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Assessment of refinery effluents and receiving waters using biologically-based effect methods

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Prepared by the Special Task Force on Biological Effects Measures (WQ/STF-32):

G. Whale (Chairman)
P. Baldoni-Andrey
A. Basseres
T.K. Frost
I. Fotiadou
L. González Bajos
I. Keresztenyi
T. Parkerton
M. Paumen
M. Comber (Consultant)
A. Girling (Consultant)

K. den Haan (Technical Coordinator)

I

ABSTRACT

Within the EU it is apparent that the regulatory focus on the use of biologically-based effects methods in the assessment of refinery effluents and receiving waters has increased in the past decade. This has been reflected in a recent refinery survey which revealed an increased use of such methods for assessing the quality of refinery effluents and their receiving waters. This report provides an overview of recent techniques used for this purpose. Several case studies provided by CONCAWE member companies describe the application of biological methods to effluent discharge assessment and surface water monitoring.

The case studies show that when biological methods are applied to refinery effluents and receiving waters they raise different questions compared with those obtained using physical and chemical methods. Although direct measurement of the toxicity of effluent and receiving to aquatic organisms is the most cited technique, more recent efforts include tests that also address the persistence of effluent toxicity once discharged into the receiving water.

Similarly, ecological monitoring of receiving waters can identify effects of effluent inputs arising from species interactions and other secondary effects that would not always be apparent from the results of biological tests conducted on single aquatic organisms.

In light of recent and proposed regulatory developments the objectives of this report are therefore to:

- Discuss the application of biologically-based effects methods (including ecological monitoring) to refinery discharges and receiving waters,

- Assess the implications of such methods for future regulation of refinery discharges and

- Provide guidance on good practice that can be used by refineries and the downstream oil industry to carry out and interpret data obtained using biologically-based effects methods.

While the emphasis is on the toxic effects of effluents, other properties will also be covered because of their interdependency in determining potential effects in the environment. In particular, the properties of effluent constituents that determine their persistence and potential to accumulate within organisms will also be considered.

KEYWORDS

Refinery effluent, receiving water, biologically-based effects methods, toxicity; bioaccumulation, persistence, ecological monitoring, Whole Effluent Toxicity, Whole Effluent Assessment

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SUMMARY

Earlier CONCAWE reports describe the environmental impact of refinery effluents (CONCAWE, 1979) and ecological monitoring of refinery effluents (CONCAWE, 1982). Since these initial reports there have been a number changes in EU regulations, most notably the EU Water Framework Directive, that have placed increasing emphasis on ecological quality of receiving waters. This in turn has led to increased interest in more ecologically relevant measurements of effluent and receiving water quality which include direct measures of biological effects. This development has implications for refineries as highlighted by the results of a recent refinery survey which revealed that biologically-based effects methods[1] (based mainly on toxicity but also on persistence and potential to bioaccumulate) are being increasingly applied to refinery effluents and receiving water.

This report gives an overview of the types of biologically-based effect methods which are being considered and/or have been used in the assessment of effluents and receiving waters. Specific examples of the application of such methods to refinery effluent discharges are described in the Case Studies and the implications of using such methods for the future regulation of discharges are assessed and discussed. The overview is based on a review of the literature coupled with feedback from a CONCAWE refinery survey. Reference is also made to case studies which describe the application of some of these methods to effluent and surface water monitoring investigations that CONCAWE and CONCAWE member companies have undertaken.

The key findings of the report are as follows:

- Requirement for the use of biologically-based effects methods to assess the quality of effluents and receiving waters is increasing in the EU due to developing legislation, in particular the focus of the Water Framework Directive on improving biological quality of receiving waters. However, the associated costs and animal welfare considerations mean that clear and practical guidance is required so that the objectives of the legislation are achieved effectively and without wastage.

- Based on the findings of the refinery survey and the case studies, biologically-based methods provide a logical, complimentary approach to traditional physical and chemical-based effluent and receiving water quality criteria.

- Whole effluent toxicity tests with single aquatic organisms serve as the principle biological effect measure in current use; selection of test species, test design and confounding issues that can complicate data interpretation are important issues that need careful consideration.

- Although the basic scientific principles have not changed from those identified in the earlier reports (CONCAWE, 1979; CONCAWE, 1982), the range of methods that are now available, and the sensitivity of some of the assessment endpoints that are utilised, has increased significantly.

- The current review indicates that effluent toxicity is not a major issue for refineries in the EU. This generalization is consistent with existing regulations /

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[1] A 'biological effects method is one that evaluates the potential biological impact of a whole effluent on organisms commonly found in the receiving water environment and the causatives for these.

risk management measures that have progressively reduced conventional and chemical-specific pollutant discharges. In cases where effluent toxicity is observed, application of new methods described in this report indicates that toxicity is not typically persistent or refractory in nature.

- Solid phase micro-extraction, or biomimetic extraction, provides a promising analytical screening tool to assess the potential for bioaccumulation and additive toxicity of non-ionic organic pollutants such as hydrocarbons in refinery effluents. When coupled with effluent biodegradation studies, this approach can also be used to evaluate toxicity persistence. Advantages of this method include its: mechanistic basis, good precision, low cost and avoidance of animal use in testing. A key limitation is that the technique does not address potential concerns associated with inorganics.

- While the use of biologically-based in-vitro methods (e.g. biomarkers) appears to be increasing, difficulties in standardization and linkage to effects at organism level limit practical benefit. These methods are not generally recommended for use in decision-making.

- Biologically-based methods should be used as a tool in risk-based management that also considers exposure potential and receiving water quality considerations. The trigger for use should be where initial screening identifies a potential concern. The methods should not be used only for hazard assessment as appears to be happening in some EU countries.

- Best value of using biological methods can be realized by targeting sites that have low dilution and that are potentially contributing to impaired biological quality as evidenced from relevant field monitoring studies.

- If the results of applying biological methods indicate that a site effluent discharge poses a concern, it can be further applied to source investigation/control so that risks can be mitigated to improve biological quality in the local receiving water.

- Biologically-based methods are not always suitable or needed for continuous effluent monitoring since they can add significant costs without clear benefits. Advantages and disadvantages in their use therefore need to be weighed before deciding whether they are applicable to a specific situation. Whether they bring benefits or additional burden will ultimately depend on the study design, specific test methods used, and interpretation of the results.

1. INTRODUCTION

European effluent discharges have traditionally been controlled and regulated by assessment of chemical and physical parameters. These parameters have typically included dissolved oxygen, suspended solids, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and concentrations of specific substances (e.g. ammonia). Such an approach provides a good method for control of relatively simple effluents containing a limited number of constituents or a very well characterised effluent with known substances present. The use of this approach has resulted in the reduction of the discharge of hazardous substances leading to an improvement in the quality of receiving waters. A recent overview, outlining the chemical approach and its application, has been published (ECETOC, 2004). Despite confounding factors and other limitations, this "chemical-based" approach is generally well understood and accepted by both industry and authorities.

Implementation of the associated regulations has led to a steady reduction in the discharge of hazardous substances to receiving waters and improvements to the quality of receiving waters as shown in recent surveys (CONCAWE, 2004; CONCAWE, 2011). However, recent EU initiatives such as the Water Framework Directive (WFD, 2000/60/EC), have focussed on improving the ecological condition of the receiving water (EU, 2000). The stimulus for this has been situations where receiving water 'health' (e.g. biological diversity) has been found to be unexpectedly poor even though chemical parameters remain in compliance. The focus has therefore shifted from the physical and chemical characterisation of water quality to biological quality.

In the past, studies aimed at assessment of biological 'health' as a consequence of oil refinery discharges were focussed on receiving water quality (CONCAWE, 1982). Typically this included the determination of species diversity in a given ecosystem using standard techniques. These approaches are still used but increasingly a range of other methods have been employed to estimate or predict the ecosystem's response to a discharge. For example, tests which assess the toxicity (T) of an effluent are conducted with species, representative of the aquatic environment, either *in situ* or as a laboratory simulation (SETAC, 2000).

Other parameters such as the potential to bioaccumulate (B) or to be persistent (P) have also been incorporated in an approach frequently referred to as Whole Effluent Assessment (WEA) or sometimes PBT assessment (OSPAR, 2005). The test methods used have initially come from assessing single chemical substances and in some cases they are used without modification for the specific application to complex whole effluent discharges (ECETOC, 2004).

In the future, refineries within the EU are likely to encounter WEA as a result of a number of regulatory developments at both national and international level. For example:

- WEA is referred to as a mechanism to demonstrate Best Available Techniques (BAT) in the Integrated Pollution Prevention Control (EU, 1996) Waste Water/ Waste Gas and the draft Economics and Cross Media Issues Best Available Technique Reference (BREF) Document;

- Several European countries (e.g. Germany, Ireland, UK, and Sweden) use some aspects of WEA in a regulatory context and many others are developing such approaches (Power and Boumfrey, 2004);

- WEA approaches can potentially be used as tools to support the assessment of Good Ecological Status as required in the WFD (Allan et al, 2006);

- The Oslo, Paris Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR) is studying the use of WEA as a means to reduce or eliminate the presence of Priority Substances from the marine environment (OSPAR, 2005).

In addition to the regulatory context, WEA can potentially be used by refineries and other downstream facilities (e.g. distribution/depots) to validate that their operations are acceptable and/or to improve their environmental performance by:

- Providing a more holistic assessment of their effluent quality which may be more easily understood by other stakeholders (e.g. demonstrate a lack of toxicity to key organisms);

- Providing a cost effective mechanism to assess all potentially hazardous substances in their effluents rather than undertaking individual substance assessments;

- Assessing process effluent streams within a refinery to identify problematic sources of toxicity and target them for management at source. This approach can also be beneficial in addressing effluent treatment plant problems by identifying those streams that may be adversely affecting performance.

- Providing additional data which could be used to demonstrate that REACH[1] environmental exposure predictions may be overly conservative on the basis that product handling and waste water treatment systems may be more effective in removing/reducing toxicity than default assumptions would suggest.

To understand the current status of WEA within the industry, CONCAWE has undertaken a survey amongst member companies to assess the current level of use of biologically-based effect methods by its member companies. The last time that the application of toxicity assessments and environmental monitoring relevant to oil refineries was assessed was almost 30 years ago (CONCAWE, 1979 and 1982). The new survey was designed to provide data to identify the trends in both the application of biologically-based effect methods by regulators and also refinery experience in the use of such methods.

The results of the questionnaire showed that 44% (28 of 64) of the facilities that responded are required to do some form of biological assessment on their effluents. There are a myriad of testing requirements across the group, but the most common test parameter is acute (short-term) toxicity. Some sites conduct chronic (longer-term) toxicity in lieu of or in combination with acute testing, but only 4 sites test for bioaccumulation. Overall, the testing regimes are not the same across the EU and in many cases not all refineries within the same country are required to conduct biological testing. This indicates that the regulators implement this on a site-by-site and case-by-case basis.

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[1] REACH is the European Union regulation (EC) No 1907/2006) concerning the Registration, Evaluation, Authorisation and restriction of Chemicals. It came into force on 1st June 2007 and replaced a number of European Directives and Regulations with a single system.

Although there are variations in local use, regulators in 11 countries implement it in some form. The countries include: Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Norway, Spain, Sweden and the United Kingdom. The expectation is that biological testing will increase in order to meet the provisions or the WFD (EU, 2000).

The objectives of this report are to discuss the application of biologically-based effect methods in the context of applying WEA to refinery discharges, to assess the implications of such methods for future regulation of refinery discharges and to indicate good practice that can be used by refineries and the downstream oil industry to carry out and interpret data from biological effects assessments.

The contents of the appendices to this report are as follows:

APPENDIX I: Case Studies

- Case Study 1. Assessing Refinery Streams – addressing the impact of treatment on toxicity and assessing environmental impact (provided by Shell).

- Case Study 2. Effluent toxicity at Mongstad refinery (provided by Statoil)

- Case Study 3. Ecological monitoring of the marine environment at Mongstad refinery (provided by Statoil).

- Case Study 4. A study on the ecotoxicity of Mol Danube Refinery effluents (provided by Mol).

- Case Study 5. Predicting the effect of refinery effluents (provided by ExxonMobil).

- Case Study 6. Whole Effluent Assessments on Refinery Effluents (provided by CONCAWE).

- Case Study 7. A new biotic index for non-specialists, developed by Repsol, as a tool for water quality control in Spanish rivers (provided by Repsol).

- Case Study 8. Methodology for measuring the impact of treated waste water discharged in an estuary (provided by Total).

APPENDIX II: Framework for the quality assessment of aquatic toxicology laboratories (provided by BP).

With the exception of Case Study 6, the content of **Appendices I** and **II** has been provided by CONCAWE member companies. No further review or revision of the case study reports has been undertaken. References cited in the case studies are not held by CONCAWE.

From here onwards the case studies will be referred to in this report by their number (e.g. Case Study 1) with no further reference to **Appendix I.**

2. BACKGROUND & TRENDS

The composition of refinery effluent discharges will vary depending on a number of factors including crude feedstock and the extent of the hydrocarbon processing. However a similar range of process operations are involved (CONCAWE, 1999) and this leads to the production of effluents that contain similar contaminants albeit at differing concentrations.

The main constituents of refinery effluents that contribute to contamination of aqueous effluents are un-dissolved oil, solid particulates and dissolved substances, both organic and inorganic. The dissolved substances include metals (which may originate from the crude and/or catalysts), hydrogen sulphide, ammonia, phenols and cyanides (CONCAWE, 1999). In a normal refinery, certain process water streams, which contain high quantities of sour gases (mainly hydrogen sulphide and ammonia), are stripped to lower the concentration of these gases before discharging the water to the effluent treatment plant.

The use of specific process chemicals (e.g. to support specific refinery processes and for blending in some final products) can also lead to site-specific differences in the composition of refinery waste water streams and final effluents.

Except where site-specific differences apply, effluent property data from one refinery location will be indicative of issues that are likely to be present at other refinery locations. This is important because it means that refinery effluents may be able to be assessed using WEA methods and the data read across from one site or operation to another of broadly similar characteristics. The fact that these similarities exist can be demonstrated when the sources and historical methods for treating refinery wastewaters are examined, as described in the following sections.

Sources of waste water in a refinery

In order to manage refinery effluents effectively, it is important to identify the sources of waste water that contribute to the final effluent. Refineries use significant volumes of water for heating (steam) and as a coolant. The water (with the exception of "closed loop" refineries) is ultimately discharged in refinery effluents. Refinery effluents can also include ballast water (which comes from product tankers arriving at the refinery), water derived from the crude oil itself and from rainwater run-off.

The water that contributes to a refinery effluent can become contaminated with hydrocarbons to varying degrees. For example, cooling water should not, under normal circumstances, be contaminated with hydrocarbons but it can be if there is a failure in heat exchanger tubes. At the other end of the scale process water (originating from a number of sources including the drainage of water from crude oil and product tanks, from steam used in the distillation and conversion units, wet chemical treatment or from water washing of crude and oil products) which has been in intimate contact with hydrocarbons, can contain significant levels of dissolved and free hydrocarbons.

The main sources of hydrocarbon-contaminated water at a refinery are likely to include some or all of the following:

- Desalter units

- Distillation units

- Hydro-treatment units

- Sour water strippers

- Visbreakers (thermal cracker)

- Catalytic cracking units

- Hydrocracking units

- Lube oils

- Spent caustic

Refinery wastewaters will contain various hydrocarbon constituents (aliphatic and aromatic), phenols, sulphides and mercaptans, ammonia and amines, heavy metals and various salts that originate from these process-related sources.

In addition to cooling and process water, natural rain water can also be contaminated when it falls on hydrocarbon contaminated surfaces. Owing to the potential for spills and leaks during maintenance and operations, rain water run-off from process areas is likely to be the most heavily contaminated. Treatment of this water can be difficult owing to the volumes and spikes that can occur.

Ballast water from crude oil transport by tanker will be contaminated with hydrocarbons and will require some form of treatment before it is finally discharged to the environment.

A more detailed explanation is provided in CONCAWE (1999) and an example of an analysis of sources at a specific refinery is provided in Case Study 4.

One of the main concerns with refinery effluents has been their hydrocarbon content. Consequently, generic characteristics such as oil in water content have historically been used to control refinery discharges (CONCAWE, 2004) although other factors are now being given increased consideration (CONCAWE, 2011). Over the past three decades these generic controls have led to an increase in both procedural and technical measures and water treatment facilities. These have significantly reduced the total amounts of hydrocarbons (measured as oil) discharged from refineries as shown in **Figure 1**. The measures used include segregation of waste streams based on the degree of final effluent water treatment required and improved maintenance procedures to reduce spills and surface contamination.

Refineries are also subject to regulation under the Industrial Emissions Directive (IED) (EU, 2010) which came into force on 6 January 2011. The IED brings several separate pieces of EU legislation on industrial emissions under one directive. The Integrated Pollution Prevention and Control (IPPC) Directive (EU, 2008) is one of those now included in its coverage. The IPPC Directive aims to ensure that particular industries consider the environment as a whole, and the impacts of both routine and accidental releases. Consequently, releases to wastewater arising from minor spillage and any oil contaminated surface water run-off (from process areas)

are captured by interceptors/catchment facilities. The runoff goes to clean up along with other waste streams for appropriate treatment (e.g. by the main refinery wastewater treatment plant).

Figure 1   Trends in oil discharged versus refinery throughput 1969-2008 (CONCAWE 2011)
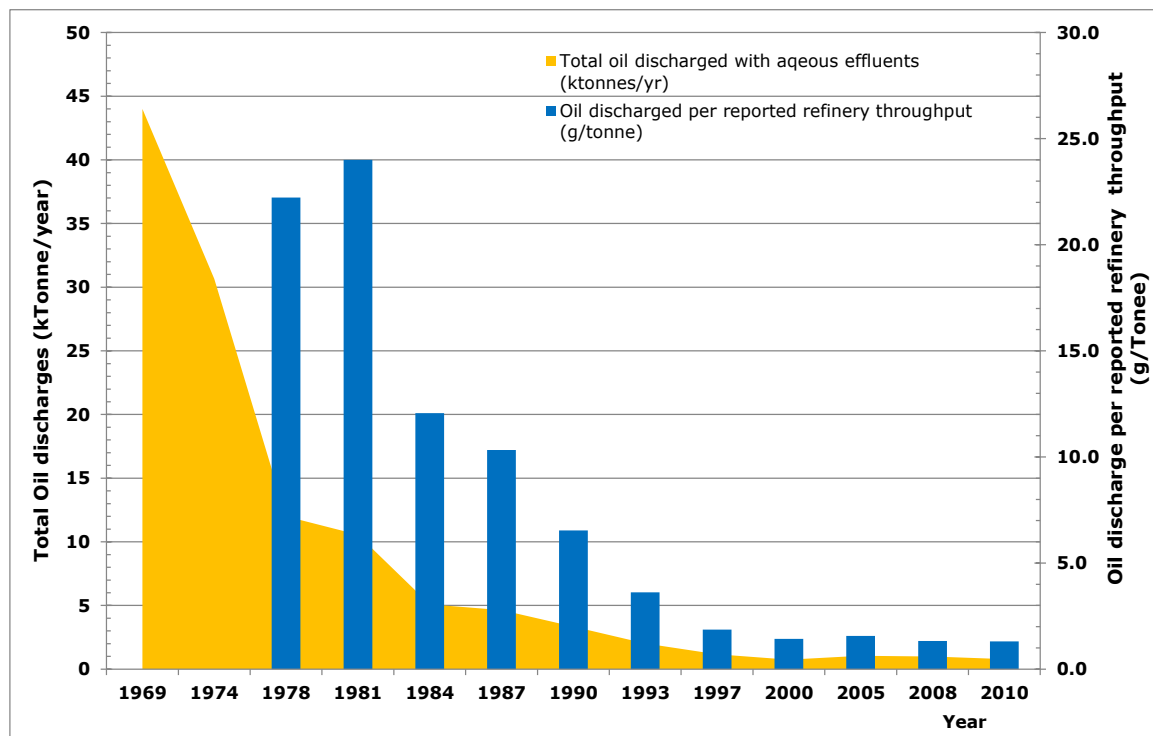
Treatment of refinery waste water

Although there is no standard refinery effluent treatment system, a number of generic features will be applicable to these systems in most refineries. The main contaminants to be removed in waste water treatment are un-dissolved oil, solid particles and dissolved organic and inorganic substances. Refinery wastewater treatment is typically a 3-stage process, including; primary, secondary and tertiary treatment. The purpose of the primary stage is to recover free oil and remove gross solids. In the secondary stage, dispersed oil and fine solids are removed, while in the tertiary stage, dissolved oil and other dissolved organic contaminants are removed.

There are a number of water treatment processes, which have been used singly or in combination, to remove oil and other contaminants from waste water for many years and are still essentially the same as the methods described by CONCAWE (1979). These include:

- Gravity separation, e.g. API separators, plate interceptors, tank separation, etc.;

- Advanced treatment, e.g. flocculation, gas flotation, sedimentation, filtration, etc.; and

- Biological treatment, e.g. bio-filters, activated sludge, aerated ponds, etc.

There has been a trend towards increasing complexity. Since the CONCAWE (1979) report, additional categories of further effluent treatment have been recognised as being used at refineries (CONCAWE, 2004). These are:

- Biological treatment followed by additional polishing treatment;

- Physical treatment followed by biological treatment in shared offsite facilities.

Biological treatment is now an important component of most refinery effluent treatment systems.

In 2008, 14 of the 125 refinery locations reported that their effluents were given biological treatment in external multi-user Waste Water Treatment Plant (WWTP) facilities, most commonly after partial on-site treatment. The other 111 refineries perform an on-site final treatment before discharging their process effluents, for which 103 sites apply a three-stage biological system. The majority of refineries (78) have an aerated activated sludge reactor as the biological unit (**Table 1**). Hence, of the 125 refineries, 117 (94%) subject their process effluents to biological treatment before discharge.

Table 1   Summary of EU oil refinery wastewater treatment processes used in 125 refinery locations in 2005 (CONCAWE 2011)

Treatment	Number	Type of biological treatment	Number
3 stage biox	103	Trickling filter	16
Mechanical	2	Aerated lagoon	5
Chemical	2	Activated sludge	78
Physical	4	Non aerated lagoon	1
API	0	Fixed bed bio-film reactor	1
External WWTP	14	Aerated tank	1
none	0	other	1
Total	125	Total	103

Toxicity assessment of refinery effluents

The issue of refinery effluent toxicity was first addressed by CONCAWE in 1979 (CONCAWE, 1979) and in subsequent environmental impact assessments of refinery effluents in 1982 (CONCAWE, 1982). However, since then there have been many studies both of effluent toxicity and the status of the receiving water environment. These have been undertaken both by the industry itself (See case studies) and by others (ECETOC, 2004; OSPAR, 2007a). A recent questionnaire (CONCAWE, 2004) has provided additional information with respect to how biological assessment tools such as toxicity or bio-accumulation potential have been applied to refinery discharges.

One of the concerns facing refineries is that the use of biologically-based effect methods, such as described in this report, will inevitably raise new questions and potential issues regarding their operations and discharges. Existing water treatment facilities may be suitable for meeting defined chemical specific criteria but, on the basis of biological measures, their effluents may still be regarded as hazardous and be subject to more stringent controls. It may also prove difficult to assess the nature

of the contaminants causing the adverse effects. For example, small changes to an operation, such as the change of a process additive, may result in this additive being carried over in small quantities to the effluent which would not be picked up in measures such as BOD and COD but could be detected by measures of toxicity or bio-accumulation potential. Another potentially confounding factor when assessing refinery discharges can come from non-process operations such as cleaning at turnarounds or local housekeeping objectives.

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3. METHODS FOR ASSESSING TOXICITY, PERSISTENCE AND BIOACCUMULATION OF WHOLE EFFLUENTS

A summary and review of established methods for assessing toxicity, persistence and bioaccumulation of effluents has been published by ECETOC (ECETOC, 2004). The key findings of the ECETOC report and highlight those issues of particular interest to the petroleum refining industry are summarised in the following sections.

A more recent project conducted within Europe over the period 2006-2010 and referred to by the acronym NoMiracle (Novel Methods for Integrated Risk Assessment of CumuLative stressors in Europe) had the objective of developing new methods and models for better and more integrated risk assessment of chemicals (see: http://nomiracle.jrc.ec.europa.eu). The project involved researchers from 17 countries and looked not only at the effects of single chemical and mixtures, but also at their interactions with other factors such as climate change, disease and allergens. The final outputs of the project are still being compiled but a "tool box" of methods has been described for assessing exposure and effects and for assessing and managing risk. The methods described include sampling techniques and models for assessing exposure, novel toxicity screening and assessment tests for assessing hazard and biological monitoring techniques for assessing impacts. Models for assessing risks and statistical techniques for taking account of variability are also described.

3.1. TOXICITY

There are essentially three reasons to conduct toxicity studies on effluents;

- To comply with regulations and identify the toxicity of the effluent;

- To investigate whether the effluent is impacting the environment and to carry out studies that are designed to confirm this and explore the type/extent of the toxicity;

- To initiate an internal assessment and toxicity identification process in the event that toxicity in the effluent is observed and requires action.

The purpose and objectives of conducting a study must be clearly assessed and identified before it is undertaken. Acute (short-term) or chronic (long-term) toxicity may be tested for and, given the variability of effluents, the tests need to be designed, conducted and interpreted with great care. It is essential that the guidance available on sampling, handling of samples and conduct of the test is followed and that each step and manipulation is accurately documented. A key part of conducting a toxicity testing programme is the 'learning by doing' approach; the test programme may need to be refined and tailored as it progresses, depending on individual site conditions and objectives.

The ECETOC report (ECETOC, 2004) also covers issues that relate to:

- Sampling: detailed reviews on sampling are available (Whitehouse, 2001; US EPA, 1994). Procedures used for the collection, storage and preparation of samples should ensure that measured parameters such as toxicity do not significantly change before testing is conducted. This may require taking several sets of samples for different purposes. Aspects of sampling that may have significant influence on the results if not dealt with properly include the method of collection (for example the need to use inert containers and rinsing

procedures), the required volume of samples in relation to testing ((semi)–static, flow-through) and homogeneity and pooling of samples. The measurement of basic physical-chemical properties of samples includes pH and dissolved oxygen, conductivity or salinity, colour, physical state (e.g. emulsion) and suspended solids. All steps should be adequately documented. Sample storage requires very careful consideration to ensure that properties do not change over the period before the tests are carried out. Depending on the protocols followed, the time between sampling and testing should be kept to a minimum and should not normally exceed 24-48 hours.

- Test selection: the test selection criteria (e.g. duration and species) will depend on the objectives of the project and the stage of the project where testing is employed. Screening tests e.g. SPME (see below) or Microtox[®] (*Vibrio fischeri)* are usually fast and cost effective but may be unsuitable for regulatory requirements. The use of fish in ecotoxicity testing is the subject of an ECETOC review (ECETOC, 2005), and, given the likely non-polar narcosis mode of action of refinery effluents, fish are unlikely to be more sensitive than other organisms (McGrath et al, 2004 and 2005). In the example given in Case Study 4, Microtox[®], daphnids and algae were found to be more sensitive with respect to acute toxicity than fish. The combination of Microtox[®] and algae also correlated well with chemical parameters in the effluent. The ECETOC review identifies two approaches to avoid the use of fish toxicity testing that are applicable to the petroleum industry;

  - <u>Biomimetic devices</u> - Sherren et al, 2001, described the use of lipophilic solid phase samplers (biomimics) in refinery and petrochemical effluents to simulate the uptake of organic contaminants into aquatic organisms. They addressed uptake, quantified as Total Body Residue (TBR), which was defined as the total molar concentration of organic compounds that can be absorbed by an 'organism' when exposed to complex organic mixtures. Toxicity tests with Microtox[®] and *Daphnia magna* were conducted on the effluents at the start of the exposure period and a close correlation was found between toxicity and TBR when TBR values were within the range 10-100 mmol/kg of lipid. Effluents giving a TBR of greater than 100 mmol/kg of lipid were always very toxic to the organisms tested and effluents with a TBR of less than 10 mmol/kg were not acutely toxic.

    Solid phase micro-extraction (SPME), has also been described in a case study funded by CONCAWE (Leslie et al, 2002; Parkerton et al, 2000; Leslie et al, 2005 and see also Case Study 6). In this study assessments of refinery effluents were conducted in support of an OSPAR demonstration project. A comparison of SPME and aquatic toxicity was included. This approach is recommended within a strategy for addressing petrochemical effluents (**Section 5**).

  - <u>Use of existing invertebrate and algae data, in conjunction with alternatives to fish, e.g. cell lines</u> - This is described by Whale et al (2003), Hutchinson et al (2003), Jeram et al (2005) and is the subject of a research programme sponsored by the CEFIC Long Range Research Initiative (CEFIC LRI).

- Factors influencing toxicity test endpoints – Effluent sample toxicity can be influenced by a range of physical-chemical test variables that interfere with the organism itself, the measurement technique and the bioavailability of possible toxicants. Examples from the literature include test temperature, pH, buffer solutions, hardness and salinity (Vasseur et al, 1986), dissolved

oxygen concentrations (Rattner and Heath, 2002), the photoperiod (Ho and Quinn, 1993), the dissolved organic matter concentration (Ghillebaert et al, 1996) and the diet of the test organisms (Belanger et al, 1989). To negate these effects many of the standard tests describe water quality criteria. 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.

- Applicability of test organisms: The choice of using freshwater or marine test organisms should be guided by the salinity of the receiving water and the salinity of the effluent. While it may be unclear which to use when addressing situations where a saline effluent is discharged into a freshwater environment, it has been argued that the receiving water should take precedence when determining the type of test organisms to be used (Whitehouse, 2001). However, sometimes it may be desirable to test the effluent in its original state using organisms adapted to the resulting physical-chemical conditions.

  Studies have shown that, for many chemicals and for many taxa, the sensitivity of freshwater and marine species is similar (ECETOC, 2003). However, the complexity of this has been demonstrated in Case Study 6, (CONCAWE sample #9), where the interpretation of chronic toxicity was impacted by the salinity of the receiving water.

- Expression and interpretation of the results: The results of whole effluent toxicity tests may be expressed as a volume percentage or dilution factor of the effluent that results in an effect (lethal or sub-lethal) on a defined percentage (e.g. 50%) of the test organism population within a prescribed time period. Alternatively, it may be expressed in terms of the highest effluent concentration (or lowest dilution) in which survival or the response of a sub-lethal endpoint was not statistically significantly different to that of the control. The resulting test parameters are reported as ECx or LCx values (where EC and LC stand for lethal and effective concentration respectively and x is the percentage of affected organisms e.g. 50) or no observed effect concentrations (NOEC).

  Toxicity may also be expressed in terms of toxic units. Either as Acute toxic units (Tua) defined as 100/L(E)C50 from an acute test (when toxicity is expressed as % effluent by volume); or as chronic toxic units (Tuc) defined as 100/NOEC or EC10 from a chronic test. An example of the use of Toxic Units is shown in Case Study 4.

3.2. PERSISTENCE

Slow degradation of a chemical substance increases the potential for it to induce toxic effects following long-term exposure. This potential is further increased if the substance also has the potential to bioaccumulate. Such effects may be widespread as a result of transport processes and even occur in areas that are remote from the source. As a consequence persistent substances are of particular regulatory concern.

Persistence cannot be measured directly, only inferred from continued presence in the environment or the lack of observed degradation in the laboratory after extensive experimentation. In principle the assessment of persistence in the environment should be based on actual half-life data.

There is still considerable scientific debate over how the persistence of a single chemical substance can be assessed. It is even more difficult to define what

'persistence' really means in relation to complex effluents that may contain many substances. OSPAR have concluded that it is incorrect to refer to the 'persistence of effluents' (OSPAR, 2005), and, supporting this, ECETOC (ECETOC, 2004) concluded that persistence should only be addressed in the context of persistence of toxicity or of potential bio-accumulative substances. Therefore, the methods discussed should always be assessed alongside these other parameters.

