DELIVERABLE

Project title

TESTNET

Towards European Sectorial Testing Networks for Environmental Sound Technologies

D 3.1a Evaluation Report Test case 1a: TOXcontrol BioMonitor for Surface water

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PP	Restricted to other programme participants (including the Commission Services)	
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CO	Confidential, only for members of the consortium (including the Commission Services)	

Planning

Deadline	Date	Comments
Due date of delivering final version	05-2007	
Planned date of delivering final version	10-2007	*Postponed further due to missing field test*

Version Management

Ver.	Date	Editor	Comments
0.1	20-09-2007	Anders Lynggaard-Jensen	Close to final – only missing results from field tests and revised offer from testing lab to producer – all Annexes produced as draft finals.
final	01-03-2008	Anders Lynggaard-Jensen	Field test included and Annexes edited

Accompanying documents of the deliverable:

Nr.	Title	Editor	Remarks

Executive summary of the deliverable

Test Case 1a: TOXcontrol BioMonitor for Surface Water.

A suggested scheme for Environmental Technology Verification including a Verification Institute has been tested for water monitoring using technology where a test protocol was not readily available and the test work was not considered as straightforward – hence the use of a Verification Institute (VI).

The Test Protocol builds upon two existing standards:

- A "generic" standard, ISO 15839:2003 "Water Quality – on-line sensors/analysing equipment for water - specifications and performance tests"
- A more specific standard ISO 11348-1:2004 "Water Quality – Determination of the inhibitory effect of water samples on the light emission of Vibrio Fischeri (Luminescent bacteria test) – Part 1: Method using freshly prepared bacteria"

The deliverable contains comments to all the steps to be performed according to the suggested verification scheme including time schedule and involved costs as well as recommendations for changes and improvements of the verification scheme. All documents produced are commented and included in Annexes. Finally the stakeholders for this test case are listed. The annex include:

A: Quick Scan report giving an overview of the technology (Done by the VI)
B: Offer from Verification Institute to produce protocol and from Test lab. to perform test
C: Verification Protocol (Done by the VI)
D: Test Plan (Done by the Test lab.)
E: Test Report (Done by the Test lab.)
F: Verification Report (Done by the VI)
G: Minutes from meetings in the Board of Experts (BoE) and the Task Group (Done by VI/Test lab.)
H: The originally proposed verification scheme to be tested

Summary of findings

Case no/technique	1a/ Surface water - Biomonitoring
Type of scheme	With Verification Institute (VI)

The case study 1a has been devoted to verification within the area of Water Monitoring. The profile of the test is:

- Equipment: microLAN TOXcontrol, Biomonitor
- Verification scheme: TESTNET scheme with Verification Institute
- Verification protocol based on standards: EN ISO 15839 and EN ISO 11348
- Application: Surface water at water intake,
- Measurement: Luminiscence (inhibition %) in lab. and field

Findings of the case study can be summarised as follows, more details are mentioned in the evaluation report:

- Having used both verification schemes (see also Deliverable 3.1b), it is recommended to use a two path verification scheme with a common start and conclusion administered by a Thematic Verification Organisation, who delegates the actual work to be done during the verification to be headed either by a Verification Institute (if no protocols are available) or by a Verification Laboratory (a “fast track”, if protocols are available).
- The starting definition of technology within the scope of verification was that it should either be ready to market or an advanced prototype – we think it should be at least ready to market and that existing technology on the market also should be allowed to be verified.
- The Quick Scan becomes very important for checking if technology is ready to market and for checking for available protocols.
- If Standards or Guidelines are available it is recommended to use them as starting point for verification protocols.
- A draft protocol can be prepared within a short time by the VI. Therefore, it will be better to ask the VI to write a draft of the protocol, before sending material to the BoE
- It is expensive and time consuming to include a too large group as “Board of Experts” – on the other hand it could be a forum for stakeholder involvement
- It is efficient to have a task group consisting of the VI, Test Lab and producer (possibly also a member of the BoE) – this will inolve the Test lab earlier in the process
- Testing biomonitors is more time consuming than foreseen in the planning guidelines of EN ISO 15839, however it is considered possible to verify this type of monitors.
- The calendar time needed for verification of biomonitors will be between 9 and 12 months – protocol work taking half of the time.
- The estimated effort spent for verification of biomonitors is between 70 and 170 days – the actual test work only covering one third of this
- Close contact to the producer during the tests is deemed necessary – possibly through the VI, who also should be informed frequently of results obtained during the testing
- A simplified verification scheme – compared to the scheme to be tested – is suggested based on the experience gained

Table of Contents – Test Case 1a: TOXcontrol BioMonitor for Surface water

Basic data

Case no/technique(s)	1a/ Surface water – biomonitoring
Date for report	15-12-2007
Type of scheme (with/without VI)	With VI
Partner(s): (lead+other), role (VI, Test lab)	DHI (lead+Test lab/lab tests), KIWA (subcontracted lab/field test), EXERA (VI)
Author of this report	Anders Lynggaard-Jensen, DHI Dominique di Benedetto, EXERA
Status (which step in scheme)	Finished

The steps performed

The following list summarises the different steps to be tested in the suggested verification scheme with a Verification Institute (included in Annex H) and below is given the comments to each of the steps as they have been carried out, including suggestions for improving the verification scheme.

Step	Text in Box in Scheme	Status
1	Producer selects VI	Done
2	VI performs Quickscan	Done
3	VI makes cost estimate for protocol	Done
4	BoE Available	Done
5	TVO installs a BoE	Done
6	BoE examines docs & appoints Task group	Done
7	BoE evaluates protocol	Done
8	Task group makes protocol fit for use	Done
9	VI decide on the tests to be done	Done
10	VI & Producer select Test lab.	Done
11	Test lab. develops test plan and makes an offer.	Done
12	Test lab. tests performance	Done
13	VI evaluates test results	Done
14	VI makes Verification report	Done
15	VI sends Verification report, test report & advice to TVO	Done
16	TVO evaluates verification procedure	Done
17	TVO awards Statement & allows use of Verification Logo	N/A

1: Producer selects VI.
In this case a branch organisation (TESTNET) selected the technology, contacted the producer (microLAN) and appointed the VI (EXERA). However, the suggestion is that a producer, an end user organisation or a branch organisation contacts the TVO, who then – if a suitable protocol does not exist - will appoint a qualified VI from a list. If a suitable protocol is available and can be used without too many changes, the scheme without a VI should be used. This is based on the fact that the TVO in time will have information of all previously developed protocols and tests performed, and therefore can suggest coordination/timing of tests to be done. It is seen as unlikely that one single producer can pay all costs for protocol and test work – especially if the producer is an SME in the phase of bringing a new product to market.

2: VI performs Quickscan.
Done and sent to the TVO. Have requested/received documents from the producer. VI approved the technology as fit for test. An important comment: It says in the flow sheet comments, that technology within the scope is either ready to market or an advanced prototype – we think it should be at least ready to market and that existing technology on the market also should be allowed to be tested. We have to remember that this ends up with a logo, and it will be unfair competition to existing products, if they are not allowed to go for this. Further, if this test scheme will get any support from the Commission – it will be against the rules to give support to some suppliers and not to others. The obvious (and realistic – at least for monitoring equipment) example is that an end user organization wants to test available Nitrate sensors (and possibly pay for some, if not all the costs) – should only ready to market sensors or prototypes then be included ? The Quick Scan Report is attached as Annex A.

3: VI makes cost estimate for protocol.
Not done, but again the comment is that it will be too expensive for one producer, and far too expensive if no protocol nor standard which can be adapted exists at all. However in this case we do have two existing standards – one of them even giving a protocol – so it would have been possible to give a fairly good estimate – we shall include the actual costs instead.

4: BoE available.
The VI has to consider if a BoE is available - otherwise the VI asks the TVO to form the BoE. In fact it should always be the TVO that installs the BoE (see comment 5), however, here we agreed that the answer from the VI should be yes, and that the BoE should consist of: VI (Staff from EXERA), EXERA stakeholders (EXERA is a French/Italian stakeholder organization, which as one of its tasks is testing monitoring equipment), DHI (as developer and end user), ISO TC147 WG2 (who has produced the standard/protocol: EN ISO 15839: Water Quality – Online Sensors/Analysing Equipment for Water – Specifications and Performance Tests and here represented by the convenor), EUCETSA (as end user organization) and the German/Dutch Expert Group on BioMonitoring (Corina de Hoogh, KIWA as contact). See also the section “Stakeholder list”.

5: TVO installs a BoE:
Should always be the TVO, which is aware of possible participants for a BoE and therefore appoint this. The VI is born member and can of course give advice to the TVO. Other

members should be end users and experts, who has been involved in the work with standards/protocols/testing within the actual technology area. It has been discussed if the producer(s) should be member(s), and no agreement has been reached. In this case the producer(s) is not a member of the BoE, but everybody agrees that the producer(s) shall be member of the Task Group. Finally the issue concerning payment of the work done by the BoE has been discussed – who shall pay and who shall get payment - without any result.

6: BoE examines docs & appoints Task group.
These documents consist of the QuickScan report incl. docs from the producer and possible existing protocols/standards – which in fact has been collected by the VI. Therefore it is proposed to have a box in the flow scheme saying that the VI prepare the documents to be sent to the BoE members, who then can assess the documents before a BoE meeting – the BoE meeting being another box.

If possible the VI suggests a draft protocol to be considered at the BoE meeting – especially if some standards/protocols exist, which can be adapted. In this case the VI made a suggestion based on the two standards:

- EN ISO 11348-1: Water Quality Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) – Part 1: Method using freshly prepared bacteria.
- EN ISO 15839: Water Quality – Online Sensors/Analysing Equipment for Water – Specifications and Performance Tests

At the meeting the BoE can discuss the documents/draft protocol or look for an existing (might need some adaptations) protocol/give recommendations for a new protocol and appoint a Task Group (which should be a smaller group than the BoE in order to be operational) - if it is clear that no protocol exist, the task group will produce this according to BoE recommendations.
The BoE meeting was held at the VI (EXERA) in Paris on January 15th 2007. The minutes from the meeting and the participant list are included as Annex G. Members of the BoE being almost the same as the involved stakeholders. It was also decided at the meeting that the members of the Task Group should be: VI (chairman), Producer, Test lab, and 1 or 2 of the other BoE members with special knowledge/interest of/in the system to be tested. In this case this mean: VI (Dominique Di Benedetto), Producer (Joep Appels), Test lab (Anders Lynggaard-Jensen), BoE-members (Corina de Hoogh and Paul Ockier).
As the Test lab is suggested as a member of the Task Group, it is suggested to move the procedure for involving a Test lab to this point in the flow scheme (see also item 10).

7: BoE evaluates protocol.
Either the existing draft protocol or the new draft protocol shall be evaluated by the BoE (if existing, it can be done on the mentioned BoE meeting). This also means that it is suggested to move the arrow from the loop, where the Task Group develops a new protocol, to a position just before the box “BoE evaluates protocol”.
Depending on the evaluation the draft protocol is passed on to the Task Group either for further work or for making the draft protocol fit for use. In this case the BoE decided to ask

the Task Group to make the draft protocol provided by the VI before the BoE meeting fit for use – and of course include comments from the BoE meeting.

8: Task group makes protocol fit for use.
This is suggested to be done at a meeting chaired by the VI, but held at the Test lab., because the equipment to be tested should be set up before the meeting by the producer(s) and the Test lab. As this step is considered to be a practical exercise – it is believed that a better job can be done, if the Task Group members actually can see the equipment in operation and ask questions to the producer(s), who also can give a much better presentation of the equipment. Further, test bench facilities, measurement procedures, etc, can be adapted during the meeting – as long as the draft protocol is not changed. The producer shall after the meeting carry out the necessary training of the staff of the Testing lab.
In this case the Task Group meeting was held at the Test lab. (DHI) in Aarhus, Denmark on February 7th 2007. The minutes from the meeting and the participant list are included as Annex G. As the meeting was held at the Test lab, the staff, who is going to carry out the tests, and who had installed the equipment together with the producer the day before the meeting, also attended the meeting. The day after the meeting the producer used half a day to train the staff at DHI.
An important result from the meeting is that the field tests (test divided into lab. and field test according to EN ISO 15839) will be carried out at a location in the Netherlands – a surface water intake - in order to get a more realistic test site than available in Denmark. The task Group member representing the Dutch/German expert group on Biomonitoring was subcontracted to be heading the field tests (se also item 10). A closer stakeholder involvement cannot be obtained.

9: VI decides on the tests to be done.
This is suggested to be changed to: VI produces final protocol. The final protocol is a result of the Task Group decisions, and should now be fit for use. The VI sends it to the TVO and the Task Group, which is exactly what has been done in this case. The Verification Protocol is attached as Annex C. Further comments in the section “Recommendations for changes and improvements: Documents produced in the Scheme”

10: VI & Producer select Test lab.
This step is suggested to be moved in front of the appointment of the Task Group, as the Test lab. is an important partner in the task group work (the term Testing labs is suggested to be changed to Test lab., who will be in charge of the tests – the Test lab might however subcontract other labs if necessary). The procedure to select a Test lab could be as simple as choosing from a list of 3 labs provided by the TVO, who will have the knowledge of which labs. who have the expertise within the area in question. It could also be a tendering procedure based on the material, which the VI has sent to the BoE. The qualified labs from the list at the TVO can then give an offer of the costs incurred until the start of the actual test.
This step has to be further assessed, but in this case it can be said that the VI and the Producer selected DHI as the Test lab.

11: Test lab develops test plan and makes an offer.
Based on the final protocol received from the VI, the Test lab should develop the test plan including the costs to carry out the test. This should not be too difficult as a draft plan should

already have been agreed at the Task Group meeting. However, according to the scheme the VI has to finally approve the plan before it can be a part of the verification protocol and the Producer has to approve the costs before the tests are started. As the Task Group (including the producer and the VI) has discussed all this before, it is suggested that these approvals are replaced by a box saying "Task Group agrees", which was done in this case. However, The VI stated that too little time was allocated for the lab tests.
In this case the actual costs are not calculated, but the test plan is made after the guidelines in the EN ISO 15839, which requires work described for every active day of the test – so costs at least presented as person days involved is available in the plan. These costs have to be added to the costs for participating in the setup of the equipment, the Task Group meeting and the training. The Test Plan is attached as Annex D and the Offer from the testing lab., which afterwards has been made based on the gained experience, is attached as Annex B.

12: Test lab tests performance.
Laboratory tests were started following the protocol and recorded according to the Test plan. However, problems of different kind showed up causing the tests to take 4 times as long as planned ! As stated in the beginning by the VI too little time was allocated for the lab tests – only 1 test period originally, which became 4 test periods. Problems are described in the final test report together with the results obtained (Annex E1). Some of the problems originated from the instrument and some from the protocol. It will be adviseable that the Test lab has an active link to the producer and the VI – possibly going through the VI to coordinate problem solution (possibility to call for a Task Group meeting). In this case there was a link between the producer and the Test lab, which solved some practical problems – (kept the instrument running), but actual test/protocol issues should have been solved together with the VI during the test – which in the end might have saved some time.
Despite the problems encountered the instrument was delivered to the subcontracted Test lab responsible for the field test, immediately after the 4th test period in the lab. Problems were encountered to get the field test started (resource problems), but the field test was eventually finished, and the obtainable results (Annex E2) sent to the VI.

13: VI evaluates test results.
The raw results from the lab test together with a summary of the setup in the laboratory were sent to the VI just after the laboratory tests were concluded. However, as the VI stated this was not enough for him to evaluate the results completely so he could go on to the next step, therefore the Test lab. made the final Test report without the field test results included (field test was running late), and the VI accepted the Test report (for the lab. tests), and could evaluate results from this part of the test for use in the Verification report. Evaluation of field test results was done later based on the Test report from the subcontracted lab doing the field test.

14: VI makes Verification report.
The Verification Report was made from the Test Report from the lab test and updated when the Test Report from the field test became available. The Verification report is included in Annex F: Verification Report, and further comments are included in the section "Recommendations for changes and improvements: Documents produced in the Scheme".

15: VI sends Verification report, test report & advice to TVO.
The Verification Report and the Test Report (from the lab test) was sent to the TVO, together with the following advice: “The laboratory tests revealed some problems preventing a continuous measurement that can be expected for an on-line automatic system. As this instrument is a new marketed one, we think that these problems might be solved by the manufacturer with additional tests”. After the VI had evaluated the field test this advice was changed to: “The laboratory and field tests revealed some problems preventing a continuous measurement that can be expected for an on-line automatic system. As this instrument is a new marketed one, we think that these problems might be solved by the manufacturer with additional tests, improvements in plumbing design and also in stability of bacteria suspensions”.

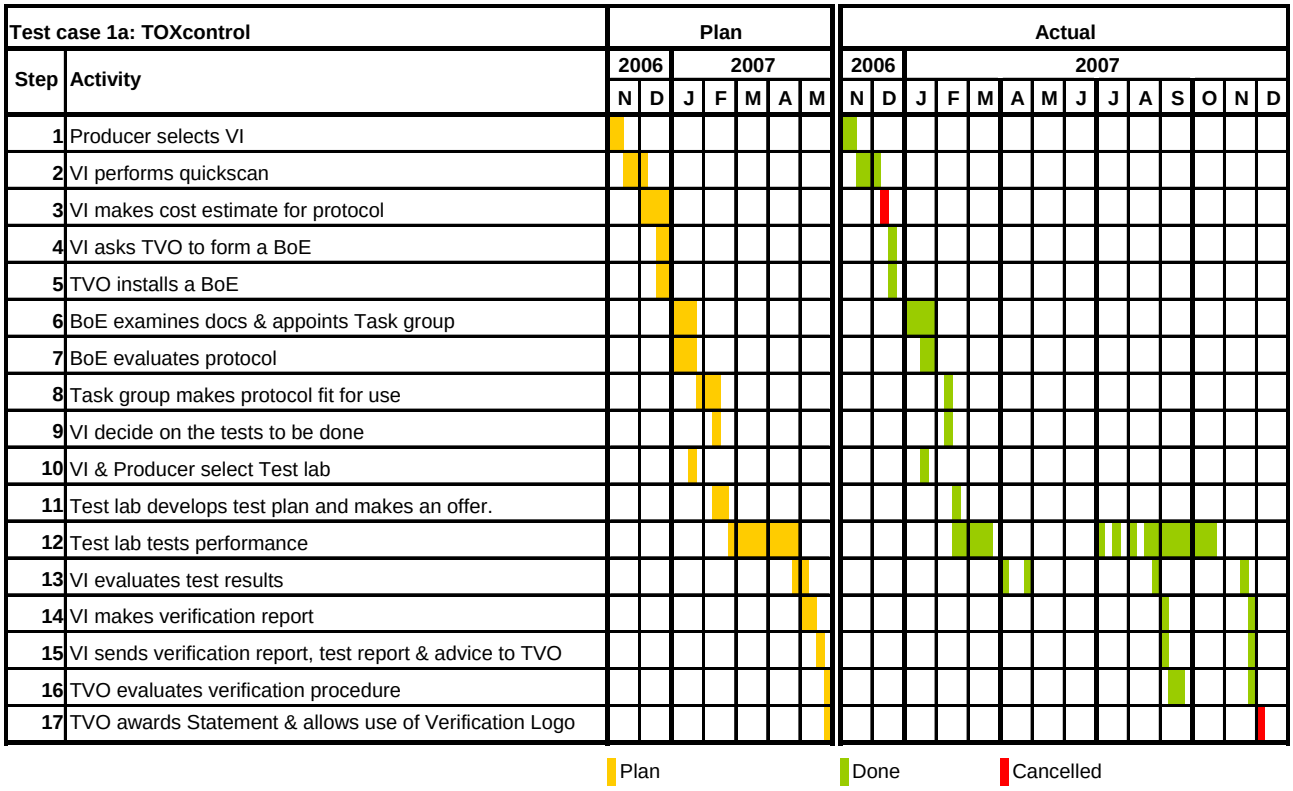
16: TVO evaluates verification procedure.
All material was sent to the TVO, who has evaluated the verification procedure and taken into account the suggested changes and the obtained results.

17: TVO awards Statement & allows use of Verification Logo.
Not done, but the step shall of course still be included in the scheme.

All above comments and suggestions to the verification scheme has been summarised in the form of an updated verification scheme presented in the section: “Recommendations for changes and improvements: The Scheme”

Time schedule

The planned timing (see time schedule below) for this case was somewhat compressed compared to the other cases, as this case had to be concluded by the end of May 2007, allowing for a calendar time of 28 weeks compared to the other cases which were allowed 40 weeks.



