Working Group of the Federal States on Water Problems
(LAWA)

Recommendation on the Deployment of Continuous Biomonitors for the Monitoring of Surface Waters

Compiled by the LAWA 'Biomonitoring' Committee 1996

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This paper is a translation from the German text, edited in 1996. All statements are on the level of knowledge at that time, except the lists of biotests run at different sites and the descriptions of the ”DF Algae test“ and the “bbe Algae Toximeter“.

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Content

Recommendation on the Deployment of Continuous Biomonitors for the Monitoring of Surface Waters

Compiled by the ”Biomonitoring” Committee of the Working Group of the Federal States on Water Problems (LAWA)
(Date: October 1995)

1. COMMISSION

The LAWA ”Biomonitoring” committee was commissioned by the LAWA Working Group ”Surface Waters“ (LAWA-AG ”O”) to draw up national recommendations for the deployment of continuous biotesting methods for the monitoring of water courses. The recommendations were to be based on both the results of the joint federal/states project ”Effects tests Rhine“ (”Wirkungstests Rhein“, WIR) and the experience gathered in the individual federal states. The brief was to set out the limitations and possibilities for their employment.

Continuous biotests are defined by the Committee as those employing living organisms including bacteria. Biosensors, in which tissue, cells, organells, membranes or biomolecules are used for detection of pollutants, are not included in this definition.

2. AIMS OF THE EMPLOYMENT OF CONTINUOUS BIOTESTS

Biotest methods have been used for several decades in a number of areas for the monitoring of water courses. They assess the effect of biologically harmful or biologically stimulating substances on selected test organisms. The aim of biological effect monitoring is to determine the effects of as many pollutants as possible. Because of the the multitude of specificities of action of pollutants, test methods with various kinds of organisms must therefore be deployed.

In the biotests, a distinction is made between static (laboratory) biotests and dynamic biotests, which are deployed in various fields of application.

Static biotests with aquatic organisms play a significant role in the Federal Republic of Germany both for the implementation of the Water Resources Management Act (Sections 7a and 19g WHG) and the Waste Water Charges Act (AbwAG), and in the approval of chemicals in compliance with the Chemicals Act (ChemG) and the Plant Protection Act (PflSchG). Generally, in static biotests the effects on the test organisms exposed to a test medium (e.g. waste water, surface water) or a chemical for a certain test period (e.g. 24 h or 48 h) are determined. In these acute tests no exchange of the test medium generally occur in the course of the test.

In contrast, in continuous (dynamic) biotests the test organisms are exposed to the test medium, e.g. river water, continuously or in semi-continuous systems in cycles of a few minutes. Changes in metabolism or of behavioural/physiological parameters induced by sublethal effects are measured using automatic detection systems. The designation ”biomonitor” characterises the main function of the system as a monitoring and warning system for the identification of combined pollutant effects. The employment of continuous biotests for the monitoring of immissions and emissions offers the possibility of monitoring waters continuously in relations to time. In the monitoring of immissions they are primarily deployed as warning systems which, for instance, indicate elevated concentrations caused by accidental discharges above the usual background concentration of the water course. In conjunction with chemical analysis and more detailed biological examinations, they assist in securing evidence of illegal discharges and accidents. Applied to the monitoring of emissions they can supplement the monitoring with standardised static biotests, as a means of continuous quality assessment.

The variuos applications of continuous biotests for the monitoring of emissions will be discussed in more detail in another recommendation report to be published by the committee.

3. THE RESULTS AND RECOMMENDATIONS FROM A COMPARATIVE STUDY TO ASSESS THE OPERATION, THE INFORMATION WHICH CAN BE OBTAINED AND THE PRACTICAL APPLICATION OF CONTINUOUS BIOTESTS

Against the background of the Sandoz accident, the International Commission for Protection of the Rhine (IKSR) was commissioned, at the 7th Environment Ministers’ Conference of the riparian states of the Rhine on 19.12.1986 in Rotterdam, to review the national research programmes in the Rhine catchment area including the biological tests. Requested by the German Commission for the Protection of the Rhine (DK), its ”Measuring Methods” working group set up the joint federal/states project group ”WIR” (Testing the Effects on the Rhine), which in November 1989 submitted an outline plan for research projects for the ”development, testing and implementation of biotests for the monitoring of the Rhine”. Test methods using organisms of various trophic levels (producers, primary and secondary consumers, and destruents) were developed and evaluated in the research program. In 8 projects, a total of 22 biomonitors were tested between 1989 and 1993, selected to cover a range of possible effects as wide as possible including those caused by bactericides, insecticides or herbicides.

The biomonitors were evaluated at the Rhine measuring stations Bimmen and Bad Honnef, in a mobile measuring container on the Rhine and the lower Main, as well as at the Federal Institute of Hydrology, during 1990 - 1991. Experiences from other monitoring stations were also incorporated into the appraisal. In addition, tests were carried out with chemicals in the laboratory with waste waters and at field stations by chemical spiking.

The results of the trials of the individual biomonitors are presented in detail in the final reports on the subprojects; the principal results of all projects were presented in a final summary report of the combined research project (UBA Reports 1/95).

The combined research project has shown that biomonitors are fundamentally suited to the detection of pollutant peak concentrations in water courses.

Especially well suited methods for water monitoring were selected from the multitude of methods tested for each trophic level. For this purpose, an assessment matrix was drawn up, on the basis of which biomonitors could be compared with each other according to objective benchmarks. The criteria used for the assessment of the scientific information content of the measured data and of the reliability of the technical equipment in routine operation are shown in the following table:

Table 1: Assessment criteria and weighting for continuous biotests

Criterion	Weighting
1. Sensitivity (surface water)	A
2. Sensitivity (waste water)	A
3. Useful life	A
4. Computer equipment	A
5. Susceptibility to faults	A
6. Availability	A
7. Plausibility check	A
8. Sensitivity (lab)	B
9. User-friendliness	B
10. Material deployment	B
11. Required maintenance	B
12. Test organisms	B
13. Required space	B
14. Refractory time	B
15. Measuring range	B
16. Acquisition costs	C

(A = very important or essential requirement; B = important; C = less important; for explanatory notes on the criteria, see UBA Reports 1/95, p. 31-41).

On the basis of the assessment criteria (classification of suitability) the ”WIR” project group tracking the project made the following recommendations on the employment and operation of the test systems for the monitoring of the Rhine (UBA Texts 34/94):

The aim is to implement a test battery of biomonitors with organisms of different trophic levels in each suitable measuring station, because biotests with organisms of differing trophic levels are not interchangeable.

a) Unless already used, the ”dynamic daphnia test” or a comparable system is proposed as the priority test.

The ”dynamic daphnia test” was recommended for implementation at water course measuring stations by the ”WIR” project group in 1989. A working group report entitled ”The dynamic daphnia test - Experiences and practical instructions” (publ.: State Environmental Office North Rhine-Westphalia (LUA-NRW), 1994) provides an overview of the functioning and measuring principle of the daphnia test, summarises the practical experience gathered to date, gives instructions on the operation of the test system, describes the existing evaluation tests, and details the space and investment required for the acquisition of the test system.

b) A fluorescence algae test is proposed as the second test system for implementation. Here the ”WIR” project group recommends the "DF algae test”.

At present only the ”biosense algae toximeter” and the ”DF algae test” of the algae tests investigated can be considered for measuring stations, because insufficient experience has been gathered so far with other test systems. Because of the superior equipment and the, although only slightly, higher sensitivity in the dose response experiment, the ”DF algae test” is recommended.

c) After installation of the automatic daphnia and algae test systems, efforts should be made to equip the measuring stations with a suitable bacteria test. Completion of the development of the automatic luminous bacteria systems should be awaited.