The ECETOC report should be consulted if there are concerns relating to abiotic hydrolysis or photo-degradation of effluents. Given the limited scale over which photo-induced-toxicity concerns are expected for refineries, the lack of an accepted methodology for quantitatively considering photo-induced toxicity and the difficulties in extrapolating laboratory results to the field, it is suggested that concerns relating to photo-induced-toxicity should initially be qualitatively assessed in any effluent assessment. This can be done by addressing the extent to which concentrations of any poly-aromatic hydrocarbons (PAHs) that are present might be expected to cause effects based on published data. If the possibility for effects occurring is indicated, further investigations could be undertaken including the collection of additional site specific data (e.g. receiving water depth and transparency) to determine if photo-induced toxicity concerns may in reality pose a true concern.

Biodegradation test methods for effluents can be based on the "Ready" or "Inherent" tests that are used for single-chemical substances. The 'ready' biodegradation tests are most frequently used as screening tests to identify substances that degrade rapidly. Their suitability for effluents can be questioned in that effluents are mixtures and the identification of all their constituents can be difficult to achieve. However, work conducted by CONCAWE (see Case Study 6) indicates that the ready tests do seem to give comparable data to those obtained from the inherent tests (see below).

'Inherent' biodegradation tests are less stringent than the ready tests; they use a higher concentration of microorganisms in the inoculum (and a higher concentration of test substance when assessing single substances). Inherent tests can potentially be used to identify effluents that do not require further investigation because of their potential to bioaccumulate and/or their toxicity. They can also be useful in assessing the treatability of the effluent components. The inherent tests with the potential to be applied to whole effluent samples will normally be one of the inherent or simulation test series (OECD 302 or 303). In Case Study 6, the Zahn-Wellens test (OECD 302B) was assessed alongside a ready style test. Both tests yielded comparable data and showed that both toxicity and the concentrations of potentially bioaccumulative substances substantially decreased within 7 days.

Based on the limited availability of data for refinery effluents, the ready and inherent biodegradation tests both appear to have the potential for application in effluent assessment and in particular can be used to identify effluents that require further studies on bioaccumulation potential and toxicity. Case Study 4 showed that the treatment of effluents from the Mol Danube refinery consistently led to large reductions in toxicity and that a reasonable correlation existed between chemical parameters (e.g. COD) and toxicity to bacteria and plants. Consequently, the effluent should not raise significant concerns regarding its potential to exert toxicity following conventional biological treatment.

3.3.   BIOACCUMULATION

The discharge of potentially bioaccumulating substances is of particular concern because the substances may accumulate to toxic levels in organisms higher up the food chain – a phenomenon known as secondary poisoning. Such substances will

tend to have an octanol/water partition coefficient (log K_{ow}) of between 5 and 8 (ECETOC, 1995). However, it is also important to realise that many such substances may also be susceptible to degradation/metabolism and that this will reduce the extent of bioaccumulation.

The bioaccumulation of persistent substances in effluents may be addressed by first biodegrading a sample and then identifying the potentially bio-accumulative substances that remain. A scoping study that was sponsored by CONCAWE as part of a larger project incorporated such an investigation and is described in Case Study 6.

A number of methods have been developed for determining the bioaccumulation potential of effluent constituents. These have been reviewed by OSPAR (2005) and de Maagd (2000) along with an assessment of their associated advantages and shortcomings. All the methods are based only on the physical-chemical characteristics of the substance, and thus only indicate a *potential* to bioaccumulate. They can therefore only be considered as indicative screening tools, the actual bioaccumulation of substances being dependent on their persistence in the environment and their susceptibility to metabolism.

The methods that have been developed are:

- High Pressure Liquid Chromatography (HPLC);

- Empore® (C18) discs;

- Semi-permeable Polymeric Membrane Devices (SPMD);

- Solid phase micro-extraction (SPME) fibres.

SPME has been demonstrated to have significant advantages over the other methods for application to refinery effluents as discussed elsewhere (Case Study 6). The key features of SPME that make it attractive are that samples can be assessed without filtration and analysis can be easily conducted by gas chromatography.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the SPME effluent extracts enables the specific chemical constituents of the effluent that are contributing to the mass of material extracted to be identified. Further analysis to determine the concentrations of constituents which may be of concern for bioaccumulation can then be used to focus in on those that might need to be subject to further investigation. For example, the SPME method was used in an OSPAR Demonstration Programme described in Case Study 6. The data from this study showed that SPME yielded information about the constituents of effluents which had the potential to bioaccumulate. It was also useful in providing information on potential effluent toxicity.

4. PREDICTING AND MONITORING THE EFFECTS OF EFFLUENTS IN THE RECEIVING ENVIRONMENT

The results of tests conducted on whole effluents in the laboratory can be utilised in the prediction of their effects in the receiving water. However, they have their limitations, for example:

- Toxicity can be misjudged because bioavailability and fate processes in laboratory tests may not reflect receiving water conditions;

- Sampling is usually discrete and therefore it is difficult to predict the consequences of temporal variability in composition for effluent toxicity;

- The methods currently available for determining the persistence and bioaccumulation properties of effluent constituents do not allow their potential for causing long-term effects to be predicted with a high level of confidence (Burton et al, 2000).

Chapman (2000) has therefore argued that the results of laboratory toxicity tests should only be used in isolation for hazard screening or for monitoring the effectiveness of measures used to control effluent quality and not for drawing definitive conclusions regarding probable effects in the field.

The advantages of chemical and biological monitoring of receiving waters 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 problematic, if not impossible to use as a risk-based management tool for receiving waters polluted by multiple inputs over a relatively large geographical scale. This is 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, impacts from non-polluting sources such as the physical quality of the habitat will have a major impact on the biological status (Dyer et al, 2000). It is therefore appropriate that WEA and receiving water monitoring be viewed as complementary techniques that can be used singly or in combination depending upon the particular circumstances.

4.1. APPLICATIONS FOR RECEIVING WATER MONITORING STUDIES

Common applications for receiving water monitoring studies are:

- To determine water quality status;

- To evaluate major episodic discharges to receiving streams;

- To evaluate receiving water exposure concentrations that equal or exceed laboratory-derived toxicity levels;

- To interpret whether laboratory responses indicate ecosystem impairment;

- To increase confidence in an assessment when the receiving water contains particularly sensitive or endangered species;

- To understand the effects of effluents that are known to contain components that are poorly evaluated by Whole Effluent Toxicity (WET) testing;

- To verify fate modelling and the role of fate processes in determining contaminant exposure (e.g. degradation, dilution, transformation, volatilisation, and sorption);

- To undertake spatial tracking of pollution-related impacts.

Field monitoring involves comparison of samples from target sites that are receiving effluent with samples taken from the same location at another time (e.g. before discharge), or with samples from a reference site not impacted by the discharge (e.g. upstream of the discharge) or with samples from a site that is presumed pristine. The techniques and methods used are described in more detail in **Section 4.2**. Examples of their application to refinery effluents are described below.

4.1.1. Assessing the biological status of receiving waters

Monitoring surveys to assess the biological status of refinery effluent receiving waters have been undertaken for many years. An overview of the methods used was given in an earlier CONCAWE report (CONCAWE, 1982).

An example of this can be seen with long-term studies of the Kinneil intertidal area of the Forth estuary in eastern Scotland. This area has been subject to the effects of industrial discharges, principally from oil refinery and petrochemical processes, since the 1920s. The inter-tidal fauna has been studied annually since 1976 using consistent methodology providing over 20 years of data. During this time discharges from the industrial sources have substantially reduced through a combination of plant closure and the installation of improved effluent treatment systems. The conclusions drawn from this monitoring study were that there has been a significant increase in species diversity which is attributed to the improvements made to the petrochemical effluents that are discharged to the area (McLusky and Martins, 1998).

An example of a long-term refinery effluent monitoring study related to the Mongstad Refinery in Norway is given in Case Study 3. Monitoring has been undertaken here regularly since 1972 and the data from several earlier years were presented in a CONCAWE review (CONCAWE, 1982). The data enabled trends and major environmental gradients to be detected. It has also shown that physical factors, particularly exposure to wave action, can play a significant role in determining faunal distribution in rocky shore locations. Such factors need to be quantified so that sites of chemically similar exposure can be compared to each other in order to assess whether adverse effects from pollution are occurring.

4.1.2. Predicting effects in receiving waters

Toxicity assessments on field collected water and sediment samples can provide an intermediate step between effluent assessments and full monitoring studies.

In Case Study 1, chronic toxicity assessments with the aquatic invertebrate, *Daphnia magna*, have been used to demonstrate lack of adverse effects in water courses which receive refinery effluents that have previously been shown to be acutely toxic to *D. magna*. It was possible to conduct the tests at water hardness values that were outside those specified in the method guideline and successful reproduction was achieved even in slightly estuarine waters (up to 5%). Under such circumstances it was important that appropriate controls were included in the range of treatments in order to assess the validity of any observed responses.

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In the same example, the Microtox® test was shown to be generally more sensitive to the effluent test samples. The test (which is based on the response of a luminescent bacterium, measured as a reduction in light output) was capable of detecting toxicity when the acute *D. magna* tests were not. There also appeared to be a link between toxicity as determined by Microtox® and the chronic *D. magna* study. When mortalities occurred in the adult *Daphnia* used in the chronic study, the contributing effluent was significantly more toxic to Microtox than on previous occasions. Furthermore, on this occasion water samples taken from the Brook just after receiving the effluent were also toxic to Microtox® and had higher than normal COD (Chemical Oxygen Demand) values.

The data in this case study showed there was no correlation between measured toxicity and the magnitude of chemical parameters that formed the basis of the existing discharge consents. The toxicity assessments therefore provided valuable additional information.

Whole sediment tests with the amphipod, *Corophium volutator,* were also successfully used in Case Study 1 to assess toxicity of both fresh and marine water sediments. These tests were capable of detecting adverse effects related to an effluent and could be used to assess the toxicity of both freshwater and marine sediments.

Both the chronic toxicity tests with *D. magna* and the sediment studies with *C. volutator* indicated that one of the effluents was having an environmental impact. Having results from two complementary studies provided weight of evidence that the quality of this effluent needed to be improved.

Effluent toxicity was variable throughout the study and ideally *in-situ* toxicity assessments, where test organisms are deployed directly into the water course, should have been used to study the overall long-term effects of effluents in the receiving environment. *In-situ* assessments have the advantage that these would capture any accidental releases or minor plant failures. However, such *in-situ* studies are difficult to interpret and, the tidal saline nature of the lower reaches of the river effectively prevented the deployment of such tests with freshwater organisms like *D. magna*.

4.1.3.   Monitoring effects in the environment

The aim of Case Studies 7 and 8 was to gain an understanding of how current markers for biological quality performed for receiving waters receiving refinery effluents. The studies related to two different receiving waters, Case Study 7 related to a freshwater river, while Case Study 8 related to an estuary.

The use of the biological index in Case Study 7 was found to be very helpful in providing a wider knowledge of the receiving water and the sensitivity of the community present to the effects of the effluent quality. The observed changes to the quality of the receiving water justified the high investment made on the waste water treatment plant. A coincidental learning point from this study was that even when the legal control parameters had been met for the refinery effluent, other factors (in this case increased salinity arising from drought conditions and associated high recycling of the municipal treated waste water) may have impacts that are outside the control of Industrial operations.

In Case Study 8 the importance of very careful pre-planning and consideration of "extreme" events was clearly demonstrated. Thus although care was taken to

ensure there were 3 "reference" points, including two up-river of the discharge, the outcome was such that none of the data for these points was usable in the final assessment. It is concluded that studies of this nature must take account of the following:

- Comparisons should be based on data for iso-salinity sampling stations;

- The organisms which are naturally present should be given primary consideration as community indicators;

- If it is necessary to expose monitoring organisms (e.g. mussels) at different locations for comparison, and these should be located under very similar physical-chemical conditions.

4.2. FIELD MONITORING TECHNIQUES

Field monitoring can involve 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). Samples may be compared in terms of analyte concentrations to validate persistence and bioaccumulation modelling, or 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 a history of exposure to different types of pollution. Monitoring usually involves repeat or regular sampling of specific sites, however, for the purpose of a whole effluent assessment, limited sampling (a survey) may be sufficient. Measurement of generic water quality parameters will almost always be part of the monitoring programme.

Some of the field monitoring techniques that can be applied are described briefly in the following sub-sections. More detailed guidance on the these and other techniques that are targeted at meeting the needs of the Water Framework Directive (see **Section 5.2.1**) can be found in a technical report published by the European Commission (EU, 2010).

4.2.1. Fate and exposure of contaminants

The following techniques are available for use in fate and exposure monitoring studies:

- Specific analyte measurement (e.g. SPMD, caged mussels, large volume *in situ* sampling);

- Other water and sediment quality parameters;

- Tissue analysis using for example mussels and/or fish;

- Solid phase extraction (SPE) techniques;

- Biomarkers;

- Dilution studies using dyes and other markers.

4.2.1.1.   Specific analyte measurements

Measurement of specific effluent constituent concentrations in receiving waters, when considered in conjunction with other parameters, including suspended solids, sediment and biota analyses, allows a fuller understanding of the environmental fate of the constituents to be developed. The fate of other constituents may also be predicted from the results of such analyses if they share similarities (e.g. in chemical structure) with those that have been studied.

Water column

The nature of water column monitoring will, to an extent, depend upon local circumstances and experience. For example, in Norway the choice of monitoring methods used to assess the fate of constituents of refinery effluents has been influenced by experience gained in monitoring effluents resulting from offshore oil and gas operations. Oil companies operating in the Norwegian sector of the North Sea have conducted field studies since the mid-1990s to monitor produced water discharges to the marine environment. As a consequence of the rapid dilution of the discharges, the methods employed to measure concentrations of constituents of exploration and production produced water discharges needed to be capable of detecting ultra-low concentrations of the chemical substances of concern. The results of these studies have been used to validate models for predicting Polycyclic Aromatic Hydrocarbon (PAH) dilution and concentration gradients arising from the discharges (Durell et al, 2006; Neff et al, 2006).

The following direct and indirect methods of monitoring concentrations of effluent discharge constituents have been tested and evaluated in the Norwegian water column monitoring program:

- Direct water sampling (spot sampling);
- In-situ large volume water sampling (time averaging);
- Solid phase micro-extraction (SPME) techniques;
- Deployed semi-permeable membrane devices (SPMDs);
- Deployed blue mussels;
- Plankton samples.

The SPMDs and mussels together with *in-situ* sampling of seawater were identified as being capable of measuring average levels of chemical constituents present in produced waters. Furthermore, predictions using mussels, SPMDs, and modelling were found to support and complement each other and the surveys demonstrated that all are valuable tools for estimating the fate and impact of chemical contaminants present in produced waters that are discharged to marine environments (Durell et al, 2006). The methods may also be applicable to programs designed to investigate the fate and effects of constituents of refinery effluents.

Chemical analysis of sediment samples has been practiced as part of refinery effluent monitoring programs for many years. Case Study 3 shows that the surface layer of sediment in the vicinity of Mongstad refinery in Norway has been analysed for oil-derived hydrocarbon content since 1985. In 1990 the analysis was extended to include the heavy metals Pb, Ni and V. In some cases the range of metals covered was extended to include Hg, Zn, Cd, Cr, Cu, Co, As and Fe. The surface layer of the seabed was also analysed for the oil related hydrocarbons; naphthalene, phenanthrene, dibenzothiophene and their alkyl homologues, fluoranthenes and pyrenes. Occasional analyses of Total Petroleum Hydrocarbons

(TPH) and the normal alkanes (n-C$_{31}$) of the sediment samples were also undertaken. Sediment samples were taken using a Van Veen grab sampler. Case Study 3 shows the utility of such monitoring in helping refinery operators distinguish the impacts of their different activities.

4.2.1.2. Other water and sediment quality parameters

Physical, chemical and biological characteristics of natural environments fluctuate within and between regions. Understanding the extent of these fluctuations is important when assessing the significance of the impacts of effluent discharges. A wide range of parameters may be relevant; pH, dissolved oxygen, temperature, salinity, density, turbidity and alkalinity for the water column; and colour, smell, organic matter content and distribution of particle size for the sediment. The relevance of each needs to be considered on a case-by-case basis.

Routine monitoring at Mongstad refinery in Norway (Case Study 3) has, since 1990, included measurement of organic matter content of the surface sediments (by measurement of ignition loss) and grain-size distribution analysis. This data has been used to classify the sediments according to standard criteria. The grain-size distribution also provides an indication of the strength of prevailing water currents at the sampling location. Additional hydro-graphic data (temperature, salinity, density, oxygen level and turbidity) are also collected from a permanent station in the vicinity of the Mongstad refinery.

4.2.1.3. Tissue analysis in mussels/fish

Analysis of the tissues of sedentary animals, such as marine mussels, in receiving water environments has been practiced for many years in effluent monitoring programs. For example, at the Mongstad refinery in Norway (Case Study 3), chemical analysis of oil-derived hydrocarbons present in the tissues of blue mussels collected in the area surrounding the effluent discharge, has taken place on a regular basis since 1990. Measurements have been made in both native wild-caught mussels and those deployed in cages for four months at 4-5 stations on the seabed. Heavy metal concentrations were also occasionally determined in the mussel tissues within the same period.

Similar analyses of fish tissues can be performed but care has to be taken when interpreting results obtained from wild-caught (as opposed to caged) individuals because of uncertainties over their history of exposure.

Analysis of tissues from caged or wild-caught invertebrates and fish can provide an indication of exposure to effluent components (Chappie and Burton, 2000). Such information may be valuable in determining the potential for bioconcentration/bioaccumulation of effluent constituents in the food chain. The information may also be combined with methods designed to assess the health and fitness of indigenous populations of organisms, For example, the Scope for Growth test (Widdows et al, 1995) can be used to assess whether growth rates of mussels are compromised by exposure to marine waters receiving effluent discharges.

If tissue analysis is 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 are fully taken into account when designing a monitoring program.

4.2.1.4. Solid phase (micro) extraction techniques – SP(M)E

In some receiving waters it may be possible to use indirect methods for monitoring the concentrations, fate or behaviour of effluent constituents. SPME is one example of such a method that is based on the relationship that exists between the chemicals octanol/water partition coefficient (log K_{ow}) of a chemical substance and its potential to bio-concentrate/bioaccumulate. Partitioning of a substance onto an SPME fibre is also related to the log K_{ow}. Exposing SPME fibres to water samples containing effluent constituents and then desorbing the bound constituents enables their molar masses to be determined using chromatography. The molar masses can then be used to determine the hydrophobicity of the constituents as an indicator of their bioaccumulation potential and their concentrations in the environment. Hydrophobicity can also be used as an indicator of their baseline (narcotic) toxicity. SPME methods are considered to be particularly relevant to receiving water studies involving refinery effluents (Leslie et al, 2002; Parkerton et al, 2000).

Case Study 6 describes a programme funded by CONCAWE, in which assessments of refinery effluents were conducted in support of an OSPAR demonstration project addressing comparability of SPME and aquatic toxicity.

4.2.1.5. Biomarkers

A biomarker is defined in the context of this report as any biochemical, physiological, or histo-pathological indicator of exposure or response to a contaminant by individual organisms (Van Gestel and Van Brummelen, 1996). The definition includes measurements made in portions of a single organism, including contaminant receptor molecules, bio-chemicals (i.e. detoxification enzymes), blood, bile, and tissues (e.g. liver, tissue).

Biomarkers have been widely used as indicators of contaminant impacts in ecosystems. However, just because a biomarker response is observed this does not necessarily imply that an adverse effect is likely to be already present or occur in the future. Biomarkers should therefore be considered to be indicators of exposure and/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).

There has been substantial progress in developing biomarker methods to assess pollution in marine benthic systems. Many of the techniques have been developed through practical workshops (Bayne et al, 1988; Addison and Clarke, 1990; Stebbing and Dethlefsen, 1992). These methods have now been incorporated into national and international monitoring programmes and have contributed towards a framework for general and contaminant-specific monitoring (OSPAR, 1997 and 2008-09).

The increasing use of biomarkers is evident when looking at water quality monitoring which has been undertaken in areas such as Milford Haven in Wales. In this example, reviews of the environmental studies that have been used to assess the ecological status of the Milford Haven area have been undertaken for the Milford Haven Waterway Environmental Surveillance Group (MHWESG) by Hobbs and Morgan (1992) and Bent (2000). These indicated that, in addition to established benthic diversity monitoring and chemical analysis studies, the use of biomarkers has increased during the 1990s. These biomarkers were used not only to extend the previous monitoring and surveillance programmes, which focused on the acquisition of baseline water quality and biological data, but also included work commissioned after the *Sea Empress* oil spill in February 1996. The biomarker methods that have been used include:

- EROD (Ethoxy-Resorufin-O-De-ethylase) activity in flatfish. This involves measurement of enzymatic activity in response to xenobiotic compounds, present. In this case it has provided a measure of exposure to PAHs in the absence of fish mortalities;

- DNA-adduct formation studies in fish and mussels. The complexation of DNA with PAH compounds is used as an indicator of exposure;

- Scope for Growth (SFG) in mussels (*Mytilus edulis*). Measurement of energetic resources available for growth provides an indication of the physiological fitness of an organism following exposure to toxic (or other) stressors;

- Mussel immunity studies. Involved the measurement of changes in the status of the immune system resulting from exposure to toxic (or other) stressors.

The study is interesting because although the data from these biomarkers and other studies are considered to be of high quality, the ecological studies undertaken in Milford Haven have not always been able to reflect change due to, for example, oil pollution. According to Bent (2000) this is due to naturally occurring gradients in biota and physical-chemical parameters that make interpretation of the results difficult. Similar difficulties with interpretation of biomarker response have been found in the studies presented in Case Study 8, where, in many instances, the results of the biomarker studies did not appear to correlate with other measures of water quality. The availability of specific wild species (e.g. flatfish) and difficulties associated with transporting and caging such organisms also presented a number of practical difficulties.

The authors of the Milford Haven study note that there has been an increasing trend since the 1990s to evaluate biomarker methods to assess impact of contaminants in pelagic ecosystems. For example, the international BECPELAG programme investigated a number of biomarker methods (Hylland, 2000; Hylland et al 2001 and 2002). Based on the results from the BECPELAG workshop a suite of biomarkers methods (ECETOC, 2004) were included in the Norwegian continental shelf's yearly water column monitoring programme. Biomarkers were also used in a 2006 environmental water monitoring program of a Norwegian onshore facility (Liquefied Natural Gas plant) and it is anticipated that a similar approach will be included in future monitoring programs for Norwegian oil refineries.

Hagger et al (2008) have proposed a biomarker response index (BRI) that can be used to classify the ecological health of aquatic ecosystems. BRI that is based on a suite of biomarkers in individual blue mussels (*Mytilus edulis*) which they believe provide an integrated relative measure of the general health status of these coastal invertebrates. They also believe that the BRI can be used to reduce uncertainty in defining risk classification and provide better evidence of existing impact and may be of value in address these issues in the context of the EU WFD.

In summary, biomarkers are being used to an increasing extent in Europe as part of ecological water quality monitoring programmes, especially in marine environments. This trend is likely to continue as the WFD is implemented. However, as the example provided in the Case Study 8 indicates, the associated techniques are not always easy to employ and as has been pointed out by Forbes et al (2006) it can be very difficult to draw definitive conclusions with respect to probable effects on whole organisms.

4.2.1.6.   **Dye studies**

Dye studies have been used for many years in water column monitoring studies is to determine flow characteristics and patterns in water bodies and associated dispersion and dilution profiles for effluents. Dye studies are also a key tool used in validating models for predicting such behaviour.

Experiments involving the addition of fluorescent dye (Fluorocene) to the main effluent from the processing unit at the Mongstad refinery were conducted to validate the dilution factor that was estimated by dispersion modelling (Golmen and Nygaard, 2006). The dye was detected by sensitive sensors in the sea surrounding the diffuser segments at 50 m depth up to 1,300 m from the inlet well. Vertical profiles of fluorescence, turbidity, salinity and temperature versus depth were determined at regular intervals each day at 30-40 stations at varying distance from the diffuser. The sensor readings represented theoretical dilution factors of 250 - 900 within the sampled area. The dye was consistently detected in the sea recipient in water layers between 30 and 40 m depth confirming the in-layering depth predicted by the model.

4.2.2.   **Biological effects**

Biological effects monitoring approaches fall into several broad categories that are applicable to both water and sediment. The sequence illustrated in **Figure 2** shows the increasing probability of predicting effects using different approaches.

Figure 2   Predicting receiving stream impacts from effluent discharge
                    (after Waller et al, 1996)

Probability of predicting effects
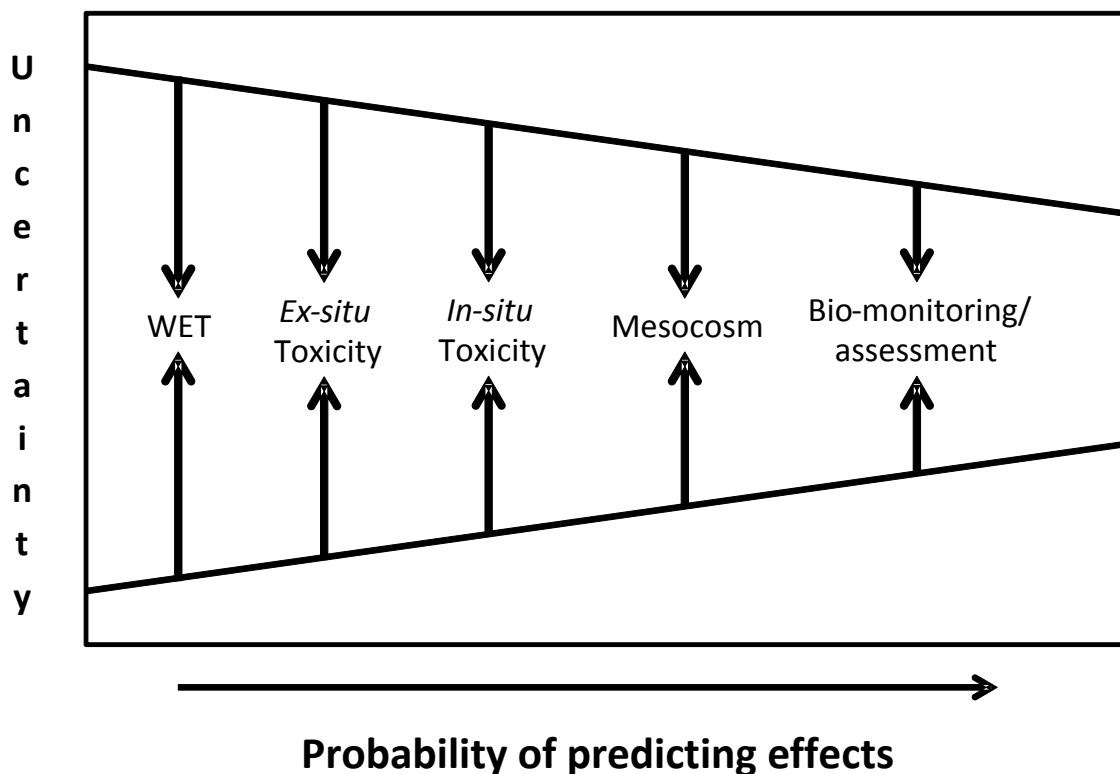
4.2.2.1. Whole Effluent Toxicity (WET) tests

See previous discussion – **Section 3.1** and ECETOC (2004). Effluent samples are tested directly to determine their toxicity. The results are used to predict effects that might arise following release to the environment.

4.2.2.2. *Ex-situ* toxicity monitoring

Samples of receiving water and/or sediment are taken and subject to testing using the approaches described in **Section 3.1** and in ECETOC (2004). Differences between WET and *ex-situ* test results are likely to result from interaction of effluent sample properties with receiving water/sediment properties in the *ex-situ* samples. In contrast, the properties of the dilution water/sediment used in the WET tests are likely to be more standardised (Waller et al, 1996) and therefore possibly less relevant to the site-specific conditions.

4.2.2.3. *In-situ* toxicity monitoring

In-situ toxicity monitoring typically involve exposing organisms at locations within the receiving environment that are chosen either to provide an indication of effects arising from exposure to effluent dilutions or as controls to demonstrate background levels of response. The approach offers significant advantages over the previous two approaches in that it takes account of all the local variables controlling exposure and can integrate the effects of discontinuous discharges. In the opinion of La Point and Waller (2000) the approach is not employed as commonly as it should be.

Holding test organisms in cages or enclosures at predetermined locations ensures that they are exposed to the desired set of conditions. This can avoid complications that may be introduced by, for example, historical contamination of sediment or impaired habitat (Waller et al, 1996). However their use still needs to be considered carefully because effluent plume location and strength can vary both in time and space, making it difficult to quantify exposure. It should also be recognised that *in-situ* studies may overestimate effects because the test organisms are unable to avoid contaminant effects using behavioural strategies.

4.2.2.4. Aquatic mesocosm studies

Aquatic mesocosms are semi-natural systems that are designed to examine the effects of contaminants in receiving environments (ponds, lakes, streams, rivers, estuaries or the open sea) under relatively natural exposure conditions in a replicated way. Replication of test systems, such as artificial streams and ponds or enclosures in lakes and marine and estuarine environments, allows multiple treatments or replication of single treatments to be applied (or both) in such a way that the results can be assessed using tests of statistical significance (see for example Girling et al, 2000).

Mesocom studies provide the opportunity to examine direct toxic effects on many targeted responses at the sub-organism, whole organism or community level or in community function endpoints. They can also be used to identify secondary responses arising from the effects of toxicants on species-species interactions.

Mesocosm studies can be expensive to commission and the large amount of data they generate can be difficult to interpret. Consequently they need to be designed and planned very carefully and resourced appropriately.

4.2.2.5.   **Bio-monitoring**

Bio-monitoring is defined here as the long-term tracking of water/sediment quality to evaluate historical trends in the status of freshwater and marine habitats. Species abundance and diversity monitoring, particularly of benthic and intertidal habitats, has been one of the cornerstones of bio-monitoring programs. Species diversity monitoring of open freshwater (e.g. lakes) or pelagial habitats is less frequently performed because of the difficulty in sampling these habitats, and the unrestricted movement of organisms into and out of potential zones of influence.

Great care is again required when designing biomonitoring programmes if results are to be obtained which can be clearly interpreted (Dyer et al, 2000; Dyer and Wang, 2002). Long-term and regular monitoring programmes are more likely to detect changes in the quality of effluent receiving waters than 'one off' studies. A well-designed long-term monitoring programme must also consider a whole host of habitat and chemical factors that will allow the results to be considered in a wider context.

Techniques used in bio-monitoring surveys were first reviewed by CONCAWE in 1982. However there have been significant additions in the period since then.

4.2.2.6.   **Condition monitoring**

Many of the bio-monitoring programmes undertaken are best classified as "condition monitoring" because they are not tied to assessing the impact of particular discharges/effluents. However, such surveys may provide an indication of where water quality is impacted and may be refined to assess the impact of effluents.

Condition bio-monitoring is not a new concept and has been practiced quite extensively in assessing the ecological status of both the marine benthic environment and fresh water streams and rivers. For example, biological monitoring has been incorporated into the UK National Marine Monitoring Programme (NMMP) since the 1980s (CEFAS, 2008). The objectives of the NMMP are to:

* Establish as precisely as practicable the spatial distribution of contaminants in UK waters and to identify their biological impact, thus identifying any areas of specific concern;

* Detect trends in contaminant concentrations and biological well-being in those areas identified as being of concern; and

* Measure long-term natural trends in physical, biological and chemical parameters in selected areas.

