# How Can the Degree of Pollutant Impact on a Stream be Documented?

**The Ecotoxicology Group at EAWAG has developed a novel two-step approach to assess the toxicity of water samples. This procedure would allow for processing of a large number of water samples and evaluation of their ecotoxicological potential. For such a procedure, it is important to consider that the water samples need to be tested with respect to all relevant toxic effects and currently known mechanisms of toxicity. The approach will be included into the Swiss modular concept and opens new possibilities in the ecotoxicological assessment of water.**

The Swiss modular concept employs methods for the assessment of stream quality [1, see article by A. Peter, p. 7]. The basic idea is the integrated assessment of running waters, bringing together hydrological/morphological, biological and chemical/ecotoxicological aspects. However, there still is relatively little information on the ecotoxicological evaluation of natural waters. A new ecotoxicology module was therefore developed by an interdisciplinary working group at EAWAG and in discussions with internationally recognized experts.

## Substances Exhibiting Ecotoxicological Effects

Of the roughly five million chemical compounds known today, approximately 80,000 are commonly in use. Each year, some 500–1000 new compounds are added [2]. During production, use, and disposal, chemical compounds are inevitably released into the environment. They represent a significant potential threat to stream and riverine ecosystems. As fish studies have shown, even relatively low pollutant concentrations can cause damage (see article by P. Holm, p. 23). One of the problems is that these low concentrations cannot always be measured by traditional analytical methods. In addition, there are chemicals which are only harmful when they occur in combination with certain other chemicals. We need, therefore, new methods for assessing the ecotoxicological effects of chemical compounds or of mixtures of compounds which go beyond classical chemical methodologies.

## Classical Test Systems

Starting in the 1950s, the toxicity of chemical compounds has been tested by using aquatic organisms. For about the last 20 years more complex environmental samples, such as wastewater and sewage sludge, have also been examined with these methods. The advantage of ecotoxicological tests over traditional chemical analyses lies in the fact that these tests address the issues of bioavailability and interactions among multiple compounds.

In classical tests, organisms such as bacteria, algae, *Daphnia* or fish are exposed to water samples for a certain amount of time. Toxicity is assessed by measuring mortality rates or growth inhibition or by observing typical behaviors. Normally these tests use short exposure times, and responses can only be observed at relatively high pollutant concentrations. These conditions may be appropriate for wastewater, but are rarely found in streams. In addition, such tests only assess direct toxicity, while the effects of other types of toxic compounds, e.g., hormone-active or carcinogenic compounds, are not detected. Such tests have only limited value for the ecotoxicological evaluation of water samples from a stream. Long-term tests, on the other hand, disclose toxic effects of chemicals at very low levels. Unfortunately, they are labor-intensive, costly, and are usually employed when some negative effects have already been observed.

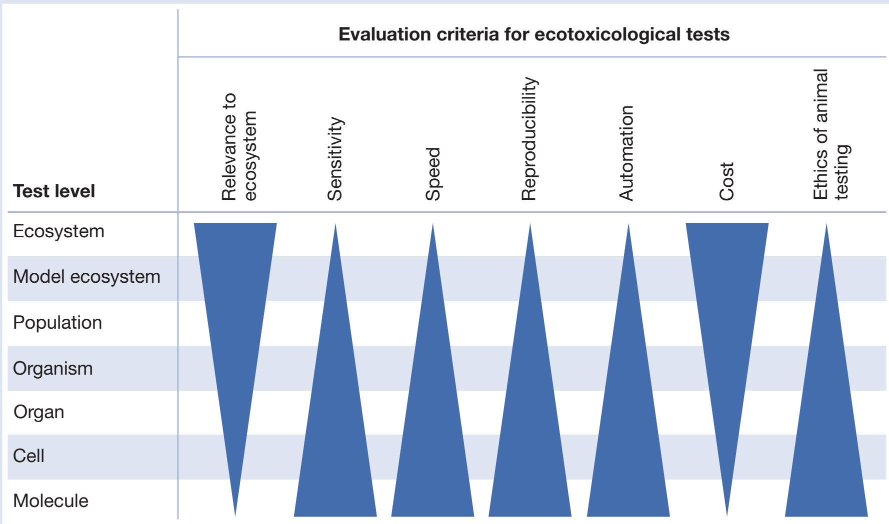


**Fig. 1: Evaluation criteria for ecotoxicological tests. Criteria are more or less well defined depending on the biological level at which the test is conducted.**

Most classical tests have been standardized on national or even international levels, particularly by cooperation among the countries of the Organization for Economic Cooperation and Development (OECD) and the International Organization of Standardization (ISO). Standardization assures consistent sensitivity of the test organisms and reproducibility of tests between laboratories.

