Hazardous Substances Series

Practical Guidance Document on Whole Effluent Assessment

Whole Effluent

Assessment

OSPAR Commission

2007

The Convention for the Protection of the Marine Environment of the North-East Atlantic (the "OSPAR Convention") was opened for signature at the Ministerial Meeting of the former Oslo and Paris Commissions in Paris on 22 September 1992. The Convention entered into force on 25 March 1998. It has been ratified by Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, Netherlands, Norway, Portugal, Sweden, Switzerland and the United Kingdom and approved by the European Community and Spain.

La Convention pour la protection du milieu marin de l'Atlantique du Nord-Est, dite Convention OSPAR, a été ouverte à la signature à la réunion ministérielle des anciennes Commissions d'Oslo et de Paris, à Paris le 22 septembre 1992. La Convention est entrée en vigueur le 25 mars 1998. La Convention a été ratifiée par l'Allemagne, la Belgique, le Danemark, la Finlande, la France, l'Irlande, l'Islande, le Luxembourg, la Norvège, les Pays-Bas, le Portugal, le Royaume-Uni de Grande Bretagne et d'Irlande du Nord, la Suède et la Suisse et approuvée par la Communauté européenne et l'Espagne.

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Executive Summary

This Guidance Document has been prepared by the Intersessional Expert Group (IEG) in the framework of the OSPAR Hazardous Substances Committee. The task of this working group was to explore the applicability of Whole Effluent Assessment (WEA) and to come forward with a description of a robust WEA tool that is applicable in practice, has an added value for the environment and is acknowledged at an international level by both Contracting Parties as well as industrial organisations; a practical tool, ready to be used for different purposes or in different kinds of policy frameworks.

In order to fulfil this task, the working group reviewed existing tests and techniques, and conducted several studies to improve technology. Two international effluent monitoring programmes were also conducted ('learning by doing'). Several background documents and reports were produced. Finally, results and experiences were used during discussions on cost-efficient yet meaningful test sequences.

This Practical Guidance Document contains the main results and conclusions of the working group. Guidelines for the pre-selection of effluents where WEA may have most added value are presented in Part A. The relevant WEA parameters (being acute and chronic toxicity, liability to bioaccumulate and persistency) are mentioned in Part B. The most optimal test sequence is presented in a WEA flowchart, together with tiered approaches and short cuts for cost-effective application. A toolbox with different tests, methods and protocols for each of the parameters is presented in Part C. Part D closes up with practical assistance.

It has been shown that WEA has an added value compared to the substance approach. Also, tests and protocols are available and used in several countries and by industrial parties. The tool is robust and ready to use. At the same time, development continues and newer, better tests are being introduced. In order to give this Guidance Document a dynamic character, those tests are mentioned in the 'optional' toolbox of this guidance document.

Although further development can (and surely will) take place, this Guidance Document indicates that at this moment WEA is ready for practical application. Decisions on the integration of WEA in different policy frameworks and/or legislation are not in the scope of the OSPAR working group, but of the national governments or the European Commission. This Practical Guidance Document may be used to help in the discussions within the relevant bodies and organisations.

I) Introduction

I-1) The principles and added value of WEA

The *'single substances approach'*[1] has been, and still is, a successful policy instrument for instance used within the OSPAR Strategy and EC frameworks like Water Framework Directive REACH and IPPC. The basis of the *'single substances approach'* is the assessment of Persistency, Bioaccumulation and Toxicity (PBT-criteria) of substances. Those criteria represent the environmental hazard of the substances and are used to derive water quality targets and emission limit values.

The *'single substances approach'* is considered to be effective, but nevertheless it is generally recognised that this approach has some shortcomings. Estimations indicate that at present over 50 000 substances exist in the world. Although a number of programmes are in place to identify substances and to characterise PBTs, there still remain many substances to be assessed and much data to review. Also this approach only addresses those substances deliberately manufactured. This means that there may be possibly harmful substances present in effluents and thus emitted to the environment, from which the risks will never be assessed. Concern that harmful substances may be present but undetected in effluents is supported by research findings, which indicate that only a limited number of the substances present in wastewater, surface water or sediments, can be analysed, identified or quantified. It is also a concern that the substance approach does not take into account the combined effects of different substances that maybe present together in environmental samples.

Those are the main reasons for (ongoing and growing) interest in the development and implementation of PBT-tests that can be applied to entire environmental samples, like effluents, surface water or sediments. This *'whole sample approach'* has already been applied in numerous investigations and these demonstrated that the substances identified by analytical methods could explain only a small proportion of the adverse effects that were measured in the samples. This means that a large fraction of the adverse effects in (waste) water and sediments is caused by "unknown" substances or by combinations of substances [2].

Whole Effluent Assessment (WEA) is the instrument for the *'whole sample approach'* developed for effluents. WEA increases the understanding of the combined effects of all known and unknown substances within effluents, especially in complex mixtures.

The advantages of the concept of WEA have led to the development of WEA tests throughout the world. In several countries (e.g. USA, Canada, Germany, Ireland, Spain, Sweden) some WEA tests are already applied in national legislation or permitting[3]. Within EU - IPPC the WEA concept has been included in monitoring effluent strategies mentioned within several BREFs, like the BREF for the Large Volume Organic Chemicals (2003) and for Organic Fine Chemicals (2006). In most of the countries the regulatory application of WEA is (until now) restricted to the parameter acute toxicity, although other parameters might be included in the future.

I-2) OSPAR and WEA

Within OSPAR the added value of WEA has also been acknowledged. An OSPAR WEA Working Group was initialised in 1999 under the Hazardous Substances Committee (HSC). The OSPAR Working Group has members representing both Contracting Parties and industrial organisations (see Annex 1 for members).

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1 Single substances approach is also referred to as 'chemical specific approach' or 'substance by substance approach'.

2 See also 'OSPAR WEA report Practical Study 2003', Gerritsen et al, 2004.

3 Several different abbreviations co-exist with WEA. In the UK 'Direct Toxicity Assessment (DTA), in the USA 'Whole Effluent Testing' (WET).

The task of the OSPAR WEA working group was to explore the added value and the practical applicability of WEA in identifying 'effluents of possible concern'. Within OSPAR's strategy this identifying currently is based on the single substances approach and WEA might be a useful, additional tool.

The scope of the working group within OSPAR is as follows[4]:

a) The application of WEA should primarily be directed to complex effluents, which contain complex mixtures of chemicals or require very detailed process specific knowledge to allow characterisation. For those effluents WEA has the most added value.

b) The WEA tool should focus on potential for long-term adverse effects (like persistency, chronic (and acute) toxicity and liability to bio-accumulate), since OSPAR is concerned with the protection of the marine environment.

c) The working group was to come forward with a description of a robust WEA tool that is applicable in practice, has an added value for the environment and is acknowledged at an international level by both Contracting Parties as well as industrial organisations. A practical tool, ready to be used for different purposes or in different kinds of policy frameworks.

d) This practical tool might be integrated into European or national policy frameworks and legislation. However, decisions on this matter are not in the scope of the OSPAR working group, but of the national governments or the European Commission[5].

The working group did not conduct scientific or fundamental research, but combined and discussed already existing tests and experiences. Some tests were technically optimised and two large practical effluent monitoring studies were conducted ('learning by doing'). This has resulted not only in practically sound WEA tests, but also in the common experience with WEA and a better understanding of the advantages and added value of the WEA approach.

I-3) This WEA Guidance Document

The Guidance Document reflects the shared opinion of experts from Contracting Parties and industry, working for the past 7 years in the IEG on WEA under the HSC of OSPAR[6]. It gives the current state of not only the relevant WEA parameters and internationally accepted (or still emerging) WEA tests, but also describes optimal test sequences and practical aspects. The ambition is to stimulate the practical application of WEA (within different kinds of policy frameworks).

The document contains 4 components:

a) *Pre-selection: When to apply WEA?*

Guidelines on how to find those complex effluents where the single substance approach may not be conclusive and where WEA probably has most added value.

b) *Flow-chart: Which WEA-parameters to apply in what sequence?*

What parameters (Persistence, Bioaccumulation, Toxicity) are relevant and what is the optimal test sequence? Is it possible to have tiered approaches, or short cuts, for cost-effective application?