Comparing actual to plan shows that until the start of the laboratory tests (first cluster of weeks in line 12), the verification procedure followed the time schedule as planned. Laboratory tests took longer than planned and then there is a gap of 4 months before the field test was up running (second cluster of weeks in line 12) – some of the delay due to lack of resources and some due to practical problems with the set up. Then some delay before the final test report without the field test results was delivered to the VI. However, it was decided to continue with the activities 13 to 16 based on the laboratory test results only, and these have then been done according to plan. Field test results were delivered to the VI in October and the Verification report updated in November.

The lesson learned is that there should be planned with more time for the laboratory and field test as well as the installation at the field site. Taking the unnessary delays into account it is concluded that the planned time schedule will need another 6 weeks for activity 12: Testing lab tests performance in order to be able to accomodate unexpected problems due to first test of an instrument and protocol

Costs

Costs needed to carry out the test in this case are recorded as the time actual spent on work mentioned in the flow scheme (step 1-17). Costs are divided on the VI, the BoE, the Test lab and the TVO. It is clear that the main part of the effort spent goes into the work with the protocol and the actual test work is responsible for less than half of the costs. In order to save costs it is obvious that it should be considered to have less persons in the BoE, and of course optimise procedures and communication.

Test case 1a: TOXcontrol		Actual effort spent (days)					Comments
Step	Activity	VI	BoE	TL	TVO	Tot.	
1	Producer selects VI	0	0	0	0	0	N/A
2	VI performs quickscan	4	0	0	0	4	
3	VI makes cost estimate for protocol	10	0	0	0	10	VI: Protocol adaptation
4	VI asks TVO to form a BoE	1	0	0	0	1	VI: Expert Selection
5	TVO installs a BoE	0	0	0	2	2	TVO: Decide on VI or VL
6	BoE examines docs & appoints Task group	3	10	0	0	13	VI: Organisation+meeting
7	BoE evaluates protocol	2	10	0	0	12	VI: Protocol adaptation
8	Task group makes protocol fit for use	3	2	3	0	8	VI: Final protocol
9	VI decide on the tests to be done	2	0	0	0	2	VI: Final protocol
10	VI & Producer select Test lab	0	0	0	0	0	N/A
11	Test lab develops test plan and makes an offer.	0	0	2	0	2	
12	Test lab tests performance (incl. subcontracted labs)	2	0	47	0	49	VI: Follow up on tests
13	VI evaluates test results	8	0	0	0	8	
14	VI makes Verification report	5	0	0	0	5	
15	VI sends Verification report, test report & advice to TVO	1	0	0	0	1	
16	TVO evaluates verification procedure	0	0	0	2	2	
17	TVO awards Statement & allows use of Verification Logo	0	0	0	0	0	N/A
Totals		41	22	52	4	119	

No offer was prepared for the work with the protocol, but the prepared offer from the testing lab to the Task Group (which was actually just a lump sum
based on the planned time schedule), has now been revised based on the experience gained.

The revised offer - presented in Annex B – actually only shows the core part of an offer: the costs and the timing of the activities involved (where activities are the steps in the new proposed verification scheme described below).

The costs are given as effort spent for this actual test case compared to estimates of what is considered to be the minimum and the maximum effort to be used in the scheme (depending on availability of protocols and stability of equipment). Costs for test benches, reference materials, etc. are not included – these can differ a lot depending on the technology tested – 0 to 10 k€.

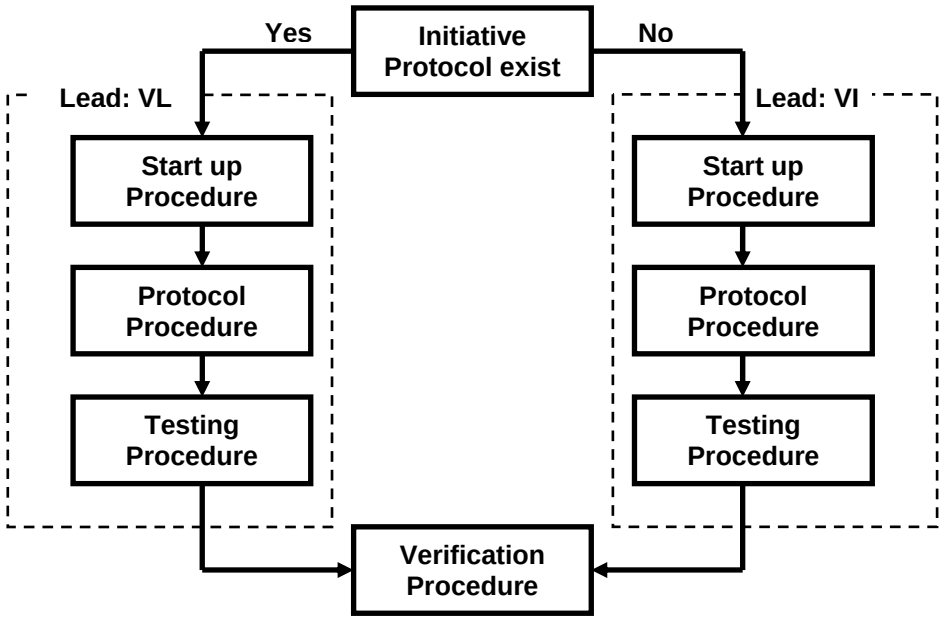
Annex B also shows the estimated time schedule for the steps to be performed – estimated from the maximum effort. As can be seen, it is anticipated that the total time needed for this verification scheme can be as long as one year – protocol work responsible for half of that. The minimum time needed might however come down to 8 – 9 months, mostly depending on the efficiency of the protocol work. .

Recommendations for changes and improvements

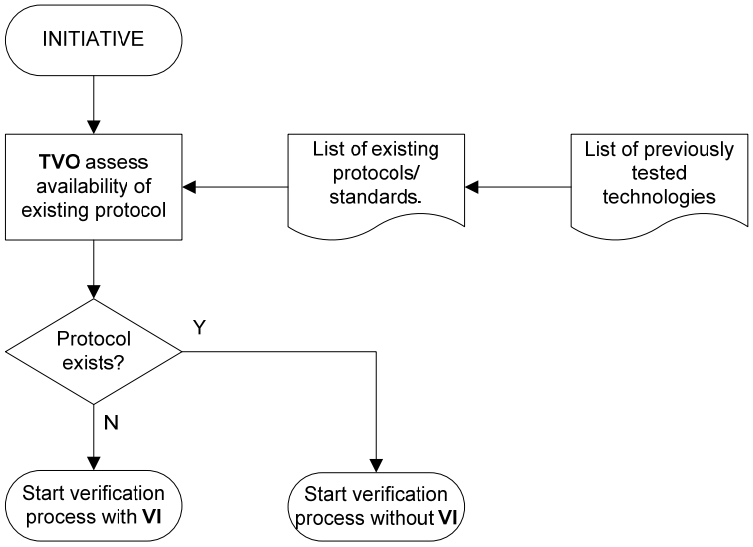
The Scheme

It is suggested to use a common start procedure for the verification process with and with out the involvement of a Verification Institute (VI). Having used both schemes and discussed with the stakeholders it certainly seems that there will be the need for a “fast track”, which involves the use of existing protocols/standards – and therefore no need for a VI. The “fast track” is operated by Accredited Test labs acting as Verification labs (VL) supervised by the Thematic Verification Organisation (TVO). On the other hand – if no protocol/standard exists or the most suitable protocol/standard really needs some adaptation – the involvement of a VI heading this work is highly recommended.

Further it is suggested that the last steps in the verification schemes, where the TVO evaluates the verification procedure, awards the verification statement and allows the use of a Verification Logo are common for the two schemes. Therefore it is recommended to use a two path verification scheme with a common start and conclusion administered by a Thematic Verification Organisation, who delegates the actual work to be done during the verification to be headed either by a Verification Institute or by a Verification Laboratory.



The recommended verification schemes therefore include 5 steps in each path – each of these steps shown below for the scheme with a Verification Institute. The scheme is taking into account the comments given above on the steps performed following the original scheme.



European ETV Scheme.

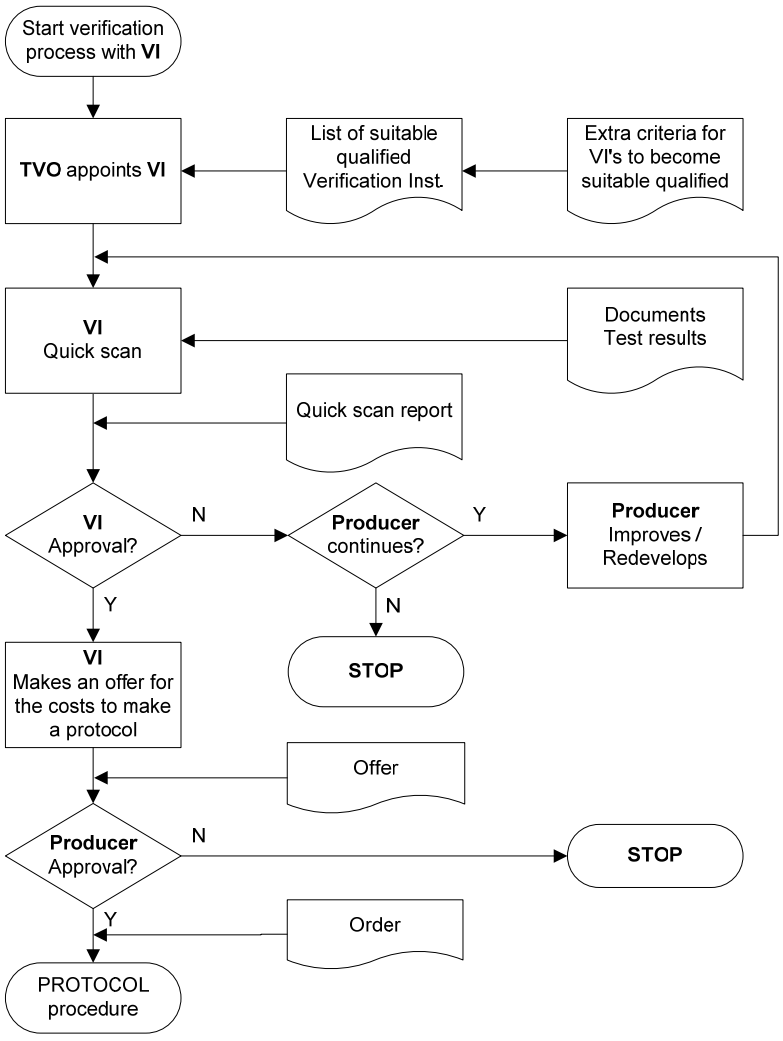
(flowchart) Test Case 1

INITIATIVE

A producer, an end user organisation or a branch organisation contacts the TVO, who then – if a suitable protocol does not exist - will appoint a qualified VI from a list. If a suitable protocol is available and can be used without too many changes, the scheme without a VI should be used.

This is based on the fact that the TVO in time will have information of all previously developed protocols and tests performed, and therefore can suggest coordination/timing of tests to be done.

To enhance a specific technology the Thematic Verification Organisation (TVO) also can call for initiatives.



European ETV Scheme – With VI

(flowchart) Test Case 1a

START UP procedure

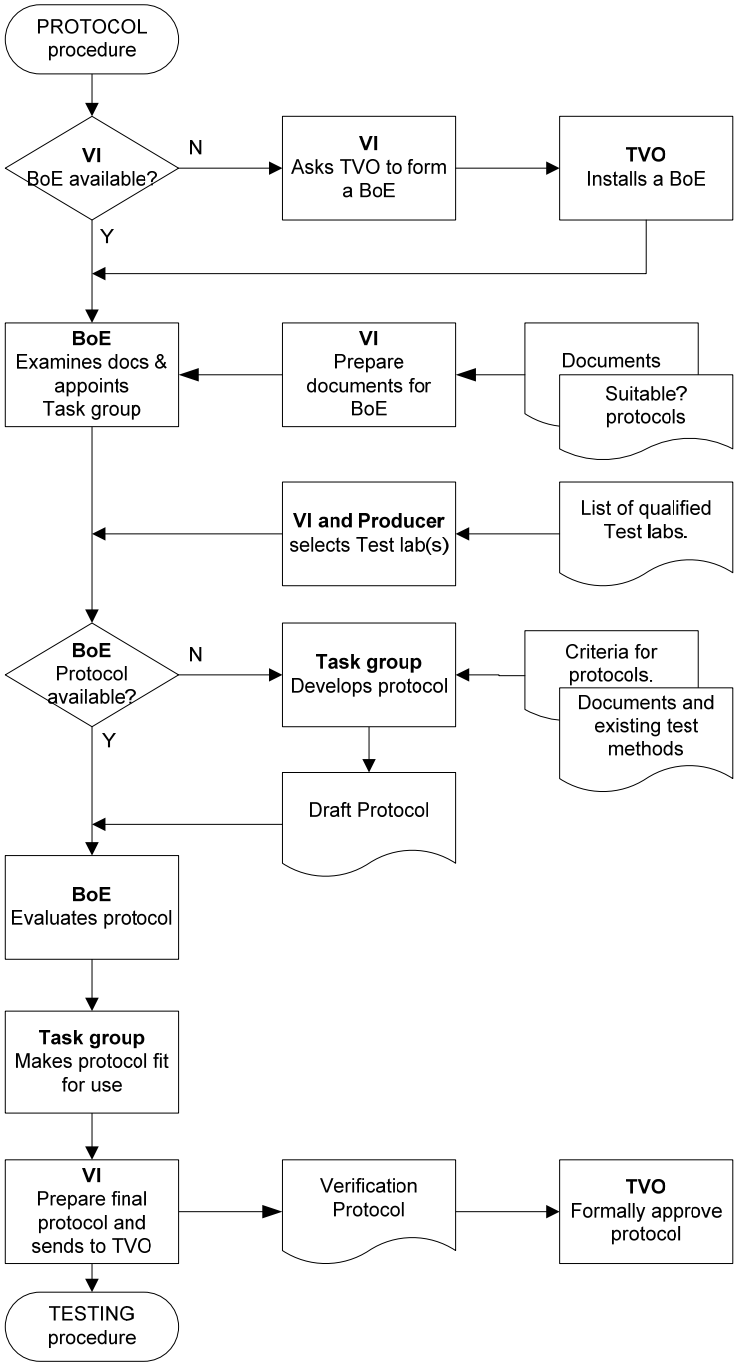
The TVO selects the Verification Institute (VI), from a list of qualified institutes
Extra criteria are added to the demands of EN 45011 to focus on the quality needed. Compliance to these criteria is audited as well by the national Accreditation body.

The VI examines if the technology is within the scope, ready to market (or an advanced prototype) and if enough and satisfying test results are available.
The VI sends the Quick scan report to the TVO.

The VI decides wether the process can go on. If not and if the producer wants to continue, he will improve the documentation or even the technology. The producer is allowed to ask for another VI.

The VI estimates the costs for developing or adjusting a verification protocol.
(The VI has examined if suitable protocols are already available; the VI gives the producer his opinion about the tests that have been done.)
The TVO gets a copy of the offer.

The producer gives an order to the VI based on an agreement about the costs.
Sometimes the order is given by a group of producers or by the branch organisation.
The VI sends a copy of the order to the TVO.



European ETV Scheme – With VI
(flowchart) Test Case 1a
PROTOCOL procedure

The VI asks, on behalf of the producer, the appropriate Board of Experts (BoE) to make a Verification Protocol. When there is no BoE (yet) for this specific field of technology the TVO forms a BoE.

The VI prepare the documents (if possible a draft protocol) to be sent to the BoE members, who then can assess the documents before a BoE meeting, where it should be assessed if suitable protocols are available

The BoE invites experts for a (temporary) Task group, also from outside the BoE. The VI chairs the group, the producer and the Test lab(s). which shall be selected at this point are q.q. member.

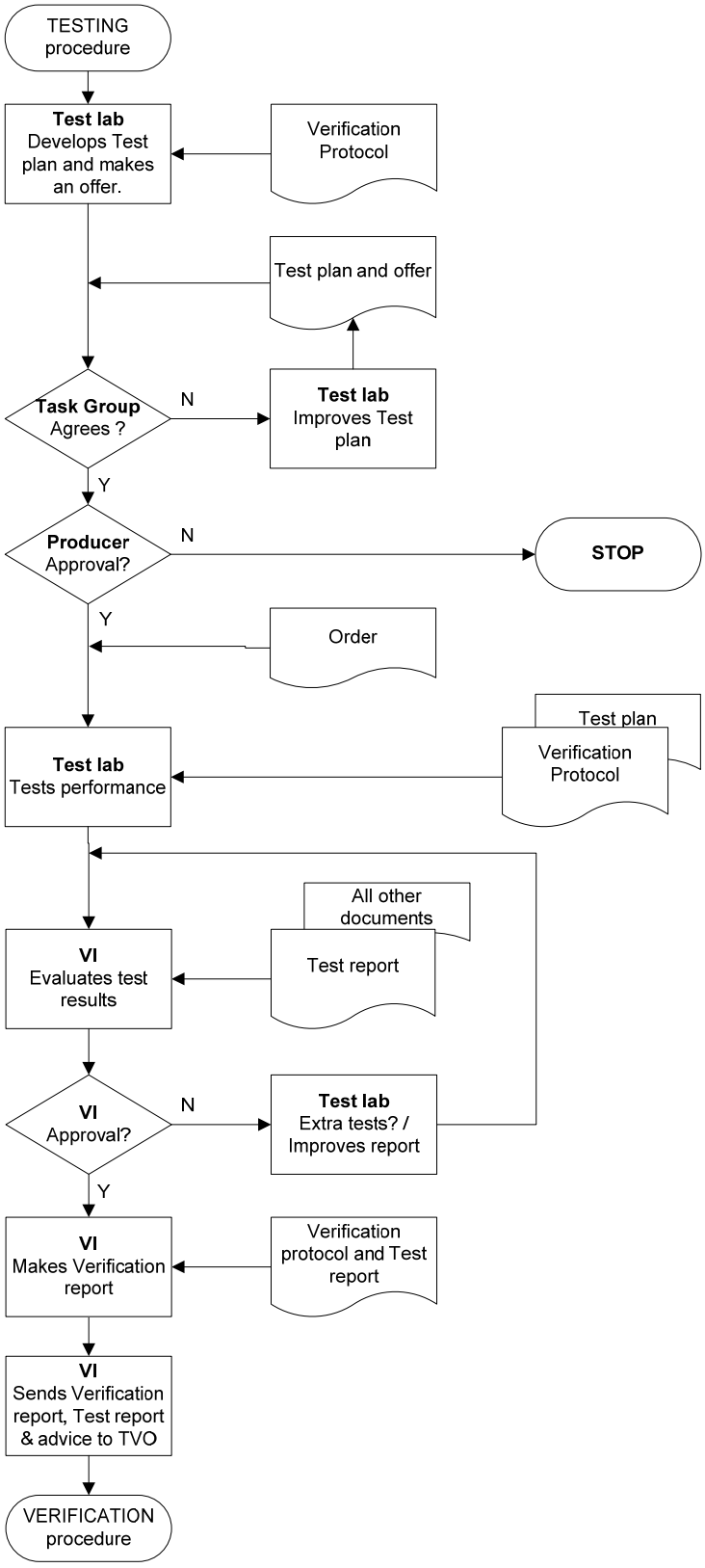
The VI and producer select Test lab.(s) from a list presented by the TVO. The list of qualified Test labs. is maintained by the Thematic Verification Organisation based on applications from Labs, which are assessed against the Criteria for Labs to become qualified.

The BoE first of all checks if the possible protocols presented by the VI available are suitable. When there is no protocol for this type of technology the Task group has to develop one.

Usually the VI in charge makes the concepts, to be discussed and approved in the Task group.
The protocol has to be as complete and detailed as possible, also with respect to the tests to be performed.

The BoE studies and comments the Draft Protocol and will approve it so it becomes a final draft, which will be made fit for practical use for the technology by the Task Group.

The VI sends the Verification Protocol to the TVO for formal approval. Only exceptionally the TVO will send the protocol back to the VI/BoE for improvement.



European ETV Scheme – With VI
(flowchart) Test Case 1a
TESTING procedure

The Test lab. develops the test plan including the costs to carry out the test. The Test plan should closely follow the requirements as set up in the Verification Protocol. Further, the Test plan focuses on quality assurance.

The Test plan forms the main part of the offer; it is the basis for judging the quality and the costs.