Of the bacteria tests, only the ”Toxiguard”, with all its weak points, could at present be proposed for deployment in measuring stations. However, instead of developing this test system further, the installation of a luminous bacteria test is recommended, due to its special importance for the monitoring of waste water discharges (draft administrative regulation according to Article 7a WHG). A conclusive judgement of the currently available test equipment cannot be made, due to the lack of experience in outdoor operation.

d) If additional personnel and financial capacities are available, the ”Dreissena monitor” or a comparable system is recommended as a further component of the test battery.

The ”Dreissena monitor” has proved its high practical adequacy at a number of locations. Since the recommended test battery comprises a test with bacteria (destruents), photosynthetically active organism (primary producers) and an aquatic animal (primary and secondary consumers), and since with the dynamic daphnia test a well suited test method is already available, the ”Dreissena monitor” is proposed only as 4th priority for use in measuring stations.

e) Fish tests are not recommended for implementation at present.

The necessity of a fish test was hotly discussed. The previously used flowthrough fish tests were not recommended, due to their low sensitivity. The ”WRc fish monitor” is indeed - like the ”Koblenz behavioural fish test” - more sensitive to pollutants, but it entails very high acquisition costs and is to be viewed as problematical in terms of the treatment of animals. Only the behavioural fish test could be considered as a potential fish test method.

The results of the combined research project and the recommendations derived therefrom were taken up by River Basin Commissions.

The **German Commission for the Protection of the Rhine** (DK) aggreed the following resolution on the employment of continuous biotests at its 99th assembly on December 8, 1993 in Mainz:

1. For implementation, both a continuous daphnia test and a fluorescence algae test (priority to the DF algae test) are recommended.
2. In addition, efforts are to be made to equip the measuring stations with a suitable bacteria test (luminous bacteria test on development through to practical adequacy).
3. In the long term, the fish tests should be replaced by other, more sensitive biotests.
4. If possible, a mussel test (Dreissena monitor or comparable systems) should additionally be run.

The ”Measuring Stations Operation” working group of the International Commission for the Protection of the Elbe (IKSE) also agreed generally with the recommendations of the ”WIR” project group in October 1994.

4. EXPERIENCES WITH THE EMPLOYMENT OF CONTINUOUS BIOTESTS IN VARIOUS RIVER BASINS

4.1 EXPERIENCES OF THE FEDERAL STATES

The experiences in the operation of continuous biotests summarised here are based on a survey carried out by the LAWA ‘Biomonitoring’ committee among the state authorities responsible for water monitoring. It shows that the dynamic daphnia test is the most frequently deployed continuous biotest. The next most widespread method is the flow fish test, followed by the Dreissena monitor and the continuous algae tests (see map p. 47). A significant increase in the use of the two last-named methods can be observed.

With regard to the alarms triggered by the continuous biotests, a decrease can be observed in recent years. Alarms which could have been linked to known accidents occurred only in isolated instances in recent years.

4.1.1 Dynamic Daphnia Test

The evaluation of the survey among the state authorities shows that the dynamic daphnia test is the most widely used of the available test systems.

Experience with the dynamic daphnia test has been available from measuring stations in North Rhine-Westphalia for more than 10 years. After the initial positive experience, additional measuring stations on the Rhine and also in other river basins were equipped with the dynamic daphnia test from the Elektron company. This allowed the operators to gather a wide range of positive and negative findings.

Biotest method	**Installed since/from**	**Measuring point, Operator**	**Water course**
Dynamic daphnia test	1990	Berlin, Mühlendamm lock, Senate Administration for Urban Development and Environmental Protection	Spree 17 km course
Dynamic daphnia test	1994	Bremen-Hemelingen, Senator for Women, Health, Youth, Social Affairs and Environmental Protection	Weser 361.1 km course
Dynamic daphnia test	1989	Bischofsheim, Hessian State Environmental Agency	Main 5 km course
Dynamic daphnia test	1993	Dessau, State Office for Environmental Protection Dessau/Wittemberg	Mulde 7.6 km course
Dynamic daphnia test	1995	Gorsdorf, State Office for Environmental Protection Dessau/Wittenberg	Schwarze Elster 3.8 km course
Dynamic daphnia test	1993 until 1996	Schmilka, Saxony State Office for Environment and Ecology	Elbe 4.1 km course
Dynamic daphnia test	1993 until 1996	Zehren, Saxony State Office for Environment and Ecology	Elbe 87.8 km course
Dynamic daphnia test	1991	Magdeburg, State Office for Environmental Protection Magdeburg	Elbe (left) 318.1 km course
Dynamic daphnia test	1988 until 1998	Bunthaus, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe 609.8 km course

Biotest method	**Installed since/from**	**Measuring point, Operator**	**Water course**
Dynamic daphnia test	1988 until 1996	Altenwerder, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe 621 km course
Dynamic daphnia test	1988 until 1998	Seemannshöft, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe (left) 628.8 km course
Dynamic daphnia test	1988	Blankenese, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe (right) 634.3 km course
Dynamic daphnia test	1995	Wandsbeker Chaussee, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Wandse
Dynamic daphnia test	1996 until 1998	Bille-Serrahn, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Bille
Dynamic daphnia test	1990	Iffezheim, State Agency for Environmental Protection Baden-Württemberg	Rhine (right) 333.8 km course
Dynamic daphnia test	1988	Karlsruhe, State Agency for Environmental Protection Baden-Württemberg	Rhine (right) 359.2 km course
Dynamic daphnia test (2 units)	1995	Worms, State Office of Water Affairs Rhineland-Palatinate	Rhine (left and right) 443.3 km course
Dynamic daphnia test (2 units)	1989	Mainz-Wiesbaden, State Office of Water Affairs Rhineland-Palatinate	Rhine 498.5 km course

Biotest method	**Installed since/from**	**Measuring point, Operator**	**Water course**
Dynamic daphnia test	1993	Koblenz, Federal Institute of Hydrology	Rhine (left) 590.4 km course
Dynamic daphnia test	before 1985	Bad Honnef, State Environmental Office North Rhine-Westphalia	Rhine (right) 640 km course
Dynamic daphnia test	1989	Düsseldorf-Flehe, State Environmental Office North Rhine-Westphalia	Rhine (right) 735 km course
Dynamic daphnia test	1995	Düsseldorf-Rathausufer, State Environmental Office North Rhine-Westphalia	Rhine (right) 744.3 km course
Dynamic daphnia test	before 1985	Kleve-Bimmen, State Environmental Office North Rhine-Westphalia	Rhine (left) 865 km course
Dynamic daphnia test	1995	Hattingen, State Environmental Office North Rhine-Westphalia	Ruhr (left) 56 km course
Dynamic daphnia test	1988	Fröndenberg, State Environmental Office North Rhine-Westphalia	Ruhr (right) 111.5 km course
Dynamic daphnia test	1988	Opladen, State Environmental Office North Rhine-Westphalia	Wupper (right) 5.5 km course
Dynamic daphnia test	1996 [1)]	Schwandorf, Bavarian State Ministry of Town and Country Planning and Environmental Affairs	Naab 36.9 km course

[1)] Mobile measuring station.

During field operation, it was found that a useful life of 7 days can be achieved before the test organisms need to be replaced. However, it may occur that the daphnia die earlier if the temperature is too high. In summer, when a lot of food is available, the animals started to reproduce. The resulting increase in the number of pulses generated may lead to false alarms. The continuous infeed of test water is not always guaranteed. For example, high suspended matter content can lead to blockage of the test chamber sieves and thus to overflowing of the test chambers. If the blockages interfere with the flow, the unit may not reliably register the occurrence. Faulty values are obtained, despite the presence of flow monitors. Temperature increases in the system (constant temperature control, illumination) cause non-specific evolution of gases. The air bubbles may interrupt the test water flow. The constant temperature control equipment is not capable of guaranteeing a temperature of 20°C under all conditions. To overcome these problems, and to clean and replace, when necessary, the otherwise almost entirely inert material, a total maintenance time of 3 to 4 hours per week should be planned for each location. The commitment for breeding and looking after the test animals is additional to this.