An overview of NMMP, which includes the collection of spatial data on chemical and ecological status as well as evidence of adverse biological effects, is given in **Figure 3**.

Figure 3   Summary of monitoring data collected under the UK NMMP scheme
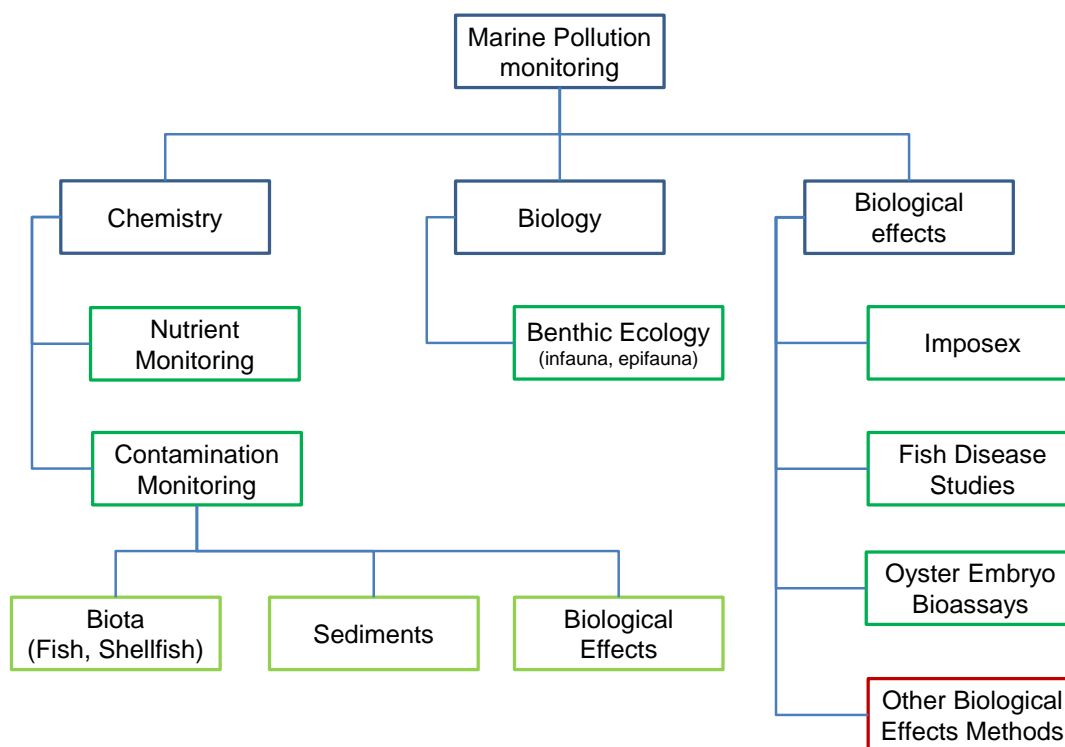
To be effective this type of monitoring programme requires cooperation between several scientific disciplines (often involving different institutions) and long-term commitments in terms of both funding and resources. In spite of this it is likely that this type of monitoring will become more important in Europe in order to establish the ecological status of water bodies as required by the EU WFD.

Benthic species diversity survey of freshwater habitats is another form of condition monitoring that has been used for many years by national regulators to evaluate the status of freshwater habitats. In the early 1970s UK scientists and water managers recognised the need for greater understanding of the ecology of running water sites and their macro-invertebrate communities so that they could develop a nationwide biological assessment programme. A four-year project led to the development of RIVPACS (River Invertebrate Prediction and Classification System) - a model that predicts the freshwater macro-invertebrate fauna expected to occur at a site in the absence of pollution. The four current RIVPACS models are based on 835 reference sites from streams and rivers through the United Kingdom (Wright et al, 2000).

The RIVPACS approach has been more widely adopted within the EU and Scandinavia by countries including Sweden (which has developed bio-assessment tools for streams and lakes), the Czech Republic and Spain. It has also had considerable influence on the drafting of the Water Framework Directive (CEH, 2008). This is manifested in the core concept of the WFD in which an ecological status target, essentially derived from the RIVPACS type approach, is set for each site. These targets are based on a fundamental knowledge of the relationship between the biota and the physical-chemical environment and involve the definition of the 'Reference Condition' for each test site.

This type of approach is also being extended to include marine waters. For example, the Norwegian Pollution Control Authorities has worked out guidelines for classification of the environmental quality applied to fjords and coastal areas, by use of "environmental condition classes" (Molvær et al, 1997).

4.2.2.7. Impact and recovery monitoring

Impact and recovery monitoring is initiated in response to incidents, the purpose being to assess both the extent and severity of the impact and the rate of the recovery over subsequent time periods.

Surveys, which incorporate many similar elements to those used in the NMMP, have been used to assess impacts of drill cutting and produced water discharged by the offshore oil and gas industry in the UK, Dutch and Norwegian sectors of the North Sea since the mid-1970s. The purpose of these sediment monitoring surveys has been to monitor impacts of the discharges and determine the magnitude and spatial extent of environmental effects of oil/gas operations (SFT, 1997 and 1999; Carroll et al, 2001, Daan and Mulder, 1996).

In these schemes assessment of disturbance of the fauna is based on a number of ecological variables, covering both the number of species and the respective individuals present, their comparative abundance, and also the presence or absence of specific species indicative of anthropogenic influence. The sea-bottom fauna is analysed using a variety of techniques, a suit of uni-variate and multi-variate statistical analysis methods (ECETOC, 2004). There are a number of ways of expressing the results, however, most are directed towards defining zones of impact. For example, in the Norwegian sector the estimates of total affected offshore area are based on biological and total hydrocarbon (THC) indicators and expressed as a proportion of the total Norwegian offshore area (Carroll et al, 2001).

The techniques used may therefore be common to those described previously. However major incidents, such as the *Sea Empress* oil spill, have provided the stimulus for developing and deploying new methods. For example, the following novel techniques were used to assess the ecological status of Milford Haven:

- Film and videotape records; used as part of the substrate survey records;

- Biomarkers such as EROD;

- DNA adduct studies;

- Mussel scope for growth;

- Mussel immunity studies;

- Sediment toxicity testing using species like *Arenicola* (lugworm) and *Corophium* (amphipod).

These are not described in detail here but a summary of the methods can be found in Bent (2000). In keeping with any assessment method their robustness and value depends upon the selection of clear initial objectives and following good scientific practice. To enhance their value such methods should be subject to peer review and be supported by relevant guidance.

4.2.3. Evolution of monitoring techniques

A characteristic of longer-term monitoring programmes is that the techniques used and sometimes their objectives can evolve over time. In most instances changes occur as monitoring programmes are tailored on the basis of past experience of where impacts occur, alter as new methods become available, respond to meet new regulatory requirements or can simply be down to changes in resource availability.

The example given in Case Study 3 is illustrative of how monitoring programmes can evolve. The methods used in the period from 1972 to the early 1980s were time and resource consuming and in 1980 a full survey of all 17 monitoring sites and 6 reference sites took four people approximately eight days at low spring tides, excluding the additional analysis of data and reporting which took one person several months. Subsequent surveys were therefore tailored to reduce the time and resources required to complete them. In the same study, the earlier faunal surveys helped to provide a baseline for future monitoring of the marine environment, and formed the basis for development of an altered monitoring programme. The monitoring surveys conducted from 1985 (baseline survey) were more comprehensive, including chemical analysis of oil hydrocarbons in sediments and biota, hydrographical measurements as well as sediment analysis. In 1990 analyses of heavy metals in sediment was introduced, and from 1994 the sediment measurements were replaced by analysis of metals in biological tissues (blue mussels).

The range of techniques used in monitoring surveys has increased considerably since the previous CONCAWE review (CONCAWE, 1982). In fact soon after the initial review was completed progress in monitoring methodology and statistical approaches to analysing survey data occurred. For example, the 1972 marine baseline survey for the Mongstad refinery (Case Study 3) that was reported by CONCAWE (1982) was updated and a new baseline survey carried out. The survey was undertaken in 1985 and 1987 and incorporated new and more efficient methods of analysing species diversity in the benthic faunal surveys.

When such changes occur it is important that the original data is not lost. In the case of Mongstad refinery survey multivariate analysis (cluster-analysis) of the fauna was carried out. This allowed comparisons to be made between the various stations in the new survey and comparison with results for previous surveys, (Johannessen and Høisæther, 1986).

In a similar way the study of the abundance of intertidal organisms at the rocky seashore, which has also been an important part of the monitoring program at Mongstad, has evolved. The seashore has been monitored since 1972. The littoral and sub-littoral zones have been subject to investigations due to their ability to respond to different types of stress and pollution. Surveys have shown that the algae and sessile animals occupying the littoral zone are sensitive and good indicators of variations in the environment. Originally, semi-quantitative measurements of distribution of selected dominating species (plants and organisms) at fixed stations (so called 'level analysis') were undertaken but this was replaced in 1986 by a new method called 'square analysis' (Johannessen and Høisæther, 1986) that is still used. This method is applied to a smaller and defined area that is more precisely investigated compared to the 'level analysis' that was applied to a larger area and studied less accurately. Two statistical methods were used for the community analysis: Multi-Dimensional Scaling (MDS) and cluster analysis as described by Hjolman and Risheim (1992). Occasionally, photographic

documentation (film and videotape records) was also included. Species diversity was shown by applying Bray Curtis similarity index (Field el al, 1982).

4.2.4. Statistical analysis

A suite of multivariate statistical methods are available for analysing biological monitoring data as shown in Case Study 3, This contrasts with the analysis of toxicity test data, where univariate approaches are applicable because only one variable (toxicant concentration) is being varied

The use of multivariate techniques has been shown to have distinct advantages for complex field datasets generated from bio-monitoring studies. Although univariate statistics can be applied to bio-monitoring results information can be lost and the potential for over-interpretation of individual measurement endpoints is introduced. To ensure bio-monitoring studies are statistically robust any design issues relating to replication and pseudo-replication must be carefully considered from the outset.

Case Studies 7 and 8 demonstrate other statistical methods for assessing the results obtained from biological (and chemical) monitoring. Case Study 7 describes an alternative approach to using biological indices. In this case the company involved set certain objectives on the biological indices, including the need to account for biological diversity, which led to a new biological index being adopted. The case study describes how they set about this and the methods used. In Case Study 8 the use of alternative statistical methods (e.g. Dunnetts circles) is described. In all these cases the different approaches were required either because the situations being assessed were different (Case Study 3 describes a marine system, Case Study 7 a freshwater river and Case Study 8 an estuarine system) or the questions being asked were different.

There have been difficulties in communicating the results of studies that have been analysed using multivariate methods between bio-monitoring practitioners and the regulators (Giddings et al, 1999). This is because the results are often not intuitive or obvious. However, these statistical tools (cluster analysis, multidimensional scaling, ordination, canonical correlation analysis, discriminant function analysis and the like) have clear scientific advantages because they can maximise the use of all the available data to provide optimum interpretive power. In all cases it is important that the difficulties with communicating results through these tools should not be used as an obstacle to undertaking bio-monitoring in the first place.

5. CURRENT REGULATORY APPROACHES

5.1. HISTORICAL EMPHASIS OF EFFLUENT CONTROL AND MONITORING IN EUROPE

For many years, regulators have carried out surveys of receiving water quality downstream of large volume chemical and refinery discharges. These surveys have most often taken the form of measurements of concentrations of chemicals of concern/interest in the water column and sediments. However, in some cases assessments of the biological 'health' of the systems have been also been carried out as described by CONCAWE (1982).

In-situ benthic species abundance and diversity studies and chemical analysis of water and sediment have been used for many years to assess water catchment quality and assess potential environmental impact of discharges (see Case Studies 7 and 8). Deployment of test species (e.g. caged fish and bivalves) and the use of surrogates such as bio-markers is less commonly used to regulate discharge inputs. However it is becoming more common as an evaluation tool used by the regulators to assess the quality of the receiving environment as discussed in section 4.2.

The use of toxicity-based methods to assess the potential for effluents to cause damage to the aquatic environment has been established for many years and continues to grow. Toxicity tests have been used to assess effluent quality in some countries such as the UK since as early as the 1950s (Alabaster and Lloyd, 1982). In these early studies large species such as fish (rainbow trout, (*Oncorhynchus mykiss*)) and brown shrimp (*Crangon crangon*) were used. Under the UK 1974 Control of Pollution Act (COPA), these became embodied in law and effluents discharged into the marine environment were assessed on the basis of their chemical composition and toxicity to the armed bullhead (*Agonus cataphractus*) and the brown shrimp (*Crangon crangon*) (Franklin, 1980).

In the 1970s/80s there was a general shift within Europe to the control of releases of specific hazardous chemical substances. These were based on "lists" of hazardous substances issued by responsible governments or agencies (e.g. the "Black", "Grey" and "Red" lists). Environmental Quality Objectives & Standards were developed for the substances on these lists which stipulated levels in the environment not to be exceeded on a short- long- or intermittent-term basis. The standards for certain substances were related to the intended use of the water (e.g. bathing water, drinking water).

In the late 1990s, it was recognized that a more integrated approach to the protection of the aquatic environment was required, which included biological and chemical criteria. This lead to the EU requiring member states to introduce legislation on Integrated Pollution Prevention & Control (IPPC) which incorporated Best Available Techniques (BAT) for controlling pollution. Furthermore, toxicity assessments are recommended as part of the IPPC monitoring strategy (EU, IPPC, 2003) where it states that:

"With toxicity tests it is possible to assess the possible hazardous character of waste water in an integrated manner and to asses all synergistic effects which may occur because of the presence of a lot of different single pollutants. Apart from the possibility of using the toxicity tests to estimate potential hazardous effects on the ecosystem/surface water these tests can help to protect or to optimise biological waste water treatment plants. Toxicity tests, when used in combination with direct

measurements of specific substances and with the measurements of sum parameters, are increasingly becoming a set part of any Whole Effluent Assessment strategy (WEA)".

In addition to IPPC the WFD (see **Section 5.2.1**) has provided overarching legislation requiring that all European receiving waters must meet biological and chemical quality criteria by 2015.

OSPAR (see section 5.2.2) has, in parallel to the EU initiatives, developed hazardous substance controls but with increasing emphasis on Persistent (P), Bioaccumulative (B) and Toxic (T) substances or very Persistent (vP) and very Bioaccumulative (vB) substances.

5.2. CURRENT NATIONAL AND EU REGULATORY ACTIVITIES

Current EU and OSPAR water quality policy and legislation is aimed at achieving 'good environmental quality' of surface waters and sediments using a substance-specific approach. Hence, the environmental hazards of chemicals are assessed on the basis of their PBT or vPvB properties (see **Section 3**). These are determined in laboratory studies using a range of test methods (including physical-chemical methods). The resulting data are used as the basis for setting environmental quality standards and targets for receiving waters (and sometimes sediments) and emission limit values for effluent discharges. However, many regulators recognise the limitations of such an approach for complex effluents and wastes and are attempting to use more holistic approaches such as WEA.

One of the principal advantages of biologically based methods, such as aquatic toxicity testing, is that they provide an assessment of the combined effects of all the components in a complex effluent. This is particularly helpful with refinery effluents, where many of the components are of a similar type and mode of toxic action in aquatic organisms. Another advantage of biological methods is they can add a degree of biological relevance that may facilitate public understanding of the impact of an effluent and demonstrate the difference between contamination (i.e. where the presence of an 'alien' substance is demonstrated but it does not have any effects) and pollution (where an effect can be observed or demonstrated).

WET tests are a form of WEA and effluent controls based on WET have been common in the USA and Canada for several decades. A number of European countries (e.g. UK, Germany and Sweden) have also extensively utilised WET methods.

5.2.1. Water Framework Directive (WFD)

The WFD is a legislative framework to protect and improve the quality of all water resources (rivers, lakes, groundwater, transitional and coastal water) within the European Union. The WFD was published and entered into force in December 2000 (Official Journal of the European Communities, L327, 22 December 2000, pages 1-72). Member States were required to incorporate the WFD into national law by the end of 2003.

The WFD was published in 2000 and it incorporates a number of stages of development/implementation, an overview of which is presented in **Figure 4**. The WFD is currently in the implementation stage with many steps required to achieve "good status" of all European waters by 2015.

Under the WFD, River Basin Management Plans (RBMP) will be produced for each District. A RBMP is a key planning document for a River Basin District, RBD[2], and sets out the specific objectives and the measures to achieve them. The RBMPs **should** have been in place by 2009 but at the time of publication of this report several had not been completed. The RBMP links the WFD and the water-related requirements of other Community legislation, including the Birds Directive (79/409/EEC), the Habitats Directive (92/43/EEC), the Nitrates Directive (91/676/EEC), the Urban Wastewater Treatment Directive (97/271/EEC), the Environmental Impact Assessment Directive (85/337/EEC), the Environmental Quality Standards Directive (2008/105/EEC) and the Drinking Water Directive (98/83/EC).

Figure 4   Overview of the Water Framework Directive
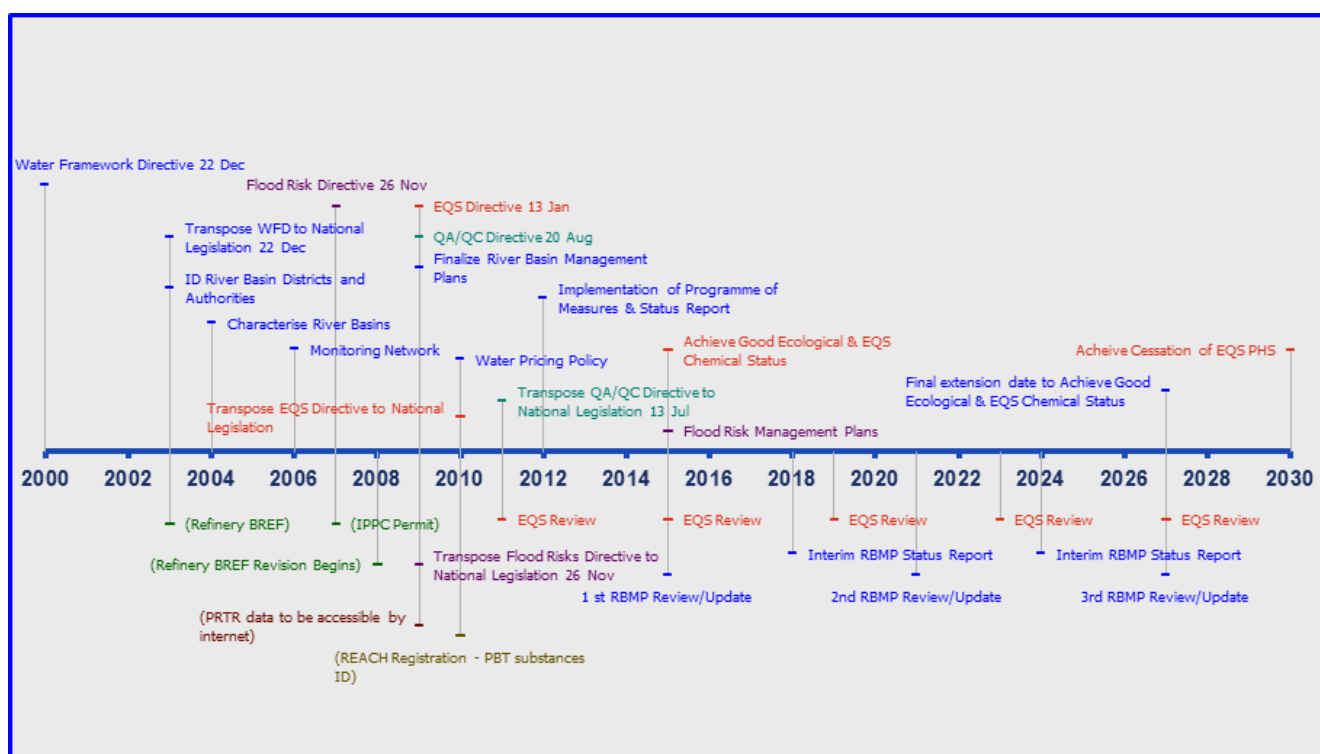
At the outset, a number of steps were required to be completed by all member states within a specified time frame:

- To identify the individual river basins lying within their national territory, assign them to individual RBDs and identify competent authorities by 2003 (Article 3, Article 24);

- To characterise RBDs in terms of status quo, pressures, impacts and economics of water uses and produce a register of protected areas within the RBD, by 2004 (Article 5, Article 6, Appendix II, Appendix III). This includes the identification of key interest features (those features which are crucial to attainment of good ecological status);

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[2] River Basin District. means the area of land and sea, made up of one or more neighbouring river basins together with their associated ground waters and coastal waters, which is identified under Article 3(1) of the WFD, as the main unit for management of river basins.

- To carry out, jointly and together with the European Commission, the inter calibration of the ecological status classification systems by 2006 (Article 2 (22), Appendix V);

- To start operating the monitoring networks by 2006 (Article 8);

- To monitor and analyse the river basin's characteristics in order to identify a programme of cost-effective measures to achieve the WFD's environmental objectives by 2009 (Article 11, Appendix III);

- To produce and publish RBMPs for each RBD including designating heavily modified water bodies, by 2009 (Article 13, Article 4.3);

- To implement water pricing policies that enhance the sustainability of water resources by 2010 (Article 9);

- To put the programme of measures into operation by 2012 (Article 11);

- To implement these measures and achieve the environmental objectives by 2015 (Article 4).

This type of management plan has already been in place in some EU countries for several years and as a consequence for many member states the majority of the steps have been completed. For example, in the UK RBMPs have been developed in the form of catchment management plans and the assessment of the environmental impact of refineries has been included in some of these (i.e. Southampton Water, Mersey Estuary, Milford Haven and Humber Estuary). Elements of these have been covered in the case studies.

The role of WEA in the WFD could be seen as proving part of the monitoring strategy in relation to the following:

(i) *Surveillance* monitoring: assessing long-term water quality changes and providing baseline data on river basins allowing the design and implementation of other types of monitoring,

(ii) *Operational* monitoring: providing additional and essential data on water bodies at risk or failing environmental objectives of the WFD,

(iii) *Investigative* monitoring: assessing causes of such failure.

WEA can form an intrinsic part of a monitoring programme and this has been recognised in the technical report, a 'directory of emerging techniques and methods for water quality monitoring', completed under the European Union's Sixth Framework Project, "Screening Methods for Water Data Information in Support of the Implementation of the WFD (SWIFT-WFD; http://www.swift-wfd.com/) project" as summarized by Allan et al (2006).

5.2.2. OSPAR

OSPAR is the mechanism by which fifteen Governments of the western coasts and catchments of Europe, together with the European Community, cooperate to protect the marine environment of the North-East Atlantic. The OSPAR Convention formed a Hazardous Substances Committee (HSC) to facilitate the implementation of a strategy for Hazardous Substances. The strategy included the development of programmes and measures to identify, prioritize, monitor and control (i.e., to prevent and/or reduce and/or eliminate) the emissions, discharges and losses of hazardous substances that reach, or could reach, the marine environment.

The HSC saw a number of advantages in the WEA approach (OSPAR, 2005) over assessment of PBT/vPvB properties of individual components that included the following:

- Incorporation of a range of methods to reveal (potential) effects of whole samples (water, sediments and effluents);

- Circumvention of the limitations of the substance-oriented approach by measuring PBT/vPvB values directly in samples;

- Providing 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;

- Offering a short cut to the substance-based approach by assessing whether an effluent is harmful;

- Providing a mechanism for identifying substances (or a combination of substances) responsible for toxic effects and/or their source using toxicity identification evaluation (TIE) methodology.

It is unlikely that WEA will replace current OSPAR methods for hazardous substance control but rather act as a safety net approach allowing checks to be made on point source discharges for potential hazards. The method is seen as contributing to the achievement of the OSPR Convention goals (OSPAR, 1992) which states:

'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'.

Many European regulators have or are considering WEA approaches as a potential tool to support both the hazardous substance strategies of OSPAR and address Water Framework Directive (EU, 2000) requirements. Additionally WEA approaches are increasingly being seen as a tool in combating emissions and identification of BAT under the IPPC Directive (EU, 1996).

With respect to WEA methodology a number of European based initiatives and development work has been undertaken both by individual countries (e.g. Germany, The Netherlands and UK) and under the auspices of OSPAR.

Table 4   Some Examples of Regulatory Approaches of WEA
                 (after Power and Boumfrey, 2004)

Country	Outline of WEA scheme
EU Generic	IPPC Directive 96/61/EC BAT, WFD and related EQS Directive 2008/105/EC. Good water quality objectives may use a WET approach for setting discharge limits and/or monitoring compliance and/or quality.
Belgium	EU approach with sector specific conditions based on BAT. Demonstration programme being used to develop protocol.
Denmark	Non statutory approach including biodegradation and bioaccumulation. Source control used to protect receiving water.
Ireland	Mandatory Emission Limit Values based on toxic units. Source control primary vehicle with some receiving water monitoring.
England, Scotland and Wales	Small number of consents in place. DTA demonstration programme (industry & regulator initiative) developed protocol for acute toxicity testing. Bioassay use expected to increase where receiving water quality is assessed as poor.
France	EU & routine monitoring. Some site specific licensing. Used as basis for taxation.
Germany	Regulatory use as hazard reduction under wastewater ordinance and wastewater charges act. Basis of taxation. Primarily source control but also uses daphnids for early warning in large rivers. Some states assess mutagenicity and endocrine effects.
The Netherlands	EU and risk based approach to account for receiving water conditions. May be used for source control following evaluations.
Norway	Can be applied as regulatory instrument. Emission Limit Values and site specific limits. Source control based upon total emission factors.
Spain	Regional use in permits. Source Emission Limit Values. Hazard based source control. Some taxation of discharges.
Sweden	Surface water protection is main goal. Bioassays used to license some discharges. Source control can include biodegradation and bioaccumulation.
OSPAR	Intersessional Expert Group has developed methodology in the context of OSPAR Hazardous Substance elimination goals. This includes assessment of Persistence, Bioaccumulation & Toxicity (OSPAR, 2007).

The schemes followed can be very detailed and much more complex than this simplistic summary implies. For example, many incorporate trigger elements (or action levels) which serve to impose restrictions on effluent discharges or require the discharger to provide additional information. For example, according to the Swedish regulations (Björklund and Undén, 1996), further investigations should be actuated if the Toxicity Equivalents (TEQ) value[3] exceeds 100. This was the case for the Mongstad refinery where initial ecotoxicological results indicated that the TEQ value was > 100. Model dispersion studies and environmental risk assessment (applying the PEC/PNEC approach) were therefore included in the ecotoxicological program (Tone Frost Personal Communication).

Since the review by Power and Boumfrey many of the countries listed have been working with OSPAR in the development of guidance on the use and application of WEA. This involved undertaking a WEA demonstration project involving a total of 25

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[3] TEQ = TU (toxicity unit) /Q (effluent volume: m^3/day)

effluents from 8 different participating parties. Nine of the effluents were provided by CONCAWE and this helped CONCAWE provide valuable input to the OSPAR project (see Case Study 6). Furthermore CONCAWE's involvement, particularly with respect to the development of flow charts and practical methods for assessing the persistence and toxicity of constituents with the potential to bioaccumulate, was incorporated into OSPAR's practical guidance document on WEA (OSPAR, 2007b). This is considered to be valuable as it seems likely that this OSPAR guidance will be used within the EU (i.e. under WFD requirements) and as mentioned previously could feature within the revised BREFs[4].

5.3. FEEDBACK ON REFINERY EXPERIENCE WITH APPLICATION OF WHOLE EFFLUENT ASSESSMENTS (WEA) BY THEIR LOCAL AUTHORITIES /REGULATORS

To assess how widely WEA methods are applied to refineries, CONCAWE's Water Quality Management Group (WQMG) surveyed CONCAWE member companies during 2005 and included a section requesting them to provide details of their experience with biologically-based effect methods in the assessment of their refinery effluents (CONCAWE, 2011).

The survey data indicated a wide spectrum of regulatory use with 23 of the 52 refineries (representing almost half of the CONCAWE member's refineries) reporting that some form of biological monitoring was being undertaken on their discharge. The majority indicated that WEA was a legal requirement of some sort, usually as part of a discharge consent or permit or part of their IPPC requirements. Some refineries indicated that WEA testing had been discussed with their local competent authority (CA).

Feedback received since the survey indicates this requirement is increasing as part of IPPC permit requirements. This view is supported by proposed revisions to the IPPC Directive where WEA is being seen as an integral part of defining Best Available Techniques (BAT) as per the conclusions of the kick-off meeting of the Technical Working Group (TWG) for the Review of the BREF for Common Waste Water and Waste Gas Treatment/Management Systems in the Chemical Sector (CWW) held in Seville, Spain in June 2008. This presentation referred to use of WEA or similar techniques in permitting/setting emission values for chemical installations/sites.

The survey results indicated that there was a greater use of receiving water monitoring (36 refineries reporting use of such techniques) although it was usually not carried out as a legal requirement. All the monitoring programs covered by the survey included chemical specific parameters and 15 refineries indicted that their programs included some form of biological monitoring. Biological monitoring covered receiving water and sediments and where specified the methods used included toxicity, bioaccumulation and species diversity assessment.

On the basis of the survey it appears that refineries undertake some of this monitoring for their internal use only and as a consequence the results are not always published. In other cases the monitoring is undertaken by the refinery and the CA to assess contaminants of concern or as a commitment to IPPC permits. In the remainder of cases the CA undertakes receiving water and sediment monitoring mainly to assess contaminants of concern but occasionally to assess biological

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[4] BREF: Best Available Techniques Reference Document

status (i.e. the type of monitoring which will be increasingly required under the scope of the EU WFD).

In terms of the WEA techniques applied to the effluents the information from the survey indicated that they were all based on methods described in section 3. These included Direct Toxicity Assessment (DTA) methods using bacteria (e.g. Microtox[®]), acute and chronic invertebrate testing and fish toxicity tests. Although species used were not always specified, the survey returns indicated that acute and chronic toxicity assessments were undertaken using both freshwater and marine species depending on the location into which the effluents were discharged. It was also apparent that many of the requirements for fish testing were based on the fish embryo test. The use of fish embryo tests is a useful development because it avoids many of the animal welfare issues associated with the use of juvenile/adult fish.

In addition, to the water column tests, at least one refinery had assessed the toxicity of sediments using an amphipod test specified by their CA. Other refineries had also undertaken bioaccumulation assessments although details of the methods used were not provided.

In the final part of the survey, refineries were requested to provide feedback on whether there had been any investment over the previous 5 years to address concerns associated with chemical and/or biological monitoring data. The feedback indicated that 18 refineries had invested between 1-10 million € on environmental improvements with one location spending >10 million €. Although most of these sites indicated that chemical concerns were the main driver, 9 sites also indicated that investments had been made on the basis of both chemical and biological monitoring results.

6. STRATEGY FOR ASSESSING EFFLUENTS

It is important that the tools and approaches used to assess the potential effects of effluents are consistent with the overall strategy for protecting the receiving water environment. Until relatively recently the focus was on control of selected chemicals. These were chosen due to concern over their toxicological properties, which were often allied to their lack of degradation and potential to bioaccumulate. In such circumstances, the strategy was to measure exposure concentrations of the individual chemical(s) (e.g. by Gas Chromatography) and interpret them relative to defined hazardous threshold concentrations. However, as noted elsewhere in this report, the focus has increasingly shifted towards strategies that take account of the combined effects of all the contaminants present in an effluent on the ecological status of the receiving water. This emphasis will continue following implementation of the WFD.

Toxicity tests conducted on whole effluent samples enable their hazard to be assessed directly without the need for integration of data for all the constituents. Only if the hazard is considered unacceptable, might it then be necessary to focus on specific chemicals. This may be a useful consideration if the conclusion from, for example, a REACH assessment indicates that a discharge from a site (e.g. a refinery, terminal, distribution depot or retail) presents an unacceptable risk to the receiving environment (i.e. the risk characterisation ratio, RCR[5], is >1).

