Whole Effluent Assessment

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Whole Effluent Assessment

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SUMMARY

There is increasing recognition by regulators that there are limitations to the substance-specific approach for assessing and controlling the environmental fate and effects of effluents. Consequently, many regulators are seeking more holistic techniques such as whole effluent assessment (WEA) to supplement existing approaches. Even in countries where whole effluent toxicity (WET) is already assessed there is growing desire to address other issues including persistence and bioaccumulation of effluent components. It is inevitable that new WEA approaches will reveal different issues from those raised by existing substance controls. However, to ensure that these approaches are capable of indicating potential environmental effects, it is important that developing WEA protocols are scientifically robust, sustainable and, ultimately, fit for purpose. This report provides an overview of WEA approaches in terms of their applicability to existing regulation, the types of tests being considered and how WEA could be tailored to meet specific objectives. In addition, case studies are provided with recommendations made regarding both the applicability of a number of WEA approaches and when these should be considered and applied to improve the environmental hazard/risk assessments.

The information reviewed suggests that WEA approaches will increasingly be incorporated into effluent assessment and control schemes. In many of these schemes WEA approaches are seen as new (developing) tools for assessing effluent quality that should be applied in combination with (and not instead of) the substance-oriented approach. Within Europe, WEA-type schemes are generally seen as supporting the hazardous substance strategies of OSPAR and the Water Framework Directive (WFD). As with any initiative there are advantages and disadvantages of WEA approaches. One of the principal advantages of WEA approaches is that these can improve the information relating to environmental hazard of poorly characterised and complex effluents (i.e. those containing unknown mixtures of chemicals) and hence help their risk assessment. Disadvantages could potentially occur if the tests are inappropriate and/or incorrectly applied and interpreted, leading to demands for unjustified risk reduction measures.

The most widely applied WEA schemes assess toxicity to aquatic organisms. These have relevance for the protection of ecosystems although the relevance and interpretation of results ultimately depends on the tests used. For example, experience in the US (Diamond *et al*, 1999) reveals that acute toxicity observed in WET tests may be traced by impact assessment in the environment. However, more refined toxic endpoints (e.g. those used in chronic toxicity) are not so easily traced to the environment as these can be of limited significance compared to other stressors (effects of shipping, diffuse inputs, etc.). The ephemeral and often intermittent nature of the chronic toxicity for many effluents can make it extremely difficult to pinpoint simple solutions (Diamond *et al*, 1999; WERF, 2000). WEA can also be extended to the receiving environment to provide additional data to complement existing analytical and biological diversity studies and thereby improve the assessment of both sediment and water quality. In many

countries methods are being developed to assess persistence (P) and bioaccumulation (B) of effluent components. Such tests can potentially improve the risk assessment process for discharges but it is important that their limitations are recognised and put into context.

The results of this review indicate that there is considerable practical experience with WET (i.e. whole effluent toxicity) testing and many of the pitfalls and practical problems have been identified. There is reasonable confidence that an appropriate set of tests (at least for acute and, to some extent, chronic toxicity assessments) exist which have guidance on limit conditions for testing and interpreting results. However, this is not the case for methods for assessing the persistence and bioaccumulative characteristics of effluent components. These tests are in a much earlier stage of development and will require more practical experience, standardisation and verification in their application to effluents to demonstrate their usefulness and feasibility. To help facilitate this process an overview of P and B tests and their suitability for incorporation into effluent control schemes has been provided in this report.

The procedures used should ensure that the test results reflect the properties of the sample rather than circumstantial conditions or confounding factors. Thus when measuring toxicity, critical parameters that should be within restricted limits include pH, temperature, dissolved oxygen, hardness, salinity, suspended solids and colour. These parameters may require different limits for different organisms and practical experience suggests that certain substances are often the cause of the toxicity in a sample (e.g. ammonia is relatively toxic and a common component of many effluents). The presence of such substances may mask or interfere with other effects of importance.

One of the key factors that must be considered for any WEA test method is its relevance to the environment to be protected. In a move to increase the sensitivity of biological monitoring and toxicity assessments over and above that seen in traditional bioassays a range of immunological and biochemical tests have been developed. These approaches are referred to as biomarkers because they measure biochemical, cellular or molecular responses (but not adverse effects) induced by exposure to certain stressors. However, it is important to balance sensitivity with environmental relevance and to recognise that not all responses of biomarkers represent adverse effects. For example, it is not surprising that biomarkers are amongst the most sensitive of assays because induction of stress proteins and detoxification systems is the natural response for an organism subjected to a toxicant. However a number of other non-toxicant factors may influence these biomarker responses. If the biomarker is intended for use in WEA, it is important to be able to differentiate whether or not the response can be related to real toxicant environmental effects. For example, in effluent assessments the quality of the water may be impacted by a number of factors in addition to contaminants (e.g. hardness, ionic composition, salinity, pH). These may induce biomarker stress responses that are not contaminant related. Furthermore, there is no scientific basis to apply screening tests for endpoints that have no relevance to an ecosystem

functioning under real world conditions. For example, while several *in vitro* genotoxicity screening tests, originally developed for human health purposes, have been applied to effluents and environmental samples, these methods are not suitable for use in WEA. Although not yet sufficiently validated for use in WEA, there are several published *in vivo* methods in aquatic organisms that could be used to assess genotoxic hazard (especially for developmental or reproductive impacts) in a WEA context. Furthermore, there is a continued lack of understanding of the implications of naturally occurring endocrine materials in the wider environment. At this moment therefore it is believed to be inappropriate to recommend the widespread use of *in vivo* endocrine disruptor testing in a whole effluent assessment programme. However, there may be specific circumstances where such tests should be considered (e.g. production of known endocrine disrupting chemicals).

While the environmental relevance of fish toxicity tests is clear, there are concerns over the relative sensitivity of fish as well as the ethics of their use in WEA. A number of studies suggest (Walker *et al* 1991; Fentem and Balls, 1993; Weyers *et al,* 2000) that fish are rarely the most sensitive species in effluent assessments. However, experience from the USA indicates that fish are the more reliable test species. Dyer and Wang (2002) and related studies showed that fish and macroinvertebrates may exhibit different levels of discrimination with fish indicating change more often than macroinvertebrates. It may be that fish and invertebrates pick up on different types of stressor and that more than sensitivity should be considered. In certain circumstances (e.g. need to protect fish spawning grounds or as potential indicators of endocrine disruption) their use will currently be unavoidable. It is therefore unclear at the moment whether or not these should be incorporated into routine WEA programmes. It is important that the range of tests selected from the battery available gives maximum protection to wild fish populations while at the same time minimises the number of fish used in effluent testing.

Site-specific considerations make it impractical to recommend a single standardised WEA testing programme for all effluents. Ultimately these will be tailor-made and influenced by the objectives (e.g. is it for local compliance, environmental impact, tracing source or nature of toxicity components), the nature of the effluent(s) being assessed (i.e. is it a single discharge point or combined discharge from different processes, batch or continuous processes, etc) and finally, the local environmental situation (is the receiving water salt or freshwater, protected ecosystem, etc.). General aspects to be considered when developing a testing strategy are discussed in Chapter 5.

Biological and chemical monitoring is part of the WEA methodology and can be applied for two different purposes. One is to monitor the receiving water to assess whether or not reduction measures have been successful. The second use is in monitoring the receiving water during in the development phase of WEA tests and programmes in order to assess whether the results of such tests are capable of predicting environmental impact. Again the relevance of the tests can be affected by many factors and there is no 'one size fits all' approach for monitoring. Nevertheless

there are good examples of a tailored approach yielding good data on discharge impacts. The UK DTA programme on the river Tees used acute toxicity in the receiving water to identify zones of impact from discharges. The oil industry in the North Sea has also utilised similar approaches to assess the very localised impact of produced water discharges.

Since a large number of WEA methods are not fully understood in terms of factors which influence their variability and reliability, the TF recommends that these need to be developed by applying them in practice. In this respect, fact-finding projects carried out jointly by industry and authorities to identify specific areas for improvement appear to produce more meaningful results and are preferable to the introduction of a strict legislative or penalty-based system. Experience with many forms of hazard and risk assessment has shown that ultimately a flexible stepwise approach is advisable when new procedures and methods are being validated.

1. INTRODUCTION

Traditionally most effluent discharges in Europe have been assessed and regulated on the basis of physical and chemical properties. These typically include parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids, pH, and concentrations of specific hazardous substances. This 'analytical approach' provides a sound basis for controlling effluents containing relatively few contaminants which themselves have well-defined ecotoxicological properties. This method has been used successfully in many countries to reduce inputs of hazardous substances and has led to significant improvements to the ecological quality of many rivers and coastal regions. This approach is regarded as generally well understood, albeit with some limitations and confounding factors, by both the authorities and industry. However, difficulties can occur if this analytical approach is used to assess the environmental significance of complex, less well-characterised and variable effluents.

For these effluents, ecotoxicity measurement may provide an additional and more holistic means of assessing their potential impact on the aquatic environment. In fact ecotoxicity assessments are already used in effluent control schemes in several countries and will play an increasing part in the regulation of effluent discharges in the EU. Power and Boumfrey (2004) have noted that there appears to be a logical progression when looking at historical trends in the use of effluent bioassays in various international jurisdictions. Most countries start with chemical hazard-based systems to which they add effluent bioassays (first lethal, then sublethal measures) and then use receiving environment evaluations to predict or measure impacts. Occasionally additional endpoints are included in assessments, such as persistence, bioaccumulation or sometimes more specific toxicity endpoints like genotoxicity or endocrine effects.

The terminology used for ecotoxicity assessments varies in applications between countries. The terms WEA and WET are used, sometimes interchangeably. In this document the term WET is taken to mean Whole Effluent Toxicity utilising solely acute and/or chronic toxicity measurements whereas WEA is taken to mean Whole Effluent Assessment utilising the broader approach of toxicity along with some or all of the additional parameters, endocrine disruption, persistence, genotoxicity and the potential to bioaccumulate. The term validation in this report does not relate to the suitability of a given test for WEA rather it refers to the ability of the test to realistically predict environmental effects.

A number of countries have started to approach this issue in national regulation. In particular the US, Germany and Sweden have been using WET or WEA for some years in some sectors of industry or in the regulation of municipal wastewater treatment plant discharges. The WEA tool is also considered in legislation such as the EU Water Framework Directive (WFD) (EC, 2000) and developments within OSPAR to control discharges of hazardous substances. There is no EU-wide legislation on effluent testing which recommends or obliges the use of certain species or

tests, although some European national authorities do request specific toxicity tests based on, for example, experience, costs, receiving water, sensitivity and organism availability. As a consequence, accepted standardised tests (international, European or national) are generally used to allow comparison of results.

The developments in recent years have been mainly focussed on the practical applicability and limitations of the tests themselves. Unfortunately there has been only limited progress in the evaluation of the predictive capacity of tests in terms of identifying real environmental impact. Information on this issue is mainly available from US experience and from investigations on discharges from the off-shore oil industry (Burton *et al,* 2000; Dyer *et al*, 1998; Dyer and Wang, 2002; Environment Canada, 1999).

WEA brings greatest value when focussed on unknown mixtures of substances. However the development of the tool in practice is not straightforward. WEA is difficult in that it is not possible to control all variables except one and then to draw firm conclusions. The experimental conditions simply cannot be controlled and are sometimes very difficult to measure. Therefore WEA can only be developed by applying it to real world conditions and carefully evaluating the outcomes. Implementing WEA into legislation at a premature stage would increase the risk that schemes are adopted with tests that do not contribute to real environmental improvement. To support the scientific development of WEA it was considered useful to evaluate and summarise the practical experience with WEA within industry and from programmes where industry has been involved. An ECETOC Task Force was therefore commissioned with the following terms of reference:

- Summarise practical experience with WEA, including acute toxicity, chronic toxicity, bioaccumulation and persistence by the regulatory community and industry worldwide;
- evaluate the usefulness of these approaches in terms of:
  - practicality and suitability of tests for effluents;
- ability of the tests to determine realistic potential effects and to define useful endpoints;
  - speed and cost effectiveness;
- describe the role of WEA in the context of water quality management and propose a rapid, cost-effective and scientifically sound strategy for such assessments.

Although there is an increasing interest in studying human health effects of effluents this was considered to be a separate area of expertise and outside the remits of this report. A brief history and background of WEA is followed by a short overview of available tests that have been applied to WEA. The practical advantages and disadvantages of these tests are discussed, as is their ability to yield meaningful results when applied to actual effluents (Chapter 2). Chapter 3 addresses the use of monitoring data to assess the impact of effluents, including the use of biomarkers. Where possible, emphasis has been placed on the relation between measured impact

and the predictive capacity of the tests. Finally, a decision scheme for WEA testing is presented, supported by practical examples (Chapter 4) taken from experience within industry and from joint activities with authorities. The strengths and weaknesses of the decision scheme are highlighted and guidance and recommendations given (Chapter 5).

1.1 Single substance approach

Both chemical analysis and ecotoxicity assessment of effluents have their own merits and disadvantages. For example, chemical analytical methods have potential advantages in that they allow total pollutant loads of specific substances to be determined. Consequently, the importance of the chemical approach will remain owing to the need to demonstrate the presence or absence and/or reduction of priority (hazardous) substances under the EU WFD. Chemical specific approaches can also be used in water basin catchment management systems to ensure that environmental quality standards for specific substances are not exceeded.

If the ecotoxicological properties and environmental fate of a single substance are understood, it is possible to carry out a risk assessment and demonstrate risk reduction. This approach is consistent with the principles defined by the European Commission for the risk assessment of chemicals as described in the TGD (EC, 1996, 2003). In the risk assessment approach the assessment of the environmental risks of a chemical consists of three steps:

1. Hazard identification: an indication of the adverse effect that a substance has an inherent capacity to cause;
2. exposure assessment: an estimation of the concentration to which environmental compartments (i.e. aquatic, terrestrial, atmosphere) are, or may be, exposed. This is expressed as the predicted environmental concentration (PEC). This entails the determination of the sources, emission routes and degradation pathways of the substance;
3. risk characterisation: the estimation of the incidence and severity of the effects likely to occur in an environmental compartment due to actual or predicted exposure to a substance (this may include risk estimation, i.e. the quantification of that likelihood).

The approach is designed to assume a cautious reasonable worst-case estimate of exposure where measured data are lacking although it recognises that there is scope for improving risk assessments by refining the exposure scenarios. For example, models can be improved by using additional measurements to feed back into model calculations. For the environmental effects assessment a predicted no effect concentration (PNEC), using the acute or chronic toxicity data and an assessment factor, should be calculated for species representative for the environmental compartment under investigation (EC, 2003).

In essence, the aim of any environmental chemical risk assessment is to carry out quantitative risk characterisation, wherever possible, by identifying or extrapolating from the ecotoxicity data, to give concentrations at which no effects are expected and comparing this level with the estimated exposure level. Where quantification is not possible, a judgement on the likelihood of risk will need to be made on a qualitative basis. For environmental protection goals, risk characterisation is expressed as the:

Predicted Environmental Concentration (PEC)/Predicted No Effect Concentration (PNEC)

Where the PEC/PNEC > 1, there is considered to be a risk. Under these circumstances, there is an option to request further testing (either monitoring data to refine exposures or more data on effects to characterise the hazard further). Because of the reasonable worst-case approach adopted, very often a risk is identified where data are lacking, and in these cases the first consideration is whether more measured data would refine the exposure scenario by demonstrating that current exposures were below the level of concern. Alternatively, if there are large uncertainties in the effects database, leading to the application of high 'Assessment Factors' it may be necessary to consider performing additional tests (e.g. a chronic ecotoxicity test with the specific chemical to the most sensitive aquatic species).

1.2 WEA approaches

For complex effluents containing many substances, ecotoxicity or whole effluent assessment methods have a role. One of the principal advantages of ecotoxicity assessments is that they provide a measure of the combined effects of all the components in a complex effluent, such as synergistic, additive or antagonistic effects. Secondly, they add a degree of biological relevance that can help public understanding of the impact of an effluent and demonstrate the distinction between contamination (i.e. presence of a substance) and pollution (i.e. introduction by man of substances which result or are likely to result in hazards to human health or harm to living resources and ecosystems (OSPAR, 1992)). Furthermore, WEA provides a mechanism for evaluating the environmental significance of a complex effluent and allows for some degree of understanding of the environmental effects of mixtures. As described later in this report, bioassay methods can also be used to assess the quality of receiving waters, identify toxic components of an effluent and track the origins of toxic chemicals within a multi-plant site.

One fundamental point is that whilst most industries and the EU support the principle of risk assessment for both chemicals and effluents this is not universal. Some countries adopt a hazard based approach in which the ultimate goal is the reduction of toxicity in effluents discharged irrespective of the environmental risk these pose. Power and Boumfrey (2004) also identified two types of applications of WEA schemes i.e. risk assessment and hazard reduction. In risk

assessment schemes, studies tended to be undertaken on a site-specific basis to protect the quality of the receiving environment (e.g. no acute toxicity outside a defined mixing zone). In hazard assessment schemes, emission limit values on toxicity were set with the overall objective of reducing the hazard of the effluents discharged irrespective of the risk posed to the receiving environment. Some schemes were based on a mixture of both risk and hazard. Other variations included the fact that some WEA schemes were required for regulatory purposes whilst others were on a voluntary or case (e.g. sector) specific basis. Examples of these schemes are summarised in Table 1.

In any approach there are a number of difficulties and limitations in the application of ecotoxicity testing including deciding which bioassays are appropriate for a given situation and understanding the limitations of specific tests for a given objective. One of the critical factors affecting the choice and application of any whole effluent assessment method is its intended application; e.g. whether results are to be used for risk assessment, monitoring or compliance. As an example, ecotoxicity assessments will be significantly different if these are to be used by industry as a quick 'self check' or are being demanded by a competent authority (CA) to regulate a specific discharge. These two cases could utilise very different test techniques with varying degrees of precision adequate in each case for their intended purpose.

In terms of the limitations of bioassays, consideration should always be given to probable differences between environmental effects indicated by laboratory bioassays performed on effluents and the actual effect of an effluent in the aquatic environment. Biodegradation and chemical loss processes (e.g. photo-oxidation, hydrolysis, volatilisation and adsorption) influence the toxicity in the receiving environment and may not be reflected in the laboratory based evaluation. Other factors needing careful consideration are the selection and relevance of the test species and methods for a given purpose. A number of schemes currently under development are also considering methods to assess the 'persistence of toxicity'. For example, a procedure for including assessment of biodegradation and its influence on toxicity has been advocated by Nyholm (1996) and more recently demonstrated by Whale and Battersby (2004). Assessment of the 'persistence of toxicity' was also a facet studied in the UK Direct Toxicity Assessment (DTA) demonstration programme (UK DTA, 2001a).

In addition to ensuring that the ultimate objective of a WEA study is clear from the outset it is also important to balance theoretical objectives with practical limitations. Experience suggests that this can be best achieved if there is a mutual trust between industry and the regulatory authorities. For example, one of the key learning points from the UK DTA programme was that such co-operation and mutual understanding was very beneficial in ensuring the development of a cost-effective and environmentally appropriate control scheme for chemically complex effluent discharges (UK DTA, 2001b).

Table 1: Examples of regulatory approaches of WEA (Power and Boumfrey, 2004)

Country	Brief details of WEA scheme
United States	Regulatory - National Pollutant Discharge Elimination System under the Clean Water Act – 'waters shall be free from toxics in toxic amounts'. Primarily source control, but some receiving environment bioassays. More than 6,500 permits with WET monitoring requirements or limits.
Canada	Regulatory - primarily source control but some receiving environment bioassays. 80% of industrial surface discharges have bioassays for compliance, most others for monitoring.
Australia	Generally not mandatory and used for monitoring but some site-specific permits. Tiered risk based approach. Primarily source control, but some receiving environment.
New Zealand	Flexible regulatory system. Risk based. Mix of bioassay and biomonitoring, may include bivalve bioaccumulation and health.
EU Generic	IPPC Directive 96/61/EC Best Available Technology (BAT) and related to EQS. Water Framework Directive (WFD) good water quality objective may rely upon WET approach.
Germany	Regulatory - hazard reduction under Wastewater Ordinance (AbwV) and Wastewater Charges Act. Used as a basis for taxation. Primarily source control but includes use of daphnids in large rivers for early warning. Includes assessment of mutagenicity and endocrine effects for some states.
Belgium	EU approach (sector specific conditions based on BAT). Demonstration programme to develop protocol.
France	EU plus routine monitoring and occasional site-specific licensing. Used as a basis for taxation.
England, Scotland and Wales	Small number of consents in place. DTA demonstration programme (industry and regulator initiative) has developed protocol for acute toxicity testing. Bioassay use expected to increase in areas where receiving water biological quality is poor.
Denmark	Non-statutory strategy for use includes biodegradation and bioaccumulation. Source control to protect receiving water.
The Netherlands	EU plus risk based approach to account for receiving water conditions. Potentially source control following evaluations.
Eire	Mandatory emission limit values (ELV) based on toxic units. Source control and some monitoring.
Northern Ireland	Mandatory emission limit values (ELV) based on toxic units. Source control and some monitoring.
Norway	May be applied as a regulatory instrument. ELV plus site-specific limits. Source control based on total emission factors (TEF).
Spain	Regional use in permits. Chemical source ELV. Source control, hazard based. Some taxation of discharges.
Sweden	Focus on protecting surface water. Bioassays used to licence some discharges. Source control may include biodegradation and bioaccumulation.

Another factor, which needs careful consideration, is how best to resolve the uncertainty about the precision of results of ecotoxicity testing and any other WEA methods. This is important as results can be influenced by effluent sampling methods, sample storage conditions, time between sample collection and biological testing, available biological testing laboratories accredited to the necessary quality control standards, inter- and intra-laboratory variability, effluent variability, level of understanding of site-specific receiving water conditions, and the influence of the latter on effluent toxicity to resident organisms. These aspects must be carefully examined to ensure that any WEA scheme is both scientifically sound and practicable.

Requirements of bioassay methods

The choice of bioassay method will depend on the intended application. Cefic (1999) recognise 3 generic applications for whole effluent toxicity assessments which are for risk assessment, monitoring and legal compliance as described below.

Risk assessment applications

For risk assessment purposes and in line with the EC (2003) the test programme should begin with standardised laboratory bioassays following a tiered approach starting initially with acute toxicity assessments. The species should be selected on the basis of knowledge of their susceptibility to known toxic effluent components or as representative of important functional groups in the receiving environment. The bioassay results are then compared with predicted or measured dilution patterns in the receiving water to assess potential risk. Application factors may be applied to the data to extrapolate from acute or chronic no-effect levels determined in the laboratory to the field, and to take account of uncertainty of the data. Further work may be required to assess the level of risk posed by a discharge if 1) the risk assessment shows that the expected effluent concentration in the receiving water is close to the predicted no-effect level, or 2) there are concerns over the potential for longer term effects resulting from the presence of persistent and toxic effluent components.

Monitoring applications

Bioassay methods for monitoring effluents differ from those used in risk assessment in that they should provide a convenient and practicable mechanism for assessing variability of effluents being discharged and give a warning if the effluent toxicity has altered significantly. Monitoring techniques need not use the most sensitive of methods but they have to be capable of adequate discrimination of changes in toxicity that can be correlated with the results of risk assessment

and/or compliance tests. To be useful, these test methods need to be inexpensive, rapid, relatively portable and easy to conduct. Field monitoring studies (see Chapter 4) can be used to provide a mechanism for checking that discharge consent parameters are achieving the degree of control and protection envisaged. Monitoring studies should, where possible, include pre- and post-discharge assessments (in both time and space). These will ensure that changes in status attributable to the effluent can be identified confidently.

Compliance applications

Bioassays conducted for compliance purposes will be determined by the competent authority. Tests for compliance that have potential legal implications need to be of a statistically robust design, yield unambiguous results and be reproducible and amenable to the closest scrutiny. If tests do not meet these criteria there is potential for operators to find themselves liable to inappropriate and unjustified legal penalties (i.e. when it is the test method rather than the operators' performance which is at fault). Because of the legal ramifications, compliance tests should always be conducted by approved laboratories with quality control accreditation for that test.

1.3 Potential application of WEA in effluent control schemes

In current EU and OSPAR thinking the aim of water quality policy and legislation is to obtain a 'good environmental quality' of surface waters and sediments. At the moment, water management in these regimes adopts a substance-oriented approach, by which the environmental hazards of chemicals are assessed on persistence, bioaccumulation and toxicity. The PBT-values for individual substances are derived in laboratories using a range of ecotoxicity, biodegradation and physico-chemical tests. These values tend to be translated into environmental quality targets (e.g. Environmental Quality Standards) for surface waters and sediments and emission limits for effluents. However, many regulators recognise that there are limitations with the substance-orientated approach and are looking increasingly to develop and utilise more holistic approaches such as WEA. For example, OSPAR (2001) cites several positive attributes of the WEA approach which include the fact that WEA:

- Incorporates a range of methods to reveal (potential) effects of whole samples (water, effluents and sediments);
- circumvents the limitations of the substance-oriented approach by measuring PBT-values directly in samples;
- provides more relevant data for hazard and risk assessment by improving the understanding of the combined effects of both known and unknown substances in a discharge or waste;

- offers a 'short cut' to the substance approach by assessing whether an effluent is harmful;
- if an effluent is found to be 'harmful' the responsible (combination of) substances can be discerned by a toxicity identification evaluation (TIE).

OSPAR currently view WEA as a safety net for the substance-by-substance approach. WEA will not replace existing approaches within OSPAR with regard to the reduction of emissions and discharges of hazardous substances. However, OSPAR believe WEA will make it possible to check point sources for their contribution (in terms of adverse effects) in emissions and discharges to the surface water and sees WEA as another tool to help achieve the 'OSPAR convention' (OSPAR, 1992) which 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'*

In principle, WEA offers a short cut in reaching OSPAR's targets, because it directly focuses on the assessment of the adverse effects of discharges to the marine environment.