Daphnia magna has proved to be a suitable test organism. The age at which the daphnia are introduced into the system must be adjusted to the conditions of each location (lack of food in winter; production of young in summer).

The threshold values for triggering the alarm are designed dynamically. The necessary programming is provided individually and independently of the use of the device. The evaluation is made more difficult by the fact that substantially differing pulse rates may occur in the right and left chambers. There is furthermore no generally accepted algorithm for alarm detection. Because of this it became clear from all users that the daphnia test can only be carried out with the support of specially qualified personnel.

In occasions, a lower sensitivity of the dynamic daphnia test was observed in comparison to the Dreissena monitor.

The problem points listed have been the subject of the second meeting on the dynamic daphnia test, held on November 15/16 1995 in Hamburg.

4.1.2 Fish Tests

Continuous fish tests were employed as the first biomonitors at measuring stations on the Rhine. The frequent pollution incidents of that time have since become less significant, so that the number of alarms has drastically decreased in recent years.

Biotest method	Installed since/from	Measuring point, Operator	Water course
Flow fish test (Ker-ren)	1979	Bremen-Hemelingen, Senator for Women, Health, Youth, Social Affairs and Environmental Protection	Weser 361.1 km course
Flow fish test (Ker-ren)	1990	Iffezheim, State Agency for Environmental Protection Baden-Württemberg	Rhine (right) 333.8 km course
Flow fish test (Ker-ren)	1989	Karlsruhe, State Agency for Environmental Protection Baden-Württemberg	Rhine (right) 359.2 km course
Flow fish test (Ker-ren)	1972	Bad Honnef, State Environmental Office North Rhine-Westphalia	Rhine (right) 640 km course
Flow fish test (Ker-ren)	1989	Düsseldorf-Flehe, State Environmental Office North Rhine-Westphalia	Rhine (right) 735 km course
Flow fish test (Ker-ren)	1972	Kleve-Bimmen, State Environmental Office North Rhine-Westphalia	Rhine (left) 865 km course
Flow fish test (Ker-ren)	1988	Wesel, State Environmental Office North Rhine-Westphalia	Lippe (right) 3.6 km course
Flow fish test (Ker-ren)	1994	Schalksmühle, State Environmental Office Hagen	Volme
Koblenz Behavioural Fish Test	1993	Koblenz, Federal Institute of Hydrology	Rhine (left) 590.4 km course

4.1.2.1 Aqua-Tox-Control (Kerren) Flow Through Fish Test

Temperature control of the test system is generally not possible, due to the high water flows. The test water flow is not monitored, which means that failures are not indicated. The device sensitivity setting is problematical and not reproducible. The required useful life of 1 week is maintained due to the low susceptibility to faults. Approximately 2 hours' on-site maintenance per week is required.

It was at times not possible to procure golden ide complying with the test requirements. Individuals of inadequate size escape from the test chamber; too large ones may injure themselves.
The alarm is indicated if the set pulse limit is exceeded.

4.1.2.2 Passavant Flow Through Fish Test

The last three fish tests of this design in operation have been decommissioned in 1995, firstly because there were a large number of false alarms, and secondly because the units did not register water pollution incidents.

4.1.2.3 Koblenz Behavioural Fish Test

The Koblenz behavioural fish test with BehavioQuant^R was developed further in the course of the Testing of Effects on the Rhine (”WIR”) combined research project, and has been operated since then at the Koblenz measuring station of the Federal Institute of Hydrology (BfG). The unit is currently running fault-free.

4.1.3 Mussel Tests

4.1.3.1 Dreissena Monitor

The Dreissena monitor is being increasingly applied in water monitoring. The following experience has been gathered by the operators:

Temperature control is not necessary. A substantially reduced flow rate does not affect the functioning capability of the monitor. Flow interference does not generally occur.

Adjustment of the individual mussels is relatively time consuming, requires a delicate touch and a lengthy training period. The maintenance time is 2 to 5 hours per week.

The aimed for useful life of 7 days is easily achieved. However, a disadvantage is that monitoring does not automatically restart after a power failure.

The mimic for adjustment corrodes relatively quickly (useful life below 1 year).

Biotest method	Installed since/from	Measuring point, Operator	Water course

Dreissena monitor (Borcherding & Volpers)	1994	Schmilka, Saxony State Office for Environment and Ecology	Elbe 4.1 km course
Dreissena monitor (Borcherding & Volpers)	1995	Domitzsch, Saxony State Office for Environment and Ecology	Elbe
Dreissena monitor (Borcherding & Volpers)	1995	Bad Düben, Saxony State Office for Environment and Ecology	Mulde
Dreissena monitor (Borcherding & Volpers)	1995	Görlitz, Saxony State Office for Environment and Ecology	Neiße
Dreissena monitor (Borcherding & Volpers)	1992	Karlsruhe, State Agency for Environmental Protection Baden-Württemberg	Rhine (right) 359.2 km course
Dreissena monitor (Borcherding & Volpers)	1995	Düsseldorf-Rathausufer, State Environmental Office North Rhine-Westphalia	Rhine (right) 744.3 km course
Dreissena monitor (Borcherding & Volpers)	1995	Hattingen, Ruhr Corporation	Ruhr (left) 56 km course
Dreissena monitor (Borcherding & Volpers)	1992	Fröndenberg, Ruhr Corporation	Ruhr (right) 111.5 km course
Dreissena monitor (Borcherding & Volpers)	1992	Bergheim, Erft Corporation	Erft
Dreissena monitor (Borcherding & Volpers)	1995 [1)]	Schwandorf, Bavarian State Ministry of Town and Country Planning and Environmental Affairs	Naab 36.9 km course
Mossel-Monitor	1993	Koblenz, Federal Institute of Hydrology	Rhine (left) 590.4 km course

[1)] Still in the trial phase. Mobile measuring station.

Since the mussels cannot be laboratory-bred, the animals are procured from the wild. This procurement can be difficult. In some cases a high mortality rate of the mussels was recorded.

An evaluation programme for the dynamic limit value setting and alarm detection is provided with the unit. Numerous plausible alarms were registered in the past years (Schmilka/Elbe). Inactive mussels are automatically excluded from measurement and indicated by the unit.

4.1.3.2 Mossel-Monitor

In Germany the Mossel-Monitor is currently only operated in Koblenz (BfG). The following experience has been gained there:

Temperature control is not necessary. A substantially reduced flow rate does not affect the functioning capability of the monitor. Flow interference does not generally occur.

Adjustment of the mussels is not necessary.

The maintenance time is 30 minutes per week. For reloading 30 minutes per mussel should be planned. The aimed for useful life of 7 days is achieved. The material is inert and corrosion-proof.

Since the mussels cannot be laboratory-bred, the animals are procured from the wild. This procurement can be difficult. Mortality of the mussels is low. Relatively few specimens are required.

An evaluation programme for the dynamic limit value setting and alarm detection is provided with the unit.

4.1.4 Algae Tests

Very little experience has been gained so far for continuous algae tests on site.

In the laboratory, no particular problems occurred to date in the DF algae test. The useful life of 7 days has not yet been achieved due to the inadequate nutrient stock solution.

In the LAR/bbe algae test operated in Hamburg, initial outdoor experiences indicate no major problems. This system is characterised by particular maintenance-friendliness. The aimed for useful life of 7 days is easily achieved.

The laboratory-breeding of algae represents no problem.

There is as yet little experience for continuous operation.