A strategy for identifying and managing the effluent chemical constituents responsible for the unacceptable hazard might then be based on a consideration of the chemical constituents of concern (determined by analysis of the effluent in conjunction with known or measured hazard data for the constituents), the processes resulting in their presence in the effluent stream and the mechanisms by which their concentrations in the effluent might be reduced. In the latter case, the first option should probably be to consider modifying process-related factors before moving on to effluent treatment options.

When assessing the data derived from effluent studies, consideration should always be given to possible differences between effects observed in laboratory tests and those that may occur in the receiving environment. This is because fate parameters (adsorption, abiotic degradation and biodegradation) can significantly influence the toxicity in the receiving environment but may not be reflected in the results of laboratory based evaluations conducted under very simplistic exposure conditions. It is also important that the toxicity of an effluent is assessed in the context of its persistency or potential to bioaccumulate. Assessing persistence or the potential for bioaccumulation, in isolation, does not yield useful information that can help prevent potential for impact in the environment.

6.1. RECOMMENDED STRATEGY FOR ASSESSING REFINERY EFFLUENTS

The strategy described below, starts with an assessment of the priority for the study to be undertaken. Subsequently, an initial assessment is carried out following a tiered approach. Further studies of toxicity and/or persistence and/or bioaccumulation may be required depending upon the outcome of the initial assessment. Toxicity test results are compared with predicted or measured dilution patterns in the receiving water to assess potential risk. The requirement for risk

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[5] RCR is the ratio of predicted exposure concentration of a substance to its predicted toxic hazard concentration. For an effluent this relates to the properties of its constituents.

management measures is decided upon on the basis of the outcome of risk characterisation.

6.1.1. Prioritisation

Prioritisation of effluents will depend upon many factors, and it is not possible to recommend a single approach. However, among the factors to be considered are:

- Flow rates of discharge;

- Dilution factors in the receiving waters;

- COD/DOC/F_b [6] values;

- Receiving water characteristics;

- Sensitivity of environment;

- International, national and local regulations.

Whichever approach is adopted, it is important that it is clearly described and justified.

6.1.2. Initial assessment

Based on the examples described in Case Studies 2 and 6, the initial stages of an effluent assessment program should cover the following parameters;

- Traditional measures e.g. pH, metals, COD, conductivity and free ammonia. The normal metals that would be addressed, other than those that might be expected due to the use of catalysts, include mercury, zinc, copper and vanadium.

- SPME screening methods – the experience described in this report, clearly demonstrate the advantages of using SPME as an initial toxicity screen, e.g. see **Figure 5**. SPME is especially useful for refineries/petrochemical plants to

  – Screen the toxicity potential of non-ionic organic chemicals in site-specific effluent samples that could collectively contribute to narcotic effects in exposed organisms;

  – Address an assessment that includes bioaccumulation potential and;

  – Gain insights into the identity of predominant chemicals in samples, especially if GC-MS is utilised.

- Toxicity screening using Microtox® – the advantages of using Microtox® are that it is quick and relatively inexpensive. Furthermore, provided the data is generated on reasonably consistent effluents, it should be expected, at least for acute assessments, that the results will correlate with toxicity to other organisms (see Case Study 4). It is particularly useful for Toxicity Identification and Evaluation (TIE), although perhaps more for stronger (more toxic) effluents than for end of pipe or weak (less toxic) effluents. Although there are concerns for the potential to identify false positives, current experience is that the Microtox® 15 min EC_{50} test is reasonably predictive and has low incidence of false positives.

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[6] F_b= fraction of organic matter that is susceptible to biodegradation (see Case Study 4)

Figure 5   An example strategy for initial assessments of refinery/petrochemical plant effluents utilizing SPME fibres
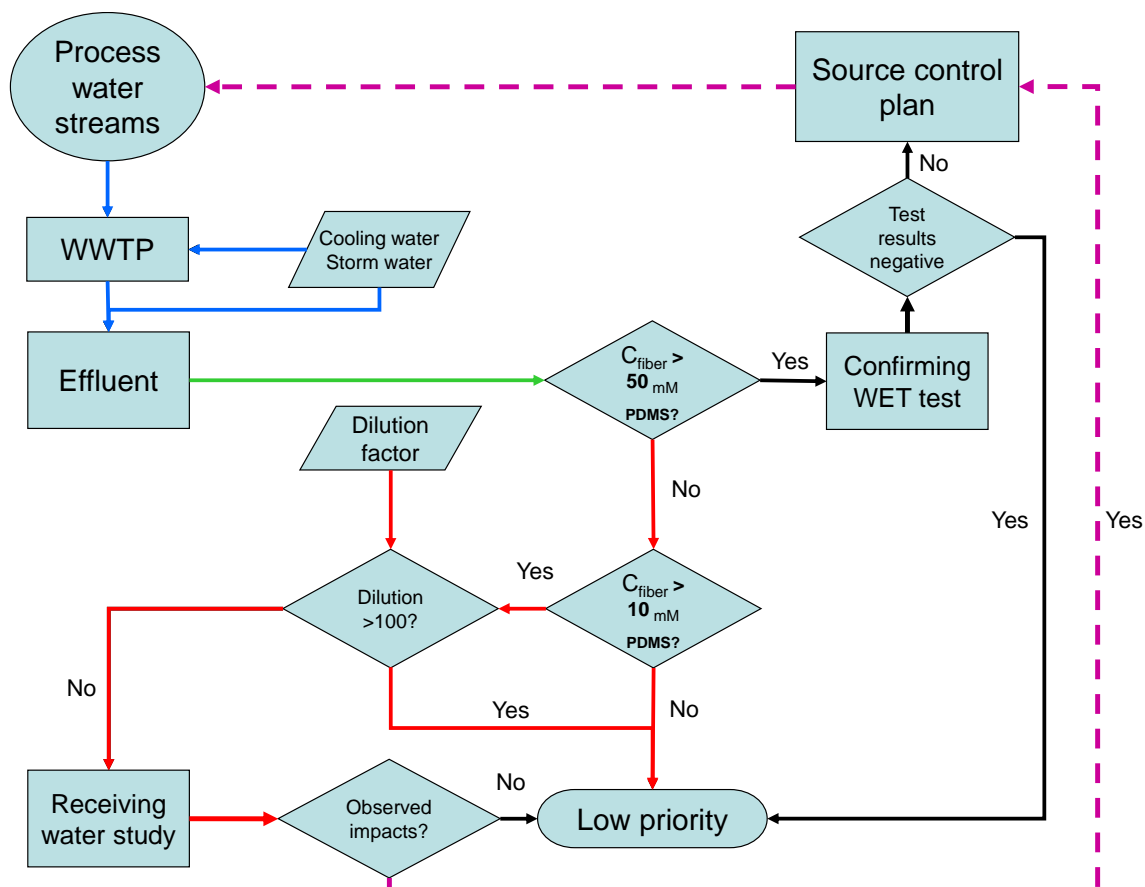
* C_{fibre} in the diagram refers to the concentration on the SPME fibres

6.1.3.   Further assessments

6.1.3.1.   Toxicity

When there is a need to further address the toxicity of an effluent, then there are a number of potential methods that can be used. In Case Study 6 a range of methods is described that can be used to address specific questions. These include acute and chronic toxicity tests that utilize freshwater and marine organisms. The actual choice will depend entirely on the purpose for the study and the data already collected.

6.1.3.2.   Persistence

One option that is clearly highlighted in Case Studies 4 and 6 that can be considered is a degradation test to address whether the toxicity observed in an effluent is likely to persist in the receiving waters. This test should be designed in such a way as to minimize the potential for confounding effects (e.g. metal induced toxicity, ionic imbalance which may cause adverse effects; see Case Study 6). Investigations of the utility of XAD® resin columns to pre-concentrate the hydrocarbon constituents in an effluent, thus enabling the toxicity to addressed independent of the original matrix, have been undertaken. These indicate that recoveries of most spiked hydrocarbons were >60% (P. Leonards communication to

CONCAWE). Consequently, the XAD extraction method has the potential to link the toxicity of an effluent to its hydrocarbon constituents.

The choice of whether a ready style (e.g. DOC) test (OECD 301 series) or a Zahn-Wellens (OECD 302B) style test is conducted is not considered to be too important for effluents that are derived from petroleum refineries or petrochemical plants.

6.1.3.3. Monitoring studies – introduction to a TRIAD approach

Where there is a need to extend investigations further out into the receiving waters, monitoring techniques are used. These 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 can be used to provide a mechanism for checking that discharge consent parameters are achieving the degree of control and protection envisaged. Taking into consideration the learning from Case Studies 7 and 8, there are a number of recommendations that should be considered when addressing monitoring studies:

- Monitoring studies should, where possible, include pre- and post-discharge assessments (in both time and space). This will ensure that changes in status attributable to the effluent can be identified confidently;

- Where possible, monitoring studies should address all three of the following aspects:

  – In-situ monitoring of the biota

  – Monitoring of the chemistry of the effluent and the receiving waters

  – Bioassays conducted in the receiving waters.

This is referred to as the TRIAD approach (see also León Paumen et al, 2007).

One of the major limitations with monitoring studies is that it is not always feasible to incorporate appropriate controls or to separate potential effects of an effluent under investigation from those which may be caused by other discharges in the vicinity. A possible approach would be to assess the relationship between acute and chronic effects measured in an effluent to adverse effects in the environment. Currently, investigations on refinery waste water streams are underway to assess whether it is possible to develop a better understanding of the link between WEA methods and effects observed in mesocosms (artificial streams).

6.1.4. Risk characterisation

Toxicity measurements made on <u>whole effluent</u> samples provide an integrated assessment of the effects of all the constituents that are present. This information coupled with known (or even default) dilution characteristics of the receiving water enables the risk potential of discharges to be evaluated. Such an approach can be of greater value for risk characterisation of complex effluent discharges than approaches based on hazard properties of individual constituents alone. Biological effects measurements made on <u>receiving water</u> samples can be used to verify risk characterisations based on effluent sample data alone. This is an increasingly important consideration with respect to implementation of the WFD and can also be useful to assess the validity of safety assessments conducted under REACH.

6.1.5. Risk management

The costs of upgrading or installing new effluent treatment facilities to address hazard concerns can be high. It is therefore important to determine first whether such measures are required or whether required reductions in hazard can be achieved using other approaches.

Once installed it is also important that such facilities are correctly operated. WEA can play an important role in this process. In the past, one approach has been to treat all effluent from a site but this can be problematic and expensive. This is particularly true for many refineries because run-off water is routed into the effluents and therefore rainfall events can affect the performance and design capacity of the waste water treatment system. More recently, the approach has been to separate effluents into process water, water which may become accidentally contaminated and surface water run-off. However, even this approach has its limitations because of the aggregation of process waters from many different operations that may not all require the same level of treatment. In these circumstances WEA methods can be used to prioritise where effluent treatment is focussed.

When a wastewater or effluent has toxicity which raises concern there are techniques already described in this report which can be used to identify the nature and potential source of this toxicity. These need to be applied in a structured way because it is recognised that WEA approaches can be of limited benefit unless there is a clear understanding of how the toxicity of effluents of concern can be reduced.

Toxicity identification evaluation (TIE) and Toxicity reduction evaluation (TRE) provide a means for identifying toxic effluent constituents and their origin within a complex process system. TRE is designed on a site-specific basis and is conducted in a stepwise fashion to narrow the search for effective effluent toxicity control measures. In common with TIE, the TRE protocols were developed by the US Environmental Protection Agency (US EPA, 1999). The first stage of a TRE is to identify the test(s) to be used for toxicity tracking and TIE. These techniques have been used in the USA for many years and guidance on steps to be taken and procedures to be followed are available at the US EPA (US EPA, 1991; 1993a; 1993b).

In the US the WET schemes are based on consents, where failure to meet the conditions of a consent can lead to significant fines. Therefore, the species and methods used in the TIEs and TREs tend to be based on those used for the compliance tests. As a result these can be both time consuming and expensive to undertake. In the EU this need not be the case and sites have used more simplistic measures based on either microbial assays or high throughput modified toxicity tests. This was shown in the UK DTA Demonstration Programme where on-site testing was carried out on three occasions using high-throughput tests with the marine diatom *Skeletonema costatum* and the embryos of Pacific oyster (*Crassostrea gigas*) to track and aid in the identification of waste streams of concern which were entering the Langholm sewage treatment plant (Hutchings et al, 2004). The two species selected were those identified to be the most sensitive during initial assessment of the discharge. Microtox[®] has also been used in refinery TIE investigations when this was shown to be as sensitive as more standard tests such as *Daphnia magna* and *Acartia tonsa* (G. Whale communication to CONCAWE).

Although by their nature TIEs and TREs are focussed on toxicity, there is no reason why these principles could not be extended to incorporate other endpoints of concern. For example, the case studies presented in this report indicate that SPME

measurements could be incorporated into effluent assessment and management strategies (e.g. SPME measurements could be used to benchmark discharges both within sites and between sites). SPME measurements could provide valuable information on the efficacy of wastewater treatment and, as a surrogate for toxicity, identify streams/discharges of concern. There is also the potential to use SPME measurements in tandem with effluent toxicity tests to provide an indication of whether there are likely to be contaminants, capable of causing toxicity, other than hydrocarbons present in the effluent.

6.2.   ASSESSING EFFLUENTS FOR COMPLIANCE PURPOSES

Where tests to determine persistence, bioaccumulation or toxicity (PBT) properties of effluents or effluent constituents are conducted for compliance purposes, the type of test will be determined by the needs of 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 they do not meet these criteria there is potential for operators to find themselves liable to legal penalties through no fault of their own. Compliance tests should therefore always be conducted by approved laboratories with quality control accreditation for that test. Guidance on factors to take into consideration when selecting testing laboratories has been provided in **Appendix III**.

7.   MANAGEMENT OF EFFLUENT ASSESSMENT PROGRAMS

There are a number of levels at which WEA can be incorporated into the management of effluent and receiving water quality related study programs. These include the management of pollution inputs at a regional level (as advocated by OSPAR), management of discharges into specific water bodies and very specific studies to assess contributions of individual waste streams to a site's effluent discharge.

Tools and strategies for the management of effluents using WEA at an international and national level have been described in **Sections 4** and **6**. These are likely to be prescriptive and sites investigated will have to follow specific procedures and guidelines. At a local level, sites could be faced with using WEA in the context of River Basin Management Plans (RBMPs) or as part of industrial sector initiatives under the auspices of the revised IPPC Directive.

7.1.   RIVER BASIN MANAGEMENT PLANS

RBMPs are an important component of how the EU WFD aims to assess and improve water quality. However, the development of management plans for areas of concern, such as estuaries, is not a new concept. In fact, initiatives to assess and improve discharges to important water bodies would most likely be the justification for the earlier investigations of impacts of refinery effluents reported by CONCAWE (CONCAWE, 1982).

Although these earlier investigations may not have referred to the same terminologies, many sites would have had to evaluate their impacts as part of initiatives to 'clean up' specific locations and these would have incorporated WEA techniques. For example, the Milford Haven area in West Wales in the UK has been subject to many environmental studies. These have included a review of the environmental status of the Milford Haven undertaken by Hobbs and Morgan (1992). In more recent years there have been initiatives to coordinate these types of activities and the Milford Haven Waterway Environmental Monitoring Steering Group (MHWEMSG) was set up in the 1980-ties. The MHWESMG was responsible for organising studies to assess the environmental status of the Milford Haven Waterway.

As large visible dischargers, oil refineries are likely to be identified as potentially important contributors to water bodies and consequently feature in RBMPs. Therefore, when such plans are developed, refinery Health, Safety and Environment (HSE) managers should be aware of the potential implications and of the steps which can be taken to ensure any actions incumbent on their locations are appropriate. One of the first steps is to ensure that any information from environmental investigations and studies (including relevant internal investigations) are made available and reviewed prior developing a RBMP. This first data collation step should also include any studies and information on the sources of aquatic pollution to the watercourse. The data should be collated in a Geographic Information System (GIS) that is compliant with the INSPIRE directive (EU, 2007). Where these data are limited it may be appropriate, as in the case of Milford Haven, to plan and undertake a series of initial studies to enable a management strategy to be developed (Kitts, 1999).

7.2. REFINERY-SPECIFIC STRATEGIES

Outside of RBMPs, refineries can take proactive steps to manage investigations to assess the potential impact of their effluents. It is apparent that a number of refineries have undertaken such studies, many of which have been conducted in agreement with (or by) the relevant authorities. It is important that such studies are properly managed, that objectives are clear and that results are appropriately reported (i.e. to avoid conclusions which are drawn from poor quality or unreliable data). Having studies that have been properly managed and that take due consideration of the factors which affect the reliability of WEA measure, will produce data that can be used to provide a robust baseline. This baseline can then be used in discussions with appropriate stakeholders (authorities, concerned members of the public etc.) to determine whether:

- improvements to effluent quality are required;

- any additional treatment employed has been effective;

- even if their water treatment systems are not as specified in new REACH Material Safety Data Sheets (MSDSs) or as specified under IPPC BAT, they are still sufficient to ensure that a discharge is of an acceptable quality;

- accidents and/or upsets that have occurred have resulted in any significant effects either in terms of effluent toxicity or adverse environmental impacts.

The baseline data can also subsequently be used to assess the efficacy of any risk reduction measures.

7.3. LESSONS LEARNED

The primary factors to consider at the outset of any effluent assessment program are its purpose and objectives. Once these have been set it is then possible to determine how these can be met and whether WEA can provide part of the solution. On the basis of the refinery survey information referred to earlier it is clear that there is an increasing use of WEA methods within refineries and a likelihood that this will continue to increase with new and developing regulation. Therefore, refinery HSE and effluent managers need to ensure they have a better understanding of WEA and how this can affect their operations.

It is evident that poor or inappropriate WEA methods and/or inappropriate interpretation of the data can occur and that this can lead to erroneous conclusions being drawn. Therefore, two of the first matters to address when considering using WEA methods are the identification of appropriate tests methods and the selection of a reliable and experienced test laboratory. Ideally these, and the objectives of the study, should be agreed with relevant authorities and other stakeholders before any practical work is undertaken.

Additional factors to consider include:

- effluent sampling methods;

- sample storage conditions;

- time between sample collection and biological testing;

- inter- and intra-laboratory variability; and

- effluent variability.

If the data are going to be used to provide an assessment of the impacts of site effluents on a receiving water body then it is also important to consider the following:

- the level of understanding of site-specific receiving water condition, and

- the influence of the latter on effluent toxicity to resident organisms.

When the WEA techniques are extended into the receiving water, it is important to understand that, in addition to all the factors outlined above, the potential for confounding factors and misinterpretation of data increases significantly. In fact, in some locations where there are multiple discharges coupled with lack of appropriate control sites it could be argued that such assessments are inappropriate.

These aspects must be carefully examined to ensure that any resulting WEA data are scientifically sound and relates to the scenario under consideration. Only then will reliable conclusions be drawn and appropriate actions identified.

8. DISCUSSION

This report provides an overview of how measures of biological effect can be used by refinery operators to assess and manage effluent discharges. It is apparent that such measures will increasingly be incorporated into the regulation of effluents under initiatives such as the EU WFD and OSPAR. Under these regulations, whole effluent toxicity measurement is seen as one tool for assessing effluent quality that should be applied in combination with (and not instead of) the substance-oriented approach. Assessments that also address persistence and bioaccumulation potential of effluent constituents will provide additional information that can be used to assess the long-term hazards and risks posed by effluent discharges.

One of the principal advantages of WEA is that they can provide a clear indication of the combined effects of all the constituents present in what are often poorly characterized and complex effluents. Such assessments can be difficult or impossible to obtain from analyses of data for individual effluent constituents. However, this should not be taken to imply that WEA techniques are simple to apply in all cases. If the methods used are inappropriate or incorrectly applied there is a high probability of drawing incorrect conclusions and this can lead to, for example, reputational issues with regulators or demands for unjustified risk reduction measures (e.g. increased water treatment).

It must be recognised that, whilst most industries and EU Member States support the principle of risk-based management of both chemicals and effluents, this is not universal. Some countries still adopt a hazard-based approach in which the ultimate goal is hazard reduction of 'dangerous substances' or reduction of toxicity in effluents discharged irrespective of the environmental risk they might pose. This is an important consideration because, in risk-based management, studies are generally undertaken on a site-specific basis to protect the quality of the receiving environment (e.g. no acute toxicity outside a defined mixing zone). However, in hazard assessment schemes, emission limit values on toxicity (or toxicity loads) are set with the overall objective of reducing the hazard of the effluents discharged irrespective of the risk posed to the receiving environment. To complicate matters further some schemes, reported in the literature, appear to be based on a mixture of both risk and hazard. Therefore, before embarking on refinery effluent investigations, it is essential to determine how the information will be used by the local authorities. In both the literature and refinery survey data it was observed that although most WEA studies were required for site regulatory purposes, others were undertaken on a voluntary or case-specific (e.g. sector) basis.

8.1. EFFLUENT ASSESSMENT

The most widely applied WEA schemes assess toxicity to aquatic organisms; for effluents this is referred to as WET testing. WET tests do have relevance to protecting ecosystems although their relevance and the interpretation of their results ultimately depends on the tests used (ECETOC, 2004).

It is important that the procedures used in toxicity assessments ensure that the test results reflect the properties of the sample rather than circumstantial conditions or confounding factors. Therefore, when measuring toxicity there are critical parameters (pH, temperature, dissolved oxygen, hardness, salinity, suspended solids and for some tests colour) that need to be maintained within restricted ranges. 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 (ECETOC, 2004). For refinery effluents, there is an increasing body of data which indicates that, in the absence of confounding factors (in this case also including toxicity attributed to metals and ammonia) the toxicity of the effluent can be predicted on the basis of the hydrocarbons present as measured using SPME procedures.

The case studies and feedback from a recent refinery questionnaire show that 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.

CONCAWE has contributed to the development and evaluation of methods to assess persistence (P) and bioaccumulation (B) of effluent components. Such tests can potentially improve the risk assessment process for effluent discharges but it is important that their limitations are recognised and put into context. In this respect CONCAWE has also contributed to developing guidance on the use of such methods and this has been incorporated into the OSPAR WEA guidance document (OSPAR, 2007b).

8.2.   FIELD MONITORING ASSESSMENTS

Field monitoring of receiving waters will increase under the requirements of the EU Water Framework Directive for member states to establish River Based Management Plans (RBMPs) and to determine the ecological status of their water bodies. However some refineries have already been undertaking such studies since as long ago as the 1970s. More recent examples are provided by the Case Studies and these have been valuable in demonstrating continual improvements in receiving water quality associated with investments made in waste water management and treatment. They have also provided environmental status baselines against which the impacts of spills or unexpected releases can be assessed.

A key learning point from the case studies is that there should be pre-evaluation of a site before embarking on a full field monitoring programme. This should include an assessment of the prevailing physical-chemical conditions because these alone will affect the biological diversity and the ability of any proposed monitoring organisms to survive. If ignored, such factors can mask other potential disruptions to the populations associated with effluent discharges. Problems can be encountered in identifying suitable reference stations particularly in estuaries where salinity gradients occur. Furthermore, physical factors (strong currents) and storm events may lead to losses of caged organisms.

The suitability of the site to support organisms imported to the site to monitor for effects *in-situ* also needs to be considered. For example, caged fish and mussels need to be submerged for 24h every day and this may limit the sites at which they can be deployed. Likewise, if biomarker responses in imported or indigenous organisms are to be used any assumptions made about the suitability of the site to support the selected organisms need to be checked.

Interpretation of the results of monitoring studies with respect to cause and effect can be difficult. These difficulties increase in large water bodies receiving multiple inputs and again in estuaries in particular. One recommendation is that the monitoring program should be tiered and sampling stations selected initially by a combination of what is already known about the other inputs and, for large water bodies, by modelling.

Diversity studies need to be carefully planned and designed to achieve the required level of detail. In some circumstances these can be tailored for an individual water course using specifically developed taxonomic keys. If more specialist taxonomic analysis is required local expertise sampling collecting and handling operations can be used prior to sending the samples to specialist laboratories for the taxonomic and biomarker determinations. This may help to prevent any deterioration of the samples prior to specialist assessment.

8.3.   IMPLICATIONS FOR REFINERIES

It is apparent that the application of whole effluent toxicity (WET) and other WEA methods to assess control and monitor oil refinery effluents is increasing in response to EU Directives and resulting regulations:

- The refinery survey feedback indicates that WET is used in site permits and in IPPC in particular. It has also been stated that toxicity limits will be introduced into the BREFs to provide reassurance that the effluent treatment is effective.

- The focus to achieve 'good ecological status' under the Water Framework Directive is leading to an increase in biologically relevant monitoring. This could either be to directly assess the impact of a refinery effluent discharge (i.e. WEA) or as part of wider initiatives to assess water quality and develop River Basin Management Plans.

Refinery effluent managers therefore need to be aware of the potential benefits and pitfalls of WET and/or WEA programmes.

In the best case scenario WET/WEA can provide additional evidence that the refinery effluents are of an 'acceptable' quality and unlikely to lead to adverse effects. Using toxicity identification and evaluation (TIE) and toxicity reduction assessment (TRA) approaches may also help sites by identifying where water treatment or management efforts should be focused. In the worst case scenarios selection of the wrong tests and failure to correctly interpret data may lead to refineries being inappropriately identified as the cause of deterioration in water quality which could potentially lead to fines, damage to reputation and implementation of costly additional water treatment that will provide no additional environmental benefits.

When faced with using such assessments it is therefore important to agree clear objectives, identify the scope of the study and only use appropriately qualified and experienced laboratories/contractors. However responsibility for such studies should not be handed over blindly. It is important that the Industry develops the resources required to become a trusted partner in the discussions on the design of the WEA and its objectives. Furthermore, appropriate resources are needed to perform the correct interpretation of the data obtained. Although a lot of this work can be performed by consultants and contractors, the availability and involvement of in-house expertise is crucial to assure that this work is executed correctly and serves the purpose of the study. This will ensure any decisions following the WEA investigation can be taken by management that has been informed free of vested interests.

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10.   GLOSSARY

API	American Petroleum Institute	USA trade association that represents all aspects of the US oil and natural gas industry
B	Bioaccumulation	Bioaccumulation refers to the accumulation of substances, like organic chemicals in an organism.
BAT	Best Available Techniques	These are the most effective and advanced stage in the development of activities and their methods of operation which indicate the practical suitability of particular techniques for providing in principle the basis for emission limit values designed to prevent and, where that is not practicable, generally to reduce emissions and the impact on the environment as a whole.
	Biomarker	Any biochemical, physiological, or histo-pathological 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, bio-chemicals (i.e. detoxification enzymes), blood, bile, and tissues (e.g. liver, tissue).
BOD	Biological Oxygen Demand	A measure of the amount of oxygen consumed when the organic components of an effluent degrade.
BREF	Best Available Reference Document	A BREF is a BAT Reference Document and is the report resulting from the exchange of information for the guidance of decision makers involved in the implementation of the IPPC Directive. The BREFs are used by the Operators of the installations (during the preparation of the application for the IPPC Permit), the Environmental Authorities (Permit writers, Policy makers) and the Public in general.
BRI	Biomarker Response Index	An index that is based on a series of biomarker responses at different levels of exposure.
COD	Chemical Oxygen Demand	A measure of the amount of oxygen consumed when the organic components of an effluent are reacted with an oxidising agent and is usually greater than the BOD. It is a measure of the maximum potential for oxygen consumption.
	Inherent Biodegradation tests	Tests that are designed to demonstrate that a chemical has the potential to biodegrade given the right conditions in the environment.
	Insult	In the context of environmental assessments an "insult" is the release of chemical contaminants into the local environment (stream/river). It normally refers to a sudden change (usually increase) of the released material.
	Iso-salinity	Points in the environment where the salinity is the same.
F_b	Bio-available fraction	The fraction of the organic matter present that is susceptible to aerobic biodegradation $$F_b = \frac{BOD_5}{(0.65 \times COD)}$$

INSPIRE		An EU directive (EC, 2007), establishing an Infrastructure for Spatial Information in the European Community.
IPPC	Integrated Pollution Prevention Control	EC, 1996 – Regulations to ensure that particular industries consider the environment as a whole, and the impacts of routine and accidental releases.
MHWESMG	Milford Haven Waterway Environmental Monitoring Steering Group	A group set up to assess the environmental status of Milford Haven and which conduct studies that address this need.
NMMP	National Marine Monitoring Programme	A UK scheme which includes biological monitoring to assess the UK waters, the impact of contaminants and identify areas of concern and long-term trends.
NOEC	No observed Effect Concentration	The concentration obtained from a chronic toxicity test at which effects are not observed, usually expressed in units of concentration, e.g. mg/l
OSPAR		Oslo, Paris Convention for the Protection of the Marine Environment of the North East Atlantic
P	Persistence	An assessment of the time that it takes for a chemical to degrade in the environment. There is rarely 1 single value as degradation is dependent on the chemical and the environment. Usually refers to the ½ life of the primary degradation of the chemical.
PBT	Persistent, Bioaccumulative and Toxic	A hazard based approach to categorising chemicals. It is part of the assessment of chemical substances under REACH
	Pelagic	Any water in the sea or river that is not close to the bottom is in the pelagic zone. . Also applies to Pelagial zones.
	Primary degradation	Primary biodegradation refers to the disappearance of the compound as a result of its immediate biotransformation to another chemical.
RBMP	River Basin Management Plan	Set up under the WFD, a RBMP describes a river basin, its status and the strategy to be adopted in order for it to meet the objectives of the WFD by 2015.
RBT	Ready Biodegradation Tests	Tests which are stringent in design, and attempt to screen out chemicals that will rapidly biodegrade in the environment.
RIVPACS	River Invertebrate Prediction and Classification System	A model that predicts the freshwater macro-invertebrate fauna expected to occur at a site in the absence of pollution. The four current RIVPACS models are based on 835 reference sites from streams and rivers through the United Kingdom (Wright *et al* 2000).
SPMD	Semi-permeable membrane devices	Designed to mimic the parts of animals that cause bioconcentration. These are usually made of plastic tubes or bags containing oil. The plastic allows contaminants to pass through, like membranes of animal cells. The oil inside is similar to a highly purified fish fat and the chemicals dissolve in this oil just as they do in the fats of a fish.

SPME	Solid Phase Micro-extraction	Fibres which allow for the extraction of chemicals which is related to hydrophobicity (which in turn is related to a chemical's potential to bioaccumulate), its bioavailability and analysis can be easily conducted by gas chromatography. Can be used to assess potential for toxicity and bioaccumulation.
	Stressors	Parameters in the environment which impact the organisms and "stress" them. These can be changes in the temperature, salinity, flow rate as well as chemical contamination.
T	Toxicity	A measure of the chemical's toxicity, usually expressed in units that are related to the concentration of the chemical, the test duration and the effects being measured, e.g. concentration causing 50% lethality after 96 h (96 h LC50).
TIE	Toxicity Identification Evaluation	An exercise that tracks where toxicity in an effluent originates in a refinery and usually involves working back through the various input streams to the effluent.
TRE	Toxicity Reduction Evaluation	Designed on a site specific basis and involves first TIE and then identified effective effluent control measures to reduce toxicity.
	TRIAD	A monitoring study that assesses the in-situ chemistry, biology and encompasses laboratory toxicity assessments with the material from the site under investigation.
TU	Toxic Units	Acute toxic units (Tu_a) defined as 100/ L(E)C50 from an acute test (when toxicity is expressed as % effluent by volume); or as chronic toxic units (Tu_c) defined as 100/NOEC or EC10 from a chronic test.
	Ultimate biodegradation	This refers to the complete conversion of a chemical to its inorganic mineral constituents, water and carbon dioxide.
WEA	Whole Effluent Assessment	This approach covers assessing the toxicity of effluents and the persistence and potential for bioaccumulation of the effluent constituents
WET	Whole Effluent Toxicity	This approach covers assessing the toxicity of effluents
WFD	Water Framework Directive	A recent EU initiative focussed on improving the ecological condition of receiving waters (EC 2000) and which stipulates that all waters must meet biological and chemical quality criteria by 2015.