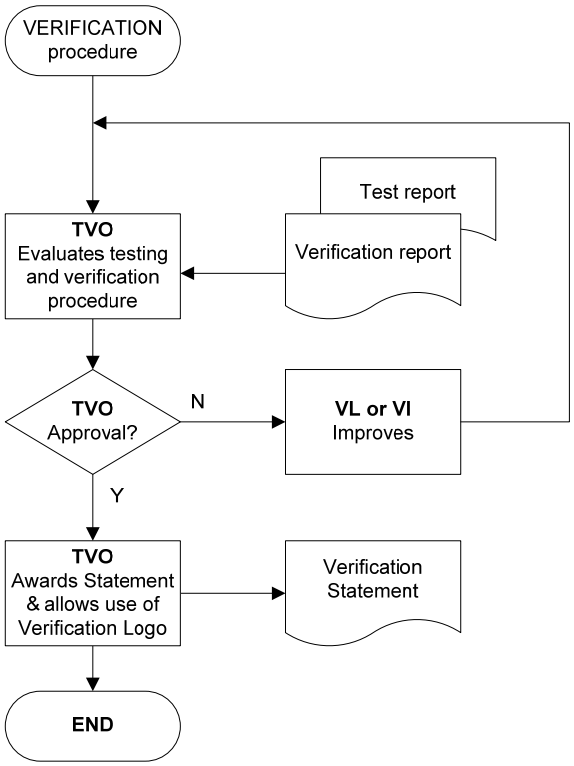
The Task group who had worked together making the protocol fit for use hs to agree on the Test Plan, and the Producer has to accept the offer, before the tests can begin.

The producer gives an order to the Test lab based on an agreement about the costs. It is possible that more than one Test lab gets orders for different tests or even for the same tests.

The Test lab performs the necessary tests, writes the Test report and submits it to the VI, who may be present during the tests performed by the Test lab(s).

The VI evaluates the tests performed and prepares a Verification Report for the TVO.

The Verification Report is a Management Summary based on the Test Report including the judgment and conclusions of the VI, and is sent to the TVO together with the Test Report and an advice on the verification



European ETV Scheme
(flowchart) Test Case 1
VERIFICATION procedure

The Verification Statement is basically made up by a diploma (declaration) and the Verification Report.

Documents produced in the scheme

A. Quick Scan (section written by the VI)
The objective of the Quick Scan report is to give a short description of the general characteristics of the product, and of the tests already performed. Information must be gathered by the Verification Institute (VI). This information step can be difficult when new technologies are concerned, due to the lack of publications and documents. This first step is useful for the TVO to decide if the technology can continue in the verification process. The form is not too difficult to fill in, except the questions regarding the test methods – reproducible/accuracy – which suppose that these characteristics are described in the standards and procedures. The Quick Scan report is included as Annex A.

B. Offer (section written by the VI and the Test lab)
Concerning the offer from the VI, some difficulties can be encountered: SMEs might refuse/not be able to pay for a protocol, especially for a new technology. Hopefully, in the monitoring domain, generic protocols generally exist, as for example the ISO 15839 Standard which was used for case 1a "Water Monitoring" of the TESTNET project. I think that the cost of the protocol – at least for new technologies of monitoring, developed especially by SMEs' - should be subsidised at this stage. On the other side, when a generic protocol exists, the cost of preparation and adaptation of a draft protocol should remain low or moderate.

Concerning the offer from the Test lab it is important to note that the main part of the effort spent at the Test lab goes into the step “Test lab tests performance”, and that this step therefore in an offer has to be more detailed. Likewise the expenses for possible test bench facilities, reference materials and other consumables. The offer shall not include the work done in the Task Group, as this should be included in the offer from the VI as a part of the protocol costs.
Costs for the work done at the Thematic Verification Organisation are not included in any of the offers, but it is envisaged that these should be a fixed amount.
The offer was not made before the work started but as the actual costs have been recorded, an offer has been made based on the experience gained (cost and time schedule – Annex B)

C. Protocol (section written by the VI)
From my point of view, a draft can be prepared within a short time by the VI. Therefore, it will be better to ask the VI to write a draft of the protocol, before sending material to the BoE. If this task is devoted first to the BoE, it needs preliminary BoE meeting(s), which are costly and time consuming for experts!
The draft protocol is a living document to which the VI has added and changed according to the comments received especially on the BoE and the Task Group meeting. The Verification protocol is included as Annex C

D. Test plan (section written by the Test Lab.)
The Test Plan is made according to the requirements given in the protocol and the guidelines given in the standard EN ISO 15839 by the Testing lab. The test plan is included as Annex D.

E. Test report (section written by the Test Lab.)
The Test Report has to be produced according to the requirements given in the Verification Protocol – which points to the requests in the standard EN ISO 15839 - by the Test lab (lab test) and its subcontracted lab (field test) and sent to the VI to be included in the Verification report. The Test reports – one for the laboratory tests and one for the field test – should strictly follow the requirements given in the verification protocol and is included as Annex E1 and E2

F. Verification report (section written by the VI)
The verification report is similar to a “management report” containing 3 to 4 pages, giving an opinion on the ability of the technology under verification (new or Environmentally Sound Technology) to ensure the use for which it is intended. The verification report is included as Annex F

G. Minutes
Meetings required by the verification scheme include a meeting in the Board of Experts and in the Task Group. The meeting in the Board of Experts should approve the protocol made by the VI and the meeting in the Task Group should confirm what has to be done in all the following steps. The minutes are included as Annex G.

H. Verifcation scheme to be tested. Version including Verification Institute.

Stakeholder list

Name	Organisation	Category (type of org)	Contact information
Appels Joep	microLAN	Producer	joep.appels@microlan.nl
Cahiere Veronique	EXERA	Stakeholder org.	veronique.cahierre.exera@wanadoo.fr
Cecile Jean-Luc	AFNOR	Org. member of EXERA	jcecile.ira@arles.cci.fr
De Hoog Corina	KIWA	Researcher. Dutch/German Expert group on Biomonitoring	corina.de.hoogh@kiwa.nl
Di Benedetto Dominique	EXERA	Stakeholder org.	dominique.di-benedetto@wanadoo.fr
Dosset Christian	EXERA	Stakeholder org.	dosset.exera@wanadoo.fr
Lynggaard-Jensen Anders	DHI	Developer, user and Convenor for the standardisation working group ISO TC147 WG2	alj@dhigroup.dk
Lachenal Jacques	LNE	Org. member of EXERA	jacques.lachenal@lne.fr
Ockier Paul	EUCETSA	End user organisation	p.ockier@eucetsa.com
Pelletier Claude	EXERA	Stakeholder org.	claude.pelletier@wanadoo.fr
Quertier François	VEOLIA WATER	End user. Org. member of EXERA	francois.quertier@veoliaeau.fr
Tran-Minh Canh	ECOLE DES MINES SAINT-ETIENNE	Research director in biochemistry University	tranminh@emse.fr
Wacheux Herve	VEOLIA WATER	End user and member of the standardisation working group ISO TC147 WG2. Org. member of EXERA	herve.wacheux@veoliaeau.fr

*) EXERA is a French/Italian user association, which as one of its tasks is testing monitoring equipment. Acts in the project as the Verification Institute, which comes close to the role in real life.

Annex A: Quick Scan Report

Verification Institute	Producer
Name: EXERA Contact: Dosset C., Di Benedetto D. Address: 4 cité d'Hauteville 75010 Paris, France Web site : www.exera.com Telephone: +33 (0) 153328008 Telefax: +33 (0) 153328009 Email: dominique.di-benedetto@wanadoo.fr Date Quick Scan: 17 december 2006	Name: microLAN B.V. Contact: Joep Appels Address: Biesbosweg 2 5145 PZ Waalwijk, Netherlands Web site: www.microlan.nl Telephone: +31 416 348090 Telefax: +31 416 347504 Email: joep.appels@microlan.nl

Previous Quick Scan:

Previous Quick Scan performed: ☒ No ☐ Yes, date:

Description of Product

The microLAN TOXcontrol biomonitor is a recently marketed product for drinking water/surface water on-line biomonitoring. It uses freshly cultivated light emitting bacteria as a biological sensor. It can be considered as an automated version of the ISO 11348 standard allowing continuous monitoring of drinking water/surface water. These new monitors must be tested following ISO 15839 standard in order to evaluate laboratory and on-line performance characteristics. Due to biological measurements performed by the biomonitor, several tests need to be adapted by the Board of Experts.

Description/principles clear:	☒ Yes	☐ No:
Declared performances described:	☒ Yes	☐ No: not completely
New Innovative Product:	☒ Yes	☐ No:
Ready-to-market:	☒ Yes	☐ No:
Prototype in advanced stage of develop.:	☐ Yes	☒ No:

Description of tests performed on product:

Tests performed on product:	☒ Yes	☐ No: rather applications than tests
Test lab suitable qualified:	☒ Yes	☐ No: DHI
Test protocol available:	☒ Yes	☐ No: ISO 15839, ISO 11348 (with some adaptations)
Test Protocol suitable:	☒ Yes	☐ No: in the early weeks of 2007
Test Methods available (Standards):	☒ Yes	☐ No: ISO 15839 and ISO 11348 standards
Test Methods described:	☒ Yes	☐ No: complete on December
Test Methods suitable:	☒ Yes	☐ No:
Test Methods reproducible:	☐ Yes	☐ No: no information from manufacturer
Test Methods accuracy:	☐ Yes	☐ No: no information from manufacturer
Test Results available:	☐ Yes	☐ No: manufacturer's literature: applications rather than tests
Test Results in line with declaration:	☐ Yes	☐ No: no information from manufacturer

Conclusions Quick Scan: The microLAN TOXcontrol biomonitor should be tested by DHI starting February 2007.

Verification Institute: EXERA
Name: DI BENEDETTO Dominique
Date: 8 December 2006
Paraphe:

☒ Copy to Thematic Verification Organization:

Annex B: Offer

Test Case with Verification Institute		Effort (days)			Time schedule (calender months max.)											
Procedure	**Activity**	**Case 1a**	**Min.**	**Max.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Initiative	TVO assess availability of existing protocol	0	1	3												
Start up	TVO appoints VI	0	1	2												
	VI performs Quick Scan	4	2	5												
	VI makes an offer for the protocol	0	1	2												
Protocol	TVO installs a BoE	2	1	3												
	VI prepare docs for the BoE (draft protocol)	10	5	10												
	BoE examines docs and appoint Task Group	10	5	15												
	VI and Producer select Test Lab	0	1	2												
	Task group develops protocol for BoE	3	5	10												
	BoE evaluates protocol (final draft protocol)	12	5	15												
	Task Group makes protocol fit for use	8	5	10												
	VI prepare final protocol and send to TVO	2	2	4												
Test	Test Lab makes test plan and offer	2	1	3												
	Test lab tests performance (sum of tasks below)	47	23	60												
	- set-up of instrument and test facilities in lab	*2*	*1*	*4*												
	- training of staff in use of instrument to be tested	*1*	*1*	*2*												
	- laboratory test and report	*22*	*10*	*25*												
	- set-up of instrument and test facilities at field site	*2*	*1*	*4*												
	- field test and report	*20*	*10*	*25*												
	VI evaluates test results	10	5	10												
	VI makes verfication report	5	3	6												
	VI sends verification reports and advice to TVO	1	1	2												
Verification	TVO evaluates verification report	2	1	4												
	TVO awards statement and verification logo	1	1	1												
Totals and documents		**119**	**69**	**167**												

Activity Task in activity Document

Annex C: Verification Protocol

Verification Protocol: TOXcontrol biomonitor, manufactured by microLAN B.V.

Introduction

This protocol was written to verify the performance characteristics of the TOXcontrol biomonitor manufactured by microLAN B.V. in Netherlands. The TOXcontrol biomonitor is an on-line monitor devoted to on-site measurement of toxicity in water. This protocol is adapted from ISO 11348-1 and ISO15839 standards. The toxicity is measured as an inhibition factor, which is calculated from the loss of luminescence of luminescent Vibrio fischeri bacteria.

Summary

The protocol describes the laboratory and field tests to be performed to verify the performance characteristics of the TOXcontrol biomonitor. The biomonitor uses luminescent bacteria to achieve the measurement of toxicity. It is claimed by the manufacturer that the monitor is an automatic version of the laboratory method described in the ISO 11348-1 Standard. As the biomonitor is an on-line instrument, tests described in the ISO 15839 Standard were selected to verify its performance characteristics. Several tests were adapted to biomonitoring. A Board of Experts (BoE) and a Task Group were set up to select and modify the tests described in the ISO 15839 standard, in order to cope with on-line biomonitoring.

Objectives

Scope
The tests envisaged in the verification protocol are intended to check the performances of an on-line biomonitor, having in mind that the measured value is a global parameter: toxicity. Consequently, we may consider the biomonitor as (part of) an Early Warning System, and only the most relevant tests have been selected and adapted be the BoE and the Task Group.

General application procedure for producer
The producer is involved in the verification process as a participant of the task group, which defines the practical tests selected by the BoE. When only one producer is concerned by the verification, he can be invited to participate to the BoE.

Table of Contents

Definitions, abbreviations and symbols used in the Verification Protocol

EWS: Early Warning System
$EC_{20,15}$: toxicity level giving a 20% inhibition factor for 15 minutes tests
CoV: coefficient of variation (relative)
LOD: limit of detection
LOQ: limit of quantification
LDC: lowest detectable change
BoE: Board of Experts
ISO: International Standard Organization
nm: nanometer = 10^{-9} meter
µl: microliter = $10^{-9}m^3$ ml: milliliter = $10^{-6}m^3$
FNU: formazin nephelometric unit

Documents

List of existing protocols referred to
At the present time, no protocols are described for on-line biomonitoring

List of Standards and requirements
The present protocol is based on:

- ISO Standard 11348-1 : "Water quality – Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) – Part 1: Method using freshly prepared bacteria (Revision of ISO 11348-1: 1998)"
- ISO Standard 15839:"Water quality – On-line sensors/analysing equipment for water – Specifications and performance tests"

General description of the technology/field of application

The TOXcontrol biomonitor is an on-line water quality monitoring system. It is an automatic instrument, which uses freshly cultivated light emitting bacteria (Vibrio fischeri) as a biological sensor. Toxic substances present in the water sample destroy some amount of bacteria, leading to a decrease of the light emitted by the bacteria. The instrument calculates the inhibition factor as described in the ISO 11348-1 Standard. Measurement parameters can be selected according to the parameters described in the standard: dilution ratio, exposure time…More information can be found in the Web site of the manufacturer (www.microlan.nl).

The instrument uses a test suspension of bacteria stored at 4°C. 50 µl of the suspension is simultaneously mixed with a 4.5 ml of a control solution (pure water + NaCl), and the same volume of water sample. A dilution step of 1:1 is made on control solution and sample with the control solution. Mixtures are allowed to homogenize at 15°C, and a measurement of light is performed on the two solutions at time t = 5 minutes (stabilization time) and time t = 15 or 30 min. Then an inhibition factor is calculated using the calculations of the standard.

comment: the correction factor f_k is calculated according to the standard, but the corrected intensity of light I_{ct} is obtained from the sample intensity at the beginning of the measurement (t = 5 min), and not from the intensity of the control solution at the beginning of the measurement, as described in the standard. As the measurement is a differential one, and as the inhibition factor can be linked to a reference concentration of a toxic chemical, the calculation can be considered as relevant. Furthermore, interference effects due to colored or (and) turbid samples should be minimized by this calculation.

Requirements to the users manual

These requirements are given by the manufacturer.
Check list Installation TOXcontrol:

Requirements and connections:
a. Size:
- width: + 120 cm: needed for TOXcontrol (80 x 80 x 122
cm – l x w x h)
- with optional TOXcontrol trolley (art. no. 04TCB00601) please add: 740 mm (H)
- please keep at least 20 cm behind the instruments for connections
b. Water connections:
- Sample water: 32,6 ml/min., bore size 4,8 mm int. diameter (silicone tubing: 04TCWM913A048016)
- Reference water (not chlorinated): 32,6 ml p/min., bore size 4,8 mm internal diameter (silicone tubing: art. no. 04TCWM913A048016), optional is feed option (art. no. 04TCB00501) to dose the reference water with a magnetic valve (art. no. 04ACE10604001): + 200 ml/h (depending on the cleaning steps)
- Drain: free flow hose, 10 mm internal diameter
c. Electricity: 220V / 50 Hz – 110V / 60 Hz
d. Weight: + 150 kg.
e. Environment:
- Sample temperature: 1 - 30 degrees Celsius, when temperatures between 30 – 40 degrees can be expected use Additional Cooling option (art. no. 04TCB00302)
- Working conditions (room temperature): 1 - 30 degrees Celsius.
- Humidity: < 95%, no condensation
- Sun light: instrument should not be placed directly in sun light
f. Connections:
- telephone line (ISDN or standard): for pcAnywhere remote control & support
- RS-232: standard 9-pin plug for pc / network connections
- 4-20 mA signal (optional, art. no. 04TCB00301).

Reagents & parts needed:
- Salt solution: 200 g / l Sodium chloride (in distilled water): + 700 ml needed per week
- TOXcontrol bacteria LT: article number: 02TCB00304; bacteria 10x & cultivation media 10x; Freeze dried luminescent bacteria for low toxicity (clean water) applications, 1 vial p/wk + cultivation media for start up of culture, 1 vial p/wk for cultivation on the TOXbioshaker
- TOXtip syringes: article number: art. no. 04TCEP34813; 100x, 2 needed per week
- Culture flask (for cultivation using the freeze dried bacteria in media): art. no.: 04TCFI02000; Culture flasks, with St. steel cap and stirrer, 5x, 1 needed per cultivation (can be sterilized after usage)
- Pipettes: article number: art. no. 04TCF10300, sterile pipettes 10 ml, 50x, 1 p/wk
- Tubing: depending on application / use: pump tubing should be exchanged 1 p/wk
- Reference standard: Zinc sulphate (1.117 mg ZnSO4*7H2O in 100 ml distilled water).

Detailed description of the performances/parameters to be verified

The performance parameters to be verified are described in the ISO 15839 Standard, with some adaptations to biomonitoring using batch (discontinuous) measurements.

For the laboratory tests, the linearity test has been adapted. As toxicity is a “global” parameter, the instrument can be considered as an early warning system (EWS) for which linearity is a parameter of limited interest. The user – especially in drinking water preparation and control - is more interested in the detection of pollution episodes and toxic parameters like $EC_{20\text{-}15}$, the concentration effect relationship: concentration of toxic substance giving an inhibition factor of 20% for a 15 min measurement.

The data gathered from linearity test are normally used for the determination of the coefficient of variation (CoV), the limit of detection (LOD), the limit of quantification (LOQ), the repeatability, the lowest detectable change (LDC), and the bias. For biomonitors, results are not given in concentration units, and the bias test has no signification in this case.

During the preparation of the protocol, the Board of Experts and the Task Group agreed to modify the procedures allowing the calculation of the required parameters. Inhibition factors as described by the ISO11348-1 standard were selected instead of concentrations of chemical standards (ranging from 5 to 95% of the measurement range of the analyzer/sensor under test). Three inhibition levels were selected: 20%, 50% and 80%. The substances used in the tests are those which are given in the ISO 11348-1 Standard (except Potassium dichromate ($K_2Cr_2O_7$)):

- Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)
- 3,5.dichlorophenol (purity=99.x %)

For interference tests, a food-dye - tropaeolin O - and diatomeous earth were selected for color and turbidity tests, respectively.
Note: for these interference tests, the testing laboratory should try to obtain a measurable interference effect by using concentrations of interfering compound high enough to produce a significant change of the 20% inhibition factor (zinc sulfate). This change can be set at 3 times the repeatability calculated at 20% inhibition for zinc sulfate. As a starting point, the concentration of tropaeolin O for color interference should give an absorbance of 0.1 for 1cm path length at 490 nm. For turbidity, a 50FNU suspension of diatomeous earth can be used. Diatomeous earth can be replaced by polystyrene beads. For these tests, it is possible to use several solutions containing increasing amounts of interfering substances, in order to determine a level at which a significant difference can be seen between zinc solutions (20% inhibition factor) and the same zinc solutions containing the interfering substances. The difference is significant if it is higher than the repeatability at this level of 20% inhibition.

Some tests can be run using only one toxic substance, zinc sulfate: response time, interferences, drift and sample temperature. Memory effects should be performed with one mineral compound, zinc sulfate, and with the organic substance 3,5.dichlorophenol.

For field tests, a water plant treating Rhine water has been selected. It is equipped with all required utilities, and with on-line analyzers fed with the same water sample flowing through the TOXcontrol biomonitor. Parameters to be determined for field tests are response time, bias, long-term drift, and availability.

