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 Union 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 l'Espagne et l'Union européenne.

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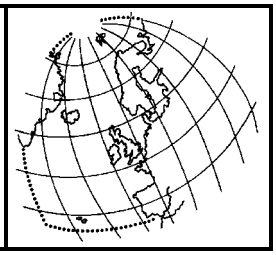

OSPAR Background Document concerning the Elaboration of Programmes and Measures relating to Whole Effluent Assessment

Contents

ANNEXES

LIST OF TABLES

DEFINITIONS/GLOSSARY

Activated sludge: Biomass produced in the aerobic treatment of wastewater by the growth of micro-organisms.

Adsorbable organic bound halogens (AOX): Group parameter used to quantify the amount of organically bound halogens in complex environmental samples which adsorbs to active carbon.

Acute toxicity test: Short exposure in relation to the life span of the organisms.

American Society for Testing and Materials (ASTM): Standards association in the United States of America.

Analysis of variance (ANOVA): A statistical method for testing the significance between means dividing the total variation into separate parts attributable to different treatments.

Application factor: A unitless factor for estimating PNEC from test results with acute and chronic ecotoxicity tests.

Association française de Normalisation (AFNOR): French standards association.

Back tracking (= toxicity source evaluation): Evaluation of the source of a biological effect in the sample by analysis of specific substances or different fractions of the sample.

Best Available Technique (BAT): The latest stage of development (state of the art), of processes, of facilities or of methods of operation which indicate the practical suitability of a particular measure for limiting discharges, emissions and waste (according to the definition of BAT in the OSPAR Convention, Appendix 1).

Bioaccumulation: Accumulation in an organism of substances from its environment.

Biochemical oxygen demand (BOD): The oxygen consumed by aerobic micro-organisms in metabolic processes, when a sample is incubated [usually 5 days at 20°C] in the presence of a nitrification inhibitor.

Bioconcentration factor (BCF): Ratio of the concentration of a chemical in an organism and in the surrounding environment.

Biomonitoring: Assessment of the biological status of populations and biocommunities or analysis of amounts of potentially toxic substances in tissues and fluids of exposed living beings.

British Standards Institution (BSI): Standards association of the United Kingdom.

Chemical oxygen demand (COD): A measure of the amount of oxygen consumed under specified conditions in the oxidation of the organic and oxidizable inorganic matter contained in water.

Chronic toxicity test: Exposure period covers a significant part of the life cycle or covers life stages (e.g. early life stages) or life processes (e.g. reproduction) considered to be especially sensitive (OECD, 1998).

Dansk Standard (DS): Danish standards association.

Deutsches Institut für Normung (DIN): German standards association.

Direct Toxicity Assessment (DTA): Approach used in the United Kingdom to assess the biological effect of whole environmental samples (e.g. effluents, receiving water, soil, contaminated land, air, leachates, sludge) employing bioassays. DTA may be applied to effluent control with toxicity tests alongside the chemical-specific assessment (cf. also WET).

Dissolved organic carbon (DOC): The fraction of TOC that is dissolved in the water sample.

Effective Concentration (EC): Statistically derived concentration at which a defined effect is observed in an organism or population. It is usually measured over a defined period of exposure (e.g. 48h) and calculated for the 10%, 50% and 90% effect level in a population (cf. also LC).

European Inventory of Commercial Chemical Substances (EINECS): List of all substances deemed to be on the Community market on 18 Sept., 1981.

Emission Limit Value (ELV): Maximum allowable release of a substance from an industrial operation to air, water or land.

Environmental Quality Objective (EQO): Statement of the desired use of a specified part of the environment. The quality to be aimed for in a particular aspect of the environment (e.g. in surface water) usually not expressed in quantitative terms.

Environmental Quality Standard (EQS): Maximum concentration of a substance which is permissible in the receiving medium (i.e. a *water quality based* parameter) consistent with the chosen EQO, incorporating a margin for safety versus possible health and environmental effects. The concentration of a potentially toxic substance which can be allowed in an environmental compartment.

Excess sludge: Activated sludge removed from biological treatment process and usually treated in anaerobic mesophilic digestion (= *surplus sludge* or *secondary sludge).*

Group parameter (= sum parameter): Analytical-chemical, biochemical or biological methods to determine a specific element or a chemically defined group (AOX etc.) or the toxicity of (complex) mixtures of organic and inorganic compounds.

Hazard assessment: Evaluation of the inherent properties of a substance or discharge with effect related data like physical-chemical characteristics, toxicity against different species, bioaccumulative potential and biodegradability to derive a NOEC and a preliminary PEC.

International Organization for Standardization (ISO): World wide federation of national standards bodies.

Lethal concentration (LC): Statistically derived concentration where a defined part of organisms are lethally affected. It is usually measured over a defined period of exposure (e.g. 48h) and calculated for the 10%, 50% and 90% effect level in the population (cf. also EC).

Long-term test: Test duration exceeds 7 days.

Lowest ineffective dilution (LID): The reciprocal value of the volume fraction of wastewater in the test at which only effects not exceeding the test specific variability have been observed.

Lowest observed effect concentration (LOEC): Lowest tested concentration at which a significant effect is observed when compared with a control or reference; sometimes EC_{10} is given as LOEC.

Nederlands Nomalisatie-instituut (NEN): Netherlands standards association.

Norges Standardiseringsforbund (NS): Norwegian standards association.

No observed effect concentration (NOEC): Test concentration immediately below the LOEC.

Oesterreichische Normen (ONORM): Austrian Organization for Standardization.

Office of Prevention, Pesticides, and Toxic Substances (OPPTS): Office of the United States Environmental Protection Agency (EPA) which publishes harmonised EPA test guidelines.

Organisation for Economic Cooperation and Development (OECD): Intergovernmental organisation in which 29 industrialised countries in North America, Europe and the Pacific are represented.

OSPAR: 1992 OSPAR Convention for the Protection of the Marine Environment of the North East Atlantic. Successor of the 1972 Oslo Convention for the Prevention of Marine Pollution by Dumping from Ships and Aircraft and the 1974 Paris Convention for the Prevention of Pollution from Land-Based Sources.

Predicted Environmental Concentration (PEC): Expected environmental concentrations derived from emission based model calculations or monitoring data.

Predicted No Effect Concentration (PNEC): Concentration where no adverse effect is expected on living communities calculated from short time or chronic test results and uncertainty factors.

Polluter pays principle (PPP): Principle that places the financial burden for the prevention and control of pollution on the party responsible for its generation, and meant to promote precautionary actions.

Potentially bioaccumulating substances (PBS): Non-biological methodologies under development which are designed to detect the fraction of substances which is liable to accumulate in organisms.

Risk assessment: A specific scenario where – in this context - the PEC of specific chemicals (or discharges) is quantified and compared with the PNEC. The PEC are derived from usage pattern, relevant compartments affected and elimination factors, the PNEC from laboratory test results applying specific assessment factors.

S9: Liver homogenate of rats containing enzymes for activation and inactivation of genotoxic compounds.

Sewage treatment plant (STP): Biological treatment plant for the clarification of municipal wastewater with activated sludge (see also WWTP).

Short-term tests: Test duration does not exceed 7 days.

Standardiseringen i Sverige (SIS): Swedish Institute for Standards.

Society of Environmental Toxicology and Chemistry (SETAC): Professional non-profit society with offices currently in North America and Europe dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to hazard/risk assessment.

SS: Swedish Standards.

Stripping: Removal of substances from the water phase to the atmosphere during the aeration process in wastewater treatment plants.

Suomen (Finland) Standard Isoimisliito (SFS): Finnish standards association.

Total organic carbon (TOC): The total amount of organic compounds, both soluble and insoluble, present in the water.

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.

Toxic Unit (TU): An expression of the toxic potency of a substance in solution or of wastewater expressed as a multiple of a standard toxicity endpoint. For a chemical this is often the concentration divided by LC_{50}. For a wastewater it is often 100% divided by the endpoint as a percentage (e.g. 100%/LC_{50} of 10% equals 10 TU).

Toxicity reduction evaluation (TRE): Process where the potential to reduce the emission of toxic substances at the source is evaluated.

Ultimate biodegradation: The breakdown of organic compounds by micro-organisms in the presence of oxygen to give carbon dioxide, water and mineral salts and new biomass.

Uncertainty factor (UF): A factor applied to an exposure or effective concentration to correct for identified sources of uncertainty.

Wastewater: Water that has been altered in its quality due to use in households, industries, agriculture or others and the flow off to go with it during dry periods as well as precipitation run-off from buildings and man-made ground sealing. This includes the discharge of produced water from offshore oil and gas production platforms in the sea.

Whole Effluent Assessment (WEA): analytical-chemical, biochemical or biological methods to determine:

1. a specific element; and/or
2. a chemically defined group (e.g. AOX, BOD, COD, TOC, Ntot, PBS); and/or
3. a sum parameter (e.g. PAH, BTEX, heavy metals); and/or
4. the toxicity (acute and chronic), genotoxicity, persistence, bioaccumulation, endocrine disruption, of (complex) mixtures of organic and inorganic compounds.

Whole Effluent Toxicity (WET): Evaluation of effluent toxicity with direct measurements in biological tests (see also DTA).

Wastewater treatment plant (WWTP): cf. STP.

EXECUTIVE SUMMARY

The background document in hand focuses on emission based methods of wastewater analysis as part of whole effluent assessment. The great variety of test organisms, standards, data evaluation and experience of the OSPAR's Contracting Parties are summarised and different programmes and strategies to predict adverse effects upon water quality and biological populations in the recipients are described and discussed.

There are essentially two approaches for evaluating possible adverse effects of wastewater discharges on living communities in river and marine recipients. In the water quality based approach, monitoring in the receiving waters is of primary importance. In the emission-based approach, the monitoring of effects through performance of bioassays and of chemical parameters (group parameters as well as specific substances) through analysis of the wastewater itself is of primary interest. Usually both approaches are combined. However, with regard to the use of parameters for effluent control within the contracting parties, there is up to now no common approach at hand. This background document provides the information on the state of the art and its practical use needed to support further discussions on how to harmonise the use of Whole Effluent Assessment (WEA) among the Contracting Parties.

BACKGROUND

The 1994 Working Group on Point Sources (POINT 94) took up the issue "ecotoxicological evaluation of wastewater" with a view of summarising the information on use and experience on that subject within the member states. During the relevant OSPAR workshop held on 23-24 September 1997 in Berlin, it was stated that bioassays are valuable tools in gaining additional information about wastewater quality and should especially be applied to monitor complex effluents, focussing on effluents with a potentially great environmental effect. For the conclusions of the Berlin Workshop see Annex I.

In previous POINT meetings (1995, 1998) it was agreed to use group parameters and biological effects parameters in Whole Effluent Assessment (WEA).

The previous POINT meetings revealed that there is only limited support for stipulating emission limit values by means of bioassays, but showed a need for discussing ways how to introduce bioassays in legislation and permitting (POINT 98/5/5). This led to the proposal to prepare a background document in which the current status of test development, data evaluation, and practical experience is summarised. POINT 97 decided that a background document should be prepared to serve as a basis for determining what role bioassays and chemical group parameters may play in the work of POINT.

At the workshop in Lelystad the background document was further elaborated and was identified as an important basis for the ongoing discussions on WEA (Annex II). POINT 99 agreed:

a. that WEA has the potential to be an efficient additional tool with regard to hazardous substances e.g.:
   (i) to identify and characterise individual effluents;
   (ii) to identify industrial sectors which discharge these effluents;
   (iii) to use this tool in the evaluation and development of BAT;
   (iv) to develop targets/benchmarks for effluent quality and/or quality of receiving waters;

b. to present the background document to PRAM 2000 with a view to having it published;

c. to arrange for the future work to be carried out by an intersessional expert group with the objective to further investigate existing possibilities for protecting the marine environment, and to report on the outcome of this to POINT or its successor group, aiming at the practical application and implementation of WEA in support of the implementation of OSPAR's strategy with regard to hazardous substances.

The background document gives an overview on emission based methods of wastewater analysis as part of whole effluent assessment. Test organisms, standards, data evaluation methods and experience of the Contracting Parties are summarised. The different extrapolation approaches applied to predict adverse effects upon water quality and biological populations in the recipients are discussed.

GENERAL INFORMATION

Wastewater constituents

Wastewater may contain a variety of known and unknown substances. In the EINECS inventory of existing chemicals about 100 000 chemicals presumed to be on the European market are listed. A lot of them are potential wastewater components from manifold sources. In view of the precautionary principle it would be optimal to analyse all substances in a discharge, to determine their concentrations, and to have knowledge of their effects on the environment. On the basis of such data, efficient measures could be taken to minimise harmful effects.

In most cases, however, knowledge of wastewater constituents is very limited. In chemical processes, not only the target substances and products, but also an additional large number of unknown by-products may be synthesised. Moreover, new substances may be formed during biotic or abiotic degradation in the treatment plants. It would require a

huge expenditure to analyse every single substance if it were possible at all. For most substances, there are not even any standardised analytical methods. Information about biological effects of chemicals potentially present in treated effluents would in most cases be unavailable, even if these chemicals were identified and their concentrations were known. This applies in particular to synergistic or antagonistic effects (Matthiessen *et al*., 1993; Johnston *et al*., 1996).

Effluent monitoring as regards group parameters like AOX, TOC, BOD in combination with bioassays, is appropriate to achieve both a reduction of chemical loading and a decrease of ecotoxicological effects from wastewater.

Group parameters like AOX, TOC, BOD provide valuable information about the efficiency of wastewater treatments and can basically characterise effluents from different industries. Nevertheless, specific chemical characterisation of single substances may still be required and information on the persistence and bioaccumulation of hazardous substances in effluents should not be ignored.

Results from bioassays may indicate levels of toxicity and potential environmental impact without necessarily correlating with chemical group parameters.

Bioassays

Currently the chemical specific approach plays a major role in water quality policy. However, when considering complex mixtures such as effluents, the possibilities for a chemical specific assessment are limited since:

a. there are many substances which can not be identified;

b. not all substances can be analysed/are detectable. The number of substances can be so large, that a chemical specific approach is not feasible;

c. there is a lack of data on effect-parameters for many substances. Data on the environmental characteristics are not available or incomplete;

d. micro-pollutants and degradation-products are undefined, and therefore not accounted for;

e. combined effects of substances, present in the discharges, are not being taken into account. The environmental characteristics of a mixture can differ significantly from those of single substances;

(e.g. according to Tonkes *et al*., 1997).

Some of the disadvantages of the chemical specific approach can be avoided by using chemical group parameters (e.g. COD, TOC, AOX, PBS) which provide a better picture of the constituents of an effluent as all substances are considered regardless of their chemical

specification. Commonly only a few % of the concentration measured by group parameters can be explained by the specific chemical analysis. But scientists agree, that there is no strict relation between group parameters and ecotoxic effects measured in effluents.

Bioassays have the advantage that toxic effects of bioavailable substances on aquatic organisms are measured directly and therefore all kinds of hazardous substances including their degradation products are considered.

A major objective of aquatic toxicity tests is to estimate the "safe" or "no adverse effect" concentration of test items, which is defined as the concentration that will permit normal propagation and development of fish and other aquatic life in receiving water (Klemm *et al*., 1994). The biological endpoints that have been considered in tests to determine the adverse effects of toxicants include survival, reproduction, growth, locomotor activity, gill ventilation rate, heart rate, blood chemistry, histopathology, enzyme activity, olfactory function, and teratogenesis. It is not feasible to measure all of these effects on a routine basis nor easy to link them to ecological impact. However, lethality, reproduction and growth may be combined into a population level endpoint which may be more defensible to use than others.

Acute mortality is a severe and easily observed effect. The results are usually expressed as the concentration lethal to 50% of the test organisms (LC_{50}) over a short exposure period (24 - 96 hours). For micro-organisms other endpoints such as the inhibition of cell growth or of special biological functions (nitrification, luminescence) are used. For many compounds, the toxicity increases with increasing exposure period. However, even laboratory life cycle tests are not able to accurately predict the environmentally safe concentration, because they are e.g. conducted with a limited number of species or under highly controlled, steady-state conditions.

Bioassays are used for different purposes, e.g. effluent control, effect/concentration curves for single substances, on-line biomonitoring and as biomarkers. Depending on their application they meet specific and different requirements. It should be noted, however, that the effects which may be identified are limited to those specific to the organisms, test conditions and endpoints employed.

In the early 1970s the first testing guidelines were developed. In an effort to obtain data on chronic effects of effluents in a cost-effective manner, the US-EPA began developing short-term toxicity tests for estimating chronic toxicity in 1980 (freshwater 4-7d; saltwater 1h-9d).

Since then, the number of ecotoxicology tests and the experience in performing tests has grown rapidly. The ability to detect acute and chronic toxicity plays an increasing role in identifying and controlling the toxicity of discharges to surface water.

First experience in effluent testing indicated, that even discharges, that had passed chemical quality criteria imposed by competent

authorities were acutely toxic to aquatic life (Heber *et al*., 1996). In other words, effluent limitations on specific wastewater constituents do not necessarily provide adequate protection for aquatic life. In many cases, the toxicity of wastewater constituents is not known. In contrast, by assessing WET Effluents testing with bioassays enables the detection of additive, synergistic, or antagonistic effects, and/or an evaluation of the toxicity of an effluent which has not been chemically characterised (US-EPA, 1995): In the case of positive results, detailed fractionation studies are carried out.

A first review about *Environmental Hazard Assessment of Effluents* was published by Bergmann *et al*. (1986). In 1995 a workshop on whole effluent toxicity at the University of Michigan provided a detailed overview (Grothe *et al*., 1996). The Society of Environmental Toxicology and Chemistry (SETAC) held a conference at Luton University on the 15-16th July 1996 and a major symposium and workshop was hosted by Zeneca (Brixham Environmental Laboratory), in Torquay from 29 to 31st October 1996. In 1997, an OSPAR workshop on the "ecotoxicological evaluation of wastewater" was organised by the Federal Environmental Agency in Berlin. In the recent workshop ”Effluent Ecotoxicology: A European Perspective”, held in Edinburgh from 14-17 March 1999, experience with numerous test methods was presented from different European countries. The proceedings of this workshop including the reviews of Chapman (2000) and La Point *et al*. (2000) have been published in Environ. Toxicol. Chem. 19, 2000.

For monitoring wastewater discharges, attention has focused on bioassays, that meet the following requirements:

a. internationally accepted standard with clearly defined endpoints;
b. reproducibility and comparability of the results;
c. sensitivity towards a large number of chemicals;
d. measurement of biologically relevant toxic effects using organisms representative of the aquatic environment (juridical reliability);
e. able to show clearly the success of wastewater treatment;
f. practicable for routine measurements (test organisms available throughout the year, suitable for laboratory cultivation);
g. moderately time consuming and having moderate equipment costs and able to rapidly provide unambiguous test results.

There are acute and chronic international standardised methods available which meet these requirements. The test principles are described in the chapter on Standardised methods and methods approved or proposed for testing wastewater (page 13).

While direct discharges of industrial wastewater into the receiving water may cause direct effects upon the aquatic community, indirect discharges are treated together with domestic wastewater in municipal

biological treatment plants. Municipal wastewater treatment plants usually consist of a mechanical treatment (grit removal, primary clarification), a biological treatment (TOC removal, nitrification, denitrification, phosphate precipitation) and a final clarification tank (sedimentation of activated sludge, effluent). In this context ecotoxicity tests are applied to assess possible adverse effects of effluents on the biological process. The respiration and nitrification inhibition tests with activated sludge are the most widely accepted tests for predicting impacts on purification efficiency. In addition, biodegradation tests are used to assess the behaviour of effluents in the treatment plant.

METHODS FOR WHOLE EFFLUENT ASSESSMENT TESTING

General

In a recent review paper on aquatic toxicity testing methods for pesticides and industrial chemicals about 450 pelagic and 260 benthic test methods from national and international test standards and the scientific literature have been reviewed (OECD 1998a). In addition, about 20 test methods for determining biodegradation and elimination are listed in the current ISO work programme ”water quality”. However, only a few of the described test methods have been applied in wastewater toxicity evaluation. Nevertheless, there are tests for all four trophic levels (bacteria, algae, herbivores, carnivores) available and a test battery can be used to find out the trophic level most sensitive to the effluents tested (trophic level approach).

The principles of most test standards are based on their respective OECD guidelines, which have been adopted and specified in EU and ISO as well as in national standards. The different groups of standards are related to a larger or lesser degree. The section on Standardised methods and methods approved or proposed for testing wastewater (page 13) of this BD focuses on internationally accepted standards with ISO and CEN as the most important ones, followed by OECD guidelines. National standards are only summarised if they cover specific additional aspects for whole effluent testing. In the section on Tendencies and methods under development or often employed but not internationally standardised of this BD, trends and methods under development are presented.

Test species, test methods, and the corresponding ISO, EN, OECD and national standards are summarised in Annex III. The time required to perform the tests is also estimated based on German experience and literature information. The lower value corresponds to routine testing of series of samples and/or screening, the upper value to a single sample test and/or full scale test performance. Real test costs may be calculated by multiplying the required time by the corresponding price per hour of laboratory work.

Sampling and pre-treatment

The sampling procedure as well as preservation and pre-treatment of samples are described in detail in ISO 5667-16: The choice of representative sampling points, frequency of sampling etc. is dependent on the objective of the study. The material of vessels should be chemically inert, easily 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 samples are normally stable for up to 24 hours. Deep freezing below – 18°C in general increases the stability in preservation. In general, biotests are carried out with the original sample.

Wastewater samples containing large amounts of particulate matter, sludge and sediment interfere with the behavioural requirements of the test organisms or with the detection devices (e.g. by photometry). In some tests (e.g. Ames-test) sterile conditions are required. All separation methods, however, involve the risk that active components, bound to the particulates, are removed prior to the tests. It is recommended to allow the sample to settle for 30 minutes to 2 h, if the presence of particulates causes severe problems. Only large particles are removed in the process. Centrifugation is in general preferred to filtration. In some testing guidelines for wastewater testing a sedimentation process has been introduced as a routine element (e.g. DIN 38412 T 30). Other test methods (e.g. the *Vibrio fischeri* assay) offer the possibility of determining a correction factor for parameters such as turbidity.

Wastewater organisms may interfere with the test system (e.g. bacteria with respiration inhibition, protozoa with alga growth).

As a rule, samples with extreme pH values exceeding the tolerance limits of the test organisms are 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). Neutralisation of samples is proposed e.g. in the German test guidelines for ecotoxicity testing of wastewater.

Especially when testing for genotoxicity, effluent as well as surface water samples are often highly concentrated.

Standardised methods and methods approved or proposed for testing wastewater

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. Sampling programmes for biotests in WEA are sometimes based on this guideline (e.g. in Germany). Furthermore, this international standard describes wastewater monitoring based on LID.

Ecotoxicity testing

In acute toxicity tests the test organisms are exposed for a relatively short time in relation to the generation time. Acute toxicity affects the survival/mobility of the organisms (e.g. fish or Daphnia acute toxicity test). In chronic toxicity tests the organism is exposed for a significant part of its life cycle. Chronic toxicity covers sublethal effects in the form of reduced growth (e.g. algae or bacteria growth inhibition test), or reproduction capacity (e.g. Daphnia reproduction test) or altered a development (e.g. fish early life stages test).

Acute ecotoxicity tests generally involve exposure of any test organism to different effluent concentrations and controlled water. The test duration ranges from 30 minutes (e.g. bacteria) to 96 hours (e.g. fish). The test can be performed as static, semi-static or flow-through. Flow-through tests are generally considered too costly and impractical to be conducted at off-site laboratories.

The short-term chronic methods are considered to be an effective analytical tool, since they provide a more comprehensive prediction of the effects of toxic effluents on aquatic life in receiving waters than acute tests, and since the overall level of effort is reduced compared to earlier (long term) chronic test methods (US-EPA, 1995). The endpoints generally used in chronic tests are survival, growth, and reproduction within a test duration of 16 hours to 7 days. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components that adversely affect the physiological and biochemical functions of the test organisms. In the Netherlands, there are plans to include long term toxicity tests in WEA (H.B.Pols, RIZA, pers. commun.). Correlation between different parameters is under investigation.

The test duration of long term ecotoxicity tests, usually applied in environmental risk assessment procedures for chemicals is in the range of 7 to 60 days. The endpoints generally used are survival, growth, and reproduction. Several national assessment schemes have proposed their use in WEA, when effects in the receiving water are to be expected.

All test guidelines presented below are, if not otherwise noted, most readily applied to test substances which, due to their water solubility and low volatility, are likely to remain in water, or they are designed to be applied to effluents directly. They are all used or proposed by different countries to assess and/or regulate the discharge of toxic substances into the aquatic environment (see Annexes III-1 to III-3 and the chapter on Status of whole effluent assessment for the Contracting Parties (page 43)).

ACUTE ECOTOXICITY TESTS IN FRESHWATER ENVIRONMENT

The following acute tests are most commonly used by the Contracting Parties: Fish (*Leuciscus idus*, *Brachydanio rerio*, *Cyprinus carpio*, *Dicenthrarcus palrax*, *Gasterosteus aculeatus*, *Salmo trutta* and *Salmo salar*), daphnids (*D. magna*, *D. pulex*), algae (*Scenedesmus subspicatus*, *Raphidocelis subcapitata*) and bacteria (*Vibrio fischeri*, *Pseudomonas putida*, activated sludge, anaerobic digester sludge).

In the United States, 10 freshwater and marine organisms are commonly used: *Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex*, fathead minnow *(Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), mysids (*Nysudiosis bahia* and *Holmesimysis costata*), bannerfish shiners (*Notropis leedsi*), sheephead minnows (*Cyprinodon variegatus*), and silversides (*Menida menidia*, *M. beryllina*, and *M. peninsulae*) (US-EPA, 1995).

The acute tests used by the Contracting Parties and associated countries are summarised in Annex III-1 and described below. The main differences in standard procedures are the recommended specific test organisms and the duration of the test. Other guidelines are mentioned only if standardised EN or ISO or OECD test procedures do not exit or if important differences are to be reported. The different species used are mentioned in Annex III-1. For complete experience with test methods in different countries see the chapter on Status of whole effluent assessment for the Contracting Parties (page 43).

Acute fish toxicity test

The acute fish toxicity test is one of the most commonly used biotest methods. It is well established and internationally standardised (ISO, EN, OECD). It is also applied to determine wastewater charges (”Abwasserabgabe”) (e.g. in Germany), for wastewater permits (e.g. USA), and for environmental hazard and risk assessment of industrial effluents (e.g. Sweden).

The fish are exposed to the test substance or wastewater for a period of 48 - 96 hours. Mortalities are recorded and the LC_{50} or the Lowest Ineffective Dilution (LID; Germany) is determined. In general at least 7 fish are used for each concentration and in the controls. For the LC at least five concentrations have to be tested. In Germany a national standard method with 3 fish for each concentration is applied for the determination of toxic effects in wastewater.

Acute toxicity test with crustaceans

a. *Daphnia sp.* acute immobilisation test

As is the toxicity test with fish, the test with daphnids is also widely recommended and internationally standardised (ISO, EN, OECD). Daphnids not more than 24 hours old at the beginning of the test, are used. Laboratory-bred daphnids, apparently healthy and with a known

history (breeding method, pre-treatment), are used in this test. At least 20 animals, preferably divided into four groups of five animals each, should be used for each test concentration and for the controls. The test duration is 24 or 48 hours. The percentage immobility at 24 hours and, if determined, at 48 hours is plotted against concentration on logarithmic-probability paper. The EC_{50} is calculated. The EC_0 and EC_{100} have to be reported as well. In Germany a national, more cost effective standard method with 10 animals per concentration is used to determine the Lowest Ineffective Dilution (LID). Acute toxicity tests with *Cerodaphnia dubia* are recommended by the US-EPA for assessing effluent toxicity, but no reference guideline is available (Weber, 1993).

b. Acute gammarid toxicity test

The Contracting Parties (e.g. Denmark) have reported experience with gammarids but so far the test has only been standardised by the US-EPA. The US-EPA specifies the amphipods *Gammarus fasciatus*, *G. pseudolimnaeus*, and *G. lacustris* as test organisms. The mortalities of the test organisms are recorded at 24, 48, 72 and 96 hours and the LC_{50} is determined. A minimum of 20 gammarids per concentration is exposed to five or more concentrations. The 48, 72 and 96 hours LC_{50} and their corresponding 95% confidence limits are reported, as is the 24 hour LC_{50}, if sufficient data have been generated.

Acute toxicity tests with rotifers

Rotifers are used in WET testing (e.g. in Denmark, Belgium), but to date no internationally standardised test procedures exist. The rotifer *Brachionus calyciflorus* has been applied in ”toxkit” methods, and an ASTM standard is available.

Acute toxicity tests with protozoans

Protozoans are used in WET testing (e.g. in Denmark, Belgium), but to date no internationally standardised test procedures exist. The ciliate Tetrahymena is part of the Danish risk assessment scheme for effluents, and an OECD standard protocol has been elaborated (Pauli, 1996).

Acute bacteria toxicity tests

Bacterial toxicity tests have been applied to assess indirect discharges with the objective to protect biological treatment plants. In this sector toxicity tests with activated sludge (inhibition of respiration/nitrification) are used. In addition, bacterial toxicity is measured as a routine parameter to assess impacts of specific direct discharges such as cooling water on the aquatic environment.

a. Activated sludge respiration inhibition test

This method is used to determine the toxicity of substances and effluents. It has been reported by different countries (e.g. Spain, UK) and is also used for environmental hazard and risk assessment of

industrial effluents (e.g. Sweden). The method is internationally standardised (ISO, OECD).

The method assesses the effect on micro-organisms by measuring the respiration rate. The purpose of this test is to provide a rapid screening method to identify substances, which may adversely affect aerobic microbial treatment plants and to indicate suitable non-inhibitory concentrations of test substances to be used in biodegradability tests. The oxygen consumption of aerobic sludge is measured to determine the respiration rate. At least 5 concentrations should be used. The test duration is 3 hours. An EC_{50} value is calculated.

b. Nitrification inhibition test

This test is useful to identify toxic effects on nitrification processes in aerobic microbial treatment plants. It is internationally standardised (ISO). It has not yet been in use for a very long time and for that reason only little experience with effluents has been reported so far. For determining nitrification inhibition the concentration of nitrite and nitrate is determined. Most commonly, 5 different concentrations of test substance are tested. Inhibition is calculated by comparing the nitrification rate of test vessels with that of the controls. The test duration is 4 hours.

c. Acute toxicity test with *Vibrio fischeri*

The acute toxicity test with *Vibrio fischeri* (previously known as *Photobacterium phosphoreum*) is one of the most commonly used biotest methods. It is well established and internationally standardised (ISO). It is used by many member states (e.g., Belgium, Finland, Germany, and Sweden) for environmental hazard and risk assessment of industrial effluents.