4 For more detailed scope of the working group see the progress reports (OSPAR site).

5 The regulatory use of WEA in different Contracting Parties will be evaluated by OSPAR HSC committee after several (3-5) years.

6 This shared opinion does not necessarily reflect the official opinions of Contracting Parties or Industrial organisations.

c) *Toolbox: Which WEA-tests and protocols to apply?*

For each of the WEA parameters many tests and methods have been (or will be) developed. Points to consider are which test methods are internationally applied and accepted and the relevance of each method for different effluents. The guidance document shows two levels in the toolbox: 'common toolbox' (tests that are generally accepted and applied) and 'optional toolbox' (tests that are not generally applied or still are under development).

d) *Practical aspects: How to deal with sampling and storage?*

The document provides references to practical guidelines with respect to issues such as effluent sampling, storage and how to cope with variations in effluent composition.

The structure of the Guidance Document is chosen to create a dynamic character. The flowchart indicates relevant WEA parameters and their sequence in testing. The toolbox contains tests ready for application (common toolbox) and yet open for new methods (optional toolbox) depending on developments and knowledge over time. OSPAR Contracting Parties should use this guidance in the light of their own practical experience of the application of WEA. If they have developed techniques or methodologies which are not covered in this guidance, but which they believe can produce equivalent or enhanced results, they should be free to use them as they see fit.

A) Selection of effluents where WEA has most added value

A-1) Selection criteria

The advantage of WEA compared to the single substances approach is that it measures the combined effects of all substances present in the sample: including the unknown substances or the substances that have not yet been assessed[7]. The added value of this method is greatest in complex effluents, which contain complex mixtures of chemicals or require very detailed process specific knowledge to allow characterisation.

Description of 'simple' and 'complex' effluents

Simple effluents are effluents where all the components have been identified and where the adverse effects caused by the effluent can be explained by the chemical properties of the components, including any combined effects.

Complex effluents are effluents that do not fulfil the criterion for simple effluents, being effluents containing complex mixtures of known and unknown chemicals resulting in an inadequate assessment with the single substance approach.

For complex effluents there is a realistic possibility that the single substance approach is not comprehensive and may result in the emission of potential adverse effects into the surface water. Therefore, the first objective is to identify these effluents. The OSPAR WEA working group has developed the following criteria or guidelines to aid in the selection of effluents where WEA has the greatest potential to add value:

a) Facility related aspects indicating 'complex effluents';
b) Previous WEA results of the effluent;
c) High levels of organic carbon present in the effluent;
d) Poor ecological quality of the surface water.

Criterion a) Facility related aspects indicating 'complex effluents'

This criterion reflects information on the facility (or plant) considering both the substances used or manufactured, the processes involved which generate the effluents and the water treatment system on the site. If this information indicates that the single substance approach may be inadequate, the application of WEA is advisable. Examples are:

- Facilities that use complex chemical or physical processes with the possibility of (unknown) side products (*like the organic fine chemistry, large volume organic chemistry*);
- Facilities where there is a risk of specific modes of action, like endocrine disruption or other (*for instance: hospitals, pharmaceutical industry, Municipal Wastewater Treatment Plants (MWTPs), organic fine chemicals, large volume organic chemistry, dyes from the textile industry*). In this case also the tests applied should be able to detect these specific modes of action.
- Facilities where many different substances are being used or released or where products and substances are regularly altered (*like MWTPs, waste treatment stations, tanker cleaning installations or multi purpose plants*);

Criterion b) Previous WEA results of the effluent

If previous WEA testing showed the presence of adverse effects that could not be explained by the substance approach, it is always advisable to conduct an investigation whether or not the results were due to a 'one time' processing aspect, or have a continuous character. Often a second investigation can be more directed towards causes, or can be more detailed than a first screening.

[7] The added value was demonstrated in 'OSPAR WEA Practical Study Programme 2003', Gerritsen et al, 2004.

Criterion c) High levels organic carbon present in the effluent

High levels of organic carbon in the effluent indicate the presence of high concentrations of organic substances. This means that the chance of substances that may cause an adverse effect is greater than in effluents with low levels of organic carbon. When searching for effluents where WEA will have an added value, high levels of organic carbon can be a good selection criterion or guideline.

However, it should be noted that low levels of organic carbon are not a guarantee for the absence of adverse effects (there is no clear cut-off level for DOC or TOC content). There are a range of possible explanations for these adverse effects which could include ionic composition, presence of toxic metals or highly toxic organic compounds (e.g. pesticides).

High organic carbon content as pre-selection criterion

In the OSPAR practical study 2005 the relation between organic carbon content and the presence of acute toxicity was investigated[8]. In most cases high acute toxicity levels were found when high levels of organic carbon were present. On the other hand, in some cases acute toxicity was also measured in effluents containing very little organic carbon. Therefore, high levels of organic carbon are an indicator of the presence of acute toxicity, but there is no straight cut-off level below which toxicity will surely not occur.

Criterion d) Poor ecological quality of the surface water

Another reason to apply the WEA approach is when the ecological status of the surface water is poor. One of the possible causes of a poor ecological quality is the presence of substances that cause adverse effects in one or more organisms, which may lead to effects throughout the food chain and disturb the ecological equilibrium. WEA tests measure the effects on organisms and form a link between chemistry and ecology. WEA tests could be applied also to surface water and effluents for (de)selecting effluents that need to be investigated in relation to the ecological status[9]. It must be recognised that good ecological status of the surface water - on the other hand - does not guarantee the absence of hazardous substances.

WEA and surface water quality

Case in Germany.

Several years ago in the river Wupper, a too low amount of fish was present. It turned out that one effluent discharging into this river, was not toxic for fish but for Crustacean (Daphnia). The reason for the decrease in the amount of fish was that the effluent caused the death of the crustacean, that were the food of the fish[10].

Diagnostic investigations bad ecological surface waters.

Bad ecological surface water quality can be caused by several aspects, the most relevant being hydromorphological aspects (water quantity measures, dikes, dams, etc.), climatological aspects, or the presence of (known or unknown) chemical substances. When a poor ecological status has been signalled, a diagnostic study can be started in order to find the cause(s). WEA tests might have a role in this investigative monitoring.[11]

A-2) Direct effluents and indirect effluents

WEA is applicable to both 'direct effluents' (effluents discharging directly into the surface water) as well as to 'indirect effluents' (effluents that are first being treated in a Municipal Wastewater Treatment Plant (MWTP) prior to discharge into the surface water). When undertaking screening

8 See report 'OSPAR practical study 2005 on WEA', Roex et al. 2007.

9 Furthermore, WEA tests could be used to determine the status of receiving waters and sediments in conjunction with biological and chemical surveys as a "TRIAD" based approach which have been demonstrated to be valuable for such complex investigations.

10 Reference: Presentation of Mrs. B. von Danwitz, June 2005, for the Dutch working group on WEA.

11 In the Netherlands pilot studies are carried out in order to evaluate the application and added value of WEA testing within the investigative monitoring that e.g. would be necessary for the WFD.

of effluents of possible concern (as is the goal within OSPAR) both types of effluents should be included because both have potential to cause adverse effects on the marine environment.

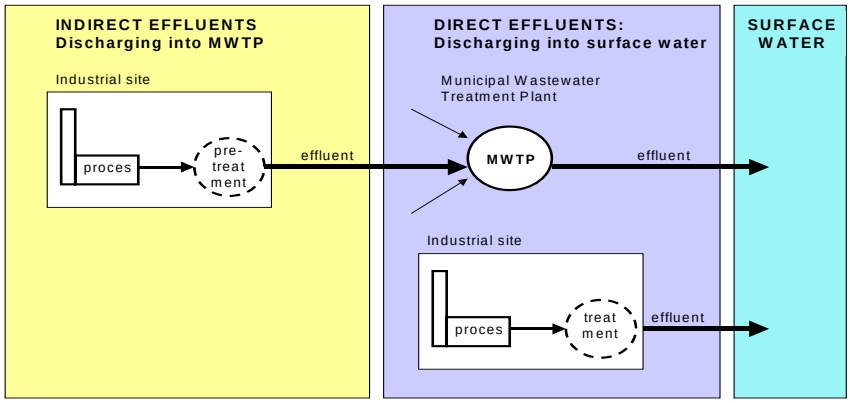


Figure 1) Indirect and direct effluents

Definitions of 'direct effluents' and 'indirect effluents'.