Effect of temperature and humidity changes (in the laboratory): tests are performed on sample temperatures and on environment monitor temperatures ranging from 1°C to 30°C, within the specification values given by the manufacturer. Temperature tests should be performed on the monitor equipped with its cabinet if significant response changes are observed. (*The instrument was delivered in the laboratory without its cabinet.)*

Detailed description of the Test Procedures and Test Methods

The TOXcontrol biomonitor is an automatic on-line measurement instrument. The inhibition time can be set to 15 or 30 min, leading to measurement times of 30 or 45 min. The 15 min option was preferred by the BoE for all the tests. The measurement described in the ISO 15839 standard was chosen as one reading delivered by the instrument - the 10 readings per measurement required by the standard should lead to prohibitive times for the duration of the laboratory tests, and for practical aspects concerning the field test. Furthermore, files containing raw data are stored into the instrument computer, allowing supplementary data treatment if necessary. The laboratory will determine first the concentration of the three substances giving an inhibition factor of 20%, 50%, and 80%.

Laboratory tests
Laboratory tests can be performed with an instrument delivered without or with its cabinet. The instrument will be installed and started with default parameters by the manufacturer. Reference and sample solutions will be prepared and used following the manufacturer recommendations. Sample solutions will be delivered from laboratory glassware directly to the sampling chamber of the instrument.
The bacteria suspension can be used for one week (or 5 working days). During this period a loss of 90% luminescence occurs. The influence of this loss on performance characteristics must be verified at least on the repeatability test (see repeatability). A bias value - expressed as the difference of inhibition factor on a zinc sulfate solution giving a 20% inhibition factor - measured the first day (fresh bacteria suspension) and the fifth day can be calculated. The bias can be said significant if it is greater than the repeatability measured during the fifth day. In case of significant bias, the coefficient of variation and the limit of detection should be calculated when the bacteria storage is filled with a new bacteria suspension, and calculated again on the fifth day.

Response time
The ISO 15839 procedure can be executed with 20% and 80% inhibition factors using zinc sulfate. The change between the 20% inhibition factor solution and the 80% inhibition factor solution can be done just before the delivery of the third measurement, for example at time = 85 min for a 15 min inhibition time (30 min measurement time). For the TOXcontrol biomonitor, which is a discontinuous-reading system, the response time should be the measurement time.

Repeatability
Standard deviation of 6 measurements at 20% and 80% inhibition factor for zinc sulfate and 3,5.dichlorophenol. The tests should be carried out just after filling the instrument with freshly prepared bacteria and after 5 days with the same bacteria suspension, on the zinc

sulfate solution. In this way, the effect of bacteria loss with time can be highlighted: the difference (bias) of measurements should be lower than the repeatability calculated on day 5 measurements. If this is not the case, tests on coefficient of variation and limit of detection should be performed on the first day (fresh bacteria), and on the fifth day.

Coefficient of variation CoV
Calculate the coefficient of variation in accordance with ISO 8466-1 for the 20%, 50%, and 80% inhibition factors for 6 successive measurements at each level.

Limit of detection LOD
Three times the standard deviation of 6 measurements at 20% inhibition factor performed with zink sulfate. In case of a relative limit of detection lower than 2%, the LOD can be calculated at a lower level, for example 10% inhibition.

Limit of quantification LOQ
10 times the standard deviation of the measurements used for LOD.

Lowest detectable change
Three times the repeatability

Day-to-day repeatability
Standard deviation of 6 measurements at 20% inhibition factor on 5 consecutive days
NB: these 6 measurements can be used to calculate the short-term drift at 20% inhibition level.

Short-term drift
Slope of the regression line obtained from 6 measurements at 20% inhibition factor, equally distributed over 5 consecutive days (shortest time period between any maintenance operation). If the confidence limits of the slope contain zero, no significant drift can be detected.

Memory effects
This test should be performed just after the repeatability test at 20% inhibition level with zinc sulfate. After the 6 measurements required for the repeatability test, expose the instrument to a solution having a concentration equal to twice the concentration of zinc sulfate giving an inhibition factor of 80%, for a period of 5 measurement times, and then change to the solution of zinc sulfate giving an inhibition factor of 20%. Calculate the mean of 3 consecutive measurements after the third measurement. Calculate the difference between the mean obtained from the repeatability test at 20% inhibition factor and the mean of the 3 consecutive measurements at 20% inhibition level performed after the high concentration step. A memory effect is found if this difference is bigger than the lowest detectable change, LDC.
The same test should be done with 3,5.dichlorophenol at 20% inhibition level, memory effects being essentially due to adsorption-desorption effects, which can be different for mineral and organic compounds.

Environmental effects
The tests will be performed with zinc sulfate at 20% inhibition level. The temperature effect will be performed:

- on samples at 1°C and 30°C, the monitor remaining at ambient (lab) temperature
- on the biomonitor at 1°C and 30°C. For this experiment, the monitor should be installed in its cabinet.

In these experiments, the differences between the means of 3 successive measurements performed at each temperature level should be lower than the lower detectable change obtained at 20% inhibition.

Field tests
The biomonitor will be installed in monitoring station according to the ISO 15839 Standard. The sampling system is an integral part of the measurement system. The biomonitor will be fed with a water sample using the existing sampling system of the monitoring station. Look at annex B and annex C of the ISO 15839 Standard for supplementary information.
The instrument is continuously fed with an unknown sample. The measurements values cannot be compared to reference values, as the response is a ”global” parameter – toxicity – but spiking techniques can be used. Zinc sulfate will be used as the spiking substance. Spiking will be realized with water samples in which known concentrations of zinc sulfate will be added. The connections between the sample input, the spiked samples storage, and the input of the sampling system will be as short as possible, with a “response time” well below the response time of the sampling system.

Response time
Response time will be derived from readings of samples and spiked samples, the spiking concentration being approximately the concentration of zinc sulfate giving 80% inhibition level. See under section: Response time, for performing the test. The response time is measured on a complete system, including the sampling system.

Long term drift
Two measurements per week during 8 weeks will be carried out on samples and spiked samples with zinc sulfate at 20% inhibition factor. A regression line will be calculated from the differences of measurements between spiked samples and unspiked samples. If the confidence interval of the slope of this regression line contains zero, no significant drift can be assessed.

Availability and up-time
Follow the ISO 15839 Standard.

Requirements to Test Plans incl. Quality assurance

The testing laboratory has been asked to deliver a test plan and the quality assurance program applied in the laboratory.

Analyses and Data Management

-

Requirements to the Test Report

The test report should be written following EWE format (EXERA) and with respect of the recommendations given in ISO 15839 Standard.

Specific requirements for Verification Institute (Verificator) and Test laboratories

The Verification system and the testing laboratory should be familiar with water monitoring.

Verification of Tests reported by or by order of the Producer
To our knowledge, no tests described in the standards have been performed on the TOXcontrol biomonitor

Dominique Di Benedetto, for EXERA as Verification Institute.

Annex D: Test Plan – Laboratory test

Testplan Lab. Test TOXcontrol	**$ZnSO_4 \cdot 7H_2O$**							
Tasks	**"Conc." (inhibtion)**	**Day No.**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
Memory effect, LOD, LOQ, LDC, CoV, Repeatability, Day to day repeatability, Short term drift	20%	1						
	2*80%							
	20%							
	80%							
CoV, Day to day repeatability, Short term drift	20%	2						
	50%							
Day to day repeatability, Short term drift, inteference1	20%	3						
	20% + int.							
Day to day repeatability, Short term drift, inteference2	20%	4						
	20% + int.							
Repeatability, Day to day repeatability, Short term drift	20%	5						
	80%							
Day to day rep., Short term drift	20%	6						
	3,5 Dichlorophenol							
Tasks	**"Conc." (inhibtion)**	**Day No.**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
Memory effect - 3,5 Dichlorphenol	20%	2						
	2*80%							
	20%							

Annex E1: Test Report – Laboratory test

TESTNET Workpackage 3

Test case 1a: TOXcontrol

Test Report

Laboratory Tests

Manufacturer:	**microLAN**	**Dok. Version**
Verification Institute:	**EXERA**	**2007-08-24**
Testing Lab.:	**DHI**	**Final**

1. Materials and Methods

The laboratory test has been performed at the Testing lab. with an instrument delivered without shielding cabinet (figure 1.1). The instrument was installed and started with default parameters by the manufacturer. Reference standard and bacteria culture was delivered by the manufacturer and sample solutions were prepared in the lab. following the description in the test protocol delivered by the Verification Institute.

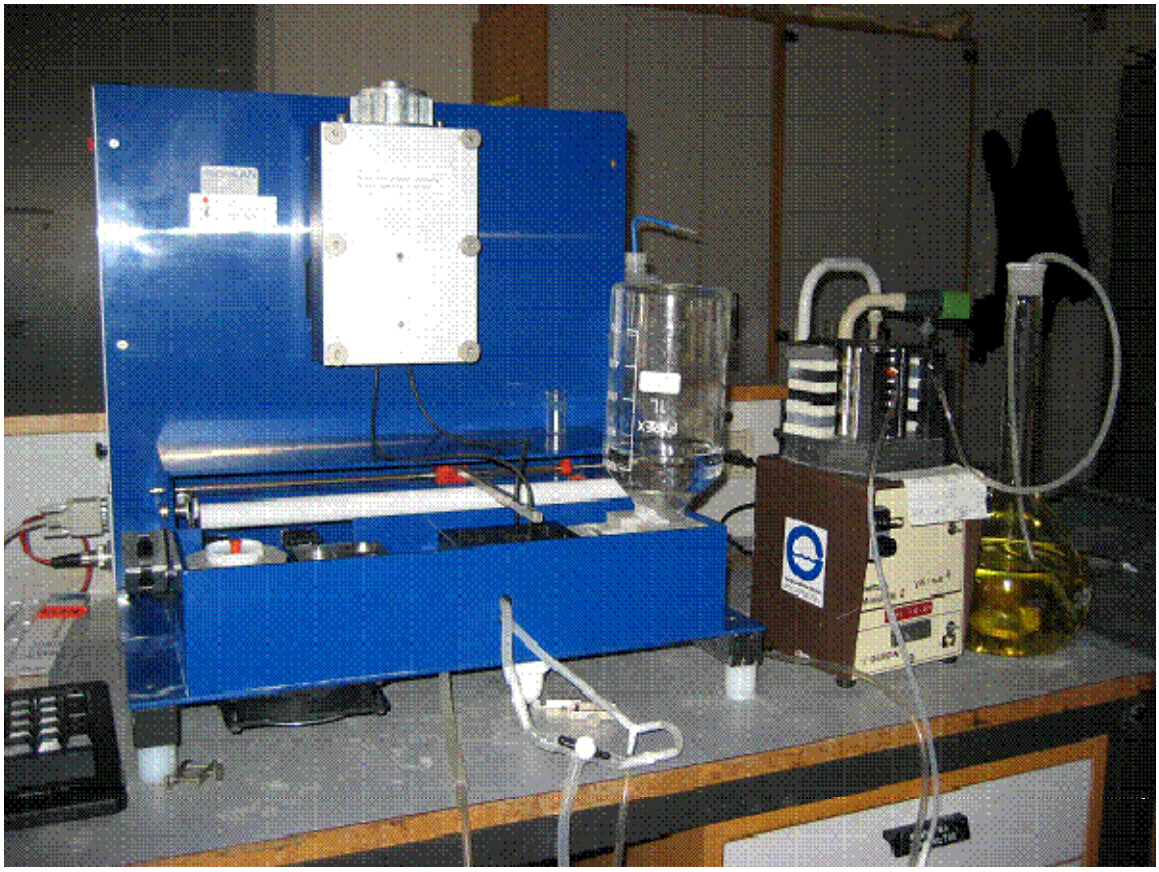


Figure 1.1: Setup of instrument (without shielding cabinet) in the laboratory.

Sample solutions was pumped directly to the sampling chamber of the instrument through a tube fitted with a 2 position valve. The valve made it possible to switch between a sample solution from laboratory glassware and tap water. Each time a new sample solution was used, a volume of 3 times the volume of the sampling tube and chamber was pumped before starting a new measurement.

The instrument has two parallel lines – a reference and a sample line - each equipped with a syringe, which also function as a measuring chamber for the bacteria produced luminescence. The instrument simulates batchwise the procedure described in the standard EN ISO 11348-1 1998 using the reference line to give the correction factor based on tap water. The correction factor is used for the calculation of toxicity in the sample line, which is fed with sample solution or reference standard. An incubation time of 15 min. was used in the tests giving a

total time for a measurement of 30 min. Every 6th measurement was using the reference standard.

The reference standard is a stock solution of $ZnSO_4,7H_2O$ fed to the reference line of the instrument. After dilution in the instrument the concentration will, according to the manufacturer, be 10 mg Zn/l (~ 44 mg $ZnSO_4,7H_2O$/l).

Bacteria suspensions was prepared in a special incubator delivered by the manufacturer, who also delivered the freeze dried bacteria. A bacteria suspension can according to the manufacturer be used for 1 week.

Sample solutions to be used for the tests are according to the protocol:

- $ZnSO_4,7H_2O$ of 4 different concentrations giving inhibition factors of approx. 20, 50 and 80%, the last hereafter doubled in concentration in order to give "2*80%". These sample solutions will be used for all tests (except interference).
- 3,5 Dichlorophenol of 2 different concentrations giving inhibition factors of approx. 20 and 80%, the last hereafter doubled in concentration in order to give "2*80%" These sample solutions will be used for testing of memory effect.
- Tropaeolin O (interferent 1) of different concentrations starting with 1 mg/l (abs 0.1 at 490 nm) in the the $ZnSO_4,7H_2O$ sample solution giving the inhibition factor of 20%, and then either made stronger or weaker according to the results of the interference tests it is used for.
- Diatomeus earth (interferent 2) of different concentrations starting with an amount giving approx. 50 FTU in the the $ZnSO_4,7H_2O$ sample solution giving the inhibition factor of 20%, and then either made stronger or weaker according to the results of the interference tests it is used for.

Experiments using the instrument with freshly prepared bacteria showed that the sample solutions listed in table 1 gave the requested responses.

Inhibition %	**$ZnSO_4,7H_2O$ mg/l**	**3,5 Dichlorophenol mg/l**
20	2.5	2
50	12.5	-
80	25	6
2*80	50	12

Table 1.1: Prepared sample solutions.

The instrument is controlled by a PC and all measurements and calculations are stored in an Excel file on the PC. The file was afterwards used as documentation as it was formatted to give an easy overlook of measurements from the different sample solutions (color coding), and how calculations were performed in the instrument. Annex 1 shows the Excel sheet before and after formatting – data from the first test day incl.

More sheets has been added to the file in order to handle the many measurements and calculations. Data to be handled are referenced from the original (and formatted) sheet to the sheets calculating the performance characteristics and checking the function of the instrument, and due to the use of color coding, time stamps, test periods, etc., the Excel file, which is attached to this report now contains the full documentation of the tests performed. The tables and plots shown below are simply cut and paste from the Excel file.

2. Results and Comments

According to the test plan (table 2.1) the test should be carried during 6 days using one bacteria suspension (which lasts for a week).

Testplan Lab. Test TOXcontrol	**$ZnSO_4 \cdot 7H_2O$**							
Tasks	**"Conc." (inhibtion)**	**Day No.**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
Memory effect, LOD, LOQ, LDC, CoV, Repeatability, Day to day repeatability, Short term drift	20%	1						
	2*80%							
	20%							
	80%							
CoV, Day to day repeatability, Short term drift	20%	2						
	50%							
Day to day repeatability, Short term drift, inteference1	20%	3						
	20% + int.							
Day to day repeatability, Short term drift, inteference2	20%	4						
	20% + int.							
Repeatability, Day to day repeatability, Short term drift	20%	5						
	80%							
Day to day rep., Short term drift	20%	6						
	3,5 Dichlorophenol							
Tasks	**"Conc." (inhibtion)**	**Day No.**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
Memory effect - 3,5 Dichlorphenol	20%	2						
	2*80%							
	20%							

Table 2.1: Original test plan

However, due to various reasons commented below (and in the Excel file), the test work lasted for 4 weeks, and therefore contains results based on measurements using 4 bacteria suspensions. This also means that the deviation between the bacteria cultures can be assessed.

Below the obtained results for each period are shown and the work commented, and the chapter concludes with an overall assessment of the performance of the instrument based on plots of the correction factors, all the measurements using the reference standard and the measurements were the instrument was operating unattended for longer periods with the sampling line connected to the tap water.

Test period no.: 1

Results Lab. Test TOX-Control		$ZnSO_4 \cdot 7H_2O$							
Date	Tasks	"Conc." (inhibtion)	Period : Day	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
# 21-02	Memory effect, LOD, LOQ, LDC, CoV, Repeatability, Day to day repeatability, Short term drift	20%	1 : 1	9,7	10,2	7,6		4,2	11,2
		2*80%		96,8	94,9	96,3	95,9	94,9	
		20%		21,5	15,1	16,2			
		80%		75,7	72,6	73,6	68,7	66,2	66,1
22-02	LOD, LOQ, LDC, CoV, Repeatability, (Day to day rep., Short term drift)	20%	1 : 2	4,9					
		50%		27,2	31,6	30,2	30,6	28,5	26,9
27-02	LOD, LOQ, LDC, CoV, Repeatability	20%	1 : 7	9,8	10,9	12,7	10,8	12,9	12,5
		80%		45,2	41,7	37,9	37,7	34,2	33,1

Day 1 and day 2 of this period strictly follows the test plan concerning the tests with Zinc Sulfate, but during the second day the lab. staff became ill and did not manage to carry out the planned tests with 3,5 Dichlorophenol. Therefore the instrument was stopped and no further test performed until day 7, where it was decided to carry out 6 measurements using respectively the 20 and 80% inhibition factor sample solutions, in order to be able to calculate the mentioned performance characteristics on the last day where the bacteria are claimed to be in order for use.

The measurements of the “20%” sample solution seems rather low at the beginning of the first day (one value of 0.9 is considered an outlier and is therefore rejected), but after the use of the “2*80%” for the memory test they are as expected. Also the measurements of the “80%” sample solution seem to be a little bit too low (for measurements this day see also Annex 1). On the second day both the “20%” and the “50%” are much lower than expected – although some decay of the bacteria might have taken place. Day 7 shows measurements at half the value, which in fact might be OK due to bacteria decay, but that would imply that the bacteria have become more active again in the end of the period.

Test period no.: 2

Day 1 of the test does not include the memory effect of the “2*80%” sample solution for Zincsulfate, as this was done during the first period. Further, the measurements of the “50%” sample solution are moved to day 1 from day 2 in order to get more time on day 2 for the test of memory effect using 3,5 Dichlorophenol.

The measurements on day 1 of both the “20%” and the “50%” sample solution containing Zinc Sulfate seem to be quite high, whereas the “80%” is within a range as expected. The measurements using 3,5 Dichlorophenol looks far too high on the “20%” – although consistent, but might be as expected on the “2*80%”. All in all the measurements using 3,5 Dichlorophenol was decided to be OK for a memory effect test.

Results Lab. Test TOX-Control		$ZnSO_4 \cdot 7H_2O$							
Date	**Tasks**	**"Conc." (inhibtion)**	**Period : Day**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
# 01-03	LOD, LOQ, LDC, CoV, Repeatability, Day to day repeatability, Short term drift	20%	2 : 1	31,9	35,4	29,9	36,7	34,8	33,6
		50%		71,5	55,4	64,5	67,5	68,6	69,9
		80%		84,2	83,3	84,1	81,8	82,1	78,4
02-03	Day-to-day rep., Short term drift	20%	2 : 2	7,1					
03-03	LOD, LOQ, LDC, CoV, Rep., Day to day rep., Short term drift	20%	2 : 3	14,4	7,6	4,0	0,9	-6,5	-1,3
		80%		36,7	34,9	31,9	36,3	37,5	35,2
04-03	Day-to-day rep., Short term drift	20%	2 : 4	17,5					
05-03	Day-to-day rep., Short term drift	20%	2 : 5	3,2					
06-03	LOD, LOQ, LDC, CoV, Rep., Day to day rep., Short term drift	20%	2 : 6	-2,1	0,1	6,1	5,2	2,3	-2,2
		80%		24,1	24,4	25,4	23,4	23,8	18,2

Results Lab. Test TOX-Control		3,5 Dichlorophenol							
Date	**Tasks**	**"Conc." (inhibtion)**	**Period : Day**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
02-03	Memory effect - 3,5 Dichlorphenol	20%	2 : 2	64,3	65,3	72,8			
		2*80%		93,1	93,5	93,8	94,7		
		20%		71,1	76,2	74,9			

On day 3, the measurements of the “20%” sample solution looked peculiar (starting quite high on the first, then decreasing to a quite low value on the second day and increasing again on the first of the measurements on the third day). It was therefore decided to make 6 measurements of both the “20%” and the “80%” sample solution, and postpone the test of interference1 to a following period (Should have been done this day according to the test plan. However, time is to short – and the day is a Saturday).