Biotest method	Installed since/from	Measuring point, Operator	Water course

DF algae test (Univ.Regensburg)	1995 [1]	Bischofsheim, Hessian State Environmental Agency	Main 5 km course
DF algae test (Univ.Regensburg)	1996 [2]	Schwandorf, Bavarian State Ministry of Town and Country Planning and Environmental Affairs	Naab 36.9 km course
DF algae test (Univ.Regensburg)	1995 [1]	Karlsruhe, State Agency for Environmental Protection Baden-Württemberg	Rhine (right) 359.2 km course
DF algae test (Univ.Regensburg)	1997 [1]	Worms, State Office of Water Affairs Rhineland-Palatinate	Rhine (left or right) 443.3 km course
bbe Algae toximeter	1997	Schmilka, Saxony State Office for Environment and Ecology	Elbe 4.1 km course
bbe Algae toximeter	1996	Bunthaus, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe 609.8 km course
bbe Algae toximeter	1996	Bille Serrahn, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Bille
bbe Algae toximeter	1997	Seemannshöft, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe (left) 628.8 km course
Algae toximeter (LAR)	1995 until 1997	Seemannshöft, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe (left) 628.8 km course
FluOx algae test	1995 [1]	Bad Honnef, State Environmental Office North Rhine-Westphalia	Rhine (right) 640 km course

[1] Still in the trial phase. [2] Mobile measuring station.

4.1.5 Bacteria Tests

The Toxiguard - with fixfilm bacteria - and Toxalarm - with freely moving bacteria - devices are currently used for water monitoring. A luminous bacteria test is currently being developed and tested.

Biotest method	**Installed since/from**	**Measuring point, Operator**	**Water course**
Bacteria test ”Toxalarm” (LAR)	1988 until 1997	Bunthaus, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe 609.8 km course
Bacteria test ”Toxalarm” (LAR)	1988 until 1997	Seemannshöft, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe (left) 628.8 km course
Bacteria test ”Toxalarm” (LAR)	1988 until 1996	Blankenese, Free and Hanseatic City of Hamburg, State Environmental Protection Authority, Office of Environmental Investigations,	Elbe (right) 634.3 km course
Luminous bacteria test (Univ. Regensburg)	1996 [1) 2)]	Schwandorf, Bavarian State Ministry of Town and Country Planning and Environmental Affairs	Naab 36.9 km course
Bacteria test “Toxiguard”	1993	Koblenz, Federal Institute of Hydrology	Rhine (left) 590.4 km course
BioLum luminous bacteria test	1995 [1])	Bad Honnef, State Environmental Office North Rhine-Westphalia	Rhine (right) 640 km course

1) Still in the trial phase.
2) Mobile measuring station.

4.1.5.1 Toxiguard and Toxalarm

Problems occur because bacteria grow in the tubing. As a result the maintenance time required for rinsing is approximately 3 h per week. The useful life is between 3 and 7 days; daily maintenance is recommended.

Bacterial populations are attracted from the test water, which are unstable with regard to their physiological characteristics during the course of a year. In the winter months the growth of the biofilm is often very slow in the Toxiguard device.

Special evaluation programmes are currently not available; on the other hand, alarms are easily detectable.

The present experiences show that the bacteria toximeter „Toxalarm“ is very expensive concerning maintenance with a tendency to develop faults. The weak spots of this biomonitor are the thin tubes which tend to block up. Daily rinsing (10 minutes) all testwater tubes and/or of all tubes enhancing the growth of bacteria with citric acis (2 %) can reduce the growth in the tubes to an acceptable level. The maintenance time required is longer than that which is assumed for continuously working monitoring stations.

4.2 EXPERIENCES IN THE NETHERLANDS (DBW/RIZA)

Continuous biotests for water monitoring have been employed in the Netherlands since the 1970s. In the 1980s the initially emeployed fish test systems developed by to SPOOR *et al.* (1977) and POELS (1977) were replaced by the flow through fish test developed by JUHNKE and BESCH (1971) (for listed literature: see UBA reports 1/95).

In 1988 biotests systems were jointly subjected to a review by the DBW/RIZA (Inland Waterways Service/Institute for Inland Water Management and Waste Water Treatment) and a number of drinking water supply companies. Only the flow through fish test and the dynamic daphnia test emerged from this as suitable test systems. Both biomonitors were installed in control stations on the Rhine and Maas, whereby the flow through fish test was already in operation within the framework of the ongoing monitoring programme.

The flow through fish test employed in the Lobith measuring station on the Rhine, and on the Maas, showed low false alarm frequency, simple operation and a comparatively low maintenance requirement of some 6 hours per week. Failings were the lack of calibration facilities for the sensitivity setting and the lack of computerisation.

In 1988 and 1989 the flow through fish test indicated some 20 alarms. In some cases increased concentrations of various substances were detected. For the alarm registered at the Lobith measuring station in February 1989, increased concentrations of fluoranthene, pyrene and trioxane were measured simultaneously. However, analyses were not carried out in response to each biotest alarm, or produced negative results. In 1990 three alarms were registered on the Rhine, and only one case on the Maas. As a consequence of the improved water quality, the flow through fish test increasingly proved to be too insensitive. In 1991 two alarms were registered on the Maas. Chemical analysis showed concentrations of 7 µg/l tributyl phosphate and 18 µg/l trichloromethane, respectively.

The dynamic daphnia test was evaluated in addition to the flow through fish test at Dutch measuring stations. The experience showed that the dynamic daphnia test required extensive expertise with the test system, due to its more difficult handling. When the suspended matter content was high, problems occurred with the test which were eliminated by the installation of filters. At water temperatures of around 20°C within the one-week test phase, reproduction was observed which could be delayed at 17°C +/- 1°C. Forever, as a result of the temperature reduction air bubbles were formed.

To prevent false alarms the alarm criteria were modified. An alarm was only triggered when the dynamic alarm thresholds were exceeded, or values fell below them, in both chambers.

The required maintenance for the dynamic daphnia test is approximately half a day per week, according to the experience at the Dutch measuring stations.

5. POSSIBILITIES AND LIMITATIONS OF CONTINUOUS BIOTESTS - RECOMMENDATIONS FOR EMPLOYMENT

5.1 POSSIBILITIES AND LIMITATIONS OF MONITORING OF SURFACE WATERS WITH CONTINUOUS BIOTESTS

Mainly chemical/physical methods are currently employed for the monitoring of the water quality. The effects of substances in the water, including synergistic and antagonistic effects of substances, can only be inadequately estimated on the basis of the concentrations of the analysed substances. No account is taken of the potential effects of unknown or chemically undetected substances. This can only be assessed by biological systems. Biotests can also detect pollutants with biological effects which are not included in analytical matrices, or have very high detection limits.

The reactions of the organisms observed in the biotest system are generally non-specific (integrated test criteria such as swimming behaviour, luminescense, oxygen production etc.). No conclusion can therefore be drawn regarding the responsible substance or substances for the indicated effect. If the important information regarding the presence of a pollution event in the water is to be supplemented by qualitative and quantitative description of the event, chemical analysis is essential. The substances causing an alarm can frequently not be determined, however; because despite sophisticated analytical techniques, only a certain portion of the range of substances occurring in water courses can be analysed. Furthermore, the detection limits of the analytical method may be above the concentrations which are harmful to organisms, or pollutant peaks may be distorted by the strategy of sampling. Combination of effects of substances in which individual concentrations are not noticeably increased, or of substances influenced by the water characteristics, may also be the causes of alarms.

Lack of identification of the substance which has an impact on the organisms is unsatisfactory as two aims of a time-based monitoring system can only partially be achieved: the search for the responsible party is difficult because immission and emission samples cannot be analysed retrospectively with the same methods; and the lack of identification makes it difficult for water utilities to assess whether, and which, measures may need to be taken to protect drinking water if the water abstractions, for example where water is removed by bankside filtration, can not be stopped.