It is not only OSPAR that see such advantages. WEA type schemes have been utilised in many countries (principally North Americas) for several decades and the recent review by Power and Boumfrey (2004) indicates that there are a number of different terminologies for approaches involving effluent assessment which are similar to WEA (see Table 2). In the majority of these approaches effluent assessments are ecotoxicity based but many approaches go further to look at persistence, mutagenicity and bioaccumulation potential of the effluent contaminants.

Table 2: Examples of approaches similar to WEA

Country/organisation	Terminology used for effluent assessment approach
OSPAR	WEA (Whole Effluent Assessment)
USA (and some European Countries)	WET (Whole Effluent Toxicity)
Canada	ETT (Effluent Toxicity Test)
Germany	ICE (Integrating Controlling of Effluents)
The Netherlands	WEER (Whole Effluent Environmental Risk)
New Zealand	AEE (Assessment of Environmental Effects)
UK and Australia	DTA (Direct Toxicity Assessment)

In many of these schemes toxicity based effluent assessments are seen as a new (developing) tool for assessing effluent quality which should be applied in combination with (and not instead of) the substance-oriented approach. Within Europe, WEA type schemes are generally seen as supporting the hazardous substance strategies of OSPAR and the WFD (EC, 2000). For example, WEA has potential to contribute to WFD targets for priority substances and it is expected that WEA could be used within Integrated Pollution Prevention and Control (IPPC) for the elaboration of emission controls for priority substances. Some European countries also believe that WEA targets will feature in periodical revision of best-available-techniques reference documents (BREFs) for certain industrial activities.

Sector approach

Developing generic methodologies for specific industry sectors would appear at first glance to be a further possible method of application for WEA. It would, for instance, mirror the IPPC sector approach or the generic TGD release scenarios for each industry sector. For sectors where the release scenarios have considerable similarity, e.g. pulp and paper production, a sector-wise approach would initially appear to offer advantages. However, in reality this is often far from the case. In the early stages of the OSPAR investigations into WEA the Intersessional Expert Group (OSPAR, 2001) used a sectoral approach with the pulp and paper and pharmaceutical industries as examples of sectors with similar operations and hence of discharges. It proved impossible to learn very much from this approach with most studies taking different methodological approaches because of local circumstances. In particular the pulp and paper sector data, which perhaps had the greatest likelihood of comparability, was impossible to interpret and OSPAR took the decision to go ahead on a non-sector basis.

In most cases the local conditions are very specific. As the OSPAR study highlighted, the actual manufacturing and treatment processes are varied and this will affect the choice of methods and tests as well as the results. Examples of such conditions are:

- Multi-sites (different production units at one site, complex discharge situation, mixing of effluents);
- different processes within one sector;
- variability between different production phases (batch wise specifics, etc.);
- different salinities or ionic strength of effluents;
- different salinities of receiving water;
- use and mixing of cooling water with process releases;
- variable receiving water sensitivity and flow characteristics.

Ultimately the success of WEA schemes will depend upon their scientific merit, practical application and ability to deliver environmental benefits in a transparent and cost-effective manner. Some of the factors that need to be considered for WEA schemes to be of value are discussed in this report.

2. TESTS USED IN WEA

2.1 Introduction

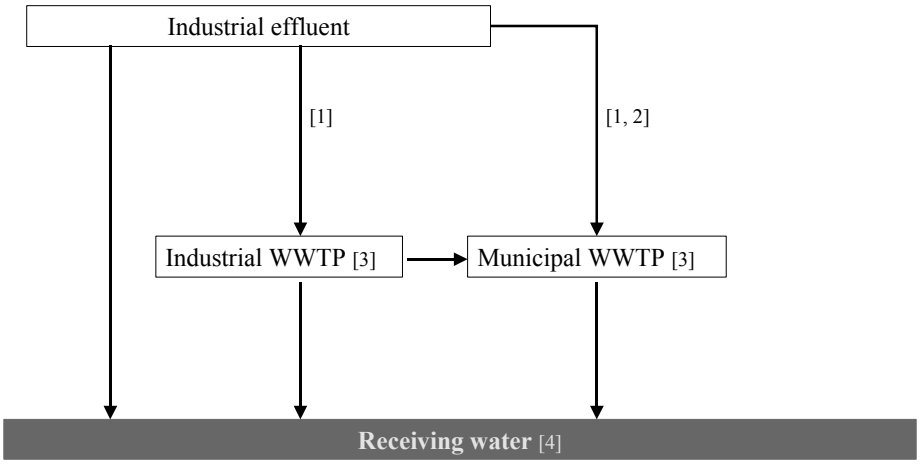
One of the foundation pillars in the WEA concept is the measurement of effluent ecotoxicity. The use of ecotoxicity testing as a mechanism for environmental protection is based on the assumption that the results from laboratory ecotoxicity tests are indicative of a potential for environmental effects. Experience has shown that this is hard to demonstrate.

WEA methodology must take account of diversity in the environment, including different trophic levels: decomposers, primary producers, primary consumers and secondary consumers (although animal ethics considerations may militate against this approach in the instance of fish testing – see Section 2.2.2).

WEA methodology must also take account of different industrial wastewater discharge scenarios. In general there are a number of scenarios (see Figure 1).

In each case, for the purpose of environmental protection, the effluent to be considered under WEA should be that which is discharged into the receiving water (i.e. the raw wastewater in the case of direct discharge, but the treated effluent in all other cases). In many cases this may cause problems in generating representative samples for testing (e.g. discharge of multiple industrial discharges into a municipal sewage treatment plant.

Figure 1: Routes of effluents into receiving water



[1] May have undergone primary (pre-) treatment step(s);
[2] Municipal WWTP may receive more than one industrial effluent (*i.e.* effluents from different industrial sites);
[3] Physico-chemical and/or biological (secondary and sometimes tertiary) treatment;
[4] Freshwater, estuary or coastal water.

The variability of discharge conditions, effluents and test results has always been an important consideration in the development of WEA methodology. These conditions have resulted in a number of practical solutions that have been included in test guidelines that are specifically designed for effluent testing. Additionally, it has proven essential to follow strict guidance in sampling and handling of samples and the proper documentation of all steps. In this section such procedures will briefly be reviewed, followed by an overview of available effluent tests and their feasibility in practice. Actual practice is still difficult, as demonstrated by 43% of false positives that were observed in a blind round robin test carried out by 16 US laboratories with the highly standardised chronic *Ceriodaphnia dubia* test (Moore *et al,* 2000a). Such studies illustrate why many of the available tests still have to be applied in practice and their findings understood. 'Learning by doing' will benefit most from an open and constructive dialogue between the stakeholders (UK DTA, 2001b).

2.2 Ecotoxicity tests

WEA has traditionally relied on tests developed for assessment of the hazards posed by single substances. Certain considerations however need to be taken into account when using such tests for WEA. An initial consideration is that effluent samples are generally complex mixtures, which often vary in composition over time. A second consideration is that samples may change with time after they have been taken. Thirdly, test conditions need to fulfil certain criteria before test organisms can be introduced and the addition of the effluent sample into a test medium may change the medium such that it does not fulfil these test conditions e.g. lowering the pH beyond a physiologically acceptable range. Other considerations include selection of dilution media and temperature and the timescale over which tests should be conducted. Whilst these additional complicating factors need to be carefully thought out and assessed, experience from programmes in a number of countries around the world has shown that WEA can be carried out using suitably modified toxicity tests. Experience also suggests that a key part of conducting a WEA programme is the 'learning by doing' approach and that it is very difficult, if not impossible, to rigorously prescribe what a WEA programme should look like at the outset. Instead, the approach needs to be tailored as the programme progresses, depending on individual site conditions and objectives and each individual programme should seek to build, as far as possible, on the experiences of others who have undertaken similar programmes in the past.

2.2.1 Sampling procedures

The complication posed by the variability of samples requires careful consideration of the sampling in terms of where, when and with what frequency sampling has to be carried out to allow meaningful results to be generated. It is outside the scope of this report to give detailed

recommendations on this topic, since it will depend on the purpose and the specific discharge, nevertheless, detailed reviews on sampling are available (DTA, 2001; Crane, 2004; US EPA, 1994).

To control potential problems posed by the instability of samples it is important that appropriate procedures are adopted for the collection, storage and preparation of samples to ensure that measured parameters such as toxicity do not significantly change before testing is conducted. Most of the general sampling and sample preparation recommendations are based on experience with ecotoxicity testing but some may also be useful for other tests, such as those to determine bioaccumulation or persistence. In the following sections, we will only briefly discuss some of the key points that are important when carrying out and understanding whole effluent testing.

Several aspects of sampling may have significant influence on the results if not dealt with properly. For the collection of samples these include the use of inert containers, rinsing procedures, homogeneity and pooling of samples and determining the required volume of samples in relation to testing (semi-static, static or flow-through). The measurement of basic physico-chemical properties of samples includes pH, dissolved oxygen, temperature history from sampling until testing, conductivity or salinity, colour, physical state (e.g. emulsion) and suspended solids may also be required. All steps should be covered by the chain of custody/duty of care documentation. In many cases it has proved useful to split samples in order to store a sufficiently large sub-sample for later use if necessary. Such sub-samples may be used, for example, when practical problems arise during testing or when interpretation of results raises difficulties. In all cases it is desirable to keep the length of time between sampling and initial testing to a minimum, preferably less than 48 hours (DTA, 2001; Crane, 2004). If the samples are kept for a significant period then it is important to verify the influence of the storage conditions on the sample integrity.

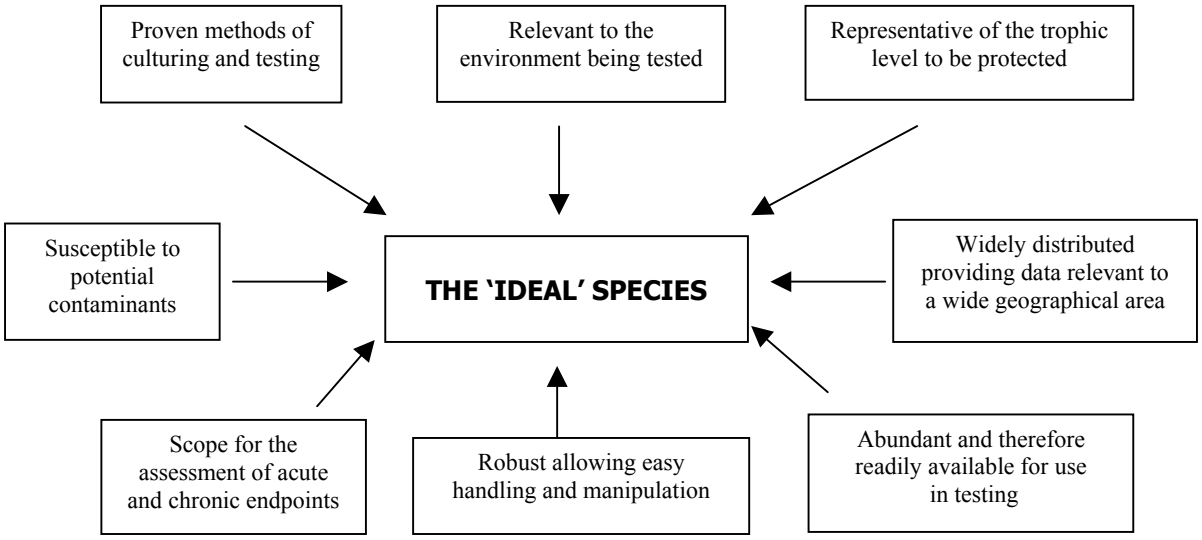
2.2.2 Test selection

WEA comprises a battery of tests. The selection of which tests to use in WEA will depend on the target or objectives of the testing. For example, screening tests are usually fast and cost effective but may be unsuitable for regulatory requirements. Other factors such as the available resources, the requirements of the test organisms, and effluent characteristics such as toxicity fluctuation may influence selection of test type. All the above-mentioned issues should be considered when selecting the type and the conditions of the toxicity tests. A diagrammatic representation of the factors that influence the selection of test organism is given in Figure 2.

When running toxicity tests, the nature of the dilution medium to use is an issue. In general, in the laboratory, it is the norm that specified synthetic media are used. There may be conditions and

targets where it is possible to use natural waters rather than synthetic dilution water. For example upstream receiving water may be used as the dilution water. However, in such a case, extreme care should be taken with the interpretation of the results due to decreases in the level of control over the test variables. The issue of test/dilution medium is discussed further in Section 2.2.5.

Figure 2: Criteria for the 'Ideal' toxicity test species



The use of fish for WEA represents a dilemma between the need to ensure that this important taxonomic group of organisms is protected, and legitimate and widely felt concerns about animal testing. It is important that the number of fish used in effluent testing is kept to a minimum. From the review by Whale *et al* (2003) it appears that fish could potentially be replaced by the use of a multi-trophic test battery incorporating the use of fish cell lines with existing *Daphnia* and algae test procedures. Currently there is very little experience of testing complex effluents using cell lines and there is no standardisation and validation of these tests internationally (e.g. by European Centre for Validation of Alternative Methods). In practice, where fish have historically been used for effluent schemes, it has proved difficult to switch to alternative ecotoxicological endpoints. However there is no scientific basis preventing this. The use of fish in both existing and developing schemes should be challenged and viable alternatives sought where possible. One example of such an alternative is the use of solid phase micro-extraction (SPME), although this use requires assumptions to be made about the mode of action of the constituents of an effluent (Leslie *et al*, 2002; Parkerton *et al*, 2000). In the longer term, genomic technologies may offer potential to diagnose a range of adverse effects on fish (effects on specific organs, etc.) that may replace/reduce reliance of fish tests. The use of fish in ecotoxicity testing is the subject of an ECETOC Task Force and workshop (ECETOC, 2004a,b).

2.2.3 Acute tests

Acute toxicity refers to the adverse effects of a sample that are demonstrated within a short period of exposure. The term 'short' is generally understood to cover a period of up to 12 hours for single celled organisms and up to one third of the time taken from 'birth' to sexual maturity for invertebrates provided that the species could survive in good condition without feeding for such a period. Typically this would be 2-4 days for standard tests with higher organisms.

Although there is a large set of existing test organisms, it is recommended that standardised tests should be used wherever possible. These cover the taxonomic groups: bacteria, algae, invertebrates and fish and a selection of the most commonly used standardised tests is given in Table 3.

The acute toxicity of an effluent is measured through mortality/immobility (fish and invertebrates) or decreased growth or metabolic rate (algae and bacteria).

The objective of acute toxicity tests is to identify discharges of toxic effluents, which have an immediate detrimental effect in the receiving environment. They may be applied to intermittent or continuous discharges and because of their relatively low cost and rapid timescale, are applied much more frequently than chronic tests. These tests are generally conducted in the laboratory but may on occasion be conducted in the field.

2.2.4 Chronic tests

Chronic toxicity is defined as the adverse effects of a sample, which are demonstrated only after a long-term exposure in relation to the life of the test organism, which may include, in a number of cases, reproduction of the organism. Because of this extended exposure time, external factors such as water hardness, ionic balance, etc. are usually much more critical in chronic tests than in acute tests. The chronic toxicity of effluents is usually measured through sublethal effects such as reduced fecundity or decreased growth rate.

The objective of chronic toxicity tests is to identify discharges of toxic effluents, which have a detrimental effect over a longer period of time. They are thus usually only applied to continuous discharges and, because of cost and timescale issues, are applied much less frequently than acute tests. These tests can, in general, only be conducted in the laboratory. Usually WEA is pursued in a stepwise fashion. Thus chronic tests on effluents are usually only applied, because of their higher costs, after a full acute testing regime has already been carried out.

Table 3: Examples of the most commonly used ecotoxicity tests and their applicability to WEA

Ecotoxicity test	Endpoint	Applicability to WEA and comments	Test guideline [a]
Acute			
Inhibition of light emission of *Vibrio fischeri*	EC_x/NOEC	Applicable as a screen. Regulatory acceptance may be an issue. Possible issues with pH, colour and turbidity	ISO 11348
Algal growth inhibition	EC_x/NOEC (biomass and growth rate)	Applicable. Possible issues with metal chelation, colour, turbidity and microbial contamination	OECD 201, C.3 (92/69/EEC), NFT 90-375, ISO 10253, OPPTS 850.5400, USEPA OW 1003.0
Lemna spp (duckweed) growth inhibition	EC_x/NOEC (frond number/size)	Applicable - algal tests usually preferred	ISO/CD 20079, OECD in draft, OPPTS 850.4400
Daphnia spp	EC_x/NOEC (immobility)	Applicable	OECD 202(I), EN ISO 6341, C.2 (92/69/EEC), OPPTS 850.1010
Gammarus spp	EC_x/NOEC (immobility)	Applicable as a screen. Regulatory acceptance may be an issue	OPPTS 850.1020
Bivalve embryo larval development	EC_x/NOEC (% normal development)	Applicable - issues with control viability at certain times of year	OPPTS 850.1055, OPPTS 850.1025
Penaeus spp (shrimp) and others	LC_x/NOEC (survival)	May be cannibalistic. Applicable as a screen. Regulatory acceptance may be an issue.	OPPTS 850.1045
Copepod e.g. *Tisbe* spp	EC_x/NOEC (immobility)	Applicable	ISO 14669
Americamysis bahia (shrimp) (previously *Mysidopsis)*	LC_x/NOEC	Applicable - warm water species	OPPTS 850.1035
Fish	LC_x/NOEC (survival)	Applicable	ISO 7346, OECD 203, C.1 (92/69/EEC), OPPTS 850.1075, USEPA OW 1000.0

Table 3: Examples of the most commonly used ecotoxicity tests and their applicability to WEA (cont'd)

Ecotoxicity test	Endpoint	Applicability to WEA and comments	Test guideline [a]
Chronic			
Daphnia spp	EC/LC_x/NOEC (mortality, impaired fecundity, neonatal growth)	Applicable - possible issues with pH, metal chelation, colour, turbidity and microbial contamination	ISO/FDIS 10706, OECD 211, OPPTS 850.1300, C.20 (2001/59/EC)
Ceriodaphnia dubia	EC/LC_x/NOEC (mortality, impaired fecundity, neonatal growth)	Applicable - possible issues with metal chelation, colour, turbidity and microbial contamination	NF T 90-376, ISO/CD 20665, USEPA OW 1002.0
Americamysis bahia (shrimp) (previously *Mysidopsis)*	EC/LC_x/NOEC (mortality, impaired fecundity, growth)	Possibility of cannibalism	OPPTS 850.1350
Tisbe battagliai (copepod)	EC/LC_x/NOEC (mortality, impaired fecundity)	Applicable	Draft OECD
Inhibition of population growth of *Brachionus calyciflorus*	EC/NOEC population growth inhibition	Applicable - possible issues with colour, turbidity and microbial contamination	NF T 90-377, ISO/CD 20666
Pseudomonas putida growth inhibition test	EC/NOEC population growth inhibition	Applicable as a screen. Regulatory acceptance may be an issue. Possible issues with pH, colour and turbidity	EN ISO 10712
Fish 14 day	LC_x/NOEC survival	Applicable	OECD 204
Fish short-term embyo/larva	EC/LC_x/NOEC hatch survival growth	Applicable	OECD 212, C.15 (2001/59/EC), OPPTS.850.1400

[a] See Appendix A for details of test guidelines

In addition, these test types can be further divided into static, semi-static or flow-through depending on whether, or how often, the effluent being assessed is replaced with fresh sample during the test. Effluent replacement is more often carried out during longer tests (i.e. usually chronic tests) but a semi-static regime (i.e. where the effluent is replaced at discrete intervals) is not uncommon in vertebrate or invertebrate acute tests. Some of the advantages and disadvantages of the various test protocols are given in Table 4.

Table 4: Advantages and disadvantages of test types *(adapted from EPA 821-R-02-12, US EPA, 2002)*

	Advantages	**Disadvantages**
STATIC TESTS	1. Simple and inexpensive. 2. Limited resources (space, manpower, equipment) required permitting staff to perform more tests in the same amount of time. 3. Smaller volume of effluent required than for static renewal or flow-through tests.	1. Dissolved oxygen (DO) depletion may result from high chemical oxygen demand (COD), biological oxygen demand (BOD), or metabolic wastes. 2. Possible loss of toxicants through volatilisation and/or adsorption to the exposure vessels. 3. Generally less sensitive than static renewal or flow-through tests, because the toxic substances may degrade or be adsorbed, thereby reducing the apparent toxicity. Also, there is less chance of detecting slugs of toxic wastes, or other temporal variations in waste properties.
SEMI-STATIC TESTS	1. Reduced possibility of DO depletion from high COD and/or BOD, or ill effects from metabolic wastes from organisms in the test solutions. 2. Reduced possibility of loss of toxicants through volatilisation and/or adsorption to the exposure vessels. 3. Test organisms that rapidly deplete energy reserves are fed when the test solutions are renewed.	1. Require greater volume of effluent that non-renewal tests (only really an issue in fish tests). 2. Generally less sensitive than flow-through tests, because the toxic substances may degrade or be adsorbed, thereby reducing the apparent toxicity. Also, there is less chance of detecting slugs of toxic wastes, or other temporal variations in waste properties. 3. When using large volumes for effluents there may be a problem in maintaining sample integrity. if fresh samples are taken they may not be identical to earlier samples.
FLOW-THROUGH TESTS	1. Provide a more representative evaluation of the toxicity of the source, especially if sample is pumped continuously directly from the source and its toxicity varies with time. 2. DO concentrations are more easily maintained in the test chambers. 3. A higher loading factor (biomass) may be used. 4. The possibility of loss of toxicant due to volatilisation, adsorption, degradation, and uptake is reduced.	1. Large volumes of sample and dilution water are required. When using large volumes for effluents there may be a problem in maintaining sample integrity. If fresh samples are taken they may not be identical to earlier samples. 2. Test equipment is more complex and expensive, and requires more maintenance and attention. 3. More space is required to conduct tests. 4. Because of the resources required it would be very difficult to perform multiple or overlapping sequential tests.

It is worth noting that there has been significant investigation into the use of *in situ* monitoring using caged or sessile organisms immersed directly into receiving water (e.g. Goldberg *et al,* 1978; Snyder-Conn, 1993; Barjaktarovic *et al,* 1995). Whilst this type of monitoring may provide valuable information on the state of the environment in proximity to an effluent discharge, discussion of *in situ* testing is deferred to Section 3.2.5.

2.2.5 Factors influencing toxicity test endpoints

Toxicity tests were developed originally to assess effluents in the early 1940s (Rand *et al,* 1995) however they have been mainly used for the assessment of single substances. Recently they have been used more extensively for the assessment of wastewater and a number of limitations have been identified. A wastewater sample may well be a complex mixture, containing soluble as well as insoluble organic and inorganic substances. These can interfere with:

- The organism itself;
- the measurement technique;
- the bioavailability of possible toxicants.

Some of the most common factors interfering in WEA ecotoxicity testing are discussed below and summarised in Table 5.

The importance of having appropriate water quality for specific aquatic toxicity tests has been recognised for many years and there are several references in the literature indicating that water quality (physico-chemical parameters) of the test medium influences the results of acute toxicity tests. For example, Vasseur *et al* (1986) state that test temperature, pH, buffer solutions, hardness and salinity must be considered when analysing toxicity data. Rattner and Heath (1995) cite the importance of considering dissolved oxygen concentrations, Ho and Quinn (1993) the photoperiod, Ghillebaert *et al* (1996) the dissolved organic matter concentration and Belanger *et al* (1989) the diet of the test organisms. The concern is that organisms stressed by physico-chemical conditions will have increased susceptibility as increased energy metabolism associated with organism stress is often combined with more rapid toxicant uptake. For example, Rattner and Heath (1995) have shown that rapid temperature change coupled with toxicant exposure has more profound effects on fish than responses observed in fish which have been acclimated to temperature prior to exposure. In toxicity tests for hazard assessment there is a need to identify the toxicity of substances under standard conditions to enable comparison of the hazard of different substances. Consequently, over the course of test method development, limits to water quality factors have been incorporated into international test guidelines to reduce the effects of stress on test organisms. However, although most test guidelines cite acceptable conditions, little has been done to study the effects that may occur if these guideline limits are not met.

Table 5: Test interferences and limitations

Test type	Typical interference* or limitation of tests use for effluents
Algae	The presence of nutrients in the effluent may cause an accelerated growth of the organisms. The presence of particles may interfere with the growth measurement (unless removed at the start of the test by filter sterilisation). Chemicals adsorbing light in the range 400-700 nm may interfere with algal growth for physical reasons rather than by toxic action (there are now standardised methods to overcome this effect). Test sterility must be preserved to obtain meaningful results, however, this is not recommended for effluents as sterilisation may cause changes in effluent chemistry. EDTA is a normal constituent of the test medium, but it may interfere with the bioavailability of metals. Volatile substances may be stripped by agitation of the test flasks although there are now methods to minimise this. pH adjustment may be needed to distinguish pH effects from chemical toxicity.
Bacteria	For tests using luminescent bacteria e.g. *Vibrio fischeri* turbid samples may interfere with the measurement of the luminescence. pH adjustment may be needed to avoid pH effects when measuring toxicity. Testing with *V. fischeri* requires sample salinity addition for freshwater samples, which may affect bioavailability. The range of sensitivity for reference substances differs according to the preparation of the bacteria (freshly prepared, freeze-or liquid-dried). Highest possible test concentration: 80 %.
Plant tests e.g. *Lemna minor*	The presence of nutrients in the effluent may cause an accelerated growth of the organisms. EDTA is a normal constituent of the test medium, but it may interfere with the bioavailability of metals. pH adjustment may be needed to avoid pH effects when measuring toxicity.
Invertebrates e.g. *C.dubia* or *D. magna*	Difficulty counting the organisms in turbid or coloured water. Presence of other organisms may interfere with metabolic processes and possibly kill organisms by infection. pH adjustment may be needed to avoid ph effects when measuring toxicity. Effluents containing surface-active materials may exhibit surfactant effects such as clumping or flotation of organisms causing physical effects and subsequent organism death, although means exist to minimise this effect.
Fish	Requires larger volume of effluent and larger area to perform the tests. Animal ethics considerations are encouraging reduction in the number of chordate organisms used in ecotoxicity testing (limit testing, after other trophic level testing has been performed is becoming more favoured). pH adjustment may be needed to avoid pH effects when measuring toxicity. In many cases fish tests have been found to be less sensitive than tests with algae or invertebrates. this may be because historic effluent testing often used fish species and therefore effluents that affect fish have already been managed to remove the toxicity. there are also important exceptions to this generalisation e.g. effluent testing where factors such as ammonia are very important.
Marine tests in general	May require salinity adjustment when testing high concentrations (>10%) of effluent. This may alter toxicant expression.