Industrial effluents usually (but not always) have received a treatment at the site. The effluents can be discharged into the surface water directly (so-called 'direct effluents'), or first be transported to a Municipal Wastewater Treatment Plant (MWTP). In the MWTP the effluent is cleaned, together with other industrial effluents, municipal wastewater and/or rain water. Those industrial effluents are called 'indirect effluents', since they are discharged indirectly, through the MWTP and together with other wastewaters.

For indirect effluents the treatment in the MWTP will be taken into account in the WEA assessment[12]. This can be performed by the choice of persistency step for direct and indirect effluents (see chapter B).

12 This is also in line with the WFD 2000/60/EC and IPPC directive (see next chapter).

B) WEA flowchart: test sequence and tiered approaches

B-1) Flowchart with relevant WEA parameters

Parameters most relevant to the marine environment, that can cause long term effects, are: persistency, bioaccumulation and chronic toxicity. However, often acute toxicity should also be addressed for effluents (to freshwater or marine waters), especially in early screening assessments and prioritisation (see tiered approaches, paragraph B-2). Persistency, bioaccumulation, chronic and acute toxicity are part of the current WEA flowchart.

Other relevant parameters that may be of importance for the marine environment are endocrine disruption and mutagenicity. These parameters are increasingly being used in the assessment of single substances. However, the tests are still under development and the relevancy of the parameters to environmental impacts is still being discussed. Mutagenicity and endocrine disruption are not (yet) included in the flowchart. Tests and methods for those two parameters are included in the optional toolbox (see chapter C).

The basic WEA flowchart shows the sequence in testing of the parameters persistency, bioaccumulation and toxicity. It is most important that persistency in this test scheme should not be assessed as a separate parameter, but combined instead with other parameters[13]. The flowchart starts with a persistency test (degradation test) to remove the majority of non-persistent substances. After this 'pre-treatment step', the treated sample is used for testing toxicity and bioaccumulation. This combination of tests reveals the persistent levels of respectively acute toxicity, chronic toxicity and bioaccumulation. Those are considered to be the important effects, since they occur over a long period (persist).

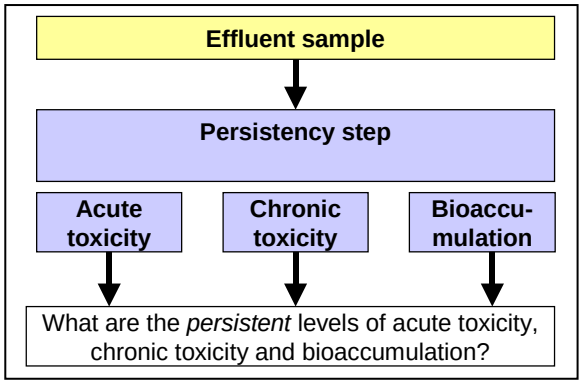


Figure 2: The principle of the basic WEA flowchart: Persistency is combined with the other WEA parameters.

Persistency within WEA is combined with bioaccumulation and toxicity.

Within the single substance approach, persistency, bioaccumulation and toxicity are measured in separate tests. The three results (P, B and T) are used to give an overall estimation of the environmental characteristics of the substance. Persistency is tested in degradation tests and the level of persistency is related to the rate and extent of DOC removal, oxygen uptake or CO_2 evolution during the tests. In single substance tests, these parameters reflect the decrease in concentration and give an indication of the persistency of this substance.

For whole effluent samples it is more complicated to interpret results expressed as DOC removal, since it does not allow to differentiate between the substances present in the sample. High DOC removal might be caused by only one or a few substances, while other, more toxic or bio-accumulative substances may still be present. The practical studies from the OSPAR working group showed that no strong relationship exists between DOC removal and the remaining acute toxicity, supporting the above assumptions [14].

[13] In some countries, persistency in itself is considered as a reason for concern.

[14] OSPAR WEA products: Practical study 2005, Practical study 2006, Background report on persistency (progress report SPDS, December 2004).

For the parameters persistency and toxicity, important distinctions can be made in the type of tests to be used. When the different tests results are included, it results in a more extended flowchart.

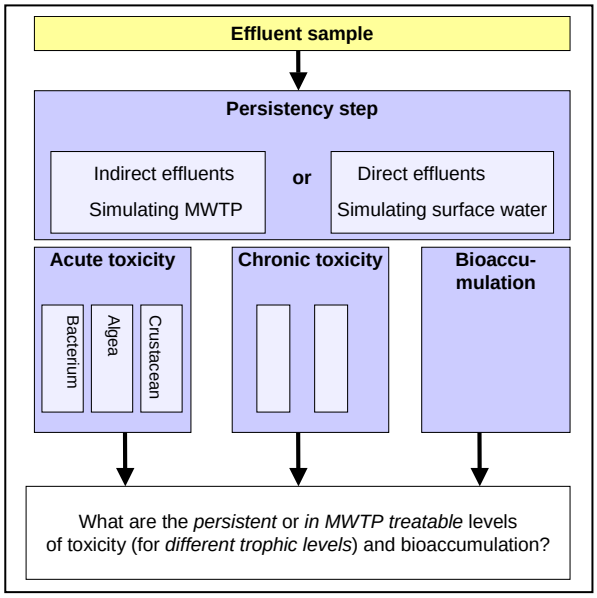


Figure 3: Basic WEA flowchart with different tests for persistency and toxicity.

For persistency there is a basic difference between the tests to be applied for direct effluents and for indirect effluents. The type of persistency test to be applied to effluents directly discharging into the surface water, should simulate the events occurring in the surface water. For indirect effluents the persistency test should simulate the events occurring in the MWTP, in line with IPPC and the WFD. The different tests should attempt to identify the extent to which toxicity or bio-accumulative potential will be removed in the receiving waters on the one hand or in a MWTP on the other hand.

Assessment quality of indirect discharges in line with international guidelines

Taking into account the processes in the MWTP for indirect effluents, is in line with the IPPC directive and with the Water Framework Directive.

IPPC article 2 definitions of 'emission limit values' of the IPPC directive, where it is stated that

'… the effect of a water treatment plant may be taken into account when determining limit values, the flowchart for indirect effluents also takes the biodegradation in the MWTP into account…'

WFD (2000/60/EC, article 2 Nr. 40 includes:

'… for indirect releases into water, the effect of a waste-water treatment plant might be taken into account when determining the emission limit values'.

This leads to the application of different types of tests (different strategies) depending on whether the effluent is direct or indirectly discharged. While choosing a test within the two categories (simulating surface water or simulating MWTP), the type of treatment an effluent has received prior to discharge should also be assessed, as part of the decision as to which type of test is used (see chapter C for tests).

For acute and chronic toxicity the distinction lies at the trophic level of the test organisms applied. In line with the single substances approach, toxicity should preferably be tested at more than one trophic level (e.g. bacteria, algae, crustacean, or fish) in order to obtain a broad insight in the effects on the different levels within the ecosystem. Tests that measure acute toxicity are more developed than those that address chronic toxicity. For acute toxicity generally three trophic levels are usually investigated (bacteria, alga, crustacean) and given the state of the tests, these trophic levels are included in the flowchart. Measurement of chronic toxicity is currently less well developed and while some tests are being used, no specific trophic levels are recommended at this stage. More than one trophic level may need to be investigated depending on the type of effluent (see chapter C for tests).