## Alternative Testing Systems

In addition to the classical test systems, there are procedures for the evaluation of ecotoxicological characteristics of streams which approach the problem at a range of levels (Fig. 1). While the toxic effect of a chemical compound expresses itself in the organism, and ultimately in the ecosystem as a whole, the primary damage is done on the molecular level. Initial effects may include damage to proteins, DNA, or membrane lipids. If the damage is not repaired the effects carry on to higher levels and, with a certain delay, will affect the cell, the organs, and eventually the whole organism. Ultimately, the damage may manifest itself in populations, communities, or in the entire ecosystem. Typical symptoms include suppressed populations, increased frequency of certain diseases, shifts in predator-prey relationships, or changes in species composition. Unfortunately, for most of these "alternative" test systems, no standardization schemes have yet been developed.

**Molecular and cellular methods:** Since molecular and cellular effects manifest themselves very rapidly, the toxicity potential of water samples can be assessed in a short time span. Molecular and cellular test systems typically exhibit good reproducibility and are often more sensitive than classical tests. Costs are comparatively low, and the tests can be performed rapidly and easily. In the context of aquatic ecotoxicology, these types of tests have so far only been used in research and have been routinely used in the assessment of toxicity to mammals. These tests have the additional benefit that

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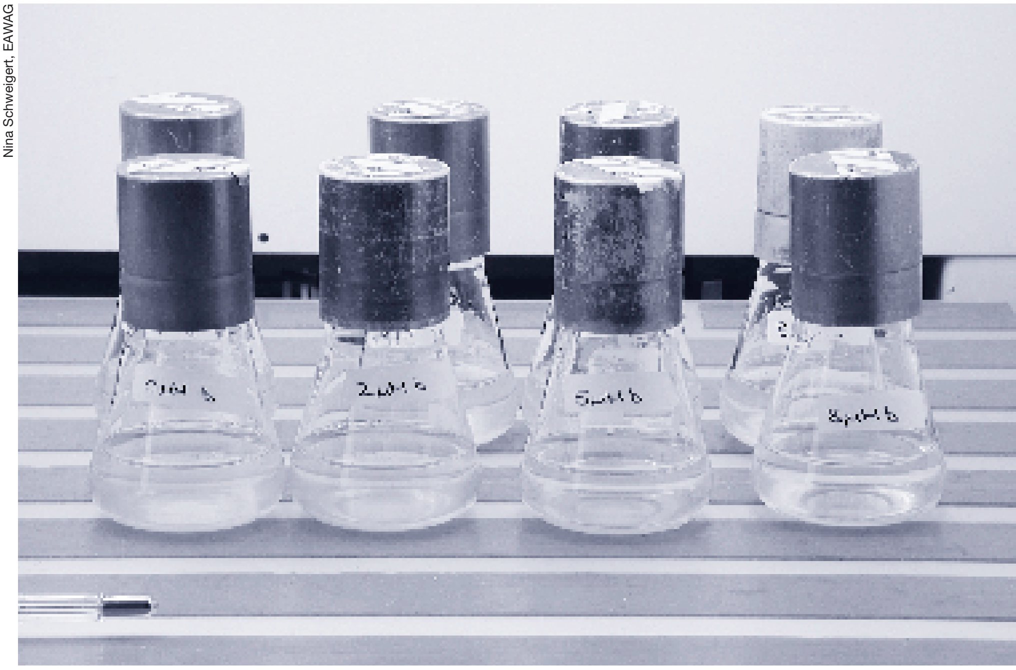


Many single-celled organisms (ex. green algae) and cell lines are easy to cultivate.