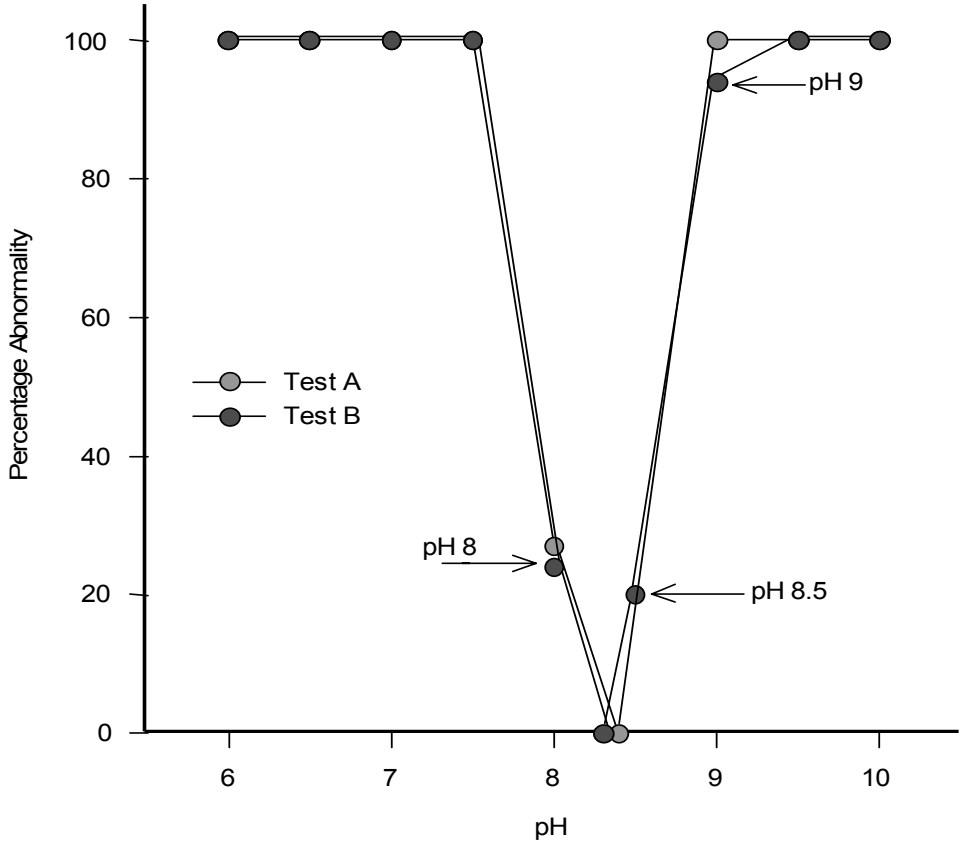
*Interference is not exclusive to effluents

One concern with the whole effluent assessment approach is that the intrinsic water quality (e.g. salinity, pH, hardness) of effluents can differ greatly from standard test conditions and this can affect the outcome of toxicity tests. In some guidelines problems of water quality affecting the test endpoints are addressed (e.g. US EPA, 1985) by recommending that the water quality parameters of the effluent test solutions be adjusted (e.g. neutralising acid or basic solutions) prior to testing to meet the required limits. Advocates of this approach recommend pre-test treatment since they believe that this ensures that observed effects give a better indication of the toxicity of the sample. However, this approach has limitations because such pre-test treatment steps may alter the ionic state and bioavailability of potentially toxic components (e.g. ammonia, phenols, transition metals) and therefore may not give a true representation of the impact to receiving waters. The implications of pre-treatment need to be considered when carrying out WEA tests.

Ideally the use of freshwater or marine test organisms is representative both for the salinity of the receiving water and the salinity of the effluent. However, in some cases these issues may conflict, for example, when a saline effluent is discharged into a freshwater environment. It has been argued that the receiving water should determine the type of test organisms to be used (UK DTA, 2001a), but in other circumstances the counter argument may prevail that it is desirable to keep the effluent in its original state as much as possible and hence to use organisms adapted to the physico-chemical conditions of the effluent. As an example of this latter argument when discharging a saline effluent to a freshwater environment, the use of freshwater organisms might mean that the effluent needed to be very significantly diluted to comply with the tolerable salinity range of the organism. In such cases any potential toxicants present in the effluent would also be strongly diluted and might not be detected. Then it might be more meaningful to use marine organisms, even though the receiving environment is freshwater. In this context it is worthwhile mentioning that in a number of studies it has been shown for many chemicals and for many taxa that the sensitivity of freshwater and marine species is similar (ECETOC, 2003b).

It is recommended that a better understanding of the effects of water quality parameters on selected toxicity tests is achieved. US EPA has recommended tests based upon the toxicity of common ions to the test species. There is little guidance elsewhere and this was recognised by Whale *et al* (1999) who generated effect curves for salinity, pH and suspended solids for some of the toxicity tests being considered for incorporation into the UK DTA scheme. An example of the influence of pH on test results is presented below. At the time, the latest guidance provided by the UK Environment Agency recommended pH 6.0 to 8.5 as an acceptable range for oyster embryo larval development test. Whale *et al* (1999) stated that although guidance on pH was developed for test acceptability with Microtox™ and *Artemia tonsa,* these were inappropriate for the oyster *Crassostrea gigas* where acceptable levels of development only occurred within a narrow pH band (8.0 to 8.5) – see Figure 3. If the pH was outside this band, development would not occur and the effluent (or dilution thereof) would be considered toxic. By comparison, US EPA (1985) recommended pH adjustment for their toxicity tests if the pH of the samples fell outside of the range of 6.0 to 9.0.

Figure 3: Effect of pH on Crassostrea gigas embryo development



2.2.6 Microbiotests

Microbiotests are of interest to WEA because they may potentially provide easier, more rapid and more cost effective ecotoxicity tests. One form of invertebrate microbiotest receiving increasing attention and being adopted for some environmental monitoring programmes are the Toxkits. These use resting stages (cysts) of certain aquatic invertebrates from which the organisms can be hatched when needed. These cysts can be stored for long periods of time without losing their viability. Details on the development and application of Toxkits are reported in Persoone (2001). Tests utilising cysts from two marine anostracan crustaceans *A. salina* and *Streptocephalus proboscideus* have also been developed.

Microbiotests typically use small test species and ideally should have the following characteristics:

- No loss of precision, reproducibility, or sensitivity when compared to conventional bioassays;
- predictive of real world effects;

- inexpensive and cost-effective;
- generally not labour intensive;
- have a high sample throughput;
- cultures that are easily maintained or maintenance free;
- modest laboratory and incubation requirements;
- low sample volume requirements.

Many of the above characteristics make microbiotests very well suited for incorporation into a multi-trophic test battery for the routine screening of effluents. Willemsen *et al* (1995) argued that microbiotests hold an advantage over conventional bioassays for routine environmental screening and that the latter are impractical for such a task. Persoone (2001) also believes that the application of conventional tests is seriously held back by the need for continuous culturing and/or maintenance of live stocks of the test species in good health and sufficient numbers, the space required to do so, and the high costs associated with this. These problems are increased by the need for specially equipped laboratories to facilitate conventional tests.

Other small-scale screening tests have also been suggested for effluent testing. CerioFAST^{TM} is a rapid assay based on the suppression of feeding activity in *C. dubia* in the presence of toxicants. It was evaluated and applied to industrial effluents by Jung and Bitton (1997). In this assay ceriodaphnid neonates are exposed to the toxicant for 1 h after which they are allowed to feed on fluorescent-stained yeast cells for 20 minutes. The presence or absence of fluorescence in the daphnids gut is used as a measure of toxic stress. Similar test procedures using rotifers (*Brachionos calyciflorus* and *B. plicatilis*) or daphnids feeding on fluorescent beads are proposed by Juchelka and Snell (1994, 1995) and De Coen *et al* (1995).

A basic consideration when developing a new toxicity test method is whether or not the test itself is fit for purpose. There are a wide variety of toxicity tests for various purposes including, tests for screening, regulatory or hazard assessments, and each has different requirements. These include test precision, bioassay organism choice, exposure time, reproducibility, sensitivity and cost (Persoone and Janssen, 1994). Theoretically, these kits could make a lot of the current thinking and technology in aquatic toxicity testing obsolete. Unfortunately, it is often the case that ecological relevance, reproducibility, reliability, robustness and sensitivity of the bioassay are not established within the context of the purpose of the method (Janssen *et al*, 2000). A few, such as the Microtox and some Toxkits may have regulatory acceptance in some countries, but such acceptance is not universal. It is possibly due to the inadequacy of the descriptions of the standard operating procedures in the literature, as pointed out by Janssen *et al* (2000). Where these have been used in demonstration programmes (for example Microtox and Eclox used in UK DTA programme on the Tees (UK DTA, 2001a) where the use of modified oyster embryo tests was quicker and more cost effective) there is evidence that these do not have any advantages over modified procedures using standard test organisms. Such tests may have value for internal (i.e. self) monitoring of effluents.

2.2.7 Biomarkers and genomics

A biomarker is defined in this report as any biochemical, physiological or histopathological indicator of exposure or response to a contaminant by individual organisms (Van Gestel and Brummelen, 1996). They have, for a number of years, been advocated as useful endpoints for the determination of biological responses to stress. It is possible that ecotoxicogenomics (the study of gene and protein expression in response to environmental toxicant exposures) may provide tools in the future to assist our understanding of how chemicals and effluents can impact on ecosystem health (Snape *et al,* 2004). The main concern with respect to the use of these tests has been the relevance of these biological endpoints to ecosystem effects and how they may be correlated. Work continues in this area and recent studies show that multi-biomarker approaches are moving into the mainstream of environmental assessment (Galloway *et al*, 2004) and that biomarkers are also being assessed for taking an evidence-based approach to regulating pollutants. There is also information available to suggest that standardised biomarker assessments are being actively considered by environmental regulators for inclusion in regulations for environmental monitoring (Pelley, 2004).

2.2.8 Expression and interpretation of the results

In WEA, the results of toxicity tests may be expressed in several different ways:

1. As a volume percentage or dilution factor of the effluent having an effect on a percentage (e.g. 50%) of the population of the test organisms (EC_{50}) within the prescribed period of time (e.g. 24-96 h), or the highest effluent concentration in which survival is not statistically significantly different from the control – the no observed effect concentration (NOEC). EC values (e.g. EC_{10}) are however statistically preferred to NOEC values since the absolute value of a NOEC is more affected by the test design. A draft guidance has been provided on the use of statistical analysis of ecotoxicology data (OECD, 2003).
2. In terms of toxic units of which there are two types:

- Acute toxic units (Tu_a) defined as $100/EC_{50}$ from an acute test (when toxicity is expressed as % effluent by volume);
- chronic toxic units (Tu_c) defined as 100/NOEC or EC_{10} from a chronic test.

An on-going trend in chronic aquatic toxicology has been the movement from NOEC estimation to expressing results as an effective concentration at a specified level of impairment and time (e.g. 7-day $EC_{20,}$ i.e. the concentration that is predicted to inhibit the population tested by 20% relative to the control over a 7-day period). Stephan and Rogers (1985) and Bruce and Versteeg (1992) discuss the advantages and disadvantages of this approach. A significant advantage of using the EC_x approach in whole effluent assessments is in the ability to interpolate response

between exposure concentrations and putting this in the context of constantly varying receiving system dilution scenarios.

In tandem with experimental design considerations (locations, replication, expected variance estimation, etc.), a clear description and justification of the statistics to be used is imperative. Results from whole effluent toxicity tests can be described by a wide variety of statistics familiar to risk assessment practitioners. Faithful adherence to assumptions and understanding the available alternative methods is necessary to provide the most appropriate interpretation of data. Whole effluent assessments are known to be especially vulnerable to non-linear, low-dose effects including the so-called 'hormetic response'. A family of statistics has arisen to properly express non-linear dose-response patterns (Bruce and Versteeg, 1992; Bailer and Oris, 1998). To date, it appears that microbial and daphnid assays are more likely to display these patterns and that flexibility in choosing exposure concentrations and statistical models is critical. Many effluents can be variable and, as a consequence, parametric statistical models may not be appropriate. Non-parametric models, such as trimmed Spearman-Karber may prove useful when the data structure does not allow probit, binomial and other common techniques for acute toxicity effect estimation (i.e. EC_x or LC_x) (Ellersieck and LaPoint, 1995). The International Standards Organisation (ISO), in cooperation with OECD, has recently published a draft guidance document on the statistical analysis of ecotoxicity data (OECD, 2003).

2.3 Bioaccumulation

A significant concern is the discharge into the environment of bioaccumulating substances of unknown identity. If these substances persist after release into the environment they may exhibit toxicity to organisms at different levels in the food chain (secondary poisoning). Substances for which this route of exposure is relevant will have a combination of three important characteristics:

- A log K_{ow} between 5 and 8 (ECETOC, 1995) (i.e. generally considered to have a potential to bioaccumulate);
- metabolism within the organism subsequent to uptake. The faster the rate of metabolism of the substance, the less it will accumulate;
- the substance must have a sufficiently long residence time in the environment to be available to partition into organisms.

This combination of characteristics should be taken into account to identify potential risks from substances through bioaccumulation.

One way to include the bioaccumulation of persistent substances in whole effluent assessment is to biodegrade the sample in activated sludge for an appropriate period of time (usually several weeks before toxicity testing) to be sure that only biodegradation resistant substances are left. In practice the combination of bioaccumulation and degradation testing can fit efficiently into a WEA testing scheme in combination with triggers for toxicity testing (see Figure 4 and Chapter 5).

To establish the bioaccumulation potential of mixtures of chemicals such as effluents, several tests have been developed, each with their own advantages and shortcomings (OSPAR, 2002a; de Maagd, 2000). A common characteristic of all methods is that they are based on the physico-chemical characteristics of the substances and thus will only indicate a *potential* to bioaccumulate. These methods must therefore be considered as indicative screening tools, the actual bioaccumulation of substances will depend on their persistence in the environment and their susceptibility to metabolism.

Since bioaccumulation is determined for components of effluents, the concept of the bioaccumulation potential of an effluent is debatable. The current bioaccumulation potential tests will give some response with many effluents. Therefore there may be a need for some form of threshold value in relation to effluent management decisions. Whether and where to set such a threshold, and whether it should be scaled by the magnitude of the bioaccumulation factor (BAF) and toxicity requires further discussion.

The different tests that have been developed are described below, together with their key characteristics.

2.3.1 Tests for bioaccumulation potential

There are a number of surrogate methods that have been applied including:

- High pressure liquid chromatography (HPLC);
- Empore (C_{18}) disks;
- Semi-permeable membrane devices (SPMD);
- Solid phase micro-extraction (SPME) fibres.

Table 6 summarises the approaches adopted in each procedural step associated with each of these approaches. The procedures for the extraction of organic substances from effluents vary in duration from $\leq$1 day for the SPME method to 14 days or longer for the C_{18} Empore disk and SPMD. The staff time involved in conducting the procedures varies from approximately 1 hour for the SPME method to approximately I day for the HPLC, SPME and Empore disk methods.

De Maagd (2000) evaluated the different procedural steps of the various methods given in Table 6 against a series of objective selection criteria (selectivity, recovery, practicality, cost-effectiveness, applicability and sensitivity). It was suggested that the ideal method for general screening of potentially bioaccumulating substances in effluents in a cost-effective manner consists of:

- No pre-treatment steps;
- a validated SPME procedure;
- no pre-treatment of the extract;
- gas chromatography (GC) or HPLC connected to a mass spectrometer for detection, with the parallel use of additional detection techniques where appropriate for further investigation.

Table 6: Summary of the procedural steps adopted with different types of laboratory-based chemical methods for assessing potentially bioaccumulating substances

Method and reference	Procedural stage					
	Pre-treatment	**Extraction**	**Pre-treatment before analysis**	**Separation**	**Detection**	**Expression of result**
High performance liquid chromatography - Stenz *et al* (1999)	Filtration	Solid phase extraction	Sample cleaned up with ultrafiltration and preparative gel permeation chromatography	Collection of fractions from C18 HPLC separation, lyophilisation of fractions	Gravimetric and TOC analysis	Weight % or proportion eluting after chemicals of set log kow
Empore disk method - Van Loon *et al* (1996)	Adjustment of sample to pH < 2 and addition of bacteriocide	Exposure of disk to sample for 14 days[a] Extract disk with cyclohexane	Evaporation under nitrogen	Minimal separation by gas chromatography	Mass spectrometry or vapour pressure osmometry	Mass material absorbed relative to a reference chemical – absorbs in proportion to its log kow
Semi-permeable membrane device (SPMD) - Södergren (1987)	-	Exposure of sample to trioliene filled SPMD for > 14 days	-	Separation by gas chromatography	Electron capture detector	Amount absorbed into an oily material inside a membrane - as Empore etc
Solid phase micro-extraction (SPME) - Verbruggen et al (1999)	Adjustment of sample to pH 7.5	Exposure of polyacrylate SPME to sample for 1 day	-	Minimal separation by gas chromatography	Mass spectrometry	Similar to empore issue – mass of material absorbed

[a] The Empore disk is exposed to the sample for 7 days before renewal of the sample and exposure for a further 7 days

Many effluent samples require specific pre-treatment steps before they can be used in some of the methods (Table 6). Some of the problems and solutions will be briefly discussed.

Filtration may be needed for samples with high particulates to prevent clogging in the extraction and chromatography steps of the isolation and identification stages. However, filtration is best conducted through inert substances, e.g. glass fibre, as some filters will remove substances from the effluent thus leading to a reduced response. Furthermore, for some samples (e.g. weak organic acids or bases), the adsorption onto effluent particulate matter or the filter, will be affected by the pH.

Extraction is used to separate similar groups of components and thus help their analytical determination. All methods of extraction suffer from incomplete extraction of the chemicals being targeted, differential extraction across groups and extraction of interfering chemicals. Therefore extraction techniques need to be assessed on a case by case basis to ensure that the approach is suitable in each circumstance to which it is being applied.

Analytical separation is the stage that leads to the separation of the chemicals in an effluent or extract of an effluent. Methodologies for separation will be chosen dependent upon the required discrimination, the most common method is chromatography. Gas chromatography separates primarily based on boiling point. Liquid chromatography may separate primarily based on hydrophobicity (reverse phase) or polarity (normal phase). The main problems with chromatography are that chemicals may co-elute, leading to misinterpretation (dependent on the method of determination) or interact strongly with the chromatography column, frequently leading to under-estimation.

Analytical detection is the final process leading to either identification and/or quantification of the chemicals that elute from the chromatography column. These methods can be non-specific, e.g. UV (for HPLC) or FID (for GC) or specific e.g. mass spectrometry. All the methods will need calibration and, depending upon the knowledge of the chemicals in the effluent and the pre-treatment, this may be chemical specific or chemical type (e.g. normalisation to a specific hydrocarbon on FID). The latter is obviously less accurate but sometimes more encompassing.

2.3.2 Experience with classical extraction and solid phase micro-extraction

Seven effluent types were studied in the Netherlands using two approaches (OSPAR, 2002a). The first approach was to use an SPE/HPLC method consisting of filtration, SPE extraction of the filtrate, Soxhlet extraction of the particulates, reverse phase HPLC of both extracts and drying of the potentially bioaccumulating substances (PBS) fraction. The other approach used SPME, carried out by inserting a SPME fibre into an effluent sample, maintaining a contact time of 24 hours with the effluent and analysis by putting the fibre directly in the injection chamber of a gas chromatograph. The peaks were quantified in millimoles by taking 2,3,5-trichlorotoluene as

external standard. The SPE/HPLC method collected more PBS than the SPME method. Furthermore the relative levels of PBS in the seven effluent types were different for the two methods. These findings need to be interpreted in terms of actual bioavailability of the substances. In addition, the study highlights that some methodological issues should be taken into account, for example salt may have an impact on the partitioning between the water and the SPME-fibre.

Another example is the use of the SPME approach in an OSPAR Demonstration Programme (Droge, 2001). As part of the overall study of the effectiveness of the methods the substances extracted and identified by the fibres following exposure to an effluent were compared with those accumulated and identified in the freshwater cladoceran *D. magna*. For the study, a dilution series of an effluent was prepared, the PBS concentration in each dilution was measured by SPME and this was then matched to the organic compounds bioaccumulated in *Daphnia* exposed to the same dilution. The daphnids were extracted with hexane and the hexane extract was analysed. As expected the organic compounds from the daphnids themselves were dominant in the extract. However, by looking at specific masses that were also found in the SPME extract a comparison could be made between SPME PBS data and that for *Daphnia* body burdens. The correlation between the response of polyacrylate in the SPME fibre and concentrations in *Daphnia* was found to be statistically significant ($P<0.01$) (Droge, 2001). The study summarised some distinct advantages of the SPME fibre method such as it being rapid, requiring only one day of exposure to test materials, easy to use and predictive (to a degree) of bioaccumulation in whole organisms.

2.3.3 Conclusion on whole effluent assessment testing for bioaccumulating substances

Although several promising screening tests have been developed, testing for substances that have the potential to bioaccumulate is not a simple routine procedure when working with effluent samples. Numerous technical difficulties are encountered in performing the tests and in evaluating test results, as briefly outlined above. It must also be remembered that current bioaccumulation tests applied to effluents are only based on the physico-chemical properties of the components and therefore should only be considered as screening tests for indicating bioaccumulation potential. This implies that any final conclusion on bioaccumulation will have to be based on substance specific information, and would thus include other factors such as the potential for metabolism.

Based on the current state of knowledge on bioaccumulation testing and because this characteristic should only be considered in combination with persistence and toxicity (P, B and T) it is not useful to test bioaccumulation in isolation. The TF therefore recommends that any test on bioaccumulation potential be conducted within an integrated testing scheme to allow a meaningful interpretation (see Figure 8, Section 5.2).

2.4 Persistence

2.4.1 Introduction to persistence of whole effluents

Persistence is of regulatory concern because it increases the potential for long-term exposure and adverse effects either directly or in combination with bioaccumulation even in remote areas. Persistence can be defined as the resistance of a substance to degradation by environmental processes (i.e. biodegradation, hydrolysis and photolysis) – it is the inverse of degradability. Persistence cannot be measured directly, only inferred from continued presence in the environment or the lack of data indicating degradation in the laboratory after extensive experimentation. Ideally the assessment of the potential for persistence in the environment should be based on measured half-life data and include assessment of metabolites.

Criteria for persistence of single substances have been proposed by a number of organisations such as OSPAR, UNEP, EC, Environment Canada (ECETOC, 2003a) and are generally based on degradation half-lives (e.g. >50 days) even though in reality, degradation mechanisms may not necessarily follow first order kinetics, (often they are mixtures of different kinetics). However there is still considerable scientific debate surrounding the justification of how the persistence of a single substance is assessed (in particular, how results from standard tests are interpreted). Furthermore, since industrial effluents are very rarely composed of a single substance it is even more difficult to define what persistence means in relation to effluents (i.e. mixtures). The TF agree with the conclusion that it is incorrect to refer to the 'persistence of effluents' (OSPAR, 2002a). An explanation is therefore needed of what is meant by the determination of persistence in the framework of WEA and how this can be done in a practical and meaningful way. The TF recommends including persistence in a conceptual approach (see Figure 8, Section 5.2).

2.4.2 Methods in use to determine degradation

Standardised tests (biotic and abiotic) are available to measure the degradation of single substances. Knowledge of the abiotic degradation of an effluent should be considered when assessing the overall degradation (persistence). The principal abiotic degradation methods available for single substances are for hydrolysis and photolysis. However, these methods are specific for single substances. In contrast to most biodegradation tests they rely upon substance specific measurements, sometimes including metabolite identification, and consequently their applicability in an assessment of effluent is limited. Organic carbon is not eliminated by hydrolysis or photodegradation; the molecules are only altered. Any such changes to the molecule may be a first step facilitating further bacterial degradation, so in this way hydrolysis may, to some extent, be incorporated in a biodegradation step.

The assessment of photodegradation and a reasonable approach to its inclusion when addressing a chemical's environmental behaviour is more problematic. Nevertheless, in theory a combination of a photodegradation study followed by a biodegradation study, compared with a biodegradation

study on its own, would indicate the potential for this mechanism to affect the environmental fate of some components in an effluent.

None of the standardised ready or inherent biodegradability tests were designed for testing whether or not a substance is persistent, (i.e. ready and inherent tests were designed to show potential for degradation which is very different to potential to fulfil criteria for persistence), nor were they designed to measure the biodegradability of mixtures.

Ready tests

The most frequently used biotic tests are the 'ready' tests which require that a substance undergoes 60 or 70% degradation (depending on the test and endpoint) within ten days of degradation starting (i.e. after the lag phase) to be classified as readily biodegradable. While this approach is assessing whether a substance should be considered as persistent or not (EC, 2003), the misgivings and uncertainties associated with this approach are discussed in detail by ECETOC (2003a). Irrespective of the suitability of using the results of standard ready tests to estimate half-lives, the issue is further complicated by the fact that industrial effluents are mixtures containing a number of unknown substances and hence the pass level assigned for single substance tests is wholly inappropriate.

The TF concludes that ready tests are inappropriate methods to use as the basis for an assessment of whether or not there are persistent substances/fractions in an effluent.