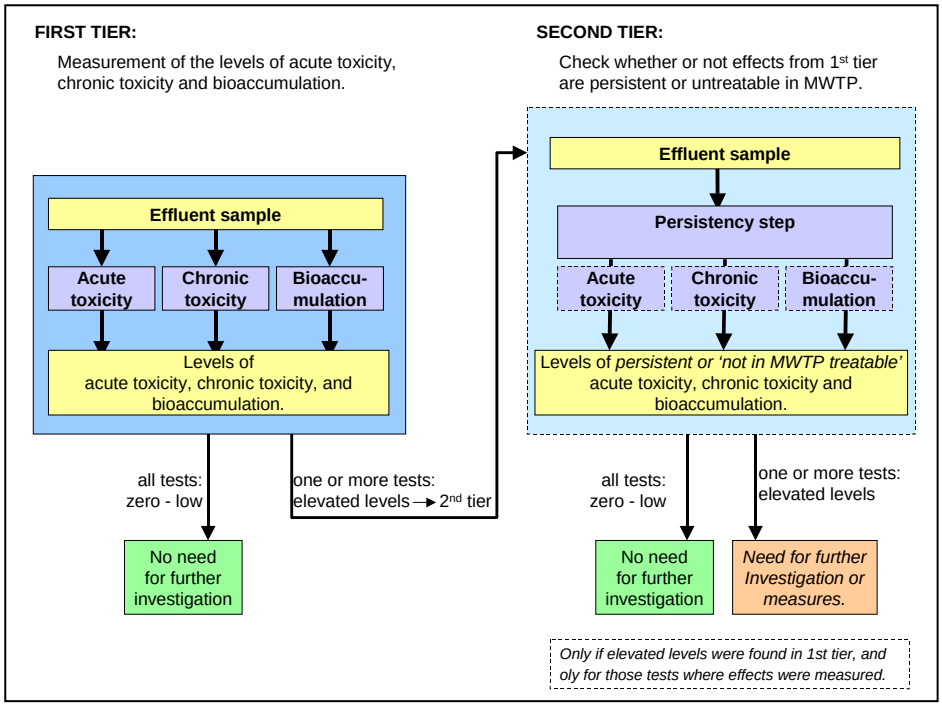
B-2) Tiered approach and shortcuts in the flowchart

For cost-effective (and time-saving) reasons, it is sometimes possible to apply a tiered approach, or shortcut, within the flowchart. This is possible when relations between the WEA parameters do exist, or when the type of process and effluent indicate that shortcuts might be possible. The following shortcuts or tiered approaches may be possible: a) Tiered approach for persistency step, b) Shortcuts for chronic toxicity tests.

Tiered approach for persistency step
The basic flowchart combines a persistency step in combination with the other WEA parameters and thus results in the relevant combination of persistent toxicity and bioaccumulation. However, the persistency step has some practical disadvantages. First of all the persistency step is time consuming (minimally 7- 28 days), which hampers a rapid analysis of the effluent quality. Second, the persistency step normally means that the sample is changed with buffers or other additives and diluted – which often results in a considerable reduction of the sensitivity.

Therefore this tiered approach (see figure 4) starts with a first screening for the presence of acute toxicity, chronic toxicity or bioaccumulation, without conducting a persistency step. If the measured levels of toxicity or bioaccumulation potential give raise to concern, the second tier can be performed. This tier starts with a persistency step; the type of test to be chosen depending on whether the effluent is discharging directly or indirectly into the surface water. After the persistency step, only those toxicity or bioaccumulation tests should be performed that showed elevated levels in the first tier. The second tier will show whether or not the toxicity and/or bioaccumulation found in the first tier, are persistent (for direct effluents) or will not be removed within the MWTP (for indirect effluents).

Figure 4: Tiered approach for persistency



This tiered approach may always be used and relatively rapidly, gives a first impression of the effluent quality and of the possible relevant WEA parameters for the effluent in question. There are certain situations where this tiered approach is especially recommended. First, this might be a cost-effective option in cases where the information on substances used and processes applied give only little indication of the added value of WEA. The first screening is used as a check on whether or not WEA has an added value for the effluent. Second, the type of treatment that the effluent received is an important factor for choosing this tiered approach. Plants where the effluent has had a biological treatment, the effluent will often contain low levels of biodegradable components. Consequently, the persistency step could be omitted in initial investigations[15]. However, biological treatment plants are not always designed to achieve 100% removal of biodegradable compounds. Therefore, the second tier can be carried out to check whether or not the levels of toxicity and bioaccumulation are persistent or will be degraded in the MWTP.

Shortcuts for chronic toxicity

Chronic toxicity is an important, long term effect and therefore relevant to the environment. On the other hand, the tests for chronic toxicity usually are costly and require much time (up to several weeks) which hampers a rapid analysis of the effluent quality. For cost- and time-effective reasons shortcuts for chronic toxicity testing may be used in certain situations.

For specific sectors, the results of bioaccumulation tests can be used as an indicator for chronic toxicity. Within a homogeneous group of effluents (for example the petrochemical refinery sector[16]) where mostly substances with a non-specific mode of action are present, chronic toxicity can be caused by bio-accumulative substances only (no other causes for chronic toxicity). When this relation has been demonstrated in a specific effluent, first a bioaccumulation test is performed and testing of chronic toxicity is only necessary when previous testing of bioaccumulation was found. However as this relationship must be proven sector-wise, this is no typical shortcut. The practical study of 2005 did not find a general relationship for all effluents within the sectors tested[17].

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15 The Practical Study of 2005 confirmed this hypothesis for acute toxicity in biologically treated effluents.

16 See for instance, Di Toro at all (2000) Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. In water and tissue. Environmental Toxicology and Chemistry. Vol 19, pp 1951-1970.

17 See report 'OSPAR Practical Study 2005 on WEA', Roex et al. 2007.

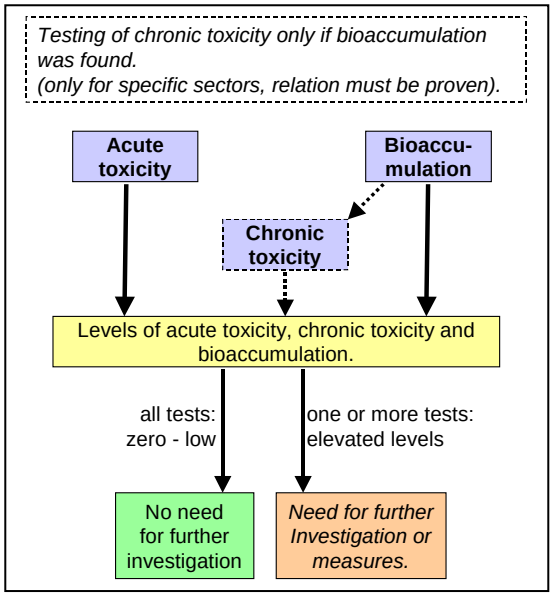

Figure 5: Shortcuts for chronic toxicity – based on bioaccumulation

Another possible tiered approach, is to use the results of acute toxicity tests as an indicator for chronic toxicity testing. Here more discussions are required before this shortcut can be recommended.

Acute toxicity a possible indicator for chronic toxicity.

It is generally accepted by both scientists and policy makers that there is a relation between the concentrations at which substances cause acute toxicity and chronic toxicity. When looking at all substances, the concentrations where acute toxicity occurs is in average a factor 10 higher than the concentration where chronic toxicity occurs. Acute toxicity in this sense can be seen as an indicator of chronic toxicity and as a motivation to conduct chronic toxicity tests. A possibility (still to be further discussed) is the application of acute testing in undiluted samples to omit chronic testing of diluted samples. Another option (also still requiring further research) is to perform acute toxicity tests in (factor 10?) concentrated samples as an alternative for chronic testing. The validity of this approach, in particular the limitations and reliability of concentration techniques for effluent samples, is currently being investigated.

On the other hand, if no acute toxicity is found, this is not a guarantee for the absence of chronic toxicity. Care should be taken for some sites (e.g. pharmaceuticals and pesticides) that may produce chemicals with specific modes of actions and have high acute to chronic ratios. Those substances are usually known and covered with the single substance approach and are not commonly present in effluents.

The WEA working group also discussed a possible short cut between the COD removal during a persistency step and the removal of toxicity and bioaccumulation. No correlation was found.

DOC removal during persistency step is not related to removal of toxicity or bioaccumulation.

In general, there is no relation between the removal of organic carbon and the removal of toxicity and bioaccumulation, during the persistency step. If high DOC removal (or low remaining levels of DOC) would correlate with low toxicity or bioaccumulation, testing of toxicity or bioaccumulation would not be necessary after the persistency test. The practical study of 2003 showed that there is no such a correlation for the majority of effluents. Some effluents showed a good removal of organic carbon but very poor removal of toxicity and bioaccumulation. In other effluents the opposite occurred: poor removal of organic carbon but a large reduction of toxicity and bioaccumulation took place during the persistency test.