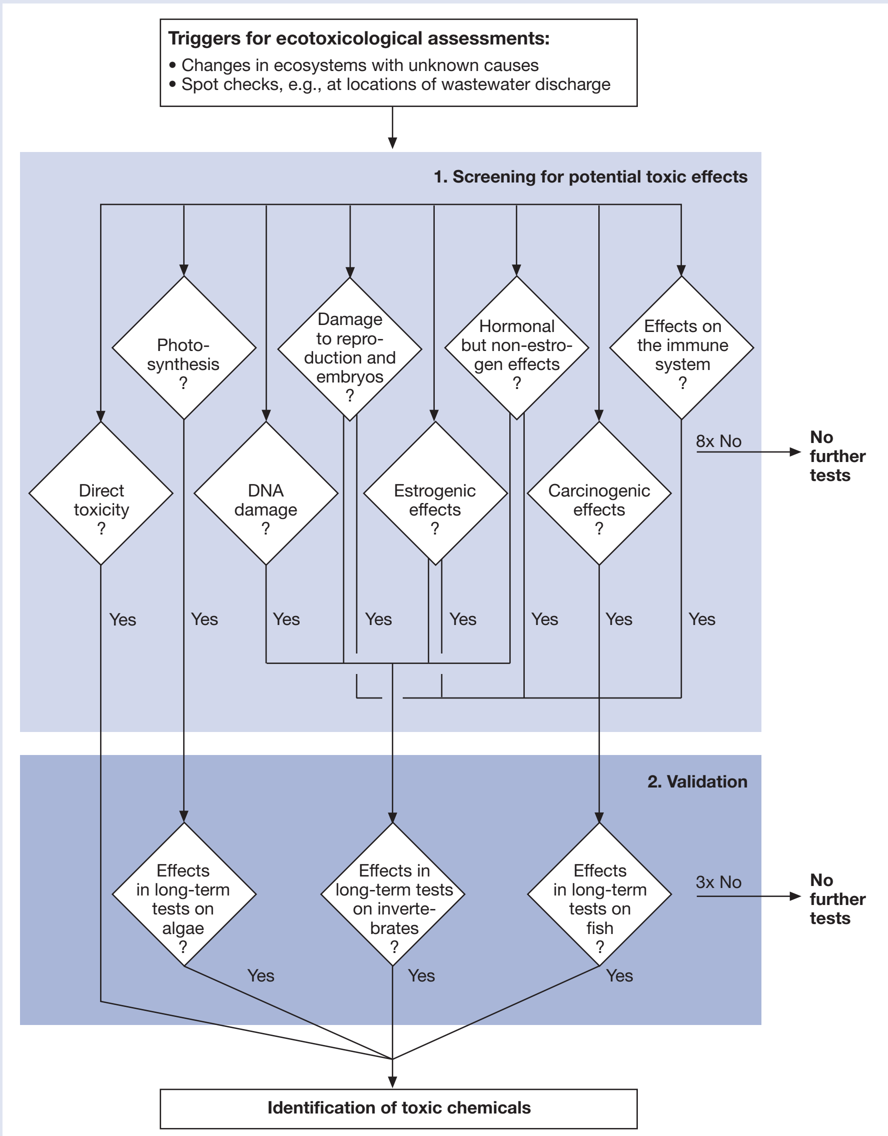
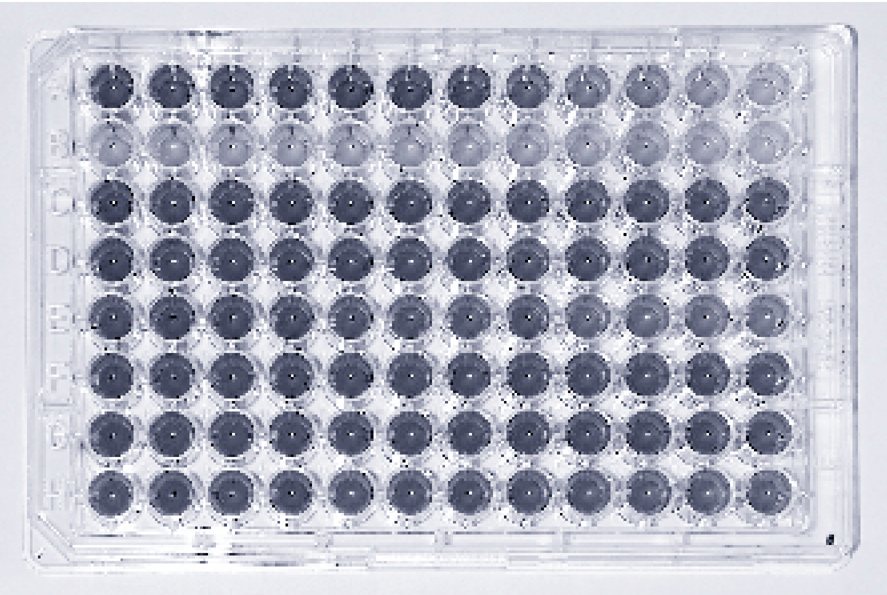