The method uses inhibition of light emission by the marine bacterium *Vibrio fischeri* as endpoint. The test is performed using a specially designed apparatus. Light emission is measured photometrically using a suspension of bacteria. The test duration is most commonly 30 minutes. A number of publications report a duration of only 5 minutes. EC_{50} values or Lowest Ineffective Dilutions (LID) are determined. The procedure is used to assess aqueous effluents, leachates, surface water and also chemicals.

SHORT TERM CHRONIC ECOTOXICITY TESTS IN THE FRESHWATER ENVIRONMENT

The endpoints generally used in short-term chronic tests are survival, growth, and reproduction. Most test guidelines especially adapted for effluent testing have been developed in the United States in recent years. In the countries of the Contracting Parties the following short-term methods are used to measure the chronic toxicity for freshwater organisms:

Chronic fish toxicity test

a. Fathead minnow (*Pimephales promelas*) survival and growth test

Larvae (preferably less than 24 hours old) are exposed in a static renewal system to control water and at least five concentrations of effluent or receiving water for seven days. Survival and weight of the larvae in the test solution is compared to that in the controls. Toxicity endpoints are NOEC (no adverse effect on survival or growth) and IC_{25} (inhibition concentration, for a 25% effect). US-EPA recommends the IC_{25} for regulatory use.

b. Fathead minnow (*Pimephales promelas*) embryo larval survival and teratogenicity test

Fathead minnow embryos are exposed in a static renewal system to control water and at least five different concentrations of effluent receiving water from shortly after egg fertilisation to hatch. The larvae are exposed an additional four days posthatch (total of eight days). Test results are determined for the combined frequency of both mortality and gross morphological deformities (terata) in test solutions compared to the controls. The test is useful for screening for constituents teratogenic to organisms exposed to them during embryonic development. Toxicity endpoints are NOEC, with no adverse effect on survival, growth, or reproduction observed, and IC_{25} (inhibition concentration for 25% effect). US-EPA recommends the IC_{25} for regulatory use.

Chronic toxicity test with crustaceans

***Ceriodaphnia dubia* survival and reproduction test**

Ceriodaphnia is closely related to *Daphnia*, but is smaller and has a shorter generation time of three to five days, compared with six to ten days for *Daphnia* (Weber, 1993). For that reason, *Ceriodaphnia* is increasingly being used to determine the reproduction toxicity of test items. *Ceriodaphnia* neonates are exposed to control water and at least 5 different concentrations of effluent or receiving water in a static renewal system for a maximum of eight days. Test results are based on survival and reproduction in the test solutions compared to that in the controls. Toxicity endpoints are NOEC (no adverse effect on survival, growth, or reproduction) and IC_{25} (inhibition concentration for 25% effect). EPA recommends the IC_{25} for regulatory use. Another prolonged test procedure with three broods of young is described in the guideline for long term ecotoxicity.

Chronic rotifer toxicity test

A 48hr reproduction test with *Brachionus calyciflorus* is currently being ring-tested by 12 laboratories in France. The standard is expected to be published early 2000.

Chronic bacteria toxicity tests

a. *Pseudomonas putida* growth inhibition test

The growth inhibition test with *Pseudomonas putida* is internationally standardised (ISO, OECD) and also recommended in Denmark for investigation and assessment of hazard/risk to freshwater environments. The test is used to determine the growth inhibition of *Pseudomonas putida* in relation to a control culture. The test duration is 16 hours. The test is performed with dilution factors of 2, 4, 8, 16, 32, 64. The results are given as EC_{10} and EC_{50}.The bacterium *Pseudomonas putida* is used as a representative of heterotrophic micro-organisms in freshwater. The test is used to determine the toxicity of water, wastewater and water-soluble substances. The test procedure is not suitable for the testing of strongly coloured or highly turbid samples.

b. *Vibrio fischeri* growth inhibition test

In addition to the acute toxicity test with *Vibrio fischeri* an inhibition growth test has been developed to determine chronic toxicity effects. Bacteria are incubated for 7 hours with the test item and the inhibition of growth is determined. In Germany a national standard guideline is available.

c. Anaerobic bacteria inhibition test

This standard (ISO draft) prescribes a screening method for assessing the potential toxicity of substances, mixtures, wastewater, effluents, sludge or other environmental samples to the production of gas from anaerobic digestion of sewage sludge over periods of up to three days. Aliquots of a mixture of undiluted anaerobically digesting sludge and a degradable substrate are incubated alone and simultaneously with a range of concentrations of the test material in sealed vessels. The amounts of gas produced by anaerobic degradation of yeast extract in exposure to the various concentrations of the test material are calculated from the amounts produced in the respective test and control bottles. The EC_{50} and other effective concentrations are calculated. This is an important toxicity test regarding digestion sludge in wastewater treatment plants but up to now it has not often been used.

d. Growth inhibition of activated sludge micro-organisms

This method is also internationally standardised (ISO), but only few test results have been reported up to now. The test method is applicable to water, wastewater and chemical substances. Flasks containing organic test medium and test material are inoculated with an overnight culture of activated sludge. The biomass of these cultures and of controls is determined. The recommended method involves the measurement of the turbidity in a spectrophotometer at a wavelength of 530nm and expression in relative units (OD530). The test provides information on inhibitory effects on the micro-organisms

over incubation periods of up to 6 hours. EC_{50}, EC_{20} or EC_{80} is calculated.

Chronic algae toxicity test

Growth inhibition test

The algae growth inhibition test is one of the most commonly used biotest methods. It is well established and internationally standardised (ISO, OECD). It is also used for environmental hazard and risk assessment of industrial effluents (e.g. Sweden) and was reported by many countries. Exponentially growing cultures of selected unicellular green algae are exposed to various concentrations of the test substance over several generations. The inhibition of growth in relation to control cultures is determined over a fixed period.

For each concentration of test substance, the cell concentration is determined at least 24, 48 and 72 hours after the start of the test. The measured cell concentrations in the test cultures and controls are tabulated together with the concentration of the test substance and the times of measurements. The percentage inhibition of the cell growth and the average specific growth rate is calculated. Both endpoints are given as EC_{50}. In addition NOEC is calculated.

In Germany a cost-effective standard method with fewer replicates is used, which is especially designed for the examination of wastewater samples to determine the Lowest Ineffective Dilution (LID). A French AFNOR standard has also been published. Such adapted protocols for wastewater testing will be incorporated as an annex in the next revision of the ISO standard.

Chronic tests with higher plants

***Lemna sp.* toxicity test**

Up to now only US-EPA and ASTM standards as well as a French AFNOR standard (ref. NFXP T 90-337 "Testing water – Determination of the inhibitory effect on the growth of *Lemna minor*") are available, although this test method was reported by many countries (e.g. Sweden, Netherlands). The OECD recommended the method for inclusion in the OECD Test Guidelines Programme (OECD, 1998a). The recommended procedure consists of exposure of *Lemna sp.* species to series of chemical concentration and determination of theEC_5, EC_{50}, EC_{90}, LOEC and NOEC for Lemna growth based on total frond number, growth rate, and/or frond mortality. Other endpoints (optional) include dry weight and chlorophyll and pheophytin pigment analyses. At least five concentrations are tested. The test duration is 7 days. Observations should be made on day 0,3,5, and 7. The test species are *Lemna gibba* and *Lemna minor*.

LONG TERM ECOTOXICITY TESTS IN FRESHWATER ENVIRONMENT

Long term ecotoxicity testing methods are not often used for effluent testing. The Swedish and Danish Environmental Protection Agencies recommend chronic tests with fish and Daphnia for environmental hazard and risk assessment of industrial effluents.

Chronic fish toxicity test

a. Prolonged toxicity test

The prolonged toxicity test with fish has been standardised by the OECD. The test is used to measure lethal and other observed effects (including all effects observed on the appearance, size and behaviour of the fish that make them clearly distinguishable from the control animals) in fish exposed to test substances. The fish are inspected at least once a day. Threshold levels and NOEC are determined at intervals during the test period (at least 14 days). If necessary, the test period should be extended by one or two weeks.

b. Early life stage toxicity test

The early life stage toxicity test with fish has been internationally standardised by the OECD. Tests with the early life stages of fish aim to define the lethal and sub-lethal effects of chemicals on the stages and species tested. These are exposed to a range of concentrations of the test substance, preferably under flow-through conditions, or where appropriate, semi-static conditions. The test is started by placing fertilised eggs in the test chambers and is continued at least until all the control fish are free-feeding. Lethal and sub-lethal effects are assessed and compared with control values. The LOEC and NOEC are determined. Freshwater and saltwater fish species are recommended: *Oncorhynchus mykiss*, *Pimephales promelas*, *Brachydanio rerio*, *Oryzias latipes*, *Cyprinodon variegatus*. Examples of 15 other well-documented fish species are given in table 1B of the guideline. The recommended test duration is 28 to 32 days depending on the test organism.

Chronic toxicity test with crustaceans

a. *Daphnia magna* reproduction test

The *Daphnia magna* reproduction test is internationally standardised (ISO Draft, OECD). The primary objective of the test is to assess the effect of chemicals on the reproductive output of daphnids. To this end, daphnids less than 24 hours of age are exposed to the test substance. At least five concentrations are examined. The test duration is 21 days. The number of living offspring produced per parent animal is determined, as are the LOEC and the NOEC. In addition, as far as possible, the data are analysed using a regression model in order to estimate the EC_x (e.g. EC_{50}, EC_{20}, EC_{10}).

b. Renewal toxicity test with *Ceriodaphnia dubia*

The test principle is described in the section on Chronic toxicity test with crustaceans (page 25) dealing with short-term chronic ecotoxicity methods. The only difference is that the test duration here is prolonged to encompass three broods of young (about 9 to 15 days). ASTM and US-EPA standards are available. The test can with appropriate modifications also be used with other Cladocera.

Chronic toxicity test with higher plants

The objective of this test is to determine effects on plants during critical stages of development. It is performed under natural conditions and in the environment. It is a multiple dose test designed to evaluate the phytotoxicity of substances, notably pesticides. The test duration, according to the US-EPA guideline, should continue for the entire life cycle of the test plants, with observations every two to four weeks. The ASTM-guideline is also designed to examine effluents, leachates, sediments and surface water. At least five concentrations are tested. Aquatic plants representative of the following plant groups are to be tested: Dicotyledonae, Monocotyledonae, vascular Cryptogamae, algae, Bryophyta or Hepatophyta. The test duration should be long enough to assess multiple applications. Observations should continue for the entire life cycle of test plants, with observations to be made every 2 to 4 weeks.

ACUTE ECOTOXICITY TESTS FOR BRACKISH AND SALTWATER ENVIRONMENTS

In general marine toxicity tests are not used as often as freshwater tests. They are nevertheless very important to assess the hazard of effluents to the marine environment.

Acute fish toxicity test

Acute toxicity tests with marine fishes are recommended by several agencies (e.g. Danish and Swedish EPA, US-EPA) for risk evaluation of industrial effluents to marine environments. The test is also applied by other member states (e.g. The Netherlands). Up to now only a US-EPA guideline and a guideline of the Environment Agency of England and Wales for *Scophthalmus maximus* are available. Currently an ISO working group is preparing a test guideline with *Scophthalmus maximus*. The following saltwater species are recommended: Atlantic silverside (*Menidia menidia*); sheepshead minnow (*Cyprinodon variegatus*); tidewater silverside (*Menidia penisulae*). The LC_{50} and NOEC are determined in static, semi-static or flow-through tests. The duration of the test is 96 hours. At least five concentrations are used.

Acute toxicity test with crustaceans

a. Acute lethal toxicity to marine copepods

The application of this test was reported often (e.g. Belgium, Netherlands) and the test is also recommended by the Danish Environmental Protection Agency as a procedure for investigation and assessment of hazard/risk to marine environments from industrial effluents. Recently an ISO-guideline with the test species *Acartia tonsa*, *Tisbe battagliai* and *Nitocra spinipes* has been published. The guideline for *Tisbe battagliai* has been ring-tested by the Environment Agency of England & Wales.

Copepods are exposed to a range of concentrations of a chemical substance in a seawater, effluent or water sample. Mortality is recorded after 24 and 48 hours. The LC_{50} is determined. Optional determination of NOEC and LC_{100} is recommended. A limit test can be performed at 100 mg/l or at a lower maximum concentration at which the substance is soluble or is in stable dispersion under the conditions of the test.

b. Mysid acute toxicity test

Belgium reported the application of the acute mysid toxicity test. Up to now only US-EPA guidelines are available. The test is used to determine the acute toxicity of chemicals. The mysids should be exposed at least to 5 concentrations. A minimum of 20 mysids should be exposed to each concentration for up to 96 hours. LC_{50} is determined at 48 and 96 hours. Each test chamber should be checked for dead mysids at 24, 48, 72, and 96 hours. In addition to death, any abnormal behaviour or appearance should be reported.

Acute toxicity test with bivalves

a. Bivalve acute toxicity test (embryo larval)

The Environment Agency of England & Wales has developed a *Crassostrea gigas* embryo larval guideline that has been ring-tested. Existing US-EPA and ASTM guidelines prescribe methods for the evaluation of the acute toxicity of chemicals and mixtures to different bivalves: eastern oysters (*Crassostrea virginica*), pacific oysters (*Crassostrea gigas*), quahogs (*Mercenaria mercenaria*) or bay mussels (*Mytilus edulis*). The ASTM guideline also recommends the use of the test with appropriate modifications for aqueous effluents, leachates, oils, particulate matter, sediments and surface water. The test starts about 4 hours after fertilisation while embryos are in the 2- to 4-cell stage. At least five concentrations are tested in a static system. The endpoint for this test is the determination of the 48h $EC_{50.}$ An LOEC and NOEC are also to be calculated.

b. Oyster acute toxicity test (shell deposition)

An US-EPA- guideline prescribes tests to be used to determine the acute toxicity of chemical substances and mixtures to the Eastern

oyster (Crassostrea virginica). At least 20 prepared oysters are placed in each of the test chambers and exposed for a period of 4 days. At least five test concentrations should be used. The oysters are inspected at least every 24 hours. Shell deposition, i.e. the measured length of growth that occurs within the test period, is the primary criterion. At the end of the test the EC_{50} is determined.

Acute toxicity test with rotifers

In addition to the acute toxicity test with the freshwater rotifer *Brachionus calyciflorus* the ASTM standard describes another toxicity test with the newly hatched rotifer *Brachionus plicatilis* for estuarine and marine waters. The procedure is applicable to most chemicals and also for testing of aqueous effluents, leachates, oils, particulate matter, sediments, and surface water.

Acute toxicity test with protozoans

The Danish EPA has proposed another test system with the marine ciliate *Uronema marinum*, but no test guideline is available.

Acute bacteria toxicity test

Vibrio fischeri assay

The acute toxicity test with the marine bacterium *Vibrio fischeri* is also applied for freshwater effluent toxicity testing. The test method is described in the section on Acute bacteria toxicity tests (page 16).

SHORT TERM CHRONIC ECOTOXICITY TESTS FOR BRACKISH AND SALTWATER ENVIRONMENTS

Marine algae growth inhibition test

Monospecific algae cells are cultured for several generations in medium containing a range of concentrations of the test substance. The method is available as ISO draft. The minimum test duration is 72 hours, during which the cell density in each sample is measured at least every 24 hours. Inhibition is measured as a reduction in growth and growth rate. The $EC_{10,}$ EC_{50} and the NOEC are determined. Recommended algal species are *Skeletonema costatum, Phaeodactylum tricornutum* and red macroalgae.

Chronic fish toxicity test

a. Larval survival and growth test

This method has been recommended by US-EPA for evaluating the chronic toxicity of effluents and receiving waters to sheepshead minnow (*Cyprinodon variagatus*), using newly hatched larvae in a seven-day, static renewal test. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components which adversely affect the physiological and biochemical functions of the test species. This method is commonly

used in one of two forms: (1) a definitive test, consisting of a minimum of five effluent concentrations and a control, and (2) a receiving water test, consisting of one or more receiving water concentrations and a control. In a similar test toxicity to inland silverside (*Menidia beryllina*), is determined. The only difference is that 7 to 11-day old larvae are exposed. Results are based on the survival and weight of the larvae.

b. Embryo-larval survival and teratogenicity test

This method has been recommended by US-EPA for evaluation of the chronic toxicity of effluents and receiving waters to the sheepshead minnow (*Cyprinodon variegatus*), using embryos and larvae in a nine-day, static renewal test. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components which adversely affect the physiological and biochemical functions of the test organisms. The test is useful in screening for teratogens because organisms are exposed during embryonic development. This method is commonly used in one of two forms: (1) a definitive test, consisting of a minimum of five effluent concentrations and a control, and (2) a receiving water test, consisting of one or more receiving water concentrations and a control.

Chronic toxicity test with crustaceans

Mysid shrimp chronic toxicity test

The US-EPA recommends a short-term test using mysids, to determine the chronic toxicity of effluents using survival (Klemm *et al*., 1994). Another test method applied for chemicals is the long-term chronic toxicity test with mysids, which is performed as a flow-through test. At least five concentrations are used. The test duration is 28 days. The LC_{50}, MATC (Maximum-acceptable toxicant concentration) values, and effects on growth and reproduction are determined.

Genotoxicity testing

Under genotoxicity generally all effects that damage the DNA are summarised. DNA damage might be repaired enzymatically so that changes are not inherited by daughter cells and may lead to a change in DNA sequence (mutation).

There are three reasons for considering genotoxic effects in effluents (de Maagd, 1998):

a. Genotoxicity can affect fitness and reproduction of organisms

   I. Higher mutation frequencies can increase the instability of ecosystems

   II. Genotoxic compounds might be relevant for humans when contaminated surface water is used

downstream for other purposes such as agriculture, recreation or drinking water.

The first two arguments are based on a few studies (Lynch *et al*., 1995) but have not been proved in a clear cause/effect relationship. Up to now is not clear what relevance genotoxic effects have at the ecosystem level (de Maagd, 1998, de Maagd, 1999b, Depledge, 1998). The third argument can be broadened to encompass the claim that genotoxic effluents always indicate that compounds are used within the sector which may be considered to be of safety relevance to humans.

Often effluent as well as surface water samples are highly concentrated on solid phase or extracts in order to enhance sensitivity. But that might lead to unrealistically high and ecologically irrelevant exposure concentrations and there is no agreement as to what concentration factor would be acceptable (de Maagd, 1998, 1999b). Also, each concentration procedure recovers different fractions of the sample, and volatile substances may be lost. So testing crude samples should be favoured to get a realistic estimate of the genotoxicity of an effluent (de Maagd, 1999b).

There are numerous test procedures for genotoxicity testing of wastewater, but only a few of them are based on standardised international test guidelines. The most frequently applied test procedures are summarised in Annex III-4.

BACTERIAL TESTS

Ames assay

The Ames assay is a bacterial in vitro test with mutant *Salmonella typhimurium* strains that have lost their ability to grow in the absence of histidine. Reverse mutations caused by exposure to mutagenic compounds can reactivate their ability to form colonies in the absence of histidine. The number of colonies at different concentrations of the test compound is compared with that of the negative controls and is a measure of mutagenicity. The most commonly used *Salmonella* strains in wastewater screening are TA 98 and TA 100, designed for detecting frame shift mutations and point mutations respectively. Usually the test is performed in the absence and the presence of S9 liver homogenate in order to activate promutagens. The Ames-test has been the most widely used method in wastewater mutagenicity testing (Stahl *et al*., 1991, Houk, 1992) but in the last decade other genotoxicity tests were established, which are faster and easier to handle. Recently a microplate version of the Ames-test based on colour changes has been developed (Hubbard *et al*., 1994).

umuC-assay

The umuC-assay was originally developed by Oda *et al*. in 1995. The assay is based on the use of a genetically modified *Salmonella typhimurium* strain TA 1535 that contains plasmids with the umuC gene and the lacZ gene, which encodes for ß-galactosidase. The

activation of the umuC gene, as a part of the SOS pathway, by DNA-damaging agents is measured by an increase of ß-galactosidase induced colour reaction at 420 nm. The test is carried out with and without S9. Bacterial growth is measured as turbidity at 600 nm and biomass factors are considered in the test results.

Experience with the umuC test for wastewater testing is reported by Rao *et al*. (1995) with extracts of bleached kraft mill effluents in Canada. In Switzerland and Germany hospital and municipal wastewater has been investigated (Hartmann *et al*. 1998, 1999, Gartiser *et al*., 1999). The test method has been introduced for routine regulatory testing of chemical and pharmaceutical effluents (Miltenburger, 1997, Wastewater Ordinance of Germany).

CHROMOSOME DAMAGE

There are several standards for determining chromosome damages in eukaryotic cells. The OECD guidelines contain 13 tests for genetic toxicology testing of chemicals. For wastewater testing several approaches have been pursued, which are summarised in section on Genotoxicity (page 33), but no broadly accepted standards or procedures exist.

Elimination and Biodegradation

REMOVAL BY BIODEGRADATION

The biodegradability of wastewater samples is most commonly estimated by determining the biological oxygen demand over 5 days (BOD). The BOD is compared with the chemical oxygen demand (COD) and a BOD/COD-ratio of about 0,5 is assumed to indicate biodegradability of wastewater. As a parameter for readily degradable organic substances the BOD serves as an important criterion for choosing the dimensions of sewage treatment plants. Nevertheless, the short test duration involves the risk that too much weight might be given to the BOD as a criterion in the evaluation of overall biodegradability. For that reason, standard 28-day biodegradation tests for ready biodegradability with low inoculum concentrations (about 30 mg/l suspended solids) have also been occasionally applied to complex mixtures over. Standardised procedures are available from the OECD 301 test series and from ISO guidelines (Annex III-5). Endpoints of ultimate biodegradation are oxygen consumption and CO_2-evolution. Other endpoints such as the DOC-elimination are also used, but strictly speaking this can be interpreted as biodegradation only when degradation follows a typical curve with a lag-, a degradation- and a plateau-phase. Nevertheless the test design of the ready biodegradation tests assumes relatively low-test concentrations of 10 to 50 mg/l TOC, so that in the future standardised adaptations for wastewater applications should be considered. It is known that longer term BOD testing is interfered with by the oxygen consumption of nitrification processes, and this reduces accuracy, especially for wastewater samples with high ammonium loads. Other test systems with CO_2-evolution as an endpoint avoid this problem, but up to now these have seldom been applied due to the greater effort

involved in performing the test. In Sweden a modified DOC die away test according to EN ISO 7827 is used to determine degradability of wastewater (”Stork project”, Swedish EPA, 1997). In the Netherlands Tonkes *et al*. (1997) followed a similar approach using a modified OECD 301 E procedure with surface water as inoculum (Tonkes, personal communication 1999). In both studies the test duration was 28 days. It should be noted that such bulk parameters, yielding integrated estimates of biodegradation, may overlook the significance of more persistent chemicals which, though comprising a small proportion of total organic carbon, may nevertheless be of high toxicological significance (see also the chapter on Determination of elimination with group parameters (page 29).

REMOVAL BY SORPTION

Sorption to activated sludge

The sorption of wastewater constituents to activated sludge in biological wastewater treatment systems is an important clarification process. The sorbed fraction might be removed from the system with the excess sludge or might be degraded in the adsorbed phase.

There is still no internationally accepted test guideline for determining the adsorbable fraction of wastewater. Most commonly this fraction is estimated by the Zahn-Wellens test method according to EN ISO 9888 where the ”three hour value” is used to estimate sorption processes (see the chapter on Determination of elimination with group parameters (page 29). But strictly speaking, this test is not designed to distinguish between adsorption and biodegradation. One method, developed by the EPA for determining the sorption of chemicals on activated sludge, uses common model kinetics such as the Freundlich or Langmuir isotherm. The activated sludge is washed, settled and lyophilised into a dry powder prior to use as a sorbent (EPA OPPTS 835.1110 draft Activated sludge sorption isotherm April 1996).

Other methods described in the literature use fresh (Pagga *et al*., 1994) or dried activated sludge at different water hardness classes (Kördel *et al* (1996). Adsorption kinetics and isotherms are determined by DOC or chemical analysis over 2 to 48 hours using laboratory shakers or stirrers.

It must be noted that adsorption tests cannot be used to assess the fate of chemicals as chemicals might undergo biodegradation in the adsorbed phase. In this context the retention time of chemicals adsorbed to activated sludge is determined by the sludge retention time (usually 20 to 30 days) and not by the hydraulic retention time (usually less than 8 hours) in WWTP.

Sorption to solids and sediments

The possibility that hazardous wastewater constituents may be adsorbed to suspended solids and deposited in rivers has not been considered yet. First experimental approaches were discussed by

Pardos *et al* (1999). Degradation tests with suspended sediments which are under development, such as the shake flask batch test according to ISO/CD 14592, consider adsorption processes but are not practicable for wastewater testing as ^{14}C labelled substances are added.

REMOVAL BY EVAPORATION

There is no accepted standard to determine the removal by evaporation of wastewater samples. The Zahn-Wellens-Test provides for an additional abiotic control without inoculum but with a biocide to inhibit biodegradation. DOC-elimination in the abiotic control may be interpreted as stripping or other physical-chemical processes (see the chapter on Determination of elimination with group parameters below).

DETERMINATION OF ELIMINATION WITH GROUP PARAMETERS

The behaviour of wastewater in municipal treatment plants can be simulated by determining the elimination of organic sum parameters and by combining (bio)degradation test with ecotoxicity tests.

a. Zahn-Wellens-test

The Zahn-Wellens-test is the most commonly used test for determining inherent biodegradability of chemicals. International (ISO, EN, OECD) as well as national standard guidelines (EPA, ASTM, DIN) are available. The principle consists of an activated sludge static test with a high inoculum concentration (200 – 1 000 mg/l suspended solids). The test concentration is relatively high compared with the biodegradation tests discussed above (50-400 mg/l DOC). DOC/COD-elimination is determined for the filtered samples over a period of 28 days. Along with the test vessels with the test compound, blank vessels are assayed and an abiotic degradation check (abiotic control) is carried out.

In Germany three modifications of this test are part of the Wastewater Ordinance. Here the inoculum concentration has been fixed at 1 000 mg/l suspended solids and the test duration varies between 3 and 28 days according to the respective requirements in the different wastewater sectors. A DOC/COD-elimination of 80% (less the part eliminated in the abiotic control) is considered to indicate treatability in municipal treatment plants. The test is also used to determine elimination of other group parameters, such as AOX. Since strictly speaking the amount eliminated by biodegradation and that eliminated by adsorption cannot be distinguished, especially in the case of complex mixtures, results are given as elimination (=bioelimination).

b. Treatment plant simulation model

A laboratory sewage flow-through treatment plant is used to determine degradability of organic compounds. This test is also known as the Coupled units test or OECD confirmatory test. The test item is dissolved in a synthetic sewage matrix and continuously dosed

into the activated sludge vessel (3-litre capacity). A control unit is only fed with the synthetic sewage. Both units might be coupled by interchanging a defined volume of activated sludge once a day. DOC is measured in the effluent, and the daily DOC-elimination is calculated after correcting for the material transfer due to the transinoculation procedure. ISO, OECD and EPA methods are available. In a recent modification the concentration of synthetic sewage was halved in order to guarantee stable nitrification conditions (DIN 38412 L26, ISO /NP 16821). The test has been occasionally used to assess elimination of effluents in sewage treatment plants (Gartiser *et al*. 1996), but the considerable effort involved prevents its broader application. Further extensions of the test method with an additional anoxic vessel for denitrification processes are under development (Deutsche Einheitsverfahren, DEV L 41).

c. Elimination of biological effects

Degradability of wastewater constituents may be of special interest if effluent samples indicate ecotoxic or genotoxic effects. Usually this additional information is obtained by coupling degradation tests with the respective effect test. Effects can then be classified as ”inherent degradable” or ”hard”. This very useful approach may be considered as a part of a TIE-procedure. Up to now there is no international accepted guideline for combining degradation tests with effect tests.

In the Netherlands Tonkes *et al*. (1997) combined a DOC die away test with effect tests and also determined the degradability of potentially bioaccumulating substances (PBS).

In Germany, hospital and textile effluents have been assessed with a combination of elimination and genotoxicity and ecotoxicity tests. The Zahn-Wellens test and the treatment plant simulation model have been used as a degradation system and the practicability of the Zahn-Wellens-test has been confirmed (Jäger *et al*., 1995, 1996a, 1996b, Gartiser *et al*., 1996b, 1997).

The combination of a treatment plant simulation model with ecotoxicity tests has been integrated in the German Wastewater Ordinance for the sector ”landfill leachate”. Here the limits regarding effluent toxicity may be achieved after the biological treatment process.

In the United States of America a guideline for "Assessing microbial detoxification of chemically contaminated water .... " exists using a degradation test not specified and the *Vibrio fischeri* assay. The percentage difference between the EC_{20} of the treated and the untreated sample is used to assess the progress of detoxification (ASTM D 5660-96).

De Groot (1999) proposed to combine a 28 day biodegradation test with the chronic Daphnia reproduction test and the early life stage test with fish, but up to now no test results are available and the effort involved is considered enormous. Whale *et al*. (1999a) used a

respirometer biodegradation test to assess the recalcitrant ("hard") or readily biodegradable ("soft") toxicity of three effluents.

Tendencies and methods under development or often employed but not internationally standardised

Ecotoxicity

In the section on Standardised methods and methods approved or proposed for testing wastewater (page 13), the standardised and most commonly used methods are described. A short summary is given in Annexes III-1 to III-3. Although these approved test methods meet the general objectives of effluent and water toxicant control, alternative test methods may be needed for special areas.