C) WEA Toolbox

C-1) Common toolbox and optional toolbox

For each of the WEA parameters, different kinds of tests and methods are available (or are being developed) throughout the world. For instance, acute toxicity for bacteria can be tested with different species of bacteria, or can be performed using different endpoints and practical methods. Each method or test has specific advantages or relation to the effects in the environment. Therefore, a toolbox with a variety of tests for each WEA parameter is required for tailor-made application on effluents with different characteristics and different situations in the receiving surface water.

As the result of the inventories and experiences of the OSPAR WEA working group, two different toolboxes are presented in this guidance document: a common toolbox and an optional toolbox. The common toolbox includes methods that are generally accepted, applied by many parties and are the most technically robust. The optional toolbox contains methods which are less generally applied or less well developed, but might be useful for specific situations or after more development in the future. Thus over the course of time and development, tests from the optional toolbox may enter the common toolbox. For mutagenicity and endocrine disruption the place and relevance within WEA is not yet defined by the OSPAR WEA working group. The tests are now placed in the optional toolbox.

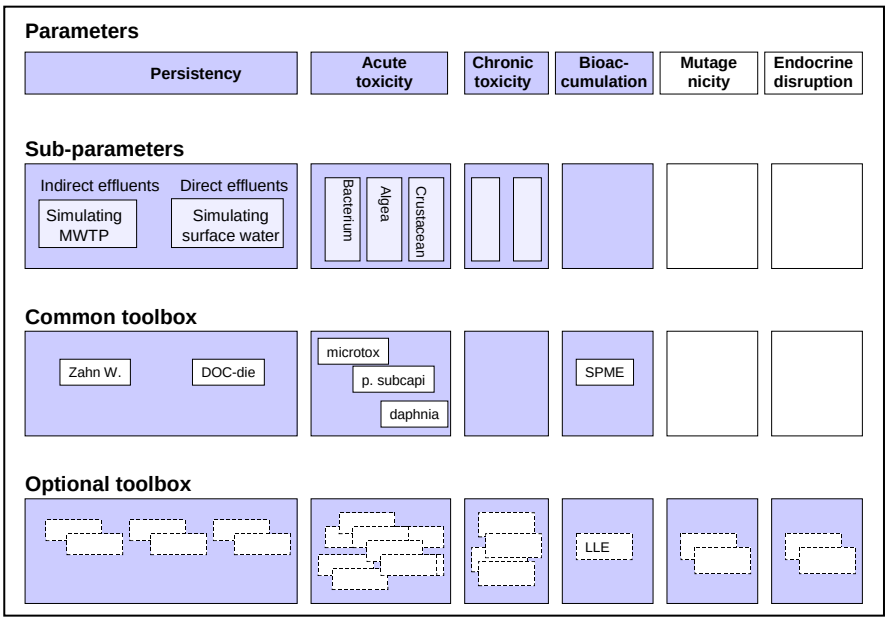

Figure 6: Common and optional toolbox for the WEA parameters

Below a short description of the tests are given, while an overview of tests, methods and protocols is presented in tables 1 – 6 in paragraph C-3.

C-2) Short description of the tests

Acute toxicity (table 1)

Acute toxicity is the parameter that has been most widely used and developed and for which the most protocols are available. Many countries and parties apply tests for bacteria, algae and crustacean, almost always with the same organism or protocol. Those tests are placed in the common toolbox. Fish tests are available, but less commonly applied due to ethical and cost

reasons. The fish egg/larvae test[18] is sometimes used to solve this matter. Besides the common tests several other tests might be applied optionally.

The toolbox contains tests with fresh water organisms and marine organisms. Effluents can have a saline, brackish or fresh character and be discharged in marine, brackish or fresh water system. Ideally, the test organism used would be in line with the receiving water system (e.g. tests with marine organisms for discharges to marine water systems, tests with fresh water organisms for discharges to fresh water systems). When the nature of the effluent is different from the one of the receiving water system (fresh water effluent discharging into marine waters / saline effluent discharging into fresh waters) a choice must be made. Either the effluent is altered (addition of salinity for fresh water effluents discharging into marine waters) or another test protocol is chosen (application of fresh water organisms, even for effluents discharging into marine waters). Both choices have their pros and cons and no general preference can be expressed at this time[19].

Chronic toxicity (table 2)
During the summer of 2006 a questionnaire with regard to the state of the art of and experiences with the tests was set out within the OSPAR WEA working group (quick scan). Chronic tests are less common than acute tests. Information from the practical study and the quick scan resulted in 15 chronic tests, from which 9 were ISO standardised. The organisms tested belong to the following taxonomic groups: Bacteria, Algae, Crustaceans, Rotifers, Bivalves, Echinodermata, Fish and Plants. Time exposure ranges from 16 hours to 90 days depending on the life-cycle of the test organism. Most tests use the growth inhibition as test endpoint, and are static tests, being only 30% semi-static. Concerning marine and freshwater tests, 9 tests can be applied to freshwaters and 7 tests to marine and brackish waters.

From the quick scan, the number of chronic tests applied per country are: 8 in Sweden, 6 in the Netherlands, 6 in Germany and 4 in Portugal, assuming that tests with marine and freshwater algae and with *Lemna* are chronic tests. All the replying countries use Algae and Crustacean tests, and Echinodermata tests are only used by one country.

Bioaccumulation (table 3)
For bioaccumulation two tests are available, both based on extraction and giving an indication of the potential for bioaccumulation. The LLE method is applied in Sweden on a routine basis for many years and reflects the total extraction of potentially bio-accumulative substances, including the fraction bound to particulate matter. The SPME method is a more recent method, and reflects more closely the possible bioaccumulation in the ecosystem, and only measures bio-available substances. The SPME method is placed in the common toolbox because of its technical advantages (simpler, shorter and cheaper) and because it will better reflect the potential to bio-accumulate in an ecosystem. The LLE method is placed in the optional toolbox and can be useful in assessing the total environmental load[20].

Mutagenicity and genotoxicity (table 4)
For genotoxicity and mutagenicity testing of wastewater several international standardised methods exist. (Genotoxicity accounts for any potentially harmful effects on genetic material while mutagenicity accounts for permanent transmissible changes of the genetic material). Although international standardised methods are available, the application within the participants of the OSPAR practical studies is still very limited.

During the summer of 2006 a questionnaire on the state of the art and experiences of the tests was elaborated within the OSPAR WEA working group (quick scan). Only few answers were submitted and thus the results of the enquiry can not be considered as representative. Therefore additional

18 The fish egg tests are recently implemented in German wastewater evaluation procedures. Egg/larvae tests have been used for several years in Sweden.

19 This pragmatic approach could be agreed upon as recent reviews show that there are few systematic differences between the sensitivities of freshwater and marine water fish, invertebrates and algae/plants.

20 See also the Progress Report of the WEA Working Group, for HSC meeting in April 2006 and the underlying report 'Parts 1 & 2 of Final Report on Measuring Potentially Bioaccumulative Substances in Effluents', RIVO, Commissioned by CONCAWE, March 2006.

references from literature have also been evaluated. At the moment, genotoxicity (umu-assay) is included in regulatory routine measurement (e.g. effluents from chemical industry) in Germany only. The test is also applied in the Netherlands. In Sweden genotoxicity is no common parameter. In Portugal the MUTATOX test is applied. The micronuclei test with V79 is foreseen as a new parameter in the German wastewater ordinance. A ring test with 10 labs and 4 samples was successfully terminated. The micronuclei test with amphibian larvae is applied in France.

Endocrine disruption (table 5)
During the summer of 2006, a quick scan was performed for endocrine disruption. Tests for endocrine disruption are less far developed and standardised than tests for the mutagenicity. by Sweden, Germany and the Netherlands responded to the quick scan. In those countries the tests for endocrine disruption are applied within R&D programmes and Sweden applies some tests in the second tier (after bioaccumulation and toxicity) in the processes of issuing permits and for the paper and pulp sector as a guidance for choosing the best possible treatment for the effluents. In all three countries in vitro tests (with modified yeast or human cells) are being developed. In Sweden, in vivo tests with Zebrafish are applied.