Fig. 2: A two-step approach to the ecotoxicology module for the assessment of streams.



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**Estrogenic effect of wastewaters from different sources on recombinant yeast cells. Dark test tubes: estrogenic impact; Light test tubes: no estrogenic impact.**

they not only measure direct toxicity, but also expose other relevant mechanisms by which a certain chemical may do harm.

## A New Concept for Stream Assessment

Based on the rationale discussed above, we have developed the following concept (Fig. 2): Water samples are examined in a two-step approach. First, molecular and cellular techniques are used to determine potential toxic properties. In a second step, positive responses are validated; i.e., it is verified that observed toxic effects also manifest themselves on the level of the whole organism. Assessments may be triggered by indications of wastewater discharge or signs of biological disturbance. In addition, spot checks can be made to identify locations that are exposed to a higher risk.

**Step 1:** The toxicity potential of a water sample is assessed with cell cultures and single-celled organisms. In addition to direct toxicity, the test is also expected to uncover more subtle toxic effects. For this reason the assessment does not rely on a single test; instead, the water sample is subjected to a battery of cellular test systems. On the molecular level, recombinant cell lines of invertebrates or fish are examined for carcinogenic effects, hormone-like activity or damage to the immune system. Single-celled algae serve as representatives of plants and are evaluated for effects on the photosynthetic apparatus. Direct toxicity is assessed using bacteria. In the first step, no organisms higher than *Daphnia* or fish are used. If all tests in step 1 are negative, the water samples can be considered harmless, and no further tests are performed.

With time, the test battery will have to adapt to perceived needs and technical capabilities. Individual tests may be replaced by newer or more sensitive tests, or the number of tests may be expanded in order to include toxicity mechanisms which were previously unknown.

**Step 2:** Only the group of organisms which exhibits toxic effects in step 1 is subjected to further tests in step 2, thereby eliminating unnecessary testing on animals. Test organisms for a particular group should be selected such that they are typical representatives of the stream or lake from which the water sample was obtained. It should, however, be a species which is relatively sensitive and can be maintained in the laboratory. These organisms are then used in long-term tests which focus on the effects observed in step 1. A negative result at this point indicates that there is the potential for toxic effects at the cellular level, but the effect is not strong enough to manifest itself at the level of the organism. Again, the water samples can be considered harmless; however, if the toxic effects are confirmed, the specific chemical compounds responsible for the damage need to be identified in order to devise appropriate mitigation measures. If the observed toxic effects are the result of an interaction between two or more compounds, identification can become difficult or even impossible. In such cases, one must resort to a more pragmatic solution by reducing contaminant levels across the board.

## Conclusions

The new concept presented is a promising approach to the ecotoxicological evaluation of streams. A large number of water samples can be examined for the complete range of relevant toxic effects. In addition, the number of tests on animals is reduced dramatically. Long-term tests need to be conducted only for water samples which indicate toxic effects in the first set of tests. The challenge now lies in developing this approach to the point where it can be applied on a routine basis. The next steps include finding answers to questions that remain open and testing the approach in case studies.

**Nina Schweigert, biologist, participated in the development of this new concept for ecotoxicological assessments of streams as part of her postdoctoral research. Since April 2001, Nina Schweigert is back at EAWAG in order to develop the concept into an application.**

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[1] BUWAL (1998): Modul-Stufen-Konzept. Mitteilungen zum Gewässerschutz Nr. 26, 43 p.
[2] Fent K. (1998): Ökotoxokologie. Thieme Verlag, Stuttgart, 288 p.