As direct information on short-term effects of intermittent water pollution events cannot be obtained with other methods, continuous biotest methods are, in the view of the LAWA ”Biomonitoring” committee, despite the restrictions cited, a necessary supplement to physical/chemical monitoring:

- They permit continuous, time-based recording of the effect of intermittent impacts.
- Both positive (alarm) and negative (all-clear) test results can be directly applied for the assessment of analytically detected intermittent impacts.
- The detection of a pollution incident in the continuous biotest is always an indication of potential damage to the aquatic ecosystem. It can therefore serve to initiate more detailed biological examinations in order to ascertain effects on the aquatic ecosystem, of which the test organisms are after all only individual trophic levels. For example, artificial substrates were deposited at various points in the water course which, in the event of an alarm, can immediately be checked for colonisation irrespective of the water level.
- In smaller rivers, for which no comprehensive chemical monitoring programmes can be carried out because of their low background concentrations, continuous biotests can be employed to detect intermittent pollution events, as ”triggers” for more detailed chemical analyses.

– Continuous monitoring also serves as a not-to-be-underestimated deterrent for illegal dischargers in all surface waters.

5.2 RESULTS AND RECOMMENDATIONS

To expand continuous biological water monitoring, and based on the results of the combined research project and the additional experience gathered in the individual federal states, the LAWA ”Biomonitoring” working group makes the following recommendations for the employment and operation of test systems for surface waters:

Continuous biotests are fundamentally suited to the detection of pollutant peak concentrations in surface waters. They are essential for the initial immediate estimation of the effects of pollution incidents.

Since biotests with organisms of differing trophic levels are not interchangeable, efforts should be made to install a battery of tests with organisms of different trophic levels at each suitable monitoring station.

The general requirements to be made of biological test systems include plausibility of the measured data and technical equipment reliability in routine operation. The experience gathered to date in the operation of individual continuous biotests does not permit the unqualified recommendation of specific devices or methods. Rather, there is a need for development and testing of methods at all trophic levels.

Based on the current knowledge the following recommendations for the employment of continuos biotests for the individual trophic levels can be made:

Destruents (bacteria):

None of the bacteria tests currently on the market is suitable for the monitoring of surface waters. Completion of the development of automatic luminous bacteria systems should therefore be awaited.

Producers (algae):

Here the results of the combined research project indicate the suitability of the fluorescence algae tests. Recommendations for a specific algae test device can not be made at present. The results of the ongoing practical trials of the various devices on the market must be awaited.

Primary consumers (daphnia, mussels):

The observation of the swimming behaviour of the daphnia as a test criterion has proved valuable. There are still substantial shortcomings in the measurement of swimming behaviour in the devices currently on the market. This results in occasional problems in the evaluation of the monitoring data and, thus, in automatic alarm detection.The technical reliability of the equipment also needs to be improved substantially by the manufacturer of the dynamic daphnia test [1].

The existing mussel tests (Dreissena monitor, Mossel-Monitor) have so far worked for the most part reliably at their various deployment locations. A problem may be the procurement of test organisms from the wild. The differences in design and technical specifications of the two tests must be re-assessed for every new potential location. No clear preference can be stated at this point.

Secondary consumers (fish):

The flow fish tests deployed in the past no longer meet today's requirements. On the other hand, the results obtained during development and deployment of the Koblenz behavioural fish test with BehavioQuant® are promising. The test needs to be assessed at a different location before a recommendation can be made.

5.3 MARGINAL CONDITIONS ON THE OPERATION OF CONTINUOUS BIOTESTS

Based on experience the following demands must be made for the operation of continuous biotests:

Experience has shown that adaptation of the test equipment to local conditions is essential. This should therefore be one of the elements of the installation and operation of the tests. Such adaptation includes addition of food for the test organisms in the event of food shortage, or the setting of the sensitivity of the alarm, which should be seen as the optimum balance between ”false alarms” and inadequate response.

The conditions in the monitoring station (climate, lighting etc.) must not impair the test organisms.

Continuous online transmission of the biotest data to the supervisory personnel, or to the alarm centre, must be ensured for the early-warning system.

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[1] State of knowledge in 1995. Based on more recent evaluations of the Dynamic Daphnia Test the doubts concerning this test's practicability have increased. At present, new methods (based on video analysis) are developed.

Alarm situations must be interpreted taking into account a number of environmental parameters which effect organisms but which are not necessarily harmful (e.g. temperature, pH value, electrical conductivity, turbidity).

In searching for the causes of alarm events, one should rely neither on the usual control samples (daily composite sample) nor on event-controlled sampling alone. It is advisable to operate a computer-controlled sampling facility (e.g. self-emptying control sampler) parallel to the continuous biotests, in which composite samples are taken over short periods (e.g. 1 hour) and are not rejected in the case of an alarm event.

Specially qualified personnel are essential, even with almost full automatic monitoring and data preparation, to ensure proper operation (care and maintenance) and to be able to expertly analyse the data delivered by the systems.

Whereas the equipment should be designed such that the routine maintenance can be carried out by technical personnel, such as technicians, more highly qualified personnel are required during installation and in assessing alarm situations. In case of alarm, this involves the interpretation of the biological effects, assessment of the level of potential damage to the ecosystem, and establishment of the interfaces to chemical monitoring. The installation and adaptation at a new monitoring location is likewise not possible without the expert appraisal and, where necessary, modification of all parameters influencing the test organisms and their responses.

5.4 OUTLOOK

The need for development and testing set out above shows clearly that this paper will need to be updated in future. When the outstanding problems have been solved, it will be possible to reach concrete recommendations for the use of continuous biotests.

There should be a regular exchange of technical information between the relatively small number of users, so that detail solutions are immediately made available to all users, for example.

Proposals could also be drawn up on how continuous biotests could be operated in mobile measuring stations on small surface waters.

With the possibility to monitor emission sources directly, a new, broad field of application opens up both for the continuous biotests examined in detail here and for the devices which have proven to be too insensitive for surface waters. The assessment of this important aspect for water protection will be the subject of a further paper of the LAWA ”Biomonitoring” Working Group.

APPENDIX A

DETAILS OF OPERATING COSTS AND MAINTENANCE REQUIREMENT OF THE RECOMMENDED CONTINUOUS BIOTESTS

(Date: April 1995)

Dynamic Daphnia Test

Manufacturer:

Elektron GmbH
Magdeburger Straße 19
D-47800 Krefeld

Acquisition cost:

47,250 DM (+ VAT)

Operating costs (excluding personnel):

Approx. 100 DM per month
(electricity, test chambers, sieve elements, pump hoses)

Space requirement:

100 x 60 x 65 cm (computer installed separately) or 140 x 60 x 65 cm (with integral computer), plus space for installation of suspended matter separators and tempering equipment.

Maintenance requirement:

Approx. 3 hours per week on site.
(Cleaning of the unit, the preliminary filters, and restocking of test chambers)
Approx. 5 hours per week in total (including cleaning of the equipment in the laboratory and evaluation).
Plus commitment for daphnia breeding.

Useful life:

7 days

Test organisms:

Daphnia magna STRAUS, available all year round; cloned culture also available from the Federal Environmental Agency (UBA-WaBoLu) in Berlin.
The prerequisite for operation of the dynamic daphnia test is daphnia breeding in the laboratory under standardised conditions. The daphnia breeding, and the operation and maintenance of the device, should be undertaken by specialist personnel.

Computer equipment:

A serial interface can transmit the data to a central system in ASCII format. A computer with software for evaluation is available together with the test system, but evaluation with different software on central systems ist also possible.

The operation and maintenance of the device should be undertaken by specialist personnel.