Persistency (table 6)
Persistency is a complicated parameter, as is known from the single substance approach. Although biodegradation is used here to assess persistency (as shown in Figure 8) there are other factors (e.g. hydrolysis) which can influence the persistency of chemicals. One of the difficulties is that 'persistency' is defined in fixed cut-off levels, expressed as half-lives, while in reality persistency represents a gradient going from easy degradable to hardly or non-degradable substances. In test results this goes from 'readily degradable' (non-persistency) going to 'degradable with some effort' (persistent or non-persistent) to 'non-degradable'(persistent).

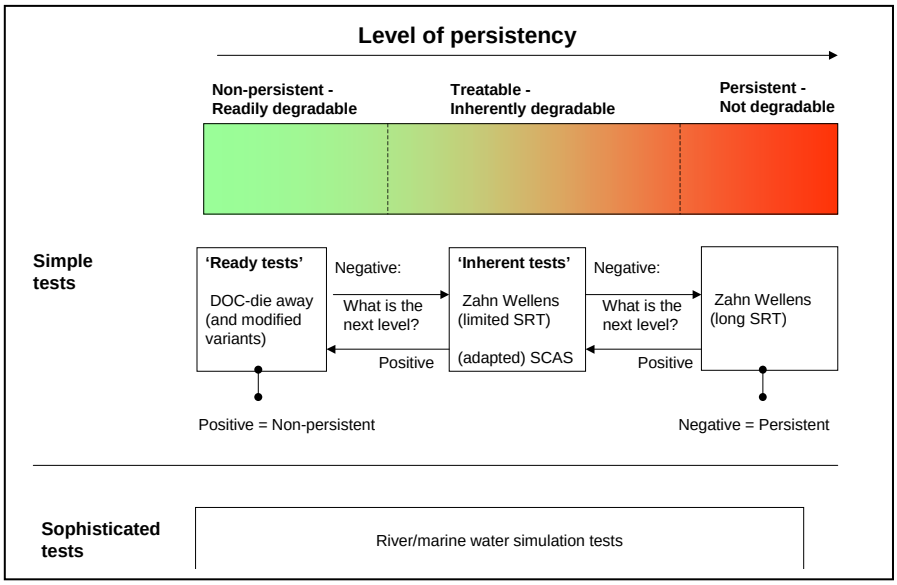

Figure 7: Gradient of persistency with available tests

Tests, such as, simulating different conditions for degradation, sorption and/or chemical reactions are available. Those tests can be applied to provide information on the different levels of persistency. A distinction is made between relatively 'simple' biodegradation tests (ready type and inherent type) and more 'sophisticated' surface water simulation tests that are generally less well developed. In terms of their application within a WEA framework it is advised to work with a tiered approach, starting with relatively simple tests and, as and when required followed by more sophisticated tests. Both types of tests have their specific characteristics as well as their limits for interpretation and understanding the results in terms of 'persistency'.

At present the common toolbox contains relatively simple biodegradation tests to be applied in a first tier. The practical study 2005 indicated that the two relatively simple tests (one for direct effluents and one for indirect effluents) give valuable information, but need further work with regard to test optimisation and understanding. Some care is needed as for how the tests are undertaken and for application to complex effluents, these could be technically improved.

C-3) List of test protocols

Table 1: Acute toxicity

Trophic level	Iso guideline	Test species	***Fresh water or Salty water***		**Applied by**								**Common or optional toolbox**	
			Fresh water	*Salty water*	**Belgium**	**Ireland**	**Portugal**	**Germany**	**Netherlands**	**Sweden**	**UK**	**Arkema**	**Concawe**		
Bacterium	ISO 11348 - 2	*Vibrio Fischeri*	*F*	*S*	■	■	■	■	■	■	■	■	■	Common	
Algae	ISO 8692 [21]	*Pseudokirchneriella subcapitata,*	*F*		■	■	■		■	■	■	■	■	Common	
	DIN 38412-33	*Scenedesmus subspicatus*	*F*					■							Optional
	ISO 10523	*Skeletonema costatum*		*S*		■					■				Optional
Crustacean	ISO 6341(Germany DIN 38412-30)	*Daphnia magna*	*F*		■	■	■	■	■		■	■	■	Common	
	ISO 14669	*Tisbe battagliai*		*S*		■					■				Optional
	ISO 14669	*Acartia tonsa*		*S*									■		Optional
	SS 02 81 06	*N. spinipes,*								■			■		Optional
Fish	OECD 203	*Oncorhynchus mykiss*	*F*		■	■		■							Optional
	DIN 38412-6 and ISO/DIS 15088 standard	Fish egg test, *Danio rerio*	*F*					■							Optional
Other	OECD 1998, (Portugal: ISO 20079 (2005)	*Lemna minor*	*F*				■								Optional

[21] 1-can be regarded as acute test (applying to ISO 8692 and DIN 38412-33, ISO 10523, ISO 20079); 2-can be regarded as subchronic test (applying to OECD 212 and ISO/DIS 15088).

Table 2: Chronic toxicity

Trophic level	Test and guideline	***Fresh water or Salty water***		**Applied by**									**Common or optional toolbox**	
		Fresh water	*Salty water*	Belgium	Ireland	Portugal	Germany	Netherlands	Sweden	UK	Arkema	Concawe		
Bacteria	*Pseudomonas putida* growth inhibition test (Pseudomonas cell multiplication inhibition test) (ISO 10712:1995)	F												Optional
	Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms (ISO 15522:1999)	F	S											Optional
Algae	Freshwater algal growth inhibition test with unicellular green algae (ISO 8692:2004) [22] or Growth inhibition of *Desmodesmus subcapitata* (DIN 38412-33:1991)	F											Common	
	Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum (*ISO 10253:2006)		S										Common	
Crustacean	*Daphnia magna,* Reproduction Test (OECD 211) or Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea) (ISO 10706:2000)	F											Common	
	Harpacticoid Copepod development and reproduction test *(Nitocra sp.)* (OECD draft)		S											Optional
Fish	Short term toxicity test on Embryo and Sac-Fry Stages (OECD 212) or Determination of the acute toxicity of wastewater to zebrafish *Danio rerio* eggs (ISO/DIS 15088)	F											Common	
	Determination of toxicity to embryos and larvae of freshwater fish -- Semi-static method (ISO 12890:1999)	F											Common	
	Subchronic toxicity to fish	F	S											Optional
Other	Determination of the chronic toxicity to *Brachionus calyciflorus in 48 h* (ISO/CD 20666)	F												
	Oyster larvae development *Crassostrea gigas* (Bequalm protocol, 2001)		S										Common	
	Toxicity to eggs and larvae of *Mytilus edulis* (Gramno *et al.*)		S											Optional
	Fertilization and Embrionic Development Test with *Psammechinus miliaris* (ASTM guideline)		S											Optional
	Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*) -- Duckweed growth inhibition test (ISO 20079:2005)	F											Common	

[22] 1-can be regarded as acute test (applying to ISO 8692 and DIN 38412-33, ISO 10523, ISO 20079); 2-can be regarded as subchronic test (applying to OECD 212 and ISO/DIS 15088).

For Chronic toxicity the following guidelines or standardsare in preparation:

OECD draft TG	Harpacticoid Copepod development and reproduction test*(Nitocra sp.)*
ASTM guideline / Bequalm protocal 2001	Larval development test with *Crassostrea gigas*
ASTM guideline	Fertilization and Embrionic Development Test with *Psammechinus miliaris*

For Chronic toxicity the following internal procedures are available:

Granmo *et al*.[23]	Toxicity to eggs and larvae of *Mytilus edulis*
	Subchronic toxicity to fish

Table 3: Bioaccumulation potential

	Test and guideline	Applied by									Common or optional toolbox	
		Belgium	Ireland	Portugal	Germany	Netherlands	Sweden	UK	Arkema	Concawe		
SPME method	References from OSPAR working group[24].	RD		RD	RD	RD			RD	RD	Common	
LLE method	References from Sweden and from OSPAR working group[25]	RD				RD	R					Optional

R: Regulatory purposes RD: Research and Development

[23] Granmo, Å., Ekelund, R., Berggren, M., Brorström-Lunden, E., and Bergqvist, P.-A. Temporal Trend of Organochlorine Marine Pollution Indicated by Concentrations in Mussels, Semipermeable Membrane Devices, and Sediment. (2000) Environmental Science and Technology 34, 3323-3329.