APPENDIX I: CASE STUDIES
INTRODUCTION TO SUBSTANTIVE APPENDICES

The following eight case studies describe projects undertaken by CONCAWE or CONCAWE member companies. The studies were designed to trial the application of biological methods to the assessment of the hazards and impacts of refinery effluents in receiving waters. The case studies are referred to in the main body of the report and learning from them is used to support the recommendations and conclusions of the report.

Case Study 1: **Assessing Refinery Streams – addressing the impact of treatment on toxicity and assessing environmental impact (Shell).**

Case Study 2: **Effluent toxicity at Mongstad refinery (Statoil).**

Case Study 3: **Ecological monitoring of the marine environment at Mongstad refinery (Statoil).**

Case Study 4: **A study on the ecotoxicity of Mol Danube Refinery effluents (Mol).**

Case Study. 5: **Predicting the effect of refinery effluents (ExxonMobil).**

Case Study 6: **Whole Effluent Assessments on Refinery Effluents (CONCAWE).**

Case Study 7: **A new biotic index for non-specialists, developed by REPSOL, as a tool for water quality control in Spanish rivers (Repsol).**

Case Study 8: **Methodology for measuring the impact of treated waste water discharged in an estuary (Total).**

CS-1. CASE STUDY 1: ASSESSING REFINERY STREAMS – ADDRESSING THE IMPACT OF TREATMENT ON TOXICITY AND ASSESSING ENVIRONMENTAL IMPACT

CS-1.1. KEY POINTS AND LEARNING

There was no correlation between measured chemical parameters on which the consents were based and toxicity for the effluent streams assessed. Thus toxicity assessments provide valuable additional information over the normal consent parameters. In fact toxicity assessments provided information to help make management decisions regarding waste water treatment options, and the case studies cited here, helped focus where additional effluent treatment was required. These studies also provided some reassurance that offsite water treatment for some of the waste water streams, was an acceptable option and that the other acutely toxic effluents were not having significant environmental impacts.

Toxicity assessments on field collected water and sediment samples can provide an intermediate step between effluent assessments and full monitoring studies.

Daphnia magna chronic toxicity assessments have been used to successfully demonstrate lack of adverse effects in waters which receive refinery effluents which have been shown to be acutely toxic to *D. magna*. Furthermore, the tests could be undertaken outside recommended water quality values for water hardness and although mortalities were higher than recommended limits successful reproduction even occurred in slightly estuarine waters (up to 5‰). However, to aid interpretation of results it is important that appropriate saline controls are used.

The Microtox test was generally more sensitive (i.e. capable of detecting toxicity) as the acute *D. magna* studies and was even capable of detecting toxicity in the brook. There also appeared to be a link between toxicity as determined by Microtox and the chronic *D. magna* study. When mortalities occurred in the adult *D. magna* the contributing effluent was significantly more toxic than on previous occasions. Furthermore, on this occasion water samples taken from the Brook just after receiving the effluent were also acutely toxic to Microtox and had higher than normal COD (Chemical Oxygen Demand) values.

Whole sediment tests with amphipods, *Corophium volutator* were successfully used to assess toxicity of both fresh and marine water sediments. These tests were capable of detecting adverse effects related to an effluent and could be used to assess the toxicity of both freshwater and marine sediments.

Both the chronic toxicity and sediment studies indicated that one of the effluents was having an environmental impact. Having results from two studies provided weight of evidence that the quality of this effluent needed to be improved and, on the basis of these studies an additional biological effluent treatment was installed after the dissolved air floatation (DAF) unit treatment.

In initial effluent toxicity studies the effluent flows were visibly greater than during the course of this study in which predominantly dry weather prevailed. Ideally studies should have been conducted under both wet and dry conditions but this is difficult to plan. The ability to accurately determine effluent and river flows would have considerably enhanced the value of this study.

The effluent toxicity was also variable throughout the study and ideally in-situ toxicity assessments, where test organisms are deployed directly into the water course, should have been used to study the effects of effluents in the receiving environment. In situ assessments have the advantage that these would capture any accidental releases or minor plant failures. However, such in-situ studies are difficult to interpret and, the tidal nature of the lower reaches of the River effectively prevented the deployment of in-situ tests with freshwater organisms like *D. magna*.

Wherever practical pretreatment of samples should be avoided and any dilutions made with recipient water to maximize environmental relevance of the test media. When considering the conduct of these tests, it is important also to assess whether to take one large sample, and preserve it (which opens up discussion on the preservation methods) or take a series of samples at the renewal times, which opens up the potential for confounding/changing factors. In this study, the latter approach was adopted as it was considered that the effluent would degrade during the long period of holding. This did, indeed, however, introduce a confounding factor when the plant providing the effluent experienced problems with the effluent DAF unit.

A number of ecotoxicity based studies were undertaken by Shell in the late 1990s to provide more relevant data to assess whether refinery effluents had potential to cause adverse environmental impacts. The case study reported here is based on some of the ecotoxicological investigations of effluents from a refinery that had invested heavily in water treatment systems to improve the quality of the final effluents discharged into a predominantly fresh water environment. In all cases the refinery effluents met their authorisation (consent) limits, which were based on generic chemical properties (biological oxygen demand, pH, total oil) and total suspended solids.

In the first stages of the investigations the acute toxicity of a wide range of oil refinery waste streams and refinery effluents was assessed. Discharges were mainly assessed using a rapid bacterial bioluminescence test (Microtox™) and acute toxicity tests with the freshwater invertebrate *Daphnia magna.*

Microtox tests were conducted using a Microbics Corporation Model 500 Microtox Analyser, following the test protocols for either the 100% screening test or basic test protocols given in the instruction manual supplied with this instrument (Microbics, 1992).

Tests with *D. magna* were 48 h acute tests using 10 *D. magna* less than 24 hours old per vessel without renewal of media. The first two tests were carried out in unsealed conditions in 150 ml glass crystallising dishes, containing 100 ml of the effluent test concentration. Owing to concerns that volatile solvents and other components may have been present (based on odours from test solutions during the first tests) the subsequent tests were carried out in sealed, 150 ml Erlenmeyer flasks, completely filled with the effluent test concentration. Each test consisted of two replicate dishes or flasks for each effluent test concentration plus two replicates containing control media. The tests were carried out in a temperature controlled room set at a nominal 20 ± 2°C with artificial illumination on a 16h light, 8h dark, automatic cycle following standard laboratory procedures with water quality recorded during the tests.

CS-1.2. INITIAL LABORATORY STUDIES OF EFFLUENTS AND WASTE WATERS

CS-1.2.1. Effluent toxicity studies

The results of the initial acute studies indicated that, although the refineries met their consent limits, many of the effluents discharged into the environment were acutely toxic to Microtox, ranging from <1.1 to 180 toxic units (i.e. requiring up to a 180 fold dilution to reduce their toxicity to the Microtox 15 min EC_{50} value) as shown in Table 1. The same effluents were also toxic to *D. magna* up to 70 toxic units, (i.e. requiring up to a 70 fold dilution to reduce their toxicity below the 48h EC_{50} value to *D. magna*), for this species as shown in Table 2. Consequently some of the oil refinery effluents that discharged into a small brook and river would be considered to be hazardous and have potential to cause adverse impacts in the receiving environment.

CS-1.2.2. Waste water toxicity studies

In addition to assessing the effluents that were discharged directly into the environment, studies were undertaken of waste waters being discharged for offsite water treatment. This was to determine whether these streams may be affecting the operation of the waste water treatment plant and the quality, (toxicity), of its final effluent. These initial studies, which are also summarised in Tables 1 and 2, reveal that the waste streams were also acutely toxic to Microtox, at up to 360 toxic units.

As part of this investigation, the toxicity of samples before and after waste water treatment was assessed and the results are summarised in Table 3. These data indicate that the waste water treatment plant is effective in removing the toxicity of the refinery waste streams. However, at the time when these studies were undertaken, there were some discussions as to whether the offsite waste water treatment plant would remain the best disposal option, or would continue to accept these streams. There was also some debate as to whether the toxicity of the waste water could be reduced before being discharged to the offsite treatment works. In order to assess whether any particular waste water stream contributed to the majority of the toxicity, a snapshot "one-off" Microtox toxicity assessment of the major streams contributing to the waste waters was undertaken. These data are summarised in Table 4 and show that many of the contributing streams had a similar level of toxicity and could be treated as combined waste water.

Table 1   Summary of Microtox tests of samples from Refinery effluents

Effluents are discharged direct to the environment. Wastewaters sent for offsite treatment

Location	Toxic units (based on Microtox 15 minute EC$_{50}$ values) 95% confidence limits given in parentheses						Summary of results	
Date sampled	09/05/97	28/05/97	03/06/97	10/06/97	19/06/97	22/12/97	Range	Mean
Effluent A	**< 1.1**	-	**200** (130 – 250)	-	-	-	**< 1.1**	**-**
Effluent B	**7.1** (5.9 – 9.9)	-	-	**23** (20 - 26)	-	-	**7.1 - 23**	**11**
Effluent C	**< 1.1**	-	**180*** (130 - 250)	**<2.2**	-	-	**< 1.1 - 180**	**-**
Effluent D	**17** (9.1 - 20)	-	**12** (12 - 13)	**15** (14 - 17)	-	**22** (17 - 28)	**12 - 22**	**15**
Effluent E	**< 1.1**	-	-	-	-	-	**< 1.1**	**-**
Waste water 78	**10** (5.6 - 20)	**13** (10 - 16)	**16** (13 - 19)	**360** (300 - 430)	**170** (120 - 230)	-	**10 - 360**	**20**
Waste water 1402	**220** (170 - 280)	**26** (11 - 40)	**21** (20 - 24)	**67** (65 - 71)	**330** (290 - 380)	-	**21 - 330**	**45**
Waste water DAF	-	**125** (120 - 130)	**180** (110 - 300)	**71** (70 - 72)	**56** (45 - 71)	**110** (90 - 125)	**56 - 180**	**91**

* Possible solvent contamination of effluent. High COD content recorded (22 g/l)

Table 2   Comparison of Microtox and Daphnia magna acute toxicity tests of Refinery effluents

Location	Toxic units (based on Microtox 15 min EC$_{50}$ or 48h D. magna EC$_{50}$ values for each effluent) with 95% confidence limits given in parentheses							
Date collected	28/05/97		03/06/97		10/06/97		22/12/97	
Test Start Date	Microtox 28/05/97	*D. magna* 28/05/97	Microtox 03/06/97	*D. magna*[#] 04/06/97	Microtox 10/06/97	*D. magna* 11/06/97	Microtox 22/12/97	*D. magna* 22/12/97
Effluent A	-	-	**200** (130 – 250)	**40** (23 – 67)	**<2.2**	**2.4*** (2.0 - 2.9)	-	-
Effluent B	-	-	-	-	**23** (20 - 26)	**6.2** (4.3 - 13)	-	-
Effluent D	-	-	**12** (12 - 13)	**< 1***	**15** (14 - 17)	**10** (7.7 - 13)	**22** (17 - 28)	**15** (12 - 18)
Waste water 78	**13** (10 - 16)	**67** (50 – 91)	**16** (13 - 19)	**1.4*** (0.97 – 1.8)	**360** (300 - 430)	**31*** (23 - 34)	-	-
Waste water 1402	**26** (11 - 40)	**18** (13 – 24)	**21** (20 - 24)	**3.0*** (2.3 - 4)	**67** (65 - 71)	**71*** (48 - 100)	-	-
Waste water DAF	**125** (120 - 130)	**56** (42 – 77)	**180** (110 - 300)	**56** (42 - 77)	**71** (70 - 72)	**62** (45 - 83)	**110** (90 - 125)	**31** (26- 38)

#: *D. magna* test on the 28/5/97 and 4/6/ 97 were conducted in open vessels which may have led to lose of toxic volatile components

*: surface trapped (but mobile) *D. magna* found in test vessels

Table 3   Results of Microtox and Daphnia magna acute tests on feeds to and from the offsite (third party) waste water treatment plant

Description of sample used in the toxicity assessment	Toxic Units (based on EC_{50} values of neat sample) 95% confidence limits given in parentheses	
(All samples collected on 16/05/97)	Microtox (based on 15 min EC_{50})	*Daphnia magna* (based on 48 h EC_{50})
Combined refinery effluent to Waste Water treatment works (WWTW)	**31** (28 - 34)	**9.1** (6.2 - 14)
Domestic effluent to WWTW	**2.3** (1.2 – 4.2)	**< 1**
Combined Refinery /domestic effluent	**3.3** (2.5 - 4.3)	**2.3** (2.0 – 2.8)
Final WWTW effluent to Brook	**< 1.1**	**< 1**

Table 4   Results of Microtox tests on waste streams contributing to the discharge to the offsite waste water treatment works (WWTW)

Sample	Toxic Units (based on 15 minute EC_{50} values with 95% confidence limits given in parentheses)
Pipe line to waste water treatment works	**58** (43 - 83)
DAF	**125** (100 - 142)
MH51	**< 2.2**
MH59	**1.0** (0.37 – 2.9)
MH96 (94)	**7.7** (6.7 – 9.1)
AENP	**> 71**
V6584	**> 71**
V8691	**> 18**
V8951	**> 18**
S22	**15** (10 - 23)
S39	**45** (5.6 - 360)
S40	**> 18**
S1590	**12** (10 - 14)
T1402	**62** (32 - 120)
SCA005	**18** (13 - 24)

CS-1.2.3. Recipient water and sediment studies

The initial laboratory studies revealed that the refinery effluents were acutely toxic, thus raising concerns that these discharges may be having an adverse environmental impact. Therefore, to assess the potential for adverse effects to occur as a consequence of the refinery discharges, additional ecotoxicity studies were undertaken. These included 1) chronic toxicity studies of water samples taken from a small brook and river into which the refinery effluents were discharged, and 2) an assessment of the toxicity of sediments in the vicinity of these discharges which would assess the potential historic and current impact of the discharges. In the chronic toxicity assessments a reproduction study with the freshwater invertebrate *Daphnia magna* was undertaken in which neonate *D. magna* were exposed for 21 days to water, sampled on several occasions throughout the course of the study, from the brook and river both upstream and downstream of discharges. The study was conducted using a semi-static test procedure based on OECD (1984, 1995) guidelines. The survival and reproduction of *D. magna* exposed to the water samples during the test were compared with those of *D. magna* exposed to appropriate controls, (i.e. culture, saline and clean river water conditions). These assessments were conducted during the late summer, when the water flow in the river and brook were relatively low and consequently the potential for environmental impact from the effluent discharges was considered to be the greatest. Samples had to be taken close to low tide to minimise interference from saline intrusion in the lower river sections.

In addition to the *D. magna* chronic test, Microtox® was used to assess the toxicity of test samples and contributory refinery effluents to allow comparisons of the toxicity with those found in the earlier effluent toxicity studies.

To provide some additional information on the effluent quality, the Chemical Oxygen Demand (COD) was measured in all fresh samples and effluents using Dr Lange LCK 314 and 114 cuvette test kits (ranges 15 - 150 and 150 - 1000 mg/l respectively), following the instructions supplied with the test kits. Ionised ammonia NH_4^+ (as total N) was measured using Dr. Lange, LCK 304 and 303 cuvette test kits (ranges 0.015 - 2.0 mg/l and 2 - 47 mg/l respectively).

In the sediment tests, toxicity was assessed over 10 days using the benthic amphipod *Corophium volutator*. Although this may not appear an obvious species for freshwater sediments, the advantage of using *C. volutator* is that this species is tolerant to wide ranges of salinity fluctuation enabling one bioassay to be used to assess both the freshwater and saline stretches of the river into which the refinery effluents were discharged. This method has also been shown in Shell internal company studies to be a sensitive method for assessing hydrocarbon contaminated sediments, and has been used in Triad type studies to monitor the impact of historic drilling mud discharges in the North Sea.

Details of the sites sampled for the reproduction and sediment tests are summarised, together with a brief rationale for why the sites were selected is provided in **Table 5**.

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Table 5   Description of the sites sampled for sediment and water toxicity assessment

Sample Code	Description of sampling location	Description of sediment	Significance of sample to the study
R1	River, upstream of landfill site and Refinery	Freshwater area, anoxic sand rich in organic material	River control no known inputs
R2	River, upstream of refinery but downstream of potential contamination from landfill site	Freshwater area, anoxic sand rich in organic material	To assess if water quality immediately prior to entering refinery complex
R3	River midway inside refinery prior to any consented discharge	Estuarine, anoxic mud	To assess water quality of the River before confluence with Brook
R3A[*]	As R3 but sediment visibly contaminated with oil sheen	Estuarine, anoxic mud with oil sheen	To assess effect of oil contamination on sediment
R4	River immediately after confluence with Brook	Estuarine, anoxic mud	To assess quality of River after Brook confluence prior to receiving effluents A, B and C
R5	River after receiving effluents A, B and C	Estuarine, anoxic mud	To assess impact of effluents A, B and C on the River.
R6	Sediment only sample taken from edge of effluent C outfall	Semi-anoxic estuarine mud	Assess sediment quality in vicinity of effluent C discharge into the River
B1	Brook prior to entering refinery.	Freshwater, anoxic, organic rich muddy clay	Brook control
B2	Brook after receiving treatment works effluent but prior to receiving Effluent D.	Freshwater, anoxic, organic rich muddy clay, smell of hydrogen sulphide, *Chironomids* present	To assess the impact of treatment works effluent and quality of brook water prior to receiving effluent D
B3	Brook after receiving Effluent D	Freshwater, anoxic, organic rich muddy clay. Slight 'oily' smell	To assess the ecotoxicological impact of effluent D on the Brook
B4	Brook prior to confluence with river (sediment only)	Freshwater, anoxic, organic rich muddy clay with some sand present	To assess if sediment was toxic ~100 m from effluent D discharge
E1	River after leaving site and prior to entering main estuary. Surface sheen of oil visible on mud	Semi-anoxic unconsolidated estuarine mud	Assess sediment quality of River as it leaves site
E 2	Further down River prior to River channel merging with main estuary.	Semi-anoxic unconsolidated estuarine mud with some animal burrows	Assess sediment quality of River prior to merging with main estuary
E 3	Unconsolidated mud from River bed as it merges with main estuary	Semi-anoxic unconsolidated estuarine mud	Assess sediment quality of River as it merges with main estuary
E 4	River sediments in main estuary at point channel divides	Semi-anoxic consolidated estuarine mud	Assess sediment quality of Estuary at furthest point where channel is visible and can be sampled at low water

*: Oil visible at River site 3 is suspected to have originated from an old disused outfall (non Shell) which has previously caused oil contamination problems.

Figure 1 Schematic showing locations of sampling sites in relation to discharges
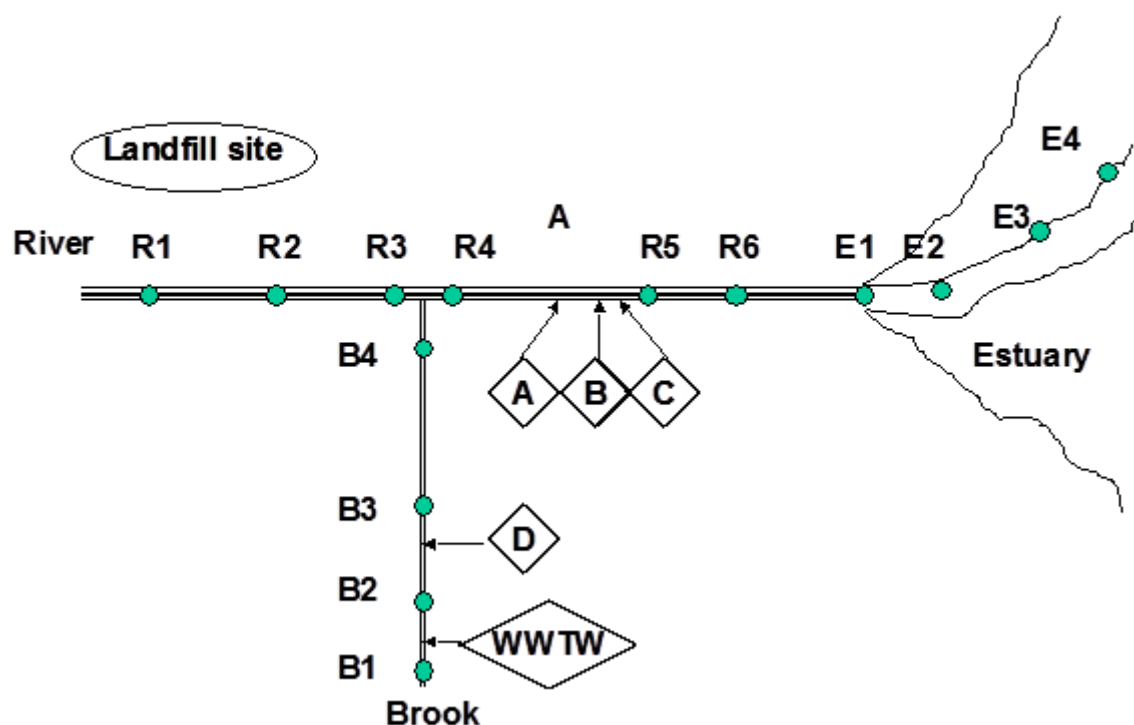
Water and effluent samples- collection and preparation

Spot samples were collected from each location twice weekly throughout the test duration using a 10 litre stainless steel bucket. Sub-samples were taken from the bucket (taking care to avoid any surface film) using acid washed; hexane rinsed and dried 2.5 litre amber glass Winchester bottles. These were completely filled and sealed prior to transport to the laboratory. Even though samples were not taken close to low tide some river samples were found to have slightly elevated levels of chloride compounds (1-5 ‰). As saline water could potentially be detrimental to the survival and reproduction rate of the fresh water species *D. magna*, saline controls were incorporated into the study. These were prepared by dissolving 2 g/l of artificial sea salts, (Tropic Marin, Aquatechnik, Wartenberg, West Germany), into the river water control. Prior to the tests, water was siphoned from the central region of the 2.5 litre sample bottles to avoid removal of surface oil and sediment, which could potentially interfere with the toxicity assessment. Soil extract, prepared by autoclaving 100 g of general loam compost in one litre of reconstituted water for 15 minutes at 120°C and then vacuum filtered through Whatman GF/C paper, was added at 20 ml/l to each of the samples. Reconstituted water (prepared by dissolving salts into reverse osmosis water using the U.S. Environmental Protection Agency (1975) recipe to produce a 'hard' water") with soil extract added was used to provide control media.

During the tests, pre-treatment of samples prior to media renewal was avoided wherever possible. Only one silt laden sample, collected from sample point R5 on Day 18 of the test, was pre-treated by filtering through a 10 μm GFC filter paper before use in the study.

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Observations about the conditions when the samples were collected show that the weather was predominantly fine during the time of the study (Table 6). The observations also indicate that flow rates from Effluent A and B were variable, and on some occasions were non-existent. As with the case of the majority of effluents from a refinery, surface water does contribute to the effluent flows. This makes it difficult to assess receiving water impact on the basis of effluent dilution alone.

Table 6   Observations made at the time of water sampling

Date	Weather Conditions	Observations
16/7/97	Fine	Flow from Effluent A into the River was low
21/7/97	Fine	Effluent A was not flowing into the River at time of sampling
23/7/97	Fine	Flows from Effluents A and B into the River were low. Water samples from Brook site 1 and River sites 4 and 5 were silty. This was attributed to drainage work being carried out just South of the Refinery
28/7/97	Fine	Flow from Effluent A into the River was low and not sampled on this occasion. Water samples from River sites 4 and 5 were still silty but Brook 1 was clear
30/7/97[1]	Fine	Effluent D was not flowing therefore no samples were collected from Brook sites 2 and 3. Effluent C from the interceptor had a white, frothy scum. This was also seen in the River downstream of the Effluent C outfall.
31/7/97[2]	Slight rainfall	Effluent D was discharging oily effluent with a strong odour. Oil was visible in Brook downstream of the Effluent D outfall.
4/8/97	Fine	Effluent A was not flowing into the River at time of sampling. River site 5 was silty with oil visible on surface

Notes: 1)   It was not possible to collect samples from the Effluent D as the DAF unit was not operating therefore samples were collected on the next day when the unit had been repaired.

2)   At the time of collection following the repair the effluent from the DAF had a strong odour and there was a visible oil sheen on the surface of the Brook downstream of the discharge

3)   *Daphnia magna reproduction*

The *D. magna* chronic test was based on OECD 1984 and 1995 guidelines for a semi-static test procedure. However, owing to practical constraints in obtaining water samples, the test media was renewed only twice weekly and not at least 3 times a week as recommended in the OECD (1995) guidelines. In these toxicity assessments, water samples collected from sites R1, R2, and B1 were tested undiluted (neat). Water samples from the remaining locations were tested undiluted (100%) and at a dilution of 10%. River control water was used to prepare the dilutions, as this was considered to be more representative of conditions in the receiving environment.

The test was conducted in 150 ml glass tall form glass beakers containing 100 ml of media for the first four days, and increased to 125 ml for the remainder of the test. Ten replicates per treatment were prepared. After Day 4 of the test, any male *D. magna* present were removed. As a consequence of removing males the number of organisms (females) in at least one of the treatments was reduced to 7. To ensure comparability all treatments were therefore reduced to 7 even where the number of males was <3. As a result the number of individuals was below the OECD (1995) recommended number of 10 per treatment. Although not ideal, this was not considered to be a problem, as this study was designed to screen the chronic toxicity the river and brook samples, as opposed to providing data for regulatory submission for hazard assessment of a chemical.

Throughout the test duration *D. magna* were fed daily with the unicellular algae *Chlorella vulgaris* at a concentration of 0.1×10^6 cells/ml. The following observations were also made and recorded on a daily basis:

i. Adult mortality (i.e. *D. magna* that were not seen to swim during a 15 second observation period).

ii. the number of live young produced

iii. the presence of ephippia or white eggs in adult brood pouches

iv. The presence of dead young or aborted broods.

Young were removed daily after scoring and live, adult *D. magna* were transferred to fresh test media twice weekly.

The test was carried out in a temperature controlled room set at ± 20°C with artificial illumination of the test vessels on a 16h light 8h dark, automatic cycle. Water quality of the test media (pH, dissolved oxygen concentration, water hardness, temperature and salinity) was assessed throughout the test.

The following reproductive endpoints were calculated on the basis of the numbers of young *D. magna* produced during the course of the study:

i. The mean number of young produced by adults in 21 days. (Only young born to adults that survived the duration of the test were included in this calculation).

ii. The mean number of young produced per adult per day using the calculation given below:

$$m_d = \frac{\sum_{x=0}^{x=d} n_x}{d}$$

where:

m_d = the mean number of offspring on day 'd'.

n_x = the number of offspring produced on day 'x'.

x = 0, is day 0, the beginning of the test

Young produced per day were calculated in this way for days 9, 15 and 21.

The results of the 21 day *D. magna* have been summarised in Table 7. These data show that there was no mortality in the control (ASTM culture media) or the Brook

control (site B1) over the 21 day test. The mortality in the river water control (site R1) by day 21 was 14 % and, therefore, is lower than the 20% maximum mortality for controls recommended in the OECD (1984) guidelines. However, mortality in the saline control after 21 days was 43% indicating that the saline control media was less suitable for maintaining the *D. magna* over 21 days than the other control media.

During the *D. magna* chronic tests all water quality determinations, with the exception of water hardness, were within the OECD (1995) guidelines. The hardness of the control media was within the anticipated normal range of between 150 - 190 mg/l $CaCO_3$ for this type of media. However, the hardness of the water samples assessed in the study was much greater than the controls and increased rapidly in the tidal regions. For example, the water hardness of the upper River sites (R1 - R3) and all the Brook sites were similar within the range of 250 - 350 mg/l $CaCO_3$. However, for the tidally affected sites R4 and R5 the hardness was generally between the range 290 - 590 mg/l $CaCO_3$ although one "silty" sample from R5 had a hardness of 1000 mg/l $CaCO_3$. Although the water hardness of most of the samples were outside the OECD (1995) recommended range for *D. magna* tests this could not be addressed (apart from the inclusion of the River and Brook upper sites as controls) without affecting the integrity of the samples. This did not cause any significant problems because the tidal samples collected from R4 and R5 did not appear to have any deleterious impact on *D. magna* survival or reproduction rate.

Table 7   Summary of 21 day D. magna chronic toxicity assessments of Brook and River water samples

Sample description	Adult mortality during the study (%)	Number of adults surviving to day 21	Reproductive rate (number of young produced per surviving adult at the end of the test)	
			Mean	Standard deviation
ASTM Control	0	7	103	13
Saline Control	43	4	135	7
River 1	14	6	76	44
River 2	17*	5	70	45
River 3 Neat	0	7	96	20
River 3 10%	14	6	82	42
River 4 Neat	0	7	126	20
River 4 10%	14	6	85	47

Sample description	Adult mortality during the study (%)	Number of adults surviving to day 21	Reproductive rate (number of young produced per surviving adult at the end of the test)	
			Mean	Standard deviation
River 5 Neat	0	7	148	7
River 5 10%	29	5	111	33
Brook 1	0	7	88	10
Brook 2 Neat	0	7	91	33
Brook 2 10%	43	4	94	36
Brook 3 Neat	100	0	0	0
Brook 3 10%	29	5	136	8

Notes: All samples tested neat (100%) unless stated otherwise

The only treatment in which mortalities in the test exceeded those found in the saline control, was for neat water samples collected from the Brook site B3 (just downstream of the Effluent D outfall). In these samples all of the exposed *D. magna* had died by Day 21 with the majority of these mortalities occurring after the day 15 renewal. This coincided with the time when problems were experienced with the operation of the Effluent D DAF unit.

Mortality in the remaining water samples over the test was variable. On many occasions greater mortalities occurred in the diluted (10%) samples (e.g. for samples from R3, R4, R5 and B2) than the neat samples. This observation suggests that the mortalities were unlikely to be attributed to contaminants present in the water samples although there is no obvious explanation for this observation.

The OECD (1995) guidelines recommend that reproductive output is expressed as total juveniles per parent *D. magna* alive at the end of the test. Therefore the mean number of young born to adult *D. magna* surviving to Day 21 has been calculated and is summarised in Table 8. These results reveal that although there were only 7 (as opposed to 10) replicates the coefficient of variation in the ASTM controls was only 13% and therefore lower than the maximum value of 25% recommended in the OECD (1995) guidelines.

Following the OECD (1995) recommendations, using results based on surviving *D. magna* effectively removes the influence that variable mortality would have had on the reproductive rates. For example, the data in Table 7 show that although adult mortality was high in the saline control, the mean number of young produced per surviving adult over 21 days, (135) exceeded the mean number produced by adults in the ASTM control (103). This suggests that although the low levels of salinity appeared to have an impact on the long term survival of the adults, these were not

detrimental to the reproduction rate of the surviving *D. magna.* The corrected data also show that although there was 43% mortality in the 10% dilution of site B2, the mean reproductive rate per surviving adult (94) is almost identical for the neat sample (91) where no adult mortality was observed.