Inherent tests

Inherent biodegradability tests were not designed to determine half-life data needed to assess whether a substance is persistent nor were they designed to measure the biodegradability of mixtures. However they could be used as the biodegradation step to identify effluents that do not require further investigation of their potential to bioaccumulate. The tests may also be helpful in determining the treatability of the effluent components and thus indirectly give some indication of persistence. The tests with potential application for whole effluents will be normally one of the inherent or simulation tests (OECD, 1981a,b, 1992a, 2001). Consideration could be given to the use of adapted inocula for continuous discharge situations.

The most accurate estimation of the persistence of effluent components would be based on knowledge of all the different components present in the effluent. If all the components are known and there is biodegradability data for them all then an estimate of the persistence can be made. This is very rarely the case.

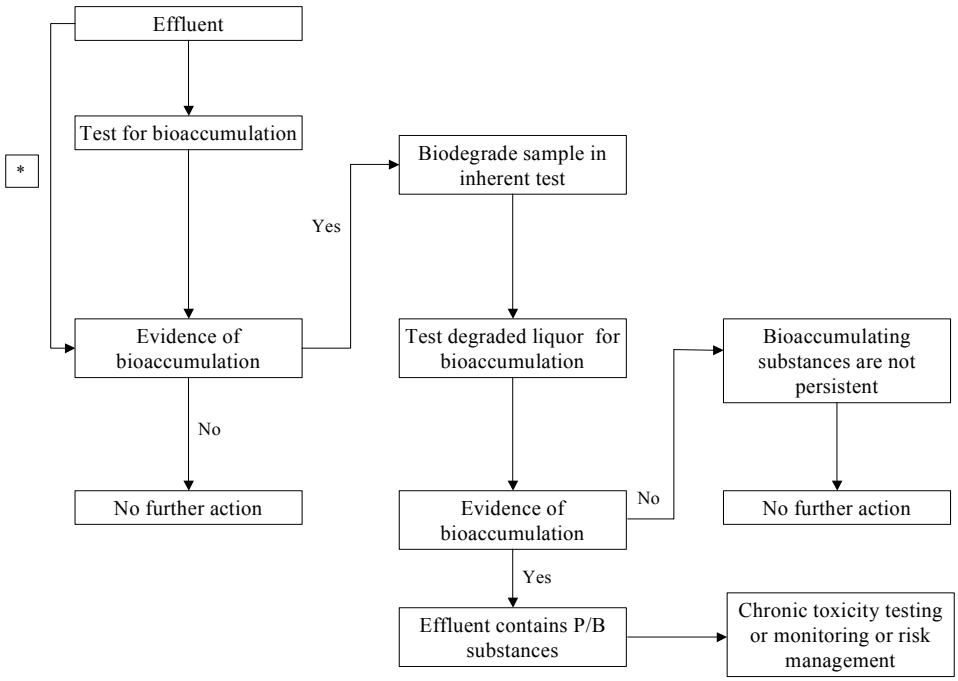
Another approach to estimate persistence combines degradation and chemical analysis. For this purpose a biodegradation test is performed together with chemical analysis of the degraded

stream. Normally all biotreated streams, including those receiving only municipal wastewater, will contain a recalcitrant fraction of organic carbon, composed of natural materials that resist treatment in the plant. Such a fraction is also found in inherent tests, for example in semi-continuous activated sludge (SCAS) tests. To assess whether the undegraded fraction contains hazardous persistent substances it is necessary to identify, by analytical methods, the nature of this organic carbon, i.e. identification of the individual substances. Such an assessment will normally require an effort considerably beyond the scope of whole effluent testing.

2.4.3 Integration of biodegradation testing into WEA scheme

For the reasons given above, unless comprehensive compound analysis can be performed in parallel, conducting tests for biodegradability in isolation from bioaccumulation potential and toxicity is not considered to have any scientific merit. The TF recommends that given the current limitations in the state of the science, the most pragmatic approach would be that inherent tests be used (together with information on abiotic degradation) as the basis for an assessment of whether further studies should be carried out on bioaccumulation potential and toxicity (Figure 4). Confirmation of the predicted P and B/T should be linked where appropriate to some form of monitoring in the receiving environment.

Figure 4: Integration of the assessment and evaluation of 'P' and 'B' within a WEA scheme



* If components of effluent are known

2.4.4 Conclusions

None of the currently available standard biodegradation tests were designed to measure the biodegradation kinetics of mixtures and they are not suitable for such a purpose.

The most effective way to identify possible persistent substances is to characterise the wastewater components from knowledge of the processes and chemicals used at the site, however this often may not be possible, particularly for complex effluents and extensive processing within the site.

For the time being the inherent biodegradation tests seem to have the greatest potential for application in WEA. They can be used to identify effluents that require further studies on bioaccumulation potential and toxicity. However, persistent substances will always be identified in both domestic and industrial effluents and should not be automatically considered as harmful.

The specific characteristics of an effluent discharge (e.g. continuous, intermittent) should be considered when deciding which biodegradation test to use and how to interpret test results.

Due consideration should be given to the contribution of abiotic processes to the overall degradation of effluents/effluent components.

The TF concludes that within the scope of WEA, investigating persistence in isolation is not meaningful. When working with effluents, persistence is only meaningful when tested within the context of bioaccumulation and toxicity.

2.5 Endocrine disruption

Endocrine disruption (ED) is singled out for mention in this report because of widespread scientific concern that conventional risk assessment (traditionally relying on acute studies with fish, algae and invertebrates) may fail to predict chronic reproductive and developmental effects caused by this mode of action.

Endocrine disrupting chemicals are defined as:

- Exogenous substances that cause adverse health effects in an intact organism or its progeny, consequent to changes in endocrine functions (EU, 1997), or;
- exogenous agents that interfere with the production, release, transport, metabolism, binding and action or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and regulation of developmental processes (Kavlock *et al*, 1996).

This area has attracted major and increasing regulatory attention since it became clear in the 1990s that endocrine disruption had the potential for significant disturbance of ecological systems up to the population level (e.g. oestrogenic effluents, organochlorines and tributyltin substances). Moreover, there is scientific concern that current environmental risk assessment methods fail to protect against the potential for chronic (reproductive or developmental) effects due to the limited scope of acute lethality tests periodically used for effluent hazard assessment. Research into the area of improved hazard assessment has increased rapidly and now continues at a significant rate in Europe, North America and the Asia-Pacific region.

It is impossible in a short space to adequately describe the tests available and their various endpoints although a discussion of the subject has been compiled in a draft Background Document, commissioned by the German Federal Environmental Agency, for the OSPAR Intersessional Expert Group on Whole Effluent Assessment (OSPAR, 2003).

The conclusions stated in the OSPAR (2003) document are:

- Endocrine disrupting chemicals cause adverse health effects in organisms or their progeny as a consequence to changes in endocrine functions;
- the regulation of endocrine processes in invertebrates differs considerably from that in vertebrates;
- several *in vitro* and *in vivo* test methods have been developed in order to detect the endocrine-disrupting potency of chemicals, but up to now only draft standard test procedures have been established;
- among the *in vitro* tests the 'E-Screen test', based on the human breast cancer cell line MCF-7 and the 'yeast assay', which measures the induction of gene transcription following hormone receptor activation have been applied to wastewater samples. *In vitro* tests are time and cost-effective and thus appear applicable within whole effluent assessment. However, *in vitro* models only cover single endpoints and have limitations as predictive tools in risk assessment;
- among the *in vivo* tests, endpoints such as gross morphology and induction of vitellogenin in fish and amphibians have been used for wastewater assessment, although the considerable amount of effort involved limits their applicability for routine measurement;
- currently no single test system, which detects specific endocrine-disrupting effects, can be recommended for wastewater evaluation. Work to generate suitable test systems is on-going and OSPAR should monitor developments in this area;
- at the moment OSPAR (2003) recommend incorporating chronic (partial life-cycle and/or reproduction) methods for ecotoxicity testing in WEA, which increases the probability of determining endocrine effects in effluents.

In addition to these conclusions it should be noted that:

Endocrine disruptor research was initiated by the investigation of complex mixtures (e.g. sewage effluents) in Europe, Japan and North America followed by TIE. This led to an initial focus on oestrogenic chemicals in surface waters. However, today this has broadened to include aromatase inhibitors, anti-oestrogens, androgens and related xenobiotics.

While many countries are continuing to address the endocrine disrupting potential of complex effluents, there is now a growing effort toward investigation of single chemical substances on the endocrine systems of aquatic organisms.

The tests used to date to investigate complex effluents (or those that are likely to be adopted in the future after completion of OECD validation) are likely to be significantly more expensive than traditional acute lethality tests. Unless investment is made to develop and validate rapid and cost-effective *in vivo* screens, the widespread use of expensive methods (developed for substance risk assessment) will therefore place a substantial financial burden on those deploying them.

OSPAR proposed two methods for ecotoxicity testing of potential EDs as part of WEA and marine risk assessment:

1. A chronic invertebrate test (partial life-cycle and/or reproduction) using a small scale marine crustacean test (e.g. 21-d copepod life-cycle test) (for example see Hutchinson *et al*, 1999a,b; Breitholtz and Bengtsson, 2001);
2. a 21-d non-spawning fish screening assay using marine or estuarine fish. For example Thorpe *et al* (2000) have developed a juvenile rainbow trout vitellogin screening assay that could be used in such instances (for many years UK laboratories have used these organisms for estuarine testing as they can be adapted to low salinities).

Together, these would give an increased probability of determining potential developmental and reproductive effects due to endocrine disruptors present in effluents and complex mixtures. There is some evidence that both these methods could be abbreviated in the future by the inclusion of new ED-specific endpoints.

To the Task Force's knowledge, no country in the world currently requires that ED testing be mandated for all industrial discharges. However, the pulp and paper industry in North America and Scandinavia is required to address the reproductive (including the ED) issue. More recently, the US State of Indiana has proposed that ED assessments be incorporated into the Whole Effluent Toxicity (WET) programme, while the Environment Agency for England and Wales requires ED screening with fish for at least one municipal discharge (Colchester in Essex).

Examples of endocrine disruptor research on a national scale are exemplified by the LOES project in the Netherlands (RIZA, 2004) and current research in the UK (UK Environment Agency, 2004). In the Netherlands, in 1997-2000, a large-scale baseline study entitled 'National Investigation into Oestrogenic Compounds in the aquatic environment' (Dutch acronym, LOES) was carried out. The LOES project aims were:

1. To investigate the occurrence and sources of various natural oestrogens and xeno-oestrogens in the Dutch aquatic environment including wastewater, drinking water and (inland, estuarine and marine) surface waters;
2. to assess oestrogenic/reproductive impacts on sentinel fish species inhabiting this environment. Target chemicals included: natural hormones; ethinyl-oestradiol; alkylphenols; alkylphenol ethoxylates; bisphenol-A; phthalates; polybromobiphenyls (PBBs) and polybrominated diphenylethers (PBDEs).

Further information on current research being carried out in Europe can be found at: http://europa.eu.int/comm/research/press/2002/pr1505en.html

While standardised tests are now available there is as yet little experience of their use in the field and they are relatively expensive and not easy to carry out. There is also a continued lack of understanding of the implications of naturally occurring endocrine materials in the wider environment. At this moment, therefore, it is believed to be inappropriate to recommend the widespread use of *in vivo* endocrine disruptor testing in a whole effluent assessment programme. There may be specific circumstances where such tests should be considered (e.g. production of known endocrine disrupting chemicals).

2.6 Genetic toxicology

This term covers two areas of toxicity, which are often confused.

Genotoxicity addresses potentially harmful effects on genetic material (e.g. DNA damage and repair mechanisms) which are not necessarily reflected by direct evidence of mutation and are therefore not transmissible and mutagenicity, which refers to the causation of permanent transmissible changes in the genetic material of cells or organisms. The assessment of genotoxicity in the context of ecotoxicity is sometimes referred to as eco-genotoxicology (Wurgler and Kramers, 1992). In contrast to mammalian risk assessment where carcinogenicity is the major driver for genotoxicity testing, in ecosystems scientific concerns are primarily focused around developmental and reproductive impacts (Anderson and Wild, 1994; Depledge, 1994; Jha *et al*, 1996). Focusing on important genetic factors that influence the ecological fitness of a

population, Kurelec (1993) broadened this perspective into the 'genotoxic disease syndrome' that includes several non-cancerous conditions caused by genotoxins.

This area of mammalian toxicology and ecotoxicology has been well studied. A survey report on applied methods and the development of methods and tests for genotoxicity/mutagenicity was prepared by Germany (OSPAR, 2002b) (in conjunction with Hydrotox GmbH, a German consultant) for the OSPAR Intersessional Expert Group on WEA.

In the context of environmental risk assessment, a large number of tests are available using both prokaryotic and eukaryotic organisms both *in vitro* (e.g. using bacteria or unicellular eukaryotes, often genetically modified in some way) and *in vivo* using higher organisms (e.g. plants, invertebrates and fish).

The area of genotoxicity/mutagenicity testing is in general far more mature than that for ED. As applied to microbial (e.g. Ames test) and mammalian systems, there is extensive standardisation and validation of genotoxicity test methodology through the OECD and other organisations. For many years governments and industry have used these systems for the evaluation of agrochemicals, industrial chemicals, pharmaceuticals and other applications of chemicals. However genotoxicity test methods applied to aquatic plants and animals are less developed. The most promising are being developed using the principles established for testing mammalian systems.

The OSPAR report (OSPAR, 2003) illustrates the numerous tests available that have been used in many different studies on many different types of effluent.

Some examples of the tests available are:

Genotoxicity tests

umuC assay and SOS chromo-assay – these assays use the fusion of a reporter gene, which encodes for the production of an enzyme, to part of the cellular DNA repair mechanism of either an engineered strain of *Salmonella* or a particular strain of *E coli*. When the DNA repair mechanism is activated in response to DNA damage the enzyme is produced and the degree of enzyme formation can be used to infer the level of damage response.

Comet assay – In this test, cells exposed to potential mutagens are lysed and the DNA extracted and examined by electrophoresis. Damage to the DNA caused by mutagenic activity results in differential dispersion patters of the DNA in the electrophoresis chamber, which can be assessed to infer the degree of damage caused.

Mutagenicity tests

Ames test and *E. coli WP2* assay – these tests rely on the use of a mutant *Salmonella* strain, unable to grow in the absence of the amino acid histidine or a strain of *E. coli* unable to synthesise the amino acid tryptophan. Mutagenic compounds can cause the reactivation of the ability of the organisms to produce histidine or tryptophan and hence grow in media free of these compounds.

Mutatox assay – this test uses a form of the luminescent bacterium *Vibrio fischeri* that has lost the ability to emit light. Mutagenic compounds can cause this ability to be reactivated and the degree of mutagenic impact measured directly by measurement of light emission.

Eucaryotic tests – There are a number of mutagenicity tests available using eucaryotic cells, many of which use microscopic analysis of genetic material after appropriate treatment or staining techniques.

The Hydrotox GmbH report concluded (*inter alia*) that:

- Bioassays for detecting genotoxic and mutagenic effects provide additional information about the quality of wastewater and should be implemented in WEA;
- no genotoxicity should be acceptable in wastewater samples unless the origin has been explained and further tests show harmlessness of effluents;
- in case of positive genotoxic results in surface waters, from a scientific point of view extensive monitoring programmes should be performed to identify industrial or municipal sources of the genotoxic substances;
- genotoxicity tests should be implemented in discharge permits for those industrial or municipal sectors which are thought to use, process or discharge genotoxic substances;
- a test battery of bacterial (umuC assay or SOS chromo-assay and Ames test) and eucaryotic cells (micronucleus or Comet assay with permanent cell lines or suitable organisms) should be considered.

The critical issue with respect to eco-genotoxicity testing appears to be the environmental relevance or significance of positive genotoxicity/mutagenicity tests carried out on effluents. Many published papers on eco-genotoxicity research have concentrated on the most sensitive *in vitro* or *in vivo* endpoint for a particular situation, without significant discussion on whether the endpoint was of ecological environmental relevance. Despite the lack of understanding of relevance, one genotoxicity test, (the umuC assay), has been applied by authorities in Germany to wastewater discharge consents since 1999. While this method is used in some industries for human risk assessment purposes, the rationale behind this application by German regulators for environmental risk assessment purposes remains unclear.

Whilst the implementation of widespread eco-genotoxicity testing has been defended on the grounds of adherence to the precautionary principle, it should be realised that 'genotoxicity' is a mode of action and not a toxicological endpoint. Moreover, there remains the issue of which tests to use and the lack of understanding of what implications positive results may have. An additional difficulty in the interpretation of umuC and other genotoxicity test results in an environmental context is the fact that there is a natural background of genotoxicity (BUWAL, 1996). For example, in terms of DNA damage, the oxidants produced by normal human metabolism are estimated to be 10,000 DNA hits per cell per day (Ames and Gold, 1990; Ayala and Kiger, 1980). Authors who advocate the implementation of eco-genotoxicity testing recognise the general shortcoming of the tests, i.e. that there is an absence of a quantitative causal relationship with significant genotoxic endpoints at the population/ecosystem level (de Maagd and Tonkes, 2000).

Because of the difficulties in the interpretation of current tests and the lack of understanding of their relevance at population and ecosystem level outlined above, the TF does not recommend the inclusion of *in vitro* genotoxicity testing as a routine component of WEA testing. If there is concern over potential genotoxicity for a given discharge, probably the best way forward would be to use *in vivo* genotoxicity screens validated in terms of their sensitivity to a range of compounds and using biologically significant cytogenetic endpoints such as chromosomal aberrations or micronuclei (Jha *et al*, 1996).

3. CHEMICAL AND BIOLOGICAL MONITORING OF RECEIVING WATERS

3.1 Introduction

WEA is a tool that can be applied to predict effects of effluents in the environment. However, it has limitations. For example, toxicity can be misjudged because bioavailability and fate processes in laboratory tests may not reflect receiving water conditions, and because sampling is usually discrete it is difficult to predict the effects of temporally variable effluents. Even an intensive sampling effort, for example taking 24 h composite samples every month for WEA assessment, would mean the effluent was is being sampled for approximately 4% of the time. In addition, given the state of the science for determining the persistence and bioaccumulation potential of effluents, it is difficult to determine whether effluents may cause long-term effects (Burton *et al*, 2000). Chapman (2000) pursues this point, arguing that WET testing should be used for hazard screening, but risk assessment to check that WET is providing the degree of control and protection envisaged, requires biological monitoring of receiving waters. The advantages of chemical and biological monitoring of receiving waters (field monitoring) are that, by comparison with WEA there is greater realism of exposure conditions and a more diverse biological community can be examined. However, field monitoring has its own limitations and complications, for example, it may be hard to use as a risk management tool for grossly polluted receiving waters, because of the difficulty of attributing toxicity to a particular source or it may be hard to discern subtle effects because of natural biological variability. In addition, impact from non-pollution sources such as the physical quality of the habitat will have a major impact on the biological integrity (Dyer *et al,* 2000). It is therefore appropriate that WEA and field monitoring be viewed as complementary techniques both or either of which are most appropriate in different circumstances. LaPoint and Waller (2000) conclude that field monitoring can augment WET testing to:

- Evaluate major episodic insults to receiving streams;
- evaluate receiving water exposure concentrations that equal or exceed laboratory-derived toxicity levels;
- interpret whether laboratory responses indicate ecosystem impairment;
- increase confidence in an assessment when the receiving water contains particularly sensitive or endangered species;
- understand the effects of effluents that are known to contain components that are poorly evaluated by WET testing.

A strategy for organising such considerations is suggested in Section 3.2.6.

Field monitoring can include:

- Taking samples from the environment to use in laboratory bioassays (ambient toxicity testing in US parlance - see Appendix B);
- conducting *in situ* bioassays;
- monitoring biological communities in the receiving water/sediment;
- chemical analyses for specific chemicals and/or generic water quality parameters to:
  - validate fate modelling;
  - understand the potential impact of bioavailability.

Field monitoring, with respect to WEA, involves comparison of samples from target sites receiving effluent with samples taken from the same location at another time (e.g. before and after discharge), or with samples from a reference site not impacted by the discharge being investigated (e.g. upstream of the discharge) or with samples from a site presumed to be pristine. Samples may be compared in terms of analyte concentrations to validate persistence and bioaccumulation modelling, in terms of toxicity, or in terms of structure of the biological community to validate toxicity tests. Biological community structure may either be compared to reference sites or to pollution indices derived from the species typically observed in sites with similar hydrology, topography, geomorphology and exposure to different types of pollution. Monitoring usually involves repeat sampling of specific localities, but for the purpose of WEA, limited sampling (a survey) may be sufficient. Monitoring may also be performed that is unrelated to specific discharges. Such so-called 'condition monitoring' (or 'state-of-the-environment' monitoring, or surveillance monitoring in the WFD) is based on the premise that understanding of the impact of stressors on the environment will be incomplete. Understanding may be inadequate regarding: stressor interactions; mechanisms of action; bioavailability; discharges (especially diffuse discharges). Thus, condition monitoring acts as a safety net for more focused monitoring and it may identify a need for further investigation (forensic ecotoxicology). Condition monitoring does not specifically address WEA, but the methodology used is applicable to *in situ* toxicity and biological community monitoring and so some examples are given. Condition monitoring is also addressed by ECETOC (1999).

3.2 Methodologies

3.2.1 Pre-study considerations

The design of any monitoring study needs careful up-front consideration of objectives. Common objectives will be:

- Determination of water quality status (e.g. as could be implemented under the WFD). This can be considered equivalent to condition monitoring as discussed later;
- determination of driving factors and causation in environments that receive effluents (e.g. partitioning effects of point versus diffuse pollutant sources, Suter *et al,* 2002);

- validation of WET testing predictions (Diamond *et al,* 1999). In this context, validation needs to be viewed in terms of clarifying the expression of toxicity under receiving water conditions, rather than supporting or disproving the results of effluent testing;
- spatial tracking to validate fate modelling and role of fate processes in pollutant exposure (e.g. degradation, dilution, transformation, volatilisation, and sorption).

Such considerations will drive the type of samples (chemistry, biology, environmental compartment) to be taken and the position of sampling. In practice, a monitoring study may have multiple objectives and different methodologies may address more than one objective. Indeed, to derive the best information about the environmental condition, and to track changes in the environment, a variety of sampling and monitoring techniques may be required (SPMD, caged mussels, large volume *in situ* sampling, collection of zooplankton) (Durell *et al,* 2000; Johnsen *et al,* 1998; Utvik *et al,* 1999; Utvik and Johnsen, 1999).

The importance of bioavailability of substances in receiving waters can only be assessed, via monitoring, when an appropriate environmental characterisation has been made and the monitoring methods have been very clearly designed. This arises, for example, with many metals because they interact with ions and particulates in the environment and the fate, behaviour and effect of such metals is altered by these interactions (Hall and Anderson, 1995; Brown, 1968; Birchall *et al,* 1989). What defines an appropriate environmental characterisation, will depend upon the substance. For example to understand the behaviour of chromium it would be necessary to include iron and manganese measurements (Fendorf and Zasoski, 1992; Nakayama *et al,* 1981), whereas zinc would not interact with these metals and thus the measurement would be unnecessary.

3.2.2 Sampling frequency

The frequency with which samples are taken will depend on the objectives of the monitoring (ECETOC, 1999). If the objective is to understand steady-state exposure conditions, then sampling frequency will need to consider:

- Seasonal variation (for example flow rates, where variability may justify repeat sampling in different seasons);
- variability in discharge patterns which will be process-specific but may be critical especially for batch production processes.

To understand whether the biological community in the receiving water has been impacted, sufficient numbers of samples need to be collected to take account of special and temporal variability in the community. Spacie (1986) concluded that dynamic models of exposure are needed to determine if WET-tested effluents are having an impact on receiving waters. Temporal

variability needs to take into account all of the points listed above with respect to exposure and additionally:

- The rate at which the community may compensate, through immigration or species' intrinsic rates of increase. This will be especially important in the case of batch discharges;
- ambient seasonal variation in community composition. The important issue is to ensure that exposed communities are sampled at the same stage of seasonal development as control communities. Thus, if a community is to be compared with historical, pre-discharge data, the equivalent stage of seasonal development should be sampled.

In practice, the above factors will need to be considered for condition monitoring, but sampling for regulatory compliance may be dictated by the timing of discharges, especially if they are intermittent.

3.2.3 Sampling position

The issue of where to take samples relates to both the zone of potential impact and to the choice of a control site.

Monitoring for regulatory purposes should involve sampling the potentially impacted waters at the edge of the discharge mixing zone. Comparing the results of WET testing with monitoring at the edge of the mixing zone requires care, since a number of factors related to the receiving environment can influence the toxicity of the effluent components. The assumption is that the toxicity of the effluent in the receiving environment can be predicted on the basis of dilution as occurs in the WET test when calculating the volume percentage of effluent that causes an effect). However, the receiving water properties may not be the same as the diluent media used in the WET tests and processes such as sorption and volatilisation which can occur in the receiving environment are not normally taken into account in WET testing. These processes have however on occasions been addressed (Belanger *et al,* 1988).

The choice of a control site is determined by the objectives of the monitoring and in particular the trigger for risk management action. For example, action might be triggered if the community differs from that present at a benchmark site with equivalent hydrology, topology and geochemistry. The benchmark site is often selected as representing a pristine or near-pristine environment and the aspiration is absence of pollution. Alternatively, action might only be taken if a change in the community is attributed to a particular discharge. Under this latter scenario a discharge is acceptable as long as it doesn't lead to further deterioration of the biology of the receiving water, i.e. the aspiration is for improvement to the biological condition of receiving waters. Here, the relevant control samples would be taken upstream of the discharge, with, ideally, pre-discharge sampling to show that the downstream and upstream communities are equivalent. Establishing benchmark conditions is a requirement of the WFD, although here the objective is not a pristine or near-pristine environment (termed a high quality habitat), except for

those waters already having this status, but good ecological status (the next category down regarded as showing 'low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions').