[24] Internal report from OSPAR working group, annex to progress report for HSC 2006 (1): ''SPME as a tool in WEA _CONCAWE Contribution to OSPAR Demonstration Project 2005-2006 (Parts !&2) of Final Report on Measuring Potentially Bioaccumulative Substances in Effluents: Interlaboratory Study Workshop and Review', Leslie and Leanords, 2005.(both LLE and SPME, protocols included)

[25] See reference above (nr. 24) and Hynning, P-Å (1996). Separation, identification and quantification of components of industrial effluents with bioconcentration potential. Wat. Res. 30(5), 1103-1108.

Table 4: Mutagenicity and genotoxicity

		Applied by:							Common or optional toolbox	
	Test	Belgium	France	Portugal	Germany	Netherlands	Sweden	UK		
In vitro										
ISO 13829:2000	Determination of the genotoxicity of water and waste water using the *umu*-test				R	RD				optional
ISO 16240:2005	Determination of the genotoxicity of water and waste water -- Salmonella/microsome test (Ames test)				RD					optional
MUTATOX test	*Vibrio fischeri (Photobacterium phosphoreum)* non luminescent variant			RD						optional
VITOTOX test	*Salmonella typhimurium (SOS* bioluminescence variant)	RD								optional
ISO/DIS 21427-2	Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 2: "Mixed population" method using the cell line V79				RD					
In vivo										
ISO/FDIS 21427-1	Evaluation of genotoxicity by measurement of the induction of micronuclei -- Part 1: Evaluation of genotoxicity using amphibian larvae (ISO/DIS accepted)	RD	RD							optional

R: Regulatory purposes RD: Research and Development

It should be noted that far more tests are being and have been used in research and development projects some of which have been described[26] References:

Corbisier, P. Barcelo, D.: Report on the bioset technical workshop on genotoxicity biosensing. Bioset: Biisensors for Environmental Technoloigy Newsletter No. 8, March 2001

Mouchet F., Gauthier L., Mailhes C., Jourdain MJ., Ferrier V., Devaux A.: Biomonitoring of the genotoxic potential of draining water from dredged sediments, using the comet and micronucleus tests on amphibian (Xenopus laevis) larvae and bacterial assays (Mutatox and Ames tests). J Toxicol Environ Health A. 2005, 68(10), p. 811-832

Reifferscheid, G., 2006: Untersuchung von Abwasserproben auf Gentoxizität - Ergebnisse eines Ringversuchs mit dem in vitro-Mikrokerntest im Rahmen der Standardisierung nach ISO. UWSF submitted

Van Hummelen P, Zoll C, Paulussen J, Kirsch-Volders M, Jaylet A.: The micronucleus test in Xenopus: a new and simple 'in vivo' technique for detection of mutagens in fresh water. Mutagenesis. 1989 4(1), p. 12-16.

[26] in the “Survey on Genotoxicity Test Methods for the Evaluation of Waste Water within Whole Effluent Assessment” (OSPAR Hazardous Substances Series 156 (2002)

Table 5: Endocrine disruption

					Applied by:									`Common/Optional toolbox	
	Test							**Germany**	**Netherlands**	**Sweden**					
In vitro	*YES test*	*Yeast modified*						RD	RD	R					Optional
	YAS test	*Yeast modified*								R					Optional
	E-screen Assays with MCF-7cell line							RD							Optional
	ER-calux test	*Human cell line, modified*							RD						Optional
	Estrogenic and androgenic effects [27]	*Modified yeast cells*								R					
In Vivo	*Zebrafish, two generation*	*Fish test*								R					Optional

R: Regulatory purposes RD: Research and Development

[27] Sumper et. al.

Table 6: Persistency

		Test variations			Applied by:									**Common/Optional toolbox**	
Guideline	**Test**	***Inoculum used***	***Mineral medium added?***	***Effluent diluted?***	Belgium	Ireland	Portugal	Germany	Netherlands	Sweden	UK	Arkema	Concawe		
Direct effluents – 'simple tests'															
DOC-die away	OECD 301A	Effluent of an MWTP	Yes	10% inoculum, but 90% effluent										common	
	OECD 301E	Secondary effluent of MWTP	yes	Yes, Depending on TOC										common	
		Reference surface water	Only if TOC<20 mg/l	Yes, 1:1										common	
		Reference surface water	Yes	Yes and no										common	
	Novel Passive Biodegradation test	none	no	Concentration range											Optional
Indirect effluents – 'simple tests'															
Zahn Wellens	OECD 302B, DIN EN 29888	Activated sludge (1 g/L)	Yes, according to DIN EN 29888	Depends from inoculum concentration, about 20% activated sludge, but 80% effluent										common	
		Activated sludge		Yes, depending on TOC										common	
	ISO 9888	Activated sludge (0.5 g/L)	yes	Yes, depending on TOC										common	
		Activated sludge	yes											common	
'More sophisticated tests'															
Biodegradability in seawater	e.g. OECD 306, ISO 16221	Biodegradability in sea water													Optional
Aquatic simulation tests	e.g. ISO 14592, ASTM E 1279-89(95), OPPTS 835.3170	River die-away tests													

Tests for persistency have been described in the IEG products ' OSPAR Background Document on Persistence and Bioaccumulation Determination in Whole Effluent Assessment, IEG, Sweden, 2003 and was elaborated by the United Kingdom in December 2004 (Annex to progress report to OSPAR SPDS meeting).

D) Practical aspects

D-1) Tailor made monitoring

The tool-box includes a large variety of different tests, to allow assessment of different kinds of effluents in a tailor-made way. Sometimes – based on previous WEA testing or knowledge of substances, processes and treatment technologies – an estimation of the relevant WEA tests can be made for the effluent in question. At other occasions the characteristics of the effluent are insufficiently known or changing regularly.

The approach might be to start with a complete WEA test to know what kind of parameters and tests are relevant for the effluent in question. When the applied substances, processes and treatment have a continuous nature, the relevant set of tests might be used for future tailor-made and cost-effective assessment. When changes occur, another broad screening with the entire WEA set is necessary.

The choice of representative sampling points, frequency of sampling etcetera is depending on the objective of the study and should reflect the variability of the effluent. It is crucial that effluent samples are representative in both local and temporal respects. In order to check whether the WEA results vary considerably or are reasonably constant, several subsequent testing moments should be chosen. In Germany the so-called '4 out of 5' approach is chosen: from five subsequent WEA tests, 1 may exceed the standards. In the UK the standard variation between the results in time must be below a certain level[28].

D-2) Sampling, preservation and pre-treatment of samples before testing

Many detailed documents are available on sampling and testing. In this guidance document, only the most important highlights are presented.

- *ISO 5667-2:1991. Water quality -- Sampling -- Part 2: Guidance on sampling techniques*
- *ISO 5667-3: 2003. Water quality -- Sampling -- Part 3: Guidance on the preservation and handling of water samples*
- *ISO 5667-16: 1998. Water quality – Sampling Part 16: Guidance on biotesting of samples*

> Standard ISO 5667-16. An extract of the background document from 2000 is given here:
>
> *"Details concerning sampling, pre-treatment of samples, test performance and data evaluation in the context of biotesting are prescribed in the international standard ISO 5667-16:1998. Guidance is given on how to cope with problems encountered in biotesting due to the nature of the water sample and on the test design. Special emphasis is placed on ecotoxicological testing with organisms. This standard also includes general remarks on how to carry out biotests, how to test" difficult substances", how to evaluate procedures and to present the results".*

The sampling procedure is considered to be the most susceptible step of effluent testing because failures can not be corrected. Usually routine testing takes place at the end of pipe, but partial wastewater streams inside the plant are also assessed, especially if the origin of effects is to be ascertained. The use of automatic time of volume proportional samplers should be preferred. Usually 2 or 24 hours mixed samples are more representative than manually drawn random samples. If sampling takes place for a longer period (e.g. 24 h) the samples should be cooled.