Day 4 - which is a Sunday – the only measurement carried out was the planned using the “20%” sample solution. Interference test using interferent 2 was postponed to a following period – to be done together with interferent 1. The instrument was left to measure automatically on tap water in order to get an idea of the standard deviation on blank measurements.

On day 5 the instrument broke down after the first measurement on a “20%” sample solution. The software on the PC did not respond, not even after resetting the PC. The supplier was

contacted and he fixed the problem the next day, where 6 measurements of both the “20%” and the “80%” were carried out - although they should have been that already on the fifth day according to the test plan.

After evaluation of the results obtained in this period, it was decided to run a third test period for interference tests using interferent 1 and 2, and at the same time get more measurements carried out using the “20%” sample solution, as the results form this period looks quite strange, whereas the results from the “80%” sample solution seem to be as expected.

Test period no.: 3

Results Lab. Test TOX-Control		**$ZnSO_4 \cdot 7H_2O$**							
Date	**Tasks**	**"Conc." (inhibtion)**	**Period : Day**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
# 07-03	Inteference Tropaeolin O - abs 0.1 at 490 nm (1mg/l)	20%	3 : 1	11,9	15,9	18,1			
		20% + int.		31,6	34,7	21,9	25,0	23,1	25,7
		20%		19,4	16,1	20,1	11,2	24,8	24,4
13-03	LOD, LOQ, LDC, CoV, Rep.	20%	3 : 7	5,5	6,3	2,9	8,0	9,6	4,6

Day 1 started with interference tests using Tropaeolin O in a concentration of 1 mg/l (known to give an absorbance of 0,1 at 490 nm) in the “20%” sample solution of Zinc Sulfate. The results clearly shows that a concentration of 1 mg/l causes interference, so dilution is necessary in order to find the limit. However as this was now known to work, it was decided to carry out the determination of the limit later, and instead do the first interference tests with the other interferent - Diatomeus earth in a concentration giving a turbidity of ~ 50 FTU. The instrument was therefore left to measure automatically on tap water during the period with producing the correct sample solution – “20%” Zinc Sulfate + 50 FTU caused by the Diatomeus earth.

However, it was more difficult than expected to get the correct concentration – keeping the Diatomeus earth suspended while measuring turbidity – and therefore it was not until late on the third day the first tests were made giving strange results on both the “20%” sample solution with and without Diatomeus earth – nothing could be seen.

On day 4 and 5 the instrument was closed down (Saturday and Sunday), and on day 6 measurements were carried out using “20%” Zinc Sulfate sample solution with and without Diatomeus earth – concentration of Diatomeus earth increased to cause 100 FTU. There seemed to be an effect, however the syringe started leaking – and it was uncertain if the Diatomeus earth really was kept in suspension for as long as the measurement lasted.

In order to check if an effect can be seen, if the Diatomeus earth be kept in suspension and if it is the Diatomeus earth that wears the syringe and thereby make this leaking, the syringes were changed on day 7 and measurements were carried out using 20%” Zinc Sulfate sample solution with and without Diatomeus earth – concentration of Diatomeus earth increased to cause 1000 FTU.

The results from the 6 first measurements on the 20%" Zinc Sulfate sample solution without Diatomeus earth seemed to be OK, and the first measurements with Diatomeus earth also demonstrated a clear effect of turbidity, but then the syringe started to leak again, and the 6 following measurements on the 20%" sample solution without Diatomeus earth also were much higher, as if the turbidity was not cleaned from the syringe.

After this it was decided to cancel the interference test using Diatomeus earth and not to use any of the measurements except the 6 measurements of the 20%" Zinc Sulfate sample solution without Diatomeus earth, which were carried out just after the change of the syringes. However, it was demonstrated that turbidity has an effect on measurements, but the value which can be said to cause this, can not be given.

Test period no.: 4

Due to the problems in the previous period with using Diatomeus earth, a period more is required in order to complete the interference test with Tropaeolin O.
On day 1 the test using a concentration of in a concentration of 1 mg/l in the "20%" sample solution of Zinc Sulfate was repeated together with more measurements of the "20%" sample solution itself – and comparable results with the previous test were obtained.

Results Lab. Test TOX-Control		**$ZnSO_4 \cdot 7H_2O$**							
Date	**Tasks**	**"Conc." (inhibtion)**	**Period : Day**	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6
# 15-03	Inteference Tropaeolin O - abs 0.1 at 490 nm (1mg/l)	20%	4 : 1	13,0	9,6	9,6	10,4		
		20% + int.		27,1	23,8	22,1	23,9	22,6	32,0
16-03	LOD, LOQ, LDC, CoV, Rep.	20%	4 : 2	11,3	12,0	11,1	8,4	11,7	12,4
20-03	Inteference Tropaeolin O concentrations used 0.8, 0.25 and 0.4 mg/l	20%	4 : 6	8,2	6,5	8,3			
		20% + int.		21,6	21,1	20,0	20,6	19,6	22,0
		20% + int.		12,8	10,6	12,0	8,5	12,4	10,6
		20% + int.		15,0	15,1	15,7	16,4	14,3	14,8
21-03	LOD, LOQ, LDC, CoV, Rep.	20%	4 : 7	6,3	7,4	6,7	4,7	5,2	5,8

On day 2, 6 measurements of the "20%" sample solution without Tropaeolin O were done and the instrument closed down for the weekend. However, later this day it was decided to leave it running unattended on tap water for the next 3 days (day 3, 4 and 5), because it was not possible to do further work before day 6, but at the end of day 5 the instrument was taking air in due to a failure on the tap water supply line - therefore there are no measurements before on day 6 where the interference test was completed.

On day 7, 6 extra measurements using the "20%" sample solution without Tropaeolin O were done and the laboratory test was stopped, because the instrument had to be packed for delivery to the field test site.

Overall performance of instrument

Figure 2.1-2.3 shows other available measurements/calculations from the instrument from each period plotted as a function of the bacteria age (counted from the first measurement of the period). The plots clearly shows that period 4 is somewhat different tan the other 3 periods, and this becomes even more clear when the trends of the measured Toxicity of the Ref.standard (~ 44 mg/l Zincsulfate, heptahydrate) vs. the age of the bacteria are calculated as shown below.

Period no.	Trend inhib% / day	
	All data	Day 1-3
1	-4,5	-16,8
2	-12,5	-13,6
3	-9,3	-12,0
4	-1,6	-0,3

These plots are used in the overall assessment of the measurements to be used for the calculations according to the protocol – as described in chapter 3. Further, they constitute a good basis for an assessment of the operation of the instrument – which should be discussed with the manufacturer.

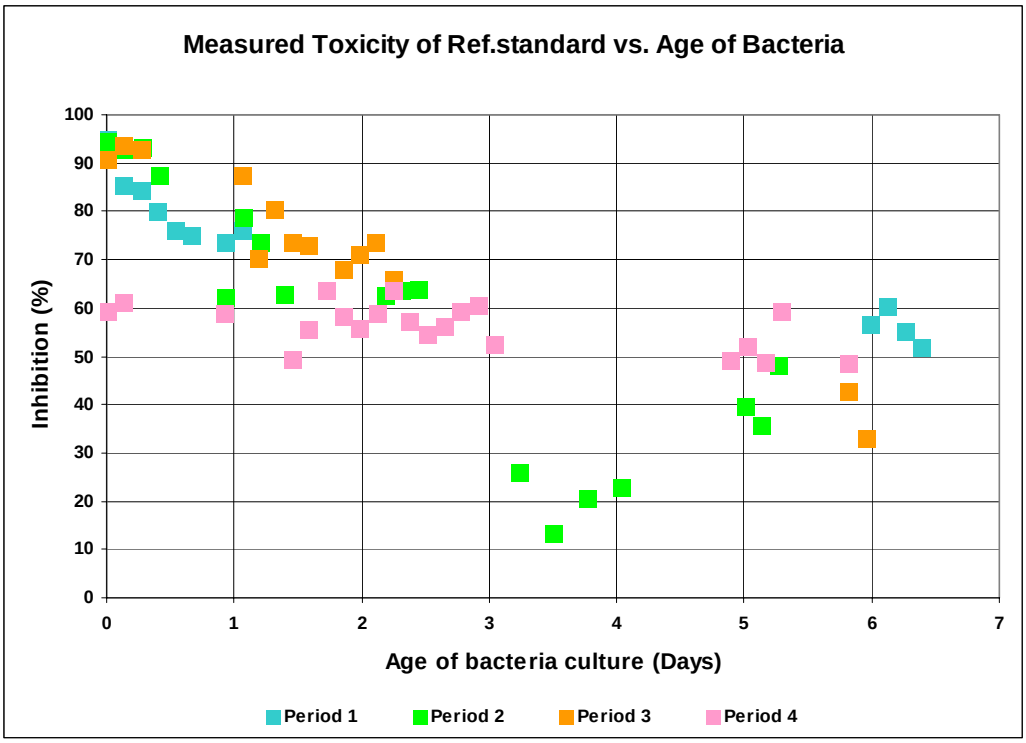


Figure 2.1: Measured Toxicity of Ref.standard vs. Age of Bacteria

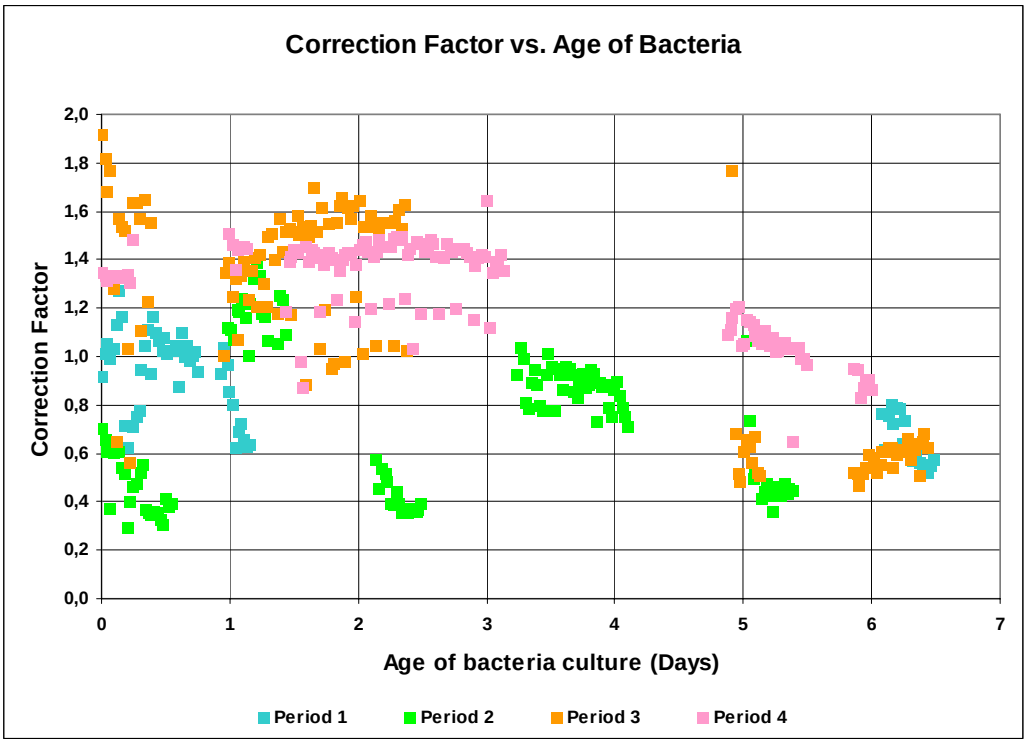


Figure 2.2: Correction Factor vs. Age of Bacteria

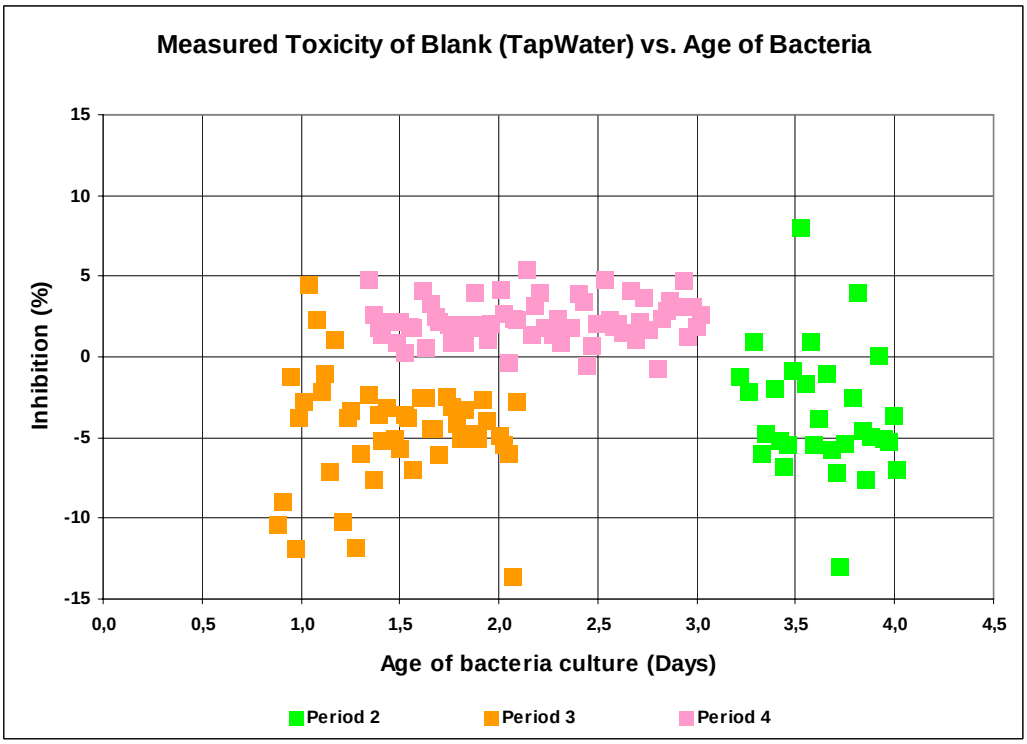


Figure 2.3: Measured Toxicity of Blank (Tap Water) vs. Age of Bacteria

3. Datahandling and Performance Characteristics

Response time

Figure 3.1 (ref. ISO 15839) illustrates how a batch instrument like the TOXcontrol can have a response time – which mostly for batch instruments are known as "carry over" from one sample to the next.

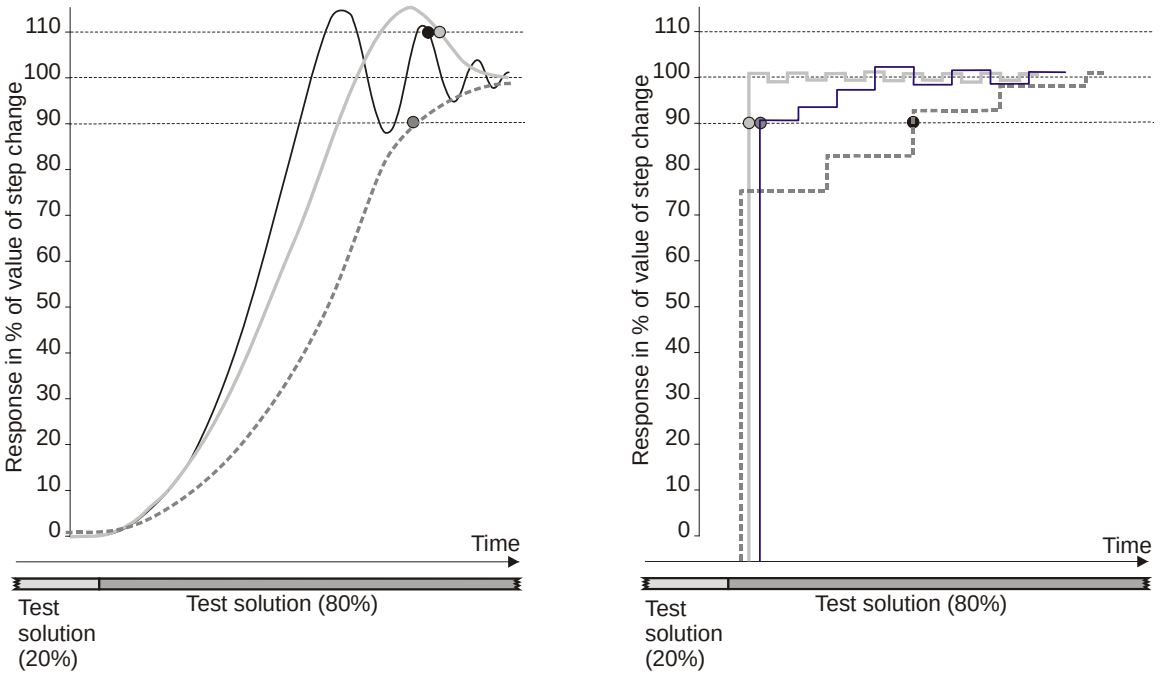


Figure 3.1: Response of continuously working instruments and batch instruments

The measurements from the first day of the first period (also used for memory effect of the Zinc Sulfate) clearly demonstrates that no significant "carry over" can be detected – neither upwards nor downwards in concentration, which means that the response times (both + and -) are equal to the measurement time, which is set to 30 minutes. This is reported in the final result table in chapter 4.

CoV, LOD, LOQ, LDE and Rep.

The Coefficient of Variation (Cov), Limit of Detection (LOD), Limit of Quantification (LOQ), Limit of Detectable Change (LDE) and Repeatability (Rep.) are calculated according to the protocol using the first and last full data sets (6 measurements) for all 4 periods (see below).

The final results for these performance characteristics are then taken as the "worst case" scenarios (marked with yellow), which in this case means:

- Period 1 is used for results concerning "80%" Zinc Sulfate sample solution
- Period 2 is used for results concerning "50%" Zinc Sulfate sample solution

- Period 3 is used for results concerning “20%” Zinc Sulfate sample solution

$ZnSO_4 \cdot 7H_2O$		Avg.	Stddev.	CoV	LOD	LOQ	LDC	Rep.
"Conc." (inhibtion)	Period : Day	inhib.%	inhib.%	-	inhib.%	inhib.%	inhib.%	inhib.%
80%	1 : 1	70,5	4,0	5,7	12,1	40,5	12,1	4,0
50%	1 : 2	29,2	1,9	6,6	5,8	19,2	5,8	1,9
20%	1 : 7	11,6	1,3	10,8	3,8	12,5	3,8	1,3
80%		38,3	4,5	11,8	13,6	45,2	13,6	4,5
20%	2 : 1	33,7	2,5	7,4	7,4	24,8	7,4	2,5
50%		66,2	5,8	8,7	17,4	57,9	17,4	5,8
80%		82,3	2,1	2,6	6,4	21,5	6,4	2,1
80%	2 : 6	23,2	2,5	10,9	7,6	25,4	7,6	2,5
20%	3 : 1	19,3	5,2	26,7	15,5	51,6	15,5	5,2
20%	3 : 7	6,1	2,4	39,3	7,2	24,1	7,2	2,4
20%	4 : 2	11,2	1,4	12,8	4,3	14,2	4,3	1,4
20%	4 : 7	6,0	1,0	16,5	3,0	9,9	3,0	1,0

The marked results are filled into the final result table in chapter 4.

Day to Day Repeatability and Short Term Drift

Calculations are performed according to the protocol (see below), but although the protocol states that these performance characteristics should be calculated using the “20%” Zinc Sulfate sample solution, this is done using the “80%” Zinc Sulfate sample solution from period 2 (even if it is based on 3 data sets only), because the results from this period based on the “20%” are assessed as unuseable for this calculation (se comments in section 2.2).

$ZnSO_4 \cdot 7H_2O$		Avg.	Stddev.	Day to Day rep.	Short term drift
"Conc." (inhibtion)	Period : Day	inhib.%	inhib.%	inhib.%	inhib% / day
80%	2 : 1	82,3	2,1	Based on 80%	
80%	2 : 3	35,4	2,0	31,2	-11,2
80%	2 : 6	23,2	2,5		
20%	4 : 1	10,7	1,6	Based on 20%	
20%	4 : 2	11,2	1,4	2,5	-0,8
20%	4 : 6	7,7	1,0		
20%	4 : 7	6,0	1,0		

However, the results for the “20%” Zinc Sulfate sample solution is as an alternative based on the 4 available datasets from period 4, although this period behaves differently than the other

3 periods (see section 2.5). It should be considered if the result can be included in the final result table.

Memory effect

Calculations are done as stated in the protocol (see below), and for Zinc Sulfate the calculated difference is compared to the highest value for LDC from the "20%" Zinc Sulfate sample solution, which is 15.5. For the 3.5 Dichlorophenol the LDC is calculated as 3 times the highest calculated standard deviation, which gives a value of 14.0. In both cases the calculated difference is less than the LDC, which means that no significant memory effect can be detected.