The criteria recommended for selecting alternative test species and/or test design include the following topics (Weber 1993, Klemm *et al*. 1994, Chapman *et al*. 1995, OECD, 1998):

I. the proposed species should be ecologically, commercially and/or recreationally important within the receiving water's trophic structure

II. the species should be at least as sensitive to toxic substances as the current test species representing that phylogenetic category

III. an early life stage should be used because an early life stage is usually the most sensitive stage

IV. the early life stage of the alternative species should be readily available throughout the year

V. the alternative species must be easy to handle in the laboratory

VI. the alternative species must give consistent and reproducible responses to toxicants

VII. the toxicological endpoints should be easily quantifiable and amenable to statistical analysis

VIII. inter- and intralaboratory validation of the test procedures should be performed

IX. the test method should be of practical feasibility.

The stringent requirements necessarily placed on suitable test organisms render WET tests suitable as monitoring tools and indicative screens only. Such organisms are, necessarily, commonly among the more robust components of ecosystems. Absence of measured effects on test organisms exposed to an effluent cannot, therefore, be assumed to imply an absence of possible impacts on receiving water communities as a whole.

Comparing the most commonly used test methods as mentioned in the section on Standardised methods and methods approved or proposed for testing wastewater (page 13) with the activities in the different countries of the Contracting Parties, not much experience with procedures under development has been reported. Most of the

methods mentioned in addition to the most commonly used are toxkits (e.g. Daphtoxkit, Rotoxkit) and the use (in standard procedures) of different species of known taxonomic groups (see the section on Status of whole effluent assessment for the Contracting Parties (page 43)).

Recommendations for guideline revision and development are summarised in the Detailed review paper on aquatic testing methods for pesticides and industrial chemicals" published under OECD Series on Testing and Assessment (No. 11, 1998)". A total of 449 pelagic and 258 benthic methods have been compiled. OECD recommended the following tests with a high priority for OECD Guideline development:

a. Pelagic tests

(i) Crustacea, saltwater sp., acute and reproduction tests;

(ii) Higher plant, Kormophyta (*Lemna*), growth test;

(iii) Fish, full and/or partial life cycle test;

(iv) Microalgae, freshwater and saltwater sp., growth test;

(v) Mollusca saltwater sp., acute on ELS and shell deposition tests;

(vi) Bacteria, sludge bacteria, nitrification test;

b. Benthic tests

(i) Insecta, *Chironomus*, acute and chronic (growth and emergence tests);

(ii) Crustacea (amphipod), saltwater sp., acute and reproduction tests;

(iii) Annelida, saltwater sp., acute test;

(iv) Crustacea, freshwater sp. (*Hyalella* acute and growth tests).

In the "Proceedings of the International Workshop on the protozoan test protocol with *Tetrahymena* in aquatic toxicity testing" the UBA recommended the establishment of an internationally recognised test guideline with protozoans, due to the importance in the aquatic environment (Pauli *et al*., 1996).

The development and use of cost-effective toxkits has also been a widely discussed subject. There are a lot of advantages but the comparability of the results with those of known and evaluated test methods is an essential prerequisite. This aspect must also be taken into account in the application of test methods using "parts of organisms" e.g. cells, cell organelles or enzyme activities.

Enzymatic assays and biochemical group parameters

Enzymatic reactions at the suborganism level have been used occasionally as biological endpoint in WET testing. In Germany the

cholinesterase inhibition test is standardised (DIN 38415-1) and other enzymatic activity tests are under development (E-DIN 38411-10).

Test methods using the inhibition of acetylcholine-esterase to test complex mixtures are described in the literature (van Loon *et al*., 1995). However most commonly, enzymatic assays are performed in surface water monitoring. The enzymatic activities of biocenoses in activated sludge are currently being determined in several tests as a part of a European research project. Here the alanine aminopeptidase assay showed the most sensitive reaction (Alte *et al*., 1999). An ATP-luminescence method has also been developed within this project (Dalzell *et al*., 1999).

The induction of cytochrome P450 by major groups of aromatic and chlorinated chemicals is another endpoint used for toxicity testing of complex effluent using the EROD-assay (7-ethoxy-resorufin O-deethylation). In this assay microsomes prepared from rat liver may be exposed *in vitro*, or may already have been exposed *in situ* and P450 is detected fluorimetrically (van Loon *et al*, 1995).

Genotoxicity

Numerous test methods have been applied for effluent genotoxicity testing, they have been summarised by Stahl, 1991, Houk, 1992 and Helma *et al*, 1997.

BACTERIAL TESTS

SOS Chromoassay

The SOS chromotest originally was developed by Quillardet *et al* (1982). The test detects induction of the SOS genes, which are involved in DNA repair. The principle is similar to that of the umuC-test. The SOS genes are fused with a reporter gene, lacZ, that encodes for ß-galactosidase. If genotoxins induce the SOS function, the reporter gene is also activated and the formation of ß-galactosidase is quantified photometrically by its ability to form a yellow-colour metabolite. While the umuC assay uses *Salmonella typhimurium* as test organism, the SOS chromotest uses *Escherichia coli K12* bacteria. There is some evidence that the umuC test detects lower genotoxic responses than the SOS chromotest for two reasons: First the outer membrane of the Salmonella tester strain used is made more permeable to genotoxins and second the umuC reporter gene is placed on a multicopy plasmid while in the SOS chromotest it is placed on a single bacterial chromosome (Oda *et al*., 1995; de Maagd, 1999b). But there are only few comparative studies about the sensitivity of tests.

Wastewater studies using the SOS chromotest were performed in Canada (White *et al*., 1996; Legault *et al*., 1996), Austria (Helma *et al*., 1996) and Germany (Janz *et al*., 1990).

Mutatox ™

The Mutatox assay uses a dark variant of the luminescent saltwater bacteria *Vibrio fischeri.* (*Photobacterium phosphoreum*), also used for determining acute bacterial toxicity. Genotoxic damage induces the return of luminescence, which is used as a measure of genotoxicity. In contrast to the SOS chromotest and the umuC test the activation of the SOS pathway is determined by formation of a protease, that breaks down a repressor protein of the lux pathway thus leading to luminescence (de Maagd, 1999b). The test is used especially in the United States (Johnson, 1992).

Other bacterial tests

Helma *et al*. (1996) reported results of concentrated water samples (including wastewater) from other bacterial genotoxicity test systems applied to wastewater samples. The Microscreen phage-induction assay with *E. coli* strains was developed by Rossmann *et al*. (1984). The activation of the SOS system results in the release of lytic phages from *E. coli* [WP2s], which are detected following infection of a second (indicator) *E. coli* strain [TH-008]. The genotoxic potency is evaluated by counting the plaques in the bacterial layer. The DNA-repair assay with *E. coli* K12 strains enables the detection of (repairable) DNA-damage by comparison of the differential survival of strains differing in their DNA-repair capacity.

CHROMOSOME DAMAGE

In vitro and in vivo testing of genotoxicity at a higher level of biological organisation with eukaryotic cells might be more relevant for human and ecological risk assessment. But often the sensitivity of the test systems used is reduced. Generally test performance is much more time-consuming compared with the bacterial tests. So up to now chromosome damage testing of wastewater has been evaluated only in research projects. There are different endpoints in testing chromosome damage:

I. *sister chromatid exchange (SCE):* symmetric exchange between DNA segments of sister chromatids within one chromosome

II. *micronuclei formation* chromosome fragments that were not incorporated in the daughter nucleus and appear in the cytoplasm

III. *aneuploidy* unequal segregation of homologue chromosomes during cell divisions leads to numerical chromosome changes in cells

IV. *chromosome aberration* macro-damage of chromosomes including SCE, strand breaks, intercalations and micronuclei formation (=clastogenesis)

In Germany the chromosome aberration test with Syrian hamster cells V79 has been used in different studies to assess mutagenicity effects on eukaryotes (Göggelmann, 1989, Jäger, 1995, Gartiser, 1996,

Miltenburger, 1987). The test is well established in mutagenicity testing of chemicals according to OECD 476.

However, in 1992–1996 a joint program of the German Environmental Agency (UBA) and the Association of the German Chemical Industry (VCI) to select appropriate methods for assessing the mutagenic potential of native industrial wastewater showed that eucaryotic test systems like chromosome aberration with V79 CHO cells (CA), alkaline filter elution method with hepatocytes of Leuciscus idus (AFE-test), DNA-inhibition test using HeLa cells (DIT), as well as sister chromatid exchange test (SCE) often give unreliable and unsystematic results, which cannot be reproduced. One reason could be the partially high osmotic potential of some wastewater, which could lead to cellular dysfunction and damage. Only the CA-test produced reliable concentration dependent results if single substances were tested, but turned out to be not sensitive enough and too expensive. Hence the steering group of the joint UBA-VCI project focused on the umu test, which showed good correlation to the Ames test, especially with respect to not genotoxic samples.

In recent years the COMET assay has gained broad attention, because the test is relatively easy to handle and can be applied with different cells (e.g. Singh *et al*., 1988). The principle is that strand breaks of DNA lead to the formation of DNA fragments, that are differentiated in gel electrophoresis. The resulting comet like structure is quantified by measuring the length of the tail. As single cells (e.g. blood cells) can be used, one organism might be studied multiple times, so that a time-curve can be obtained. A disadvantage is the absence of a clear dose-response relationship, so that a quantitative evaluation is difficult (de Maagd, 1999). One test version with hepatocytes from fishes has been extensively used to determine genotoxicity of wastewater samples (Hollert *et al*, 1997).

In the *Allium*-test (onion) root cells are exposed and dividing cells are analysed for chromosome aberration (number of chromosomes, micronuclei) by light microscopy (Nielsen *et al*. 1994; de Maagd, 1998). Steinkeller *et al*. (1999) used another plant test, the *Tradescantia-micronuclus* assay (spiderwort), to determine mutagenicity of different surface water samples collected near industrial effluents. The endpoint is the number of micronuclei in meiotic pollen mother cells.

Elimination and Biodegradation

As mentioned in sections on Removal by biodegradation and on Determination of elimination with group parameters (see pages 27 and 29), the methods used to determine DOC-elimination in wastewater elimination tests in principle do not distinguish between biodegradation and elimination by adsorption, since the transfer of hazardous substances to sewage sludge and, subsequently, to farmland cannot be excluded. On the other hand, the methods commonly used to determine ultimate biodegradability use low inoculum concentrations and therefore underestimate adsorption processes and

elimination in sewage treatment plants. For that reason, new methods were developed combining both endpoints: CO_2 and DOC (Strotmann *et al*., 1995, Baumann *et al*., 1998). Currently DOC/CO_2 tests with high inoculum concentrations (around 200 mg/l suspended solids) are under development by ISO and further research is being performed in several projects of the German "Umweltbundesamt". Application of these test systems would facilitate knowledge and interpretation of behaviour in sewage treatment works. Low concentrations of persistent and highly active substances may not be ignored in such approaches.

Degradability of substance in marine ecosystems has been ignored for a long time. Recently, ISO working groups have adapted 5 fresh-water methods for use to determine the ultimate aerobic biodegradability of organic compounds in the marine environment in static aqueous test system. These methods are the DOC die away test, the closed bottle test (ISO 10707), the two phase closed bottle test, the CO_2 evolution test (ISO 9439) and the CO_2 headspace test. An ISO draft has been distributed, in which the optimal conditions for testing biodegradation in marine water are described. (ISO TC 147: Guidance for the determination of biodegradation in the marine environment 1998-28-04).

Endocrine disrupting effects

Currently several test methods suitable for detecting estrogenic or endocrine disrupting effects of chemicals in the aquatic environment are under development. But there are only few studies that consider possible effects of wastewater.

The induction of vitellogenin synthesis both in vivo and in vitro has proven to be a reliable biomarker for assessing the estrogenic activity of individual substances and the more complex effluents of sewage treatment plants. The first study, from the UK, was presented by Purdom *et al*. (1994), who exposed rainbow trout as tests organisms to WWTP effluents. A comparable German study using monoclonal antibodies confirm vitellogenin to be a suitable biomarker in WEA (Hansen & Dizer, 1998). In the Netherlands, Gimerno *et al*. (1999) exposed a population of male carps (*Cyprinus carpio*) to industrial effluents and determined the level of vitellogenin, a typical female protein in the plasma. With the aim of developing a practicable, reliable and cost-effective bioassay suitable for routine testing, a combined dot-blot/RNAse protection assay utilising digoxigenin-labelled cRNA transcripts of plasmid psg5Vg1.1 was used by Islinger *et al*. (1999) for the quantification of vitellogenin-mRNA in isolated rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Trout hepatocytes were also employed by Gagne *et al*. (1999).

In Belgium, Tanghe *et al* (1999) used the widespread recombinant yeast estrogen assay to screen aquatic environmental samples without any pre-treatment and found cross reactions with humic acids. In Germany Rehmann *et al*. (1999) found positive test results with a sewage sludge extract and concluded that the modified yeast estrogen assay was applicable for environmental monitoring.

Up to now there are no adapted test methods for determining endocrine disrupting effects of wastewater. Moreover, the majority of tests under development focus on estrogenicity as a mechanism, whereas numerous other potential pathways of endocrine disruption remain poorly researched. To date, for example, very little research has focussed on the ability of chemicals to interfere with endocrine function as a result of stimulation or inhibition of olfaction and other chemoreception mechanisms, despite the fact that olfactory reception is, for example, vital to trigger many aspects of secondary sexual development in salmonids (Waring & Moore 1997).

Potentially bioaccumulating substances (PBS)

Bioaccumulation describes a process whereby a chemical accumulates in plants and animals by diffusion, adsorption, active transport or by intake with food. In consequence higher concentrations of these chemicals are measured in organisms than in the surrounding environment (Pedersen *et al*., 1994). Factors that favour potential bioaccumulation are a low polarity, a low solubility in water, a high lipid solubility and a low degree of biodegradability (Pedersen *et al*., 1994). Usually the bioaccumulation potential of chemicals is evaluated by experimentally measuring the bioconcentration factor (BCF) of exposed fishes with analytical devices. For complex mixtures this substance specific approach is not practicable for routine assessment in *in-situ* studies due to the input it requires, and matrix problems (de Maagd, 1999a). Simple extraction methods with organic solvents (e.g. EOX-measurements) do not fit the purpose, because these also consider high molecular compounds, that owing to their hindered transport across biological membranes are hardly bioavailable. That is why different methods are under development to determine PBS as a sum parameter for effluents (see table 1). Other approaches include the identification of (single) substances.

TLC-METHOD

In 1985, Renberg *et al*. published the first method for the determination of PBS. A sample is applied to a reversed-phase thin layer plate (TLC) and chromatography is done with an acetone-water-mixture. The most lipophilic fractions are then isolated and quantitatively estimated using chromatography procedures such as GC, HPLC or GC-MS (Adolfsson-Erici *et al*., 1992; Hynning, 1996; Swedish EPA, 1997; Unden, 1997).

EMPORE™ DISK METHOD

Verhaar *et al*. (1995) and van Loon *et al*. (1996) published a method for a surrogate parameter for baseline toxicity simulating bioconcentration. Silica-based C18-coated teflon filter disks, the so-called Empore™ disks, were put into 2-10 litre samples of wastewater. An equilibrium is reached within 10 to 14 days. After extraction the amount of lipophilic substances on the empore disk can be detected by GC-MS or VPO (vapour pressure osmometry).

The empore disk is a biomimetic extraction procedure. In contrast to the quantitative extraction the biomimetic extraction seeks to identify the bioavailable fraction of the lipophilic organic compounds in the sample. It can be interpreted as a model of the water-biota system (Verhaar *et al*. 1995).

SPME (SOLID PHASE MICRO EXTRACTION) METHOD

The SPME method is similar to the Empore^{TM} disk but much easier to handle. A C8-, C18- or polyacrylate-coated fibre is added to the sample and the sample is then stirred for 24 hours. The accumulated compounds are extracted on solids and can be measured directly in the GC-MS without using a solvent, by injecting the fibre like a syringe (de Maagd, 1999a). The SPME is also a biomimetic method that is very simple, cost-effective and requires a shorter equilibration time compared with the Empore^{TM} disk (de Maagd, 1999a).

HPLC METHOD

Klamer *et al*. (1995) were the first to publish a HPLC based method for PBS in wastewater and sediment samples. Liquid/liquid extraction is performed on a 10-litre sample (e.g. hexane). After cleaning the extract is concentrated and fractionated in a C18-HPLC-column with gradient elution. Detection was performed with a fluorescence and a UV/VIS detector mounted in series. The retention times of 55 standard compounds correlated very well with their log P_{OW} and BCF. In their strategy for effluent long-term toxicity assessment, Boutonnet *et* al. (1999) use liquid/liquid extraction followed by semi-preparative HPLC to separate a fraction containing potentially bioaccumulating substances, which are identified by GC/MS.

SEMI PERMEABLE MEMBRANE DEVICE (SPMD)

Södergren (1987) used a plastic polyethylene tube (dialysis membrane) filled with hexane as passive extraction tool to assess the bioavailable PBS of effluents. Particle-bound compounds do not pass the membrane. In later studies purified fish lipids were used (Huckins *et al*., 1990). Depending on the type of hydrophobic phase equilibration times up to 3 weeks are required (de Maagd, 1999a).

PREPARATIVE HPLC

In Germany a PBS method based on HPLC fractionation is under development for the Federal Environmental Agency (Metzger *et al*., 1999). A methodology was developed for a sum parameter which includes dissolved and suspended-solids-bound compounds. The samples are filtered and suspended solids on the filter are extracted by an organic eluant. Together with the filtrate these are accumulated by SPE (solid phase extraction), eluted and separated by preparative HPLC. The log P_{OW} fraction of 3 to 8 is collected, lyophilised and detected by weighing or TOC.

Table 1 Proposed methods for determining potentially bioaccumulating substances (after de Maagd, 1999)

Method	Pre-treatment	Extraction	Time	Separation / Detection	Reference
TLC	pH <2	Reversed-phase thin layer plate Acetone-water-mixture		TLC / GC	Renberg *et al*., 1985
EmporeTM disk	pH 7.5	C18-coated teflon filter disk	10-14 d		Verhaar *et al*., 1995 Van Loon, 1996
Solid phase micro extraction (SPME)	pH 7.5	C8-, C18- or polyacrylic-coated fiber	24 h	GC-MS	Verbruggen, 1999
HPLC	pH 2	liquid/liquid e.g. hexane		HPLC fluorescence UV/VIS	Klamer *et al*., 1995
Semi permeable membrane device		polyethylene tube filled with organic solvent (hexane) or lipids	up to 3 weeks		Södergren, 1987
Preparative HPLC	Filtration, extraction of solids with organic solvent	Solid phase extraction of filtrate and extract		HPLC, lyophilization / TOC or weighing	Metzger *et al*., 1999

GC: gas chromatography
TLC: thin layer chromatography
HPLC: high performance liquid chromatography
MS: mass spectrometry
UV/VIS: ultraviolet or visible photometry

TEST DESIGN AND DATA PROCESSING

Selection of test organisms

In general, species which occur or at least could occur in the receiving water should be used in order to reflect real-environment conditions as close as possible. On the other hand, in some cases material from native species may not be available all year round and maintenance of the test organisms will then be much more difficult and costly. Discharges that go into the brackish or marine environment should be tested with methods employing marine species. This demand may entail to modify standard methods. Greater environmental realism is also the reason for using a test battery with one species from each of the four trophic levels, i.e. bacteria, algae, herbivores, and carnivores (trophic level approach), in order to find out the trophic level most sensitive to the effluents to be assessed. The test organism chosen within a taxonomic group may have a drastic influence on the test result. For example, in a comparison of EC_{50} values the variability in the sensitivity of 7 algae species to 3 metal compounds was found to be as high as 5 orders of magnitude (Padrtova *et al*., 1999). There are two opposing philosophies in the choice of test organism. One

tendency is to standardise test organisms as far as possible (low genetic variation) to reduce variability and increase reproducibility and comparability. The other tendency is to use geographically and biologically representative organisms and/or wild populations to have a better basis for interpretation of results based on environmentally realistic conditions. (Pedersen, 1994).

Test procedure

The ISO, EN and OECD guidelines for toxicity testing originally were designed to assess biological impact of chemicals in full-scale tests. They include the possibility of performing ”range finding” tests with critical concentrations or ”limit tests” with one concentration. For economical and ethical reasons the number of higher organisms exposed at each concentration should be kept to a minimum (Pedersen, 1994). But only a few national standards exist, in which full-scale tests were adapted especially for wastewater investigations. In Germany national standards that include screening tests of fish, Daphnia, algal and bacterial toxicity have been standardised for use in wastewater testing. Here fewer individuals (e.g. three fish instead of seven, 10 daphnids instead of 20) are exposed per concentration. Furthermore test procedures are simplified in order to save time and effort (e.g. algal growth is determined only at the end of the test and not daily).

Test results are also dependent upon the exposure time, which varies from one standard to another. The test duration of the acute toxicity tests with *Daphnia* spec. varies e.g. from 24 hours (DIN 48412 T 30) up to 48 hours (OECD, 92/69/EEC.). Other tests with *Ceriodaphnia dubia* are performed from 24 to 96 hours (Weber *et al*. 1993). From single chemical toxicity testing it is known, that effect concentrations, i.e. the EC_{50}, decrease with exposure time. Time-related data analysis might be another approach for data processing (Pedersen, 1994).

Threshold concentrations (NOEC/LOEC/LID)

The No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) are well-established parameters, especially for estimating chronic toxic effects. The data processing method usually applied is the ANOVA approach (analysis of variance) where mean response at each concentration is compared with the mean of the control using a number of statistical tests such as the t-test (hypothesis testing) (OECD, 1998). A concentration-response relationship is not essential.

In Germany the Lowest Ineffective Dilution (LID) is used for the interpretation of test results from bioassays. In principle LID is 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). So LID may be interpreted as a No Observed Effect Concentration (NOEC).

The most important argument for NOEC data processing is that for routine measurements in toxicity evaluation of wastewater special screening versions of the test guidelines may be used which are adopted to environmental samples and may be used on a smaller number of parallel batches and concentrations. Thus, however, it may not be possible in all cases to calculate a concentration/effect relationship. Nevertheless, experience shows that only cost-effective and rapid test methods that do not exceed costs for chemical analysis by orders of magnitude have a chance to be implemented in legislation.

In contrast, statisticians recently concluded that the NOEC is inappropriate as a summary of toxicity of chemicals as (OECD, 1998):

I. No information can be given on the precision, such as confidence limits

II. Data are wasted in the determination of values such as the NOEC

III. The variability of test results is greater than for determining the EC_{50}.

Concentration/response relationship

Data showing a clear concentration/response relationship may be treated statistically. Probit analysis is the most widely used data analysis method. The method assumes that response data are approximately normally distributed so that the concentration/response curve may be linearized transforming effect data into probits (i.e. the standard normal distribution plus a factor of 5 to avoid negative values) and taking the logarithm of exposure concentrations. Other transformation procedures such as the arc-sine square root transformation of data may also be useful (Pedersen, 1994). EC_{50} or LC_{50} values can also be determined graphically or with other statistical means such as the Spearman-Karber method (Weber, 1993) The lower and upper limit of effects (0 and 100 % response, respectively) or positive effects (e.g. growth stimulation) can not be transformed into probits due to the statistical distribution applied. So in some cases there is not a sufficient number of value pairs to fit the model. In addition, the sensitivity of test methods can be too low to derive appropriate dose/response effects, especially for ecotoxicity testing of effluents after treatment in biodegradation tests (Swedish EPA, 1997). However, the fitting of dose-response models based on magnitude of effects within the concentration ranges examined cannot account for incidences of hormensis (e.g. U-shaped dose response relationships) beyond this range (Chapman, 2000).

Usually EC_{50} or LC_{50} values are calculated from concentration/response relationships, but other point estimates such as the EC_{10} might also be calculated. Sales (1999) concluded that practical problems with the use of NOEC estimation might be avoided by using other points such as the EC_{20} in addition to the EC_{50}.

Generally the variation in the EC_{10} or EC_{20} values, expressed as the coefficient of variation (relationship between the variation and the mean), is considerably larger than the variation of the corresponding EC_{50} values. (Pedersen, 1994). The reproducibility of EC_{50} test results, expressed as the coefficient of variation, is reported to be usually less than 30% depending on the kind of chemical tested (Pedersen, 1994; Dorn, 1996). So it can be pointed out that a biological test can compare with chemical analysis reproducibility, which shows coefficients of variation of approx. 20% (Pedersen, 1994). Crane (1999) stated that standard methods for estimating EC values also do not efficiently use data and he proposed time–course models for a detailed analysis of toxicity.

NATIONAL STRATEGIES AND EXPERIENCE ON WEA

General

The Contracting Parties have developed different approaches to applying bioassays for wastewater evaluation. The emission-based approach requires that wastewater discharged into a receiving water must be treated to meet certain defined limiting criteria based on BAT. The water quality-based approach starts out from the actual or desirable state of the receiving water (Tonkes *et al*., 1995). Some of the national strategies applied combine both approaches in tiered assessment. Among the Contracting Parties, it is widely accepted that a battery of toxicity tests covering the different trophic levels is needed for WET evaluations. This battery should be defined according to the intended purpose (e.g. screening, characterisation and regulation of wastewater discharges).

WEA is routinely carried out in Germany. In France, Ireland, the Netherlands and the United Kingdom corresponding research and development projects are under way (Villars, 1995). Ambient toxicity (close to the outlets) is monitored in freshwater and saltwater in Denmark (unofficially), the Netherlands and the United Kingdom.

In the section on Status of whole effluent assessment for the Contracting Parties (page 43), the status of WEA in the Contracting Parties' countries is summarised on the basis of the information given in the literature and the results of the survey performed by the German Federal Environmental Agency. First the current regulatory practice is shown, second the experience in different wastewater sectors are given including research and development projects, and third the different overall strategies are described.

Status of whole effluent assessment for the Contracting Parties

Belgium

CURRENT REGULATORY PRACTICE

Wastewater regulatory practice in Belgium is organised in their three regions: Flemish, Brussels and Walloon. The Industrial wastewater discharge in the Flemish and Walloon region is subject to three levels of mandatory conditions (Vlarem, 1995, Goenen, 1996): There are:

I. General conditions for discharge of wastewater into surface waters and into sewers (limits on pH, BOD, Temperature, suspended solids, extractable substances, dangerous substance according to 76/464/EEC)

II. Sectorial conditions based on chemical analysis have been adopted in the Flemish Region for several wastewater sectors to describe BAT

III. Particular conditions are more stringent than the former two and are aimed to protect the particular ecological equilibrium of the receiving water.

The Brussels and the Walloon regions apply the federal legislation to establish permits for wastewater discharges for different sectors including chemical, pharmaceutical and as well as petrochemical industry.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

In some studies on WET testing alternative tests (toxkits, microtiterplate test with algae) were used for which standard procedures from the developers exist. These were compared with OECD- or ISO-standardised acute toxicity tests *with Vibrio fischeri, Daphnia magna* and *Oncorhynchus mykiss*. Some non-acutely toxic effluents were evaluated for chronic toxicity (Daphnia reproduction and zebrafish short-term ELS test), genotoxicity and persistence (Vandenbroele *et al*., 1998).

Studies for endocrine disrupting effects with a recombinant yeast estrogen assay (Tanghe, 1999) and comparative work with this same yeast estrogen assay and the human recombinant breast cancer cell line (MVLN) (Witters *et al*., 1999) with environmental samples have been performed, but up to now no results with effluents from industrial sectors are reported.

Table 2 Experience with whole effluent testing in Belgium

	Test system	Endpoint	Samples	literature/comments
Wet textile	*Vibrio fischeri*	AT	8	Witters *et al*., 1999
Organic chemical	*Raphidocelis*		12	Van Sprang *et al*. 1999
industry	*subcapitata*	CT		Van den Broele *et al*., 1998
(Flemish region)	(microtiterplate,			
	toxkit & standard)			The Daphnia toxkits can be
	Daphnia magna	AT		recommended as an alternative for
	(toxkit and standard)			the standard test.
	Thamnocephalus			
	platyrus (toxkit)	AT		
	Brachionus			
	calyciflorus (toxkit)	AT		
	Oncorhynchus			
	mykiss (standard)	AT		
Industrial effluents	*Vibrio fischeri*	AT	7	Heijerick, 1999
(artificially salted)	*Daphnia magna*	AT		
	Tisbe battagliai	AT		*M. bahia* could be a useful tool for
	Mysidopsis bahia	AT		rapid screening of marine samples.
Industrial effluents	*Vibrio fischeri*	AT	100	Persoone *et al*, 1993
	Daphnia magna	AT		
	Crustacean toxkit	AT		The *Vibrio fischer*i assay and
	Rotifer toxkit	AT		crustacea toxkit were the most
				sensitive tests
Industrial effluents	*Vibrio fischeri*	AT		Van der Wielen, 1994
(Walloon region)	*Daphnia magna*			
Selected Industrial	*Vibrio fischeri*	AT		Van der Wielen, 1995
effluents: "black	*Algae growth*	CT		
points"	*inhibition*			
(Walloon region)	*Daphnia magna*	AT		
	Rotoxkit	AT		
	Thamnotoxkit	AT		

AT = Acute toxicity
CT = chronic toxicity

STRATEGIES

A Flemish research programme has been set up in order to develop a strategy of whole effluent testing. The second phase has just been accomplished. LC_{50} and EC_{50} were checked for algae growth inhibition, *Daphnia magna* immobilisation and rainbow trout mortality at the end of the pipes of 8 plants of the wet textile processing industry and 12 plants of the organic chemical industry, with the algae test being most sensitive in the first case and the algae or the fish being the best indicator in the latter case (Van den Broele *et al*., 1998). Towards 2000, a new Flemish demonstration programme for whole effluent testing is planned for a three-year period. Discussions have currently started between government, scientists and industrial delegates in order to prepare a protocol for this future programme.

Denmark

CURRENT REGULATORY PRACTICE

The discharge of industrial wastewater is regulated according to the consolidated Environmental Protection Act (625/1997). About 100 industrial companies are discharging wastewater directly into surface waters. Direct discharge permits for wastewater are issued by the county councils. Discharge into municipal sewers has to be licensed by the local municipalities. In both cases, BAT as well as potential ecological risks are stated to be regarded. Toxicity testing was implemented into practice in the county council as an unofficial guideline in the 1990s according to a strategy described below. For a number of enterprises discharge permits including ecotoxicological tests with crustacean and algae species have been applied, but currently the requirement to use biotests in the control program has been dropped (Pedersen *et al*., 1994). There are regulations for effluent limit values according to BAT, potential ecotoxicological risk of discharge, content of nutrients and organic matter. (Pedersen *et al*., 1999).