The objectives of 'absence of pollution' and 'improvement to the biological quality of receiving waters' are recognisable in OSPAR's policy and have contributed to a move within OSPAR to more biological effects measures for tracking the state of the environment. The proposed use of biomonitoring in the context of WEA has stemmed from this approach and its use is being given serious consideration. Sampling locations have yet to be clarified but there will be a need to identify representative locations in the same manner as for the WFD.

3.2.4 Fate (exposure)

Monitoring may be required to validate various elements of fate/modelling, bioconcentration, dispersion/dilution and loss mechanisms such as photolysis, biodegradation, volatilisation, sorption and transformation. The following list of available techniques is discussed below: specific analyte measurement; water and sediment quality (measurement of water chemistry parameters other than specific analytes); SPE techniques; biomarkers, tissue analysis including caged mussels/fish; dye studies.

Specific analyte measurement

While an advantage of WEA is that there is less need to focus on specific chemicals, there will be occasions when specific measurements will be very helpful in monitoring studies and hence in interpretation of WEA data. For example, using specific analytes it is possible to address background levels (upstream versus downstream measurements) and dilution/current effects. When considered along with other environmental measurements, including suspended solids, sediment and biota analyses etc., it may also be possible to address bioconcentration and degradation of components of effluents, depending upon their similarity to the analyte used.

Water and sediment quality (measurement of chemical parameters other than specific analytes)

The natural background levels for physical, chemical and biological parameters fluctuate within and between regions. Knowledge of the natural background levels and how these fluctuate in time and space is desirable to identify the presence and the extent of influence from effluent discharges. Parameters that may be monitored include pH, dissolved oxygen, alkalinity for water and colour, smell, organic matter content and distribution of particle size for sediment.

SPE techniques

For some situations it may be possible to use non-specific methodologies. One example of such a method is the assessment of effluents and receiving waters using SPME. This approach has been used to assess baseline toxicity of effluents, waters and sediments (Leslie *et al*, 2002; Parkerton *et al*, 2000), and is of particular value where the main source of the chemicals is from petroleum products. The approach depends upon the relationship that exists between the log K_{ow} of a chemical and its potential to bioconcentrate and the ecotoxicity exerted as baseline toxicity. The method relies on the partitioning of the chemical onto the SPME fibre that is also related to the chemicals log K_{ow}. The chemicals are desorbed and using chromatography to obtain a molar mass and the hydrophobicity of the chemicals adsorbed to the SPME. It is possible, from these data, to estimate the potential toxicity of the sample being assessed.

Biomarkers

Biomarker is defined in the present report as any biochemical, physiological, or histopathological indicator of exposure or response to a contaminant by individual organisms (Van Gestel and Brummelen, 1996). This use of the term includes the response of almost any kind of bioassay measured from portions of a single organism, including contaminant receptor molecules, biochemicals (e.g. detoxification enzymes), blood, bile, and tissues (e.g. liver tissue). A bioindicator or ecological indicator represents organism responses at the population-level or higher, thereby restricting biomarker to its more widely accepted organism and sub-organism use (Van Gestel and Brummelen, 1996).

Biomarkers have been widely used to monitor contaminant impacts in ecosystems. Biomarkers often respond to chemical mixtures with a single response, which eliminates the need to investigate assumptions of additivity when interpreting the significance of multiple stressors. In most cases, samples are collected along gradients of predicted chemical contamination, with biomarker responses being correlated to exposure concentrations. Most biomarkers are considered as biomarkers of exposure, which is used to indicate either the presence of a contaminant in biological tissues/organs or that some biochemical receptor or site of potential action has responded to the presence of the contaminant (e.g. Kloepper-Sams *et al,* 1994). Few biomarkers represent biomarkers of effects, enabling quantitative and mechanistic understanding of an adverse biological response or clinical sign of stress/disease in an organism or population. As the field of genomics develops this situation is likely to change. There has been substantial progress in developing biomarker response methods to assess pollution in marine benthic systems. Many of the techniques used have been developed through practical workshops (Bayne *et al*, 1988; Addison and Clarke, 1990; Stebbing and Dethlefsen, 1992; and Table 7). These methods have now been incorporated in national and international monitoring programmes and have contributed towards a framework for general and contaminant-specific monitoring (JAMP, 1997, 2002). There is less agreement on biomarker methods to assess impact of contaminants in

pelagic ecosystems but some methods are being investigated under the BECPELAG programme[a] (Hylland, 2000; Hylland *et al,* 2002a,b).

Even though biomarkers are used in general monitoring surveys, limited information is available on biomarkers as supplements to whole organism responses in whole effluent toxicity tests. One exception is Choi and Meier (2001) who evaluated a fish DNA damage assay and compared responses to metal-plating wastewater effluent in tests with Microtox, *C. dubia*, and *Pimephales promelas.* They showed correspondence within a factor of 0.5.

Even greater debate remains on the interpretation of most biomarkers regarding their relevance to interpretation of effects on populations. A need still exists to extend standardisation and interpretive guidance to most biomarkers, especially in a regulatory context. Consequently, biomarkers should not be used in isolation, but as another tool to probe the presence of selected contaminants known to be associated with specific exposure profiles and in concert with well-accepted measures of biological impairment such as population and community measurement endpoints (taxonomic richness, species diversity, abundance) (Lam and Gray, 2003).

Tissue analysis including caged mussels/fish

Analysis of tissues from caged or wild-caught invertebrates or fish may provide an indication of exposure to effluent components (Chappie and Burton, 2000). This information may be valuable to determine potential for bioconcentration through the foodweb. The information may also be combined with indications of biological fitness, for example through Scope for Growth tests which measure mussel growth (Widdows *et al*, 2002). However, for monitoring to be useful, it is important that the limitations of analytical methods, choice of species (sentinel versus indicator), duration of exposure and caged versus free- swimming animals be understood.

Dye studies

The main use and benefit of dye studies in monitoring is to establish currents, flow patterns and dilution of water bodies. They are also a useful technique for validating dilution or site-specific models.

[a] BECPELAG – ICES multi-national, multi-discipline biological effects monitoring workshop. During seven research cruises in 2001, pelagic organisms were collected in order to assess the ability of selected methods (field sampling, caging and *in situ* extraction/bioassay testing) to detect biological effects of contaminants in pelagic ecosystems under uniform and standardised conditions.

Table 7: Biomarker techniques used for assessing pollution in marine benthic systems (mainly based on the BECPELAG steering group's recommendations)

Chemical family	Organism	Method(s)	References
PAH* and other synthetic organics (nitro organics etc.)	Fish	Bulky DNA adduct formation**	Dunn *et al*, 1987; Varanasi *et al*, 1989a,b; Maccubbin and Black, 1990; Liu *et al*, 1991; Stein *et al*, 1991; Johnson *et al*, 1998.
Organophosphates and carbamates	Fish Bivalve molluscs	AChE-inhibition** AChE-inhibition**	Bocquené *et al*, 1990; Finlayson and Rudnicki, 1985; Burgeot *et al*, 1993; Galgani *et al*, 1992. Bocquené *et al*, 1995.
Metals (Zn, Cu, Cd, Hg, Pb)	Fish *Mytilus* sp. Fish	Metallothionein induction** Metallothionein induction** ALA-D inhibition* (26-27)	Hogstrand and Haux, 1990, 1992; Killie *et al*, 1992; Chan *et al*, 1989; Hylland, 1999. Roesijadi *et al*, 1988; Viarengo *et al*, 1997. Hodson, 1976; Schmitt *et al*, 1984.
Planar organic contaminants (PAH, planar PCBs, dioxins)	Fish Fish	EROD or P4501A induction** PAH bile metabolites	Burke and Mayer, 1974; Eggens and Galgani, 1992; Galgani and Payne, 1991; Courtenay *et al*, 1993; Stagg and McIntosh, 1998; Kraemer *et al*; 1991. Ariese *et al*, 1993; Stein *et al*, 1993.
General	*Mytilus* sp. Fish *Mytilus* sp. Oyster Fish (Flounder)	Lysosomal stability** Lysosomal stability Lysosomal neutral red retention** DNA expression array	Ringwood *et al*, 1999. Köhler, 1991; Lowe *et al*, 1992; Moore, 1990. Ringwood *et al*, 1998.
Oil sands sediment	Fish	EROD	Tetreault *et al*, 2003

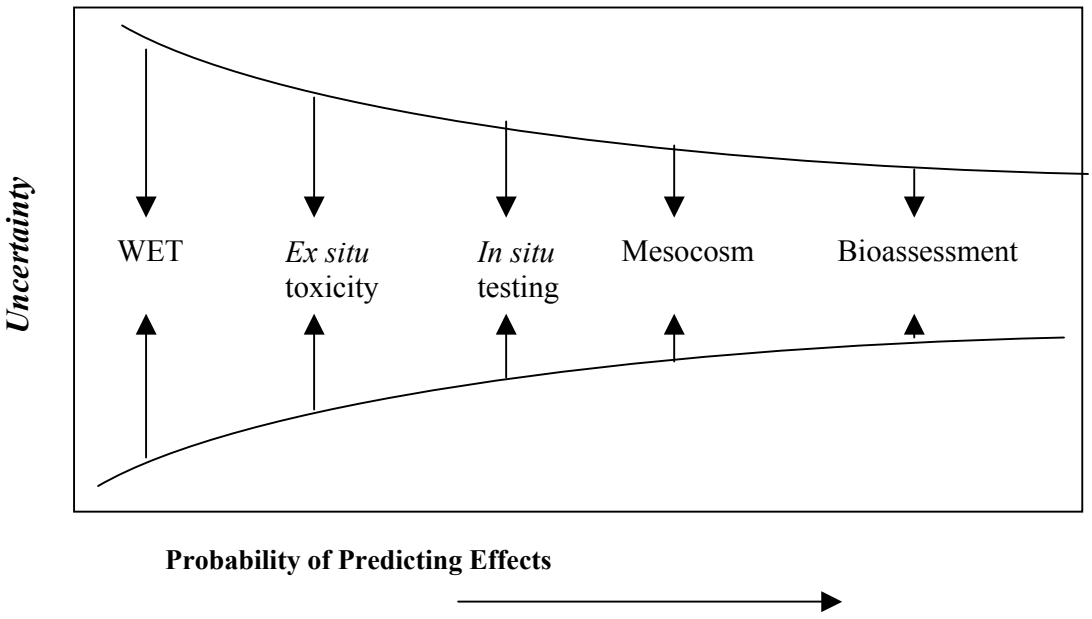
*PAH: Polynuclear aromatic hydrocarbons

**QA – method has been subject to an international Quality Assurance activity and/or inter-calibration.

3.2.5 Biological effects

Biological effects monitoring falls into several broad categories, each applicable to both water and sediment, *ex situ* (or ambient) monitoring, *in situ* monitoring, and biomonitoring. The sequence shows increasing probability of predicting effects (Figure 5).

Figure 5: Predicting receiving stream impacts from effluent discharge (after Waller et al, 1996)



Ex situ toxicity monitoring

In its simplest form, samples of receiving water or sediment are taken and used in the sorts of assays described for WET testing in Chapter 2. However, the toxicity of sediment removed from the receiving waters may also be assessed. Suitable methods are shown in Table 8.

Table 8: Sediment tests

Ecotoxicity Test	Endpoint	Applicability to WEA	Test Guideline/ Reference*
Freshwater invertebrate, *Hyalella azteca* and *Chironomus tentans*	Survival and growth	Applicable	OPPTS 850.1735
Marine invertebrate and marine amphipods	Emergence, survival and growth	Applicable	OPPTS 850.1740
Chironomus sp (freshwater midge)	Emergence/ survival 28 days	Applicable	OPPTS 850.1790 OECD 218 (spiked sediment) OECD 219 (spiked water)
Gammarus sp (freshwater shrimp)			OPPTS 850.1020
Marine amphipod e.g. *Corophium volutator*	10 day survival	Applicable. Test organism collected from wild hence possible availability issues	ASTM E 1367 EPA 600/R-94/025 PARCOM Part A
Freshwater amphipod, e.g. *Hyalella azteca* (shrimp)	Survival, growth, brood production ≥28 days	Difficult. Highly labour intensive and variable chronic endpoints	ASTM E 706-00
Lumbriculus variegatus	Survival, growth, reproduction	Reproduction is asexual and shows high variability	Phipps et al, 1993
Genotoxicity using *Salmonella typhimurium* microsome	EC_x	Ecological relevance concerns	ISO/CD 16240
Genotoxicity using umu test (*Salmonella typhimurium*)	EC_x	Ecological relevance concerns	ISO 13829

* See Section 'List of guidelines' for details

Differences between WET and *ex situ* test results can be caused by differing concentrations of food in the tests (causing differences in reproduction, disease or chemical persistence) or by differences in water quality parameters (Waller *et al*, 1996).

In situ toxicity monitoring

In situ toxicity monitoring offers significant advantages in that it addresses discontinuous discharge situations and is less affected by habitat structure of the receiving water. Nevertheless, *in situ* testing is not employed as commonly as it should be (LaPoint and Waller, 2000).

In situ pelagic tests are usually confined to fish (for example, as in caging studies) because fish can be restricted in a spatial context (Chappie and Burton, 2000). Recently, caged fish studies have been extensively used in research to identify effects due to endocrine disruption in wastewater effluents (Sheahan *et al,* 2002). Cage studies have the advantage of ensuring

organisms are exposed to effluent, removing complications that may be introduced by historical contamination of sediment or impaired habitat (Waller *et al*, 1996); however, their use and interpretation should still be considered cautiously. While a caged fish may be static in its position, effluent plume location and strength can vary making it difficult to quantify exposure. It should also be recognised that *in situ* fish studies may overestimate effects because fish are unable to avoid contaminants or periodically enter cleaner water where depuration processes could remove toxins. Furthermore, caged fish may not have access to their usual diet which may have important consequences. For example in the EDMAR studies reported by Matthiessen *et al* (2001), experiments with male flounder showed that they did not produce vitellogenin (VTG) when caged in oestrogen-contaminated estuaries. However, flounder experienced mild VTG induction when fed on mussels (*Mytilus edulis*) that had been held in an oestrogen-contaminated estuary (the Tees) for 3 months.

At present there exist few internationally accepted standardised methods that can be used to monitor biological effects related to discharges to sea from the petroleum industry (JAMP, 1997, 2002). However, development and testing of *in situ* techniques has been included as one of three components within biological effect techniques of the BECPELAG programme (Hylland, 2000; Hylland *et al,* 2002a,b). Guidelines for environmental monitoring in the vicinity of offshore petroleum installations (OSPAR, 1990) and JAMP Guidelines for monitoring contaminants in sediments (JAMP, 1999) should be referred to for detailed information on offshore sediment monitoring methods.

The vast majority of freshwater biomonitoring studies of effluents have addressed responses in the benthic environment and sediment. Bivalves have been heavily used in this regard. Belanger *et al* (1990) evaluated growth patterns of the Asiatic clam (*Corbicula fluminea*) exposed to power plant cooling tower blowdown containing heavy metals. Growth impairment, reduced survival, and copper and zinc accumulation were highly correlated with water column and sediment contamination as well as reduced health of benthic communities (Cherry *et al*, 1991; Clements *et al,* 1992). Chappie and Burton (2000) reviewed the use of *in situ* fish, bivalve, and other invertebrates such as cladocerans, chironomids, and amphipods in assessment of sediment contamination. Test species and exposure systems have been designed to identify stressors, determine bioaccumulation and understand episodic events.

Biomonitoring

Biomonitoring is practiced quite extensively although many of the programmes are best classified as condition monitoring since they are not tied to assessing the impact of particular effluents. For example, in the UK there are more than 1,000 condition biomonitoring studies. (Depledge, 2002). In its simplest form, species diversity monitoring involves taking samples of water or sediment

using methods that minimise disturbance or loss of the biota. Nets, core samplers and artificial substrates may be used. The biota are then counted and the presence/absence or abundance of specific taxa or of all taxa is analysed. Species diversity monitoring of open freshwater or pelagic habitats is less frequently performed than for benthic systems. This has been primarily due to the types and location of discharges, difficulty in sampling these habitats, and the unrestricted movement of organisms into and out of potential zones of influence. However, species diversity monitoring of freshwater pelagic habitats has been useful for long-term condition monitoring. Excellent examples of this type of monitoring include long-term tracking of water quality changes in the Laurentian Great Lakes. Carrick *et al* (2001) evaluated historical trends from the 1970s to present in Lake Michigan phytoplankton to interpret species composition trends related to reduction in phosphorous loadings, invasion of exotic species, and important changes in food web structure. Similar surveillance and trending assessments have been performed for crustacean zooplankton (Barbiero *et al*, 2001) and fish (Brazner and DeVita, 1998). Attention to fish species has focused on bioaccumulation of organic residues and populations of key forage and predator species such as sculpins and salmonids.

Species diversity monitoring to determine the effects of pollution often combines the presence/absence of taxa (less commonly their abundance) in a sample with their known pollution sensitivity to determine a pollution index score. Such indices are most frequently developed for macroinvertebrates, due among many reasons to their low mobility compared to many fish, high population densities, differential susceptibility to pollutants and ease of taxonomic identification at least at higher taxonomic levels. However, other groups have been used, for example diatoms. Most pollution-based indices were derived primarily for organic pollution and the score attributed to groups may be inappropriate for other types of pollution, for example, Plecoptera (stoneflies) are very sensitive to organic enrichment, but relatively insensitve to metal pollution.

In the UK, the ecology and pollution tolerance of aquatic macroinvertebrates can be used to provide a quantitative measure of the pollution in waterbodies of different types. To assess the pollution status of a waterbody, macroinvertebrate kick-samples are taken, taxa are identified and the reference scores attributed to each of the 82 groups included in the Biological Monitoring Working Party (BMWP) Score system are summed. Two indices of site condition are derived, the number of groups present (richness of taxa) and the average pollution tolerance of the groups (Average Score Per Taxon, ASPT). These scores are then compared with the score expected for that type of waterbody if unpolluted, based on the RIVPACS (River Invertebrate Prediction and Classification System) methodology. RIVPACS contains statistical tests that estimate the probability that a change in score is due to chance (www.defra.gov.uk/wildlife-countryside/cs2000/07/03.htm)

The BMWP score system was developed in the UK, but similar approaches have been used elsewhwere, for example, Indice Biotique (France), Belgian Biotic Index, Chutter's Biotic Index

(South Africa), Hilsenhoff's Biotic Index (USA). Australia has adapted RIVPACS to produce AusRivAS www.lifesciences.napier.ac.uk/wmfiles/101/L9.htm

If samples are taken upstream and downstream of a discharge, or before and after discharge, indices can be compared that do not make *a priori* assumptions about the pollution tolerance of taxa, although their interpretation may very well do so. Such measures include diversity indices such as species richness and evenness, and similarity/loss indices such as the Bray Curtis Index.

An alternative approach to assess the biological condition of a waterbody has been proposed by Karr *et al* (1987). The approach defines an array of measures (also commonly called metrics) that individually provide different types of information on biological attributes at the individual, population, or community level which, when integrated, indicate the overall condition of a system. No one single metric is capable of detecting or discerning all forms of degradative activity so the combined information provides a broad-based indicator of biological integrity. These multi-metric indicators of biological conditon have been combined with biogeography and regional landscape models to define thresholds for water protection goals and attainment status in numerous states of the U.S. (Simon and Davis, 1995).

Depending on the management goals of biological monitoring activities, different statistical approaches of varying robustness and validity may be employed. Therefore, it is imperative that the purposes and goals of the monitoring be understood. Biological variability requires special consideration in the context of biomonitoring. Without a proper accounting for variability in the design of a field-monitoring programme the objectives of the work may be jeopardised. The ecological protection goals and endpoints chosen for assessment should be explicitly known. Based upon these choices, the level of variability in an endpoint should be used to project minimum sampling intensity required to measure an agreed level of protection (or discernment of a level of impact). Study of spatial variability within the ecosystem being monitored should address micro- and macro-habitat features that influence biological sampling and results. These features will be specific to the system being sampled and vary tremendously from small freshwater streams to larger rivers, lakes, estuaries, and oceanic environments. Temporal features and variability become especially important in the consideration of long-term sampling programmes where the need is to establish trends of improvement or degradation. For example, Carpenter and Kitchell (1987) demonstrated that fish year-class strength varies widely year-to-year with strong year-classes of predators driving food-web dynamics for years. Spatial scale also influences interpretation of trends and causative factors such as that observed in the Tees bay and estuary, UK (Warwick *et al*, 2002). Clear identification of measurement endpoints may range from protecting specific target species (e.g. endangered taxa) to integrated community structure attributes (diversity, richness, trophic structure, etc.). Endpoints will differ in their fundamental variability. Often aspects of population and species-level measurements vary much more than community structure thereby influencing the sampling needed to meet management goals.

The need to consider statistical power with regard to meeting the goals of any programme is well established in the biomonitoring literature. Statistical power is the ability of a test statistic to detect a true difference between two treatments. In biological monitoring this is fundamentally important as it relates to the probability of obtaining a Type II error (i.e. a failure to detect a real environment effect when one is present). Johnson (1998) examined environmental perturbations in 16 Swedish lakes and determined that statistical power varied by habitat, measurement endpoint (metric), and type of pollution. Carlisle and Clements (1999) identified taxonomic richness indicators as more powerful and sensitive from a larger suite of metrics that were more heavily influenced by spatial, seasonal, and annual patterns. In many of these and similar discussions, taxonomic resolution and proficiency is a central issue. Increased resolution of the taxonomy employed in biomonitoring is well known to increase the richness of information provided in the assessment (Lenat and Resh, 2001). At the same time, variability and power are often not optimised at the lowest level of resolution and depending on the goals of the biomonitoring use of family or higher taxonomy may be an appropriate option (Ammann *et al*, 1997). In the end, cost, availability of credible and proficient taxonomists, the type of system, and protection goals should be considered in determining the level and extent of taxonomic resolution employed.

In recent years, evidence has accumulated that indicates the importance of dissociating the influence of effluents from other environmental changes, habitat alterations, and non-point perturbants in the interpretation of biomonitoring data. Long-term monitoring programmes (those that exceed 10 years) have demonstrated a great deal of value in this regard (Warwick *et al*, 2002). Because population and community responses are determined by local micro- and macrohabitat conditions as well as regional ecosystem health, great care is required when interpreting monitoring results (Dyer *et al,* 1998; Dyer and Wang, 2002). A well-constructed biomonitoring programme, therefore, must not only consider the biological sampling needs, but also a whole host of habitat and chemical factors that can be used to provide the context for the specific localised interpretation that extend well beyond characterising the effluent(s) in question.

A suite of statistical approaches is available for biological monitoring. Whereas univariate approaches are superior for toxicity testing where one variable is being manipulated and associated with a small number of response variables, the use of multivariate techniques has been shown to have distinct advantages for complex field datasets. Biomonitoring results may be probed by univariate statistics as well, but most often information is lost and the potential for over-interpretation of individual measurement endpoints is introduced. Experimental design considerations are known to be especially difficult with biomonitoring and issues regarding replication and pseudo-replication must be considered. Communication of results through multivariate statistics has been especially difficult for biomonitoring practitioners to the regulatory community (Giddings *et al,* 2002) as the explanations are often not intuitive or obvious. Yet, these statistical tools (cluster analysis, multidimensional scaling, ordination,

canonical correlation analysis, discriminant function analysis and the like) have clear advantages for using all the data available to provide maximum interpretive power. Further, the difficulties with communicating results through these tools should not be a reason for not performing biomonitoring in the first place. Monitoring of natural systems is the means to place toxicity tests into the correct framework, results obtained by biomonitoring are real results, and thus extrapolation to the ecosystem of concern is unnecessary.

3.2.6 Method integration

In practice it has proven most effective to use a suite of different techniques to determine the effects of effluents on receiving water communities. Several frameworks for such integration have been developed.

Scroggins *et al* (2002a,b, 2004) describe the Canadian Environmental Effects Monitoring (EEM) methodology that integrates sublethal WET tests, chemical measurements in the effluent, the receiving water, the sediment and fish tissue, *in situ* caged testing and bioassessment of receiving water sediment and pelagic communities. The approach has been successfully used with effluents from the pulp and paper and metal-mining industries to determine zones of community impact around discharge of effluents from these two industries.

To improve the environmental impact assessments of sediment the Sediment Quality Triad approach and concept, which is an effect-based approach to describing sediment quality using toxicity testing, chemical analyses, and measures of *in situ* benthic community structure has been developed (Chapman, 1992). This approach relies on combining assessment of the intrinsic toxicity of sediments with chemical analysis and benthic diversity in seabed surveys. The Triad approach was tested in European waters for the first time by it originators as part of a workshop, The Bremerhaven Workshop (Chapman, 1992; Chapman *et al,* 1992; Stebbing and Dethlefsen, 1992). The overall aim of the workshop was to compare available biological effects monitoring techniques for marine pollution. A secondary aim was to determine the most suitable techniques for such monitoring in Europe. Toxicity of sediments to amphipods (*Rhepoxynius abronius*, *Corophium volutator and Bathyporeia sarsi*), polychaete (*Neanthes arenaceodentata*) and bacteria (Microtox) and toxicity of pore waters to bivalve larvae (*Crassostrea gigas*) were assessed in *ex situ* monitoring during the workshop. Based on the results from this assessment, amphipod mortality and oyster larvae (*Crassostrea gigas*) abnormality test techniques were recommended for use in Europe (Chapman *et al,* 1992). The TRIAD approach has been widely used in assessment of sediment quality (Chapman and Wang, 2001; Del Valls *et al*, 1998; Borgmann *et al*, 2002). For example, in a recent Dutch study (Lahr *et al,* 2003), the approach was used to check if observed toxicity in sediment bioassays can be explained by routinely monitored priority pollutants. Standard acute bioassays were carried out with the bacterium *Vibrio fischeri*,

the rotifer *Brachionus calyciflorus* and the anostracan *Thamnocephalus platyurus*, together with chronic standard tests using *Daphnia magna* and larvae of the midge *Chironomus riparius* on a large number of samples with different degrees of contamination taken at various locations in the Netherlands. Most toxic effects observed could be partly explained by toxic concentrations of known persistent priority pollutants, mainly heavy metals and occasionally PAHs. In some of the samples, ammonia toxicity was a confounding factor during testing (Lahr *et al*, 2003).