$ZnSO_4 \cdot 7H_2O$		Avg.	Stddev.	Memory Effect	
"Conc." (inhibtion)	Period : Day	inhib.%	inhib.%	Diff. inhib.%	yes/no
20%	1 : 1	8,6	2,8	9,0	Yes: If Diff > LDC
2*80%		95,8	0,8		
20%		17,6	3,4		

3,5 Dichlorophenol		Avg.	Stddev.	Memory Effect	
"Conc." (inhibtion)	Period : Day	inhib.%	inhib.%	Diff. inhib.%	yes/no
20%	2 : 2	67,5	4,67	6,6	Yes: If Diff > LDC
2*80%		93,8	0,68		
20%		74,1	2,64		

Interference

$ZnSO_4 \cdot 7H_2O$		Avg.	Stddev.	Inteference		Memory Effect	
"Conc." (inhibtion)	Period : Day	inhib.%	inhib.%	Diff. inhib.%	yes/no	Diff. inhib.%	yes/no
20%	3 : 1	15,3	3,1		Yes: If Diff. > LDC		
20% + 1		27,0	5,0	11,7			
20%		19,3	5,2				
20%	4 : 1	10,7	1,6				
20% + 1		25,2	3,7	14,6			
20%	4 : 2	11,2	1,4				
20%	4 : 6	7,7	1,0				
20% + 0.8		20,8	0,9	13,1			
20% + 0.25		11,2	1,6	3,5			
20% + 0.4		15,2	0,7	7,5			
20%	4 : 7	6,0	1,0				

Interference test is only available for Tropaeolin O, due to the failed attempt to use Diatomeus earth in period 3. The results used are from period 4, although this period behaves differently than the other 3 periods (see section 2.5). However, as this can be regarded as a relative measurement – comparing "20%" sample solution with and without Tropaeolin O at the same day - at least the with the measurements using dilution of the interferent, and that the

interference levels seems to be consistent (also with the measurements carried out the first day in the period) calculations are done according to the protocol (see below).

The LDC values to compare with are those found for the “20%” Zinc Sulfate sample solution, which are 15.5 (start) and 7.2 (end). As the test is performed at the end of the period, the value to compare with is selected to be 7.2, which means that the significant interference level is lower than 0.4, but higher than 0.25. Therefore, the level reported in the final result table is 0.25.

Environmental Conditions

The protocol states that a temperature test shall be performed:

1. on samples at 1°C and 30°C, the instrument remaining at ambient (lab) temperature
2. on the instrument at 1°C and 30°C. For this experiment, the instrument should be installed in its cabinet.

None of these has been done as time was not available. Further the cabinet was not available either.

4. Final results form the laboratory

Taking the comments given in the previous chapters into account, the final result of the laboratory test can be reported as shown below.

Performance Characteristic TOXcontrol	Unit	Result	
Response^{+}time, Response^{-}time	Min.	30	30
Coefficient of variation (20% $ZnSO_4.7H_2O$) start, end	-	26,7	39,3
Coefficient of variation (50% $ZnSO_4.7H_2O$) start	-	8,7	
Coefficient of variation (80% $ZnSO_4.7H_2O$) start, end	-	5,7	11,8
Limit of Detection (20% $ZnSO_4.7H_2O$) start, end	inhib. %	15,5	7,2
Limit of Detection (50% $ZnSO_4.7H_2O$) start	inhib. %	17,4	
Limit of Detection (80% $ZnSO_4.7H_2O$) start, end	inhib. %	12,1	13,6
Limit of Quantification (20% $ZnSO_4.7H_2O$) start, end	inhib. %	51,6	24,1
Limit of Quantification (50% $ZnSO_4.7H_2O$) start	inhib. %	57,9	
Limit of Quantification (80% $ZnSO_4.7H_2O$) start, end	inhib. %	40,5	45,2
Lowest Detectable Change (20% $ZnSO_4.7H_2O$) start, end	inhib. %	15,5	7,2
Lowest Detectable Change (50% $ZnSO_4.7H_2O$) start	inhib. %	17,4	
Lowest Detectable Change (80% $ZnSO_4.7H_2O$) start, end	inhib. %	12,1	13,6
Repeatability (20% $ZnSO_4.7H_2O$) start, end	inhib. %	5,2	2,4
Repeatability (50% $ZnSO_4.7H_2O$) start	inhib. %	5,8	
Repeatability (80% $ZnSO_4.7H_2O$) start, end	inhib. %	4,0	4,5
Short term drift (20% $ZnSO_4.7H_2O$)	inhib%/day	-0,8	
Short term drift (80% $ZnSO_4.7H_2O$)	inhib%/day	-11,2	
Day-to-day repeatability (20% $ZnSO_4.7H_2O$)	inhib. %	2,5	
Day-to-day repeatability (80% $ZnSO_4.7H_2O$)	inhib. %	31,2	
Memory effect Dichlorphenol	diff inhib. %	6,6	No
Memory effect $ZnSO_4.7H_2O$	diff inhib. %	9,0	No
Interference caused by: Tropaeolin O	mg/l	0,25	Yes

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	TimeStamp	Alarm	Cor. Factor	Incub. time	Lumi Ref. T1	Lumi. Ref. T0	Lumi. Ref. T1 (Test)	Lumi. Sample T0.	Lumi. Sample T1.	Lumi. Sample T1. (Test)	Temp. Bacteria vessel	Temp. Incubation unit	Toxicity	Toxicity (Test)
2											°C	°C	%	%
3	21-feb-2007 12:13:05	0	0,92	15	1718713	1865164		2405699	2002327		6,0	15,0	9,7	
4	21-feb-2007 12:44:53	0	1,01	15	1229415	1214891		1551438	1409363		5,5	14,9	10,2	
5	21-feb-2007 13:16:38	0	1,05	15	1013863	962675		1222469	1190075		5,9	14,9	7,6	
6	21-feb-2007 13:48:23	0	0,99	15	871953	880271		1134025	1112994		5,4	15,8	0,9	
7	21-feb-2007 14:20:24		1,04			637813	661043	821331		44327	5,6	15,0		94,8
8	21-feb-2007 14:52:09	0	1,13	15	802174	708810		857645	929842		5,9	15,6	4,2	
9	21-feb-2007 15:23:54	0	1,28	15	763411	597535		802372	909791		5,4	15,7	11,2	
10	21-feb-2007 15:55:38	0	1,17	15	631519	540671		693184	26123		5,9	14,9	96,8	
11	**21-feb-2007 16:27:23**	1	0,72	15	348853	486041		624368	22911		5,5	15,8	94,9	
12	**21-feb-2007 16:59:07**	1	0,63	15	226085	361710		466325	10903		5,4	15,3	96,3	
13	21-feb-2007 17:32:53		0,72			435151	312566	550355		57179	5,7	15,0		85,5
14	**21-feb-2007 18:04:39**	1	0,71	15	234021	329578		425803	12287		5,9	14,9	95,9	
15	**21-feb-2007 18:36:24**	1	0,75	15	336761	448365		584063	22256		5,5	14,9	94,9	
16	**21-feb-2007 19:08:08**	1	0,78	15	323998	417192		547337	333729		5,9	14,9	21,5	
17	21-feb-2007 19:39:52	0	0,95	15	401017	423803		553971	444813		5,9	14,9	15,1	
18	21-feb-2007 20:11:36	0	1,05	15	434159	415377		543562	475891		5,4	15,6	16,2	
19	21-feb-2007 20:43:35		1,12			401513	448173	525483		91819	5,4	15,0		84,3
20	21-feb-2007 21:15:20	0	0,93	15	279167	299671		392561	394611		5,4	15,6	-7,9	
21	21-feb-2007 21:47:04	0	1,17	15	470461	403541		522644	148245		5,9	15,0	75,7	
22	**21-feb-2007 22:18:48**	1	1,10	15	439818	400469		521264	156667		5,3	15,5	72,6	
23	**21-feb-2007 22:50:32**	1	1,07	15	506607	475354		611881	172387		5,9	14,7	73,6	
24	**21-feb-2007 23:22:14**	1	1,03	15	379375	369550		465410	149717		5,8	15,5	68,7	
25	21-feb-2007 23:54:10		1,08			346076	372898	476404		101449	5,8	14,9		80,2

Raw Result file from Monitor incl. extra headings and color coding.

Operation conditions (documented in hidden columns)

Incubation time	15 min.	Col.D
Bacteria vessel temp.	5.3 - 6.0 de	Col.K
Incubation temp:	14.6-15.6 d	Col.L

Ref. Standard every 6th measurement

Ref. Standard conc. after dilution: 10 mg/l Zn (~ 44 mg/l Zincsulfate, heptahydrate)

Color coding of type of sample

- 2,5 mg/l Zincsulfate, heptahydrate ("20 % inhibition")
- 12,5 mg/l Zincsulfate, heptahydrate ("50% inhibition")
- 25 mg/l Zincsulfate, heptahydrate ("80 % inhibition")
- 50 mg/l Zincsulfate, heptahydrate ("2*80 % inhibition")
- Standard from microLAN
- 2,5 mg/l Zincsulfate, heptahydrate + interferent (Tropaeolin O)
- 2,5 mg/l Zincsulfate, heptahydrate + interferent (Diatomeus earth)
- 2 mg/l 3,5 Dichlorophenol (memory effect "20 % inhibition")
- 12 mg/l 3,5 Dichlorophenol (memory effect "2*80% inhibition")
- Tap Water (idle operation)
- Change of sample - result not used
- Obvious outlier - result not used
- If Toxicity marked red it is assessed as unuseable

	Reference (Tap water) line for calculation of f_{kt} $f_{kt} = I_{kt}/I_0$				Sample/ref.std. line for calculation of H_t (Toxicity of sample and ref.std.) $H_t = 100 * (I_{ct} - I_{Tt}) / I_{ct}$, where $I_{ct} = f_{kt} * I_{T0}$					Comments of the day
Heading ISO 11348-1	f_{kt}	I_{kt}	I_0	I_{kt} (Test)	I_{T0}	I_{Tt}	I_{Tt} (Test)	H_t	H_t (Test)	
TimeStamp	Corr. Factor	Lumi Ref. T1	Lumi. Ref. T0	Lumi. Ref. T1 (Test)	Lumi. Sample T0.	Lumi. Sample T1.	Lumi. Sample T1. (Test)	Toxicity	Toxicity (Test)	
21-feb-2007 12:13:05	0,92	1718713	1865164		2405699	2002327		9,7		New bacteria and syringes. Effort: 10h
21-feb-2007 12:44:53	1,01	1229415	1214891		1551438	1409363		10,2		
21-feb-2007 13:16:38	1,05	1013863	962675		1222469	1190075		7,6		
21-feb-2007 13:48:23	0,99	871953	880271		1134025	1112994		0,9		
21-feb-2007 14:20:24	1,04		637813	661043	821331		44327		94,8	
21-feb-2007 14:52:09	1,13	802174	708810		857645	929842		4,2		
21-feb-2007 15:23:54	1,28	763411	597535		802372	909791		11,2		
21-feb-2007 15:55:38	1,17	631519	540671		693184	26123		96,8		
21-feb-2007 16:27:23	0,72	348853	486041		624368	22911		94,9		
21-feb-2007 16:59:07	0,63	226085	361710		466325	10903		96,3		
21-feb-2007 17:32:53	0,72		435151	312566	550355		57179		85,5	
21-feb-2007 18:04:39	0,71	234021	329578		425803	12287		95,9		
21-feb-2007 18:36:24	0,75	336761	448365		584063	22256		94,9		
21-feb-2007 19:08:08	0,78	323998	417192		547337	333729		21,5		
21-feb-2007 19:39:52	0,95	401017	423803		553971	444813		15,1		
21-feb-2007 20:11:36	1,05	434159	415377		543562	475891		16,2		
21-feb-2007 20:43:35	1,12		401513	448173	525483		91819		84,3	
21-feb-2007 21:15:20	0,93	279167	299671		392561	394611		-7,9		
21-feb-2007 21:47:04	1,17	470461	403541		522644	148245		75,7		
21-feb-2007 22:18:48	1,10	439818	400469		521264	156667		72,6		
21-feb-2007 22:50:32	1,07	506607	475354		611881	172387		73,6		
21-feb-2007 23:22:14	1,03	379375	369550		465410	149717		68,7		
21-feb-2007 23:54:10	1,08		346076	372898	476404		101449		80,2	

ANNEX2

Results Lab. Test TOX-Control				$ZnSO_4 7H_2O$																				Inteference		Memory Effect	
Date	Tasks	"Conc." (inhibtion)	Period : Day	inhibi% 1	inhibi% 2	inhibi% 3	inhibi% 4	inhibi% 5	inhibi% 6	Avg. inhib.%	Stddev. inhib.%	CoV -	LOD inhib.%	LOQ inhib.%	LDC inhib.%	Rep. inhib.%	Day to Day rep. inhib.%	Short term drift inhib% / day	Diff. inhib.%	yes/no	Diff. inhib.%	yes/no
# 21-02	Memory effect, LOD, LOQ, LDC, CoV, Repeatability, Day to day repeatability, Short term drift	20%	1 : 1	9,7	10,2	7,6		4,2	11,2	8,6	2,8	32,6	8,4	28,0	8,4	2,8					9,0	Yes: If Diff > LDC
		2*80%		96,8	94,9	96,3	95,9	94,9		95,8	0,8											
		20%		21,5	15,1	16,2				17,6	3,4											
		80%		75,7	72,6	73,6	68,7	66,2	66,1	70,5	4,0	5,7	12,1	40,5	12,1	4,0						
22-02	LOD, LOQ, LDC, CoV, Repeatability, (Day to day rep., Short term drift)	20%	1 : 2	4,9																		
		50%		27,2	31,6	30,2	30,6	28,5	26,9	29,2	1,9	6,6	5,8	19,2	5,8	1,9						
27-02	LOD, LOQ, LDC, CoV, Repeatability	20%	1 : 7	9,8	10,9	12,7	10,8	12,9	12,5	11,6	1,3	10,8	3,8	12,5	3,8	1,3						
		80%		45,2	41,7	37,9	37,7	34,2	33,1	38,3	4,5	11,8	13,6	45,2	13,6	4,5						
# 01-03	LOD, LOQ, LDC, CoV, Repeatability, Day to day repeatability, Short term drift	20%	2 : 1	31,9	35,4	29,9	36,7	34,8	33,6	33,7	2,5	7,4	7,4	24,8	7,4	2,5	Based on 80%					
		50%		71,5	55,4	64,5	67,5	68,6	69,9	66,2	5,8	8,7	17,4	57,9	17,4	5,8						
		80%		84,2	83,3	84,1	81,8	82,1	78,4	82,3	2,1	2,6	6,4	21,5	6,4	2,1						
02-03	Day-to-day rep., Short term drift	20%	2 : 2	7,1																		
03-03	LOD, LOQ, LDC, CoV, Rep., Day to day rep., Short term drift	20%	2 : 3	14,4	7,6	4,0	0,9	-6,5	-1,3	3,2	7,3	229,1	21,8	72,8	21,8	7,3	31,2	-11,2				
		80%		36,7	34,9	31,9	36,3	37,5	35,2	35,4	2,0	5,6	5,9	19,7	5,9	2,0						
04-03	Day-to-day rep., Short term drift	20%	2 : 4	17,5																		
05-03	Day-to-day rep., Short term drift	20%	2 : 5	3,2																		
06-03	LOD, LOQ, LDC, CoV, Rep., Day to day rep., Short term drift	20%	2 : 6	-2,1	0,1	6,1	5,2	2,3	-2,2	1,6	3,6	228,4	10,8	35,8	10,8	3,6						
		80%		24,1	24,4	25,4	23,4	23,8	18,2	23,2	2,5	10,9	7,6	25,4	7,6	2,5						
# 07-03	Inteference Tropaeolin O - abs 0.1 at 490 nm (1mg/l)	20%	3 : 1	11,9	15,9	18,1				15,3	3,1											
		20% + 1		31,6	34,7	21,9	25,0	23,1	25,7	27,0	5,0								11,7			
		20%		19,4	16,1	20,1	11,2	24,8	24,4	19,3	5,2	26,7	15,5	51,6	15,5	5,2						
13-03	LOD, LOQ, LDC, CoV, Rep.	20%	3 : 7	5,5	6,3	2,9	8,0	9,6	4,6	6,1	2,4	39,3	7,2	24,1	7,2	2,4						
# 15-03	Inteference Tropaeolin O - abs 0.1 at 490 nm (1mg/l)	20%	4 : 1	13,0	9,6	9,6	10,4			10,7	1,6						Based on 20%				Yes: If Diff. > LDC	
		20% + 1		27,1	23,8	22,1	23,9	22,6	32,0	25,2	3,7								14,6			
16-03	LOD, LOQ, LDC, CoV, Rep.	20%	4 : 2	11,3	12,0	11,1	8,4	11,7	12,4	11,2	1,4	12,8	4,3	14,2	4,3	1,4						
20-03	Inteference Tropaeolin O concentrations used 0.8, 0.25 and 0.4 mg/l	20%	4 : 6	8,2	6,5	8,3				7,7	1,0						2,5	-0,8				
		20% + 0.8		21,6	21,1	20,0	20,6	19,6	22,0	20,8	0,9								13,1			
		20% + 0.25		12,8	10,6	12,0	8,5	12,4	10,6	11,2	1,6								3,5			
		20% + 0.4		15,0	15,1	15,7	16,4	14,3	14,8	15,2	0,7								7,5			
21-03	LOD, LOQ, LDC, CoV, Rep.	20%	4 : 7	6,3	7,4	6,7	4,7	5,2	5,8	6,0	1,0	16,5	3,0	9,9	3,0	1,0						

#: Bacteria culture changed

Results Lab. Test TOX-Control		3,5 Dichlorophenol								Avg.	Stddev.	Memory Effect		LDC
Date	Tasks	"Conc." (inhibtion)	Period : Day	inhib% 1	inhib% 2	inhib% 3	inhib% 4	inhib% 5	inhib% 6	inhib.%	inhib.%	Diff. inhib.%	yes/no	inhib.%
02-03	Memory effect - 3,5 Dichlorphenol	20%	2 : 2	64,3	65,3	72,8				67,5	4,7	6,6	Yes: If Diff > LDC	14,0
		2*80%		93,1	93,5	93,8	94,7			93,8	0,7			
		20%		71,1	76,2	74,9				74,1	2,6			

Annex E2: Test Report – Field test

TESTNET Workpackage 3

Test case 1a: TOXcontrol

Test Report

Field Tests

Manufacturer:	**microLAN**	**Doc. Version**
Verification Institute:	**EXERA**	**2007-11-28**
Testing Lab.:	**KIWA**	**Final**

1. Materials and Methods

The field test has been performed at a water intake over a period of two months with the same monitor as used in the lab test, but now installed in its cabinet and checked by the manufacturer before installation. Reference standard and bacteria culture was delivered by the manufacturer and the biomonitor was fed with water samples using the existing sampling system of the monitoring station.

Figure 1.1: Setup of instrument at monitoring station

Spiking technique using zinc sulfate shal be used for determination of the response time and the longterm drift as described in the Verification protocol. For the response time, the spiking concentration being approximately the concentration of zinc sulfate giving 80% inhibition level, and for the long term drift two measurements per week during 8 weeks will be carried out on samples and spiked samples with zinc sulfate at 20% inhibition factor

2. Results and Comments

The biomonitor measured for a period of 62 days at the monitoring station. However, it was not possible to carry out spiking experiments with zinc sulphate solutions during this period, and therefore the only computable results from the field test will be those giving the information concerning availability and up-time.

3. Datahandling and Performance Characteristics

Response time
The response time was not measured using spiked samples. However, the response time can be calculated as the sum of the TOXcontrol biomonitors measuring cycle + the retention time of the sample in the sampling line at the monitoring station, meaning that the response time always will be greater than 30 minutes.

Long Term Drift
N/A. Figure 3.1 shows the biomonitors response as measured thelast month of the field test period. The variation in the Toxicity shows the same nature as in the lab test.