Single substances discharged via industrial wastewater are regulated according to EU Directive 76/464/EEC about pollution with hazardous substances (list I and II substances) – this is implemented by the Statutory Order 921/1996 of the Ministry of Environment and Energy establishing water-quality standards. Concentrations of these substances in the aquatic environment must be below the water quality standards after initial dilution. The guidelines of the Danish Environmental Protection Agency state, that there must not occur acute toxic effects in the recipient after initial dilution. Furthermore it is presumed that no chronic effect may occur outside a defined impact zone around the discharge point. No official guideline for the practical implementation of these principles exists, but the detailed report of Pedersen *et al*. (1994) is presently used as an unofficial guideline (Pedersen *et al*., 1999). The detailed environmental hazard and risk assessment scheme taken from OECD Series on Testing and Assessment Nr. 11 Part 2, 1998 is shown in Annex V-1.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

Since the 1980s an ecotoxicological characterisation survey of 23 industries has been performed covering the major Danish industrial enterprises (Pedersen *et al*., 1994). Usually 3-5 species were used, representing algae, crustacean and fish species. In some studies toxicity upon bivalves (Blue mussel), bacteria (*Vibrio fischeri*, inhibition of respiration/nitrification of activated sludge) and plants (Cress, *Allium*) was also evaluated. Unfortunately no test results are reported in the detailed study of Pedersen *et al*. (1994).

Table 3 Experience with whole effluent testing in Denmark

	Test system	Endpoint	Samples	Literature/comments
Chemical industry Container washing Influent WWTP	activated sludge nitrification inhibition	AC AC	6	Winter-Nielsen *et al*., 1996 Interlaboratory study, role of sludge
Pulp mill wastewater	BOD, DOC shake flasks tests with DOC analysis Activated sludge respiration Inhibition *Vibrio fischeri* ^{14}C-assimilation inhibition test with natural phytoplankton *Skeletonema costatum* *Nitocra spinipes* (copepod)	BD BD AT AT AT CT AT	3	Nyholm, 1996 Assessment of degradable and persistent toxicity
Polymer binder production	nitrification test	AT		Winther-Nielsen *et al*., 1999 TIE procedure

AT = acute toxicity CT = chronic toxicity BD = biodegradation

STRATEGIES

In the 1990s a detailed strategy for effluent toxicity testing was developed for freshwater and the marine environment (Pedersen *et al*., 1994) and is currently used as an unofficial guideline. The current risks for the receiving aquatic environment are assessed by comparing the Predicted Environmental Concentrations (PEC) with the Predicted No-Effect-Concentration (PNEC) of the effluent. The principles for investigating industrial wastewater and performing environmental risk assessments are based on three levels (Pedersen *et al*., 1999):

a. Evaluation based on existing knowledge (inventory of the chemicals, mass balances, emissions);

b. Standardised investigations of the wastewater (acute toxicity tests with 3 different species, aerobic stabilisation, HPLC screening for bioaccumulative substances, evaluation of initial dilution);

c. Specialised investigations, of the wastewater or prioritised substances contained therein (chronic toxicity, toxicity to organisms from specific compartments, biodegradation, bioaccumulation tests with fish).

At each investigation step an environmental risk assessment is performed focussing on single substances but also considering the complex wastewater itself. Assuming the same principles as for assessing the risks of chemical substances based on EU technical guidance documents, a PEC/PNEC approach is performed. Herein a

set of uncertainty factors depending on the available information is considered for deriving acute and chronic PNECs:

$$UF_{PNEC}=UF_{data}*UF_{Qualiy}*UF_{Tox}$$

UF_{Data} reflects the number of taxonomic groups from which data are available and may be compared with assessment factors. For $PNEC_{acute}$ UF_{Data} range from factor 100 for test results from one taxonomic group to factor 5 for test results from 5 taxonomic groups (see table 4).

Table 4 Uncertainty factors for deriving $PNEC_{acute}$ and $PNEC_{chronic}$ for complex wastewater (the Danish approach according to Pedersen *et al*., 1999)

Available information	UFdata ($PNEC_{acute}$)	UFdata ($PNEC_{chronic}$)
Lowest EC/LC_{50} for acute toxicity	100	200
Lowest EC/LC_{50} determined in screening tests with at least one alga, one crustacean and one fish species	10	20
Lowest EC/LC_{50} for acute toxicity to species from5 or more groups of organisms	5	10
Lowest NOEC determined by tests of chronic toxicity towards at least one alga, one crustacean and one fish species	-	5

$UF_{Quality}$ reflects the presumed quality of test performance and the relevance of test organisms. For accredited laboratories with an implemented quality system and indigenous test organisms $UF_{Quality}$ is assigned a value of 1. The variability of the toxicity in the course of time can be considered by comparing the 95% and 50% quantiles of toxicity in time series if available ($UF_{Tox.}$). Similar uncertainty factors for PEC-estimations were derived taken the varying volumes of wastewater, the flow conditions in the receiving water and the reliability of estimation method into account (Pedersen *et al*., 1999).

If no significant risk is detected at the respective investigation level, no further documentation is necessary. The acute toxic unit ($TU_{acute}=1/PNEC_{acute}$) describes how much the wastewater shall be diluted in order to avoid any acute toxic effects. Any indication of a risk implies a more detailed assessment or restrictions on the discharge. Genotoxicity or mutagenity is not considered.

European Union

FUTURE WATER FRAMEWORK DIRECTIVE (WFD)

The key aims of the future Water Framework Directive (Common Council, 1999) are:

a. to incorporate all requirements for management of water status into one single system, the river basin management;

b. to coordinate all the different objectives for which water is protected (ecology, drinking water, bathing water, particular habitats) and to fill any gaps. The aim is to reach a good status (ecological and chemical) for all waters (surface water, ground water) at the latest 16 years after the date of entry into force of the WFD;

c. to coordinate all the measures (Programme of Measures, River Basin Management Plan), taken on individual problems and sectors to achieve the objectives so defined, and to define the relationship between emission limit values and quality standards;

d. to increase public participation.

Member States, according to Article 8 of WFD, shall ensure the establishment of programmes for the monitoring of the ecological and chemical status for surface waters and the chemical and quantitative status for ground waters. These programmes shall be operational at the latest 7 years after the date of entry into force of the WFD. The monitoring of ecological and chemical status is outlined in detail in Annex V of WFD. The need to carry out Whole effluent Assessment is not explicitly mentioned, but may be recommended if good ecological status is not reached in surface waters and the reasons for non-compliance are not known. In this case a whole effluent assessment of major discharges into the river may be reasonable.

The chemical status of surface waters is monitored by surveying the quality standards of the substances of the Priority List of Chemicals (about 30 - 35 substances), which will be published in spring 2000.

COUNCIL DIRECTIVE 96/61/EC CONCERNING IPPC
(Integrated Pollution Prevention and Control)

The purpose of this Directive is to achieve integrated prevention and control of pollution arising from the industrial activities listed in Annex 1. It lays down measures designed to prevent or, where that is not practicable, to reduce emissions in the air, water and land from the above mentioned activities, including measures concerning waste, in order to achieve a high level or protection of the environment taken as a whole.

According to Article 6 (Application for permits) *"Member States shall take the necessary measures to ensure that an application to the competent authority for a permit includes a description of e.g. the*

nature and quantities of foreseeable emissions from the installation onto each medium as well as identification of significant effects of the emissions in the environment."

This provision is in line with the concept of Whole Effluent Assessment.

According to Article 9 (conditions of the permit), paragraph 3:

> *"All permit shall include emission limit values for pollutants, in particular, those listed in Annex III. Where appropriate, limit values may be supplemented or replaced by equivalent parameters or technical measures."*

Since Annex III contains general substance groups as being:

> *"4. Substances and preparations which have been proved to possess carcinogenic or mutagenic properties or properties which may affect reproduction in or via the aquatic environment*
>
> *5. Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances*
>
> *9. Biocides and plant health products"*

which are defined by their effects, whole effluent assessment parameters are the appropriate means to reduce those substances.

OTHER ACTIVITIES

Between 1993 and 1995 the EU supported a holistic programme for quality assurance for marine environmental monitoring (QUASIMEME). The purpose of the project was:

a. to develop a broad based quality assurance system for marine chemistry in Europe;

b. to provide appropriate test materials and an assessment for the performance of laboratories involved in marine monitoring;

c. to obtain comprehensive knowledge of the quality of chemical measurements in the marine environment (Wells, 1999). The project was presented at the SETAC conference on Effluent Ecotoxicology at Edinburgh in March 1999. Since 1995, QUASIMEME has been a self-supporting system for continuous external quality assurance in marine monitoring.

Another research project on "Direct Toxicity Assessment of Complex Industrial Effluents Discharged to Sewer (DTOX)" funded by the EU 4th framework's standards, measurement and testing programme focuses on the predictability of possible impacts of the biological treatment process. The aim is to prepare drafting standards for five bioassays that are to be adapted for effluent testing (personal communication of Prof. Nick Christofi). Some results of this project

were presented at the SETAC conference in 1999 by Exebarria *et al*. 1999, Alte *et al*., 1999, Dazell *et al*., 1999 and Aspichueta *et al*., 1999. The method protocols include the activated sludge inhibition respiration test of biocenoses and a luminescence method for ATP decrease in activated sludge in response to toxicant exposure. Test results were compared with the established *Vibrio fischeri* assay. Up to now no detailed results from the research project are available.

Finland

CURRENT REGULATORY PRACTICE

Internationally (ISO) standardised biotests with Daphnia magna, *Vibrio fischeri, Pseudomonas putida* and algae are used occasionally in compliance monitoring. Results are presented as $EC_{50.}$ The monitoring takes place at partial streams and the outlets after mixing with cooling water.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

WET testing in Finland focuses on pulp and paper mill effluents. A wider range of biotests (freshwater fish acute and chronic toxicity, *Daphnia magna* chronic toxicity) is used in research and development projects aimed at determining the wastewater situation in various industrial sectors (Uhlmann, 1997).

Table 5 Experience with whole effluent testing in Finland

	Test system	Endpoint	Samples	Literature/comments
Pulp and Paper	Ames-test Fish hepatoma cell line	M Cytotoxicity	4	Lindström-Seppä *et al*., 1998
Pulp bleaching effluents	*Vibrio fischeri* *Daphnia magna* *Selenastrum carpricornutum* *Pseudomonas putida* *Branchdanio rerio*	AT AT CT CT Early fry stage	18	Ahtiainen *et al*. 1994
pulp mills	*Vibrio fischeri*, luminescence and mitochondrial particle test (screening) *Vibrio fischeri* growth *Raphidocelis subcapitata* *Daphnia magna* *Brachydanio rerio* embryo and sac-fry test	AT CT CT AT development	92 (screening) 18	Ahtiainen *et al*., 1999 Toxicity of pulp mill wastewater correlate with organic carbon content of the samples, but not with the AOX.

M = mutagenicity
AT = acute toxicity
CT = chronic toxicity

5.2.4.3 STRATEGIES

There is no information available about general strategies in WEA so far.

France

CURRENT REGULATORY PRACTICE

In France industrial effluents are regularly monitored for acute toxicity with daphnids. The toxicity data are used as a base for discharge taxation (de Zwart, 1995). Both the dilution capacity of the receiving water and the potential use of the water are taken into account. This means, a combination of the emission-based and the water-quality approach is applied. Discharge permits also depend on EU Directives. For more polluting industries national limit values based on BAT and BATNEEC (best available techniques not entailing excessive costs) have been issued, which are considered as minimum values. Group parameters (AOX, metal, BOD) are also included (Tonkes *et al*., 1995).

A wide spectrum of nationally (AFNOR) or internationally (OECD, ISO) standardised acute and chronic biotests including bacteria, algae, *Lemna*, rotifers, various daphnid species, freshwater as well as marine fish species is used to determine LC_{50} and EC_{50} at the outlets after mixing with cooling or other water and in the receiving waters close to the outlets. Most of these are employed only occasionally in discharge permit procedures, in water quality monitoring or in the framework of research and development programs. Acute toxicity for *Daphnia magna* and inhibition of bacterial luminescence of *Vibrio fischeri* are also determined at the outlets of treatment facilities prior to mixing.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

There are only few reports on WET testing available focussing on *Daphnia magna* and *Vibrio fischeri*. (see table 6):

Table 6 Experience with whole effluent testing in France

Sector	Test system	Endpoint	Samples	literature/comments
Chemical and textile industries	*Vibrio fischeri* *Daphnia magna*	AT AT	39	Vasseur *et al*. (1984)
Industrial plant (outflow, end of sewage basin)	*Vibrio fischeri* *Brachydanio rerio* *embryo larval stage* *Ceriodaphnia dubia* *Selenastrum capricornutum* Ames-test P450 induction	AT development CT (7d) CT M E	2	Naudin *et al*., 1995
Treated municipal wastewater	biodegradable DOC	B	5	Percherancier *et al*., 1996 BDOC represents a significant proportion of treated effluents
Industrial effluents	*Vibrio fischeri* *Daphnia magna* *Themnocephalus platyrus* *Pseudokirchnelleria subcapitata* *Ceriodaphnia dubia* *Xenopus laevis* micronuclei *Vibrio fischeri* dark mutant	AT AT AT CT CT M GT	30	Babut *et al*., 1997 optimal battery: *Daphnia magna* (24h), *Pseudokirneriella subcapitata* (72h), *Ceriodaphnia dubia* (7d). Genotoxicity assays were excluded because of difficulties in displaying the results according to the other tests.
Effluents of wastewater treatment plants	Fish benthic macroinverte-brates	AT, CT		Kosmala *et al*., 1999 seasonal monitoring
Industrial and urban effluents	Microtox Algae Daphnids	AT	16	Isnard, 1999 real dilution rate is compared to a no-effect dilution rate

M = mutagenicity AT = acute toxicity CT = chronic toxicity

STRATEGIES

Along with the acute toxicity test with daphnids it is proposed to add the photobacterium assay, chronic toxicity and a test on mutagenicity to the set of required bio-criteria in regulatory practice (de Zwart, 1995).

Furthermore a test scheme based on a series of ecotoxicity tests and analytical identification of organic pollutants of concern is under development by industrial researchers. Usually two samples should be assessed: the existing whole effluent for characterisation of the current impact of the treatment plant, and a modelled future effluent, based on available information on the new process or on pilot studies. Results of these risk assessments are used by the companies to evaluate the contribution of the new process to the impact of the plant on the receiving system and, if necessary, to make any improvements (Boutonnet *et al*., 1999). The environmental hazard and risk assessment scheme taken from Tonkes *et al*. (1995) is shown in Annex V-2.

Germany

CURRENT REGULATORY PRACTICE

In Germany, the assessment of WEA (Integrating Controlling of Effluents, ICE) has been put into routine practice since 1976. The environmental policy emphasises the emission-based approach. The water quality-based approach has been developed in parallel. According to § 7a of the German Federal Water Act (WHG), discharge permits shall be granted only if the waste load is kept at least on the current BAT level (Best Available Technology). The requirements based on BAT are established by the federal government in the appendices of the Wastewater Ordinance (AbwV) for the different industrial branches and processes and updated according to further development of BAT.

There were two legal regulations where WEA is applied in wastewater evaluation:

a the AbwV (Ordinance on Requirements for the Discharge of Wastewater into Waters, Wastewater Ordinance - AbwV) based on WHG. Within the AbwV (Annex) 10 freshwater biotests are included for which wastewater adapted national standards or EN / ISO standards exist. Included are 5 tests dealing with (bio)degradation (e.g. BOD, modified Zahn-Wellens-tests with 3 to 28 day test duration, treatment plant simulation model) as well as short term toxicity tests with *Leuciscus idus*, *Daphnia magna*, S*cenedesmus subspicatus* and *Vibrio fischeri*, representing different trophic levels in the aquatic environment. Since 1999 the umu genotoxicity test is also included;

b. the Wastewater Charges Act (Act pertaining to Charges levied for Discharging Wastewater into Waters, Abwasserabgabengesetz – AbwAG). In the AbwAG an acute fish toxicity test is implemented for industrial and municipal direct discharges to a receiving water body. Charges are based on COD, heavy metals, nitrogen, phosphorus, AOX and fish toxicity. Specific charges are calculated from pollution units. For example one pollution unit (about 40 Euro) corresponds to a load of 20 g mercury or 500 m^3 wastewater with an acute fish toxicity with a LID (Lowest Ineffective Dilution of 6). For a limit LID of 2 no charge based on fish toxicity is imposed.

Discharge limits to different wastewater sectors are set in about 50 annexes of the Wastewater Ordinance. Depending on the emission spectrum, chemical analysis of 12 anions/elements, 24 cations/ elements, 38 individual substances, and group parameters including AOX, TOC, COD as well as total nitrogen are measured. In about 30 wastewater sectors the fish toxicity test is part of the licensing of wastewater permits. BOD measurements are required in most of the wastewater sectors.

The following respective biotests are given in the Wastewater Ordinance:

Table 7 Regulatory practice including biotests in Germany

Annex	Wastewater source sector	*Leuciscus idus* [LID]	*Daphnia magna* [LID]	*Scenedesmus subspicatus* [LID]	*Vibrio fischeri* [LID]	UmuC Genotoxicity [LID]	Elimination
22	Chemical and pharmaceutical	2 (DC)	8 (DC)	16 DC)	32 (DC)	1,5 (DC)	80% - 95% TOC (DC, ID)
25	Leather and fur	2-4 (DC)					90% COD 98% BOD
30	Manufacture of sodium carbonate	32 (ID)					
31	Cooling water				12 (DC)		
40	Metals	2-6 (DC)					
51	Landfill leachate	2 (DC, ID after treatment)	4 (ID after treatment)		4 (ID after treatment)		75% DOC (ID)
57	Raw wool washing	2 (DC)	2 (DC)				

LID: lowest ineffective dilution
DC: direct discharge to a receiving water
ID: indirect discharge via public sewers to a wastewater treatment plant

Currently the most developed concepts including biotests in discharge limits are those covering the chemical industry and the landfill leachates. For the latter the limits for aquatic toxicity for indirect discharges may be reached after treatment in a laboratory activated sludge treatment plant considering that degradable toxicities (e.g. due to high ammonium concentrations) do not affect surface water. In regulatory practice the Zahn-Wellens-Test is also used as a model for elimination processes in wastewater treatment plants. Additionally other biotests can be demanded by local authorities within the discharge permit procedure in a case by case consideration.

Unlike the testing of substances, the German approach for wastewater regulation is not based on risk assessments for the receiving waters.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

In Germany WEA has a long tradition. Recently WET results of more than 10 000 samples from various industrial sectors were documented in detail in the proceedings of the OSPAR workshop held in Berlin in 1997 (Hagendorf *et al*., 1997, Diehl *et al*., 1998, 1999). Extensive research projects for the evaluation of wastewater qualities in the textile, chemical, and pharmaceutical industries as well as in hospital wastewater's have been performed. In these activities, biotests were applied to assess possible risks regarding ecotoxicity, degradability and genotoxicity of the wastewater. Only a small number of test results have been generated in German with the umu-test, since this test has only recently been implemented and employed. The current

focus is on wastewater from hospitals, chemical industry and municipal wastewater treatment plants.

Table 8 Experiences with whole effluent testing in Germany

Wastewater source sector	Test procedure / organisms	End-point	Samples [n]	Reference /comments
domestic and communal	*Leuciscus idus*	AT	125	Göggelmann *et al*., 1989
Wastewater	*Daphnia magna*	AT		
industrial treatment plants	*Vibrio fischeri*	AT		mutagenicity in raw samples
landfill leachate	Ames-test TA 98/TA100	M		
surface water	V79	M		
	Xenopus laevis	M		
chemical industry	*Leuciscus idus*	AC	21	Irmer *et al*., 1990
pulp, paper industry	*Daphnia magna*	AC		
	Vibrio fischeri	AC		
	Scenedesmus subspicatus	AC		
chemical industry	Zahn-Wellens-Test	E/BD	129	Schönberger *et al*., 1991
	BSB	B		
chemical industry	*Leuciscus idus*	AT	25	Knie, 1992
pulp, paper industry	*Daphnia magna*	AT	59	Knie, 1992
coke plant	*Vibrio fischeri*	AT		
cooling water	*Pseudomonas putida*	AT		
	oxygen consumption			
	cress	growth		
domestic and communal	*Pseudomonas putida*	AT	239	Zander-Hauck, 1992
	oxygen consumption			
Wastewater	activated sludge	AT		
	nitrification inhibition			
pulp, paper industry				nitrification inhibition
metal industry				has greatest sensitivity
landfill leachate	*Leuciscus idus*	AT	18	Zander-Hauck, 1993
	Daphnia magna	AT		
	Vibrio fischeri	AT		elimination of
	treatment plant simulation model	E/BD		ecotoxicological
				effects after treatment
chemical industry	*Leuciscus idus*	AT	ca. 300	Gellert *et al*., 1993
cooling water	*Daphnia magna*	AT		
metal industry	*Vibrio fischeri*	AT		daphnia-test showed
mineral oil industry				greatest sensitivity
textile finishing	Zahn-Wellens-test	E/BD	8	Killer *et al*., 1993
communal wastewater				evaluation of "hard DOC" in sewage treatment plant

Wastewater source sector	Test procedure / organisms	End-point	Samples [n]	Reference /comments
coke plant	*Vibrio fischeri* *Scenedesmus subspicatus*	AT CT	12	Peter *et al*., 1995 evaluation of success of different treatments
textile finishing	*Daphnia magna* *Vibrio fischeri* *Scenedesmus subspicatus* Ames-test TA 98/TA100 V79 Zahn-Wellens-Test	AT AT CT M M E/BD	78	Jäger *et al*., 1995, 1996 mutagenicity in raw samples due to azo dyes
sewage treatment plant	Ames-test TA 98/TA100 umu-test	M G	33	Fenn *et al*., 1996 two samples from inlet of industrial treatment plant E31positive
hospital wastewater	*Daphnia magna* *Vibrio fischeri* Ames-test TA 98/TA100 V79 umuC-test BSB Zahn-Wellens-Test	AT AT M M G BD E/BD	42	Gartiser *et al*., 1996 mutagenicity in raw samples Hartmann *et al*, 1998
textile finishing landfill leachate municipal wastewater	Zahn-Wellens-Test treatment plant simulation model	E/BD E/BD	131	Gartiser *et al*., 1996
municipal and industrial wastewater effluents pulp production landfill leachate	Ames-test Microscreen phage induction assay SOS chromotest differential DNA repair assay	M G G G	9	Helma *et al*., 1996 Microscreen assay was least sensitive
industrial and municipal wastewater	*Vibrio fischeri* *Daphnia magna*	AT AT	364	Münzinger *et al*., 1996
chemical industry	*Vibrio fischeri* cell growth *Pseudomonas putida* cell growth	CT CT	14	Gellert *et al*., 1996 no difference between classical method and micoplate
textile finishing hospital wastewater	Zahn-Wellens-Test treatment plant simulation model *Daphnia magna* *Vibrio fischeri* Ames-test TA 98/TA100	E/BD E/BD AT AT M	33	Gartiser *et al*., 1997 elimination of ecotoxicity and mutagenicity
outlet of industrial treatment plants cooling water	*Leuciscus idus* *Vibrio fischeri* *Scenedesmus subspicatus*	AT AT AT	62	Gellert *et al*., 1997 fish-test can be replaced by other acute toxicity tests

Wastewater source sector	Test procedure / organisms	End-point	Samples [n]	Reference /comments
chemical and pharma-ceutical industry	Ames-test TA 98/TA100 V79 umuC-test IDIT-test (immunological DNA inhibition with HeLa cells) AFE-test (alkaline filter eluation with hepatocytes from *Leuciscus idus*)	M M G G G	218	Miltenburger, 1997 highest sensitivity with Ames E101and umu-test
chemical, metal, pulp and paper, textile industries laundries sewage treatment plants	*Daphnia magna* *Vibrio fischeri* *Scenedesmus subspicatus* Ames-test TA 98/TA100 Comet-assay	AT AT CT M G	66	Hollert *et al*., 1997 additionally tests for cytotoxicity
coke plant	Ames-test TA 98/TA100 umuC-test treatment with activated sludge	M G		Siersdorfer *et al*., 1998 fractionation of mutagenicity
bottle and tank cleaning in food industries	*Daphnia magna* *Scenedesmus subspicatus* *Vibrio fischeri*	AT CT AT	ca. 50	Pluta *et al*., 1998
hospital wastewater / disinfectants	*Daphnia magna* *Vibrio fischeri* Ames-test TA 98/TA100 umuC-test Zahn-Wellens-Test	AT AT M E/BD	59	Gartiser *et al*. 1999 genotoxicity in raw samples due to quinolone-antibiotics
data compiled from 40 sectors	*Leuciscus idus* *Daphnia magna* *Vibrio fischeri* *Scenedesmus subspicatus* umu-test	AT AT AT CT G	3882	Diehl *et al*., 1999 about 10000 test results from official evaluations reported

M: mutagenicity
G: genotoxicty
AT: acute toxicity
CT: chronic toxicity
E/BD: elimination/biodegradation
BD: Biodegradation

STRATEGIES

The guiding philosophy for implementation of biotests in WEA is the Precautionary Principle which holds that all that can reasonably be expected should be done to prevent unnecessary risks, and the Polluter Pays Principle (PPP), demanding that the financial burden for the prevention and control of pollution be transferred to the party responsible for its generation. So the German's approach emphasis is on emission reduction at source.

Therefore no case by case risk assessment considering the flow capacity of the receiving river is performed. Mixing or dilution may not achieve the limit values set in wastewater discharge permits for the different parameters. German experience over the last 23 years shows that this approach promotes the further development of BAT and has supported its use considerably. Coupling WET with the BAT guarantees equal treatment of discharges in the different branches of industry regardless of the water quality of the receiving waters.

The guiding concept in emission control is the combined use of chemical group parameters, measurement of single substances and biotests. Requirements for the discharge of wastewater into waters are laid down in the appendices of Wastewater Ordinance as follows:

I. Scope of application

II. general requirements

III. requirements for wastewater at the point of discharge

IV. requirements for wastewater prior to blending

V. requirements for wastewater at the site of occurrence and

VI. requirements for existing discharges.

Requirements in the appendices refer to analysis and measurement techniques specified in the annex to the Wastewater Ordinance (DIN EN ISO standards). Identical standard protocols for the parameters liable to taxation are described in and used within the scope of the Wastewater Charges Act. The charges levied under the Wastewater Charges Act are used for the improvement of wastewater treatment plants and for measures to minimise pollutant loads in wastewater (article 13): The revenue accruing from wastewater charges may only be used for specific purposes connected with measures for maintaining or improving water quality. The *Länder* (provincial state governments) may stipulate that the administrative expenditure associated with the enforcement of the Wastewater Charge Act and of the *Länder's* own supplementary provisions shall be paid for out of the revenue accruing from wastewater charges.

In emission control the first aim is to avoid the presence of hazardous substances and undesirable effects in wastewater. Standardised biotests developed and used for that purpose must be capable of detecting effects clearly, rapidly and cost-effectively. The results from these biotests are not expected to provide final evidence of an effect at ecosystem level and consequently they are not used for risk assessment procedures. Therefore the evaluation of toxicity tests follows the concept of Lowest Ineffective Dilution (LID) according to the informative annex of EN ISO 5667-16, which up to the present is exclusively applied in Germany.

The LID refers to the batch with the highest test concentration at which no inhibition, or only effects not exceeding the test specific variability, had been observed. D is expressed as the reciprocal value of the volume fraction of wastewater in the test batch.

The wastewater control concept described is in accordance with the strategy paper of the German Association of the Chemical Industry (VCI).

The standardised methods mentioned above will be complemented by the *Lemna*-test in the near future. However this test, which is currently being standardised by OECD, is designed for single substance testing only and needs to be modified and verified for effluent testing. Moreover, the acute fish test with *Leuciscus idus* will be replaced by the fish egg test with *Danio rerio* for animal care reasons (Animal Protection Act). Owing to the small amount of direct discharges into the marine environment in Germany, only freshwater biotests have been implemented until now.

Currently biotests for other endpoints such as bioaccumulation, endocrine disruptors, immunotoxicity and mutagenicity with eucaryotic cells are under development in the framework of research and development projects.

Apart from the emission-based approach described here, water quality surveys using bioindicators and active as well as passive monitoring for water quality control became routine in Germany in the 50s. In the 70s, coastal areas were also included in the monitoring programmes. Recently, chemical quality assessment has been implemented in addition to the biological quality assessment, which also describes water quality by means of 7 categories.

In special cases, ambient toxicity close to the effluents is determined, but not routinely. In large rivers (Rhine, Elbe), continuous biological monitoring devices (daphnids, *Dreissena*) with early warning systems are in operation.

Iceland

There is no information about regulatory practice and experience in WET testing so far.

Ireland

CURRENT REGULATORY PRACTICE

The Environmental Protection Agency Act, 1992 introduced an integrated licensing system for controlling emissions from large/complex and other processes with significant polluting potential known as Integrated Pollution Control (IPC). Activities covered by the IPC licensing system are listed in the First Schedule of the EPA Act 1992 and the various sectors (13) are given in Table 9 below.

Table 9 Activities licensed by the EPA under IPC

Number	Sector	Number	Sector
1.0	Minerals and other materials	8.0	Wood, Paper, Textiles and Leather
2.0	Energy	9.0	Fossil Fuels
3.0	Metals	10.0	Cement
4.0	Mineral fibres and glass	11.0	Waste
5.0	Chemicals	12.0	Surface Coatings
6.0	Intensive Agriculture	13.0	Other activities
7.0	Food and Drink		

The Integrated Pollution Control licences issued by the EPA have, where appropriate, requested acute aquatic toxicity monitoring of effluent emissions which discharge to water or to sewer. The requirement for chronic aquatic toxicity monitoring is assessed on a case by case basis.