It is evident from the foregoing discussion that it is very difficult to be prescriptive about the conditions under which monitoring should be used as part of an effluent assessment programme, since its value and the form it should take will be determined by site-specific conditions. Professional judgement and consultation with regulatory authorities will be needed, but a decision to include monitoring is more likely if the receiving water is pristine (rather than contaminated), high in biodiversity or used for amenity purposes (rather than industrial ones) or has a low flow rate. Similarly, a decision to include monitoring in a WEA programme is more likely if the composition of effluents being discharged is highly variable or if the whole effluent toxicity PEC/PNEC is close to one.

3.2.7 Confounding factors, the influence of historical contamination

In some of the investigations previously cited it was difficult to relate effects caused by current discharges to those seen in the field monitoring studies. One of the main complicating factors is the role played by contaminated sediments. Sediments can form a repository for anthropogenic chemicals and, in addition to exhibiting direct toxic effects to benthic organisms, chemicals released from sediments have potential to cause detrimental effects to organisms in the overlying water. This can either be by release of contaminants from the sediment or via secondary poisoning (consuming benthic fauna containing toxic bioaccumulative chemicals). Consequently, there is potential for historic sediment contamination to cause adverse effects in the receiving water even though the inputs from currently discharged effluents are essentially 'clean'. This was felt to be the case in the River Aire assessments undertaken in the UK DTA programme where initial WET and chemical tests of effluents did not appear to relate to the poor biological class of many sections of the river (UK WIR, 2001). The influence of sediments was also seen by Hartwell *et al* (2000) in their studies on the South River, Maryland, USA. Their work demonstrated the importance of sediment studies since they revealed that compared to water column tests, which indicated only low-level toxic effects, the sediments in the upper stations of the South River demonstrated significant toxicity to animal (but not plant) species tested. A third example in the marine environment is provided by the programme on endocrine disruption in the marine environment (EDMAR) which was set up in the UK in 1998 to investigate in more detail the implications of earlier observations of strongly oestrogenic effects in flounder (*Platichthys flesus*) from several estuaries. In an overview of the results obtained from this programme

Matthiessen *et al* (2001) described how a TIE scheme of sewage effluents was conducted to identify substances considered likely to cause the adverse effects seen in the flounder. The EDMAR programme indicated that the majority of oestrogenic activity in sewage effluents and estuarine waters was attributable to oestradiol, although a small proportion is being caused by other natural steroids, as well as synthetic substances such as nonylphenol. However, the EDMAR studies indicated that the overwhelming majority of the oestrogenic activity in the investigated estuaries (Tees and Tyne) was found in the sediments. Owing to the complexity of undertaking sediment analyses most of the sediment-bound activity could not be identified but little appeared to be attributable to oestradiol.

3.3 Role of biological and chemical monitoring in receiving waters

Chapter 3 describes options for chemical and biological monitoring in receiving waters and illustrates that there are many options available involving chemical monitoring of specific analytes to track fate and distribution, biomarker monitoring to determine organism exposure, *in situ* toxicity tests, taking of samples for *ex situ* toxicity tests and survey of biological communities to validate the effects of WET testing. A few examples of application of these methods are given in Chapter 4 followed by conclusions including a scheme that describes, as a flow-diagram, the series of choices that need to be considered in deciding whether to monitor the receiving water.

A comparison of WEA testing and the various field-monitoring techniques illustrates that they can be complementary (Table 9) and both should therefore be considered when designing discharge management programmes. Clearly, the objectives and subsequent design of the monitoring programme are crucial to realise the success of monitoring programmes.

The many options available must be tailored to individual circumstances. Most important is to consider the nature of the receiving water, in terms of its quality, the uses to which it is put and the dilution. The quality of the receiving water needs to be considered with respect to the ability to discriminate effects caused by the effluent from those of background contamination. For example, if the water upstream of the discharge sustains poor biological diversity, biomonitoring, with either *in situ* or *ex situ* toxicity tests or through community surveys will be of little value for it will be unable to discriminate between background and effluent induced effects. However, chemical monitoring to track the distribution and fate of specific analytes in the receiving water might be desirable. The use of the water may also determine the desirability of biomonitoring. For example, if the receiving water is subsequently used as drinking water or has high amenity value for fishing or recreation the value of biomonitoring will be greater than if the receiving water is used only for industrial cooling water. The dilution of the effluent also needs to be

considered, for if it is large and mixing is rapid, it may be difficult to discriminate effects caused by the effluent.

It is also important to consider uncertainty in the WET evaluation of the effluent. Thus, if the effluent contains bioaccumulative, highly sorptive, low solubility components and/or components with low degradation potential, then precautionary chemical or biological monitoring may be desirable.

Table 9: Relative merits of different approaches

Parameter affecting the fate and effects of a discharge	**WEA testing**	***Ex situ* (ambient) toxicity testing**	***In situ* toxicity testing**	**Biological community monitoring**	**Analytical measurements in receiving waters**
Behavioural responses of organisms in receiving water (e.g. avoidance of discharge)	Not taken into account	Not taken into account	Not taken into account	Taken into account, although distinction between avoidance and mortality will rarely be possible	Not taken into account
Long-term variability in discharge composition (e.g. discharge from batch production processes)	Only taken into account by careful timing of sampling	Only taken into account by careful timing of sampling	More likely than WEA or *ex situ* testing to be taken into account but still unlikely unless more or less continuous testing	Taken into account	Not taken into account
Able to establish cause-effect relationships between contaminants and receiving water communities	Not with confidence, although in combination with monitoring and chemical analysis in receiving water confidence is increased	Not with confidence, although in combination with monitoring and chemical analysis in receiving water confidence is increased	Not with confidence, although in combination with monitoring and chemical analysis in receiving water confidence is increased	Not with confidence, although in combination with WEA testing and chemical analysis in receiving water confidence is increased.	Not with confidence, although in combination with monitoring and chemical analysis in receiving water confidence is increased
Sorption mediated toxic effects	Slow sorption may not have time to occur. Sediment as a sink is normally excluded	Can be taken into account if sampling is well designed	Can be taken into account if sampling is well designed	Taken into account	Extraction can be designed to take account of bioavailability, but assumptions still need to be applied (e.g. bioavailability of sorbed material)
Short-term discharge of toxics	Likely to be missed unless continuous sampling	Likely to be missed unless continuous sampling	May well be missed unless continuous sampling	Long-term impact of short-term discharges should be determined, although effects will be compensated by immigration and so long-term impact may be slight	Likely to be missed unless continuous sampling

Table 9: Relative merits of different approaches (cont'd)

Parameter affecting the fate and effects of a discharge	**WEA testing**	***Ex situ* (ambient) toxicity testing**	***In situ* toxicity testing**	**Biological community monitoring**	**Analytical measurements in receiving waters**
Potential to predict, and hence avoid, impact	Nature of technique is proactive, although time delay between sampling and toxicity results will mean some impact is likely	Timing of sampling compared to WEA testing means some impact is more likely than for WEA	Timing of testing compared to WEA testing means some impact is more likely than for WEA	Determination of effects is unavoidably retrospective	Timing of sampling means some impact is likely, but results may be available more quickly than for biological tests
Ability to detect effects	Ability to detect impact may be limited by low diversity of species used in toxicity tests	Ability to detect impact may be limited by low diversity of species used in toxicity tests	Ability to detect impact may be limited by low diversity of species used in toxicity tests	Ability to detect impact may be limited by different factors e.g. low population numbers of large proportion of taxa in receiving water. Not clear how the impact of WEA and monitoring limiting factors compare	Assumptions needed to compensate for bioavailability
Seasonality in fate processes	Requires extensive simulation testing to understand properly	Timing of testing should disclose	Timing of testing should disclose	Timing of sampling should disclose	Timing of sampling should disclose
Ability to determine fate (degradation rates)	Degradability crudely estimated by non-discriminating generic methods (BOD/COD), or by applying default values to pass/fail tests (OECD, 1992b) on specific analytes	Can be indirectly determined by measuring toxicity	Can be indirectly determined by measuring toxicity	Taken into account, provided sampling strategy and bioavailability are adequately taken into account	End-product of fate processes can be determined by specific analysis

Table 9: Relative merits of different approaches (cont'd)

Parameter affecting the fate and effects of a discharge	**WEA testing**	***Ex situ* (ambient) toxicity testing**	***In situ* toxicity testing**	**Biological community monitoring**	**Analytical measurements in receiving waters**
Ability to determine fate (bioaccumulation)	Bioaccumulation crudely estimated using conservative models that inadequately take account of biotransformation and/or bioavailability (e.g. log K_{ow}, or laboratory BCF studies)	Can be indirectly determined by measuring toxicity	Can be indirectly determined by measuring toxicity	Biotransformation and bioavailability addressed, but identification of exposure concentrations may be difficult unless caged animals are used	Can be indirectly measured using simulation devices (SPME, etc)
Tidal conditions	WEA cannot take it into account. Implication is that cannot discharge 2 h either side of low tide	Can take it into account through design of sampling strategy	Integration of changes in dilution over tidal cycle can be taken into account (see ECETOC, 1999)	Integration of changes in dilution over tidal cycle can be taken into account	Can be taken into account through sampling strategy
Confounding factors	Results can be confounded by process upsets	Results can be confounded by process upsets	Results can be confounded by process upsets	Results can be confounded by for example algal blooms and physical perturbations	Results can be confounded by process upsets

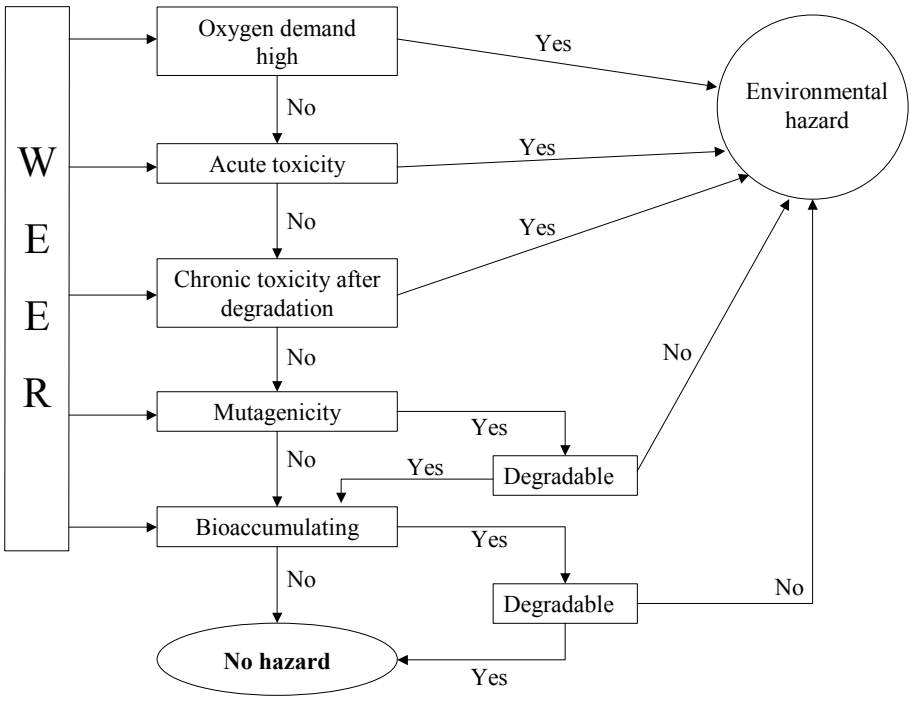
4. EXAMPLES OF WHOLE EFFLUENT ASSESSMENT

In this section the principles and tools discussed in earlier sections are illustrated with practical examples from recent WEA studies.

4.1 Experiences at two industrial sites in the Netherlands

Tonkes and Baltus (1997) published the first study on whole effluent assessment in the Netherlands. This study was initiated by the Institute of Inland Water Management and Wastewater Treatment (Lelystad, Netherlands) and carried out in close cooperation with the Department of Waterways and Public Works in the Province of Limburg and with the Water Management Administration Limburg. At that time it was called the Whole Effluent Environmental Risk (WEER) methodology. The rationale of WEER is presented in Figure 6.

Figure 6: WEER rationale



Two effluents originating from chemical plants will be discussed in more detail. Plant 1 produces intermediates for the pharmaceutical industry and Plant 2 is a conglomerate of many petrochemical and raw materials producing plants. Both plants discharge their wastewater following biological treatment.

The effluents were studied in the following tests:

- Bacterial test (*V. fischeri* in the Microtox test);
- algal test (*S. capricornutum*);
- acute crustacean test (*D. magna*);
- acute fish test (*B. rerio*);
- Tox kits (rotifer *Brachionus calyciflorus* or *B. plicatilis* or the crustacean *Artemia salina* or *Thamnocephalus platyurus*);
- chronic crustacean test (*D. magna*) after biodegradation for 28 days;
- chronic fish test (*B. rerio*) after biodegradation for 28 days;
- mutagenicity in the reverse mutation assay with *Salmonella typhimurium* TA98 and TA100 (Ames test) before and after degradation for 28 days;
- analysis of bioaccumulating substances by HPLC on apolar C_{18} column and by absorption using the Empore disk method after biodegradation.

The following results were found:

- Neither effluent was considered adverse for the environment on the basis of BOD and COD;
- the effluent from pharmaceutical Plant 1 appeared to be highly toxic in the Microtox test. The EC_{20} in the Microtox test was 0.8% effluent (125 fold dilution). However, the biological wastewater treatment plant at the pharmaceutical plant was running perfectly, with low suspended materials in the effluent and a high BOD and COD removal efficiency. The EC_{20} of Plant 2 effluent was 42% effluent (2.3 fold dilution);
- both effluents inhibited algal growth by up to 80%, although the test was performed according to OECD guidelines, a dose-response relation was not observed;
- neither effluent was acutely toxic to *D. magna* or to *B. rerio* (zebrafish);
- effluent 2 was toxic in the chronic *Daphnia* test. It did not affect mortality, but reproduction was inhibited by 50% at a dilution of 25%;
- effluents 1 and 2 increased the mortality of zebrafish eggs and larvae in the Early Life Stage (ELS) test at a dilution of 5% and lesser dilutions (i.e. more concentrated effluent);
- effluents 1 and 2 were mutagenic before and after a period of biodegradation. The mutagenicity increased after biodegradation, particularly in effluent 2;
- the presence of bioaccumulating substances was studied with an HPLC gradient method according to Klamer and Beekman (1995) and the Empore disk method (Verhaar *et al*, 1995; Van Loon *et al*, 1996). The results from the two methods were not identical. After biodegradation 65% to 90% of bioaccumulating compounds disappeared. According to the

HPLC method both effluents contained (highly) bioaccumulative substances. With the Empore disk method, both effluents contained bioaccumulating substances but the sum of molar concentrations normalised by BCF was below the NOEC estimated for organics with a non-polar narcotic mode of action.

These results were communicated to the management of both chemical plants. The management of Plant 2 pointed to the time of sampling in the middle of the summer of 1995, when the flow of the receiving river was very low. Since river water is a major component of the process waters at Plants 1 and 2 the WEA testing procedure could have identified some existing toxicity of the river water itself. Further, the wastewater treatment plant at Plant 2 had some problems with bulking sludge during the time of sampling. The effluent from Plant 2 was sampled again in January 1997 and studied in the ELS test with the zebrafish (*B. rerio*), before and after filtration (Notox, 1997a,b). The filtered and unfiltered effluent did not induce any visible effects on the development of zebrafish embryos and did not significantly affect time for hatching or for the development of the larvae during the yolk-sac period.

Conclusion

This study clearly illustrates that variability is an inherent part of many steps of the WEA process and underscores the necessity for regulators and regulated community to interact constructively to understand the observations and to interpret obtained results correctly. Furthermore this example illustrates the following points:

There is a close link between specific local conditions and the observations made in WEA, which illustrates why WEA needs to be tailor-made to deliver meaningful results.

Bioaccumulation testing is still in development and needs much more practical application to develop it into a well-understood and feasible part of WEA testing.

The increased mutagenicity following biodegradation was surprising and poses questions regarding potential for real effects in the receiving environment. Given the uncertainty over the ecological relevance of *in vitro* genotoxicity discussed earlier (Section 2.6), further work using validated *in vivo* eco-genotoxicity tests is required in order to understand the potential significance of the *in vitro* data.

The interpretation of tests may differ from single substance testing and has to be done with care, as illustrated by the lack of dose-response in the case of the algal test.

In this study, persistence was not used as a parameter in isolation, but in what is thought to be currently the best way to get meaningful results i.e. in combination with bioaccumulation and ecotoxicity.

4.2 Testing lipophilic fractions of an effluent for aquatic toxicity based on HPLC separation techniques

The aim of this study was to develop a method to evaluate the long-term environmental hazard of an effluent. The method consisted of separation of substances in the effluent by gradient-HPLC into fractions containing substances with different log K_{ow} values and testing of these fractions in aquatic toxicity tests.

The effluent selected for this project was an untreated effluent (which is not discharged as such into the environment) and thus contained a high concentration of lipophilic compounds. This therefore made it a suitable sample for addressing the HPLC methodology for assessing the potential to bioconcentrate.

The following procedures were carried out:

- The unfiltered effluent was extracted with dichloromethane and the solvent evaporated;
- the residue was dissolved in methanol for HPLC fractionation;
- the lipophilic fractions were isolated according to their log K_{ow} values using reference compounds (log K_{ow} <3, 3 >log K_{ow} <5 and log K_{ow} > 5);
- methanol was removed by extraction with dichloromethane;
- dichloromethane was evaporated;
- the residue was dissolved again in methanol.

The methanol fractions before and after HPLC-separation were used for ecotoxicological tests with algae, *Daphnia* and egg and sac-fry stages of zebrafish, which were conducted according to OECD guidelines. The results show, that the fraction containing substances with log K_{ow} > 5 was the most toxic fraction for all test organisms in this study.

It was not possible in this study to fully recover the original toxicity by combining the separated fractions. A study of possible losses during the different steps of extracting and dissolving the constituents from the effluent sample will be part of future studies with a more realistic effluent. The method was considered to be a valuable and cost-effective tool in risk assessment and biological monitoring of effluents.

This example illustrates some of the problems with the techniques that are being developed to assess bioaccumulation potential. At the same time it demonstrates that such testing needs further validation and is of limited value when used in isolation. Combining testing for bioaccumulation potential with toxicity tests is considered a meaningful approach. For example, it is important to recognise that not all of the toxicity of an effluent can be attributed to bioaccumulative substances. Therefore undertaking analyses for common contaminants responsible for toxicity (e.g. ammonia, metals) in combination with toxicity and bioaccumulation potential assessments will significantly increase the value of effluent studies.

4.3 Lessons from the SETAC publication 'Whole Effluent Toxicity Testing'

Volume 19 of Environmental Toxicology and Chemistry (SETAC, 2000) is a special issue devoted to WET testing. The papers published in this issue provide useful information on details of testing and valuable learning from the past that will benefit future projects. Important learning from these papers is summarised below and appears to support the WEA testing approach (testing scheme) recommended by the TF (Section 5.2), and the conclusions and recommendations (Section 5.3).

A comprehensive study by Chapman (2000) to assess whether WET testing achieves its generally accepted purpose of identifying, characterising and eliminating toxic effects of effluents on aquatic resources concluded that it is useful, but not perfect. The author emphasises that in WET testing, perfect tools do not exist. To a large extent this is due to the inherent variability of these tests caused by biological and anthropogenic factors, the different species used in the laboratory and those found in the field and the differences between laboratory and receiving environments. Despite the observed imperfections the study identifies the usefulness of WET testing as a tool for the first stage in a risk assessment process. As such it only represents hazard identification and results have to be interpreted in context.

The TF recommends that WET testing should be combined with an assessment of the relevant environmental parameters that influence the expression of an effluent's toxicity in receiving waters. Further, the WEA scheme suggested later in Chapter 5 is one such approach that attempts to achieve this objective. Several other contributions to the special issue (SETAC, 2000) also deal with the high variability in test results (Warren-Hicks *et al,* 2000; Moore *et al,* 2000b; Markle *et al,* 2000) that highlight the necessity for careful interpretation of results and the need to apply suitable statistics to identify significant responses.

Sarakinos *et al* (2000) discuss the relation between the toxicity of individual substances and observed whole effluent toxicity. In general the toxic action of substances was additive, single substance toxicity calculations underestimated WET of pulp and paper effluents and

overestimated WET when many heavy metals were present in the effluents. The latter could partly be explained by acknowledging the reduced bioavailability of metals.

Bailey *et al* (2000) described a survey on sewage treatment plant effluents in New South Wales, Australia, using *C. dubia* and *S. capricornutum*. Fifteen of eighteen effluents showed toxicity and observed toxicity could often be explained by ammonia and pesticide concentrations. In industrial effluents, compounds such as ammonia may also contribute to observed effects that may reduce the chance of drawing conclusions on the presence of specific hazardous substances. Diamond and Daley (2000) indicated the relative importance of effluent discharges as compared to other factors impacting the receiving environment. They observed that effluents showing toxicity (acute) in WET testing had a very low probability of causing impairment in the receiving water when the discharge flow was less than 20% of the in-stream flow. This illustrates the need to interpret WET test results within the local context, taking account of other factors influencing receiving water quality.

4.4 Examples of use of biological and chemical monitoring in receiving water

Water and sediment quality monitoring

OLF (2001) summarised the results from the background assessments and regional monitoring assessments carried out in the North Sea during the period 1990-1998, to determine the area of influence of off-shore platforms. The area of influence is based on the environmental distribution of barium, total hydrocarbons (THC) and the benthic fauna. Barium is a conservative marker used as an indicator of a general influence from the drilling activities. THC indicates influence from drilling with oil-based drilling fluids or other discharges, while benthic fauna are used as an indicator for general influence on the sediment community.

Tissue analysis

Analysis of the tissues of animals in receiving waters has been practiced for a long time. Much attention has focused on mussels (e.g. Widdows *et al*, 1985; Widdows and Donkin, 1988; Widdows *et al*, 2002). For example in Tampa Bay, Florida, oysters from 16 sites were collected during winter 1993 and analysed for both biological characteristics and tissue chemical concentrations (Fisher *et al*, 2001). Chemical analysis showed tissue concentrations at some of these sites to be higher than US national averages, as reported by the National Status and Trends Mussel Watch Programme, for total PAH, total PCB, total chlordanes, DDT, Cu, Pb, Sn and Zn. Measures of oyster internal defence, including hemocyte density, rate of locomotion and superoxide generation, varied significantly among sites. They were generally higher at sites with higher tissue concentrations of xenobiotic chemicals. Potential associations between oyster

defence characteristics and accumulated chemical contaminants, either singly or in chemical classes, were explored using correlation analysis and a composited ranking procedure. Positive relationships were found for hemocyte characteristics with certain trace metals (Cu, Sn and Zn) and PAH analytes, whereas negative relationships were found with certain PCB and pesticide analytes.

Determination of the levels and significance of key components that may concentrate through the food web will, until more standardised methods are determined, be a major element in the environmental monitoring around the oil installations in the Norwegian Sector. Sampling and chemical analysis techniques have been tested to obtain optimum data for different interpretation objectives (Durell *et al,* 2000). The field methods included sampling *in situ*, large volume waters, SPMD, caged mussels and plankton. Samples were collected near to and distant from produced water discharges and state of the art trace level analytical methods were applied to determine a suite of organic contaminants, including THC, and more than 60 parent and alkylated PAHs, phenols, decalins, benzothiophenes and selected metals. The concentrations of contaminants derived from produced water decreased rapidly with increased distance from the platforms, to near background levels 5 km from the discharge.

Biomarkers

The BECPELAG (see Section 3.2.4) provides a good example of the use of biomarkers in a monitoring programme. Atlantic cod (*Gadus morhua*), stickleback (*Gasterosteus aculeatus*) and blue mussels (*Mytilus edulis*) were deployed in cages in two areas with inputs of contaminants into the pelagic ecosystem: a coastal area (German Bight) and an offshore oil-production area (Statfjord, North Sea) plus a reference area (Hylland *et al*, 2002a,b). Buoys with SPMDs and diffuse gradient in thin films (DGTs[a]) were also deployed at each site. Both cod and blue mussels survived the deployment well, but all sticklebacks died. Later work has shown that the cage construction was not optimal for the stickleback. The results from these studies showed clear differences along transects for histopathological changes in the hepatopancreas of blue mussels and there was also reduced lysosomal stability at stations in the inner German Bight and close to the oil platform. Responses were also detected along the transect in both the German Bight and the offshore-area using acetyl cholinesterase (AChe), benzo(a)pyrene hydroxylase (BaPH), EROD-activity and glutathione *s*-transferase (GST) as biomarkers. There were low concentrations of DNA adducts in cod from both areas and no significant differences in vitellogenin levels (although there appeared to be a gradient).

[a] DGT – Diffuse gradient thin films - used to estimate integrated accumulation of metals from water

Table 10: Biomarker methods recommended by the BECPELAG steering group for biological effect monitoring using fish and mussels

Group	Organism	Method(s)	Comment
PAH	Fish	Bile PAH-metabolites	QA*
	Fish	EROD (liver)	QA*
	Fish	GST (liver)	
	Fish	DNA damage**	QA*
	Blue mussel	BaPH (hepatopancreas)	
Alkyl phenols	Fish	Plasma vitellogenin	QA*
	Blue mussel	None available	
General	Fish	Histopathology/histochemistry (liver)	QA*
	Blue mussel	Scope for growth	QA*
	Blue mussel	Lysosomal stability	QA*
	Blue mussel	Histochemistry	
Other contaminants	Fish	AChE (muscle)	QA*
	Fish	MT (liver)	QA*
	Fish	ALA-D (red blood cells)	QA*
	Blue mussel	AChE	
	Blue mussel	MT (gills)	

*QA – method has been subject to an international Quality Assurance activity and/or inter-calibration.
**Short-term response for caged fish; adducts or similar for field-collected fish

Based on these and other results from the BECPELAG workshop (Hylland *et al*, 2002b), a suite of biomarker methods will be included in the yearly monitoring programme of the Norwegian continental shelf. The BECPELAG steering group recommended a selection of methods (Table 10), but at present, a decision on which of the recommended methods to use for the monitoring programme has not been taken.