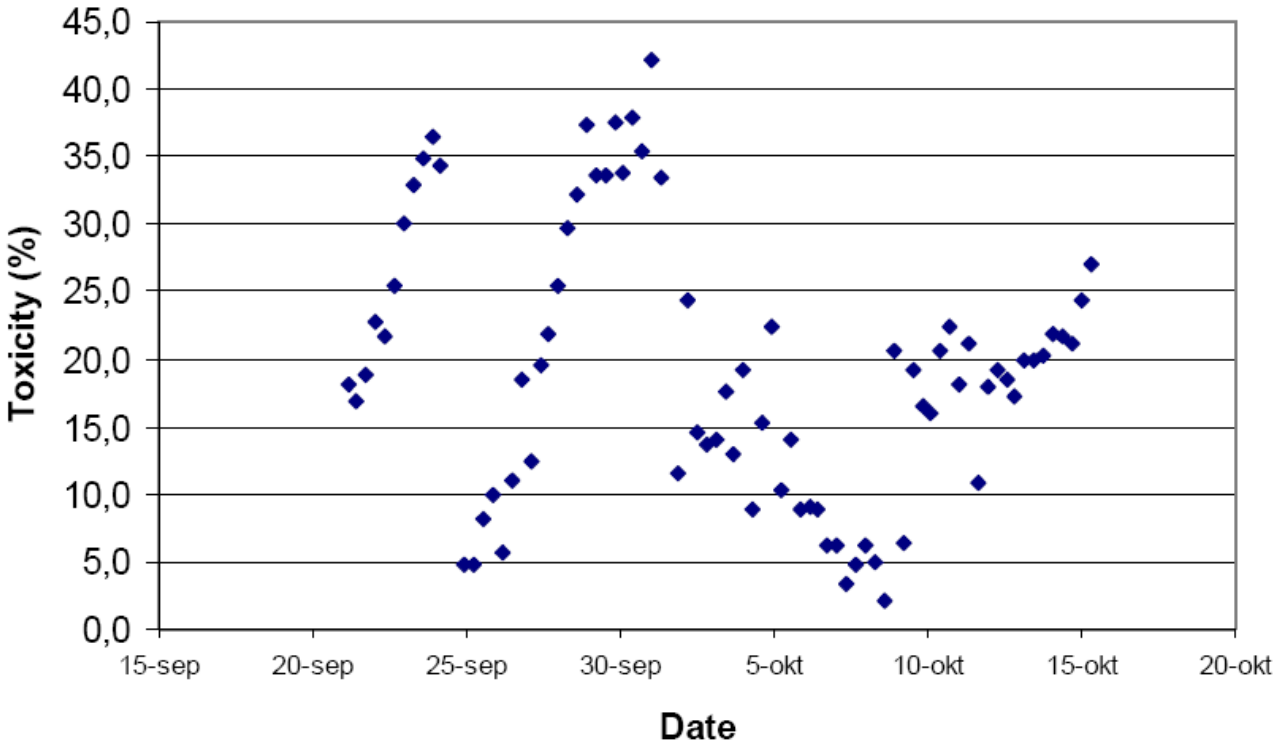


Figure 3.1: TOXcontrol response in the last month of the field test

Availability and Up-Time
As the scheduled maintenance is 4 hours pr. week, the availability becomes 97.6 %. The up-time was estimated from unusable measurements due to low luminescence levels, and separately to out-of-range temperature of the bacteria suspension. As this occurred during 25 days in total of the 62 day the biomonitor was operating at the water intake, the up-time becomes 59.7 %.

4. Final results from the field

Taking the comments given in the previous chapters into account, the final result of the tests can be reported as shown below.

Performance Characteristics TOXcontrol	Unit	Result	Effect
Response time for positive change, $t_{Response}^{+}$	Min.	>30	
Response time for negative change, $t_{Response}^{-}$	Min.	> 30	
Bias based on (absolute/relative) differences	mg/l	N/A	
Long term drift (% of working range/day)	%/day	N/A	
Availability	%	97,6	
Up-Time	%	59,7	

Annex F: Verification Report

EU-ETV VERIFICATION REPORT

TOXcontrol Biomonitor for detection of toxicity in drinking or surface water

Manufacturer: microLAN BV Mr.V. Coothstraat 60 PO box 644
5141 ET Waalwijk Netherlands
Tel: +31416540775 Fax: +31416540776
info@microlan.nl / http://www.microlan.nl/

Verification Institute: EXERA

Author: Di Benedetto Dominique

date: November 2007

Introduction

This report describes the results obtained from the verification of the TOXcontrol Biomonitor manufactured by microLAN BV in the Netherlands, and devoted to the detection of toxicity in drinking, surface and waste water. Only drinking or surface water was concerned by this verification. The verification was set up by applying the verification scheme described in the TESTNET project. The scheme with a Verification Institute (EXERA) and a test laboratory (DHI) was selected. The manufacturer did not choose the Verification Institute and the test laboratory: this was the only difference with a ”normal” verification procedure.

Technology description

The TOXcontrol biomonitor uses freshly cultivated light emitting bacteria (Vibrio Fischeri) as a biological sensor. It is an automatic instrument used for on-line measurement of toxicity in water. The instrument can run unattended for one week. It can be considered as an automatic version of the ISO 11348-1 standard describing the manual measurement of toxicity. The intensity of luminescence is measured simultaneously in the sample and in a reference (pure water) at time t=0 - when mixing the sample and reference with the bacteria suspension - and at time t=15 min. The presence of toxic material in the sample leads to an inhibition of luminescence, which can be compared to the variation of luminescence in the reference by applying a correction factor. One measurement is performed every 30 minutes. The results are calculated from the corrected light loss and given in % inhibition. The instrument is controlled by a computer. Detailed technical description can be found on the web site of the manufacturer: http://www.microLAN.nl
The bacteria are obtained from a bioreactor provided by the manufacturer. The verification of this bioreactor was not envisaged in the protocol.

The verification process

The verification process is based on the verification scheme adopted by TESTNET for the setting up of a European Environmental Technology Verification system (EU-ETV). The process including a Verification Institute (VI) was selected. A short description of the verification process is given below; for a detailed description see references [1] and [2].

- Verification Institute fills out a Quick scan form
- Verification Institute, test laboratory and stakeholders - the Board of Experts - prepare a verification protocol
- Task group (VI+laboratory+manufacturer+2/3 stakeholders) suggests tests
- Test laboratory develops a test plan
- Laboratory performs tests and writes a test report
- Verification Institute writes a Verification Report and send it to TVO
- TVO awards logo

The verification protocol

The verification protocol [3] is based on two ISO standards:

- A “generic” standard, ISO 15839:2003 “Water Quality – on-line sensors/analysing equipment for water - specifications and performance tests”
- A more specific standard ISO 11348-1:2004 “Water Quality – Determination of the inhibitory effect of water samples on the light emission of Vibrio Fischeri (Luminescent bacteria test) – Part 1: Method using freshly prepared bacteria”.

Agenda

A draft version of the protocol based on the two standards was prepared by the Verification Institute and proposed to the Board of Experts on January 15, 2007.
The verification protocol was adapted for biomonitoring, and some specifications could not be verified, as for instance linearity and bias.
The test laboratory (DHI) prepared a test plan and submitted the test plan to the task group.
The laboratory tests were performed at DHI during March 2007 and then the instrument was installed at a KIWA site for the field tests.
The test report was delivered on September 2007 by DHI. Only results of laboratory tests are described in the test report.

Test results

Test results (laboratory only) are given in the test report produced by DHI. This report must be considered as a whole. In this verification report, only significant figures are described.
This instrument can be considered as an Early Warning system for the detection of toxicity in water, rather than an analyser. Most of the performance characteristics applying to on-line sensors/analysing equipment described in the ISO 15839 standard are not given in the manufacturer’s manual and documentation.
The instrument was delivered without its cabinet, which should not cause problems for laboratory tests, except for the effect of ambient temperature variation.
The manual can be considered as a draft, containing calculation errors and spelling mistakes.

Laboratory tests
The laboratory tests were performed according to the test plan developed by the laboratory, the instrument being considered as a “black box”[1].
A table of performance characteristics is given in annex. Some results should be considered with care (remembering that no values are given by the manufacturer), as the intensities of luminescence varied a lot, even when taking account of the “natural” decrease of luminescence – 90% - during a week of unattended operation. Moreover, statistical computation can be applied only when results are not dispersed too much: thus the limit of quantification given in the table is not realistic.

[1] Using the « black box » model, the raw results are gathered until the tests are finished, which can lead to problems in case of instrument default or malfunction.

Some performance characteristics, which should have been calculated from the 20% inhibition level (2.5mg/l Zn), are not given, probably because of the large variations of luminescence intensity[2].
If outliers are removed from reference data (zinc solution at 10mg/l), a significant negative drift can be observed on the inhibition=f(time of laboratory tests) curve.
Correction factors are often outside the limits (0.6-1.3) given in the ISO 11348-1 standard (NB: A variation of 0.1 of this correction factor leads to a variation of more than 50% of the % inhibition).
The interference due to turbid samples could not be determined, due to leaks in the syringes.
The effect of ambient temperature variation was not performed, as the instrument was delivered without its cabinet.

Field tests
Field tests were performed on a KIWA site - from July 16th to September 21st -with the same monitor installed in its cabinet and checked by the manufacturer before installation. Spiking experiments with zinc sulphate solutions were not performed, and long term drift could not be computed from spiking. A value of 97.6% was obtained for availability, calculated from ISO 15839 standard, and of 59.7% for up-time. Up-time was estimated from unusable measurements due to low luminescence levels, and separately to out-of-range temperature of bacteria suspension.

Recommendations

When considering the results, and as the instrument worked correctly during short periods, some additional tests should be performed on a new instrument provided by the manufacturer, especially the tests at 20% inhibition level. Raw results should be checked in real time. The intermediate values of luminescence should be recorded, as they are available from the computer files. If possible, the concentration of zinc in the sample mixture contained in the syringe should be verified after the measurement, in order to test the homogeneity of the mixture obtained from the dilution step. This homogeneity is insured by a small magnetic stirrer, and as there are sucking-discharge steps realized by the syringes, this can lead to erratic movements of the stirrer, preventing a good mixing of bacteria suspension and sample. Perhaps it would be good to perform some tests on the bioreactor, in order to see if bacteria suspensions prepared from this bioreactor are stable and emit enough light from batch to batch.

Conclusions

The TOXcontrol biomonitor manufactured by microLAN B.V. is a tentative to develop an automatic on-line instrument – adapted from the manual method described in the ISO 11348-1 standard - which can be used as an Early Warning System for the detection of toxicity in

[2] Some uncertainties calculated from the tests are higher than the uncertainties found in the manual method using the same principle (luminescence of Vibrio Fischeri bacteria).

drinking, surface and waste water. The measurement principle follows the manual method. It uses an interesting differential arrangement to detect toxicity in water samples.
The laboratory and field tests revealed some problems preventing a continuous measurement that can be expected for an on-line automatic system. As this instrument is a new marketed one, we think that these problems might be solved by the manufacturer with additional tests, improvements in plumbing design and also in stability of bacteria suspensions.

References

[1]: draft of a European system for Environmental Technology Verification (ETV) KIWA 2007 January.
[2]: TESTNET Scheme with a Verification Institute Version 1 KIWA 15-04-2007
[3]: Verification Protocol: TOXcontrol biomonitor manufactured by microLAN B.V. Di Benedetto for EXERA as Verification Institute January 2007

Dominique Di Benedetto, November 2007

Annex1: Final results from the laboratory

Notes:

- Some results should be considered with care, as the computation of some performance characteristics using the statistical tool should not be used, when dispersion of results is too high: see for example the limit of quantification at 57.9%.
- When the instrument worked properly, it was possible to compute some performance characteristics from results at the 20% inhibition level.

Performance Characteristic TOXcontrol	Unit	Result	
Response^{+}time, Response^{-}time	Min.	30	30
Coefficient of variation (20% $ZnSO_4.7H_2O$) start, end	-	26,7	39,3
Coefficient of variation (50% $ZnSO_4.7H_2O$) start	-	8,7	
Coefficient of variation (80% $ZnSO_4.7H_2O$) start, end	-	5,7	11,8
Limit of Detection (20% $ZnSO_4.7H_2O$) start, end	inhib. %	15,5	7,2
Limit of Detection (50% $ZnSO_4.7H_2O$) start	inhib. %	17,4	
Limit of Detection (80% $ZnSO_4.7H_2O$) start, end	inhib. %	12,1	13,6
Limit of Quantification (20% $ZnSO_4.7H_2O$) start, end	inhib. %	51,6	24,1
Limit of Quantification (50% $ZnSO_4.7H_2O$) start	inhib. %	57,9	
Limit of Quantification (80% $ZnSO_4.7H_2O$) start, end	inhib. %	40,5	45,2
Lowest Detectable Change (20% $ZnSO_4.7H_2O$) start, end	inhib. %	15,5	7,2
Lowest Detectable Change (50% $ZnSO_4.7H_2O$) start	inhib. %	17,4	
Lowest Detectable Change (80% $ZnSO_4.7H_2O$) start, end	inhib. %	12,1	13,6
Repeatability (20% $ZnSO_4.7H_2O$) start, end	inhib. %	5,2	2,4
Repeatability (50% $ZnSO_4.7H_2O$) start	inhib. %	5,8	
Repeatability (80% $ZnSO_4.7H_2O$) start, end	inhib. %	4,0	4,5
Short term drift (20% $ZnSO_4.7H_2O$)	inhib%/day	-0,8	
Short term drift (80% $ZnSO_4.7H_2O$)	inhib%/day	-11,2	
Day-to-day repeatability (20% $ZnSO_4.7H_2O$)	inhib. %	2,5	
Day-to-day repeatability (80% $ZnSO_4.7H_2O$)	inhib. %	31,2	
Memory effect Dichlorphenol	diff inhib. %	6,6	No
Memory effect $ZnSO_4.7H_2O$	diff inhib. %	9,0	No
Interference caused by: Tropaeolin O	mg/l	0,25	Yes

Annex2: Final results from the field

It was during the test period not possible to use spiked samples.

Performance Characteristics TOXcontrol	Unit	Result	Effect
Response time for positive change, $t_{Response}^{+}$	Min.	>30	
Response time for negative change, $t_{Response}^{-}$	Min.	> 30	
Bias based on (absolute/relative) differences	mg/l	N/A	
Long term drift (% of working range/day)	%/day	N/A	
Availability	%	97,6	
Up-Time	%	59,7	

Annex G: Minutes

Minutes of the meeting of Board of Expert of project TESTNET WP3 case 1a "Biomonitoring".
EXERA January 15, 2007.

Objectives:
The working group WP3 of project TESTNET concerning the feasibility of the installation of a European ETV (ETV: Environmental Technology Verification) chose a certain number of technologies on which could be tested the scheme defined for the verification of these technologies. The case 1a "biomonitoring" corresponds to a solution with a "Verification Institute" (VI) which is ensured by the EXERA. The VI wrote a project of protocol intended for the checking of an apparatus of the company microLAN, the TOXcontrol biomonitor. A "Board of Expert" (BoE) was made up. This BoE met in the buildings of the EXERA on January 15, 2007 with the following agenda:

- final proposals for the protocol
- practical tests to be done in the lab and one-site
- meeting of the task group At DHI
- follow-up of the tests
- comments one the operation, improvements at this step
- …

Members of the BOE:

Cahiere Veronique EXERA	Ockier Paul EUCETSA
Cecile Jean-Luc WILL GO	Furrier Claude EXERA
De Hoog Corina KIWA	Quertier François VEOLIA WATER
Di Benedetto Domenica EXERA	Naerssen Edu Van KIWA WP2 leader
Dosset Christian EXERA	Tran-Minh Canh EMSE
Lynggaard-Jensen Anders DHI	Wacheux Herve VEOLIA WATER
Lachenal Jacques LNE	

Apologies for absence: J.L. Cecile, C.De Hoog, J. Lachenal

Christian Dosset welcomes the participants and presents the activity of the EXERA, he gives to the participants a file containing documents relating to TESTNET organization and the description of case 1a, object of the meeting.

Edu Van Naerssen presents the TESTNET project (presentation available at the EXERA). A discussion is committed on various points of the project, before passing to the technical aspect concerning the checking of TOXcontrol biomonitor. Let us quote for example:

- Obligation for the users to use a verified technique: difficulties for the processes for which it is necessary to leave the decision to the user.
- The tests should be chosen by the users: this is carried out by their presence in BoE and the “Task Group ”.
- The producers prefer to have only one European logo.
- During the tests, the producer is informed of the difficulties encountered by his technology.

Do not hesitate to supplement because I could forget points.

TOXcontrol biomonitor must be regarded rather as an alarm (EWS: Early Warning System), and not like an analyzer. It is adapted to raw waters being used for the preparation of drinking water, but it presents little interest for drinking waters of the distribution networks/[network], except the cases of ill will and terrorism, not very probable in Europe. In drinking waters, the contents of toxic substances are indeed very small, and much lower than the limits of detection of the apparatus.

The interest to test this type of on-line apparatus is to confront its specifications with the standards ISO 11348 and ISO 15839 which relate to it.

A discussion is committed on the tests to realize on the apparatus.
The substances to be used will be those of the standard ISO 11348:

- zinc Sulfate (ZnSO4,7H2 O)
- 3,5-dichlorophenol (C6H4OCl2)
- Potassium Dichromate (K2Cr2O7)

The complete system (apparatus + bioreactor) will be tested at the laboratory and on site.
One can consider a test *of interference* with presence of chlorine (test to be defined).
A test of *turbidity* will be carried out with diatomeous earth with increasing concentration to define a threshold for which a modification of the answer obtained with a zinc sulfate solution appears.
Effect of the *color of the sample*: a coloured solution will be used whose absorption band will be in the band of emission of light of the bacteria, provided that the coloured substance has a low and negligible toxicity compared to that of zinc sulfate.

One can draw up a list of the tests being able to be realized on TOXcontrol biomonitor while following the two standards ISO 11348 and ISO 15839:

- *Response time*: conform to the standard ISO 15839; for an apparatus with discontinuous (batch)measurements, it should be checked that this response time is that of time necessary for a measurement.
- *Coefficient of variation*: calculated starting from the standard deviation obtained on 6 measurments carried out to 20%, 50% and 80% of the range of measurement (on the three products?)
- *Limit of detection (LOD) and limit of quantification (LOQ):* calculated starting from 6 measurements carried out with a zinc concentration equal to 20% of the range of measurement.

  LOD: 3 times the standard deviation
  LOQ: 10 times the standard deviation

- *Repeatability*: standard deviation calculated on 6 measurements with 20% and 80% of the range from measurement for zinc.
- *Smaller detectable change*: 3 times the calculated standard deviation with 20% and 80% of the range of measurement
- *Short-term Drift*: slope of the straight regression line built starting from 6 measurements with 50% of the range of measurement, also distributed between two maintenance actions.
- *Effects of memory*: to follow the standard ISO 15839
- *Operating conditions and environment*: One can consider tests to highlight effects on the answer caused by the temperature of the sample and the ambient temperature and moisture of the site where the apparatus is installed. These effects will be carried out with zinc solutions at a concentration equal to 50% of the range of measurement. Tests to be defined by the task group.

It will be requested from the manufacturer to make appear in his documentations of information on the parameters of operation like electric and reagent consumption , waste…

A test with *aldicarb* (pesticide) with a concentration close to the LOQ can be considered: it will be discussed within the Task Group .
Interferences from chlorine, color, turbidity: the task group will specify the conditions, but proposals can already be sent by mall to Dominique Di Benedetto.

The tests on site could be carried out on a KIWA site, with the agreement of Corina de Hoog. One can envisage a test using a method of zinc additions (spiking) to a concentration equal to 5 times the LOQ.
One can carry out the tests envisaged in the standard ISO 15839 for the tests on site.

Anders Lynggaard-Jensen hopes that the apparatus will be delivered the first days of February.
If Corina accepts that the tests on site are carried out at KIWA, a meeting of the Task Group could be organized on the site February the 7, or 8 2007. The experts of VEOLIA WATER could take part voluntarily in the meeting.

D. Di BenedettoEXERA, January 20, 2007.

Task Group meeting Case 1a
7 February 2007 at DHI, Aarhus, Denmark

Agenda:

1. Welcome and short introduction of the participants.
2. Presentation of the ToxControl Monitor.
3. Evaluation of the suggested protocol concerning practical details
4. Decision/approval of tests to be done
5. Possibilities for having field tests done by KIWA at monitoring station at water intake
6. Draft plan for lab.tests and field test (final plan in the minutes from the meeting)

Participants:

Corina De Hoogh, KIWA	
Dominique Di Benedetto, EXERA	
Joep Appels, microLAN	
Ida Rasmussen, DHI	
Niels Eisum, DHI	
Anders Lynggaard-Jensen, DHI	

SUMMARY OF MEETING sent as e-mail 14-02-2007

1. Welcome and short introduction of the participants.

Beside the introductions we also went through the flow chart for the verification procedure (A1).

2. Presentation of the ToxControl Monitor.

Joep presented the TOXcontrol monitor - powerpoint and live - as it was installed in the lab. the day before the meeting. Joep is going to present the TOXcontrol monitor at a WFD workshop in Lille, France (Announcement attached) - suggests to make a poster describing the test so he can present that at the workshop - all participants at the workshop are expected to be stakeholders - more or less... (A2)

3. Evaluation of the suggested protocol concerning practical details
Domonique went through the present version of the protocol - discussions had focus on adapting reading/measurement to the ISO15839 definition, calculation of the inhibition, interferences....