When characterising an effluent/wastewater, the licensee is required to undertake an initial toxicity screening test against species from a minimum of four different trophic levels. The licensee must ensure that the tests are undertaken using accepted procedures (ISO, BS etc.) by a testing laboratory which must be agreed with the EPA. The four trophic levels can be broadly categorised as bacteria, plants/algae, crustacean and fish and Table 10 outlines a list of species which are available for effluent toxicity testing in Ireland. Having identified the most sensitive species, future monitoring is then carried out on the two most sensitive species. In addition to the requirement for toxicity monitoring, the licensee may also have to comply with a toxicity limit expressed in Toxic Units (Tu) which also takes into account the dilution available in the receiving system. The number of toxic units is equal to 100/x hour EC/LC_{50} in percentage vol/vol where higher Tu values reflect greater levels of toxicity.

In most cases, testing is carried out on a 24-hour flow proportional composite sample but where effluent variability occurs it may be necessary to undertake testing on several 24-hour composite samples. Where a wastewater is identified as being highly toxic, a Toxicity Identification Evaluation (TIE)/Toxicity Reduction Evaluation (TRE) is employed to identify the likely toxic elements in the wastewater stream and a corrective action programme is put in place to reduce or eliminate the toxicity.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

Although the EPA has not published any data in relation to the various tests undertaken for the sectors covered by the Integrated Pollution Control system, all information pertaining to the IPC licences is available for viewing by interested parties at the Agency offices.

Table 10 Suggested species for monitoring wastewater toxicity

	Receiving Environment		
Test Species	Freshwater	Estuarine or Coastal Water	Treatment Plant
Bacteria	*Vibrio fisheri*	*Vibrio fisheri*	*Vibrio fisheri*, Activated sludge (inhibition of respiration, nitrification) Anaerobic Sludge (inhibition of CH_4 & CO_2 production)
Crustaceans	*Daphnia magna* *Brachionus calyciflorus*	*Tigriopus brevicornis* *Tisbe battagliai* *Brachionus plicatilis* *Crangon crangon*	*Daphnia magna*
Fish	*Oncorhynchus mykiss*	*Pleuronectes flesus* *Scophthalmus maximus*	
Plants/Algae	*Lemna minor* *Chlorella vulgaris* *Selenastrum capricornutum*	*Skeletonema costatum*	*Lemna minor* *Chlorella vulgaris* *Selenastrum capricornutum*

STRATEGIES

The regulatory control of wastewater discharges in Ireland relies on the application of aquatic toxicity monitoring in conjunction with the requirement for testing of the chemical and physical constituents of the wastewater. Compliance with emission limit values for toxicity and other parameters is required and this is verified by monitoring submitted by the licensee and also by spot-checks carried out by the EPA.

Luxembourg

There is no regulatory practice concerning wastewater effluent ecotoxicology and no experience or test results are reported.

Norway

CURRENT REGULATORY PRACTICE

I. Land based industry

Whole Effluent Assessment (WEA), including chemical and ecotoxicological characterisation of effluents is applied on a case by case basis, and used as guidance for issuing discharge permits. Such assessments are normally performed on composite samples of the final effluent from the industry. The chemical analysis programme includes common general water quality and summary parameters as well as specific analysis of selected pollutants. Tests for acute toxicity are performed on algae, crustacean and fish. Marine or freshwater organisms are used depending on the nature of the receiving water.

Quantification of potentially bioaccumulative compounds is performed using TLC/GC. Toxicity and bioaccumulation potential may be assessed also after a biological stabilisation of the wastewater performed as a 28 days biodegradation test.

For regulation of wastewater emphasis is put on the "total emission of toxicity" expressed as the Toxicity Emission Factor, TEF. In addition a risk assessment is performed on the basis of the toxicity data and predicted recipient concentrations.

II. Offshore installations

Ecotoxicological documentation shall be submitted for all chemicals and drillings fluids used offshore. There shall be complete documentation of the potential biodegradability and bioaccumulation of the individual organic components in products that consist of several substances.

All applications for discharge permits for offshore chemicals and drilling fluids shall be accompanied by a HOCNF (harmonised offshore chemical notification format) for the products used in connection with drilling and production, including products used in closed systems. HOCNF and Guidelines for Completing the HOCNF from OSPAR 1995 shall be used. As OSPAR Guidelines for Completing the HOCNF are incomplete according to Norwegian requirements, SKIM (Co-operative forum for Offshore Chemicals, Industry and Environment authorities) has prepared Supplementary Guidelines for Completing HOCNF for the Norwegian sector.

In the case of products that consist of several substances, complete documentation shall be presented for the biodegradability of each component. The substances shall be tested according to seawater test OECD 306. Other seawater tests that are accepted are *marine CO_2 evolution test* (mod. Sturm), *marine BODIS test* (for insoluble substances) and *marine CO_2 headspace test* (mod. ISO N182), which have all been included in PARCOM ring testing (ref. Biodegradability of chemical substances in seawater, Results of OSPARCOM ring test, Elf 1996) and which give almost the same result.

Complete documentation of the bioaccumulation potential of each organic component shall be submitted for products that comprise several substances. The substance's bioaccumulation potential shall be tested according to OECD method 107 or 117.

Offshore chemicals on the Norwegian continental shelf shall be toxicity tested at product level, but SFT will also accept tests at component level, provided that data for all components is given. SFT requires the following three marine toxicity tests:

I *Skeletonema costatum*

II *Acartia tonsa*

III *Corophium volutator (*not required if *Abra alba* has already been carried out) or *Scophtalamus maximus.*

When choosing between alternative methods, emphasis shall be placed on testing the most relevant species as regards the fate of the product in question.

The operator shall carry out environmental assessments of all products discharged from the installation in connection with drilling and production activities. The environmental assessments shall be carried out when entering into new contracts concerning products that will be discharged to sea. The assessment shall also be carried out at least every third year for all products. The operator shall have a list of the products he wants to replace with less environmentally hazardous alternatives and a plan for implementation of this. New products shall be assessed against existing products. The assessment shall be carried out annually for products that are on the priority list for phasing out in accordance with phasing-out criteria given by the authorities. A pre-assessment shall be carried out on all products, and alternative products shall be ranked using the CHARM model's hazard module. Guidelines have been issued for use of the CHARM model.

The operator may replace a product in use with another product if he can document that the new product's expected environmental risk is equal or lower than that of the original product. Comparisons of alternative products' risk to the environment shall include use of the CHARM model where this is possible. (For detailed information, see www.sft.no/3504.doc "Requirements for ecotoxicological testing and environmental assessment of offshore chemicals and drilling fluids").

STRATEGIES

For land-based industry, WEA is used on a case-by-case basis for risk assessments when issuing emission/discharge permits. However, biodegradation, persistency or toxicity values are not used as emission limit values. A WEA guidance document for the authorities will be worked out which might increase a more systematic use of WEA.

Ecotoxicological documentation for all production chemicals, drillings fluids and utility chemicals (detergents, hydraulic fluids, etc) used offshore is required by the environmental authorities. All discharges require a discharge permit.

Portugal

CURRENT REGULATORY PRACTICE

In Portugal there is no legislation on bioassays on effluent monitoring (Brito, 1999). Bacteria, algae, and crustaceans (LC_{50} and EC_{50}) are routinely employed for monitoring water quality and occasionally at the outlets in authorisation procedures for special branches of industry focusing on the pulp and paper sector. There is no legislation on bioassays for wastewater monitoring. (Morbey *et al*., 1997). The Directorate-General for the Environment carries out bioassays on samples collected by the Inspectorate Body in industrial and hospital wastewater treatment plants, and on drinking water supply systems.

On the contrary Brito (1999) stated, that until 1996 the only bioassay performed was the *Vibrio fischeri* bioluminescence test.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

INETI (National Institute of Industrial Engineering and Technology) has carried out two projects: The first project 1990/91 dealt with the development of two tests for the evaluation of acute toxicity for industrial effluents (*Vibrio fischeri, Daphnia magna*). The samples were taken from a surface treatment industry with two ends of the pipe: alkaline and acid discharges. A good correlation between the results of the *Daphnia magna* test and the 5 minute *Vibrio fischeri* test were observed. Nevertheless the correlation between the Daphnia and 15 minute *Vibrio fischeri* test was lower (Morbey *et al*., 1997).

The second project dealt with the AEC (adenylate energy charge) in the polychaete *Lanice conchilega* at different sampling points in the vicinity of a cellulose effluent discharge (Morbey *et al*., 1997).

Table 11 Experience with whole effluent testing in Portugal

	Test system	Endpoint	Samples	Literature/comments
pulp and paper	*Daphnia magna*	AT, CT		Morbey *et al*. (1997)
metallurgical	*Selenastrum capricornutum*	CT		Test results of about 30 samples were reported considering *Vibrio fischeri* and *Daphnia magna*
tannery	*Vibrio fischeri*	AT		
food and cork	*Vibrio fischeri*	GT		
Industrial effluents	*Artemia* (Arc-test)	AT	5	Boia *et al*. (1992) described in Morbey *et al*. (1997) Arc-test is suitable for the control of effluents discharging into salting waters.
Cork industry	various tests	AT, CT		Mendonca *et al*. (1999) High toxicity levels were found

AT = acute toxicity CT = chronic toxicity GT = genotoxicity

STRATEGIES

In 1998 the DGA (Directorate-General for the Environment) developed a joint project with ISA (Institute of Agronomy) in order to evaluate the acute toxicity of pesticides used in paddy fields in the Sado River estuary. Tests were conducted with *Daphnia magna, Thamnocephalus placyurus* and *Rhaphidocelis subcapitata* (freshwater) and *Artemia saline* (saltwater). The project was the first step for the implementation of these tests in routine. For 1999 it is planned to extend the study to the effluents of pulp and paper, tannery, food and pig breeding industries. The objectives are to find a battery of tests for application to the different sectors and to have a scientific study for the elaboration of a legislation framework in the field of ecotoxicological bioassays (Brito, 1999).

Spain

CURRENT REGULATORY PRACTICE

The Spanish monitoring strategy for effluents is directed at the effects for the receiving water bodies and is therefore water quality-based (Tonkes *et al*., 1995).

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

Table 12 Experience with whole effluent testing in Spain

	Test system	Endpoint	Samples	Literature/comments
Fish-canning factory	RTG-2	Cytotox	4	Vega M. *et al* (1994)
Industrial effluents	Fish *Daphnia magna* Chlorella vulgaris RTG2	AT AT AT Cytotox	60 60 60 60	Tarazona JV *et al*. Laboratory of Ecotoxicology. Personal communication. Unpublished.
Industrial effluents	*Artemia* nauplii RTG-2	AT Cytotox	12 12	Tarazona JV *et al* (1991)
Urban sewages	Low volume *Daphnia magna* modified test RTG-2 *Chlorella vulgaris*	AT Cytotox AT	11 11 11	Pablos MV *et al* (1999)

STRATEGIES

A special program about organic-fraction toxicity (OFT) testing it's been carried out. For municipal wastes, usually rich in ammonia, nitrites, etc., the specific toxicity testing of the organic (lipophilic) fraction, can be more valuable for the identification of non-expected highly toxic pollutants than Whole Effluent Toxicity Testing.

Table 13 Experience with whole effluent testing in Spain (2)

	Test system	Endpoint	Samples	Literature/comments
Olive oil mills	*Azotobacter chiroccum*	CT (96 h)		García-Barrionuevo *et al*., 1993
Aeronautics industry fish-processing factory	RTG-2	Cytotox		Castano *et al*. (1994) Fish-cell-test as replacement for in vivo tests, allowing smaller quantities to be tested.
Sewage samples	RTG-2	Cytotox	3	Vega *et al*. (1996) Combination of cytoxocity testing and HPLC fractioning
Industrial effluents	activated sludge respiration inhibition test Vibrio fischeri	AT AT	The project is not finished yet	Extebarria *et al*. (1999) Part of DTOX project Microtox more sensitive, but activated sludge better represents the point of entry into WWTP
	nitrification inhibition	AT	The project is not finished yet	Aspichueta *et al*. (1999) Part of DTOX project

M = mutagenicity
E = enzymatic
AT = Acute toxicity
CT = chronic toxicity

Sweden

CURRENT REGULATORY PRACTICE

Sweden focuses on the prediction of effects by effluents for the receiving water, i. e. the water quality-based approach. (Tonkes *et al*., 1995). Industries have been advised to follow the Characterisation of Industrial Discharges (CID) guidelines for an evaluation of their effluents and for the supervision and allocation of permits since 1989 (Swedish EPA, 1997). Therein a combination of biological tests and chemical analyses are recommended to detect substances that are not readily degradable, that are toxic, and/or that bioaccumulate in wastewater. But characterisation according to CID is considered too expensive for small and medium-sized industries (Tarkpea, 1998). According to Swedish law a municipal treatment plant has no obligation to accept industrial wastewater. Each municipality can set its own restrictions regarding the substances received into the treatment system. (Tarkpea, 1998).

Wastewater is classified as acutely toxic if the concentration after initial dilution exceeds $0{,}1*EC_{50}$. The Swedish proposal for biotests in WEA includes acute fish and crustacean toxicity, algae as well as tests with higher plants (*Lemna minor, Allium cepa*). A main point is bacteria toxicity which is measured by the activated sludge respiration/nitrification inhibition assay and *Vibrio fischeri* (Swedish

EPA, 1997, Pedersen *et al*., 1994). The detailed environmental hazard and risk assessment scheme was described by Pedersen *et al*. (1996) and is shown in annex II-4 taken from OECD Series on Testing and Assessment No. 11 Part 2 (1998).

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

WET test results from different research projects were reported considering short-term algal, bacterial and crustacean tests as well as prolonged biodegradation tests with a modified OECD Screening test (DOC-Elimination) and potentially bioaccumulating substances (PBS).

Table 14 Experience with whole effluent testing in Sweden

	Test system	Endpoint	Samples	Literature/comments
Industrial effluents	*Vibrio fischeri* Inhibition of nitrification *Selenastrum capricornutum*	AT AT AT	169	Andrén *et al*. (1998) Complementation of biological toxicity tests and chemical determination (PCA)
Industrial effluents	Nitrification *Vibrio fischeri* *Selenastrum capricornutum* Ceriodaphnia dubia	AT AT AT AT	164	Tarkpea *et al*. (1998)
Chemical industry and other industrial effluents "The STORK-Project"	*Vibrio fischeri* Inhibition of nitrification Activated sludge respiration Inhibition *Ceriodaphnia* *Daphnia magna* *Nitocra spinipes* *Selenastrum capricornutum* DOC-elimination	AT AT AT AT AT AT CT B	ca. 60	Swedish Environmental protection Agency (1997) Undén (1997) Assessment of toxicity and PBS before and after stabilisation in a biodegradation test according to EN ISO 7827 Biological/chemical characterisation including AOX, EOX, PBS as sum parameters
pulp mill metal works photographic processing washing powder production	*Salmo trutta* *Ceriodaphnia dubia* *Nitocra spinipes* Algae batch test4 *Allium cepa* *Vibrio fischeri* Algal battery test	AT AT CT CT AT AT CT	10	Wängberg *et al*. (1995) Relative sensitivity is dependent on the endpoint for the tests. Algae battery test most sensitive

	Test system	Endpoint	Samples	Literature/comments
Chemical-pharmaceutical plant	*Vibrio fischeri* *Brachydanio rerio* Inhibition of nitrification Activated sludge respiration Inhibition *Ceriodaphnia dubia* *Selenastrum capricornutum* DOC-elimination PBS	AT AT AT AT AT CT B	5	Brorson *et al*., 1994 Degradation of ecotoxic effects, both the group parameter based and the single substances based strategies were useful to predict environmental effects of wastewater
Tapioca-starch wastewater	*Vibrio fischeri* *Lemna minor*	AT AT	ca. 15	Bengtsson *et al*., 1994 Elimination of ecotoxic effects, duckweed toxicity still remains
Pulp Textile Organic chemicals Mine Refinery	*Nitocra sinipes* *Vibrio fischeri*	CT AT	11	Tarkpea *et al*. (1986)
Textile food metal pharmaceutical detergent mechanic	*Vibrio fischeri* standard *Vibrio fischeri* 100%	AT (EC_{50} 5') AT (EC_{50} 5')	15	Tarkpea *et al*. (1989) In the 100% method bacteria are added directly to the sample to avoid dilution

M = mutagenicity E = enzymatic AT = acute toxicity CT = chronic toxicity B = biodegradation

STRATEGIES

From 1989 to 1996 an extensive research programme on the characterisation of discharges from the chemical industry (The STORK-project) was carried out. The proposed strategy based on that experience has three successive levels of investigation. Each level takes into account chemical characterisation, degradability, bioaccumulation (BCF > 1 000) and toxicity and the corresponding tools for evaluation. Basic information is compiled from the production process and from previous studies. At the first level, COD, BOD_7, AOX, TOC, pH, conductivity, P, N, and suspended solids are measured and biotests are employed (LC_{50} or EC_{50} for bacteria, higher plants, algae, crustaceans, and fish) in freshwater and saltwater. Investigations must be continued on the next level if no decision can be made concerning changes in the production process, the replacement of chemicals, purification measures and control programmes. In this decision making, technical as well as economic factors have to be considered. The second level includes chemical analyses using more advanced techniques (GCMS, HPLC etc.), screening tests on biodegradability, bioaccumulation, and toxicity. Herein the modified OECD Screening Test (DOC elimination in

28 days according to ISO 7827) and longer-term BOD tests (i.e. 14 days duration) are recommended in Sweden and Denmark (Swedish EPA, 1997; Nyholm, 1996).

The third level includes a wider range of toxicity tests in cage and field experiments also considering physiological and morphological alterations, population levels and ecosystem/multispecies models (Swedish EPA, 1997).

The discharged quantity of toxic substances in effluents is expressed as "Toxicity Emission Factor" (TEF), that is the Toxic Unit (TU) multiplied by the 24-hour flow

$$TEF = [100/LC(EC)_{50}]*\text{24-hour flow } [\text{vol-\%}*m^3]$$

Thus a $LC(EC)_{50}$ at 100 volume percent and a flow of 100 m^3/d corresponds to 100 TEF units. TEF values lower than 100 are deemed acceptable (Swedish EPA, 1997).

Switzerland

The assessment of effluent in Switzerland is focused on the effects on receiving water bodies Standard requirements, such as fish toxicity and non-disturbance of the biological purification process are considered. Also differentiation of limits is made between discharges into sewers and those discharged directly into surface waters (Tonkes *et al*., 1995). There are no regulations concerning the ecotoxicology of wastewater effluents. Within research projects genotoxicity of hospital wastewater was evaluated with the umuC-test and a genotoxicity identification evaluation confirmed, that fluoroquinolone antibiotics cause genotoxic effects in hospital wastewater (Giuliani *et al*., 1996; Hartmann *et al*., 1998). No further information regarding WET testing is available.

The Netherlands

CURRENT REGULATORY PRACTICE

Within the Dutch emission policy, the assessment of Dutch wastewater effluents currently is focused on the reduction of specific pollutants or substances at the source (precautionary principle). Depending on the characteristics and the environmental hazard of a substance, the polluter has to take remedial measures based on BAT or BEP (Best Environmental Practice; see IPPC) with respect to the discharges (Tonkes *et al*., 1999). The WEA (formerly called WEER, i.e. Whole Effluent Environmental Risk) testing approach including biotests is under development, but up to now no official testing methods or criteria have been established.

The Netherlands water quality policy distinguishes two approaches: the emission based and the water quality based approach. The emission approach is directed at the assessment of effluents at the source (precautionary principle), the water quality approach is directed at the effects in receiving waters (Tonkes *et al*., 1994, 1995).

Within the general emission approach three phases (also called part A) are distinguished:

a. Prevention of pollution

I. Re-use of water and substances, where possible

II. Treatment (end-of-pipe-treatment).

Within the third phase (WEA) the same assessment parameters are used as in the second phase, including mutagenicity, acute and chronic toxicity, bioaccumulation, persistence and oxygen demand. The WEA method is not meant to predict the effects on the receiving water body, but to complement the assessment of unknown components (Tonkes, 1995).

The substance specific approach (also called part B) focuses on BAT, and further demands are based on certain national criteria (e.g. Maximum Permissible Risk). This stands for an emission part. Separate from this, there is a water quality approach, which is based on environmental quality criteria.

Finally a stand-still approach is used for new discharges or the extension of existing discharges.

The use of WEA might become an extension of this policy Strategy. But that is still not for certain.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

Lots of conceptual work and literature reviews on WEA and some exemplary studies with industrial wastewater among others have been performed. Bioaccumulation was also part of this study.

In the most detailed study of Tonkes *et al*. (1997) test results of 10 complex effluents with fish (*Danio rerio*, acute and ”early life stage”), crustaceans (*Daphnia magna,* acute and chronic), algae (*Selenastrum capricornutum)* and bacteria (*Photobacterium phosphoreum*) toxicity tests are reported. Toxkits and Genotoxicity (Mutachrome test with *Salmonella typhimurium*) were also included and effect parameters are tested before and after an additional 28-day degradation step.

Moreover, there was an extensive study on cooling water carried out by Baltus, Kerkum and Kienhuis (to be published very shortly).

By now, about 100 effluents discharging into surface waters or sewers have been tested for acute toxicity. 50 effluents were investigated for genotoxicity and bioaccumulation in cooperation with German institutes.

Monitoring surface water toxicity with algae, bacteria, crustacean and fish tests are also reported (Hendriks *et al*., 1994a, 1994b; Polman *et al*., 1994).

Table 15 Experience with whole effluent testing in the Netherlands

	Test system	Endpoint	Samples	Literature/comments
Chemical effluents tank cleaning tank transhipment tank car transport oil refinery food production	*Vibrio fischeri* *Selenastrum capricornutum* *Daphnia magna* *Brachydanio rerio* rotifer – Toxkits crustacean – Toxkits	AT AT AT AT AT AT	17	Tonkes *et al*. (1999), Graaf *et al*. (1996) Evaluation of acute toxicity tests Toxkits seem to be not sensitive enough. Use is not recommended
Chemical industry Paper industry Food products	*Vibrio fischeri* *Selenastrum capricornutum* *Daphnia magna* *Brachydanio rerio* Rotifer – Toxkits crustacean – Toxkits Mutachromoplate-test	AT CT AT and CT AT and early life stage AT AT M, bioaccumulation, persistence	10	Tonkes & Baltus (1997) Elimination of PBS, toxicity and mutagenicity after treatment in an biodegradation test
Artificial wastewater	Duckweed species *Azolla filiculoides*	AT AT	2	Vermaat *et al*. (1998)
Paper mill	*Vibrio fischeri*	AT		Berbee *et al*. (1999) TIE-approach in identifying the nature of the toxic substances. Attempt at identifying the toxicants is very difficult.
Pharmaceutical industry	*D. magna* *S. capricornutum* Toxkits Fish	AT, CT AT CT		De Groot *et al*. (1999) Development of a tiered approach by Solvay pharmaceuticals, consisting of AT, bioaccumulation, CT after biodegradation
Several industrial effluents	*Cyprinodon variegatus* *Lebistes reticulates* *Scophthalmus maximus* *Menidia menidia*	AT, CT	5	Hooftman *et al*. (1999)
Industrial effluents	*Cyprinus carpio*	EE		Gimeno *et al*. (1999)

M = mutagenicity E = enzymatic AT = acute toxicity CT = chronic toxicity EE=estrogenic effects

STRATEGIES

Up till now the anthropogenic effects from effluents are only monitored at the end of pipes and in the tributary within the process. Attributing the effects in receiving water to the discharge of certain specific effluents is only under debate. If and how this will be done is not yet known. Next to this there is (limited) monitoring, for developmental reasons, of surface waters. Till now this is not related or connected to the effluent policy. It is not related to effluents.

United Kingdom

CURRENT REGULATORY PRACTICE

UK water quality management policy requires, on the whole, that consideration is taken of the quality of receiving watercourses, i.e. the 'water quality approach'. Environmental quality standards (EQS) are used to protect the ecosystem and maintain the quality for specific use, taking into account dilution and dispersion (Tonkes *et al*., 1995). There are no regulations stipulating ecotoxicity testing for effluents on a national basis, but biotests are occasionally used in compliance monitoring. There are approximately 20 toxicity-based consents in place (Tonkes *et al*, 1995), but these will be reviewed and standardised once appropriate guidelines have been developed.

Recommendations have been made to include Direct Toxicity Assessment (DTA) along with chemical-specific assessment in the evaluation of effluents and currently a demonstration project is taking place to assess the use of DTA in a regulatory context. This will lead to the phased and consistent introduction of DTA controls to appropriate discharges. However, to date, other whole effluent measures such as bioaccumulation and persistence have not been developed.

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

Most of the published results on effluent toxicity use the well-established *Vibrio fischeri*, *Daphnia magna* and algae toxicity tests. Nevertheless, great efforts have been made to develop and establish many new test systems which are described in detail in various Research and Development Reports (e.g. The Environment Agency, 1998c & 1999e, WRc/The Environment Agency, 1999). In some industrial sectors the operators have gained much experience of WET testing, but many results have not been published yet. *Crangon crangon* and *Oncorhynchuss mykiss* acute toxicity tests have been used as part of the effluent permitting process on two sites: a chemical manufacturer and a pesticide manufacturer. Table 16 gives some examples of results.

Table 16 Examples of experience with whole effluent testing in the United Kingdom

Sector	Testsystem	Endpoint	Samples	Literature/comments
none - Marine receiving water monitoring	Oyster embryo-larval development test in 3ml multiwell plates (*Crassostreas gigas)*	Development	>100	Johnson *et al*. (1999) The test is used in the DTA and in the National Marine Monitoring approach. The 'micro' version is comparable to the conventional method. A software supported evaluation is possible.
STW effluents	*Daphnia magna* *Oncorhynchus mykiss* *Tisbe battagliai* *Skeletonema costatum* *Crassostreas gigas* *Selenastrum capricornutum* Vibrio fischeri	AT AT AT CT	40	Thomas *et al*. (1999) TIE-approach The Environment Agency (1996a)
Oil refinery petrochemical effluent	Combined biodegradation and toxicity studies *Daphnia magna* *Oncorhynchus mykiss* *Tisbe battagliai* *Skeletonema costatum* *Crassostreas gigas* *Selenastrum capricornutum* Vibrio fischeri	AT	6	Whale *et al*. (1999a) Toxicity should not be taken as sole basis. The Environment Agency (1996a)
Industrial effluent	*Daphnia magna* *Selenastrum capricornutum*	AT CT		Hutchings (1999a) case study in the attempt to compare end of pipe laboratory-based toxicity tests and effects within the receiving aquatic ecosystem.
Industrial effluent	ATP luminiscence	Energy charge of cells	up to now work focuses on single substances	Dalzell *et al*. (1999) Part of DTOX project
Industrial wastewater	Activated sludge testing facility AmtoxT	B AT	1	Burgess *et al*. (1999) The addition of a wide range of micronutrients reduces the toxicity of reactor effluents.
Industrial effluent	Miniscale algal method	CT	1	Hutchings *et al*. (1999b) Phase I TIE Procedure was used to compare the miniscale method to the conventional algal test. The results were comparable.

Batch processing industry	*Daphnia magna* *Chlamydomonas* *Paramecium*	AT AT AT		White *et al* (1999) short time toxicity tests are necessary for batch production industries. The acute toxicity of effluents can be established in a 90 minutes period. TIE-procedure
Textile effluent	*Daphnia magna* Bioluminescence Chemiluminescence Amtox Cellsense Yeast biosensor system	AT AT AT		Hayes (1999) the rapid test systems under development were compared to Daphnia magna 48h
Sewage treatment works wastewater Industrial wastewater	Vibrio fischeri Aquatox		43 9	Rippey *et al*. (1999) Comparison of the two tests
Several industrial effluents focusing on chemical industry	*Daphnia magna* *Oncorhynchus mykiss* *Tisbe battagliai* *Skeletonema costatum* *Crassostreas gigas* *Selenastrum capricornutum* Vibrio fischeri	CT EC_{10} development AT LC_{10}	45 >50	Wharfe (1997) The Environment Agency (1996a)
Papermill effluent	*Pseudomonas fluorescence* luminescence and respiration inhibition *Daphnia magna* *Oncorhynchus mykiss* *Tisbe battagliai* *Skeletonema costatum* *Crassostreas gigas* *Selenastrum capricornutum*	 AT AT	14	Brown *et al*. (1996) The Environment Agency (1996a)
Offshore oil production	*Skeletonema costatum* *Tisbe battaglia* *Acartia tonsa* Microtox	AT	20	Stagg *et al*. (1995)
Offshore oil and gas production	*Skeletonema costatum* *Acartia tonsa* Microtox	AT	7	Flynn *et al*. (1995)

M = mutagenicity E = enzymatic AT = acute toxicity CT = chronic toxicity B = biodegradation

STRATEGIES

Direct Toxicity Assessment has been used widely in the context of research, development and demonstration, and numerous projects have been completed to support the use of DTA to monitor and control effluents. These include projects to:

I. **Develop and evaluate methods e.g.** *Daphnia magna* reproduction test, enhanced chemoluminescence (ECL), chlorophyll fluorescence, marine bacterial luminescence, aquatic invertebrate fluorescence (The Environment Agency 1999c, 1998c & 1999d, 1999a, 1998b, & 1999e)

II. **Improve and standardise methods e.g.** Using Image Analysis in the Oyster Embryo-Larval Development and *Daphnia magna* growth tests (WRc/The Environment Agency, 1999), ring-testing the OECD *Lemna* growth inhibition and the 48h *Tisbe battagliai* lethality test (Environment Agency, 1999f & 1999g). producing method guidelines for effluent and receiving water assessment (Environment Agency, 1999h), Standardising procedures for the Microtox® test system: Acute, Chronic, Solid-phase and Mutatox® (Environment Agency, 1999i)

III. **Develop quality control and assurance procedures e.g.** Developing a proposed scheme to ensure the quality of data generated by laboratories undertaking regulatory ecotoxicological testing (Environment Agency, 1999b) and developing performance standards for ecotoxicity tests (WRc,1996)

IV. **Improve the way in which ecotoxicity test data are used in risk assessment, e.g.** Statistical analysis of effluent bioassays, (Environment Agency, 1998a), the analysis and use of limit tests (Environment Agency, 1997) and developing a risk framework for direct toxicity assessment of effluent discharges (Environment Agency, 1999j)

V. **Demonstrate the use of the tests in the management of effluents e.g.** Toxicity Based Consents – Pilot Study, (the Environment Agency, 1996a), the direct toxicity assessment demonstration programme, (the Environment Agency, Ongoing work) and toxicity reduction evaluation case summaries for the pulp and paper industry and the chlor-alkali industry, (the Environment Agency, 1996b & 1996c).