The only samples where there was a clear impact on mean reproduction rate, were neat samples from Brook site B3, where the rate was zero owing to the fact that none of the exposed *D. magna* survived for the test duration. For all the remaining locations, the mean reproductive rates were between 70 and 148 (i.e. 68 to 144% of the ASTM control rate). For the River the mean reproductive rates for sites R3 - R5 within the refinery complex were between 82 and 148 (i.e. 108 - 194% of the G1 control). For the Brook site B2 the mean reproductive rate was between 91 and 94 (i.e. 103 - 107% of the T1 control). These data indicate that, apart from site B3, no significant adverse chronic toxic effects of the refinery effluents discharged into the River and Brook could be detected at the time of the study.

A possible explanation for the observed increase in mean *D. magna* reproductive rate with increasing distance downstream in the River, is that increasing salinity (or water hardness) of samples stimulates reproduction rates. Evidence that this may occur is supported by the high, (relative to the ASTM control) mean reproductive rates of the surviving *D. magna* in the saline control.

One problem with using the OECD (1995) recommendations is that it is difficult to ascertain whether any effects on *D. magna* reproduction occurred after any of the renewals of the water samples. This is important since environmental water samples, unlike dosed solutions used in normal OECD tests, are potentially variable and consequently can have differing impacts during the course of the study. Therefore, the total cumulative number of *D. magna* produced following exposure to undiluted River and Brook water in comparison to controls over 21 days have been represented graphically in Figures 2 and 3 respectively. These plots show that the pattern of young production for the neat River samples and all but the neat sample from Brook site B3 were similar to the controls. For the neat B3 sample, *D. magna* young production appears to lag behind and is lower than all the other samples between days 7 to 14 and ceases altogether when all the adults died after the day 15 renewal.

This impairment in *D. magna* reproduction in the B3 sample can also be seen in the analysis of mean number of young produced per adult per day for Days 9, 15 and 21 of the test, which has been summarised in Table 8. For example, the maximum number of young produced per adult per day by days 9, 15 and 21 in neat B3 (100%) sample were 0, 1.6 and 1.4 compared to the corresponding respective Brook B1 control values of 1, 3.4 and 4.2. These data therefore suggest that, even prior to the operational problems which occurred on day 15 of the study, Effluent D was adversely affecting the water quality of the Brook.

Table 8   Reproductive rates (number of young per adult per day) of D. magna exposed
to Brook and River water samples for 9, 15 and 21 days

Sample	Day 9			Day 15			Day 21		
	n	Mean	s.d.	n	mean	s.d.	n	mean	s.d.
ASTM Control	7	2.1	1.4	7	4	0.4	7	4.9	0.6
Saline Control	7.	1.9	1.8	6	5.1	1.3	5	6.1	0.8
River 1	7	0.5	1.4	6	3.5	1.8	6	3.6	2.1
River 2	6	0.4	0.9	6	2.6	1.6	6	3.2	1.9
River 3 (Neat)	7	2.2	1.5	7	3.4	1.7	7	4.4	0.9
River 3 10%	7	1	1.5	7	2.9	2.2	7	3.7	1.9
River 4 (Neat)	7	0.8	1.3	7	5	0.5	7	6	0.9
River 4 10%	7	1.5	1.7	7	3.5	2.6	7	4	2.3
River 5 (Neat)	7	0.8	1.4	7	5.1	0.6	7	7	0.3
River 5 10%	6	1.7	1.9	6	4.7	1.6	6	5.3	1.4
Brook 1	7	1	1.3	7	3.4	0.5	7	4.2	0.5
Brook 2 (Neat)	7	0	0	7	2.9	0.9	7	4.4	1.6
Brook 2 10%	4	1.3	1.8	4	3.7	1.5	4	4.5	1.7
Brook 3 (Neat)	7	0	0	6	1.6	0.7	6	1.4	0.6
Brook 3 10%	7	2.3	1.6	7	4.2	1.7	7	5.3	1.7

Notes: Values are the reproductive rate during the life of the adult *D. magna.* If an adult *D. magna* died before Days 9, 15 or 21, the number of young produced would be divided by the number of days that the *D. magna* lived. For example, if an adult *D. magna* died on Day 14, the number of young to Day 15 would be divided by 14 to give the number of young per adult per day.

n = number of adult *D. magna* used to calculate reproductive rate.
Mean = mean number of young *D. magna* produced per day per adult by the stated day.

s.d. = standard deviation associated with mean number of young.

CS-1.3. SUPPORTING ASSESSMENTS

CS-1.3.1. Microtox toxicity

Samples of effluents that were being discharged into the Brook or River at the time of sample collection were taken, using the methods previously described, for chemical analysis and Microtox toxicity assessments in the laboratory. The Microtox toxicity tests were carried out to provide comparative toxicity data on all water samples and effluents collected on each sampling occasion. Whenever possible, these tests were carried out on the same day as sample collection to minimise the possibility of sample deterioration. However, if this was not practical (i.e. due to time constraints) samples were stored in the dark at 4°C prior to testing.

The results of the Microtox toxicity tests presented in terms of toxic units are summarised in Table 9. These show that, with the exception of samples collected from site B3, no Microtox toxicity was detected in any of the water samples used for *D. magna* tests. For the water samples collected from B3 the first four caused a slight reduction (approximately 30%) in the light output of the 90% concentration relative to the controls. At the time these samples were taken, the Effluent D toxic unit values were between 2.5 and 5 (i.e. 2.5 - 5 fold dilution required to reduce toxicity below the 15 min EC_{50} value). Water samples collected from site B3 on the fifth and sixth occasions were found to be significantly toxic with calculated Microtox toxic unit values of 2.6 and 5.6. At the time these effects were detected, the Effluent D toxic unit values were found to be 8 and 125 respectively, (this compares to toxic unit values of between 12 and 22 found in the initial studies). The effects seen with the Microtox toxicity assessments of samples taken from site T3 appear to match the adverse effects observed in the *D. magna* study. For example, the light output reduction in the first four site T3 samples was matched by impairment of reproduction with acutely toxic effects found by both the Microtox and remaining *D. magna* for the day 15 sample.

The results of the Microtox toxicity screening of the other contributory effluents (i.e. besides Effluent D) during this study were variable (Table 9). Effluent A was not toxic to Microtox (<1.1 toxic units) whereas the toxicity of Effluent C throughout the test period ranged from non-toxic to Microtox (<1.1 toxic units) to highly toxic (43 toxic units). Effluent B was consistently toxic to Microtox with toxic unit values of between 4.8 - 11. Although the toxicity of the effluents was variable, their overall Microtox toxicity was similar to that found previously. This suggests that the spot samples taken from the receiving water courses during this study, were representative and valid for assessing the potential impact of the refinery effluents on the River and Brook. Ideally in-situ toxicity assessments, where test organisms are deployed directly into the water course, would have been used to study the effects of effluents in the receiving environment. In situ assessments have the advantage that these would capture any accidental releases or minor plant failures. However, such in-situ studies are difficult to interpret and, the tidal nature of the lower reaches of the River prevented the deployment of in-situ tests with freshwater organisms like *D. magna*.

Table 9   Toxicity of River, Brook and effluent samples to Microtox

Sample	Toxic units based on 15 min EC_{50} values for samples (95% confidence intervals given in parentheses)					
	Sample date (day of renewal of media for *D. magna* study in parentheses)					
	16/7/97 (Day 0)	21/7/97 (Day 4)	23/7/97 (Day 7)	28/7/97 (Day 11)	31/7/97 (Day 14[+])	4/8/97 (Day 18)
River 1 100%	>90	>90	>90	>90	>90	>90
River 2 100%	>90	>90	>90	>90	>90	>90
River 3 100%	>90	>90	>90	>90	>90	>90
River 4 100%	>90	>90	>90	>90	>90	>90
River 5 100%	>90	>90	>90	>90	>90	>90
Brook 1 100%	>90	>90	>90	>90	>90	>90
Brook 2 100%	>90	>90	>90	>90	>90	>90
Brook 3 100%	>90*	>90*	>90*	>90*	5.6 (4 - 7.7)	2.6 (1.7 – 3.8)
Effluent A	>90	>90	>90	>90	>90	-
Effluent B	4.8 (4.3 – 5.3)	7.7 (5.3- 12)	6.2 (4.3 – 8.3)	7.7 (5.9 - 10)	5 (4.2 – 5.9)	11 (6.7 - 20)
Effluent C	<1.1	2.2 (1.5 – 3.1)	1.3 (0.97-1.7)	<1.1	43**	1.5 (0.63 – 3.6)
Effluent D	3.4 (2.0 – 6.2)	2.6 (1.9 – 3.6)	5 (2.3 - 11)	4 (2.5 – 6.2)	125**	7.7 (3.6 - 17)

+ Day 15 for T3 sample
* Reduction in light output (~30%) noted but insufficient to calculate an EC_{50}
** extrapolated values

The data from the Microtox and *D. magna* reproduction studies indicate that, under the low flow conditions of the Brook found in late summer, Effluent D will have an impact on aquatic life in the Brook. However, even on the occasion when Effluent D and the site T3 sample had their greatest toxicity, a 10 fold dilution with clean river water was sufficient to reduce the toxicity of the site T3 sample below that which causes a significant effect on the survival and reproduction of *D. magna*. Therefore, in reality any environmental impact would be anticipated to be localised and effects removed as the Brook water is diluted with river water a few hundred metres downstream of site T3. This was found to be the case, since no Microtox toxicity or

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adverse effects on the reproduction rate of *D. magna* were detected in samples collected downstream of the Brook/River confluence (i.e. G4 and G5).

CS-1.3.2. Chemical Analysis

The Chemical Oxygen Demand (COD) concentrations (measured as mg/l of O_2) of test samples and contributing effluents are presented in Table 10. COD in the test samples ranged from less than 15 mg/l in River water prior to joining Brook to 81 mg/l measured in T3 water collected on the fifth sampling occasion. The latter high value is felt to be due to the influence of Effluent D and a direct consequence of problems with the DAF unit prior to the day 15 water samples being collected.

Effluent B had consistently high COD levels (>100 mg/l) throughout the test period. COD levels in the other contributing effluents varied greatly throughout the test period; ranging from 20 mg/l, measured in Effluent D at the beginning of the test to 410 mg/l in effluent from the Effluent C interceptor. A qualitative comparison of the COD values to the Microtox toxicity indicates that there does not appear to be a good correlation between COD and measured toxicity of the effluent samples.

Table 10   Results of Chemical Oxygen Demand (COD as mg/l O_2) analysis of River, Brook and effluent samples

Sample	Sample date (day of renewal of media for *D. magna* in parentheses)						Range
	16/7/97 (Day 0)	21/7/97 (Day 4)	23/7/97 (Day 7)	28/7/97 (Day 11)	31/7/97 (Day 14[+])	4/8/97 (Day 18)	
River 1	26	32	<15	17	24	22	<15 - 32
River 2	8	14	<15	20	25	23	<15 - 25
River 3	24	13	<15	21	26	21	< 15 - 26
River 4	40	31	22	37	39	38	22 - 40
River 5	38	39	26	35	45	49	26 - 49
Brook 1	28	28	35	10	25	25	10 - 35
Brook 2	49	61	56	72	60	24	24 - 72
Brook 3	49	59	54	72	81	60	49 - 81
Effluent A	23	33	58	-	37	-	23 - 58
Effluent B	250	-	140	130	130	230	130 - 250
Effluent C	52	68	66	46	410	40	40 - 410
Effluent D	20	80	50	80	200	110	20 - 200

+ Day 15 for T3 sample

The ammonium concentrations (measured as mg/l of N) in test samples and contributing effluents are presented in Table 11. The concentration of ammonium in all test samples and effluents was ≤3 mg/l except for one sample. This was collected from Effluent B on the first sampling occasion and contained 46 mg/l N. At the pH of the receiving water between 1 - 10% of the ammonia would be in its most toxic unionised (NH_3) form. The low values and the dilutions of effluents by the River indicate that toxicity attributed to ammonia was unlikely to occur during the study.

This is supported by the fact that no toxicity was seen in the initial G5 samples, when the ammonium input from Effluent B was highest.

Table 11   Ionised ammonia (NH_4+ as mg/l N) values for River, Brook and effluent samples

Sample	Sample date (day of renewal of media for *D. magna* in parentheses)						Range
	16/7/97 (Day 0)	21/7/97 (Day 4)	23/7/97 (Day 7)	28/7/97 (Day 11)	31/7/97 (Day 14[+])	4/8/97 (Day 18)	
River 1	<2	<2	-	-	-	-	<2
River 2	<2	<2	-	-	-	-	<2
River 3	<2	<2	-	-	-	-	<2
River 4	<2	<2	-	-	-	0.7	<2
River 5	<2	<2	<2	<2	<2	1.0	<2
Brook 1	<2	<2	-	<2	<2	0.1	<2
Brook 2	<2	<2	-	3.1	<2	1.5	<2 - 3.1
Brook 3	<2	<2	-	2.9	<2	1.5	<2 - 2.9
Effluent A	<2	<2	<2	-	<2		<2
Effluent B	46	<2	2.3	3.0	<2	1.8	<2 - 46
Effluent C	<2	<2	<2	<2	<2	0.17	<2
Effluent D	<2	<2	<2	<2	<2	1.4	<2

+ Day 15 for T3 sample

CS-1.3.3. Corophium volutator sediment tests

The *C. volutator* tests were conducted following the American Society for Testing and Materials (ASTM, 1991) guidelines for the 10 day toxicity tests for marine amphipods.

Approximately 200 ml sub-samples of the control and each test sediment were placed into triplicate sets of 1 litre glass beakers. The sediment in the beakers was spread evenly and 750 ml of laboratory natural sea water was gently added so as not to disturb the sediment. The contents of the beakers were then left to settle for 2 - 3 hours prior to the addition of the test organisms.

C. volutator were carefully transferred from the holding/acclimation tank into small glass beakers containing approximately 50 ml of seawater using a wide bore pipette (>5 mm). Twenty *C. volutator* were transferred to each beaker and visually inspected to assess that they were of an appropriate size, (i.e. between 5 - 10 mm) and not suffering from obvious injury or disease. After inspection twenty *C. volutator* were transferred, as above, from the small beakers to each test vessel. Test vessels were aerated throughout the study using oil free compressed air via a specially designed manifold system.

Water quality (pH, dissolved oxygen concentration and salinity) was measured in every beaker during the test on days 0, 1, 3 and 9 and with the exception of pH all of

these parameters were within acceptable limits for these tests. Although the pH was slightly above the recommended limits in most vessels, this deviation also occurred in the controls and was not considered to be significant

Test vessels were examined daily, and records made of the numbers of *C. volutator* which were swimming, crawling on the surface, immobilised (lying on the sediment surface but obviously still alive) or dead. *C. volutator* were deemed dead and removed from the test vessel if they did not respond to gentle touching with a glass pipette or recover after a few minutes in clean sea water. After 10 days the sediments were sieved and the number of live and dead *C. volutator* recorded.

The results of the sediment tests are summarised in Table 8. The only location where significant effects occurred was for sediment collected at site B3, just below the DAF discharge into the brook. In sediments from this location, mortalities and total adverse effects (mortality plus failure to burrow) of 55 and 88% were recorded after 10 days in the *C. volutator* bioassays. However, the area impacted appeared to be localised since total mortalities and adverse effects for sediment collected a few hundred metres downstream of the discharge into the brook (site B4) were only 12 and 17% respectively compared to 1.7% in the Brook control site.

Table 12

Description of test sediment	Number of *C. volutator* exposed (replicates pooled)	10 day sediment toxicity test results * Percentage values for:-		
		C. volutator mortality	*C. volutator* on surface	Total adversely affected
Control	60	0	0	**0**
River site 1	60	5	12	**17**
River site 2	60	1.7	0	**1.7**
River site 3	60	5	0	**5**
River site 3 (oily)	60	1.7	0	**1.7**
River site 4	60	0	0	**0**
River site 5	60	3.3	0	**3.3**
Brook 1	60	1.7	0	**1.7**
Brook 2	60	1.7	1.7	**3.3**
Brook 3	60	55	33	**88**
Brook 4	60	12	5	**17**
Estuary 1	60	3.3	0	**3.3**
Estuary 2	60	10	0	**10**
Estuary 3	60	5	1.7	**6.7**
Estuary 4	60	3.3	1.7	**5**
By discharge to Estuary	60	6.7	0	**6.7**

[*] Results reported to 2 significant figures

CS-1.4.   CONCLUSIONS

The initial acute toxicity tests indicated:

–   That many of the effluents were toxic and could potentially be causing adverse environmental effects.

–   That Microtox was more sensitive than *D. magna* acute test.

–   That the offsite refinery treatment of the wastewaters was effective in reducing toxicity.

–   Furthermore, a quick screen revealed that many of the wastewaters contributing to the discharge to the offsite treatment works were toxic with no single stream responsible for the overall toxicity.

The only samples in which chronic toxicity could be detected during the 21 day *D. magna* test, were those collected from Brook just downstream of the Effluent D discharge (site T3). No adult *D. magna* survived for 21 days in this treatment and reproduction rates were much lower than those of the controls throughout the study. However adverse effects were removed when the T3 samples were diluted to 10% of their original concentration using River control water.

The Microtox test was capable of detecting toxicity in the Brook samples and a cause/effect relationship between Effluent D and the toxicity of water from site T3 could be seen. For example, when the Microtox detected toxicity in the T3 sample this was accompanied by mortality of the adult *D. magna* in the reproduction study.

The implication that the water quality at Brook site B3 is impaired under normal operations is supported by the Corophium study which showed that sediments collected from the B3 site were toxic to these sediment dwelling amphipods.

In this study, no other adverse chronic toxic effects to *D. magna* could be detected at any of the other sample sites. As the study was conducted during a dry summer period where the flow rates of the receiving water were low, the chances of observing adverse effects were at their greatest. This provides some reassurance that even though effluents were acutely toxic adverse effects were not seen in the recipient water during low flow conditions. Additional reassurance is provided by the fact that no significant adverse effects were detected in sediments collected from any of the other sampling locations.

In terms of management action, the ecotoxicological data from these studies provided evidence that:-

1)   Offsite treatment was an effective mechanism for removing toxicity of the petrochemical effluents and this disposal route was maintained.

2)   One of the effluents was having an impact in the recipient water as this resulting in measurable toxicity in both the sediment and aquatic phase. As such this weight of evidence provided the justification for the installation of a bio-polishing unit (plate bio-treater) to improve the quality of water being discharged.

CS-1.5. REFERENCES

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7.   Verstraete W. and van Vaerenbergh E. (1986) Aerobic activated sludge. In *Biotechnology Vol. 8 Microbial Degradations*, ed. W. Schönborn, p. 51-54. VCH: Weinheim.

Figure 1: Cumulative number of total young produced during 21 days
exposure to controls and river water samples collected during the study
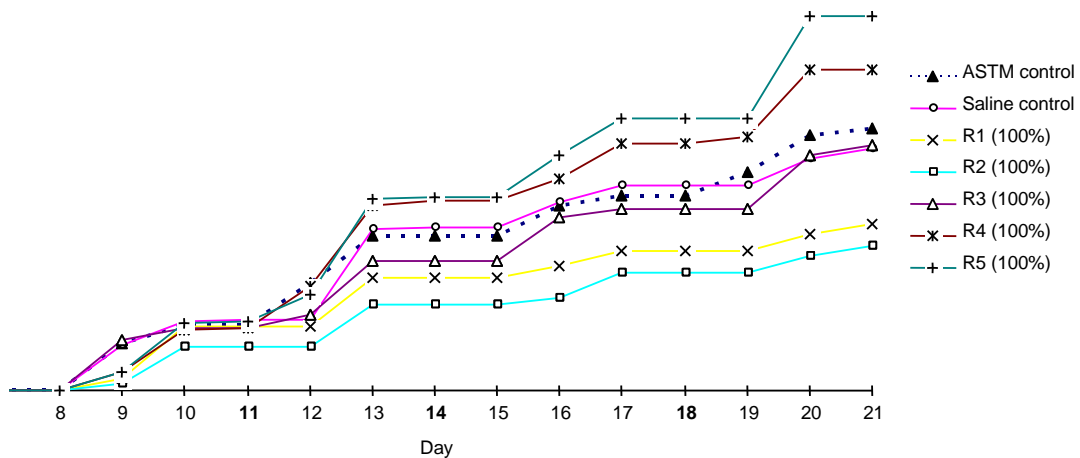
Note: Test media renewed on days depicted in bold type

Figure 2: Cumulative number of total young produced during 21 days exposure
to ASTM control and brook water samples collected during the study
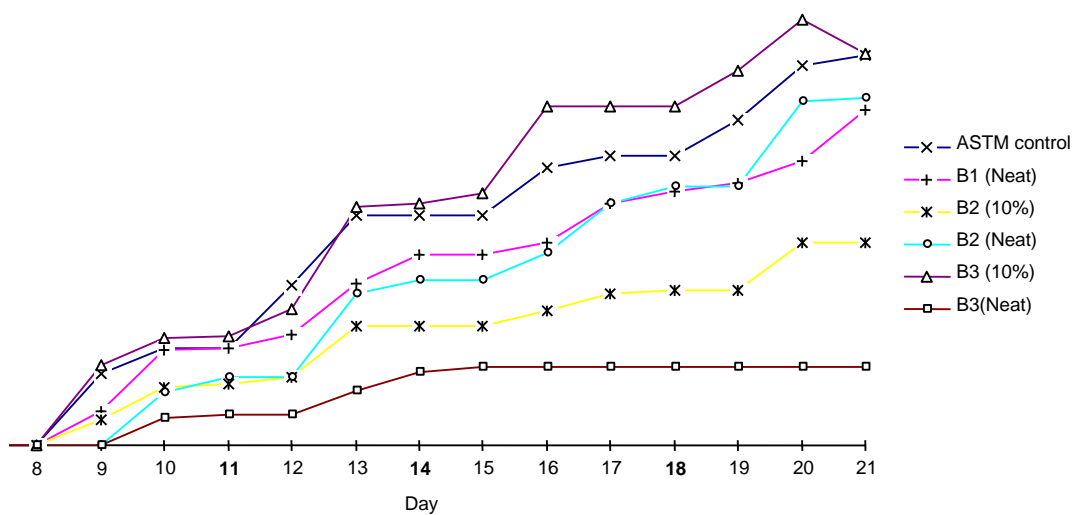
e: Test media renewed on days depicted in bold type (except for T3 media on one occasion
which was replaced on day 15 and not day 14)

CS-2. CASE STUDY 2: EFFLUENT TOXICITY AT MONGSTAD REFINERY

CS-2.1. SUMMARY AND KEY LEARNINGS

In the acute toxicity assessments of Mongstad refinery effluent conducted in both 1993 and 2004 the algae (*Skeletonema costatum)* was found to be the most sensitive species among the species tested (crustacean, fish etc.). The algal toxicity, expressed as growth inhibition, showed a significant decrease from the 1993 to the 2004 study, from a Toxic Unit value of 9.1 to 1.3. The standard algae test with *S. costatum* is therefore considered suitable as a test species at Mongstad due to its high sensitivity. Additionally, this test is inexpensive and easy to carry out and is recommended as a screening method.

The observed toxicity does not seem to be directly related to the Total Organic Carbon (TOC) level of the effluents. In fact, the TOC levels were 14.3 mg/l and 5 mg/l, respectively, in the 2004 and 1993 studies and therefore less toxicity was observed when the TOC of the effluent was higher. However, the phenol and the oil-in-water (hydrocarbon index) concentration in the effluent were lower in 2004 (0.24 mg/l and 0.64 mg/l, respectively) than in the 1993 study (0.4 mg/l and 0.9 mg/l). Additionally, the average concentration of mercury was more than 100 times higher in the effluent samples from the refinery in 1993 (0.65 μg/l) in comparison to 0.006 μg/l in 2004.

From a refinery perspective the low acute toxicity of the refinery effluent observed in the WET test conducted in 2004 provides evidence that the Effluent Treatment Plant, (which includes mechanical, chemical and biological treatment) is effective at reducing effluent toxicity. In the 1993 study this had been demonstrated by the results from the Microtox light inhibition test also performed on wastewater samples prior to and after treatment.

CS-2.2. INTRODUCTION

The oil refinery at Mongstad is situated in Western Norway. It is a modern, highly-upgraded facility with an annual capacity of 10 million tonnes of crude oil. It is ranked as the largest refinery in Norway, and is medium-sized in a European context. All crude oil refined at the plant comes from the Norwegian continental shelf. The principal products are petrol, diesel oil, jet fuel and other light petroleum products. Statoil also operates a crude oil terminal at Mongstad with a storage capacity of 9.5 million barrels. Construction commenced in 1972 and the refinery was commissioned in May 1975.

In the present case study the results from whole effluent toxicity tests at Mongstad refinery conducted in 1993 and 2004 are presented. A brief description of the effluent treatment facilities and chemical characterisation of the effluents is also presented. The toxic effect of the refinery effluent in 2004 will be evaluated in relation to the content of oil hydrocarbons, phenols, heavy metals and estimated concentration of added chemicals and the dispersion modelling of the dilution potential of the recipient environment.

CS-2.3.   EFFLUENT TREATMENT

The wastewater from the refinery includes process water from the refinery, surface/ground water and ballast water. The wastewater is given a staged approach treatment involving mechanical, chemical and biological treatment, before it is discharged to the recipient environment. The Effluent Treatment Plant (ETP) at Mongstad refinery is composed of two units, a Waste Water Treatment Plant (WWTP) and a Ballast Water Treatment Plant (BWTP). The WWTP primarily collects and treats the oily water (process water) separated in the process areas at the refinery and at the "outer area", covering the crude oil terminal. The BWTP primarily collects and treats the "ballast water" at the refinery and at the crude oil terminal, as well as clean or contaminated surface and ground water drained from the process and oil storage areas. "Ballast water" in this context is mainly settled ballast water from the oil terminal and the refinery, and settled cavern water from oil storage caverns.

CS-2.3.1.   Waste Water Treatment Plant (WWTP)

The WWTP also receives stripped sour water and neutralised NaOH from separate waste streams and the treatment plant has a maximum capacity of 300 m^3/h (Figure 1). There are criteria which the waste water stream must meet prior to being treated by the WWTP. The criteria for pH and phenol are based on the potential for toxicity of these parameters to the microbial community and the limit for ammonia is a signal of non-optimised performance of the process unit. If the waste water stream has contaminant levels exceeding the acceptance level, temporary storage is required.

Figure 1   Waste water treatment plant at Mongstad refinery
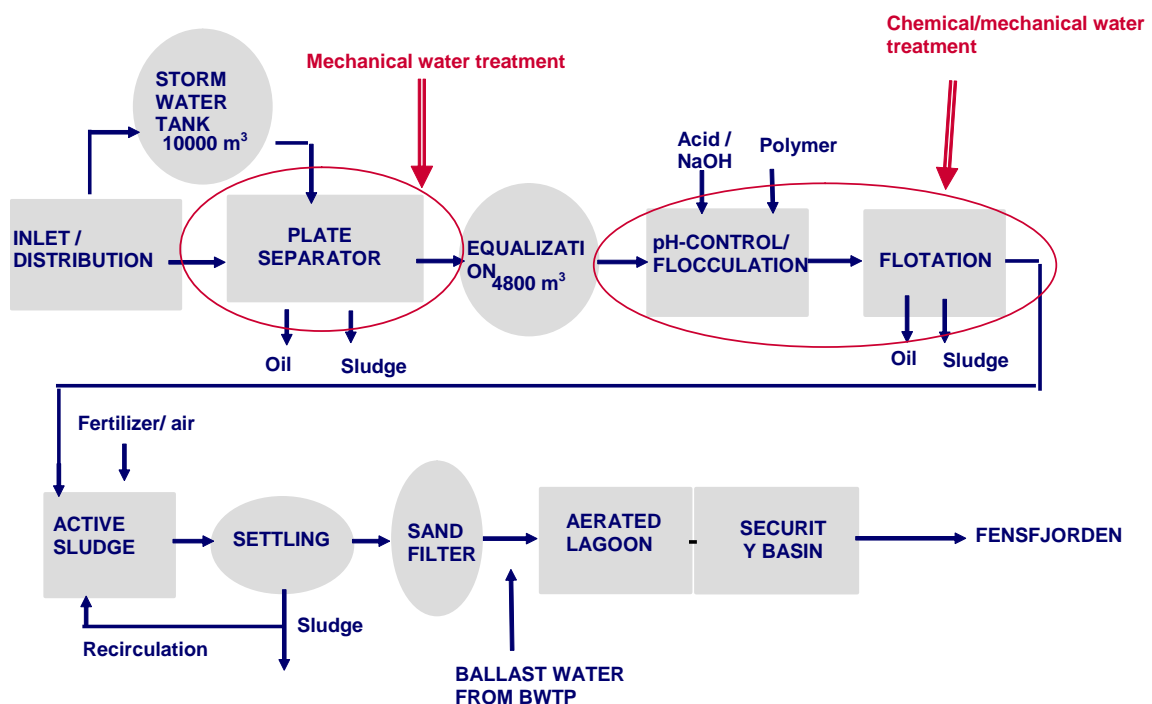
The treatment of the refinery wastewater is staged, whereby a series of physical-chemical (pH adjustment/control prior to flocculation by addition of chemicals) and unit processes are followed by biological treatment followed by further polishing steps (sand filters).

The wastewater is directed to the first stage oil and separation unit (plate separation), which includes slug removal). The secondary stage of oil removal is allowed by air flotation and oil and slug is separated before biological treatment stage. The wastewater is then pumped to the sand filter unit prior to aeration in a lagoon and to the containment basin, prior to discharge to the sea.

CS-2.3.2. Ballast Water Treatment Plant (BWTP)

The BWTP has a maximum capacity of 3000 m^3/h. The ballast water is subject to two stages of oil separation by physical-chemical processes, the secondary stage involving oil removal by flotation. The wastewater is then directed to the aerated lagoon (together with the process water) and further to the containment basin, prior to discharge to the sea (Figure 2).

The treated process water and ballast water from the refinery are released through a diffuser at 46 m water depth in Fensfjorden. At present the average wastewater volumes at Mongstad refinery (process water and ballast water) vary between 6000-23000 m^3/day. In addition there is a flow rate of cooling water to the recipient environment at an average of 29000 m^3/day (in 2003).

CS-2.4. TOXICITY OF THE OIL REFINERY EFFLUENT

The Norwegian Pollution Control Authorities demanded, through the revision of the refinery's discharge permit, for Mongstad to conduct an ecotoxicological program of the effluent at a regular basis from 2004. Therefore an ecotoxicological test program of Mongstad refinery effluent was conducted by application of a Whole Effluent Toxicity (WET) approach to the effluent in July 2004. In parallel, measurements of water quality parameters (i.e. chemical characterisation) of the effluent samples were conducted. Mongstad is presently in the process of designing an ecotoxicological program for the years to come.

The test results, from investigation of acute toxicity, will be compared to the findings from toxicity studies conducted in 1993 on the Mongstad effluent (Smith, 1993; Smith, 1997). In addition to standard toxicity tests this work also included studies addressing effects on key species on sub-lethal endpoint parameters (higher ecological relevance).