The materials of vessels used for sampling or storage should be chemically inert, easy to clean and resistant to heating and freezing. Glassware, polyethene or polytetrafluoroethene (PTFE) vessels are recommended. When cooled to between 0°C and 5°C and stored in the dark, most

[28] UK IPPC Guidance on the use of Direct Toxicity Assessment in PPC Impact Assessments, 2006, Environmental Agency.

samples are normally stable for up to 24 hours. Deep freezing below – 18°C in general increases the stability in preservation (preservation duration preferable less than two weeks, maximum two months according to ISO 5667-16).

It is known that freezing and thawing of samples may (but not always) result in loss of toxicity. When the thawing process is not controlled, volatile components may be lost. Particle size and distribution may also change, thereby influencing other tests. If samples need to be frozen, this can be achieved in polythene bottles which, even when sealed, allow for expansion. In addition, allowing particles to settle over a short period can result in a significant reduction in toxicity. If there is any concern, tests which are not sensitive to particles should be conducted to ascertain the toxicity.

The main objective of pre-treatment of samples is to obtain the original sample as far as possible and to modify the sample only as far as the test requirements demand.

a) Frozen samples should be thawed immediately before use. Homogenization should be ensured e.g. by agitation or shaking to avoid local overheating, whereas potential loss of volatile compounds should be considered.

b) In general, biotests are carried out with the original sample (see also paragraph 30). In some cases however, particles interfere with behavioural requirements of test organisms (e.g. impairment of filter feeding daphnids) or with the detection devices (e.g. by photometry). In such cases, a stepwise removal of particles is allowed. All separation methods, however, involve the risk that active components, bound to the particulates, are removed prior to the tests. Thus, as a first step it is recommended to allow the sample to settle for 30 min. to 2 h. If particles are not removable by sedimentation, centrifugation is in general preferred to filtration. If filtration is necessary, glass fibre filters are preferred. In some tests (e. g. Ames-test) sterile conditions are required and membrane filtration is needed.

c) Degradability testing of wastewater should be preferentially performed with the undiluted sample in order to assure that subsequent toxicity testing is not affected by dilution. If the sample has to be diluted before degradability testing for practical reasons (e.g. too high TOC) the dilution factor should be considered in the interpretation of results.

d) As a rule, samples with extreme pH values exceeding the tolerance limits of the test organisms need to be neutralised. Neutralisation should be omitted if the effect of pH is to be reflected or if pH adjustment is found to cause physical or chemical reactions (e.g. precipitation). Acid or base concentration used for neutralisation should be such that the volume change is as small as possible. Hydrochloric acid or sodium hydroxide are suitable neutralisation agents.

e) Several substances show an alteration in toxicity due to pH-changes (Ammonia, sulphides, cyanides, amines, phenols and organic acids). Especially the pH dependent change of ammonia toxicity to fish is notable. Increase of pH during test performance is especially observed in the Algae test due to CO_2-consumption. The pH should therefore be measured and recorded for an adequate interpretation of results. The adjustment of pH and addition of buffers can be considered.

f) Presence of nutrients in an effluent may enhance growth of algae or plant tests (e.g. *Lemna minor*). Organisms present in the samples may interfere with test organisms through physiologic or biologic means (e.g. oxygen depletion by bacteria, erasing of alga or bacteria by protozoa, infection of test organisms).

g) Presence of particles may interfere with luminescence measurements in the *Vibrio fisheri* bacteria assay, can reduce algae growth through shading, and disturb the filtration apparatus of daphnia or of fish gill.

h) Light absorption may reduce algae growth and interfere with photometrical and luminescence measurements.

i) The biological salt tolerance of the organisms used should be considered in the interpretation of results. Some tests (e.g. *Vibrio fisheri* assay, marine tests in general) need salinity addition for freshwater samples.

D-3) Expression and interpretation of toxicity tests

In WEA, the results of toxicity tests may be expressed in several different ways. Several official guidelines do exist:

- *Guidance on statistically evaluation gives OECD (2006) and ISO (2004)*
- *ISO/PDTS 20281: 2004 (draft). Water quality – Guidance on statistical interpretation of ecotoxicity data.*
- *ECETOC. Whole Effluent Assessment. Technical Report No. 94, Brussels, December 2004*
- *OECD. Current approaches in the statistically analysis of ecotoxicity data: A guidance to application. OECD Series on testing and assessment No. 54, 09-May-2006*

Threshold concentrations (NOEC/LID.) The volume percentage having no statistically significant adverse effect is the No Observed Effect Concentration (NOEC). Although often reported for acute tests NOECs are more commonly used for expressing chronic toxic effects. A concentration-response relationship is not essential. The Lowest Ineffective Dilution (LID) is comparable to a NOEC. It is defined as the reciprocal volume fraction of the wastewater sample at which only effects not exceeding the test-specific variability are observed (ISO 5667-16:1998, Annex A). However, LID is not derived through statistically analysis of data.

Concentration/response relationship EC50, EC10, LC50. The volume percentage of the effluent having a distinct (e.g. EC50 or EC10 or LC50) value can also be determined graphically or with statistical means. Statistical analysis is preferred. Usually EC50 or LC50 values are calculated from concentration/response relationships, but other point estimates such as the EC10 might also be useful.

Toxic units. An acute toxic unit (TU_a) is defined as 100/EC50 using data from acute tests, a chronic toxic unit (TU_c) as 100/NOEC or 100/EC10 with data from a chronic test. The chronic toxic unit corresponds to the LID (LID = 1/Volume fraction; TU_c = 100/NOEC = LID; example: if NOEC corresponds to 25 Vol. % effluent → LID = 1/0.25 = 4; TU_c = 100/25 = 4).

D-4) Backtracking

Backtracking the causes of WEA effects can be carried out at the level of individual waste streams within a plant, or more in detail going towards the individual substances involved (TIE method).

- *Toxicity backtracking*: Identification of the source or group of substances causing an undesired biological effect by Toxicity Identification Evaluation (TIE) or by testing tributary streams of the mixed sample.
- *Toxicity identification evaluation (TIE)*: TIE is a systematic investigation to discover what substances(s) in a mixture is/are the cause of toxicity in the mixture. Various physico-chemical pre-treatments are followed by tests for toxicity; the results providing information to the type of toxicants acting.

Toxicity backtracking down to the substances involved is time consuming and expensive and although used in the US is still regarded by many as requiring more development before it is implemented in the EU. Taking account of the costs involved there should be a strong indication on adverse effects of effluents before backtracking is undertaken. A more pragmatic approach, which includes trial and error treatment of individual wastewater streams has been shown to be more

cost-effective. Furthermore, utilising the knowledge of the plant-owner with regard to (changes in) substances/processes/treatment can often lead to localisation of potential sources of 'problem' substances within the site.

Annex 1: Members of OSPAR's working group on WEA

Members

BEL	IND	Mr. George Stalter	*CONCAWE*
BEL	IND	Mr. Dolf van Wijk	*Euro Chlor (Ecetoc)*
BEL	IND	Mr. Mike Comber	*ExxonMobil (CONCAWE)*
FRA	IND	Mr. Philippe Lemaire	*ARKEMA (CONCAWE)*
UK	IND	Mr. Graham Whale	*Shell (CONCAWE)*
UK	IND	Mr. Martin Holt	*Ecetoc*
BEL	GOV-INST	Ms. Lut Hoebeke	*Vlaamse Milieu Maatschappij (VMM)*
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Annex 2: Products of OSPAR's working group on WEA

1) Report 117/2000: OSPAR Background Document concerning the Elaboration of Programmes and Measures relating to Whole Effluent Assessment.

2) Report 156/2002: Survey on Genotoxicity Test Methods for the Evaluation of Waste Water within Whole Effluent Assessment.

3) Report 174/2003: Survey of the use of effect related methods to assess and monitor wastewater discharges - Testing of endocrine effects.

4) Report 219/2005: Whole Effluent Assessment Report:

   4.1) Justification note: Whole Effluent Assessment (WEA) within OSPAR.

   4.2) OSPAR practical study programme 2003.

   4.3) Degradability and liability to bioaccumulate – Methods used in whole effluent assessment.

5) Report 315/2007: OSPAR Practical Study 2005 on Whole Effluent Assessment