The research and development has been undertaken to investigate and demonstrate the benefits of using DTA in assessing effluents. These have been identified as, amongst others:

I. DTA provides a summary of the effects of all constituents. This includes unknown/unidentified

chemicals and chemicals which may be breakdown products

II. It can provide a measure of additivity and other combined effects and is a good way of assessing complex mixtures

III. DTA can help where known chemicals are present in the effluent, but where little or no toxicity data exists

IV. The DTA measure relates to our monitoring endpoints (receiving water biological status) better than chemical surrogates, and for some tests this relationship may be modelled

V. DTA is a proactive biological measure, which can be used to predict potential impact and as a measure of risk

VI. DTA can provide a useful summary measure for process control, and is a holistic measure for determining variability in the composition of complex effluents

VII. Some DTA tests are cost effective compared to chemical analysis, considering the relevance and holistic nature of the measurements made (Boumphrey *et al*, 1999).

Nationally (Environment Agency) and internationally (OECD) standardised acute toxicity tests with fish (*Oncorhynchus mykiss, Cyprinus carpio*), acute and chronic test *with Daphnia magna* and tests with algae (*Selenastrum, Skeletonema*), *Vibrio fischeri* and various other organisms (oyster embryo larval, *Tisbe battagliai*, *Acartia tonsa*, *Gammarus pulex* and *Lemna minor*) have been used in research and development projects (e.g. Environment Agency, 1996).

Sampling takes place at a point appropriate to the objectives of the testing. It is proposed that routine regulatory testing would take place at the end of pipe, but the way in which the result would be interpreted and used will take account of the dilution available in the receiving water, and other receiving water characteristics. During the characterisation of the effluent, sampling may take place at many different places, e.g. at the end of pipe, at a point in the receiving water, or up and down stream of the discharge outlet in order to see how the toxicity in the water changes (Environment Agency DTA Demonstration Programme, in progress). If unacceptable toxicity is found in the effluent, sampling will take place further up in the process to determine the sources and causes of the toxicity. The numerous test results available (LC_{50} and NOEC in % effluent) are published in detail in *Toxicity Based Consents Pilot Study,* Environment Agency R&D Project Record P2-493/11. 1997, and Environment Agency Technical Report P23, 1996.

The UK has developed a seven stage protocol for assessing and regulating effluents (Forrow, 1999b). This protocol has been derived as a result of previous research and development (e.g. National Rivers

Authority, 1993) and public consultation, and is currently being tested in the 'DTA Demonstration Programme'. This programme is a collaboration between the UK regulators, industry and water companies.

The protocol enables the regulator to prioritise resources, and investigate and manage complex effluents. The first stage of the protocol directs the investigation towards receiving waters where the biological quality of the aquatic system is already impaired (i.e. the existing 'worst cases'), and where there is a likelihood that this is due to toxic substances (as opposed to, for example, oxygen depletion). The effluents are then characterised using a range of toxicity tests, and a risk assessment is made and a level of toxicity is derived at which 'no harm' is thought to occur in the receiving water. If unacceptable toxicity is found, a site and process audit and TIE would be undertaken, and a toxicity reduction programme derived. This would be assessed using BAT criteria and a plan for implementation, with associated timescales, would be put forward to the regulator. The plan would be implemented, and the success of the programme in terms of toxicity reduction and changes in the receiving environment appraised and fed back into the management process.

This protocol is only one approach for using DTA in effluent management, and whilst it targets priority locations, it does not consider other objectives, e.g.

a. where a new discharge is to come on line, or where a substantial change to the process occurs which is likely to result in an increase in toxic emissions;
b. concerns where there are vulnerable/sensitive environments (e.g. habitats of special interest, fisheries);
c. environments where biological survey is difficult or not possible (e.g. marine, estuarine, constructed environments);
d. moves to more precautionary approaches (e.g. to prevent harm rather than react to its occurrence);
e. where DTA could be used in determining process or treatment BAT;
f. where voluntary activity by industry should be encouraged to:
    (i) investigate and reduce risks to the environment;
    (ii) protect treatment plants from toxicity.

Whilst the UK has not undertaken any practical demonstration in these other areas, they are considering approaches for how best to use DTA in these situations (Environment Agency, 1999j).

The UK approach focuses on the three levels end of the pipe, toxicity close to the outlet, and changes of the ecosystem related to toxicity and other anthropogenic effects. Starting point however is not the toxicity of the effluent, but the quality status of the receiving water, which is determined in ecological monitoring studies. The UK

protocol for monitoring and control of discharges from point sources, (from Tinsley, 1999) is shown in Annex V-6.

The UK DTA approach for the management of discharges from point sources focuses primarily on the quality of receiving waters for the following reasons:

I. A risk based approach, taking into account the quality of the receiving water, has been demonstrated to be an effective way to prioritise limited resources

II. Environmental conditions may change the nature of the effluent (for better or worse), and so should be taken into consideration in a risk assessment

III. The risk-based approach, which takes account of receiving water dilution and quality, allows cost and benefit to be considered, as is necessary to determine BAT

IV. BAT will not be achieved if the toxicity of the discharge is not balanced with other BAT criteria such as the need to minimise waste and the use of resources (e.g. water use)

V. BAT will not be achieved if over emphasis is given to the toxicity of the effluent, with no consideration of environmental capacity.

(Boumphrey *et al*, 1999).

Development of DTA is ongoing, and toxicity assessment methods which will better predict the effects of continuous low level exposures of chemical mixtures on populations of organisms as well as *in-situ* receiving water and rapid toxicity assessment methods are being developed and tested. Toxicity limits will not be applied in an industry sector by sector basis, but in a site-specific, case by case basis, taking into account the needs of the receiving water environment. However the UK welcomes the exchange of information regarding 'typical or achievable' toxicity levels, on an industry sector basis, as an aid to determining BAT, but not as a fixed criterion for BAT.

Other countries

United States of America

CURRENT REGULATORY PRACTICE

The USA is believed to be the most progressive country outside Europe as far as the prescription of toxicity requirements in discharge permits is concerned. Many states have legally based toxicity requirements (Tonkes, 1994). WET testing has an important role in US-EPA´s water quality program. Most industries are regulated by effluent guidelines based on the best available technology incorporating economic considerations. Heber *et al*. (1996) reported

that currently over 6500 effluent permits include WET monitoring or WET limits on a case by case basis.

The WEA Guidelines developed by the U.S. Environmental Protection Agency (EPA) were published in three detailed technical documents available on the Internet. There, test methods, ecological relevance and culturing conditions as well as statistical data analysis are described:

> Weber (ed.): Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth edition EPA/600/4-90/027F (August 1993)
>
> Lewis *et al*. (ed.): Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater. Fourth edition EPA/600/4-91/002 (July 1994)
>
> Lewis *et al*. (ed.): Short-term methods for estimating the chronic toxicity of effluents and receiving waters marine and estuarine organisms. Fourth edition EPA/600/4-90/027F (July 1994)

Since the 1980s, acute and chronic toxicity limits have been incorporated in wastewater discharge permits of industrial and municipal treatment facilities. The test methods vary geographically. There are guidelines for conducting toxicity identification/reduction evaluations of toxic effluents using BAT.

The US-EPA recommends, that the method used in any given wastewater evaluation exercise should be the method giving the highest degree of protection. The starting point for determining which wastewater investigation should be carried out is a calculation of the dilution capacity of the recipient (the mixing zone). A potential dilution factor greater than 1 000 at the minimum water flow leads to the recommendation of three types of acute toxicity tests (plant, invertebrate, vertebrate). The evaluation should enable one to set a CMC (Criteria Maximum Concentration, defined as 0,3 times the lowest LC_{50} value). For a dilution factor between 100 and 1 000 at minimum water flow either acute or chronic toxicity testing is recommended to calculate a CCC (Criteria Continuous Concentration). A factor below 100 indicates the recommendation of chronic tests for CCC calculation.

For unacceptable toxic effluents the local authorities are entitled to demand a TI/RE.

Principles for investigating and assessing the environmental risks of industrial wastewater were initially developed and implemented in the USA (US-EPA, 1987, 1991). The WEA-approach is in use. The detailed environmental hazard and risk assessment scheme is shown in Annex V-7 (taken from OECD Series on Testing and Assessment Nr. 11 Part 2: Annexes (1998)).

Current test costs are reported to range from $160 - $2240, depending upon the test method (EPA WEA: Guidelines establishing test procedures for analysis of pollutants: Final rule October 16, 1995 (Internet search).

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

No systematic literature research has been performed yet to summarise published reports. Experience exists for acute and chronic short-term tests. The *Vibrio fischeri* and the *Cerodaphnia dubia* assay have been applied commonly. The studies available are shown in table 17.

STRATEGIES

The Clean Water Act and EPA regulations authorise and require the use of an integrated strategy to achieve and maintain water quality standards considering chemical-specific analysis, biosurveys in the receiving water and WET. The WET-program gives a characterisation of the whole toxicity of an effluent without necessarily knowing all of its components and considering the effects of bioavailable substances. The strategy is completed with Toxicity Reduction Evaluations (TRE) and Toxicity Identification Evaluations (TIE, Huwer & Brils, 1999) in order to identify and reduce pollutants at the source (Tonkes *et al*., 1995).

Canada

CURRENT REGULATORY PRACTICE

Canada ranks effluents according to their environmental hazard potential and thus uses a water-quality-based approach Tonkes *et al*., 1995). ”Environment Canada” developed an evaluation system, based on effluent toxicity testing capable of ranking the environmental hazards of industrial effluents. No allowance has been made for in-stream dilution; therefore no risk assessment of environmental effects is modelled.

The ecotoxicological test systems used are *Vibrio fischeri*, *Selenastrum capricornutum*, and *Ceriodaphnia dubia*. Additionally, genotoxicity tests (SOS-chromo-test) are performed. All results are expressed as threshold values (LOECs) and subsequently transformed to toxic units. Recently a 37-effluent study was finished (De Zwart, 1995), but results are not available to the authors in detail. All test systems are considered to be necessary to describe potential risks of effluents.

Industrial sectors covered under national effluent regulation include pulp and paper, metal mining and petroleum refining. Toxicological testing is required under regulations for each of these sectors as either a compliance requirement (i.e. rainbow trout acute lethality) or as a legal monitoring requirement (i.e. battery of sub-lethal toxicity tests). At the provincial level, many industrial and municipal facilities are required to conduct aquatic toxicity testing as a condition of their effluent discharge permit. (E1).

Table 17: Experience with whole effluent testing in the USA

	Test system	Endpoint	Samples	Literature/comments
Petroleum refinery	Rainbow trout 84 strategic AT tests	AT	continuous flow-through	Arnold, 1999 Action taken after a final wastewater became toxic in fish AT-tests TI/RE strategy was successfully applied
Industrial effluents	*Vibrio fischeri* *Ceriodaphnia dubia* (1h and 48 h)	AT Suppression feeding, AT	28	Jung *et al*., 1997 similar sensitivity of both test with *Cerodaphnia dubia*
industrial effluents battery recycling factory	Cerodaphnia dubia Mutant *E-coli* (FluoroMetPlate assay)	AT AT	29	Jung *et al*., 1996 similar sensitivity of test systems
Refinery	*Vibrio fischeri* *Ceriodaphnia dubia* *Cyprinodum variegatus* *Pimephales promales* *Mysidopsis bahia*	AT AT AT AT AT	11	Beckmann *et al*. (1995) Mysid shrimps were most sensitive TIE evaluation
Petrochemical plant	*Vibrio fischeri* *Daphnia magna* *Daphnia pulex* *Gammarus pulex* *Selenastrum capricornutum* *Pimephales promales* *Cyprinodum variegatus* *Salmo gaidneri* *Mysidopsis bahia*	AT AT, 7d growth AT AT CT AT AT AT, growth AT	1 (chloroether fraction)	Dorn *et al*. (1991) Hazard assessment of toxic fraction TIE evaluation NOEC estimation of chloroether fraction Effects of chloroether fraction in outdoor streams (e.g. Gammarus feeding rate 28 d) also determined
Secondary effluent from Municipal treatment plant	*Vibrio fischeri* *Ceriodaohnia dubia*	AT AT	1	Mazidji *et al*. (1990) Wastewater fractionation with C_{18} solid extraction TIE evaluation
Several stations municipal wastewater collection system	*Vibrio fischeri* *Daphnia pulex* Dehydrogenase activity ß-galactosidase assay	AT AT E E		Logue *et al*., 1989 Decrease and degradation of toxicity by activated sludge treatment
Influent and effluent samples municipal treatment plant	Ames-test CHO test	M M	8	Waters *et al*., 1989 Ames-test was the more sensitive whereas CHO test indicates presence of cytotoxic components
Several industrial and municipal effluents	*Vibrio fischeri* several fish species	AT AT	50	Bulich *et al*. (1981) High correlation between Vibrio fischeri assay and fish toxicity

M = mutagenicity E = enzymatic AT = acute toxicity CT = chronic

EXPERIENCE IN DIFFERENT WASTEWATER SOURCE SECTORS

No systematic literature research has been performed yet to summarise published reports on WEA in Canada although WET testing is applied.

Pardos *et al*. (1999) stated, that persistent and particle bound (geno)toxic hydrophobic compounds should be more taken into account as they are of considerable importance in the delivery of genotoxins to receiving waters and deposited particle bound hydrophobic compounds contribute to longer term environmental impact. Preliminary results on 10 industrial effluents were presented at the SETAC conference at Edinburgh in 1999 but no details are given in the abstract. The Proceedings of that meeting will be published in the beginning of year 2000 in Environ. Toxicol. Chem.

Table 18: Experience with whole effluent testing in the Canada

	Test system	Endpoint	Samples	Literature/comments
Oil refinery Chemical plant Pulp and paper mill	*Vibrio fischeri* *Salmo gaidneri* *Daphnia magna* *Spirillum volutans*	AT AT AT CT	13	Qureshi *et al*. (1982)
Pulp mill effluent	Ames-test TA 100 UmuC-test fish hepatic micronucleus test V79 Mouse liver cells	A G MJ M M	1 (different extracts)	Rao *et al*., 1995 Fractionation of effects
Pulp and paper Chemical industry Metal industry Municipal wastewater	SOS chromotest	G	50	White *et al*., 1996 up to 70% of dichlormethane extracts tested were genotoxic depending on sample type and activation status
Chemical plants Pulp and paper Metallurgical plants Municipal wastewater	SOS chromotest	G	36	Legault *et al*., 1996 77% of samples induced significant response chemical data available supported positive responses

M = mutagenicity G = genotoxicity AT = acute toxicity CT = chronic toxicity

STRATEGIES

Canada has developed an evaluation system, called the Potential Ecotoxic Effects Probe (PEEP), based on effluent toxicity testing. PEEP uses the results of various toxicity tests to rank the environmental hazards of different industrial effluents. This is then used for the prioritisation of effluents for remediation. In-stream dilution is not covered by the index (Tonkes *et al*., 1995) The

environmental hazard and risk assessment scheme taken from Tonkes *et al*. (1995) is shown in annex II-8.

Slovenia

The monitoring program of effluents is mainly based on a traditional chemical-specific approach, which involves conventional chemical determinations and measurement of priority pollutants. An assessment of effluent discharging into sewerage systems includes estimation of biodegradability, but toxicity evaluation is not prescribed by regulation. Acute toxicity tests with *Daphnia magna* according to the ISO-standard are conducted when toxic substances in the effluents are expected (Tisler, 1999).

CONCLUSIONS

In general, the conclusions of the OSPAR Workshop on the Ecotoxicological Evaluation of Wastewater held in Berlin 1997 (UBA, 1997, see Annex IV) are confirmed by the findings presented in this document. It is agreed that chemical group parameters and biotests should be employed in combination in order to assess the toxicity of complex effluent mixtures.

The advantages of using group parameters (chemical group parameters in combination with biological effect parameters are as follows:

I. Whole Effluent Assessment (WEA) considers all wastewater compounds regardless of their origin and detectability by chemical analysis. The identity of compounds does not necessarily need to be known. By-products and metabolites are assessed as well

II. Toxic effects on aquatic organisms are directly displayed, combined effects are also considered

III. The sources of hazardous effluents (production steps or hot spots) inside industrial areas can be more easily identified (backtracking)

IV. The effort required to perform test in WEA is comparable with that of chemical single substances' analysis

V. The advantage of a use of chemical group parameters (AOX, TOC, COD, $N_{total,}$ PBS) is that only a small number of parameters is needed and the constituents of effluent are described in a more comprehensive way than by single chemical analysis.

Methods for whole effluent assessment testing

Bioassays are valuable instruments for obtaining additional information about the quality of wastewater and about its potential environmental impact.

There is broad agreement among scientists that ecotoxicity testing of effluents can be based on a battery of tests covering the different trophic levels. The most widespread taxonomic groups used in effluent toxicity testing are bacteria, algae, crustaceans and fishes. The organisms used most often are *Daphnia magna* and *Vibrio fischeri*. In fish and algae, different species are applied. The tests can be used directly for effluent toxicity testing, for specific screening, for biodegradation testing as well as for monitoring.

As experiences differ among the Contracting Parties, there is so far only limited support for the application of bioassays in emission control at a regulatory level using emission limit values. However, the need for discussion is recognised as to if and how bioassays should be introduced to routinely monitor hazardous effects of effluents and thereby add important information for the description of BAT.

The sampling procedure as well as preservation and pre-treatment of samples are described in detail in ISO 5667-16: The choice of representative sampling points, frequency of sampling etc. is dependent on the objective of the study. Wastewater organisms that may interfere with the test system, particulate matter and pH are factors to be taken into account.

Bacterial toxicity tests based on activated sludge respiration inhibition or nitrification inhibition consider a possible impact on biological wastewater treatment plants but they are not suitable test systems for direct discharges.

There are numerous international test guidelines to determine aquatic ecotoxicity and degradability of single substances, which can in principle be used for wastewater evaluation. But only a limited number of suitable test methods are adapted to the specific conditions of wastewater. Up to now mostly national standards are available.

There is an urgent need to create internationally accepted standards for testing ecotoxicity and degradability of wastewater. Test principles should focus on the same type of endpoints as are used for evaluation of hazardous substances in order to attain a broad acceptance of test methods. The choice of test design should be appropriate to the object of the investigation and take into account the effort to be invested in terms of time and equipment costs, so as to increase the acceptance of biotests in WET assessments.

The need to consider genotoxicity and mutagenicity testing in WEA is widely acknowledged although the potential hazard of genotoxins to the environment remains unclear. It is accepted, that no individual test covers all possible endpoints. A test battery is called for. Up to now only bacterial tests (umuC-test, Ames-test, SOS-chromo test) have been applied to a wide range of wastewater samples. The need for other test systems on a higher organism level is recognised, but currently no internationally accepted guideline exists. Mutagenicity testing of wastewater with eukaryotic cells might not be a feasible element of a routine programme because considerable input is required to perform the available test.

The need to further develop, validate, harmonise and implement test systems to test wastewater for bioaccumulation, endocrine disruptors, and genotoxicity/mutatoxicity was emphasised.

Test design and data processing

Two procedures for evaluation of raw data from toxicity tests are applied:

I. The EC/LC_{50} approach uses statistical analysis of data after transformation to an appropriate distribution (e.g. log/probit-transformation). In this approach, a concentration/response-relationship is estimated from at least 5 data pairs between 0% and 100% response

II. The result reported in the LID approach (lowest ineffective dilution) is the reciprocal value (or dilution factor) of the wastewater at which no effects are observed. Here no concentration/response-relationship is needed and the test procedure can be further simplified. However, no statistical evaluation can be done and no confidence limits can be given.

In principle both evaluation methods provide information on the hazardousness of a wastewater. In many cases, it is possible to apply both methods using the same raw data. The only advantage of the LID-approach is that cost-effective screening procedures can be performed.

National strategies and experience on whole effluent assessment

Two distinct philosophies regarding WEA testing exist:

a. The emission based (end-of-the-pipe) approach is keeping with the precautionary principle and uses, as a rule, acute and short-term chronic toxicity tests. It does not include a risk assessment regarding the recipient water. Nevertheless elimination and degradation of ecotoxic or genotoxic effects within the sewage treatment plant is evaluated in some cases to identify the toxicity relevant for surface waters;

b. The water quality based approach (i.e. DTA-approach) focuses on ambient toxicity evaluation and takes the flow capacity of the receiving river into account. It assesses the actual impacts of effluents. The risk assessment is based on methodologies developed for single substances. WEA with acute and short-term chronic toxicity tests is also performed, but more sensitive and more expensive methods including chronic and ambient toxicity are applied if adverse effects in the receiving water are expected from a tiered PEC/PNEC-procedure.

Both approaches focus on a certain type of assessment of the effluent but the ultimate goal of the two assessments differs and the samples

are also taken from different 'compartments'. The differences between the emission based and the water quality-based approach were summarised by Tonkes *et al*. (1995) as follows:

Table 19 Emission based and water quality-based approach in WET evaluation after Tonkes *et al*. (1995)

	Emission-based	Water quality based
Effluent limits	No site-specific load	Site specific concentration
Required treatment techniques	Based on intrinsic (toxic) properties of chemicals in effluent or technology based	Based on water quality criteria or preventing toxic effects in the effluent receiving water
Data requirements	Basic chemical and ecotoxicological data; technology available	Basic chemical and ecotoxicological data. Physical, chemical and biological characteristics for the receiving water and the fate of the discharged chemicals
Monitoring	Effluent	Receiving water
Competition	Uniform standards independent of site	Site specific standards
Practice	Tends to reflect worst case approach in general, but may underestimate effects of discharges in specific situations	May tend to dilution as solution in general, but stricter standards are possible when effects are intolerable in specific situations

The emission based approach results in general in a broader database for different effluents with acute toxicity endpoints while the water quality based approach leads often to TIE-procedures and detailed evaluation of threshold wastewater on special occasions. Often the water quality based approach is limited to risk evaluations for single substances identified in the effluent.

WEA can also contribute to the definition and verification of BAT. Sufficient ecotoxicity data are available from different wastewater source sectors, which can be used as general reference values.

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Annex I

27 November 1997

OSPAR Workshop "Ecotoxicological Evaluation of Wastewater"

in Berlin, 23 and 24 September 1997

- Summarised Findings -

1. Conclusion by the Lead Country Germany

Germany will present the outcome of the workshop to POINT. Further action and products will be discussed at POINT. Germany proposes to develop an OSPAR Recommendation on the basis of the following conclusion. (The findings documented in paragraphs 2 to 9 are the result of the presentations and discussions during the workshop.)

I. Bio-assays are valuable instruments for additional information about wastewater quality and their possible environmental impact. They are tools to improve the knowledge of environmental impact and to decrease the effects of toxic effluents, specifically for complex effluents.

II. Bio-assays should especially be applied to monitor complex effluents with priority for effluents having a considerable environmental impact. It is recommended to start with textile-, organic chemical- and surface treatment-industry.

III. There is a scope for co-operation on a voluntary basis which could cover all aspects of applying bio-assays, especially method development, standardisation, SOP/AQC (Standard Operation Procedures/Analytical Quality Control) and ring-tests.

2. General aspects

There was agreement that bio-assays are valuable instruments for additional information about wastewater quality and their possible environmental impact. Bio-assays are tools to improve the knowledge of environmental impact and to decrease the effects of toxic effluents, specifically for complex effluents. Nevertheless chemical characterisation normally is still necessary and information on the persistence and bioaccumulation of hazardous substances in effluents should not be ignored. Problems of correlating bio-assays with chemical analysis have been discussed. So group parameters, such as AOX, frequently bore no connection to the actual toxicity of the effluent stream.

It was mentioned that the application of bioassays may lead to a reduction of analytical effort and bio-assays have a broader span of control of toxic substances compared to the sum of chemically

measured compounds. A subject of further discussion was, whether it will be possible to reduce chemical control of effluent streams when bio-assays are applied.

3. Selection of test organisms and procedures

- It has to be recognised that the Contracting Parties have developed different approaches in applying bio-assays for wastewater evaluation.
- It is agreed that a battery of toxicity tests covering the different trophic levels is needed. This battery should be defined according to the purpose of application (e.g. screening, characterisation and regulation of wastewater discharges).
- The application of a set of international harmonised tests versus site-specific selection of test organisms has been addressed. International standardised tests would yield the advantage of comparable results and an easier co-operation between the Contracting Parties. It was mentioned that many countries are using similar tests already now. On the other hand in certain cases necessity for a site-specific selection of test organisms based on receiving surface water was seen (e.g. possible inclusion of other marine organisms).
- At present, a lack of internationally standardised and harmonised test procedures is recognised. There is great merit in further European/international co-operation in developing ecotoxicological testing procedures for whole effluent toxicity.
- There is still a need for further/ongoing method development (e.g. rapid tests).
- Appropriate endpoints are defined by currently available tests. Method development requires guidance on endpoint need (chronic, sub-lethal, rapid test etc.).
- It is agreed to replace fish as a test organism as far as possible (especially in cases of acute toxicity testing).

4. Fields of application

- Bio-assays should especially be applied to monitor/control complex effluents with priority for effluents having a considerable environmental impact.
- Bio-assays could either be applied in evaluation of new plants (e.g. pre-testing on small scale) or in the modification of existing plants. Further fields of application are monitoring, toxicity tracking (e.g. leading to the identification of toxic tributary streams or applied toxic chemical additives) and measurements in receiving waters.
- It is recommended to start with textile-, organic chemical- and surface treatment-industry (an OSPAR-BAT description or recommendation is helpful). Most relevant loads should be considered first. In medium term an industry by industry characterisation should be performed. Concerning municipal treatment plants the use of bio-assays should be directly linked to toxicity tracking for indirect industrial discharges. If toxicity is not caused by industry, additional toxicity tracking may lead

to toxic municipal/diffuse discharges (to be dealt with by OSPAR/DIFF). Another area of application is the evaluation of waste and sludge by analysing the corresponding leachates (e.g. concerning landfills).

5. Suitable emission limits for regulatory purposes

- Considering the diversity of experiences, there is only limited support for application of bio-assays as emission limit values, but a need for discussion how to introduce bio-assays in legislation and permitting. At this stage the discussion should be independent of industrial sectors because more data are needed. The data sets from Germany and Sweden can be regarded as a starting point.
- It is agreed with that the long-term goal should be no measurable (acute, sublethal, chronic) toxicity at a selected point (this might be the end of the outfall or at a point downstream in the receiving water). Further agreement is on a stepwise approach over time to achieve this goal: starting with acute toxicity and going on to chronic toxicity measurements.
- Different approaches regarding dilution of effluents in receiving waters were recognised. The merits and limitations of emission limits versus risk assessment using PNEC/PEC have been discussed.
- It is agreed with that BAT could include bio-assay monitoring/controlling requirements.
- A zero-emission approach for hazardous substances as mentioned in the Esbjerg Declaration[1], versus a zero-effect concentration derived from a bio-assay has been discussed.
- There is a strong feeling to use $LC_x/EC_x/IC_x$ in the interpretation of bio-assay results. But if no measurable toxicity as goal is accepted, there is a need to measure the No Observed Effect Concentration (NOEC).
- It is agreed with that there is a need to take costs and benefits into account (area of collaboration).

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[1] The Ministers agree that the objective is to ensure a sustainable, sound and healthy North Sea ecosystem. The guiding principle for achieving this objective is the precautionary principle.

This implies the prevention of the pollution of the North Sea by continuously reducing discharges, emissions and losses of hazardous substances thereby moving towards the target of their cessation within one generation (25 years) with the ultimate aim of concentrations in the environment near background values for natural occurring substances and close to zero concentrations for man-made synthetic substances.

Note: The United Kingdom shares the ideal of these aims, but does not accept that they are currently practicable.

6. Sampling procedure

- The sampling frequency depends on the purpose (where possible, programs should be on a statistical basis and reflect the variability of effluents).
- The sampling point depends on the specific program (e.g. inside plant, end of pipe, receiving water).

7. Quality assurance and control

- The need for quality assurance and control for both sampling and analysis was accepted. Quality assurance should consist of standard operating procedures, internal and external quality control and a quality system.
- There is a need to develop standard AQC (Analytical Quality Control) and test protocols.
- The international debate on toxicity statistics was recognised.
- Other methodical issues related to quality of data, to regulating decisions and ecological relevance were discussed.

8. Action targets, area of co-operation

- It is agreed with the aim of gaining further experience in the application of bio-assays. The member states should report these experiences to OSPAR. Experiences of other international bodies should be taken into account (e.g. OECD which already organised a workshop on this subject in 1984).
- There is a scope for co-operation on a voluntary basis which could cover sampling procedures, method development, selection of test organism, guidance on appropriate endpoints, culture-procedures, SOP/AQC (Standard Operation Procedures/Analytical Quality Control), ring-tests, accreditation schemes, analysis minimum facility and cost/benefit analysis. Based on this information and the results of co-operation, strategies to optimise the use of bio-assays within the Contracting Parties should be developed.

9. Additional remarks

- The application of bio-assays on air emissions is not within the scope of this workshop until more information is available.
- Subjects such as ELV (Emission Limit Values) versus EQS (Environmental Quality Standards) approach and the relation of the IPPC-directive and the BAT-work in the frame of OSPAR was mentioned, but not discussed.
- Further development of an OSPAR-description should consider elements such as mutagenicity, endocrine effects, meso- and microcosms and aspects such as in-vitro and in-vivo tests. Other elements are chronic toxicity tests, persistency and bioaccumulation.

Annex II

POINT 99/4/9-E (L)
Original: English
English only

OSPAR CONVENTION FOR THE PROTECTION OF THE MARINE ENVIRONMENT OF THE NORTH EAST ATLANTIC

MEETING OF THE WORKING GROUP ON POINT SOURCES (POINT)

SEVILLE: 13 - 17 DECEMBER 1999

Outcome of the Workshop on Whole Effluent Assessment

Presented by the Netherlands

Background

1. The attached document (Annex 1) contains the outcome of first part of the workshop on Whole Effluent Assessment (WEA) in Lelystad on 28-29 October. During the first day of the workshop a more general discussion took place on WEA as a tool to improve BAT decision making. It was agreed that the Netherlands would report on this part of the workshop to POINT.

The second day of the workshop was devoted to the discussion on the first draft of the Background document on the Ecotoxicological Evaluation of Wastewater within Whole Effluent assessment.