DF Algae Test

Manufacturer:

Dr. V. Gerhardt, J. Putzger
University of Regensburg
Universitätsstraße 31
D-93040 Regensburg

Acquisition cost:

50,000 DM

Operating costs (excluding personnel):

On average 100 DM per month
(electricity: 40 DM, nutrient medium: 10 DM, pump hoses: 40 DM, cleaning agent: 10 DM)

Space requirement:

The unit, including algae culture and PC, is < 1 m^2 in basic area and 1 m high.

Maintenance requirement:

5 hours per week including time commitment for algae culture.

Useful life:

7 days

Test organisms:

The DF algae test includes an algae fermenter (50 cm x 50 cm x 50 cm) with stocks of nutrient solution for one week. The breeding of the cultures takes approximately 1 hour per week. Parent cultures of the Göttingen algae collection are used. Year-round availability.

Computer equipment:

A serial interface can transmit the data to a central system in ASCII format. The unit is fitted with an alarm value identifier.

Luminous Bacteria Tests

Within the framework of the "WIR"-project luminous bacteria tests were developed by:

(1)
Dr. V. Gerhardt, J. Putzger
University of Regensburg
Universitätsstraße 31
D-93040 Regensburg
Cost: 45,000 DM
Space requirement: 60x50x50 cm (HxWxD)

(2)
Kolibri GmbH
Lohbachstraße 12
D-58239 Schwerte
Cost: 80,000 DM
Space requirement: 2 units, each 50x50x50 cm

Dreissena Monitor

Manufacturer:

Borcherding & Volpers GbR
Envicontrol
Kapellenstraße 53
D-50226 Frechen

Cost:

35,000 DM

Operating costs (excluding personal):

Approx. 20 DM per month

Space requirement:

70x120x60 cm (HxWxD)

Maintenance requirement:

Approx. 2-5 hours per week on site.
Approx. 1 hour per week in total for evaluation.

Useful life:

7 days

Test organisms:

The Zebra mussel *Dreissena polymorpha* is collected in natural habitat.

Computer equipment:

A PC with software for evaluation is part of the equipment. The software calculates a three-step alarm and allows to control the processes. A serial interface can transmit the data to a central system. It is possible to trigger a water sampler by an alarm.

Mossel-Monitor

Manufacturer:

Delta Consult B
P. O. Box 71
NL-4420 AC Kapelle
The Netherlands

Acquisition cost:

MOSSELMONITOR® 24,500 Dfl (+ VAT).

Operating costs (excluding personal):

Approx. 55 DM per month (electricity, dental glue).

Space requirement:

ca. 50x50x50 cm (HxWxD)

Maintenance requirement:

Approx. 10-15 minutes per week on site.
Approx. 1 hour per week in total for evaluation.

Useful life:

7 days

Test organisms:

The Zebra mussel *Dreissena polymorpha* is collected in natural habitat.

Computer equipment:

Only optional for external data acquisition and presentation.

The operation and maintenance of the device should be undertaken by specialist personnel.

Koblenz Behavioural Fish Test

Manufacturer:

metacom gesellschaft für datensysteme mbh
Baierbrunnerstr. 22
D-81379 München

Acquisition cost:

82,535 DM price from Munich (+ VAT).

Operating costs (excluding personal):

Approx. 70 DM per month (electricity).

Space requirement:

180x150x80 cm (HxWxD) (computer and control monitors installed seperately)
Plus space for installation of tanks for fish keeping.

Maintenance requirement:

Approx. 1- 1.5 hour per week on site.
Approx. 1.5 hours per week in total for evaluation.
Plus commitment for fish keeping.

Useful life:

7 days

Test organisms:

Golden ide *Leuciscus idus melanotus*, avaible at special breeders all year round.

The operation and maintenance of the device should be undertaken by specialist personnel.

APPENDIX B

BRIEF DESCRIPTION OF THE RECOMMENDED CONTINUOUS BIOTESTS *)

(*) Except of 5. ”DF Algae test“ and 8. “bbe-Algae Toximeter“ the descriptions were literally taken from ”UBA Texts 34/94: Continuous biotest methods for the monitoring of the Rhine. - Summary, recommendations and presentations of test methods”.

1. Dynamic daphnia test

Daphnia magna STRAUS is a crustacean and, as a low-order consumer, takes up an ecologically important position in the food chain between the destruents (e.g. bacteria) and primary producers (e.g. algae) and the higher-order consumers (e.g. fish). In the static daphnia test (to DIN 38412 part 11 and 30), the swimming capability of the daphnia after 24 h is determined for the assessment of substances and waste waters.

Fig. 1: Schematic diagram of the dynamic daphnia test

The dynamic daphnia test was developed at the State Office for Water and Waste of North Rhine-Westphalia, and has been employed as an automatic biomonitor for continuous water monitoring since 1982 in North Rhine-Westphalia and other federal states in Germany. In the dynamic daphnia test according to KNIE, the change in swimming activity is the test criterion: in the flow through system the swimming activity of daphnia is registered with the aid of light barriers. As soon as changes in the quality of the test water occur, the animals react by changing their swimming behaviour. They swim more slowly or react with increased activity. The test system of the Elektron company essentially comprises 2 test chambers with a self-priming pump system. The changes in behaviour for a specific monitoring cycle are measured with optical sensors, and electronically evaluated as pulses. The parameter ”pulses per 10-minute cycle” of the two measuring chambers is automatically transmitted to a computer. When a defined limit value is exceeded an alarm is triggered.

2. Koblenz Behavioural Fish Test

The Koblenz behavioural fish test analyses the changes in the behaviuor pattern of the fish in response to water pollutants using a computer controlled image processing system BehavioQuant®.

Fig. 2 Schematic diagram of theKoblenz behavioural fish test

In two tanks fed continuously with river water, golden ides are surveilled round the clock for two minutes at 15-minutes-intervals by two video cameras. The video cameras observe the fish with the help of two mirrors which are set at 45° angle in front of the test tanks. After digitalization of the video signals with suitable software, the movement traces of all fish are computed. These traces provide the basis for quantifying six behavioural parameters. The parameters are swimming height, mean horizontal position, number of turnings, motility (way covered per unit of time), irregularity of swimming velocity and shoaling behaviour. An larm signal is attained by statistical comparison of the current monitoring data with those of the previous 2-hour monitoring period. These measurements form the basis for calculating separate dynamic limit values for the six behavioural parameters.

3. Mosselmonitor

The Mosselmonitor® registers the distance between the two shell halves of the zebra mussel *Dreissena polymorpha* by an electromagnetic induction system. The electronic sensor consists of two small coils, clued to opposite shell halves of the mussel. All data are evaluated by a micro-computer. Four parameters are monitored for each of the eight mussels: ‘shell closed’, ‘increasing activity’, ‘decreasing shell opening’ and ‘death of the mussel’. If predetermined alarm limits are exceeded, an alarm is generated.

Fig. 3: Schematic diagram of the Mossel-Monitor

4. Dreissena Monitor

The Dreissena monitor is based on the shell movements of the zebra mussel *Dreissena polymorpha*. The basis of the test is a digital yes/no signal which is generated by the influence of a magnet attached to the mussel's shell on a reed switch. The test water flows through two river water troughs, in each of which there are up to 42 mussels. Over a time interval of 5 minutes the percentage of open mussels and the frequency of shell movements is calculated by computer, and evaluated online for possible pollutant effects. If the percentage of open mussels decreases, or the frequency of shell movements increases suddenly significantly, this indicates pollution.