Ex situ toxicity monitoring

Biological effects of contaminants in the UK coastal and estuarine environments have been monitored by use of the oyster (*Crassostrea gigas*) embryo bioassay (Thain, 1991; CEFAS, 1998; NMP, 1998). This bioassay measures both lethal and sublethal toxicity in developing embryos exposed to a water sample over a 24-h exposure period. This test has been used since the early 1990s, and gives a picture of variable and recurring toxicity in the waters of several English estuaries. As the oyster bioassays and related tests are not sensitive to the lower levels of contaminants found in waters offshore, and due to the fact that chronic bioassays are too time-consuming for general survey work, some surveys have focused on hexane-extracted concentrates of seawater using the copepod *Tisbe battagliai* exposed for 48 hours (CEFAS, 1998; Kirby *et al*, 1998; Thain and Kirby, 1996).

UK legislation also requires that controlled waters (ground water etc.) should be protected and monitored. To be able to assess the toxicity of contaminated groundwater, bioassays have been used as part of the monitoring in addition to the chemical methods. Dewhurst *et al* (2002) describe the performance of three rapid bioassays (ToxAlert™, Microtox and Eclox) compared to a *D. magna* immobilisation bioassay (48 h) when assessing the groundwater quality in an urban environment. The study showed that Microtox produced replicable results that correlated well with the *D. magna* tests, in contrast to ToxAlert and Eclox, which were not suited for this purpose. The conclusions from the study are that monitoring acute impacts in the environment with *D. magna* and Microtox are, due to their precision, range of responses and ease of use, the bioassays that appear to be most relevant for assessing groundwater toxicity of the four tests used in this study.

In a survey around a platform in the UK sector of the North Sea, sediments contaminated with oil based muds were collected and used in *ex situ* toxicity tests using an amphipod (*Corophium volutator*), a polychaete (*Arenicola marina*) and the Microtox acute test system (Grant and Briggs, 2002). Sediments were acutely toxic to *Corophium* as far as 600 m from the platform. Sediment samples taken 100 m from the platform remained acutely toxic to *Corophium* when 3% contaminated sediment was mixed with clean sediment. A concentration of 10% sediment also inhibited *Arenicola* feeding almost completely. Sediment elutriates were not acutely toxic to *V. fisheri* (Microtox) suggesting that concentrations of water-soluble toxicants were low. However, the organics extracted by dichloromethane were toxic, with the toxicity being correlated with hydrocarbon concentration. Toxicity of sediments to *Corophium* were closely related to the hydrocarbon content and metal content, but, except at sites immediately adjacent to the platform, metal concentrations were too low to explain sediment toxicity.

A comparative study of whole sediment versus elutriate and interstitial water bioassay on contaminated sediments from the River Tyne in the north east of England was performed, to evaluate their utility for routine monitoring of marine sediment quality and to assess the biological results in comparison with a comprehensive suite of contaminant analyses (Matthiessen *et al,* 1998). The bioassays for toxicity included the amphipod *Corophium* sp. and the polychaete *A. marina* whole sediment test, as well as tests on elutriates with the copepods *T. battagliai* and *A. tonsa*, the embryo of the oyster *Crassostrea gigas*, light emitting bacteria *Photobacterium phosphoreum* and the unicellular algae *Tetraselmis suecica* and *Thalassiosira pseudonana*. The results from this study showed a good correspondence between the whole sediment bioassay responses and the concentrations of the suite of measured contaminants. The elutriate and interstitial water bioassays suggested the presence of toxic materials at some stations which were apparently not harmful to the whole sediment test organisms, and thus these tests gave results which were not clearly linked to the distribution of measured contaminants. Despite this, these elutriate and interstitial water bioassays may be good candidates for pre-screening since they never gave a response when the whole sediment tests did not. Given the relative

slowness of whole sediment tests, recommendations were made on the use of elutriate and interstitial water bioassays as a first tier in a risk evaluation programme, providing that their potential limitations are borne in mind. A recommendation was also made to use whole sediment bioassays as the main tool for toxicity assessment in routine monitoring. This study also showed the value of using a battery of whole sediment bioassays for monitoring purposes because different taxa and endpoints will have varying susceptibilities to the multiplicity of contaminants present in industrialised estuaries.

In situ toxicity monitoring

In a particularly interesting study, the combined and complex interaction of urban habitat alteration, municipal wastewater treatment plant discharges (ammonia input) and salinity inputs from a stream flowing over a significant salt dome were evaluated (WERF, 2000). Fish and invertebrate communities were impaired relative to reference conditions, but it was unclear why this occurred. Fathead minnow survival and growth studies beginning with 24-hour-old fish were conducted *in situ* by exposing groups in modified perforated PVC pipe. An elaborate system was used to rotate positions of 18 replicate chambers at each site to distribute exposure to the prevailing hydrologic regime and stressors. Salinity and habitat were judged to be the most significant sources of stress and ammonia input the least. This study provides an example of the benefits accrued through collaboration between the regulated and regulator communities, in that the regulated authorities (City of Lincoln, Nebraska, USA) worked to define the most scientifically defensible site-specific water quality criteria and the Water Environment Reseach Federation acted as an independent peer-reviewer (true expert panel) drawing the best from both regulator and regulated community to the benefit of the environment.

On the Norwegian continental shelf, biological monitoring of the water column has been performed on a yearly basis since 1999 (SFT, 1999; Durell *et al*, 2000; Batelle/Sintef, 2002), with the purpose to perform direct measurements of a selection of relevant components in produced water, that can be used to predict the probability of sublethal and chronic effects in the pelagic environment caused by the petroleum industry.

Species diversity monitoring

Monitoring surveys of the sediments have been carried out in the UK, Dutch and Norwegian sector of the North Sea since the mid-1970s, with the purpose to monitor impacts, and to determine the magnitude and spatial extent of environmental effects of oil/gas operations (SFT, 1997, 1999; OLF, 2001; Daan and Mulder, 1997). The overall content of seabed surveys has varied from year to year according to the statutory requirements and the nature of research

projects proposed by oil and gas operators. In the Norwegian sector, examination of the sediments around an offshore platform is required before exploration drilling is undertaken to identify the background level of physical, chemical and biological parameters in the sediments. This is in addition to the regular surveys during the exploration drilling (SFT, 1999). Assessment of faunal disturbance is based on a number of ecological variables, covering both the number of species and individuals present, their comparative abundance, and also the presence or absence of specific species known to be indicators for anthropogenic disturbance. The estimates of total affected area on the Norwegian offshore area are based on biological and THC indicators, expressed as a proportion of the total Norwegian offshore area (OLF, 2001). The sea-bottom fauna is analysed using a variety of techniques, shown in Table 11. Surveys performed in the Dutch sector have shown that biological effects of oil based mud (OBM) discharges were detectable at up to 1,000 m by reduced abundances of a very few sensitive species (Daan and Mulder, 1997). Closer to the wells, increasing numbers of macro-fauna experienced adverse effects. In the longer term, the macro-fauna seemed to recover at distances 500 m from a drill site, but within that range the macro-fauna was still affected after 8 years. Possible effects of water-based mud (WBM) were investigated in a period of 2 months to 1 year after the discharges had terminated. Adverse effects on the benthic community were not observed, within 25 m of a discharge site.

Table 11: Parameters assessed during the analyses of fauna data from sediment samples in the Norwegian offshore monitoring programme

Univariate statistics	**Multivariate Statistics**
Number of taxa and number of individuals	Clustering analyses (Bray-Curtis dissimilarity index)
Ten most dominant taxa at each station ('top ten')	Multidimensional scaling (MDS)
Species-area curves	Correspondence analysis (CA)
Diversity index (Shannon-Wiener, H')	Canonical correspondence analysis (CCA)
Evenness (Pielou's measure, J)	
Expected number of species per 100 individuals (Es100)	

Monitoring of the macro-benthic community in UK (Tees Bay and estuary) was carried out annually between 1973 and 1999, (Warwick *et al,* 2002). Benthic fauna were enumerated and identified using the lowest possible taxonomic level. Biodiversity measures used to identify local and environmental events were traditional indices like number of species per unit area, total abundance of individuals, total biomass, Shannon diversity and Pielou's evenness, together with recently developed biodiversity measures (Warwick and Clarke, 2001) that describe the taxonomic spread of species.

5. STRATEGY FOR WEA

5.1 Introduction

The previous sections of this report describe a number of different aspects of WEA. From the examples described it is clear that the reasons for undertaking WEA and how it is carried out in practice are very diverse. The major aims of this report are to apply the experience gained to make suggestions for future WEA developments and applications and to propose a rapid, cost-effective and scientifically sound strategy for such assessments.

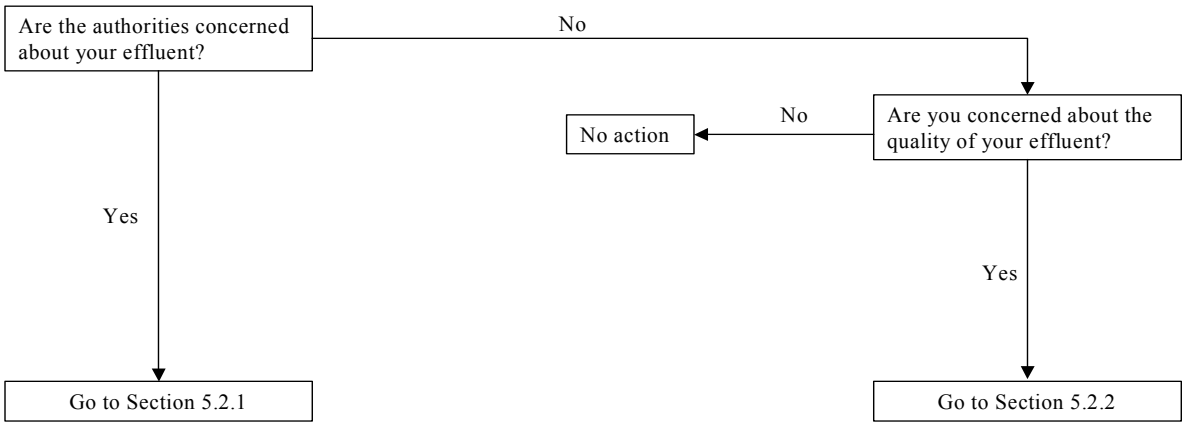
Considerable practical experience has been gained with WEA in recent years in the different areas of its application. Often these were joint activities between industry and authorities. From these activities several characteristics and the following conclusions have emerged:

- The design of WEA programmes is highly dependent on the specific purpose of testing. To analyse existing WEA results it is therefore essential to consider the specific purposes for which the WEA activity was undertaken. An obvious question would be whether these goals were achieved. In many of the studies reviewed the goals were not clearly defined;
- the application of WEA is highly dependent on the local situation. The choice of tests and the way they are carried out have to be adapted to the local conditions, such as the characteristics of the effluent (pH, salinity, oxygen content, ammonia, etc.) and of the receiving environment (freshwater, saline, environmental quality, etc.);
- in WEA, many parameters may vary continuously and together with the inherent variability of toxicity tests this often makes interpretation very difficult or even impossible. Therefore WEA is a field of science that needs to be developed by applying it in practice and to understanding and interpreting the results obtained;
- although WEA will vary, it is important that it is based on a number of appropriate validated methods (or tools). Selection of the tools used will be dependent on the specific requirements of the investigation and on local conditions. This has been referred to as the 'tools in the toolbox' approach (DTA programme, Methods Working Group);
- another important conclusion from the practical experience gained from the DTA programme is that WEA is resource intensive. It was therefore recommended to use WEA carefully and selectively and not on a routine basis;
- a study made as part of the Tees Project indicated that test for test the chemical methods were likely to be less expensive than those used for DTA. This balance shifts as the analysis of the chemicals of concern becomes less routine or they need to be measured at very low concentrations,' (UK DTA, 2001b).

5.2 Flow chart

As discussed above there are many different reasons why WEA is carried out. This section presents a simple decision tree (Figure 7) to help facilitate the choices to be made when assessing if WEA is applicable to reach the intended objectives and to decide which of the WEA toolbox to use. The approach begins by considering whether the effluent is of concern and whether WEA could be a useful tool. Then it considers the main drivers (both regulatory and for the discharger) in order to identify the appropriate approach for testing. For each driver, potential problems are identified in more detail and translated into specific objectives. Next, the potentially most successful approach is identified to reach the objective. If the objective can be tackled by WEA, the user is guided to appropriate decision schemes to identify the best possible testing strategy. In cases where WEA testing is carried out, some guidance is provided on how to evaluate the results against the objectives. In many cases, discussions between regulators and discharger to agree on conclusions will prove useful.

Figure 7: Decision tree for WEA testing



5.2.1 Regulatory drivers

The following instances from current and developing legislation may necessitate WEA testing either as a result of regulator pressure or discharger concern for new or existing plants.

- WFD monitoring indicates bad, poor or deteriorating water quality;
- regulatory concern about the PBT nature of the effluent;
- IPPC requires demonstration of use of Best Available Technology (BAT) to mitigate, alleviate or avoid harm;
- local permits under national laws, e.g. water quality.

5.2.1.1 Approach when WFD indicates water quality is unsatisfactory

5.2.1.1.1 Identification of problem

The important first step is to assess why the water quality is unsatisfactory. If it is a specific issue (e.g. EQS for priority substance is not met), identify the cause and manage appropriately. WEA is probably not helpful in this situation. If it is a broader issue (ecological quality or COD/BOD are of concern), determine the extent of the problem.

If the effluent is thought to contribute to the problem, it is important to establish whether the composition of the effluent is known. If it is, consider a substance-based assessment; if this is not feasible, consider WEA. If PBT is of concern, go to Scheme A (Figure 8), if it is only the toxicity of the effluent that is of concern, go to Scheme B (Figure 9).

5.2.1.1.2 Use of results

Apply results from the appropriate scheme as detailed in Section 2.2.8.

5.2.1.2 Concern that PBT type substances may be present

5.2.1.2.1 Identification of problem

Assess the extent of the problem and identify the specific concerns. If your effluent is thought to contribute to the problem, confirm whether the composition of the effluent is known. If yes, consider a substance-specific approach, identify if there are PBT components and manage them. If a substance-specific approach is not feasible, consider WEA - see Scheme A (Figure 8). This may include the use of parts of WEA Scheme A in a tracking study.

5.2.1.2.2 Use of results

If the results from Scheme A (Figure 8) testing indicates presence of PBT, then implement a risk management strategy. If not, discuss further with regulator if necessary, monitoring any subsequent process component changes.

5.2.1.3 IPPC

5.2.1.3.1 Identification of problem

IPPC BREF guidance is currently restricted to toxicity, so consider Scheme B (Figure 9) to demonstrate the quality of the effluent. This may apply to new operations, process changes or to an existing situation.

5.2.1.3.2 Use of results

Use the results to demonstrate that the quality of the effluent reflects the use of BAT. Regulators may use data to benchmark within sector.

5.2.1.4 Local permits

This will be site-specific; no general guidance is possible. Discussions with the local regulator should identify key concerns. If WEA testing is indicated, apply the general principles outlined in this report, using (parts of) Scheme A or B as appropriate.

5.2.2 Discharger drivers

A discharger may want to consider WEA testing to support decision-making under any of the following circumstances:

- Anticipation of developing regulation;
- comparison of impact of different processes for water treatment or production;
- charges based on toxicity/treatability of effluent;
- treatment by 3rd parties;
- direct discharge;
- environmental liability;
- local stakeholder pressures;
- reputation/responsible care issue.

5.2.2.1 Anticipation of regulation

5.2.2.1.1 Identification of problem

Identify an appropriate strategy based on the specific nature of the regulation – e.g. is it based on hazard or on risk; is the concern only on toxicity or also on PBT? Decide whether a substance-specific or a WEA approach is most appropriate. If WEA is considered and toxicity is the only concern, go to Scheme B (Figure 9). If PBT is of concern, go to Scheme A (Figure 8).

If the regulation focuses on risks it may be most appropriate to consider all effluents concerned before testing and carry out a screening level assessment to define and prioritise the problem.

5.2.2.1.2 Use of results

If the regulatory focus was on hazard and the results indicate concern, develop an appropriate management strategy. If the regulation is risk-based the results should be evaluated as described in Section 2.2.8 and appropriate action considered.

5.2.2.2 Comparison of impact of different processes

5.2.2.2.1 Identification of problem

A change in the production process (alternative technologies) is likely to affect the composition of effluent and consequently the potential to have an environmental impact. In some cases, for example when the changes are relatively simple or when the processes are well known, the impact may be assessed on a substance-by-substance basis with sufficient accuracy. However, there may be advantages in using WEA in complex situations or where the composition is unknown. If WEA is appropriate, and the potential concern is only toxicity, go to Scheme B (Figure 9). If PBT is of concern go to Scheme A (Figure 8).

5.2.2.2.2 Use of results

Analyse the data (see Section 2.2.8) to obtain a comparison to support the decision upon which process to use.

5.2.2.3 Charges based on toxicity/treatability of effluent:

5.2.2.3.1 Identification of problem

Identify the basis of the charge(s). Establish whether the concern is only on toxicity or also on PBT components? Decide whether a substance-specific or a WEA approach is most appropriate. If WEA is considered and toxicity is the only concern, go to Scheme B (Figure 9). If PBT is of concern, go to Scheme A (Figure 8).

5.2.2.3.2 Use of results

Evaluate the results to identify how and where the charges may be reduced.

5.2.2.4 Local stakeholders

This will be site-specific; no general guidance is possible. Understand their concerns and develop a response strategy that may include a component of WEA (Scheme A or B as appropriate), applying the general principles outlined in this report.

5.2.2.5 Reputation/responsible care

Identify the objectives. They will be tailored to the specific circumstance so no general guidance is possible. The resulting strategy may include a component related to effluent quality. If so apply the WEA general principles as outlined in this report.

Figure 8: Scheme A

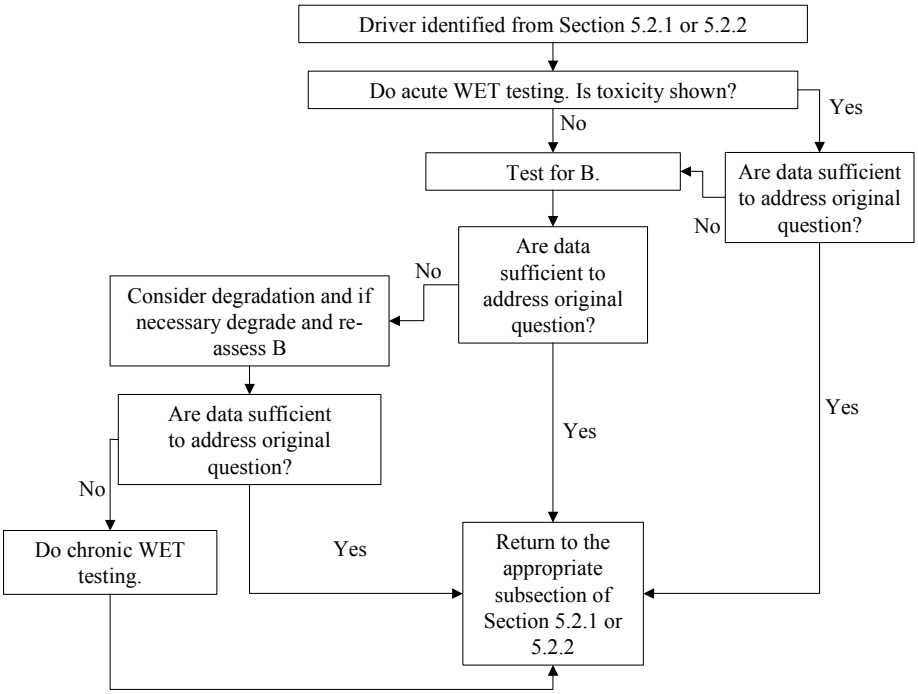
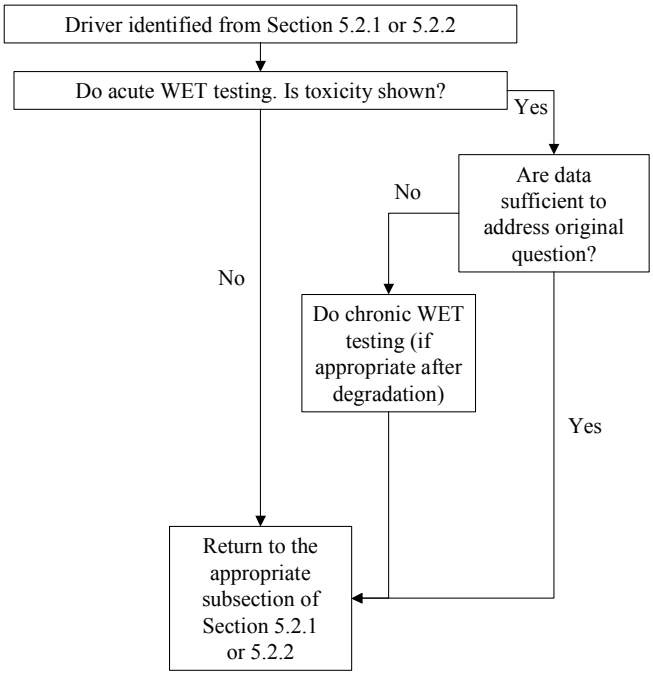


Figure 9: Scheme B



6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. WEA is a complex and developing area. With respect to WET investigations there are a few well-developed methods for assessing acute toxicity that are relatively well understood. The validity of the use of these acute tests to drive real environmental improvement has been demonstrated. However, care is still required to ensure that the acute tests selected for an investigation are appropriate in terms of the objectives.

2. There are relatively few validated chronic toxicity studies and, in comparison to acute tests, significantly more issues will be encountered when trying to interpret the implications of results from chronic toxicity assessments. For example the inherent water quality (e.g. ionic balance) in an effluent may lead to adverse effects in chronic toxicity assessments irrespective of whether any contaminants are present.

3. Evaluation suggests that it is significantly easier to predict causal acute toxic effects in receiving water than effects measured by chronic endpoints. The latter are much more difficult to discern due to the increased importance of other (background) effects. Nevertheless, Scroggins *et al* (2002a,b; 2004) integrated sublethal WET with other methods such as chemical measurements in effluents, receiving water or fish tissue and was able to identify community impact zones from paper and pulp and metal mining discharges.

4. There are significantly more issues associated with other methodologies such as those for persistence and bioaccumulation. Considerable further development of the test methodology is required. Nevertheless, because of the concerns about persistence and bioaccumulation, these issues still need to be addressed in any comprehensive assessment scheme.

5. The current suite of degradation tests (both biotic and abiotic) was designed to evaluate the fate of single substances and are not suitable, on their own, for effluents. There is a need to develop guidance on:
    - how the tests are conducted, for example, the source and concentration of inoculum;
    - how the results are interpreted, for example, the criteria used to interpret the endpoint;
    - how the results are used in combination with other properties, such as bioaccumulation and toxicity.

6. Addressing the bioaccumulation potential of components in effluents leads to similar problems to those discussed for degradation. These tests were also developed for single substances and hence their interpretation is difficult. However, techniques are available that do enable at least a fraction of potentially bioaccumulating substances to be identified.

Furthermore, it is important to recognise that most methods will only provide an indication of the potential to bioaccumulate and consequently should only be used as tools to identify effluents that may warrant further investigation.

7. Where the effluent is thought to contain substances with specific mechanisms of action (e.g. endocrine disruption) a tiered investigative approach including TIE should be adopted.

8. Although there is a significant database on *in vitro* effluent genotoxicity assessment (Houk, 1992), scientific uncertainty over data interpretation indicates that genotoxicity assessment is not recommended as a routine part of WEA. More importantly, as is the case with endocrine disruptor assays, there is a need to validate cost-effective *in vivo* eco-genotoxicity screens for application in a case-by-case approach. Until this is possible, the unjustified adoption of *in vitro* methods will have limited application given their lack of relevance to ecosystems and populations.

9. Biological response, sample variability and site-specific conditions will always make the interpretation of WEA results difficult due to the inherent limitations of the methodology. These difficulties may be overcome by using a flexible approach that incorporates:
    - a tiered and site-specific approach to WEA;
    - learning by doing;
    - early and continuing discussion with the regulatory community and other appropriate stakeholders on ways of approaching different issues.

10. Toxicity emanating from receiving water sediments may significantly complicate the assessment of the impact of effluent discharges. Sediment toxicity tests are avilable, however, it is difficult to relate the toxicity of the sediment to the quality of the overlying receiving water.

11. It is acknowledged that environmental monitoring can be difficult, time consuming and expensive and that it is subject to a high degree of natural variability and other confounding factors. Nevertheless, environmental monitoring is an integral part of the toolbox to investigate the environmental impacts of effluents and should be considered in the development of the WEA strategy since it may be the only way to identify actual impacts and environmental improvements.

12. At present, the use of biomarkers, for example genotoxic or endocrine endpoints, is not considered sufficiently well developed for routine or widespread use in WEA. This does not preclude their possible use where specific risk factors indicate that these modes of action may be important for a particular discharge.

6.2 Recommendations

1. As the scope and use of WEA expands, the value of such programmes needs to be evaluated by consideration of impacts in the receiving waters.

2. As illustrated in this report there are currently no methods available to assess the 'persistency' of effluents. The Task Force recommends that persistency should be used in an integrated way with bioaccumulation and toxicity (Figure 4). This will require the development of a suitable method that could probably be based on the current 'inherent' tests (i.e. allows for a period of microbial adaptation).

