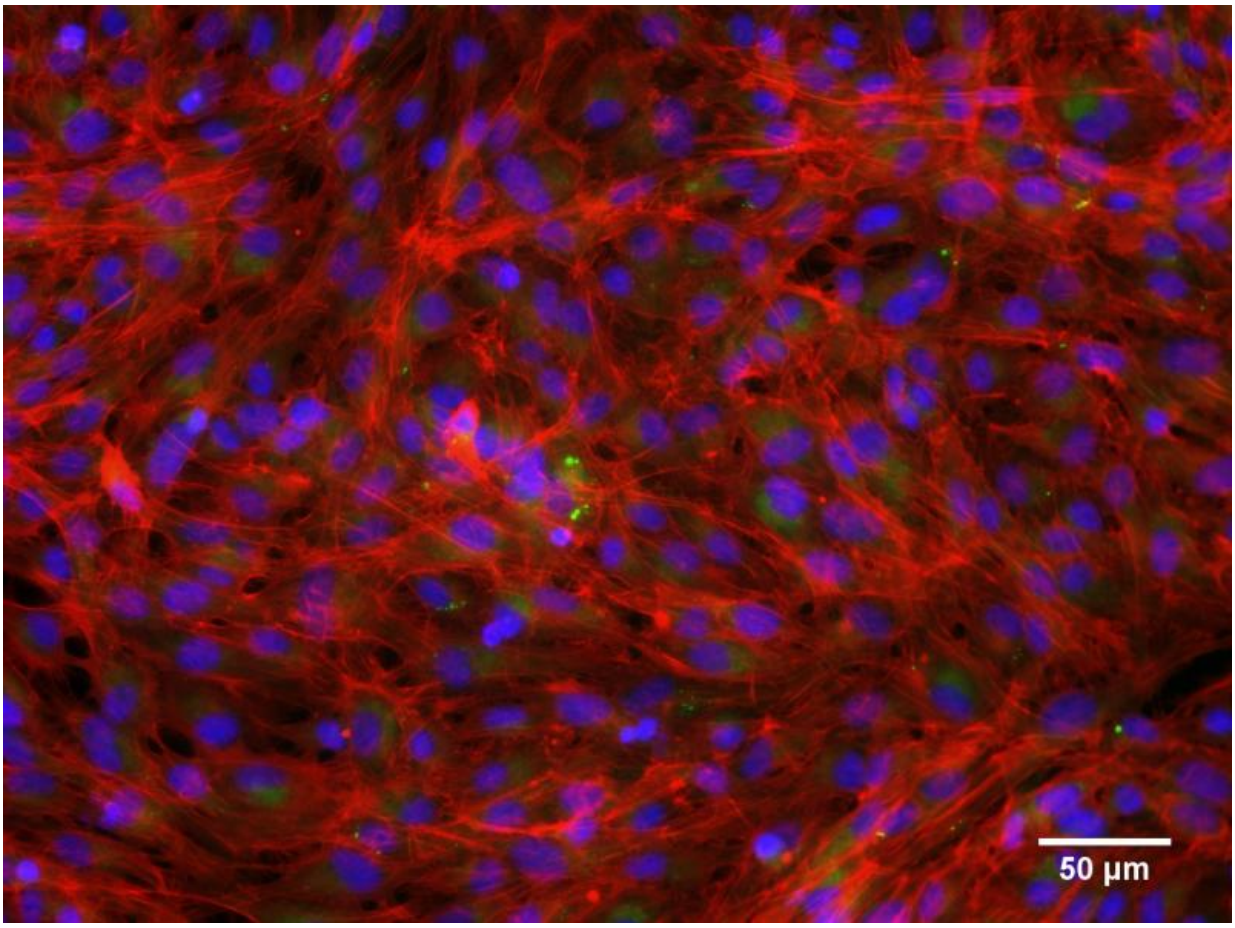
The results have been published in the journal *Science Advances* : rather than using live fish (in vivo), the tests are performed with fish cells (in vitro). After just five days, cell-population growth, inhibited to a greater or lesser extent under chemical stress - combined with modelling of toxicological effects - shows excellent agreement with data from independently conducted in vivo experiments.

Environmental toxicologist Professor Kristin Schirmer, who is leading Eawag's efforts to reduce the use of experimental animals, comments: "This is a major step towards simpler, less expensive and more rapid toxicity testing for the authorisation and use of new chemicals. It's the first time we've been able to use cell cultures to accurately predict chemical effects on growth which would only emerge after weeks or even months in live fish." The mechanism underlying the new method appears simple: the pesticides used in the study inhibit fish growth - the higher the concentrations to which the animals are exposed, the more their growth is reduced. The same effects were shown for the gill cell populations cultured in the laboratory. Kristin Schirmer explains: "The reason why the results can be extrapolated so well is that bigger fish don't have bigger cells, but more cells, and we calculate the concentration of the substance in the cells." The model thus predicts

what happens if the fish is exposed to the test substance in water - which in turn can help to refine other tests and predictive models.

The new approach is not, however, as simple as it initially appears. To determine what concentrations in cultured cells have the same effects as in live fish requires elaborate modelling and a detailed knowledge of substance properties. In addition, it is not clear whether gill cells will prove to be representative for all types of fish tissue. Other cells may react differently, and other substances may be biotransformed. Nonetheless, the study is of considerable interest for experts because of the novel approach pursued.

Dr Roman Ashauer, who initiated the study and is now working at the University of York, says: "The traditional work flow for chemical risk assessment has been 'test first, interpret later'. We've taken a different approach, by first modifying a relatively simple mathematical model of fish growth and then feeding the necessary experimental data into this model."



Rainbow trout gill cells were fixed and stained using molecular probes for nuclei (blue), lipid (green) and actin (red). These cells were healthy control cells unexposed to chemical stimuli. Credit: © Vivian Lu Tan, Eawag

The authors hope that this approach will be taken up by other scientists to test its wider applicability, and the initial signs are encouraging: at a Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC Europe) held in Glasgow, Dr Julita Stadnicka-Michalak, the study's first author, received the prestigious Young Scientist Award.

The gill cells are exposed to chemicals in varying concentrations in 24 well plates and their vitality is then analyzed. Credit: © Julian Salinas, ETH-Board

More information: Toxicology across scales: cell population growth in vitro predicts reduced fish growth; Julita Stadnicka Michalak, Kristin Schirmer, Roman Ashauer (2015); *Science Advances*. DOI: 10.1126/sciadv.1500302

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