The revised version of the document is presented by the lead country Germany under item POINT 99/4/8-E.

2. Specific attention has been given to the role and position of OSPAR in the development and policy implementation of WEA. The participants of the workshop subscribed to the viewpoint that:

> **THE OBJECTVE OF THE OSPAR STRATEGY WITH REGARD TO HAZARDOUS SUBSTANCES WILL BE SERVED BY OPERATIONALISATION OF WHOLE EFFLUENT ASSESSMENT.**

The foundation of this thesis is documented at Annex 2 of this document.

Participants of the workshop also recognised the need to internationally structure the work on WEA on a policy-oriented level and to agree upon a WEA programme in order to stimulate and facilitate the exchange of information on the development of methods within WEA.

Action Requested

3. POINT is invited to examine and discuss the attached annex on the outcome of the workshop and to decide on:

a. an informal group of experts which consists of Contracting Parties and observer organisations for the further elaboration of an OSPAR programme on WEA;

b. to discuss the terms of reference for this work.

Annex II-1

Outcome of the workshop on Whole Effluent Assessment
Part 1. WEA as a tool to improve the quality of BAT decision making

OPENING

1. **Mr. GERARD DE VRIES** of RIZA, The Netherlands and head of delegation for POINT welcomes all participants to the workshop in Lelystad. He addresses special thanks to the German Environmental Agency for their work on the background document, which has been distributed to all participants in time. The first day of the workshop will be devoted to a general discussion on Whole Effluent Assessment and more specifically, the interest of OSPAR for WEA (policy angle), the possibilities to use WEA for BAT decision making and the experiences gained with biotesting. The Netherlands will briefly report the outcome of this part of the workshop the next POINT meeting.

PRESENTATIONS

2. **Mr. JAN LEENTVAAR**, chairman of this part of the workshop and Director of Water Pollution Control of RIZA, gives an introduction on water pollution control in the Netherlands, the position of RIZA as national water research centre and today's topics in the Dutch policy making on water management. He points at his work as chairman of a working group of the international Rhine Commission, where there is also a great interest in WEA.

He shows that the substance oriented approach lacks the possibilities to fully address the negative impacts of polluting substances in the aquatic environment. Only a limited number of substances can be analysed in wastewater, surface water and sediments and just over 20 -30 % of the negative effects can be backtracked to known substances. He presents a definition and goal of WEA and emphasises the need to make international progress on the policy issues as well as on instruments and methods of WEA.

3. **Mr. HENK POLS**, of RIZA, The Netherlands, presents a way forward with WEA and POINT's work on BAT. He presents a determination of BAT based on a substance-oriented approach and draws a comparison with a determination involving biological parameters. WEA and the initial selection of substances in the strategy with regard to hazardous substances are both using the same criteria, which are persistence, bioaccumulation and toxicity. He demonstrates that the strategy on hazardous substances, as there are limits to the substance-oriented approach, will only have partial results for OSPAR's goal to prevent and eliminate pollution. There

are possibilities to apply WEA as an additional instrument to achieve OSPAR's goal. By using criteria for P, B and T with have been deduced with regard to the marine environment - the risk for the marine environment of discharges can be assessed directly.

In the discussion the following points were made:

I. It is a large step from the theory to today's regulating practice. There is a growing consensus on (acute) toxicity testing but the other area's still need a lot of scientific research

II. Nevertheless it is important to discuss the policy items concerning WEA and to develop a general outline on how WEA could contribute to the work within OSPAR. A long-term vision on WEA as an instrument to contribute to the objectives of the OSPAR strategy with regard to hazardous substances will help to structure the work and initiatives

III. Depending on the policy approach, it is open to use WEA for a local water quality assessment, for more general assessments (e.g. marine ecosystem) or to set emission limit values

IV. In principal the instrument of WEA is not restricted to discharges of wastewater. The assessment can also be applied to air emissions.

4. **Mrs. RUTH BOUMPHREY** of the UK Environment Agency, presents the current effluent control methods and experiences with WEA in the UK. For the major polluting processes the currently operating system of IPC in the UK is very similar to the EU IPPC directive. For the other discharges to controlled waters the UK operates a system of consents. WEA could be a useful instrument within these methods of effluent control. She suggests, as part of the exchange of information on BAT under IPPC, to incorporate WEA in the BAT reference documents. Until now, the UK only considered direct toxicity assessment (DTA) in defining BAT in combination with a water quality assessment. DTA has been used widely in a research and demonstration context, but as yet has been untested in a legal context. The UK wishes to bring DTA within the routine regulatory framework and will continue the development of alternative risk assessment approaches for other types of toxic effects.

In the discussion the following points were made:

I. DTA for the marine environment is difficult because there is a lack of validated methods

II. Some participants show their doubts on using DTA as a tool to assess the effects in receiving waters.

5. **Mr. GEORG MAUE** of the German Environmental Agency, presents the German experiences on using toxicity tests for legislative purposes (Wastewater Ordinance and Wastewater Charges Act). He shows results of an extensive background research and data collection on toxicity tests for industrial wastewater discharges, which involved

over 10 000 samples in 700 industrial plants. He illustrates how emission limit values - expressed as the Lowest Ineffective Dilution, LID - for acute toxicity tests have been set for several industrial branches. He emphases the positive experiences and results of the German approach and proposes an OSPAR data collection of acute toxicity tests with the aim of defining toxicity emission levels for relevant industrial sectors.

In the discussion the following points were made:

I. It is to be expected that when further toxicity tests will become available and have proven to be reliable, further legislative measures involving biological tests will be enforced in Germany

II. The German approach can be regarded as a bench marking system for industrial sectors on acute toxicity. The result of this benchmarking is subsequently being anchored by legislative measures

III. The German experiences show no problems in the application of toxicity tests with industries sectors with a large variation in water consumption. (concentration vs. toxic load).

6. **Mr. ARNO ROTHERT**, representative of CEFIC, briefly introduces CEFIC's views on ecotoxicity assessment. CEFIC aims to encourage and contribute to the development of an internationally harmonised approach to bioassay requirements. The currently available ecotoxicity assessment methods are not sufficiently reliable to be used in terms of a limit in a discharge permit. It can be used as an action level to initiate investigation; to identify sources of toxic discharges, prioritise toxicity reduction measures, plan toxicity reduction programmes and monitor improvements end of pipe / improvements in the quality of receiving waters.

WEA should be considered as one of a set of instruments to optimise BAT. For the organic chemical industry there seems no direct relationship between WEA and BAT. The question can be raised whether the objective is to reduce the COD load or, more in particular to reduce adverse effects. OSPAR should not address WEA as an instrument to assess local impacts but should make a marine consideration. Mr Rothert stresses the need for further international harmonisation and development of methods. There is a need for a structured and coherent approach of WEA, probably within OSPAR.

In the discussion the following points were made.

I. For the organic chemical industry there is a direct relation between COD and biological effects. For this reason the reduction of COD loads is a valid objective for BAT compliance.

II. The impact of an effluent in the receiving aquatic system is difficult to predict. End of pipe criteria for biological parameters are less complex and seem to be more reliable.

7. **Mr. Rob BERBEE** of RIZA, The Netherlands, gives an introduction on toxicity backtracking and the link towards Best Available Techniques. He distinguishes two general approaches for toxicity backtracking, being the elucidation of responsible source (toxicity identification evaluation) or group of substances (tributary stream) causing an undesired biological effect. He gives an overview of international experiences with toxicity backtracking. More in particular he illustrates a case study on tracking the source of toxicity in treated wastewater in a Dutch polymer plant. This study resulted in the identification of several problem areas and in measures for improvement. He concludes on the added value of toxicity backtracking to improve BAT performance.

In the discussions the following points were made.

I. Toxicity backtracking is in a stage of implementation and practicable development. Taking account of the costs involved there should be a strong indication on adverse effects of effluents before backtracking should be required

II. State of the art on backtracking makes an active role of authorities in case studies desirable.

8. **Mr. JUKKA ATHIAINEN** of the Finish Environment Institute addresses the experiences with biotests on pulp mill wastewaters and other industrial effluents. Historically the use of chlorine for paper bleaching had negative and adverse effects on the water quality. Nowadays chlorine bleaching has been phased out and the results of acute biotests in paper mill effluents show only sporadic and weak toxic responses. Moreover, and this seems contrary to the experiences in other member states, Finish waters show no adverse effects in bioassays. These findings could have been influenced by the advanced BAT measures that were imposed on industry because of the great concern for eutrophication in Finish waters.

Development of tests for genotoxicity and long term effects for discharges should be considered. Characterisation of effluents with biotests should also consider the effluent flow (toxic load) and should also take into account the quality and objectives for the receiving water.

9. **Mr HEINO FALCKE** of the North Rhine-Westphalia State Environment Agency (LUA), describes the state of the art on wastewater assessment by bioassays in the federal state. German legislation emphasis the goal of minimisation of emissions as part of the precautionary principle. The chemical industry is subjected to toxicity limitation with respect to toxicity for daphnia, luminescent bacteria or algae. A rather new issue is the monitoring of genotoxicity. A zero-mutagenicity criterion for effluents from new chemical plants has been implemented in German legislation. Mr Falcke presents a general overview and illustrates the implementation of bioassays in North Rhine-Westphalia.

In the discussion the following points were made

I. There has been no active role (participation/ facilitation) of the authorities in the toxicity reduction programmes of industrial sectors

II. There is no information available on additional measures that were taken nor on the costs involved by the German industry in order to comply with the toxicity emission limit values

III. Toxicity testing is a good control mechanism on the use and discharge of additives and biocides.

10. **Mr. AKE UNDEN** of the Swedish Environmental Agency, distributes a paper on the Swedish position on whole effluent assessment. The conclusions or proposals of the paper are:

I. The aim of WEA is to investigate whether a discharge has a negligible environmental impact or not, as a basis for a decision on the need for further measures

II. All three biohazard measurements, i.e. degradability, bioaccumulation and toxicity, should be considered, not just the latter (including effects on reproduction, teratogenicity, mutagenicity, etc.)

III. Application of a suitable set of tests or analyses should be decided upon by the national competent authority based on an OSPAR Recommendation.

FINAL DISCUSSION

11. **Jan Leentvaar** addresses the contents of the explanatory note distributed by The Netherlands before the workshop.

Taking into account:

1. the limits of the hazardous substance approach and
2. meanwhile acknowledging that both WEA and the hazardous substance approach use toxicity, persistence and bioaccumulation as parameters

the implementation of WEA as an instrument to assess risk/hazard for discharges of point sources might contribute to the objectives of the OSPAR strategy with regard to hazardous substances.

The objective of the OSPAR strategy with regard to hazardous substances will be served by operationalisation of Whole Effluent Assessment

Participants of the workshop subscribed to the viewpoint expressed in the thesis.

The conclusions of the discussion on the question of the role of OSPAR in the operationalisation of WEA can be summarised as follows.

I. Acknowledging the thesis OSPAR should play an active role in the development of WEA and should catalyse initiatives and officially adopt a pro-active attitude

II. This could be done by formulating a programme containing the OSPAR objectives for the operationalisation of WEA. This programme should also include the terms of reference for the work on WEA within OSPAR

III. The role of OSPAR could include

- the exchange information and know how on WEA
- facilitating and stimulating international co-operation
- discussing and formulating the international policy aspects of WEA
- giving guidance and publishing recommendation
- making a start on this by establishing a standing committee on WEA.

In addition the following remarks were made:

I. There is a great need to internationally structure the work on WEA on a policy-oriented level. In this way results and progress can be recorded, documented and if deemed appropriate being recommended for national implementation

II. The draft background document of Germany contains a good overview of the current practises and state of the art on biotesting in member states and could be the first of a series of documents published by OSPAR on WEA

III. In the light of OSPAR's specific concern for polluting substances that are persistent and liable to bioaccumulate specific attention should be given to tests and methods for persistence and bioaccumulation

IV. Acknowledging the need for a combined international action in order to develop methods, harmonise procedures and receive guidance for means of implementation of WEA, there is also a need for flexibility to allow contracting parties to fine tune WEA into national policies

V. WEA is one of the instruments that can be used in the process of BAT decision making. The operationalisation of WEA can also be of great importance for the work on BAT reference documents (BREF's) under the EU IPPC directive. Especially in the work on the horizontal BREF on 'wastewater and waste gas treatment and management' the need for further international development of biotests has been identified. This information exchange is however more or less restricted to proven technologies and methods. Incorporation of WEA in the work, directive or

products of IPPC could be one of the objectives of OSPAR's work on WEA

VI. Further development of toxicity backtracking is recommended as a method to identify substances and/or sources causing adverse affects in discharges

VII. The commonalties in methodology of WEA and the substance-approach should be regarded when working on WEA. WEA can be seen as an additional instrument to fulfil the objectives of the strategy with regard to hazardous substances and should not result in a shift in approach.

The possibilities for co-financing (research) projects on WEA, i.e. the Fifth Framework Programme (RTD) will increase significantly if OSPAR will adopt a pro-active approach for the WEA.

Annex II-2

Explanatory note on WEA and the OSPAR strategy on hazardous substances

1. The Convention for the Protection of the Marine Environment of the North-East Atlantic ("OSPAR Convention") states that, "Contracting Parties agree to take all possible steps to prevent and eliminate pollution and to take the necessary measures to protect the maritime area against adverse effects".

The elaboration of OSPAR's mission, as more specified objectives, is formulated in strategies:

The objective with regard to hazardous substances is to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values of naturally occurring substances and close to zero for man-made synthetic substances.

In the "*OSPAR Strategy with regard to Hazardous Substances"* the Commission will develop programmes and measures to identify, prioritise, monitor and control (i.e. to prevent/reduce and/or eliminate) the emissions, discharges and losses of hazardous substances which reach, or could reach, the marine environment.

The selection and prioritisation will be based on "PTB-criteria", i.e. criteria for persistence, toxicity (including mutagenicity) and bioaccumulation. The application of these criteria should both reflect the hazardous characteristics of substances or groups of substances and give priority to their actual or potential occurrence and effects in the maritime area.

2. The development of measures concerning best available techniques (BAT) and best environmental practice (BEP), with special consideration to the hazardous substances on the "*OSPAR List of Chemicals for Priority Action"*, is an important track for the working group on POINT sources. It is to be expected that the future work on BAT and BEP measures will be greatly influenced by the outcome of the identification and prioritisation of hazardous substances.

POINT also recognised the potential of the exchange of information on BAT under the IPPC directive for its work on measures for point sources. In examining BAT Reference Documents (BREF) POINT will examine the need to specify or select specific BAT-options taking into account the specific concern for the marine environment.

3. The methodology of Whole Effluent Assessment (WEA) is aimed to determine possible adverse effects of effluents. WEA addresses basically the same effect parameters as used in a substance-oriented approach:

I. mutagenicity (or even better genotoxicity)

II. toxicity (acute and/or chronic)

III. bio-accumulation

IV. persistence (or (bio-)degradability).

For complex effluents there is general agreement on the fact that WEA has an added value to the substance-oriented approach. Furthermore there is a growing consensus on the type of tests that should be performed, although further work on harmonisation and standardisation of methods should take place.

4. Substances identified for priority action shall be specifically addressed in the BAT work in order to fulfil the OSPAR policy goals (background/zero concentration in the marine environment). Taking into account that the selection and prioritisation of hazardous substances is based on their adverse effects, expressed in PTB-criteria, the question arises if the same objective can be served, when comparable PTB-criteria are used for effluents. Maybe it can simply be stated that such an approach results in a "short cut" in the OSPAR policy, because the aim of protecting the marine environment against adverse effects is achieved by the assessment of adverse effects in discharges.

Using WEA in this way might result in a different (additional) angle of approach for the OSPAR strategy on hazardous substances. Moreover the relevance of the discharges from specific industrial sectors for the execution of the OSPAR strategy for hazardous substances can be established.

Annex III-1: Short term ecotoxicity testing methods in freshwater environment and WWTP

Taxonomic Group	Species	Method	Endpoint	Test duration	Data	Reference of methods				National standards	Application	Time effort
						ISO	EN	92/69/EWG	OECD			
Fish	*Brachydanio rerio*	acute	survival	ST 24-96 h	LC	7346-1, 7346-2, 7346-3	7346-1, 7346-2, 7346-3	C.1	203	AFNOR T90.303; SS 28162; SS 28193; SFS 3035	France; Sweden, Finland; Norway	
	Cyprinus carpio	acute	survival	ST 96 h	LC			C.1	203		France	
	Dicenthrarcus palrax	acute	survival	ST 24 h	LC					AFNOR T90.307	France	6 h - 50 h
	Gasterosteus aculeatus	acute	survival	ST 96 h	LC					SS 28189	Sweden	
	Lepomis macrochirus	acute	survival	ST 96 h	LC			C.1	203	EPA/OPPTS 850.107		
	Leuciscus idus	acute	survival	ST 48-96 h	LC/LID			C.1		DIN 38412 T 31 (wastewater LID)	Germany	3 h
	Oncorhynchus mykiss	acute	survival	ST 24-96 h	LC			C.1	203	AFNOR T90.305; EPA/OPPTS 850.1075	France	
	Oryzias latipes	acute	survival	ST 96 h	LC	7346-1,	7346-1,	C.1	203			
	Poecilia reticulta	acute	survival	ST 96 h	LC	7346-2,	7346-2,	C.1	203			
	Pimephales promelas	acute	survival	ST 96 h	LC	7346-3	7346-3	C.1	203			6 h - 50 h
	Pimephales promelas	chronic	larval growth, survival	ST 7d	NOEC, IC25					EPA/600/4-91/002 1000.0; 1001.0		
	Salvelinus fontinalis	acute	survival	ST 96 h	LC							
	Salmo trutta	acute	survival	ST 96 h	LC				(203)		Norway	
	Salmo salar	acute	survival	ST 96 h	LC				(203)	NS 4717	Norway	
Crustaceans	*Daphnia magna*	acute	immobilisation	ST 24-48 h	EC/LID	6341	6341	C.2	202	DIN 38412 T 30 (wastewater LID); AFNOR T90.301; PRNP 4175; SFS 5062; SS 28180	Germany, France Finland, Norway, Portugal, Sweden, USA	
	Daphnia pulex	acute	immobilisation	ST (24)-48 h	EC			C.2	(202)	EPA/OPPTS 850.1010	Norway	
	Cerriodaphnia dubia	acute	immobilisation	ST 24-96 h	EC/LOEC					EPA/600/4-90/027F	USA	
	Cerriodaphnia dubia	chronic	immobilisation/ reproduction	ST 7d	NOEC, IC_{25}					EPA/600/4-91/002 1002.0	USA	3 h -25 h
	Gammarus fasciatus	acute	immobilisation	ST 96 h	EC					EPA/OPPTS 850.1020	USA	
	Gammarus pseudolimnaeus	acute	immobilisation	ST 96 h	EC					EPA/OPPTS 850.1020	USA	
	Gammarus lacustris	acute	immobilisation	ST 96 h	EC					EPA/OPPTS 850.1020	USA	

Algae	*Scenedesmus subspicatus*	chronic	inhib. growth	ST 72 h	EC/LID	8692	28692	C.2	201	DIN 38412 T33 (wastewater LID)	Germany	4 h - 30
	Scenedesmus capricornutum	chronic	inhib. growth	ST 72 h	EC				201			
	Selenastrum capricornutum	chronic	inhib. growth	ST 72-96 h	EC	8692	28692	C.2		EPA /OPPTS 850.5400	USA	
	Skeletonema costatum	chronic	inhib. growth	ST 96 h	EC					EPA /OPPTS 850.5401 (draft)	USA	7 h - 37 h
	Chlorella vulgaris	chronic	inhib. growth	ST 72 h	EC				201			
	Raphidocelis subcapitata	chronic	inhib. growth	ST 72 h	EC	8692	28692			AFNOR	France, Portugal, Norway, Finland	
Aquatic plants	*Lemna gibba*	acute	inhib. growth	ST 7d	EC, NOEC					ASTM E 141591	USA	
	Lemna minor	acute	inhib. growth	ST 7d	EC, NOEC					EPA OPPTS 850.4400	USA	
Bacteria	activated sludge	acute	inhib. respiration	ST 0,5 h	EC	8192	8192		209	DIN		
	activated sludge	acute	inhib. nitrification	ST 4 h	EC	9509	9509			DIN	Germany	
	Activated sludge (supernatant)	acute	inhib. growth	ST 6 h	EC	15522					Finland	10 - 30 h
	Pseudomonas putida	chronic	inhib. growth	ST 16 h	EC	10712	10712			DIN	France, Germany, Finland	7 h - 25 h
	Pseudomonas putida	acute	inhib. respiration	ST 0,5 h	EC					DIN 38412 T27		
	Vibrio fischeri (Photobacterium phosphoreum)	acute	inhib. light emission	ST 0,5 h	EC/LID	11348-1 11348-2 11348-3	11348-1 11348-2 11348-3			DIN 38412 T34/T341; AFNOR T90.320	Germany, Finland, France, Norway, Portugal, Sweden, USA	3 - 6 h
	Vibrio fischeri (Photobacterium phosphoreum)	chronic	inhib. growth		EC					DIN 38412 T37		
	Anaerobic digester sludge	chronic	inhib. gas production	ST 3-7 d	EC/LID	ISO/CD 13641-1 ISO/CD 13641-2						8 - 15 h
Rotifers	Brachionus calyciflorus	acute		ST 24	EC					ASTM E1440-91	USA	(ca.3 - 5 h)

ST: short term <= 7 d
EC: Effect Concentration
LID: Lowest Ineffective Dilution

Annex I-2: Short term ecotoxicity testing methods in brackish and saltwater environment

Taxonomic Group	Species	Method	Endpoint	Test duration	Data	Reference of methods				National standards	Application	Time effort
						ISO	EN	92/69/EWG	OECD			
Fish	*Scophthalmus maximus*	acute	survival	ST 72 h	LC/LOEC	ISO/AWI 15990				EPA OPPTS 850.1075		
	Cyprinodon variegatus	acute	survival	ST 96 h	LC/LOEC					EPA/600/4-90/027 F		
	Cyprinodon variegatus	chronic	larval growth/survival	ST 7 d	LC/LOEC					EPA/600/4-91/003 1004.0		
	Menidia beryllina	acute	survival	ST 96 h	LC/LOEC					EPA/600/4-90/02/027F		
	Menidia menidia	acute	survival	ST 96 h	LC/LOEC					EPA/600/4-90/02/027F		
	Menidia peninsulae	acute	survival	ST 96 h	LC/LOEC					EPA/600/4-90/02/027F		
	Menidia peninsulae	chronic	larval growth/ survival	ST 7 d	LC/LOEC					EPA/600/4-91/003 1006.0		
Crustaceans	*Acartia tonsa*	acute	death	ST 48 h	LC	14669						
	Tisbe batagliai	acute	death	ST 48 h	LC	14669						
	Nitocra spinipes	acute	death	ST 48 h	LC	14669						
	Mysidopsis bahia	acute	death	48-96 h	LC					EPA/600/4-90/027F EPA/OPPTS 850.1035		
	Mysidopsis bahia	chronic	growth/survival/ reproduction	ST 7-(28) d	NOEC					EPA/600/4-87/028 EPA/600/4-91/003 1007.0 EPA/OPPTS 850.1350		
	Artemia salina	acute	death		LC					EPA/600/4-90/027 F (method not specified)		
Algae	*Skeletonema costatum*	chronic	inhib. growth	ST 72 h	EC	10253						
	Phaeodactylum tricornutum	chronic	inhib. growth	ST 72 h	EC	10253						
	Champia parvula	chronic	inhib. growth		EC					EPA/600/4-91/003 1009.0		
Bivalves (Embryo-Larval)	*Crassostrea virginica* *Crassostrea gigas* *Mercenaria mercenaria*	acute	survival and abnormal development	ST 48 h	EC/NOEC					EPA/OPPTS 850.1055 draft ASTM E 724-98		
	Crassostrea virginica	acute	shell growth	ST 72 h	EC/NOEC					EPA/OPPTS 850.1025 draft		

Rotifers	*Brachionus plicatilis*	acute		ST 24	EC					ASTM E1440-91	USA	
Bacteria	*Vibrio fischeri (Photobacterium phosphoreum)*	acute	inhib. light emission	ST 0,5 h	EC/LID	11348-1 11348-2 11348-3	11348-1 11348-2 11348-3			DIN 38412 T34/T341; AFNOR T90.320	Germany, Finland, France, Norway, Portugal, Sweden, USA	

ST: short term <= 7 d
EC: Effect Concentration
LID: Lowest Ineffective Dilution

Annex I-3: Long term ecotoxicity testing methods in freshwater environment

Taxonomic Group	Species	Method	Endpoint	Test duration	Data	Reference of methods					Application	time effort
						ISO	EN	92/69/EWG	OECD	National Standards		
Fish	*Brachydanio rerio*	prolonged toxicity	survival	LT 14 d	NOEC				204			
	Cyprinus carpio	prolonged toxicity	survival	LT 14 d	NOEC				204			
	Lepomis macrochirus	prolonged toxicity	survival	LT 14 d	NOEC				204			
	Oncorhynchus mykiss	prolonged toxicity	growth rate	LT 14 d	NOEC	ISO 10229			204			
	Oryzias latipes	prolonged toxicity	survival	LT 14 d	NOEC				204			
	Poecilia reticulta	prolonged toxicity	survival	LT 14 d	NOEC				204			
	Pimephales promelas	prolonged toxicity	survival	LT 14 d	NOEC				204			
	diverse species	early-life stage toxicity	survival	LT 32-60d	NOEC/LOEC				210	ASTM1241-98		
Crustaceans	*Daphnia magna*	sublethal (life cycle)	immobilisaton/ reproduction	LT 21 d	EC/NOEC/ LOEC	draft ISO/ DIS 10706			211	EPAQ/OPPTS 850.1300		ca. 100 h
	Daphnia pulex	sublethal	immobilisaton/ reproduction	LT 21 d	EC/NOEC/ LOEC					EPAQ/OPPTS 850.1300		
	Ceriodaphnia dubia	life cycle	immobilisaton/ reproduction	LT	EC/NOEC/ LOEC					ASTM E1295-89		
Aquatic plants	diverse species *(*f. e. *Lemna gibba, Lemna minor)*		inhib. growth inhib. growth	14 -28 d	EC, NOEC EC, NOEC					OPPTS 850-4450 ASTM E 1415-91		

LT: long term >7 d
EC: Effect Concentration
LOEC: Lowest Observed Effect Concentration
NOEC: No Effect Concentration

Annex I-4: Genotoxicity and mutagenicity test methods used in whole effluent assessment

	Species	Strain cell line	Endpoint	Test duration	Reference of methods			National standards	Application	Time effort
					ISO	92/69/EWG	OECD			
Bacterial test systems										
Ames-test	*Salmonella thyphimurium*	TA 98 TA 100	frameshift mutations point mutations	48-72 h 48-72 h	SC5/WG9	B.14 B.14	471 471	DIN 38415-4	Germany	7-15 h
umuC-test	*Salmonella thyphimurium*	TA 1535 (genetically modified)	SOS induction	48-51 h	SC5/WG9			DIN 38415-3	Germany	1-4 h
SOS-Chromotest	*Escherichia coli K11*	*PQ37*	SOS induction	2 h					Germany	1-3 h
Mutatox™	*Vibrio fisheri*	dark variant M169	return of luminescence	24 h						1-3 h
Eukaryotic test systems										
V79-test	Syrian hamster fibroblast	V79	Chromosome aberration	ca. 24 h		B.10	473		Germany	25-50 h

Annex I-5: Biodegradation and elimination tests

Method	Concentration Test item	Inoculum	Endpoint	Test duration	Reference of methods				National standards	Application	Time effort
					ISO	EN	92/69/EWG	OECD			
Aerobic ready biodegradability											
DOC die away test	10-40 mg/l DOC	<=30 mg/l SS	DOC	28 d	7827	7827	C.4-A	301 A	EPA/OPPTS 835.3110	WW	15 - 25 h
CO_2 evolution test (modified Sturm test)	10-20 mg/l DOC	<=30 mg/l SS	CO_2	28 d	9439	29439	C.4-C	301 B	EPA/OPPTS 835.3110		20 - 30 h
CO_2-headspace test	2-40 mg/l DOC	4 mg/l SS	CO_2 (DOC)	28 d	14593				EPA/OPPTS 835.3120		10 - 20 h
MITI(I) test	100 mg/l substance	<=30 mg/l SS	O_2 (DOC)	28 d			C.4-F	301 C	EPA/OPPTS 835.3110		10 - 20 h
Closed bottle test	2-10 mg/l substance	<=5 ml effluent/l	O_2	32 d	10707	10707	C.4-E	301 D	EPA/OPPTS 835.3110		10 - 20 h
Biochemical Oxygen Demand (BOD_n)			O_2	5 - 28 d		1899-1 1899-2	C.5			WW	1 - 10 h
Two-phase closed bottle test (BODIS-test)	100 mg/l COD	30 mg/l SS	O_2	28 d	10708						10 - 20
Modified OECD screening test	10-40 mg/l DOC	0,5 ml effluent/l	DOC	28 d			C.4-B	301 E	EPA/OPPTS 835.3110		15 -25 h
Manometric respirometer test	100 mg/l substance	<=30 mg/l SS	O_2	28 d	9408	29408	C.4-D	301 F	EPA/OPPTS 835.3110	WW	10 - 20 h
Aerobic inherent biodegradability											
Modified SCAS test	20 mg/l substance	1 - 4 g/l SS	DOC	12 - 26 weeks	9887	9887		302 A	DIN, EPA/OPPTS 835.3210		
Modified Zahn-Wellens-test	50-400 mg/l DOC	200-1000 mg/l SS	DOC or COD	ca. 3 - 28 d	9888	29888		302 B	DIN, EPA/OPPTS 835.3200	WW	5 - 25 h
Modified MITI (II) test	30 mg/l substance	100 mg/l SS	O_2 (and direct analysis)	28 d				302 C			10 - 20 h
Aerobic simulation tests											
Sewage treatment simulation test (Coupled Units Test)	10-50 mg/l DOC + synthetic sewage	2500-3000 mg/l SS	DOC (and direct analysis)	ca. 21 d (6 h retention time)	11733	11733		303 A	DIN		80 - 120 h
Continuous activated sludge test with biological removal	10 mg/l substance + synthetic sewage	3000 mg/l SS	DOC (and direct analysis)	ca. 21 d (6 h retention time)	ISO/NP 16821				DIN 38412-26	WW	