3. Consideration should be given to the development of chronic toxicity studies which will be suitable for use in WEA.

4. It is recommended that a better understanding of the effects of water quality parameters on selected toxicity tests is achieved. Such studies should include the effect of confounding factors such as pH, ionic strength, natural or widely occuring toxicants etc.

5. Better understanding of the relevance and ecological implications of the release of endocrine disrupting materials in effluents is required. This should build upon the tests being developed as part of the single substance approach for endocrine disruption.

6. Testing for bioaccumulation potential of effluents is not commonly done. When it is measured, it is still not clear what such data mean in practice nor how they can be turned into a management action. A programme of work is recommended to investigate this further, perhaps using a tiered approach starting with assessment of effluents containing compounds of known bioaccumulation properties and extending to more complex mixtures.

7. Increasing pressures to reduce animal testing requires development of alternative testing methods. These are currently being developed for single substances and will need to be validated for their applicability to effluents.

8. Building on the conclusions that WEA develops primarily through practical experience and benefits from a dialogue between regulator and regulated, it is recommended to develop WEA jointly between authorities and industry at a European level. The IEG (Intersessional Expert Group) working under OSPAR SPDS would be a suitable vehicle to achieve this. It is recommended that industry coordinates its WEA activities through the IEG, develops a multi-year WEA development plan based on this report and provides active support to this plan.

GLOSSARY

Assessment	The evaluation of hazard or risk due to the toxic nature of an effluent.
Acute tests	Short-term (generally $\geq$ 96 h) tests on species, normally to determine lethal endpoints.
Bioaccumulation	The net result of uptake, distribution and elimination of a substance due to all routes of exposure.
Bioassay	A test based on measurement of the effect of a substance or an effluent on a living organism.
Chronic tests	Longer-term (generally $\geq$ 96 h) tests on species, with sublethal endpoints, e.g. measurements of effects on growth and reproduction.
Compliance test	Tests conducted to check conformance with consent conditions.
Consent	A legal authorisation to discharge effluent, usually with limits on effluent quantity and quality.
EC_x	A statistically derived concentration that, over a defined period of exposure, is expected to cause a specified toxic effect in x% of the test population.
Effluent	Any water-borne discharge to the aquatic environment, including domestic and industrial sources.
EQS	Environmental Quality Standard - the concentration of a substance in the environment that has been formally adopted by regulatory authorities as the upper limit of contamination for adequate protection of the environment and public health.
Monitoring	A series of measurements made to check the quality of an effluent, or a sector of the environment (e.g. river water) in relation to desired quality criteria.
Predicted Environmental Concentration	The concentration of a substance in the environment, predicted on the basis of available information on certain of its properties, its use and discharge patterns and quantities involved.
Predicted No Effect Concentration	The environmental concentration that is regarded as a level below which the balance of probability is that an unacceptable effect will not occur.
Receiving water	Surface water (e.g. a stream, river, lake estuary or sea) that has received a discharged waste.
Screening tests	Relatively quick (< 6 h), inexpensive indicative tests, in some cases correlated with species-specific tests, e.g. the Microtox test.
Toxicity	The inherent property of a substance to cause adverse biological effects at specific concentrations.
Whole Effluent Assessment	Whole effluent assessment utilising the broad approach of toxicity together with some or all of the additional parameters, endocrine disruption, persistence, genotoxicity and the potential to bioaccumulate.
Whole Effluent Toxicity	Whole effluent toxicity utilising only acute and/or chronic toxicity measurements.

ABBREVIATIONS

AEE	Assessment of Environmental Effects
B	Bioaccumulation
BAT	Best Available Technology
BMWP	Biological Monitoring Working Party
BOD	Biochemical Oxygen Demand
BREF	Best Available Techniques Reference Document
CA	Competent Authority
COD	Chemical Oxygen Demand
DTA	Direct Toxicity Assessment
ELV	Emission Limit Value
EQS	Environmental Quality Standard
ETT	Effluent Toxicity Test
ICE	Integrating Control of Effluents
Log K_{ow}	Logarithm of the octanol-water partition coefficient
OSPAR	Oslo-Paris Convention for the protection of the marine environment of the North-East Atlantic
P	Persistence
PAH	Polynuclear aromatic hydrocarbons
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
RIVPACS	River Invertebrate Prediction and Classification Scheme
SPE	Solid Phase Extraction
SPMD	Semi-Permeable Membrane Device
SPME	Solid Phase Micro-Extraction
STP	Sewage Treatment Plant
T	Toxicity
TEF	Total Emission Factor
TIE	Toxicity Identification and Evaluation
TOC	Total Organic Carbon
TGD	Technical Guidance Document
WEA	Whole Effluent Assessment
WEER	Whole Effluent Environmental Risk
WET	Whole Effluent Toxicity
WFD	Water Framework Directive

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APPENDIX A: LIST OF GUIDELINES

Guideline number	Guideline title	Location in report
92/69/EEC. 1992.	Commission Directive 92/69/EEC of 31 July 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal L 383, 29/12/1992 P. 0113 - 0115*	Table 3
2001/59/EC. 2001.	Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (Text with EEA relevance). *Official Journal L 225 , 21/08/2001 P. 0001 - 0333*	Table 3
ASTM E 706. 2002.	Standard master matrix for light-water reactor pressure vessel surveillance standards, E706(0). American Society for Testing and Materials, West Conshohocken, PA, USA.	Table 8
ASTM E 1367. 2003.	Standard test method for measuring the toxicity of sediment-associated contaminants with estuarine and marine invertebrates. American Society for Testing and Materials, West Conshohocken, PA, USA.	Table 8
EPA 600/R-94/025	Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA 600/R-94-025. June 1994. Narragansett, Rhode Island, USA.	Table 8
EN ISO 6341. 1996.	Water Quality -- Determination of the inhibition of the mobility of *Daphnia magna Straus* (*Cladocera, Crustacea*) --Acute toxicity test. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO 7346-1. 1996.	Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 1: Static method. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO 7346-2. 1996.	Water quality - Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] - Part 2: Semi-static method. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO 7346-3. 1996	Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)] -- Part 3: Flow-through method. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO 10253. 1995.	Water quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3

APPENDIX A: LIST OF GUIDELINES (CONT'D)

Guideline number	Guideline title	Location in report
ISO 10706. 2000.	Water quality - Determination of long-term toxicity of substances to *Daphnia magna Straus* (Cladocera, Crustacea). International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
EN ISO 10712. 1995.	Water quality - Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test). International Organization for Standardization, ISO, Geneva, Switzerland.	Table 3
ISO 11348. 1998.	Water quality - Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 1: Method using freshly prepared bacteria. Part 2: Method using liquid-dried bacteria Part 3: Method using freeze-dried bacteria. International Organization for Standardization, ISO, Geneva, Switzerland.	Table 3
ISO 13829. 2000.	Water quality - Determination of the genotoxicity of water and waste water using the umu-test. International Organization for Standardization ISO, Geneva, Switzerland.	Table 8
ISO 14669. 1999.	Water quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea). International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO/FDIS 16240. Under development.	Water quality - Determination of the genotoxicity of water and waste water - Salmonella/microsome test (Ames test). International Organization for Standardization ISO, Geneva, Switzerland.	Table 8
ISO/DIS 20079. Under development.	Water quality - Determination of the toxic effect of water constituents and waste water to duckweed (*Lemna minor*) - Duckweed growth inhibition test. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO/CD 20665. CD study/ballot initiated.	Water quality - Determination of chronic toxicity to Ceriodaphnia dubia in 7 days - Population growth inhibition test. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
ISO/CD 20666. CD study/ballot initiated.	Water quality - Determination of chronic toxicity to Brachionus calyciflorus in 48 h -- Population growth inhibition test. International Organization for Standardization ISO, Geneva, Switzerland.	Table 3
NF T90-375. 1998.	Qualité de l'eau - Détermination de la toxicité chronique des eaux par inhibition de la croissance de l'algue d'eau douce *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*). AFNOR (Association française de normalisation), Saint-Denis La Plaine, France.	Table 3
NF T90-376. 2000	Water quality - Determination of chronic toxicity to *Ceriodaphnia dubia* in 7 days - Population growth inhibition test. NF T90-376. AFNOR (Association française de normalisation), Saint-Denis La Plaine, France.	Table 3
NF T90-377. 2000	Water quality - Determination of chronic toxicity to *Brachionus calyciflorus* in 48 h - Population growth inhibition test. NF T90-377. AFNOR (Association française de normalisation), Saint-Denis La Plaine, France.	Table 3

APPENDIX A: LIST OF GUIDELINES (CONT'D)

Guideline number	Guideline title	Location in report
OECD 201. 2002.	OECD guidelines for the testing of chemicals. Revised proposal for a new guideline 201. Alga, growth inhibition test. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OECD 202(I). 2000.	OECD guidelines for the testing of chemicals. Revised proposal for updating guideline 202. *Daphnia* sp., Acute immobilisation test. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OECD 203. 2002.	OECD guidelines for the testing of chemicals. Revised proposal for updating guideline 203. Fish, acute toxicity test. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OECD 204. 1984.	OECD guidelines for the testing of chemicals. 204. Fish, prolonged toxicity test: 14-day study. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OECD 211. 1998.	OECD guidelines for the testing of chemicals. 211. *Daphnia magna* reproduction test. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OECD 212. 1998.	OECD guidelines for the testing of chemicals. Fish, short-term toxicity test on embryo and sac-fry stages. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OECD 218. OECD. 2004.	OECD guidelines for the testing of chemicals. Sediment-water chironomid toxicity using spiked sediment. Organisation for Economic Co-operation and Development, Paris, France.	Table 8
OECD 219. 2004.	OECD guidelines for the testing of chemicals. Sediment-water chironomid toxicity using spiked water. Organisation for Economic Co-operation and Development, Paris, France.	Table 8
OECD 221. 2002.	OECD guidelines for the testing of chemicals. Revised proposal for a new guideline 221. *Lemna* sp. Growth inhibition test. Organisation for Economic Co-operation and Development, Paris, France.	Table 3
OPPTS 850.1010. 1996.	Ecological Effects Test Guidelines. Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids. OPPTS 850.1010. US EPA 96-114. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1020. 1996.	Ecological Effects Test Guidelines. Gammarid Acute Toxicity Test. OPPTS 850.1020. Office of Prevention, Pesticides and Toxic Substances, US EPA 96-130. US EPA, Washington DC, USA.	Tables 3 and 8
OPPTS 850.1025. 1996.	Ecological Effects Test Guidelines. Oyster acute toxicity test (shell deposition). US EPA 96-115. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1035. 1996.	Ecological Effects Test Guidelines. Mysid acute toxicity test. US EPA 96-136. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1045. 1996.	Ecological Effects Test Guidelines. Penaeid acute toxicity test. US EPA 96-137. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1055. 1996.	Ecological Effects Test Guidelines. Bivalve acute toxicity test (embryo larval). US EPA 96-100. US EPA, Washington DC, USA.	Table 3

APPENDIX A: LIST OF GUIDELINES (CONT'D)

Guideline number	Guideline title	Location in report
OPPTS 850.1075. 1996.	Ecological Effects Test Guidelines. Fish acute toxicity test, freshwater and marine. US EPA 96-118. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1300. 1996.	Ecological Effects Test Guidelines. Daphnid chronic toxicity test. US EPA 96-120. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1350. 1996.	Ecological Effects Test Guidelines. Mysid chronic toxicity test. US EPA 96-166. US EPA, Washington DC, USA.	Table 3
OPPTS 850.1735 1996.	Ecological Effects Test Guidelines. Whole sediment acute toxicity invertebrates, freshwater. US EPA 96-354. US EPA, Washington DC, USA.	Table 8
OPPTS 850.1740. 1996.	Ecological Effects Test Guidelines. Whole sediment acute toxicity invertebrates, marine. US EPA 96-355. US EPA, Washington DC, USA.	Table 8
OPPTS 850.1790 1996.	Ecological Effects Test Guidelines. Chironomid sediment toxicity test. US EPA 96-313. US EPA, Washington DC, USA.	Table 8
OPPTS 850.4400. 1996.	Ecological Effects test guidelines. OPPTS 850.4400. Background—Nontarget Plant Testing. US EPA, Washington DC, USA.	Table 3
OPPTS 850.5400. 1996.	Ecological effects test guidelines. OPPTS 850.5400. Algal toxicity, Tiers I and II. Office of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency, Washington, DC, USA.	Table 3
OPPTS.850.1400. 1996.	Ecological Effects Test Guidelines. Fish early-life stage toxicity test. US EPA 96-121. US EPA, Washington DC, USA.	Table 3
US EPA OW 1000.0. 2002.	Fathead minnow, *Pimephales promelas,* larval survival and growth test method 1000.0. In *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. EPA-821-R-02-013 - 4th edition. US Environmental Protection Agency, Office of Water, Washington, DC, USA.	Table 3
US EPA OW 1002.0. 2002.	Daphnid, *Ceriodaphnia dubia*, survival and reproduction test method 1002.0. In *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. EPA-821-R-02-013 - 4th edition. US Environmental Protection Agency, Office of Water, Washington, DC, USA.	Table 3
US EPA OW 1003.0. 2002.	Green alga, *Selenastrum capricornutum*, growth test. method 1003.0. In *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. EPA-821-R-02-013 - 4th edition. US Environmental Protection Agency, Office of Water, Washington, DC, USA.	Table 3

APPENDIX B: VALIDATION STUDIES

Table B.1: Validation performed to assess the use of whole effluent and ambient toxicity tests to understand effects on riverine communities

Study site	Type of Effluent and Site	Single Species Tests	Matrix	In-stream biomonitoring	Reference
Scippo Creek, Ohio	Chemical resins manufacturing plant located on small (10-20 m wide) shallow (0.6 m depth) stream	*Ceriodaphnia reticulata* Fathead minnow Bluntnose minnow *8 resident species*	Effluent diluted in laboratory water and in site water Effluent diluted in laboratory water and in site water Site waters collected from various stations above and below mixing zone Site waters collected from various stations above and below mixing zone	Periphyton, benthic invertebrate, and fish populations and communities	US EPA, 1985
Ohio River at Wheeling, West Virginia	Chemical plants, oil refineries, steel mills, and POTWs on a large navigable river	*Ceriodaphnia dubia* Fathead minnow	Site waters collected from various stations above and below mixing zone Site waters collected from various stations above and below mixing zone	Phyto- and zooplankton, periphyton, and benthic invertebrate populations and communities	US EPA, 1986
Skeleton Creek, Enid, Oklahoma	Oil refinery, Fertiliser manufacturer, and POTW on a small creek (5-20 m wide, 0.3 – 1 m deep)	*Ceriodaphnia dubia* Fathead minnow	Effluents diluted in laboratory water and in site water Effluents diluted in laboratory water and in site water	Phyto- and zooplankton, benthic invertebrates, fish populations and communities	US EPA, 1986
Five Mile Creek, Birmingham Alabama	Two coke plants and a POTW on a small stream (15 m wide, 0.3 – 1.5 m deep)	*Ceriodaphnia dubia* Fathead minnow	Effluents diluted in laboratory water and in site water Effluents diluted in laboratory water and in site water	Periphyton, benthic invertebrate, and fish populations and communities	US EPA, 1985

Table B.1: Validation performed to assess the use of whole effluent and ambient toxicity tests to understand effects on riverine communities (Cont'd)

Study site	Type of Effluent and Site	Single Species Tests	Matrix	In-stream biomonitoring	Reference
Kanawha River near Charleston, West Virginia	Numerous discharges along a 125 mile large, navigable river reach	*Ceriodaphnia dubia* Fathead minnow	11 effluents diluted in laboratory water and in 34 site waters 4 effluents diluted in laboratory water and in 34 site waters	Zooplankton, periphyton and benthic invertebrate populations and communities	US EPA, 1986
Back River near Baltimore Harbor, West Virginia	POTW discharge leading into a tidal estuary	*Ceriodaphnia dubia* Fathead minnow *Microtox*	2 POTW effluents diluted in laboratory water and in site waters 2 POTW effluents diluted in laboratory water and in site waters 2 POTW effluents diluted in laboratory water and in site waters	Macrozooplankton, ichthyoplankton, benthic invertebrates, and fish populations and communities	US EPA, 1986
Ottawa River, Lima, Ohio	POTW, oil refinery, and chemical plant on a medium-sized river	*Ceriodaphnia dubia* Fathead minnow	3 effluents diluted in laboratory water and in 13 site waters 3 effluents diluted in laboratory water and in 13 site waters, fish caging study	Periphyton, zooplankton, benthic invertebrates, and fish populations and communities	US EPA, 1984
Naugatuck River, Waterbury, Connecticut	4 POTWs and several industrial discharges (a total of 28 dischargers), medium-sized river (15-100 m wide, 0.5 – 3 m deep)	*Ceriodaphnia dubia* Fathead minnow	4 effluents diluted in laboratory water and in 15 site waters 4 effluents diluted in laboratory water and in 15 site waters	Periphyton, zooplankton, benthic invertebrates, and fish populations and communities	US EPA, 1986

Table B.2: Validation studies performed to assess the use of whole effluent and ambient toxicity tests to understand effects on marine and estuarine systems

Study site	Type of Effluent and Site	Single Species Tests	Matrix	In-stream biomonitoring	References
Fernandia Beach, Florida	Pulp and paper mill, tidally influenced	*Champia parvula* *Mysidopsis bahia* *Menidia berylina* *Cyprinodon variegatus*	Effluent in upstream tidal water (all species) and ebb tide ambient water for all but *Menidia*	None	Schimmel ***et al***, 1989; US EPA, 1989
East Greenwich, Rhode Island	POTW, estuarine cove, tidally influenced	*Arbacia punctulata* (sea urchin) *Laminaria saccharina* (sea kelp) *Champia parvula* *Mysidopsis bahia* *Menidia berylina* *Cyprinodon variegatus*	Effluent in clean, natural sea water (all species except kelp) and 7 ambient site waters	Scope for growth of mussels, *Mytilus edulis*	US EPA, 1989
Panama City, Florida	POTW into an open bay	*Arbacia punctulata* (sea urchin) *Champia parvula* *Mysidopsis bahia* *Ceriodaphnia dubia* *Pimephales promelas*	Effluent diluted into river water and saltwater (effluent made to saltwater by addition of commercial sea salts); ambient marine tests at ebb, slack low, flood, and slack high tide	Seagrass community (qualitative survey)	US EPA, 1989

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No. 16	Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals
No. 19	Respiratory Allergy
No. 20	Percutaneous Absorption
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation
No. 22	Evaluation of Chemicals for Oculotoxicity
No. 23	Receptor Mediated Mechanisms in Chemical Carcinogenesis
No. 24	Risk Assessment for Carcinogens
No. 25	Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents
No. 26	Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances
No. 27	Aneuploidy
No. 28	Threshold-Mediated Mutagens - Mutation Research Special Issue
No. 29	Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment
No. 30	Genetic Susceptibility to Environmental Toxicants
No. 31	Guidance on Evaluation of Reproductive Toxicity Data
No. 32	Use of Human Data in Hazard Classification for Irritation and Sensitisation
No. 33	Application of Physiological - Toxicokinetic Modelling to Health Hazard Assessment of Chemcial Substances

Technical Reports

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans (updated by TR No. 6)
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man (updated by TR No. 17)
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man
No. 6	Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2
No. 7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8	Biodegradation Testing: An Assessment of the Present Status
No. 9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11	Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5
No. 12	The Phototransformation of Chemicals in Water: Results of a Ring-Test
No. 13	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment
No. 14	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health
No. 15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16	A Review of Recent Literature on the Toxicology of Benzene
No. 17	The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4 (updated by TR No. 64)
No. 18	Harmonisation of Ready Biodegradability Tests
No. 19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20	Biodegradation Tests for Poorly-Soluble Compounds
No. 21	Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment
No. 22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24	The EEC 6th Amendment: Prolonged Fish Toxicity Tests
No. 25	Evaluation of Fish Tainting
No. 26	The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride
No. 27	Nitrate and Drinking Water
No. 28	Evaluation of Anaerobic Biodegradation
No. 29	Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns
No. 30	Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available)
No. 31	The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment
No. 32	Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data
No. 33	Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34	Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man
No. 35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36	Biomonitoring of Industrial Effluents

No. 37 Tetrachlorethylene: Assessment of Human Carcinogenic Hazard
No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea
No. 40 Hazard Assessment of Chemical Contaminants in Soil
No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43 Emergency Exposure Indices for Industrial Chemicals
No. 44 Biodegradation Kinetics
No. 45 Nickel, Cobalt and Chromium in Consumoducts: Allergic Contact Dermatitis
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models
No. 51 Environmental Hazard Assessment of Substances
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment
No. 55 Pulmonary Toxicity of Polyalkylene Glycols
No. 56 Aquatic Toxicity Data Evaluation
No. 57 Polypropylene Production and Colorectal Cancer
No. 58 Assessment of Non-Occupational Exposure to Chemicals
No. 59 Testing for Worker Protection
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard
No. 61 Environmental Exposure Assessment
No. 62 Ammonia Emissions to Air in Western Europe
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man
No. 65 Formaldehyde and Human Cancer Risks
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs
No. 68 Assessment Factors in Human Health Risk Assessment
No. 69 Toxicology of Man-Made Organic Fibres
No. 70 Chronic Neurotoxicity of Solvents
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members)
No. 72 Methyl *tert*-Butyl Ether (MTBE) Health Risk Characterisation
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank
No. 78 Skin Sensitisation Testing: Methodological Considerations
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data)
No. 80 Aquatic Toxicity of Mixtures

No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy
No. 82 Risk Assessment in Marine Environments
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union
No. 84 Scientific Principles for Soil Hazard Assessment of Substances
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies
No. 86 Derivation of Assessment Factors for Human Health Risk Assessment
No. 87 Contact Sensitisation: Classification According to Potency
No. 88 Environmental Risk Assessment of Difficult Substances
No. 89 (Q)SARS: Evaluation of the Commercially Available Software for Human Health and Environmental Endpoints with Respect to Chemical Management Applications
No. 90 Persistence of Chemicals in the Environment
No. 91 Aquatic Hazard Assessment II
No. 92 Soil and Sediment Risk Assessment
No. 93 Targeted Risk Assessment

Joint Assessment of Commodity Chemicals (JACC) Reports

No. Title

No. 1 Melamine
No. 2 1,4-Dioxane
No. 3 Methyl Ethyl Ketone
No. 4 Methylene Chloride
No. 5 Vinylidene Chloride
No. 6 Xylenes
No. 7 Ethylbenzene
No. 8 Methyl Isobutyl Ketone
No. 9 Chlorodifluoromethane
No. 10 Isophorone
No. 11 1,2-Dichloro-1,1-difluoroethane (HFA-132b)
No. 12 1-Chloro-1,2,2,2-tetrafluoroethane (HFA-124) (updated by JACC No. 25)
No. 13 1,1-Dichloro-2,2,2-trifluoroethane (HFA-123) (updated by JACC No. 33)
No. 14 1-Chloro-2,2,2-trifluoromethane (HFA-133a)
No. 15 1-Fluoro 1,1-dichloroethane (HFA-141B) (updated by JACC No. 29)
No. 16 Dichlorofluoromethane (HCFC-21)
No. 17 1-Chloro-1,1-difluoroethane (HFA-142b)
No. 18 Vinyl Acetate
No. 19 Dicyclopentadiene (CAS: 77-73-6)
No. 20 Tris-/Bis-/Mono-(2 ethylhexyl) phosphate
No. 21 Tris-(2-butoxyethyl)-phosphate (CAS:78-51-3)
No. 22 Hydrogen Peroxide (CAS: 7722-84-1)
No. 23 Polycarboxylate Polymers as Used in Detergents
No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0) (updated by JACC No. 46)

No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27 *n*-Butyl Acrylate (CAS No. 141-32-2)
No. 28 Ethyl Acrylate (CAS No. 140-88-5)
No. 29 1,1-Dichloro-1-fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30 Methyl Methacrylate (CAS No. 80-62-6)
No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34 Acrylic Acid (CAS No. 79-10-7)
No. 35 Methacrylic Acid (CAS No. 79-41-4)
No. 36 *n*-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37 Methyl Acrylate (CAS No. 96-33-3)
No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39 Tetrachloroethylene (CAS No. 127-18-4)
No. 40 Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions
No. 41 *n*-Butanol (CAS No. 71-36-3)
No. 42 Tetrafluoroethylene (CAS No. 116-14-3)
No. 43 *sec*-Butanol (CAS No. 78-92-2)
No. 44 1, 1, 1, 3, 3-Pentafluoropropane (HFC-245fa)
No. 45 1, 1-Difluoroethane (HFC-152a) (CAS No. 75-37-6)
No. 46 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) CAS No. 2837-89-0 (Second Edition)

Special Reports

No. Title

No. 8 HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
No. 9 Styrene Criteria Document
No. 10 Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
No. 11 Ecotoxicology of some Inorganic Borates
No. 12 1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
No. 13 Occupational Exposure Limits for Hydrocarbon Solvents
No. 14 *n*-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
No. 15 Examination of a Proposed Skin Notation Strategy
No. 16 GREAT-ER User Manual
No. 17 Risk Assessment Report for Existing Substances Methyl *tertiary*-Butyl Ether

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development
No. 33	Environmental Oestrogens: A Compendium of Test Methods
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
No. 37	EC Classification of Eye Irritancy
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction
No. 43	Contact Sensitisation: Classification According to Potency, A Commentary

Workshop Reports

No.	Title
No. 1	Workshop on Availability, Interpretation and Use of Environmental Monitoring Data 20-21 March 2003, Brussels
No. 2	Strategy Report on Challenges, Opportunities and Research needs arising from the Definition, Assessment and Management of Ecological Quality Status as required by the EU Water Framework Directive based on the workshop EQS and WFD versus PNEC and REACH - are they doing the job ? 27-28 November 2003, Budapest
No. 3	Workshop on the Use of Human Data in Risk Assessment 23-24 February 2004, Cardiff
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity 2 March 2004, Berlin
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment 7-9 July 2004, Paris