Fig. 4: Construction of the Dreissena monitor

ALGAE TESTS

Substances toxic to algae (e.g. herbicides) very often intervene in the photosynthetic processes, and lead to decreases in photosynthetic activity. Toxic substances in the water can be detected from measurements of oxygen production and fluorescence. In the event of impairments of the photosynthesis, the spontaneous, variable and delayed fluorescence are altered. Photosynthetically unused energy is emitted, among others, as spontaneous in-vivo Chlorophyll a fluorescence (685 nm). Vital and intact algae give off only small levels of fluorescence, whereas algae damaged by algaecid substances (e.g. herbicides) the fluorescence increases. The property of plants of giving off light after exposure in the dark is termed delayed fluorescence (DF). The decay kinetics of the delayed fluorescence are altered by photosynthesis inhibitors, and can be applied as an index for substances toxic to algae.

5. DF Algae Test

The DF algae test (DF= Delayed Fluorescence) for the measurement of delayed fluorescence consists of a two-channel DF fluorometer, which enables a direct comparison of the decay curves of the reference sample with the polluted sample. Synthetic test water or tap water can be used as the reference sample. The weak dark red light emission of the delayed fluorescence is measured by photomultipliers.

The decay curve of the delayed fluorescence is measured at regular intervals for a sample and a reference. The analysis of the decay curves, that is, the difference in form and surface integral between the sample and reference curves, is a measure of the damage caused by toxic substances present. Test organisms are bred in a bioreactor according to the chemostatic principle. Various green and blue/green algae can be used as test organisms. The integral control processor in the unit contains a two-channel photon counter, and controls the scheduling of measurement. It is connected to a PC. This calculates the surface differential of the decay curves, displays the measured values, prints them out, and passes them, and any alarm signals, to a central processor.

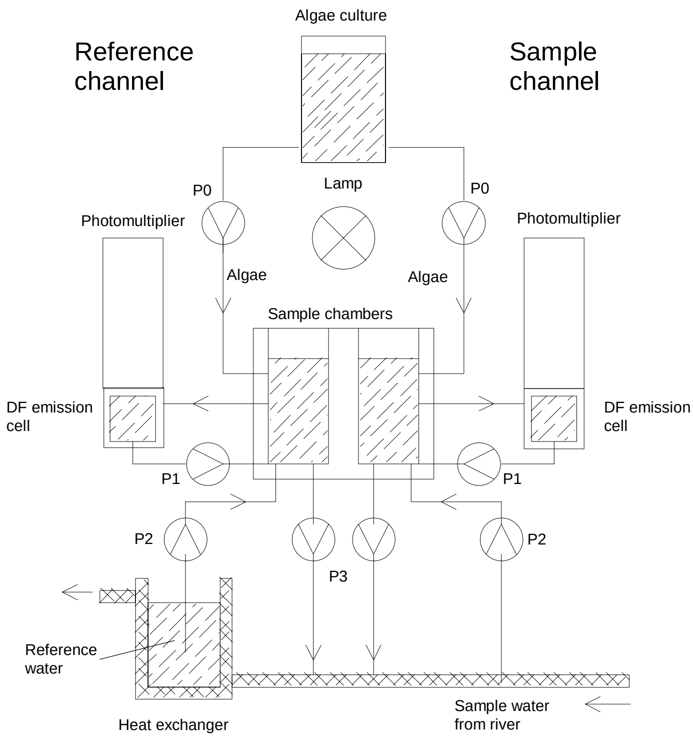

Fig. 5: Schematic diagram of the DF algae test. The system consists of an automated algae culture and a two channel DF flourometer. The DF of algae added to pure water in the reference channel is compared to algae added to the sample.

6. FluOx Algae Test

The FluOx® algae test was developed for the combined monitoring of the fluorescence signals and oxygen concentrations allowing continuous measurements of the photosynthetic activity of an algae suspension in water. A pulse-amplitude modulation fluorometer is used to generate different fluorencence signals. In addition, the algal oxygen production is measured. After excitation with modulated light, the baseline fluorescence, the maximum fluorescent yield, and the curves of the rapid increase and the slow decrease of fluorescence are measured consecutively. The oxygen production rate is recorded continuously. Finally, several successive light flashes are released in order to determine the electron flux in the elctron transport chain, the membrane voltage, and the quantal yield at photosystem II. An important vitality criterion is the amount of the maximum variable fluorescence measured as the ratio between maximum fluorescence and fluorescence in a steady state. All in all, eleven parameters are calculated which allow conclusions to be drawn regarding the nature and degree of photosynthesis impairment.

Fig. 6: Schematic diagram of the FluOx algae test.

7. Biosens Algae Toximeter

The Biosens algaae toximeter registers the instantaneous fluorencence, excited by impulse light. The test device consists of a fluorescence unit (measurement unit), an algae culture system and a computer. The intensity of fluorescence, excited by series of eight single flashes, is determined during the stationary phase as the mean value of the flashes F_4 to F_8. Each measuring cycle consists of three measurements determining the intensity of fluorescence of the water sample, of the culture (algae) and of the test cuvette. The toxicity parameter, called the ET value (**E**ffective **T**oxicity), is calculated. The measuring cycles and data recording are computer-controlled. The test algae are cultivated in a turbidostat, so that they can be used in the test always in the same physiological condition.

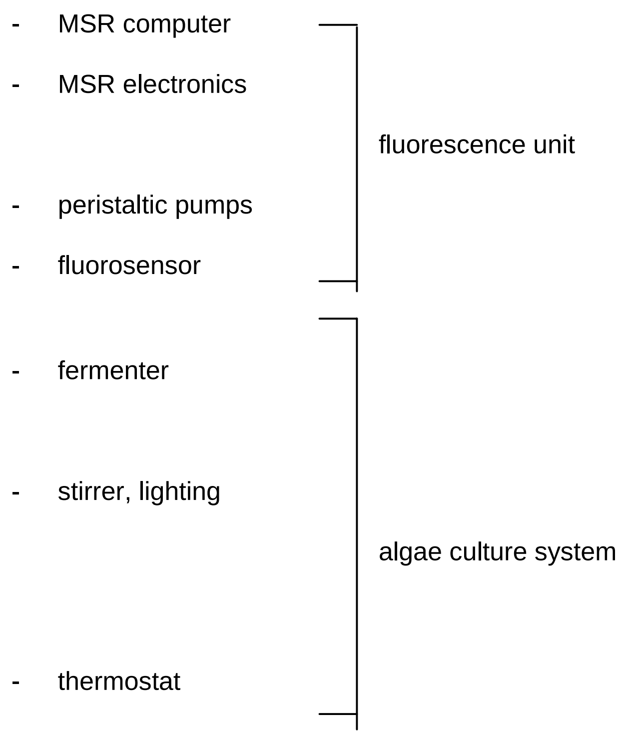

Fig. 7: Schematic diagram of the Biosens algae toximeter.

8. bbe Algae Toximeter

The bbe Algae Toximeter (1-Hz fluorescence toximeter) continually tests for toxic substances in water. The algae for the tests are cultivated automatically and independently from the test water. These standardized algae are then added to the water sample and the active chlorophyll a concentration is analysed with the 1-Hz fluorometer. If the algae are damaged, e.g. through herbicides which reduce the concentration of active chlorophyll, an alrm is induced. To enable a higher sensitivity and a better measuring precision automatically a detailed analysis of fluorescence is carried out. The 1-Hz fluorescence toximeter can also operate as an instrument for the exact measurement of algae concentration in water. This measurement corresponds to that of the bbe 1-Hz fluorometer A42A03. Different coloured LEDs assure that the algae classes are assessed correctly in their chlorophyll content. Advantages of the bbe Algae Toximeter are high sensitivity, independent cultivation of algae, control of the cultivation through the living chlorophyll measurement, no dosage mistake because of aging hoses, automatic cleansing of the measuring unit and the hoses, reutilization of the cleansing solution and no loss of time through the alarm induction. All operations are controlled by an internal computer.

Fig. 8: Schematic diagram of the bbe Algae Toximeter.