Figure 2   Ballast water treatment plant (BWTP) at Mongstad refinery
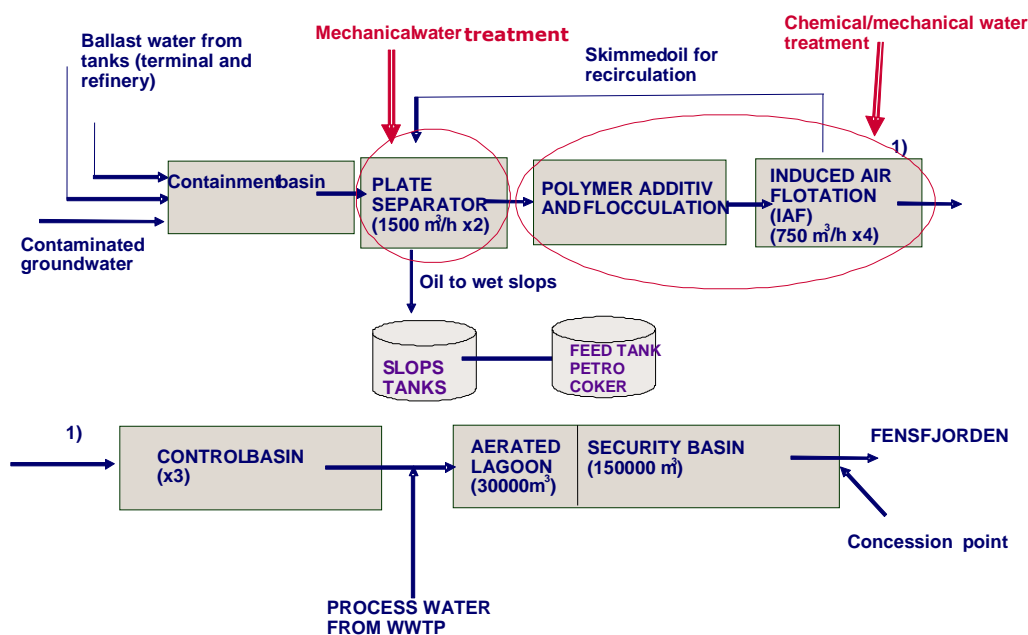
CS-2.4.1. Whole Effluent Toxicity Study - 2004

In July 2004 the WET approach as described by ECETOC (2004) for evaluation of the potential impact of the effluent on the marine environment in the Mongstad area was used.

The WET approach is a rapid, cost-effective and scientifically sound strategy for screening assessments. According to Norwegian Pollution Control Authorities guidance, it is recommended that the test programme shall begin with standardised laboratory bioassays following a tiered approach, starting initially with acute toxicity assessments (WET) on relevant test species, at minimum of three different trophic levels. In parallel with the WET testing, effluent sampling for physical and chemical characterisation of the effluent water shall take place. In addition, information on biological degradation and the potential bioaccumulation of effluent components shall be provided, followed by performance of dispersion model simulations to achieve information of the dilution potential of the recipient environment as well as input to a risk characterisation (PEC/PNEC-ratio) (SFT, 2000).

The refinery effluent was tested for acute toxicity of marine species according to test protocols developed within PARCOM (PARCOM, 1993); on the algae (*Sceletonema costatum*), on a crustacean (*Acartia tonsa*) and on the fish (*Cyprionodon variegatus*). The effluent water was also tested on the luminescent bacteria, *Vibrio fischeri*, by determination of the inhibitory effects on the light emission. The exposure regime in the toxicity tests was as following: 100, 25, 10, 2.5, 1.0, 0.25 and 0.1% effluent water. An overview of the toxicity endpoints for the different test protocols and test durations are outlined in Table 1.

Table 1   Overview of the standard toxicity tests and test species included in the ecotoxicological test program conducted in July 2004 (09.07.04-16.07.04) at Mongstad refinery (AnalyCen Ecotox, 2004)

Test species	Toxicity endpoint	Test duration (hours)	Test protocol
Micro-algea (*S. costatum*)	Growth	72	ISO 10253
Crustaceans (A. tonsa*)*	Lethality	48	ISO/CD 14669
Fish (*C. variegatus*)	Lethality	96	(PARCOM 1995 Part B & OECD 203 Guideline)
Microtox (*V. fischeri*)	Light inhibition	5/15 minutes	ISO 11348-3

The results from the toxicity tests of the refinery effluent are shown in Table 2. The effect values (EC/LC50s and NOECs) are expressed as percentage effluent of the various tests. The results showed that the effluent was not acute toxic for crustacean and fish (LC50 >100% effluent). A minor toxicity response was observed in the tests with algae and bacteria exposed to non-diluted effluent, 75% and 91% effluent, respectively. The most sensitive species tested was the algae, with the lowest NOEC value (10% effluent). Based on dilution factor of 10 of the effluent to the receiving water, no toxicity can be observed for the marine algae species.

Table 2   Results from the standard toxicity tests included in the effluent ecotoxicological test program conducted in July 2004 (09.07.04-16.07.04) at Mongstad refinery

Test species	EC/LC50-value (%)	Short term NOEC value (%)	Toxity Unit (TU)
Skeletonema costatum	75	10	1.3
Acartia tonsa	>100	25	<1
Cyprionodon variegatus	>100	100	<1
Vibrio fischerie	>91	86	<1.1

The toxicity was also expressed as Toxicity Unit (TU). This is defined either as Acute toxic units (Tu_a) defined as 100/L(E)C50 from an acute test (when toxicity is expressed as % effluent by volume); or as chronic toxic units (Tu_c) defined as 100/NOEC or EC10 from a chronic test The highest TU estimated for the effluent at Mongstad was 1.3 for the algae, based on the lowest EC/LC50 value (75% effluent) obtained in the test program.

CS-2.4.2. Investigation of acute toxicity of Mongstad refinery effluents on marine organisms - 1993

In the present study investments in development and optimisation of experimental designs have been used providing cost-effective acute toxicity information of complex effluents. Ecologically relevant and suitable test species and test

parameters were applied together with standard acute toxicity tests (Smith, 1993; Smith, 1997).

In order to evaluate the potential toxicity of the effluent from Mongstad refinery, treated wastewater was sampled from the outlet of the containment basin at the oil refinery and a number of toxicity tests were performed on selected marine species. A description of the toxicity studies and the results from the various toxicity tests are presented in the following section.

The refinery effluent is discharged to marine and estuarine waters. The daily mean wastewater volumes (process- and ballast water) varied between 7500 - 20000 m^3 in early 1990s.

CS-2.4.3. Light inhibition of marine bacteria and growth inhibition of marine algae

Effluent samples from Mongstad refinery were tested for acute toxicity on two different marine organisms, the luminescent bacteria *Vibrio fischeri* (Microtox) and the algae *Skeletonema costatum*. Wastewater samples of ballast water and process water taken before wastewater treatment and a wastewater sample collected prior to discharge (after treatment) to the containment basin from where water is discharged continuously to the marine recipient, were screened for toxicity applying the Microtox test protocol. The findings, expressed as EC50 values and Toxicity Units (TU) are reported in Table 3 for the luminescent bacteria. The samples from the containment basin, representing a mixture of treated ballast water and process water were found to have higher EC50 values ranging from 40-57%, and were thus less toxic, compared to wastewater samples taken in different water streams prior to treatment, with EC50 values ranging from 12-22%. The ballast water was clearly more toxic than the process water before treatment.

Table 3   Results from the Microtox light inhibition test performed on wastewater samples prior to and after treatment at Mongstad oil refinery sampled in December 1992 and January 1993

Effluent sample	Date	EC50 (%) 15 min.	Toxic Unit (TU)
Ballast water	09.12.92	12	8,3
(prior treatment)	09.12.92	12,6	7,9
Process water	09.12.92	21,7	4,6
(prior treatment)	09.12.92	21,3	4,7
Containment basin - Batch 0	09.12.92	53,9	1,9
(after treatment)	09.12.92	46,5	2,2
	09.12.92	40,9	2,4
Containment basin – Batch 1	05.01.93	56,9	1,8
(after treatment)	06.01.93	49,1	2,0
	07.01.93	47,4	2,1

Toxicity of the refinery effluent to marine algae *S. costatum* was determined according to the "Marine algae growth inhibition test" (ISO method DP 10253, 1988). The results from the algae test are shown in Table 4. The algae test was found 3 to 5 times more sensitive (11-15% effluent) than the bacterial Microtox test to the refinery effluent.

Table 4   Results from growth inhibition test with the marine microalgae S. costatum performed on wastewater samples after biological treatment at Mongstad oil refinery taken in January 1993

Effluent sample	Date	EC50 72-h (%)	Toxic Unit (TU)
Containment basin	05.01.93	11,6	8,6
- Batch 1	06.01.93	15,2	6,6
(after treatment)	07.01.93	14,1	7,1

CS-2.4.4. Inhibition of growth rate on marine algae

Performance of two different experiments with a mixture of marine algae exposed to different concentrations of effluents is described. In the first experiment, growth rates of cultured populations of the marine algae *Skeletonema costatum, Phaeodactylum trocornutum* and *Tetraselmis sp.* in mixture exposed to the concentration range 5 to 20% effluent water from Mongstad refinery were investigated. *S. costatum* exposed to 20% effluent water exhibited a negative growth rate, compared to no observable effect when exposed to 10% effluent water. No significant inhibitory effect on growth rates or total biomass of populations of *P. tricornutum* and *Tetraselmis sp.* was observed exposed to the range of 5 - 20% effluent water (Table 3).

In a separate experiment, the growth rate of cultured populations of *P. trocornutum* and *Tetraselmis sp.* in mixture was investigated when exposed to a concentration range of 10 to 50% effluent. No significant inhibitory effect on growth rates or total biomass of populations of *P. tricornutum* and *Tetraselmis sp.* was observed (Table 5). However, when exposed to 10-20% effluent water, a general increase in population biomass compared to the control group was observed.

Table 5 Overview of results from toxicity test with different marine species (Microtox and microalgae) performed on effluent samples after biological treatment at Mongstad oil refinery.

Test species	Exposure conditions	Effect endpoint	Effect level/effluent concentration (%)	Toxity Unit (TU)	Test protocol
Bacteria: *V. fischerie*	15 min	Light inhibition	EC50 = 40-57 EC20 = 14-18	2,5	Beckman Microtox manual, 1988
Algae: *S. costatum*	Static system 72 hours	Growth inhibition	EC50 = 11-15 EC10 = 6-8	9,1	ISO-DP 10253, 1988
Algae: *S.costatum* *P.tricornutum* *Tetraselmis sp.*	<u>Exp. 1:</u> 5, 10 and 20 % effluent	Growth rates	LOEC = 20% LOEC >20% (no effect observed) LOEC >20% (no effect observed)	-	ISO-DP 10253, 1988
Algae: *P. tricornutum* *Tetraselmis sp.*	<u>Exp. 2 :</u> 10, 20, 30, 40 & 50% effluent	Growth rates/ total biomass	LOEC >50% (no effect observed) LOEC >50%(no effect observed)	-	ISO-DP 10253, 1988

CS-2.4.5. Hatching success of the planktonic crustacean Acartia tonsa

The hatching success of the marine crustacean (copepod) *Acartia tonsa*, exposed to different concentrations of effluent water (range: 5-50%) was also investigated. The species *A. tonsa* represents an important planktonic organism in most Norwegian fjords. The results showed large batch-specific variations in hatching success, both within the control groups and the exposed groups (Table 6). This variability may be the result of errors inherent in the experimental design and counting exactness. The time from the onset of the experiment to the hatching time was not well defined. No significant effect of the refinery effluent was therefore observed.

Table 6 Overview of results from toxicity test with different to different marine species (algae and mysid) performed on effluent samples after biological treatment at Mongstad oil refinery

Test species	Exposure conditions	Effect endpoint	Effect level/effect concentration	Test protocol
Crustacean *Acartia tonsa*	96 hours exp. Jan./Feb. 93 5, 10, 20 and 50% effluent	Hatching success	No significant difference between control and exposed groups	Not available
Mysid *Neomysis integer*	96 hours exp. 5, 10, 15, and 20% effluent Salinity: 10‰, Static open system	Lethality	No lethality observed	Not available
Mysid *Neomysis integer*	24 and 72 hours exp. 50% effluent water Salinity: 10‰,	Physiological effects: stress proteins	No stress protein identified	Not available
Mysid *Neomysis integer*	10, 25 and 50% effluent 96 hours exp. Salinity: 10‰, Flow through system	Oxygen consumption	No significant difference between control and exposed groups	Not available

CS-2.4.6. Lethal and physiological effects on the mysid Neomysis integer

Adult and sub-adult specimens of the mysid *Neomysis integer* were exposed to different concentrations of effluent water. *Neomysis integer* is a brackish water species common in estuarine waters along the coast of Norway and was included in the test program due to its sensitivity to toxicants, short generation time and character as a secondary consumer, often feeding on zooplankton. The effects from exposure to an exposure regime of 5 to 50% effluent on lethality and stress proteins were investigated. No increase in lethality was registered in any of the exposed groups, and no stress proteins were found in individuals exposed to 50% effluent water in 24 and 72 hours (Table 6).

The effect of exposure to a concentration range of 10 to 50% effluent at different salinities on physiological parameters like oxygen consumption was also studied. No significant effect on oxygen consumption was observed after 96 hours exposure.

CS-2.4.7. Effects of exposure of egg and larvae on cod (Gadus morhua)

Eggs and larvae of cod (*Gauda morhua L.*) were exposed to one concentration; 50% effluent for 96 hours. The early life stage of marine fish is generally regarded as sensitive to marine pollutants. Heart rate, hatching, length/growth and mortality were measured in early life stages of cod (eggs, embryo, and larvae).

An overview of the test results of cod is presented in Table 7. The heart rate of cod embryos decreased by 15% compared to controls exposed to seawater (significant decrease) when exposed to 50% effluent. The effect found on embryonic heart rate may have consequences for the recruitment of a cod population or the amount of available larvae as food sources for other marine organisms in an ecosystem. Hatching of cod eggs was initiated at the same time in control group and the exposed group but the peak appeared earlier in the exposed group. The peak hatching time in exposed groups took place 12-24 hours earlier than in control groups. Length measurements in newly hatched cod larvae indicate that exposed larvae are shorter than larvae in control groups from exposure to 50% effluent water. However, lethality in the exposed groups of eggs/larvae was not higher than those hatched in clean seawater.

Table 7   Overview of results from toxicity test with different early life stages of cod (Gauda morhua L.) performed on effluent samples after biological treatment at Mongstad oil refinery

Test species	Exposure conditions	Effect parameters	Effect level/effect concentration	Test protocol
Fish – Cod Embryo	50% effluent 96 hours exp. Salinity: 34.5 ‰,	Heart rate	Significant effect (15% below normal) at 50% effluent	Not available
Fish – Cod Newly hatched larvae	50% effluent 96 hours exp. Salinity: 34.5 ‰,	Length	Significantly shorter at 50% effluent	Not available
Fish – Cod **Eggs**	50% effluent 96 hours exp. Salinity: 34.5 ‰,	Hatching time	Hatching was initiated at the same time but the peak appeared earlier in the exposed group	Not available
Fish – Cod Eggs/larvae	50% effluent 96 hours exp. Salinity: 34.5 ‰,	Lethality	No lethality observed	Not available

CS-2.5.   COMPARISONS BETWEEN 1993 AND 2004 WET STUDIES

Toxicity has been assessed by applying the WET method on Mongstad refinery effluent both in 1993 and 2004. The initiative to the effluent toxicity testing in 2004 was taken in order to comply with regulations as well as the need for identification of the toxicity of the effluent.

In both studies, the algae *S. costatum* was found to be the most sensitive species when exposed to effluent from Mongstad refinery. The toxicity expressed as TU values, based on the lowest EC50 values (11 and 74% effluent), tested for growth inhibition, and was estimated to give a TU value of 9.1 in the 1993 study and a TU of 1.3 in the 2004 study. This indicates that the toxicity of the refinery effluent has decreased significantly, by approximately a factor of 7, in the period from 1993 to 2004, based on the results with the algae *S. costatum*.

In the 1993 study other species, including cod, mysid and the less traditional/standardised algae species were tested for more ecological relevant and sub-lethal effect endpoints such as hatching time/success, physiological effect, oxygen consumption, heart rate etc. Furthermore, cod was tested for different early development stages (egg, embryo and larvae) viewed as more sensitive than the adult stage. These results indicate that the short term exposure for the refinery effluent although expected to be more toxic compared to the 2004 effluent, (based on the algae and the bacteria tests), did not reveal higher effects when tested for the less standardised species and sub-lethal effect endpoints. An exception was the experiments conducted with early life stages on cod that showed significant effects on length and heart rate and hatching time with short term exposure to 50% refinery effluent. However, EC50 or NOEC values could not be estimated due to limited exposure regime used in these experiments (only one effluent concentration tested).

CS-2.6.   CHEMICAL CHARACTERISATION OF THE EFFLUENT

The effluent water at Mongstad refinery comprises a complex mixture of organic and inorganic contaminants including both oil derived components and "added chemicals". The added chemicals or additives include both those consumed at the refinery and those chemicals derived from transport of crude oil (by ship/pipeline) from offshore installations. They are of concern, both due to the potential for inhibition of biological unit processes used for treatment, as well as the potential impact in receiving waters.

The Norwegian regulatory requirements, includes demands for maintaining concession limits for selected components of the Mongstad refinery effluent. These concession limits are given for daily content and/or concentration of oil-in-water, phenol, ammonia, sulphide, cyanide and pH. The refinery effluent streams are analysed daily for content of oil-in-water, phenol and ammonia, while pH, cyanide and sulphide are measured twice a week. The concentration of total nitrogen, phosphate, phosphorus and Total Organic Carbon (TOC) in the effluent is also measured on regular basis.

CS-2.6.1.   Naturally occurring components

The chemical composition of oil derived substances like Benzene, Toluene, Ethylbenzene and Xylenes (BTEX), naphthalene, Polyaromatic Hydrocarbons (PAHs), phenols and metals in the effluent is regularly analysed according to the analysis guidance applied for naturally occurring produced water constituents on the Norwegian Continental Shelf (OLF, 2003). As a part of the ecotoxicological program,

effluent water samples were taken from the outlet of the containment basin in July 2004 for chemical analysis of the above-mentioned effluent components in parallel with the conduct of the WET test. The effluent samples contained oil hydrocarbons lower than 1 mg/l, below the regulatory concession limit for discharges to Fensfjorden (5 mg/l). The total concentrations of dissolved BTEX, naphthalenes, 2-3 ring PAHs and high weight molecular PAHs (4+ ring PAHs) were measured at low ppb levels (2-6 ppb). The concentration of alkyl phenols ranged from 13 ppb for C4-C5 alkyl phenols and 26 ppb for C0-C3 alkyl phenols. Very low concentrations of C6-C9 alkyl phenols (<0.5 ppb) were detected in the refinery effluent. Analysis of metals showed concentrations close to background levels in open seawater, except for zinc.

The concentration levels of oil hydrocarbons were within the same range as in the 1993 study. The TOC (14.3 mg/l) and the ammonia (7.4 mg/l) levels in the effluent were higher in the 2004 measurements compared to 1993 (5 and 2 mg/l, respectively). However, the phenol and the oil-in-water (hydrocarbon index) concentration in the effluent were lower in 2004 (0.24 mg/l and 0.64 mg/l, respectively) than in the1993 (0.4 mg/l and 0.9 mg/l). Additionally, the average concentration of mercury was more than 100 times higher in the effluent samples from the refinery in 1993 (0.65 μg/l) in comparison to 0.006 μg/l in 2004. The alkyl phenols were not characterised in 1993 due to analytical/extraction problems.

CS-2.6.2. Added chemicals

A considerable number of chemicals are in use in the various refinery processes and in the wastewater treatment units at Mongstad refinery. Some of these chemical substances may be relatively water soluble and thus could be present in significant amounts in the water phase at process steps involving direct contact with water streams. In addition, there is a contribution from chemicals substances applied offshore at various producing facilities, derived from water residues (about 0.5 %) from crude oil via cargoes and pipeline transport. Due to the effluent complexity and lack of appropriate analytical methods for detection of chemical concentration in the effluent water, no estimation of effluent concentrations of chemicals applied at the refinery was available in 1993. However, in 2004 a chemical model system used for prediction of concentrations of "added" chemicals in different water streams prior to and after treatment in the wastewater treatment plant (WWTP) at Mongstad was developed. The difference in toxicity between 1993 and 2004 may partly be explained by the differences in the cocktail of "added" chemicals in the effluent water from the refinery, assuming hazardous chemicals that have the potential to pose harm to the environment, have become replaced by more environmentally friendly chemicals during the past ten years.

CS-2.7. DILUTION AND DISPERSION MODELLING

Numerical dispersion modelling is a useful and cost effective tool for predicting dispersion and fate of effluent water in the marine environment. The output from dispersion modelling may be further used for predictions of the dilution potential and environmental risk of discharges to the receiving environment.

In the 2004 study, dispersion model simulations were carried out using the following two-dimensional numerical models: Visual Plumes for simulating mixing of effluent discharged water with ambient water, i.e. spreading and trapping of effluent water (Frick et al. 2001), and the hydrodynamic RMA2 model and the RMA4 transport model simulation using the hydrodynamics from RMA2 (US Army, 2003a,b). Visual Plumes was used to calculate in-layering depth and near-zone dilution of effluent

from Mongstad refinery to Fensfjorden. The results from Visual Plumes have been used as input for the RMA models which is more suitable for dispersion modelling in distant areas.

The effluent is characterised with varying salinity over the year due to differences in surface water and ballast water volumes. The water temperature is also varying due to influence by the solar radiation and air temperature that change during the season. The reason for this is that the discharge water is stored in an outdoor lagoon for several days before it is discharged to Fensfjorden. The salinity in the discharge water is the most important parameter for modelling in-layering depth and dilution of submerged plumes in Norwegian fjords. Tidal currents, freshwater runoff and wind have been taken into account for an average situation.

The results from the model simulations indicated an in-layering depth in the range 35 – 40 m depth (discharge point at 46 m depth), and at this depth the discharge water on average was diluted 500 times. The models RMA2/RMA4 have been used to simulate discharge to a 5 m thick water layer. These simulations were done for a conservative constituent (decay was not included) and vertical mixing and diffusion with the overlying and underlying water masses were not taken into account in these simulations. The dilution is approximate 1000 and 1500 times respectively, 500 and 1500 m downstream of the discharge.

CS-2.7.1. Validation of dispersion modelling dye studies

Dye studies were conducted for validating the dilution modelling of the receiving environment at Mongstad. Experiments involved adding fluorescence dye (Fluorescein) to the main effluent. The dye was then detected by sensitive sensors in the sea surrounding the diffusor segments at 50 m depth, up to 1300 m from the inlet well. Vertical profiles of fluorescence, turbidity, salinity and temperature versus depth were taken at regular intervals at 30 - 40 stations each day at varying distance from the diffusor. The sensor readings represented theoretical dilution factor in the range of 250 - 900 within the sampled area (Golmen and Nygaard, 2006) and is at the same range as predicted by the two models. The dye was consistently detected in the receiving environment in water layers between 30 and 40 m depth confirming the in-layering depth.

CS-2.8.   RISK EVALUATION OF THE EFFLUENT

According to Norwegian Pollution Control Authorities guideline it is recommended that the effluent is further assessed by calculation of environmental risk by characterisation of the PEC/PNEC-ratio based on the toxicity data from the WET test, or from toxicity information on single substance level (SFT, 2000). Both approaches were conducted in the present case study. The outcome of the risk calculations based on dilution modelling with Visual Plumes and RMA2/4, showed PEC/PNEC-ratios slightly higher than 1 (when combining PEC/PNEC ratios for all components). The calculations indicated that the risk contribution from the "added" chemicals was higher when compared to the oil derived substances components (Frost et al, 2004).

Monitoring of the receiving environment was conducted in 2004. In the 2004 survey a follow-up study was conducted by analysis of oil hydrocarbons tissues of blue mussel tissues. An increased level of oil hydrocarbons and a reduced number of seashore faunal species (reduced diversity) were observed at the two outermost stations, possibly due to accidental oil spills from a ship in this area in 2003. In the regular monitoring survey in 2006 there was no significant impacts observed in the

marine recipient near Mongstad refinery. However, monitoring surveys have traditionally covered monitoring of the seashore and the sediment compartment and not addressed impacts on organisms that live in the water column.

CS-2.9.   CONCLUSION

In both whole effluent toxicity tests conducted in 1993 and 2004 the algae (*S. costatum)* was found to be the most sensitive species among the species tested (crustacean, fish etc.) when exposed to effluent from Mongstad refinery. The toxicity, expressed as growth inhibition, showed a significant decrease from the 1993 to the 2004 study, by a TU value of 9.1 and 1.3, respectively tested on the algae. The standard algae test with *S. costatum* is therefore found suitable as a test species at Mongstad due to its high sensitivity. Additionally, this test is inexpensive and easy to carry out and is recommend to be used as a screening method.

The toxicity does not seem to be directly related to the TOC level of the effluents. The TOC level was 14.3 mg/l and 5 mg/l, respectively, in the 2004 and 1993 study. However, the phenol and the oil-in-water (hydrocarbon index) concentration in the effluent were lower in 2004 (0.24 mg/l and 0.64 mg/l, respectively) than in the 1993 study (0.4 mg/l and 0.9 mg/l). Additionally, the average concentration of mercury was more than 100 times higher in the effluent samples from the refinery in 1993 (0.65 μg/l) in comparison to 0.006 μg/l in 2004.

The low acute toxicity of the refinery effluent observed in the WET test conducted in 2004 confirms the high efficiency of the Effluent Treatment Plant, including mechanical, chemical and biological treatment. In the 1993 study this is also supported by the results from the Microtox light inhibition test performed on wastewater samples prior to and after treatment. The concentration levels of oil hydrocarbons were within the same range as in the 1993 study.

CS-2.10. REFERENCES

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CS-3. CASE STUDY 3: ECOLOGICAL MONITORING OF THE MARINE ENVIRONMENT AT MONGSTAD REFINERY

CS-3.1. SUMMARY AND KEY LEARNINGS

The present case study describes the monitoring that took place covering the period of 1972-2006. The case study also addresses the extent to which the methods and analysis being used have changed, and the impact of the modifications. Traditionally, the attention in the monitoring surveys conducted until 1980 was on investigations of the rocky seashore fauna. A modification of the methods applied took place in the early 1980s. The monitoring surveys conducted from 1985 (baseline survey) were more comprehensive, including chemical analysis of oil hydrocarbons in sediments and biota, hydrographical measurements as well as sediment analysis. The surveys in 1985 and 1987 provided a baseline for future monitoring of the marine environment, and were later used for comparisons to identify faunal changes in this area. In 1990 sediment analysis of heavy metals were introduced, and from 1994 the sediment measurements were replaced by analysis of metals in biological tissues (blue mussels).

The change in the content of the monitoring programs applied to the marine environment in the Mongstad area, Norway, from study of the rocky sea shore fauna to more comprehensive programs covering a number of monitoring analysis and methods, and sampling undertaken in several environmental compartments, have been beneficial for the refinery. The current analysis and methods conducted in the "regular monitoring" surveys provide an enhanced possibility for identification and follow-up of planned discharges and spill incidences, reflecting the different activities at Mongstad refinery. For instance, identification and investigation of potential impacts from small accidental crude oil spills at shore, oil spill from ships at the harbour area, discharge from sea water scrubber outlet as well as planned discharge of process water to the marine recipient from the refinery demand for surveys including a combination of different monitoring methods and analysis. Selection from a suite of methods/analysis to be deployed dependent of the incidence and the investigations that are to be undertaken, gives the refinery flexibility with respect to design of monitoring programs.

Since monitoring surveys started in the Mongstad area in 1972, the concern has traditionally been on monitoring of the seashore and the sediment compartment, with toxicity as the primary end point parameter. In future monitoring programs, potential impacts on water column organisms as well as validation of model predictions of exposure and concentrations of effluent constituents may be addressed. Additionally, validation of model prediction of exposure of non-toxic stressors, such as inorganic nitrogen (ammonia), increase in temperature and change in O_2 level due to biodegradation of chemicals in the recipient will be evaluated introduced in future environmental monitoring programs.

CS-3.2. INTRODUCTION

Ecological monitoring of the intertidal marine environment in the vicinity of the effluent discharge at Mongstad refinery has been carried out since 1972. The monitoring data from 1972-1974, serve as the baseline before commissioning of the refinery in May 1975. The monitoring surveys in 1970`s focused mainly on community analysis of the rocky seashore fauna. In early 1980`s monitoring methodologies were subject to further development and were extended to cover chemical and physical parameters as well as monitoring of the soft sediment seabed

fauna. The updated baseline studies of 1985 and 1987, which will be described, have been used as reference for monitoring surveys conducted since 1990 to the present day. In this case study a description of the changes in monitoring survey methods through the period 1972 to 2006 is presented.

The different monitoring surveys conducted since 1972 around Mongstad refinery can be divided into four different periods dependent of the environmental monitoring methods applied:

1. Baseline/monitoring surveys 1972-1979
2. Baseline monitoring surveys 1985-1987
3. Regular monitoring surveys 1990-2006
4. Follow-up monitoring surveys 1989-2004

An overview of all elements included in the environmental monitoring surveys within the different periods is shown in the following sections. A more detailed description of the different measurements undertaken over the period 1972 to 2006 is shown in Appendix 1.

CS-3.3.   ENVIRONMENTAL MONITORING METHODS

CS-3.3.1.   Baseline/monitoring surveys the period 1972-1979

Monitoring surveys started at the site of Mongstad refinery in 1972. Surveys commissioned by BP (British Petroleum Company Limited Environmental Control Centre) in the period 1972 to 1979 were published by Syratt and Cowell, 1975, Hartley et al, 1986 and by Curtis and Grezo (1989). In addition studies undertaken in late 1970`s were reported by Dalbye et al (1978) and Dalbye and Syratt (1979).

Shore surveys in 1972, recording the vertical distribution and abundance for common intertidal species (littoral zone) were determined annually until 1974 at 18 stations in addition to five reference stations (Syratt and Cowell, 1975). At each station a belt transect was established, each extended from low water spring tides to the top of the black lichen zone. Each transect was divided into vertical intervals (0.1 m) using a simple cross staff levelling method described by Moyse and Nelson-Smith (1963) and modified by Crapp (1971). In later surveys, intervals of 0.2 m were used, approximating to about 1/10 of the extreme tidal range (1.8 m for Mongstad). The abundance of each species, (forty-five species), was recorded over an area of about 5 m to each side of the survey line and 50 mm above and below each point.

At each level on the rocky shore, the abundance scales of Crisp and Southward (1958), Ballantine (1961) and Moyse and Nelson-Smith (1963), modified by Crapp (1971) were used for the determination of the relative exposure of rocky shores (physical stress due to wave actions). It became obvious during the early stages of the monitoring program at Mongstad, that some form of shore classification for exposure was essential in order to interpret shore surveys and place them in the frame of the reference recordings. The Ballantine scale that was primarily used in Milford Haven (UK) was modified for use in Western Norway. Those species particularly sensitive to winter water temperature were excluded due to the more northerly latitude of the Norwegian site. (Dalby et al, 1978).

The abundance, shown as zonation and changes in distribution of key species of flowering plants, lichens, seaweeds, barnacles, limpets, gastropods, polychaetes, bivalves etc. were expressed as kite diagrams for the various shores. The general theory behind zonation has been adequately described by Lewis (1964) and by

Moyse and Nelson-Smith (1963). Lewis (1965) illustrated the differences in shore zonation on the south coast of Norway. Ferry and Sheard (1969) described the zonation of supra-littoral lichens on rocky shores in UK.

Limpet recruitment studies were conducted by measuring samples of limpets in situ at each station, and estimations of barnacle recruitment from spatfall. Also, the upper limit to the vertical distribution of the black littoral fringe lichen, *Verrucaria maura, at* each site was recorded during the baseline surveys of 1973 and 1974 (Syratt and Cowell, 1975). The shell characteristic (shell teeth) of the dog whelk, *Nucella lapillus*, (Cowell and Crothers, 1970; Crothers, 1971) was also studied.

The monitoring methods applied in the baseline survey (Syratt and Cowell, 1975) were also employed on data from the Mongstad survey undertaken in 1975, 1976, 1977 and 1979. Monitoring took place after a spill of light slops occurred in 1975 during commissioning. During the 1977 survey, damage to the marine flora and fauna of the rocks surrounding a small bay was detected (Dalby et al, 1978). Signs of local contamination, mainly in the form of oil on the water surface in the bay, and the presence of sewage fungus in the small stream entering the bay. Enquiries showed that there had in fact been a fractured pipe within the refinery processing area that had led to a temporary contamination of the stream (Monk et al, 1978).