4. Decision/approval of tests to be done
From the above discusions the Task Group (ie. the VI, the testing lab and the producer) agreed on the tests to be done. Dominique will from this prepare the final protocol (A3)

5. Possibilities for having field tests done by KIWA at monitoring station at water intake
Corina told that this indeed will be possible, so we focused on the practical issues like transfer of budget (A4), the exact location for the field tests and organisation at KIWA (A5), the workshop which we have discussed to take place in connection with the field tests (A6)

6. Draft plan for lab.tests and field test (final plan in the minutes from the meeting)
Overall time schedule confirmed - meaning that we will end close to the expected deadline (end of May). Detailed and final test plan wil be made after receiving final protocol from Dominique (A7), but it has already been agreed that DHI will deliver the monitor to KIWA before the middle of March - pack everything in a car and drive to Netherlands and help instaling it at the field test site.

ACTIONS:

A1: Anders. Comments and suggestions for changes were recorded on prints of the flow chart. It is difficult to report these in text, so this will be done by editng the original flow chart files and returning these to Edu. These edited charts including comments is in fact also a part of the reporting from the test case and will therefore also be included in the deliverable.

A2: Anders. This action is concerned with visibility of TESTNET at the potential stakeholders. Anders provide input from the case for the poster and take contact to Ademe, who is the lead partner for dissemination - and therefore is suggested to include some general information as well and produce the poster.

A3: Dominique. Protocol adjustments to be done in the version presented at the meeting and sent to Task Group asap. (Already done :-))

A4: Anders. As KIWA is already a partner, this action is just to make a budget transfer from one partner to another. We agreed to transfer 1 personmonth and adaquate amount of expenses (remember that it is 50% money only - KIWA will have to find the co-financing themselves). Contact Uwe (as WP-leader) and Berrie (as project coordinator) to get

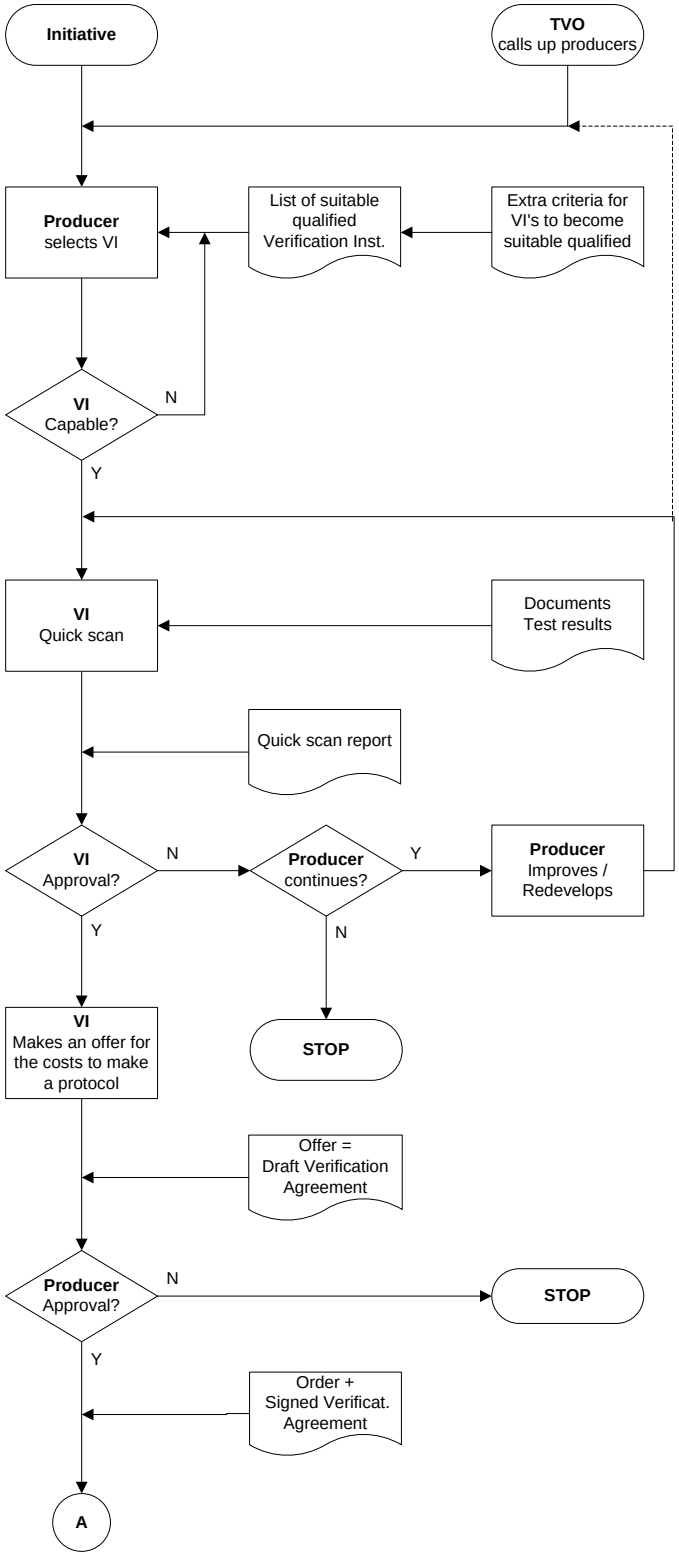
the changes into the budget and provide the justification to the SO (in the next annual report ?)

A5: Corina. Fix the location for the field test and send descripton of the site - including which other measurements/analyses already available - to the Task Group. (Dominique will include it in the Verification protocol). Look into KIWA co-financing issue and allocate the resources to do the field test. - starting middle of March and conclude early May.

A6: Anders. Originally we have suggested to have a workshop at KIWA in connection with the start or during the field tests in order to get stakeholders from the biomontoring society involved more. Due to the very short notice - we will not be able to attract enough people - instead it is suggested to have a workshop in the end of September - beginning of October with a focus on water monitoring and especially surface water monitoring. The heading for the workshop shall primarily point to water monitoring and secondarily to tests and verification in a future European ETV system. Further, it shall be arranged so interested people (the stakeholders) can see it as a continuation of their existing co-operation within the international water monitoring society. It was agreed that Anders suggests this workshop to be arranged as one of the originally planned "regional stakeholder" meetings (in fact the Task Group regards it as impossible to have a stakeholder meeting with an ETV system as the main issue (and we saw that in Stockholm) - you are not stakeholder of a general verification system, but you might be interested to hear what it can do for your working area - here water monitoring (also pointed out by Paul Ockier in a previous mail). It was also suggested to arrange the workshop together with the Techneau project (which is going to have a workshop like that anyway), and that we invite some key note speakers (also from the US EPA - they have done testing on water monitoring - se also attached brochure for the TTAP system (provided by Joep), which is yet another system). Corina (KIWA) have offered to host the workshop, and we think we will be able to attract 50-100 people (outside TESTNET) for a two day workshop. Anders take contact to responsible partners in TESTNET, in order to get the issue discussed at the TESTNET WP-leader meeting in beginning af March.

A7: Ida. Detailed test plan to be done following the suggestions from ISO15839. Starting date: Monday 19 February. Send to Task Group for final approval (and for Dominique to include in the Verification protocol, which then can be sent to the TVO (Edu)). All suggested chemicals and equipment to be ordered (already done and the training in use of the monitor has been done as well. Further, Joep can follow the tests online via the internet and communicate directly with the lab. (web camera and Skype))

Annex H: Verification scheme to be tested



European ETV Scheme - Verific. Inst.
(flowchart) page 1 (23-08-2006)
START UP procedure

The initiative to start a verification procedure is usually taken by a producer - sometimes on request of a supplier - or a branch organisation.
To enhance a specific technology the Thematic Verification Organisation (TVO) also can call for initiatives.

The producer also can contact the TVO or one of the IRC's to ask for help in selecting the Verification Institute (VI).
Extra criteria are added to the demands of EN 45011 to focus on the quality needed. Compliance to these criteria is audited as well by the national Accreditation body.

The VI asks itself if it has the required knowledge, if it is equipped and capable to develop the Verification protocol and of being the Verificator.
If not it remits the producer to more appropriate VI's.

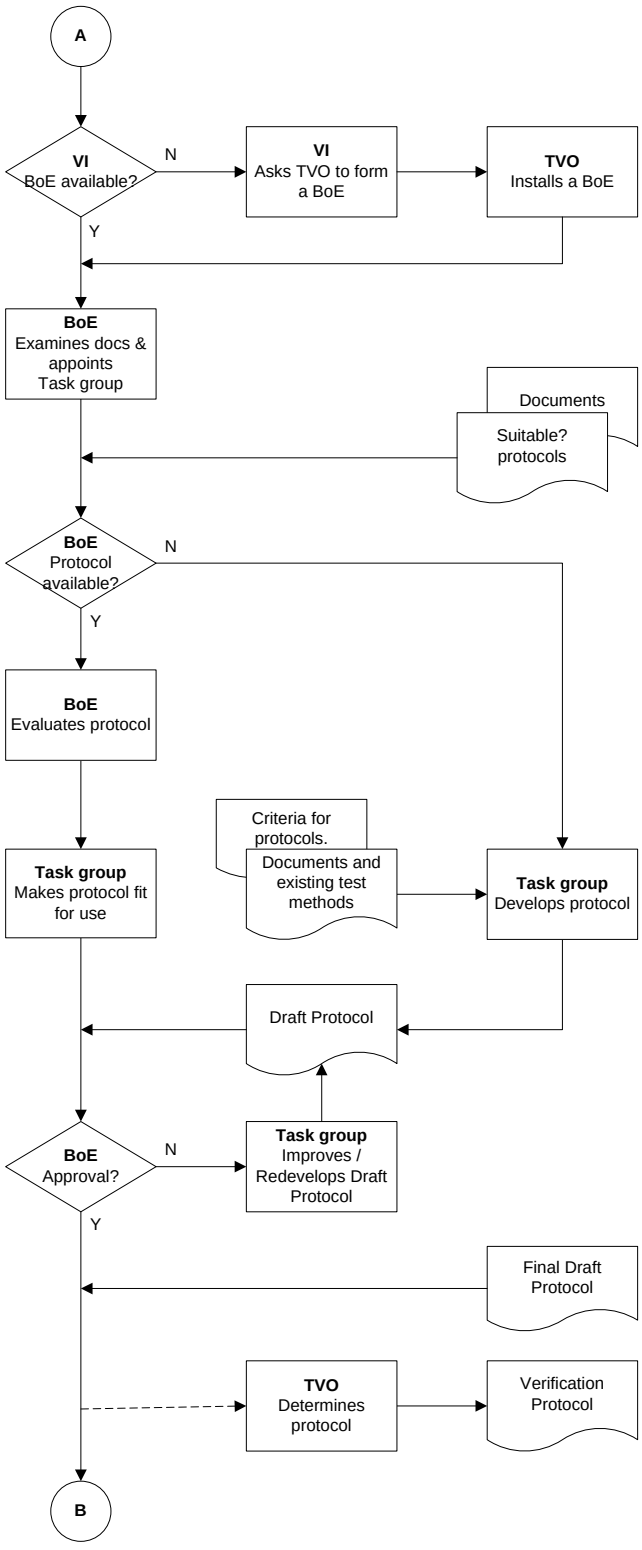
The VI examines if the technology is within the scope, ready to market (or an advanced prototype) and if enough and satisfying test results are available.
The VI sends the Quick scan report to the TVO.

The VI decides wether the process can go on. If not and if the producer wants to continue, he will improve the documentation or even the technology.
He is allowed to look for another VI.

The VI estimates the costs for developing or adjusting a verification protocol.
(The VI has examined if suitable protocols are already available; the VI gives the producer his opinion about the tests that have been done.)
The TVO gets a copy of the offer.

The producer gives an order to the VI based on an agreement about the costs.
Sometimes the order is given by a group of producers or by the branch organisation.
The VI sends a copy of the order to the TVO.

European ETV Scheme - Verific. Inst.
(flowchart) page 2 (23-08-2006)
PROTOCOL procedure



The VI asks, on behalf of the producer, the appropriate Board of Experts (BoE) to make a Verification Protocol. When there is no BoE (yet) for this specific field of technology the TVO forms a BoE.

The BoE invites experts for a (temporary) Task group, also from outside the BoE. The VI chairs the group, the producer and test laboratories who are expected to be charged with the testing are q.q. member.

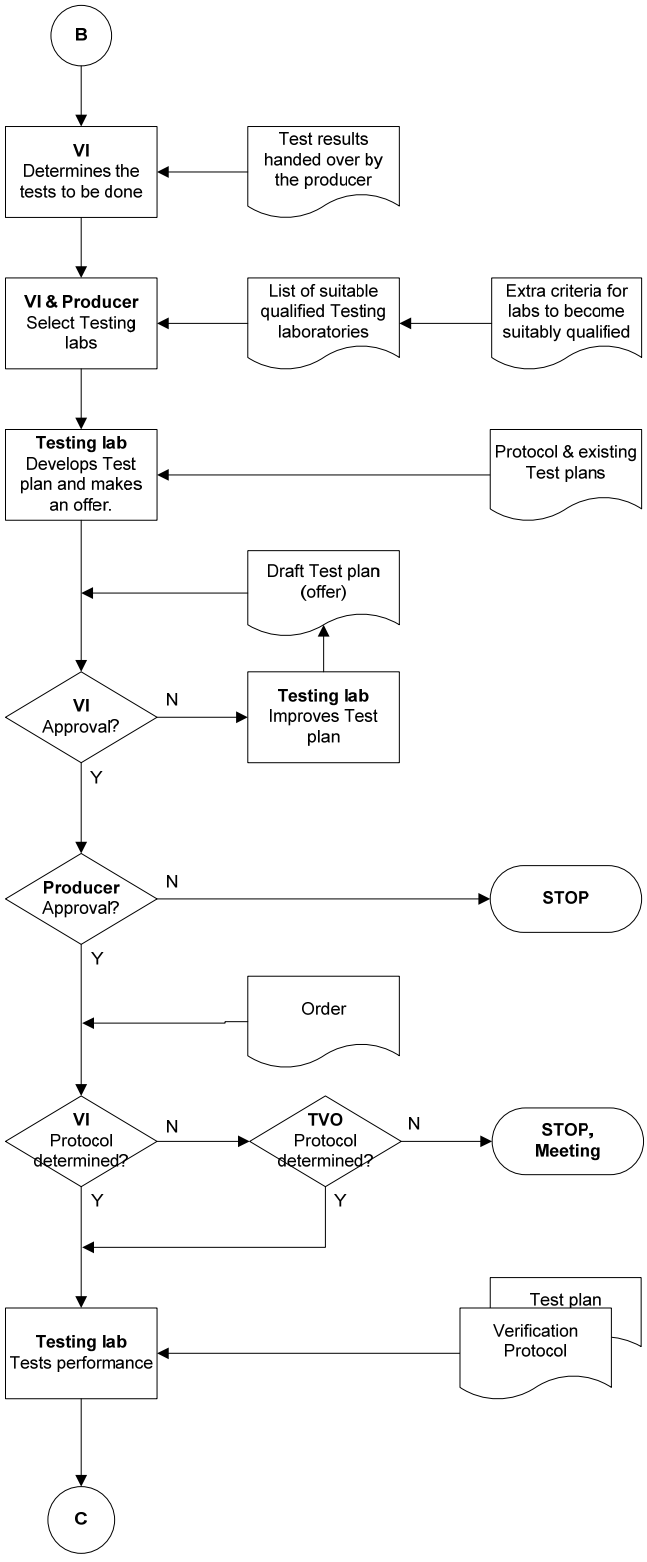
The BoE first of all checks if the protocols available are suitable. When there is no protocol for this type of technology the Task group has to develop one.

Even when a suitable protocol is available, almost always adjustments have to be made to make it fit for the type of technology/apparatus involved.
In case of small adjustments it is not necessary to install a Taskgroup. The BoE approves directly (go to connector B).

Usually the VI in charge makes the concepts, to be discussed and approved in the Task group.
The protocol has to be as complete and detailed as possible, also with respect to the tests to be performed.

The BoE studies and comments the Draft Protocol and will approve it so it becomes a final draft.
In the end the protocol has to be deter mined formally by the TVO. Only exceptionally the TVO will send the protocol back to the BoE for improvement.
To prevent delay meanwhile the testing procedure is started up.

European ETV Scheme - Verific. Inst.
(flowchart) page 3 (23-08-2006)
TESTING procedure



The first step for the VI is to verify if his judgment about the test results (and the test plan they are based on) handed over by the producer has to be adjusted.
The VI determines if and if so which tests have to be repeated/done.

When not all tests can be performed by one Test lab, the VI advises the producer in selecting laboratories. The laboratories who might perform the tests are called Testing labs from now on.
Tendering: in this case more laboratories are invited to make an offer.

The Test plan focuses on quality assurance. Concerning the tests it gives in detail extensions and other deviations of the protocol.

The Test plan forms the main part of the offer; it is the basis for judging the quality and the costs.

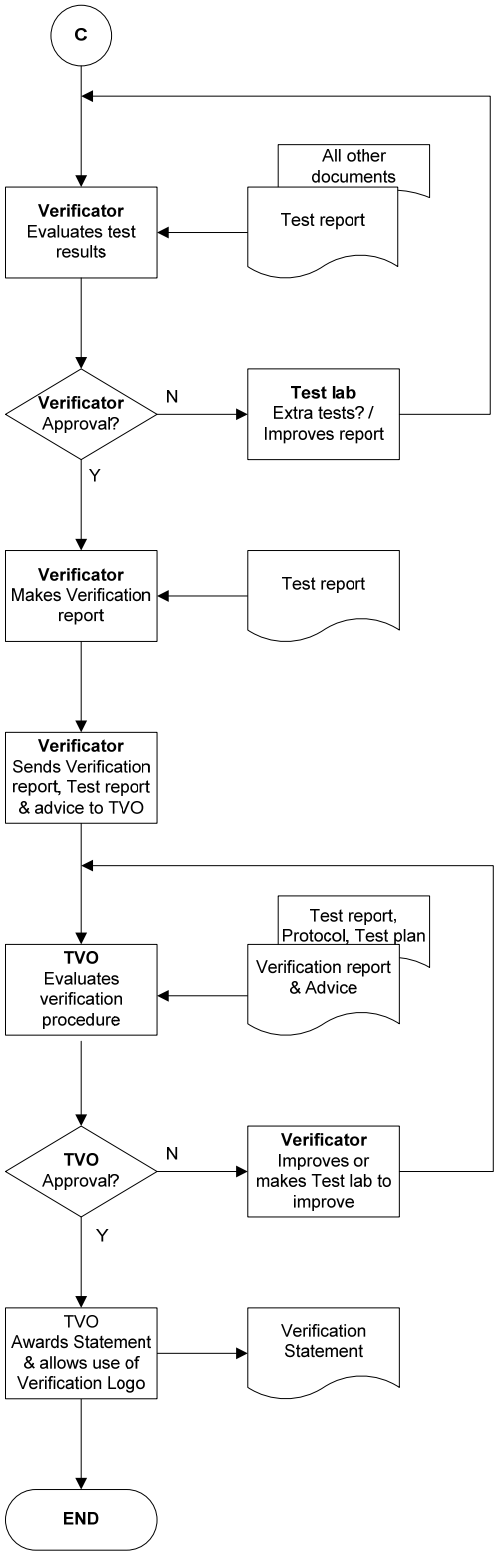
The VI criticises the Test plan together with the producer.
The Task group installed by the BoE stays stand-by to advice during the tendering and the testing procedure in case of problems.

The producer gives an order to the Testing lab based on an agreement about the costs. It is possible that more than one Testing lab gets orders for different tests or even for the same tests.

The VI sends a copy of the order to the TVO.
When the protocol has not yet been determined by the TVO, the VI urges the TVO to make a decision.
In the (exceptional) case the TVO rejects the protocol commissioning is suspended.
In this case a meeting will be arranged to examine what the consequences are:
Start all over again, redevelop the protocol or go on.

From this point on the VI has taken up the role of Verificator.
The Testing lab performs the necessary tests, writes the Test report and submits it to the VI.
The Verificator may be present during the tests performed by the Testing labs.

European ETV Scheme - Verific. Inst.
(flowchart) page 4 (23-08-2006)
VERIFICATION procedure



The VI evaluates the tests performed, prepares a Verification Report and an advice for the TVO.

The Verification Report is a Management Summary based on the Test Report(s), including the judgment and conclusions of the Verificator.

The Verification Statement is basically made up by a diploma (declaration) and the Verification Report.