BACTERIA TESTS

Bacteria play a major role as test organisms, especially in the monitoring of sewage treatment plants and waste waters. A large number of bacteria tests are based on the measurement of the breathing intensity. In the luminous bacteria test the measurement criterion is the intensity of luminescence. In the static luminous bacteria test, standardised to DIN 38412, part 34, the reduction in luminescence of *Photobacterium phosphoreum* is measured after 30 minutes relative to a control sample.

9. Regensburg Luminous Bacteria Test

The measuring technique used for registering delayed fluorescence is also suitable for recording the luminescence signal of luminous bacteria. A luminous bacteria test was developed by the University of Regensburg as a modification of the DF algae test. In a second development phase this test is going to be adapted to ISO 11348 (DIN 38412, part 34 in Germany).

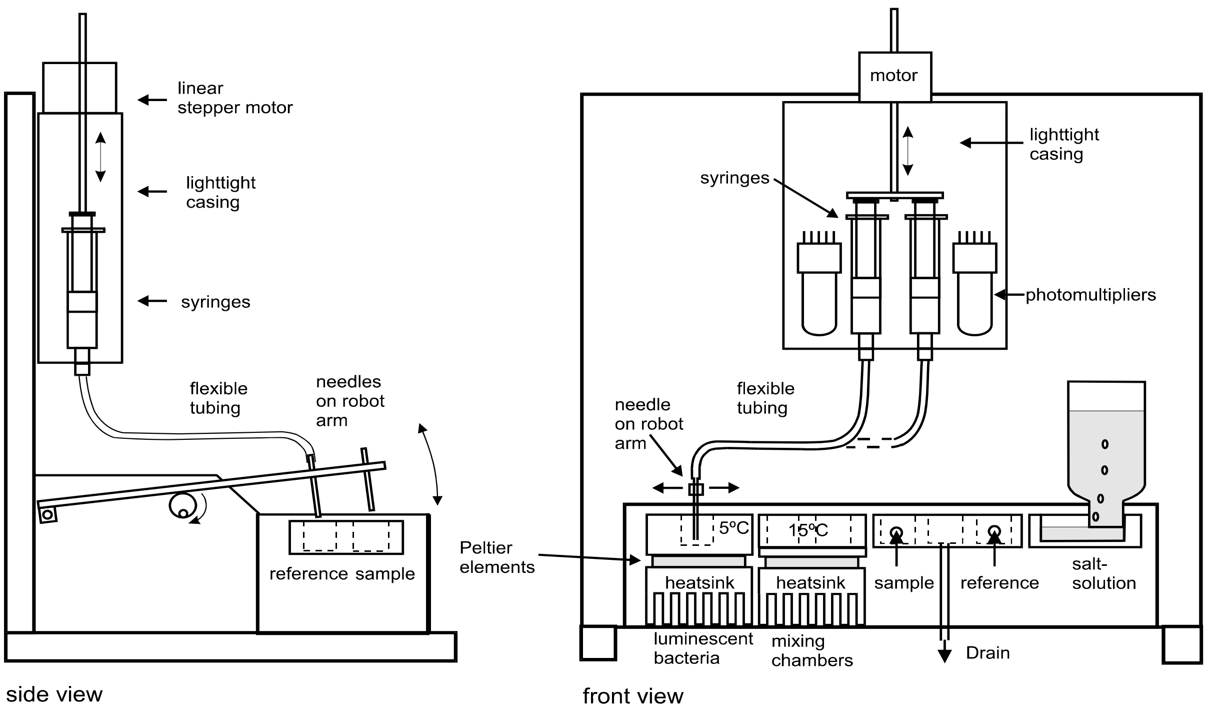

Fig. 9: Schematic diagram of the Regensburg luminous bacteria test. The side view shows how the robot arm is lowered into the sample chambers by the rotation of an excentric drum. The fluid is sucked into the syringes, and the robot arm is lifted again. The front view shows how fluids are transferred from one chamber to the other by lateral movement of the robot arm. The syringes eject the fluid then into another vessel.

The test criterion in this test is the inhibition of the luminescence of *Photobacterium phosphoreum*. Like the DF algae test, the luminous bacteria test is equipped with two measuring channels for reference and sample. Samples are taken and processed by a robot arm. Accurate dosage of the fluids is achieved by the use of glass syringes driven by a stepper motor under microprocessor control. The luminescence is detected by photomultipliers beside the syringes. The luminescence of the bacteria is measured before and after mixing with the samples once per minute during the entire incubation time. The inhibition (in %) is calculated from the luminescence intensities before mixing with the samples and at the end of the incubation time. A supply of bacteria and salt solution for one week of continuous operation is stored within the machine.

10. BioLum Luminous Bacteria Test

The automated luminous bacteria test Lumino 2000 (distributed as BioLum® by the Kolibri environmental analysis and online monitoring company, Schwerte) was developed at the State Office for Water and Waste of North Rhine-Westphalia. The test parameter is the inhibition of the bacterial luminescence of living cultures of *Photobacterium phosphoreum*.

Test water, control water and NaCl solution (3% in the test base) are pumped into a mixing vessel. The cooled luminous bacteria suspension is then added to the test sample and mixed with it at a proportion of 1:20. The luminescence is measured with a photomultiplier. After the 2-minute initial measurement period the sample is transported via a waiting position to the position at which the final measurement is made. The measured values of the initial and final luminescence are recorded by computer and the percentage inhibition of the luminous bacteria in the test sample is calculated. Finally, the sampling glass cuvette is drained and rinsed using a tipper device. All steps in the process (rinsing cycles, charging steps and measuring cycles) are automatically controlled.

Fig. 10: Schematic diagram of the BioLum luminous bacteria test

Map next page

Water quality measuring stations with continuous biotest methods
Date: September 1995

Bayern = Bavaria
Belgien = Belgium
Bodensee = Lake Constance
Dänemark = Denmark
Donau = Danube
Frankreich = France
Hessen = Hesse
Luxemburg = Luxembourg
Mecklenburg-Vorpommern = Mecklenburg-Western Pomerania
Mühlendammschleuse = Mühlendamm lock
Niederlande = The Netherlands
Niedersachsen = Lower Saxony
Nordrhein-Westfalen = North Rhine-Westphalia
Nordsee = North Sea
Österreich = Austria
Ostsee = Baltic Sea
Polen = Poland
Rhein = Rhine
Rheinland-Pfalz = Rhineland-Palatinate
Sachen-Anhalt = Saxony-Anhalt
Sachsen = Saxony
Schweiz = Switzerland
Thüringen = Thuringia
Tschechische Rep. = Czech Rep.

((Box))
Fish test
Daphnia test
Mussel test
Algae test
Bacteria test

Recommendation on the Deployment of Continuous Biomonitors for the Monitoring of Surface Waters

The continuously-operation biological tests (”continuous biomonitors“) installed in surface water monitoring stations have been found to be suitable for the detection of peak concentrations of pollutants. They constitute a necessary extension to the physical-chemical monitoring as direct information on the short term effects of pollutants can only be obtained with biological tests. Based on questions posed to the Federal States the LAWA ”Biomonitoring” committee has summarised in a report the experience gained with the different test methods applied, and their limitations and possible applications. Tables and a map provide an overview of the location and the operators of continuous biomonitors in the Federal Republic of Germany. The committee recommends, for the extension of biological monitoring of surface waters in suitable monitoring stations, the application of a battery of different biomonitors. Test methods, currently still with some reservations, are cited for each trophic level (bacteria, algae, primary predators and fish). For a number of biomonitors development and testing are still required. An appendix provides, for suitable tests, information on the test method and the capital and operating costs as well as the time required for weekly maintenance of the equipment installed in the monitoring stations. Based on the experience of the users the limitations for the operation of the different biomonitors installed in the monitoring stations are summarised. A brief description of the different biomonitors is provided in another appendix of this report.