Anaerobic biodegradability											
Anaerobic biodegradability of organic compounds in digestion sludge	20-100 mg/l TOC	10 Vol.% digestion sludge	biogas production	ca. 60 d	11734	11734			similar procedures with other dilution media are EPA/OPPTS 835.3400 and ASTM D5210-92	(WW)	10 - 25 h

ww wastewater

Annex IV: Scientific and common names of organisms

Taxonomic Group	Species	Common name	
Fish	*Brachydanio rerio*	Zebra-fish	freshwater
	Cyprinus carpio	Common carp	freshwater
	Dicenthrarcus palrax		freshwater
	Gasterosteus aculeatus	Threespine stickelback	freshwater
	Lepomis macrochirus	Bluegill sunfish	freshwater
	Leuciscus idus	Ide	freshwater
	Oncorhynchus mykiss	Rainbow trout	freshwater
	Oryzias latipes	Ricefish	freshwater
	Poecilia reticulta	Guppy	freshwater
	Pimephales promelas	Fathead Minnow	freshwater
	Salvelinus fontinalis	Splake	freshwater
	Salmo trutta	Brown trout	freshwater
	Salmo salar	Salmon	freshwater
Crustaceans	*Daphnia magna*	Waterflea	freshwater
	Daphnia pulex	Waterflea	freshwater
	Ceriodaphnia dubia	Cerio	freshwater
	Gammarus fasciatus	Scud	freshwater
	Gammarus pseudolimnaeus		freshwater
	Gammarus lacustris		freshwater
Algae	*Scenedesmus subspicatus*		freshwater
	Scenedesmus capricornutum	Green microalgae	freshwater
	Selenastrum capricornutum		freshwater
	Skeletonema costatum		freshwater
	Chlorella vulgaris		freshwater
	Raphidocelis subcapitata		freshwater
Aquatic plants	*Lemna gibba*	Inflated duckweed or windbags	freshwater
	Lemna minor	Common duckweed	freshwater
Bacteria	activated sludge		freshwater
	activated sludge		freshwater
	activated sludge (supernatant)		freshwater
	Pseudomonas putida		freshwater
	Pseudomonas putida		freshwater
	Vibrio fischeri (Photobacterium phosphoreum)	*Photobacterium*	salt and brackish water
	Vibrio fischeri (Photobacterium phosphoreum)		salt and brackish water
	Anaerobic digester sludge		freshwater
Rotifers	*Brachionus calyciflorus*	Rotifer	freshwater
Fish	*Scophthalmus maximus*	Turbot	salt and brackish water
	Cyprinodon variegatus	Sheepshead minnow	salt and brackish water
	Menidia beryllina	Inland silverside	salt and brackish water
	Menidia menidia	Atlantic silverside	salt and brackish water
	Menidia peninsulae	Tidewater silverside	salt and brackish water

Crustaceans	*Acartia tonsa*	Copepod	salt and brackish water
	Tisbe batagliai		salt and brackish water
	Nitocra spinipes		salt and brackish water
	Mysidopsis bahia	Mysid	salt and brackish water
	Artemia salina	Brine shrimp	salt and brackish water
Algae	*Skeletonema costatum*		salt and brackish water
	Phaeodactylum tricornutum		salt and brackish water
	Champia parvula		salt and brackish water
Bivalves (Embryo-Larval)	*Crassostrea virginica*	American oyster	salt and brackish water
	Crassostrea gigas	Pacific oyster	salt and brackish water
	Mercenaria mercenaria	Hardshell clam	salt and brackish water
Rotifers	*Brachionus plicatilis*	Rotifer	salt and brackish water
Bacteria	*Vibrio fischeri (Photobacterium phosphoreum)*	Photobacterium	salt and brackish water

Contents Annex V:

SOURCE	Danish environmental protection agency: Guideline for hazard assessment of industrial effluents. 1993			
APPLICATION	Procedure for investigation and assessment of hazard/risk to marine and freshwater environments of industrial effluents			
STATUS	Technical guidance document			
METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
1. Acute toxicity to fish	Freshwater: Brachydanio rerio (zebra fish), O. Mykiss (rainbow trout) Brackish water: Platichthys flesus (flounder), Clupea harengus (herring) Marine water: P. flesus, C. harengus, Scophthalmus maximus (turbot)	Tier 1: Testing of one fish, one crustacean and one alga is requested if an unacceptable risk to the environment is indicated or insufficient data or experience is provided for the assessment. Species of relevance to the receiving water should be applied. $PNEC_{acute}$ and $PNEC_{chronic}$ are estimated applying assessment factors.	96 hrs LC50	OECD TG 203
2. Acute toxicity to crustaceans	Freshwater: Daphnia magna, Gammarus pulex Brackish water: Nitocra spinipes Marine water: Acartia tonsa		48 hrs EC50 (Daphnia, Acartia) 96 hrs EC50 (other)	Daphnids: OECD TG 202, part 1 Nitocra: DS 2209 Acartia: ISO draft /5/ Gammarus: /1/
3. Toxicity to algae	Freshwater: Nitzschia palea, Selenastrum capricornutum Brackish and marine water: Skeletonema costatum, Phaeodactylum tricornutum		n.d. (72 hrs EC50)	OECD TG 201
4. Acute toxicity to microorganisms	Freshwater: Pseudomonas putida Brack. and marine w.: Photobacterium phosphoreum (Microtox)	Tier 2a: $PNEC_{acute}/PEC_{max} < 1$: Additional acute toxicity tests may be performed for refinement of $PNEC_{acute}$ (more acute toxicity data lead to a reduced assessment factor)	72 hrs EC50, growth inhibition (Pseudomonas), 0.5 hrs EC50, inhibition of luminiscens (Photobacterium)	Pseudomonas: ISO N111 (draft) Photobacterium: ISO N127 (draft)

Annex V-1 (continued) **A-2**

ENV/MC/CHEM(98)19/PART2

SOURCE	Danish environmental protection agency: Guideline for hazard assessment of industrial effluents. 1993
APPLICATION	Procedure for investigation and assessment of hazard/risk to marine and freshwater environments of industrial effluents
STATUS	Technical guidance document

METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
5. Acute toxicity to protozoans	Freshwater: Tetrahymena sp. Brack. and marine w.: Uronema marinum		Uronema: 24 hrs EC50, growth inhibition	Uronema: /11/
6. Acute toxicity to higher plant	Freshwater: Lemna minor (duckweed) Brack. and marine w.: Zostera marina		Lemna: 7 days EC50: growth inhibition and mortality Zostera: 28 days EC50: growth inhibition, photosynthesis	Lemna: /2/ Zostera: Non-published protocol
7. Acute toxicity to insects	Chironomus sp., Baetis rhodani, Cloëon bipunctata		96 hrs LC50	/3-4/
8. Acute toxicity to molluscs	Brack. and marine w.: larvae from blue mussel, oyster		96 hrs LC50	-
9. Acute toxicity to planaria	-		-	-
10. Chronic toxicity to fish (FELS/embryo-sacfry test)	as 1)	Tier 2b: $PNEC_{chronic}/PEC_{ave.} < 1$: One or more of the methods may be applied for refinement of $PNEC_{chronic}$	Embryo-sacfry test: 7-11 days EC50, NOEC, LOEC, survival, hatching, growth FELS: 28-60 days EC50, NOEC, LOEC	OECD TG 210 (FELS), OECD draft (embryo sacfry test)
11. Chronic toxicity to crustaceans	as 2)		Daphnids: 21 days EC50, NOEC, LOEC: survival, reproduction	Daphnids: OECD TG 202, part 2 Nitocra: DS 2209 Acartia: ISO draft
12. Chronic toxicity to algae	as 3)		72 hrs EC50, NOEC, LOEC: growth inhibition	OECD TG 201, ISO 8692

Annex V-1 (continued) **A-3**

ENV/MC/CHEM(98)19/PART2

SOURCE	Danish environmental protection agency: Guideline for hazard assessment of industrial effluents. 1993				
APPLICATION	Procedure for investigation and assessment of hazard/risk to marine and freshwater environments of industrial effluents				
STATUS	Technical guidance document				
METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
13. Toxicity to crustaceans	Freshwater: Gammarus pulex Brack. and marine water: Corophium volutator, C. insidiosum	Tier 2c: One or more of the methods may be applied for refining the PNEC for sediments	Corophium: 10 days LC50	Gammarus: /1/ Corophium: /6/
14. Toxicity to molluscs	Freshwater: Unio sp. Brack. and marine water: Abra alba, Macoma baltica		Abra: 5 days LC50	Abra alba: /7/
15. Toxicity to annelids	Freshwater: Tubifex tubifex Brack. and marine water: Arenicola marina, Nereis virens		Arenicola: 10 days LC50 Nereis: 10 days LC50	Arenicola: /8/ Nereis: /9/
16. Toxicity to insects	Chironomus sp.		n.d.	ASTM E 1383-90
17. Toxicity to echinoderms	Echinocardium cordatum		21 days LC50, LOLC	/10/

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Method for the assessment of acute toxicity of contaminated sediment using the borrow sea urchin Echinocardium cordatum. Ringtst protocol, Paris Commission.

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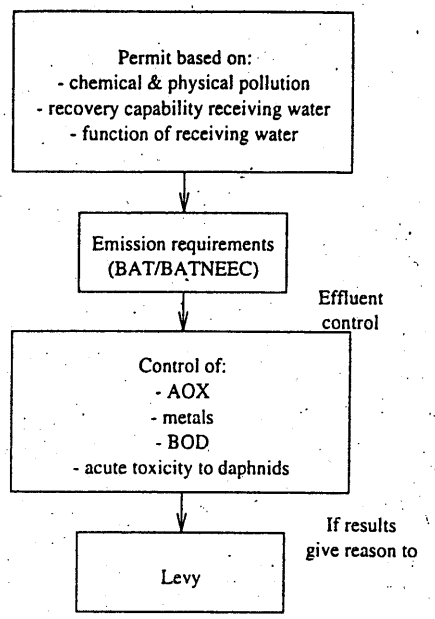
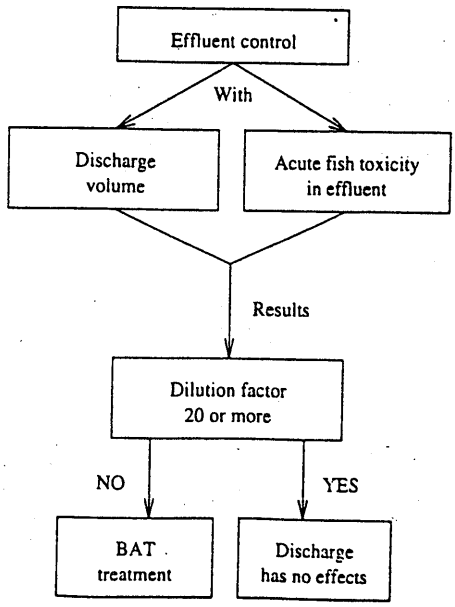
Toxic Effects of Heavy Metals upon Cultures of Uronema marinum (Ciliophora: Uronematidae). Marine Biology 54 (1979): 17-24.

Annex V-2 and V-3 **A-5**

2 **II-2: Evaluation of (complex) effluent discharges in France (in use) (Tonkes et al., 1995)**



3 **II-3: Evaluation of (complex) effluent discharges in Ireland (in use) (Tonkes et al., 1995)**



Annex V-4 **A-6**

SOURCE	Biological-chemical Characterisation of industrial waste water. Swedish Environmental Protection Agency 1990 (Naturvårdsverket)			
APPLICATION	Environmental hazard and risk assessment of industrial effluents			
STATUS	Guideline applied for the hazard identification of emissions to the aquatic environment			
METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
1. Acute toxicity to fish species	Brachydanio rerio (zebra fish), O. mykiss (rainbow trout), Salmo salar, Salmo trutta (brown trout), Alburnus alburnus (bleak), Perca fluviatilis (perch), Pimephales promelas (fat-head minnow), Gasterosteus aculeatus (stickleback), Platichthys flesus (dab), Gadus morhua (cod).	Stage 1: Requested if available data or experience is insufficient for assessing the potential hazard/risk to the environment	24-96 hrs LC50	Freshwater sp.: SS 028162 Marine sp.: SS 028189 Bleak: /1/
2. Acute toxicity to crustacean species	Daphnia magna. Ceriodaphnia dubia, Nitocra spinipes, Crangon crangon, Acartia tonsa		24-48 hrs LC50	Daphnia: SS 028180 Ceriodaphnia: /2/ Nitocra: /3/ Brown shrimp: /4/ Acartia: /5/
3. Algae growth inhibition test	Selenastrum capricornutum, Monoraphidium griphitti, Chlorella vulgaris, Scenedesmus subspicatus, Skeletonema costatum		5 days EC50	OECD TG 201
4. Vascular plant growth inhibition test	Lemna minor (duckweed), Allium cepa (onion), Lens culinaris (lentil)		5 days EC50	Lemna: /6/ Onion: /7/ Lentil: /8/
5. Activated sludge, respiration and nitrification inhibition. Microtox	Activated sludge Microtox: Photobacterium sp.		3 hrs EC50	ISO 8192 (draft), ISO/DIS 9509 (draft)

Annex 5-4 (continued) **A-7**

ENV/MC/CHEM(98)19/PART2

SOURCE	Biological-chemical Characterisation of industrial waste water. Swedish Environmental Protection Agency 1990 (Naturvårdsverket)				
APPLICATION	Environmental hazard and risk assessment of industrial effluents				
STATUS	Guideline applied for the hazard identification of emissions to the aquatic environment				
	METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
6.	Fish egg and sacfry test	Zebra fish	Stage 2: Requested on a case-by-case basis, especially. when LC50/PEC < 1. Most sensitive species/taxa identified in stage 1 is given highest priority for further testing	11 days EC50	SS 028193 (equivalent to OECD TG draft)
7.	Fish, Prolonged toxicity study	Rainbow trout		14 days LC50	OECD TG 204
8.	Fish subchronic toxicity test	Fathead minnow		7 days EC50	/9/
9.	Fish physiological effects	Salmon, rainbow trout, brown trout, perch		Physiological and biochemical	/10/
10.	Chronic toxicity to crustaceans	Daphnia magna, Ceriodaphnia dubia, Nitocra spinipes		21 days EC50 (Daphnia), 7 days EC50 (Ceriodaphnia), 14 days EC50 (Nitocra)	Daphnia: OECD TG 202 Ceriodaphnia: /11/ Nitocra: /12-13/
11.	Mussel larvae survival test	Mytilus edulis		n.d.	/14-15/
12.	Microtest for algae inhibition	algae test battery		n.d.	/16/
13.	Ames test	Salmonella sp.		revertants	/17/
14.	Extended Chronic toxicity studies with fish	Zebra fish, Clupea harengus (baltic herring)	Stage 3: Confirmatory tests. To be selected and designed depending on the specific environment.	impairment of reproduction of zebra fish, herring embryo-larvae toxicity	/18-19/
15.	Sediment toxicity tests	Daphnia magna, Tubifex tubifex		n.d.	/20/

SOURCE		Biological-chemical Characterisation of industrial waste water. Swedish Environmental Protection Agency 1990 (Naturvårdsverket)			
APPLICATION		Environmental hazard and risk assessment of industrial effluents			
STATUS		Guideline applied for the hazard identification of emissions to the aquatic environment			
	METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
16.	In-situ testing with fish	Salmon, rainbow trout, brown trout, perch		physiological effects, tainting	/21-23/
17.	Effect monitoring in-situ	Perch, dab, salmon, rainbow trout, brown trout, fourhorn sculpin		physiological and skeletal changes	/22-24/
18.	Inhibition of peri-phyton communities	n.d.		n.d.	n.d.
19.	Model ecosystem studies	n.d.		n.d.	n.d.

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Determination of the acute toxicity (LC-50) of chemical products and waste water to Ceriodaphnia dubia. SNV. Pux Report (under preparation), M. Unger. (In Swedish).

/3/ Acute toxicity (LC50) - Nitocra
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/4/ Acute toxicity (LC50) - brown shrimp

Annex V-4 (continued)	A-9

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The Allium test as a standard in environmental monitoring. Hereditas 1986, 102, pp. 99-112. G. Fiskesjö.

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A new fathead minnow (Pimephales promelas) subchronic toxicity test. Environmental Toxicology and Chemistry 1985, 4, pp. 711-718. T.J. Norberg and D.I. Mount.

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A seven day life-cycle cladoceran toxicity test. Environmental Toxicology and Chemistry 1984, 3. pp. 425-434. D.I. Mount and T.J. Norberg.

/12/ Reproduction: Nitocra
A flow through fecundity test with Nitocra spinipes (Harpactecoida, Crustacea) for aquatic toxicity. Ecotoxicology and Environmental Safety 1987, 14, pp. 260-268. B.E. Bengtsson and B. Bergström.

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/14/ Survival - bivalves: common sea mussel larvae

Annex V-4 (continued) **A-10**

Determination of the effect of industrial waste water on the early development of the common sea mussel, SNV 1983. PM 1733, Appendix 3. Åke Granmo et al. (In Swedish).

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Determination of the effects of industrial waste water on embryo and larvae development in herring and Baltic herring (Clupea harengus). SNV 1983. PM 1733, Appendix 8. Å. Granmo et al. (In Swedish).

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Toxicity of metal polluted sediments to Daphnia magna and Tubifex. Hydrobiologia 1989 (in press). T. Wiederholm and G. Dave.

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Recipient control water. SNV 1986, Report 3109, BIN NR 26, blood picture in fish. Å. Larsson. (In Swedish).

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Recipient control water, basis for methodology. SNV 1984, Report 3075. Method basis 8, taste and smell. I. Bjørklund. (In Swedish).

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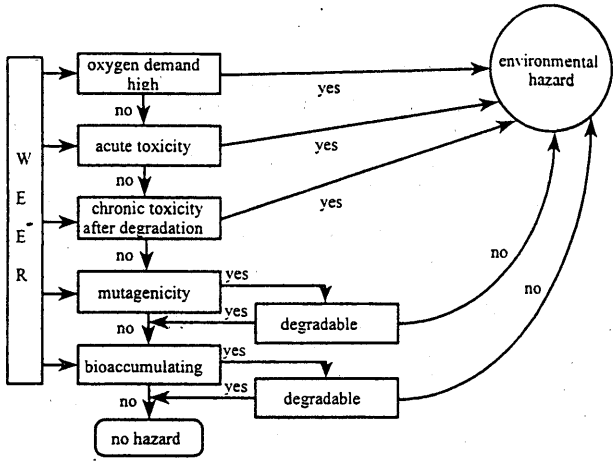
b) Recipient control water. SNV 1986, Report 3109, BIN NR27, Visible external symptoms of disease in fish. E. Neuman. (In Swedish).
c) Recipient control water. SNV 1986, Report 3109, BIN NR24, Spinal damage in fish. B.E. Bengtsson. (In Swedish).

AnnexV-5	A-11

The Whole Effluent Environmental Risk (WEER) methodology in the Netherlands (Tonkes, 1997)

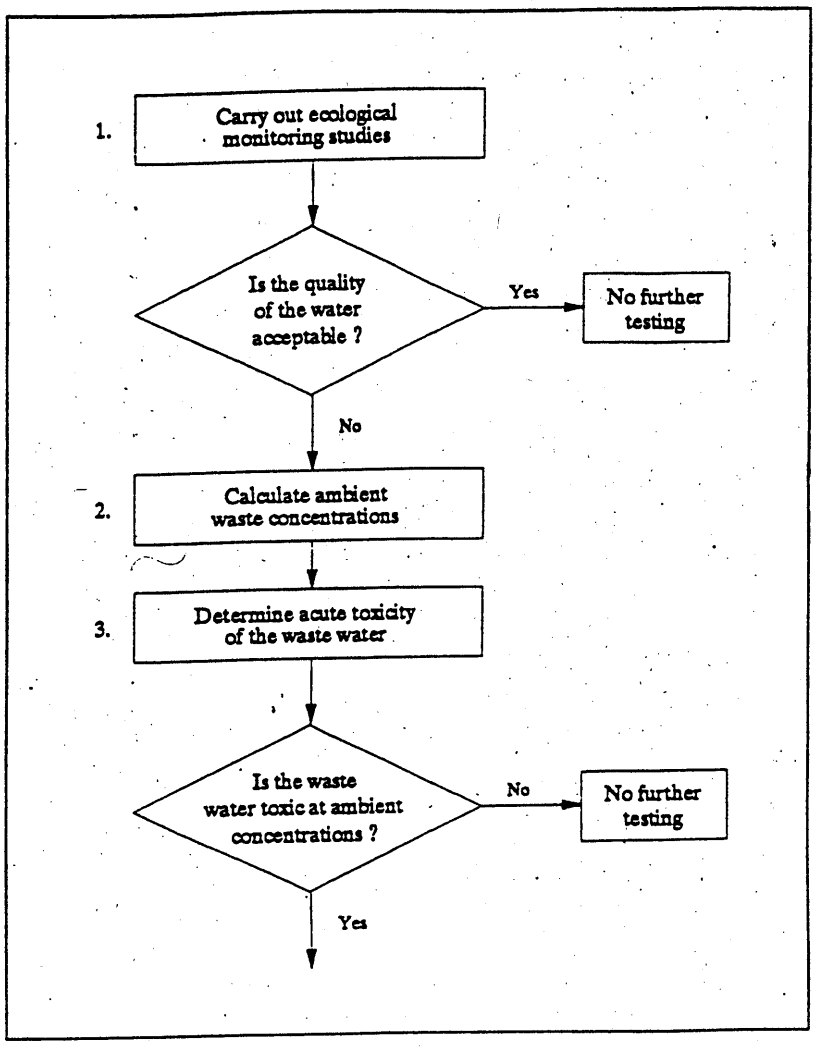
The WEER methodology is shown in the following scheme.



The Whole Effluent Environmental Risk (WEER) methodology

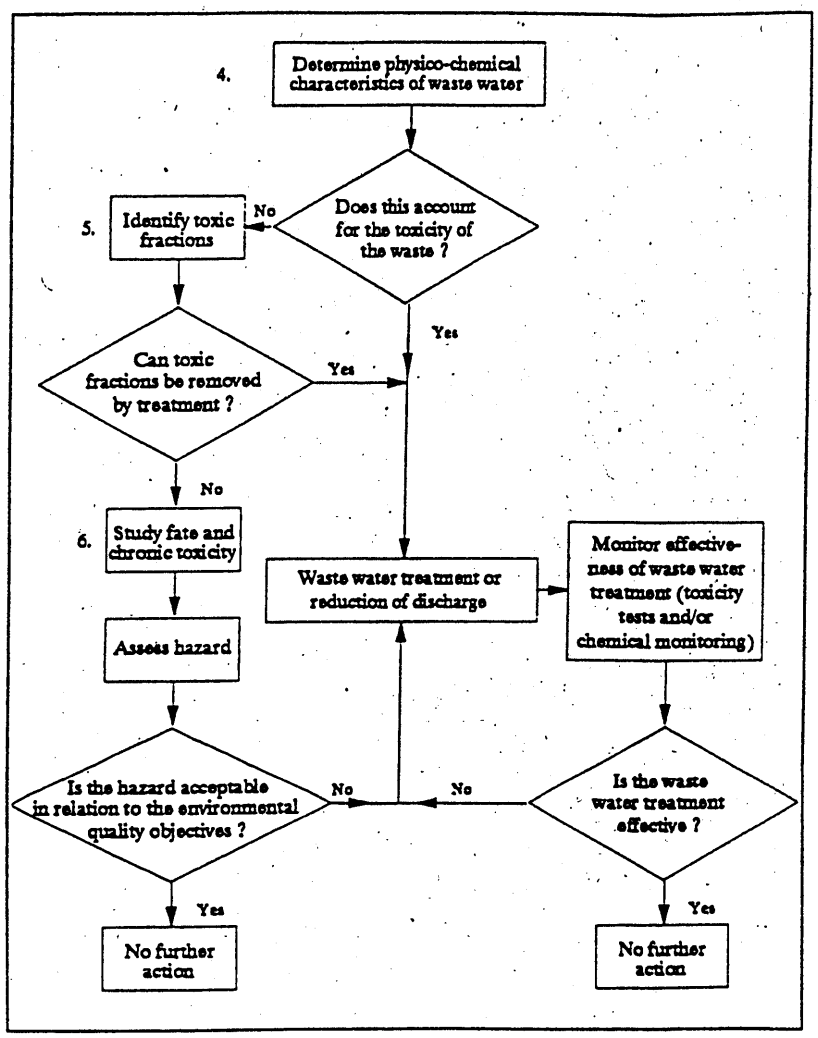
Annex V-6	**A-12**

The UK proposal for monitoring and control of discharges from point sources (Pedersen et. al., 1994)



Annex V-6 (continued) **A-13**

The UK proposal for monitoring and control of discharges from point sources (Pedersen et. al., 1994) (continued)



ENV/MC/CHEM(98)19/PART2

Annex V-7 **A-14**

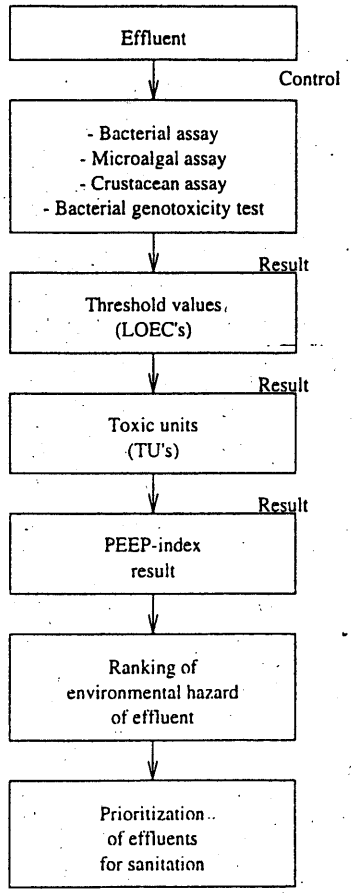
SOURCE	United States Environmental Protection Agency (US-EPA): Technical support document for water quality-based toxic control (EPA/505/2-90-001) March 1991.			
APPLICATION	Technical guidance for assessing and regulating the discharge of toxic substances to the aquatic environment (wastewater permits).			
STATUS	US-EPA guidance document to the national Clean Water Act.			
METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
1. Acute toxicity to fish	Freshwater species: Pimephales promelas (fathead minnow), Onchorhynchus mykiss (rainbow trout), Salvelinus fontinalis (brook trout) Marine species: Cyprinodon variegatus (sheepshead minnow), Menidia beryllina, M. medinia, M. peninsulae (silversides)	Whole effluent testing: Initial dilution > 1000:1: acute toxicity to 3 trophic levels (1-3) as a minimum. Chronic toxicity should be "Checked" Initial dilution > 100:1 and < 1000:1: acute or chronic toxicity to 3 trophic levels. Acute or chronic toxicity levels are to be estimated from acute/chronic ratios.	Effluents: 96 hrs LC50, LOLC Receiving water: 96 hrs LOLC	US-EPA: Methods for measuring the acute toxicity to effluents and receiving waters to freshwater and marine organisms. Fourth ed., Sept. 1991. (EPA/600/4-90/027)
2. Acute toxicity to invertebrates	Freshwater species: Ceriodaphnia dubia, Daphnia magna, D. pulex. Marine species: Mysidopsis bahia	Initial dilution < 100:1: Chronic toxicity to 3 trophic levels as a minimum. The acute toxicity level may be estimated from A/C ratios. Freshwater organisms should be applied when the actual receiving	Effluents: 24, 48 or 96 hrs LC50, LOLC Receiving water: 24, 48 or 96 hrs LOLC	
3. Toxicity to plants	n.d.	water salinity is less than 1,000 mg/l and marine organisms when above 1,000 mg/l. .....contd.	n.d.	n.d.
4. Short-term chronic toxicity to freshwater crustaceans	Ceriodaphnia dubia	Sediment toxicity testing may be required in special cases.	7 d. NOEC: survival, reproduction	US-EPA: Short-term methods for estimating the chronic toxicity to effluents and receiving waters to freshwater organisms. Second ed. March 1989 (EPA 600/4-89/001)

Annex V-7 (continued) **A-15**

SOURCE	United States Environmental Protection Agency (US-EPA): Technical support document for water quality-based toxic control (EPA/505/2-90-001) March 1991.			
APPLICATION	Technical guidance for assessing and regulating the discharge of toxic substances to the aquatic environment (wastewater permits).			
STATUS	US-EPA guidance document to the national Clean Water Act.			
METHOD	RECOMMENDED SPECIES	WHEN TO TEST	ENDPOINTS	RECOMMENDED GUIDELINE
5. Short-term chronic toxicity to freshwater fish	Pimephales promelas (fathead minnow)		7 d. NOEC: larval growth, survival; 7-9 d. NOEC: embryo-larval survival, hatchability, abnormality	
6 Algae growth inhibition	Selenastrum capricornutum		96 hrs NOEC: growth rate inhibition	
7. Short-term chronic toxicity to marine crustaceans	Mysidopsis bahia (mysid)		7 d. NOEC: growth, survival, fecundity	US-EPA: Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and freshwater organisms. May 1988 (EPA/600/4-87/028)
8. Short-term chronic toxicity to marine fish	Cyprinodon variegatus (sheepshead minnow), Menidia beryllina (inland silverside)		7 d. NOEC: larval growth, survival. 7-9 d. NOEC: embryo-larval survival, hatchability, abnormality (C. variegatus)	
9. Macroalgae fertilization test	Champia parvula (red macroalgae)		7-9 d. NOEC: cystocarp production (fertilization)	
10. Sea urchin fertilization test	Arbacia punctulata		1.5 hrs NOEC: fertilization	
11. Kelp reproduction test	Laminaria saccharina (kelp)		24 hrs NOEC: inhibition of sporophyte development	US-EPA: Biomonitoring for control of toxicity in effluent discharges to the marine environment. Sept. 1989 (EPA/-625/8-89/015)

Annex V-8	**A-16**

Assessment strategy for effluents in Canada based on the PEEP-index (Canadian proposal, partly in use) (Tonkes et al., 1995)



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