The main environmental factor separating the sampling sites at Mongstad is exposure to wave action. This needed to be quantified so that sites of numerically similar exposure could be compared to each other. A biological exposure scale, adapted from Ballantine (1961), was applied. The data was employed on a ranking scale, in which the abundance measure increases approximately logarithmically with rank, the maximum score of 7 being the maximum abundance possible in nature for a given species. Maximum abundance attained by each species at each site, was plotted against exposure grade. By use of a recursive method of curve fitting and reassessment of exposure grades, adjusted values for each site on a scale from 1, (most exposed) to 9, (most sheltered) were calculated (Dalby et al, 1978).

Limitations to the use of such abundance scales have been described by Ballantine (1961) and Lewis (1964). The shores being investigated should ideally be moderately uniform slopes of bedrock. Very uneven shores with large stacks and jagged reefs should not be considered as a whole if other more suitable shores are available. If biological monitoring is employed, recording should be quantitative and in such a form that the data can be treated statistically. More information could have been extracted with a different kind of data recording. In addition, variation in species abundance on rocky shores is often considerable from year to year, and this must be taken into account when assessing the impact of any possible contamination.

The methods applied in this period were time consuming. A full survey with focus on the intertidal marine environment could be completed in approximately eight days (at low spring tides) with four persons. In addition, analysis of data and reporting took one person about 2 months.

An overview of the methods applied in the environmental monitoring surveys in the period 1972 to 1979 is presented in Table 1. In this period the attention was only on analysis of the rocky seashore fauna.

Table 1   Methods applied in the environmental monitoring surveys conducted in the period 1972 to 1979

Year	Surveys	Rocky sea shore fauna (hard bottom)
1972-1979	Marine baseline survey/monitoring surveys	***Community analysis:***[1] _Littoral zone:_ -Vertical distribution and abundance of intertidal species - Zonation of key species - Shell characteristics (_Nucella lapillus_) - Limpet recruitment - Level analysis

I. References: Crisp and Southword, 1958; Crapp, 1973; Syratt and Cowell, 1975 (BP study); Dalby et al, 1978; Monk et al, 1978; Dalby and Syratt, 1979 and Hartley et al, 1986 (BP study).

CS-3.3.2. Baseline monitoring studies 1985 -1987

The marine baseline studies were updated in 1985 and 1987 in connection with the upgrading and expansion of the oil refinery. The main objectives of the marine baseline study were first to describe the environmental conditions in some selected marine benthic communities from various parts of the soft bottom sediment in the Mongstad area. Secondly, to provide a baseline for future monitoring of the marine environment, and form a basis for development of a new monitoring programme to be used in later comparisons to observe faunal changes in this area. In addition to the traditional survey of the shallow water populations of algae and hard bottom fauna from the littoral zone (down to 30 m), the marine baseline study comprised additional sub-investigations (Johannessen and Høisæter, 1986; Johannessen et al, 1988):

- a study of the soft seabed fauna (down to 30-40 m depth)

- sediment analysis of organic carbon and grain size distribution

- hydrographical measurements

- analysis of petroleum oil hydrocarbons and phenols in sediment, blue mussels and sea water

- counting of breeding seabirds

In addition a visual examination of the shoreline was undertaken, to record signs of oil spill pollution.

Investigations of the soft seabed fauna included benthic community abundance parameters (species assemblages and species diversity), and were useful indicators for describing the environmental status of the benthic community. Potential impacts on the fauna in the influence area were identified by estimation of species diversity expressed by diversity index (H') evenness, (J) distribution of species in geometric classes, cluster analysis (Bray-Curtis similarity index), Hurlbert species richness curve and ordination analysis (Multi-Dimensional Scaling).

The techniques included in the hydrographical measurements and chemicals analysis of the sediments and biota are further described under "regular" monitoring surveys.

The seashore has been monitored since 1972. In the baseline surveys in 1985 and 1987, the sub-littoral zone in addition to the littoral zone was subjected to investigations due to their ability to respond to different types of stress and pollution. The algae and sessile animals occupying the sub-littoral zone are sensitive and good indicators of variations in the environment. The monitoring elements and methods applied in the base line survey undertaken in 1985 and 1987 are presented in Table 2, and are further described under "regular" monitoring surveys.

Table 2   An overview of the monitoring elements and methods included in the marine baseline survey conducted in 1985 and 1987

Year	Surveys	Hydro-graphy	Sediment analysis		Contaminants analysis			Soft seabed fauna	Rocky sea shore fauna
			Grain size	Organic content	Sediment	Water	Biota		
1985-1987 [I]	Baseline study I and II	Yes	Yes	Yes	THC [VI]	THC Phenols	Hydro-carbons (NPD) in blue mussels	*Abundance:* - No. individuals - No. species *Analysis:* - Distribution of species in geometric classes [II] - Diversity index (H') [III] - Evenness (J) [IV] - E(S100) - Hurlbert species richness curve - Cluster analysis (Bray-Curtis similarity) [V] - Ordination (MDS-analysis)	***Community analysis:*** *Littoral zone:* - Level analysis [VII] - Square analysis [VIII] - No. species - Photographic documentation - Wave exposure index - Vertical zonation *Sublittoral zone:* - Square analysis - Photographic documentation - Transect analysis - Wave exposure index - No. species *Censuses of seabirds* *Analysis:* - Cluster analysis (Bray-Curtis similarity) - Ordination (MDS-analysis)

[I] References: Johannessen and Høisæter, 1986; Johannessen et al, 1988
[II] Gray and Mirza, 1979.
[III] Shannon and Weaver, 1949
[IV] Pielou, 1966.
[V] Field et al, 1982.
[VI] Total hydrocarbons
[VII] Method by Dalby et al, 1978; Hjolman and Risheim, 1992 .
[VIII] Method by Oug et al, 1985; Johannessen and Høisæther, 1986.

Semi-quantitative measurements by "level analysis" were used for determination of the distribution of selected dominating species (plants and organisms) at fixed stations at the seashore, in surveys over the period 1972-1987 (Syratt and Cowell, 1975; Hartley et al, 1986; Curtis and Grezo, 1989). A modified version of the method was applied in the baseline study in 1985 (Hjolman and Risheim, 1992). The shore at each station was divided into 15 cm vertical levels along a transect 8 m wide.

Each species was allocated an abundance value according to a scale divided into 8 categories (Dalby et al, 1978). In parallel a new method called "square analysis" was introduced into the monitoring program in 1985 (Oug et al, 1985; Johannessen and Høisæther, 1986).

CS-3.3.3. Regular monitoring surveys 1990-2006

The marine baseline studies updated in 1985 and 1987 have been used as reference for monitoring surveys proceeded since 1990. In the period 1990-2006 regular monitoring including follow-up monitoring surveys, with few exceptions, were conducted annually. The monitoring surveys included several or all elements included in the marine baseline studies (Johannessen and Høisæter, 1986; Johannessen et al, 1988). An overview of the analysis and methods applied in the regular monitoring surveys through the period 1990 to 2006 is presented in Table 3.

The monitoring surveys undertaken around Mongstad refinery since 1990 have included five main elements:

1. Soft sediment analysis and hydrographical measurements
2. Soft sediment benthic fauna survey
3. Hard bottom sediments seashore survey
4. Hydrocarbons/heavy metal analysis in sediments
5. Hydrocarbon/heavy metal analysis in blue mussels

A description of the methodologies and techniques applied in each of these is given below.

CS-3.3.3.1. Soft sediment analysis and hydrographical measurements

The soft sediment surveys include determination of organic content (ignition loss) and grain-size distribution determination of the surface sediment at a minimum of 4 stations (similar stations as sampled in the soft sediment surveys) for classification into various sediment types. The grain size distribution also indicates the current condition at the sample location.

Additionally, measured data on hydrographical conditions such as temperature, salinity, density, and oxygen level and turbidity were reported for a permanent station (Mo 61, see figure 1) included in all the surveys since 1985. Since 1990, the measurements were undertaken in the spring at different depth intervals (0, 20, 50, 100, 200 and 400 m) in the vicinity of Mongstad refinery. The hydrographical data provides information about the oxygen conditions and exchange of water masses in the marine environment.

CS-3.3.3.2. Soft sediment benthic survey

A soft benthic seabed survey has been conducted at a minimum of four stations in the period 1990 to 1993 in the regular monitoring surveys; a minimum of two stations (Mo 51 and Mo 52) are located in the vicinity of Mongstad refinery (at 24 and 40 m depth) and two stations (Mo 53 and Mo 61) are located in the Fensfjorden at the outer side of the refinery (at 330 and 455 m depth) shown in Figure 1. Two of those stations (Mo 52 and Mo 53) have been sampled over the whole period from 1990 to 2006. In addition, station Mo 55 (close to the security basin) and Mo 61 were analysed regularly in surveys in the period from 1997 until 2006.

At each station, five grab samples for determination of the benthic fauna were collected. The sediment samples were collected by use of a 0.2 m^2 van Veen grab

(the upper 5 cm) until 1997 and a 0.1 m^2 grab since 2000. Species determination was limited to organisms larger than 1 mm. Animals that live on the top of the sediment and just above the sea bottom were excluded (e.g. crustaceans). True benthic groups included in the quantitative analysis of soft corals, sea anemones, bristle worms (polychaeta and Oligochaeta), burrowing crustaceans, molluscs, echinodermata and tunicata.

Figure 1   Overview of the stations sampled and analysed in the soft sediment and hard bottom seashore surveys in the Mongstad area.
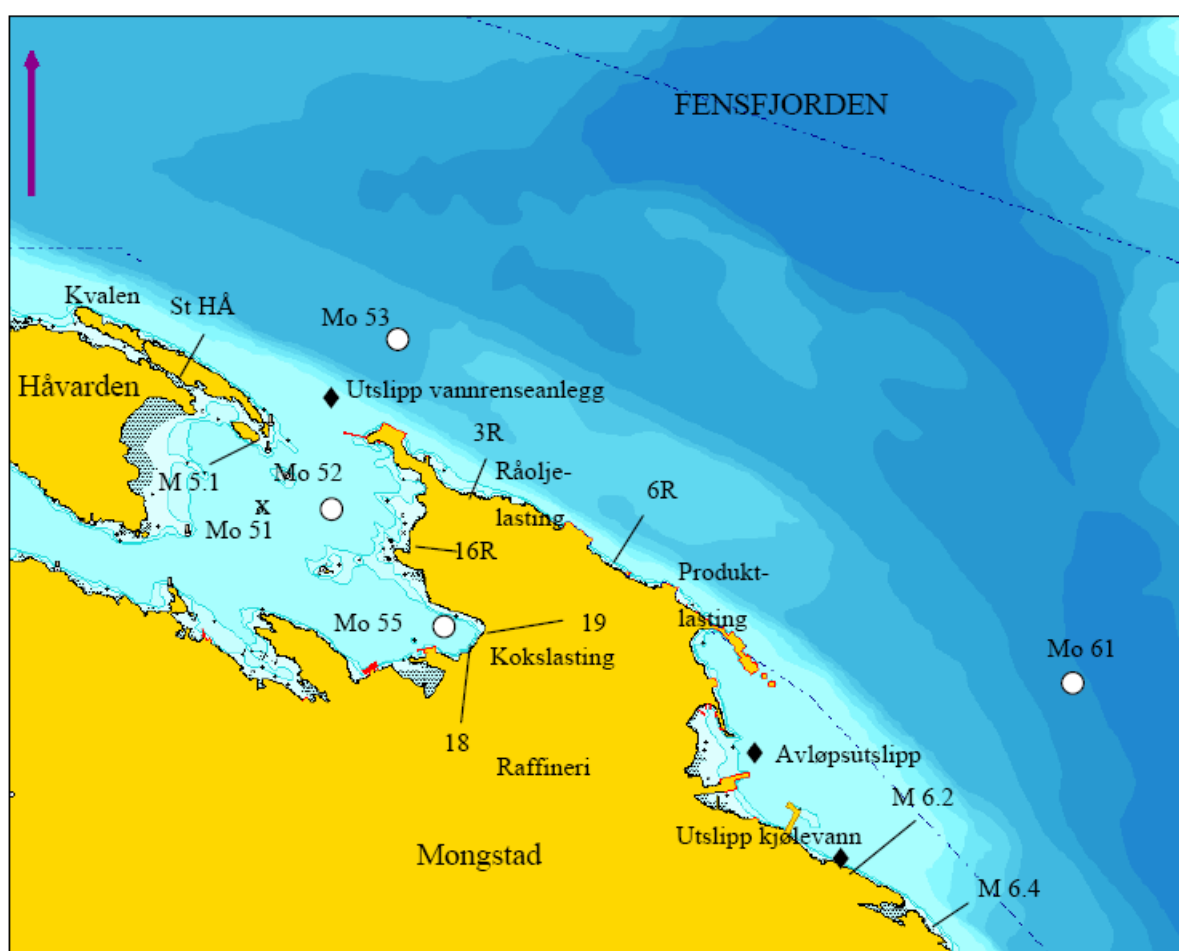
♦ "Utslipp vannrenseanlegg": Discharge point treated waste water
♦ "Avløpsutslipp": Discharge point of cooling water

Species assemblages and species diversity investigations as described for the soft seabed fauna under baseline surveys in 1985 and 1987, have also been conducted in monitoring studies of the soft sediment fauna since 1990 (diversity index, evenness, maximum diversity, species richness and ordination analysis etc.). An overview of the analysis and methods applied is shown in Table 3.

As an example, the species were grouped into geometric classes to detect major environmental changes in the fauna community (Gray and Mirza, 1979; Ugland and Gray, 1982). Cluster analysis was also undertaken to express the stress level of the community (Clarke and Warwick, 1994).

Table 3   An overview of the analysis and methods applied in regular monitoring surveys conducted in the period 1990 to 2006

Year	Surveys	Hydro-grahy	Sediment analysis		Contaminants analysis			Soft seabed fauna	Rocky sea shore fauna
			Grain size	Organic content	Sediment	Water	Biota		
1990-2006 [I]	Regular monitoring surveys	Yes	Yes	Yes	- THC - Hydro-carbons - Metals - PCB	No	- Hydro-carbons and metals in blue mussels - Oil hydrocarbons in egg wrack - PCB in blue mussels	*Abundance:* - No. individuals - No. species *Analysis:* - Distribution of species in geometric classes [III] - Diversity index (H')[IV] - Evenness (J) [V] - Hurlbert index (ES $_{N=100}$) - Hurlbert species richness curve - Cluster analysis [VI] (Bray-Curtis similarity) - Ordination (MDS-analysis [VII]	***Community analysis:*** *In littoral zone:* - Square analysis - Level analysis [II] - No. species - Length measurement of *Patella vulgate* - Vertical zonation - Coverage of groups of species - Coverage of *Pelvetia canaliculata* - Photographic documentation *In sub-littoral zone:* - Square analysis - Photographic documentation - Video documentaion - Transect analysis - Distribution of kelp and sea urchin [VIII] *Analysis*: - Bray-Curtis similarity (cluster analysis) - Ordination (MDS-analysis)

[I] Johannessen et al,1991a; Johannessen et al, 1992a,b; Botnen et al, 1993a; Botnen et al,1994a; Botnen et al,1995; Botnen et al, 1998;. Johansen et al, 2000; Johansen et al, 2003; Johansen et al, 2006.
[II] Level analysis was applied in the seashore surveys until 1992 (Dalby et al, 1978)
[III] Gray and Mirza, 1979; Ugland and Gray, 1982.
[IV] Shannon and Weaver,1949.
[V] Pielou,1966.
[VI] Field et al,1982; Clarke and Green, 1988; Carr, 1991,; Clarke and Warwick, 1994
[VII] Gray et al, 1992;
[VIII] Johannessen and Høisæter, 1986; Johannessen et al, 1992a; Botnen et al,1994a.

For comparison of the fauna between the various stations (and possible fauna gradients) and comparison with results from previous surveys, multivariate analysis (non-metric multidimensional scaling) was conducted (Johannessen and Høiseter, 1986; Gray et al, 1992). The determination of the environmental quality status of the receiving waters was undertaken by comparison of community indices with a environmental quality classification system/guideline prepared the Norwegian Pollution Control Authorities applied to fjords and coastal areas, categorising the environmental status into different "environmental condition classes" shown in Table 4 (Molvær et al, 1997).

Table 4   Criteria for classification of environmental condition or status for organic content in sediments and soft benthic fauna. The values are taken from the Guideline report for "Classification of environmental quality in fjords and coastal waters, Norwegian Pollution Control Authorities (TA-1467/1997) (Molvær et al, 1997)

	Parameters	I "Very good"	II "Good"	III "Moderate"	IV "Poor"	V "Very poor"
Sediment	Organic carbon (mg/g)	<20	20-27	27-34	34-41	>41
Diversity soft benthic fauna	Hurlberts index (ES $_{N=100}$)	>26	26-18	18-11	11-6	<6
	Shannon-Wiener index (H')	>4	4-3	3-2	2-1	<1

CS-3.3.3.3. Hard bottom seashore survey

The distribution of selected dominating species of intertidal plants and organisms at the rocky seashore has also been an important part of the monitoring program at Mongstad since 1990. In the period until the baseline study in 1985, the method "level analysis" was applied in the environmental monitoring program of the seashore (Hartley et al, 1986). This semi-quantitative method was then supported by a new method called "square analysis" that has been applied in monitoring surveys since 1986 (Johannessen and Høisæther, 1986).

The "square analysis" method is applied to a smaller and defined area, and is more precisely investigated compared to the "level analysis" that was applied to a larger area and studied less accurately. Both methods were applied in the seashore surveys for comparison until 1992. From 1992 the "level analysis" was fully replaced by the "square analysis" due to a higher accuracy of this method.

In the baseline survey (1985 and 1987), "square analysis" of the benthic algae and fauna community was undertaken in the littoral zone in three quadrates of 0.25 m^2 (0.5 x 0.5 m) per station divided into 25 sub-quadrates (1985) at the middle or lower level of the brown algae community (Hjolman and Risheim, 1992). The analysis included percentage coverage of benthic algae, lichens and sessile animals. The number of sub-quadrates covered by one species were counted and converted to percentage coverage. If one species covered less than one sub-quadrate its cover was said to be 1%. In the sub-littoral zone two quadrates (of 0.5 m^2) at fixed depths of 1, 5, 9 m and four quadrates at depth of 12 m (of 0.25 m^2) were assessed.

In the period 1991 to 1995 "square analysis" of benthic algae and sessile organism was conducted at the lower level of the littoral zone in 5 permanent quadrates (0.25 m^2) at each station. In the surveys conducted over the period 1997- 2006, "square analysis" was undertaken in 15 permanent quadrates at three different levels in the littoral zone at each station, to be sure that the selected area represented the species composition within that area. Percentage coverage of benthic algae, lichens and sessile animals, number of individuals, number of species distributed between groups of organisms (red-, brown, green- and blue-green algae) were typically registered in the square-analysis at each station.

Two statistical methods were mainly used for the community analysis in the baseline study (1985-1987) and in the regular monitoring surveys from 1990: Multi-Dimensional Scaling (MDS) and cluster analysis as described by Hjolman and

Risheim (1992). Species diversity was measured by applying the Bray Curtis similarity index (Field el al, 1982).

Other measurements typically undertaken in the littoral zone were length measurements of *Patella vulgate* as an indication of presence or absence of all year classes at each location, and vertical zonation. In the sub-littoral zone, distribution of kelp (determination of lower limit) and sea urchin in the sub-littoral zone were studied. Occasionally, photographic documentation (film and videotape records) was also included in both zones.

CS-3.3.3.4. Hydrocarbon analysis in sediments and biota

The surface layer of the sediment have since the baseline study in 1985 and through the regular monitoring surveys, been analysed for oil hydrocarbon concentrations from sampling of soft sediment on an annual basis, at a minimum of 4 stations in the Mongstad area, in the period 1990-1993, similar to those stations sampled and analysed in the soft sediment surveys. Only for two of those stations, (Mo 52 and Mo 53) is hydrocarbon analysis available throughout the period of regular monitoring (1990 to 2006). In addition, station Mo 55 (close to the security basin) and Mo 61 were analysed regularly in surveys in the period from 1997 until 2006.

In the 1985 survey, analysis of the total hydrocarbon concentration (THC) was conducted for most stations, and NPD (naphthalene, phenanthene, dibenzothiophene) analysis was performed for a few stations. Since 1990, the surface layer of the seabed was analysed for NDP and also their alkyl homologues and fluoranthenes plus pyrenes. The aromatic hydrocarbons are more directly related to oil spills as a source than the content of THC in environment. This is especially true for NPD and their alkyl homologues which are not naturally present in sediments. Occasionally, analysis of THC and the normal alkanes (n-C31) of the sediment samples were undertaken. The sediment samples were collected by use of a van Veen grab sampler.

Flame ionization detector (FID) was used for detection of aliphatic hydrocarbons until 1994 and gas chromatography mass spectrometric detection (Hewlett-Packard 5970 MSD mass selective detector) for determination of selected aromatic hydrocarbons in sediment samples as well as mussel tissues. However, improvements in the analytical methods have taken place. In 1992, the amount of internal standard in sediment samples was reduced and more efficient and complete separation of aliphatic and aromatic hydrocarbons took place.

The method for quantification of THC content in the sediments was introduced in 1994 and further optimized in 1995. Modifications in the method of preparation of the aliphatic fraction in the samples and the method of integration of the chromatograms were improved. From 1995 the THC in samples (extracts) was analysed by gas chromatography GC(FID) (HP 5890 Ser) with flame ionization detection (integration of C12-C35).

Quantification of selected aromatic hydrocarbons (PAH/NPD) in both sediment and mussel tissues were from 1995 chromatography on Fisons GC 8060 by mass spectrometric detection with Fisons MD 800 kvadropol. From 2000 accredited methods were used for analysis of PAH/NPD in the sediments and biological tissue; GC/MS gas chromatography (HP-6890) and GC/MS in SIR-mode (selected ion recordings), respectively.

The detection limit of aromatic hydrocarbons in analysis of blue mussels in the baseline survey in 1985 (30 µg/kg for NPD) was higher compared to detection limits in the method applied in later monitoring surveys. The detection limit in sediment samples was 1 µg/kg from 1990. The retention time for determination of oil hydrocarbons in sediment samples was also shorter (30%) in surveys until 2000. This may have resulted in higher levels of the heaviest oil hydrocarbons in the latest surveys (2000-2006).

Until 1997 the analysis of hydrocarbon content in sediments was related to concentrations in wet weight sediment. Due to differences in water content in samples, the method was modified, and the concentrations in sediment were expressed on dry weight basis from 1997.

Chemical analysis of oil hydrocarbons in the blue mussel tissues in the surrounding environment of Mongstad refinery was undertaken on regular basis since the baseline survey in 1985. In the baseline survey, native mussels were analysed for NPD (naphthalene, phenanthrene, dibenzothiophene).

Since 1992, the monitoring program included measurements of oil hydrocarbons in mussel tissues both in native wild-caught mussels and deployed mussels in cages (at locations where native populations of blue mussels were not found). The blue mussels were deployed in cages on the seabed at a minimum of 4 different stations in addition to a reference station (Håvarden) and were exposed in four months up to several years. Only for three of those stations (3R, 6R and 16R) is hydrocarbon analysis available through the period of regular monitoring (1990 to 2006). Three additional stations (M5.1 and reference stations M6.1 and M3.1) were included in regular monitoring surveys in the period from 1995 until 2006 as well as in the follow-up study in 1996 and 2004.

In the "regular monitoring" that commenced in 1990 the mussel tissues were analysed for similar oil hydrocarbons as for the sediment; including naphthalene, phenanthene, dibenzothiophene and their alkyl homologues, and fluoranthenes plus pyrenes. Occasionally, analysis of aliphatic hydrocarbons (n-C31) of tissues samples was undertaken.

In order to evaluate the results of hydrocarbon content in sediments and mussel tissues from the 1997 survey with results from previous surveys, the analytical results were subject to multivariable statistic methods, statistic principal component analysis (PCA) (Kvalheim and Karstang, 1987).

CS-3.3.3.5. Heavy metal analysis in sediment and biota

Since 1990, the surface layer of the sediment has been analysed for metals selected such as Pb, Ni and V at the same four stations annually in the period 1990-1993 as sampled in the soft sediment surveys, (incl. analysis of benthic fauna, sediment analysis of hydrocarbons, organic content and grain-size distribution). In some surveys, the analysis program was extended to include Hg, Zn, Cd, Cr, Cu, Co, As and Fe.

The sediment levels of metals were compared to the upper level for natural background concentrations in sediments in coastal waters (Rygg and Thelin, 1993). The content of heavy metals in sediments around Mongstad was low during this period, in the range of the natural background levels (Table 5) in coastal areas. The results from the regular monitoring surveys confirm this (Johannessen et al, 1991a. Johannessen et al, 1992 a,b; Botnen et al, 1993a; Botnen et al,1994a). In addition

this is supported by the follow-up studies (Johannessen et al, 1991b; Botnen et al, 1991; 1992; 1993b; 1994b). The heavy metal analysis of the sediment was therefore taken out of the regular monitoring program after the survey 1994 (measured at one station – Mo 55) and has since been replaced by metal analysis of biological tissues (blue mussels).

Table 5   Upper levels for natural background content of metals in sediments for marine coastal waters. The values are taken from Norwegian Pollution Control Authorities guideline for fjords and coastal waters No. 93:02 (Rygg and Thelin, 1993)

Metal	Upper level for natural content (µg/g)
Mercury	0,15
Lead	30
Zink	150
Cadmium	0,25
Chromium	70
Nickel	30
Copper	35
Vanadium	150
Cobalt	20
Arsenic	20

Recently the upper levels for natural background concentrations were revised and further extended to include criteria for classification of the environmental condition or status. The new classification system is established for metals, PAHs and some organic substances for sea water and the sediments in fjords and coastal waters, and is dependent of the potential impact on biota. The criteria for classification of environmental condition of metals in marine sediments for fjords and coastal waters are taken from the Norwegian Pollution Control Authorities Guideline report TA-2229/2007) (Bakke et al, 2007). The quality criteria or standards (Table 6) have been established following the international guidance for derivation of quality standards and risk assessment of chemicals in accordance to EU Technical Guidance Document (EC, 2003; Lepper, 2005).

Table 6   Criteria for classification of environmental condition or status of metals in marine sediments (dry weight basis). The values are taken from the Guideline report for fjords and coastal waters, Norwegian Pollution Control Authorities (TA-2229/2007)

Heavy metals (mg/kg)	I "Very good" Background level	II "Good" Non-toxic	III "Moderate" Chronic toxicity from long term exposure	IV "Poor" Toxicity (acute) from short term exposure	V "Very poor" Very high toxicity (acute) from short term exposure
Arsenic	?20	>20-52	>52-190	>190-580	>580
Lead	?30	>30-83	>83-700	>700-2200	>2200
Cadmium	?0.25	>0.25-2.6	>2.6-17	>17-160	>160
Copper	?35	>35-51	>51-120	>120-220	>220
Chromium	?70	>70-560	>560-20000	>20000-59000	>59000
Mercury	?0.15	>0.15-0.62	>0.62-0.85	>0.85-7.2	>7.2
Nickel	?30	>30-43	>43-120	>120-870	>870
Zinc	?150	>150-360	>360-1800	>1800-5100	>5100

Heavy metals (Hg, Pb, Zn. Cd, Cr, Cu, Co, As, V and Ni) were regularly analysed in blue mussel tissues (both native and caged mussels in surveys since 1994, measured at two stations in 1994 and 1995, one close to the refinery area and one station near the security basin (16R and M5.1) plus a reference station at Håvarden. In the regular monitoring surveys in the period from 1997 to 2006 an additional station was included (M6.1).

Metals in sediments and mussel tissue samples were analysed by ICP-MS (Perkin-Elmer Elan 5000) from 1994 (until 1997) and mercury was analysed with hydride AAS cold vapour technique on Perkin Elmer 3300 FIAS. In 1997 As, Cu and Zn in tissue samples were also analysed, with AAS graffiti oven and flame AAS. The method has since been under continuous revision. The method applied for tissue analysis in 2003 was a modification of the method for semi quantitative determination of metals with ICP-MS developed by Julshamn and Brenna (1999).

Until 1996 the analysis of metal content in mussel tissues was related to concentrations in wet weight. From 1996 the metal content was related to dry weight sediment (and in some instances to wet weight) (Botnen et al, 1996; Botnen et al, 1998; Johansen et al, 2000; Johansen et al, 2003 and Johansen et al, 2006).

The tissue levels were compared against criteria for classification of environmental status or condition for metals in blue mussels, shown in Table 7, as prescribed by the Norwegian Pollution Control Authorities guideline for fjords and coastal waters Rygg and Thelin (1993) and later described by Molvær et al (1997).

Table 7   Criteria for classification of environmental condition or status of metals in blue mussels (dry weight basis). The values are taken from Norwegian Pollution Control Authorities guideline for fjords and coastal waters No. 97:03 Molvær et al (1997)

Heavy metals (mg/kg dw)	I "Good"*	II "Less good"	III "Not so good"	IV "Poor"	V "Very poor"
Arsenic	<10	10-30	30-100	100-200	>200
Lead	<5	5-20	20-50	50-100	>100
Fluoride	<15	15-50	50-150	150-300	>300
Cadmium	<2	2-5	5-20	20-40	>40
Copper	<10	10-30	30-100	100-200	>200
Chromium	<3	3-10	10-30	30-60	>60
Mercury	<0.2	0.2-0.5	0.5-1.5	1.5-4	>4
Nickel	<5	5-20	20-50	50-100	>100
Zinc	<200	200-400	400-1000	1000-2500	>2500
Silver	<0.3	0.3-1	1-2	2-5	>5

* Natural content of heavy metals in blue mussels in coastal waters.

CS-3.3.4.   Follow-up monitoring 1989-2004

Follow-up monitoring is also conducted, when the results in the regular monitoring surveys indicate the need for a re-investigation within a short time period. The methods applied in follow-up monitoring surveys are identical to the methods applied in the "regular monitoring" surveys over the same time period, except that these programs tend to be less comprehensive than the regular programs and contained only those selected parts and methods required for the investigation..

In 1998 a post-investigation of the hard bottom sea shore community and blue mussel tissues analysis of aromatic hydrocarbons after an oil spill incidence 1997 at the sea shore in Mongstadvågen (Hjohlman, 1999). Additionally, heavy metal and oil hydrocarbon analysis of mussel tissues in the Mongstad area were conducted in 1996 for identification and follow-up of small oil spills in the harbour area of Mongstad oil terminal (Botnen et al, 1996). Also, follow-up monitoring was undertaken to investigate the influence of a sea water scrubber outlet (deployed in 1989) on the marine environment at Mongstad refinery (Botnen et al, 1993b; Botnen et al, 1994b).

CS-3.4.   SUMMARY

Traditionally, the attention in the monitoring surveys conducted until 1980 was on investigations of the rocky seashore fauna studying the shallow water populations of algae and hard bottom fauna from the littoral zone (down to 30 m). A modification of the methods applied in ecological monitoring surveys took place in the early 1980s. The baseline surveys conducted in 1985 were more comprehensive, including chemical analysis of oil hydrocarbons in sediments and biota as well as sediment analysis (organic carbon and grain size distribution). Hydrographical measurements were also undertaken. The surveys in 1985 and 1987 provided a baseline for future

monitoring of the marine environment and were used in later comparisons to observe faunal changes in this area. In 1990 analysis of sediments for heavy metals was introduced, and from 1994 these analyses were replaced by analysis of biological tissues (blue mussels) for the metals and since then have been undertaken in the "regular" monitoring surveys.

The change in the content monitoring programs applied to the marine receiving environment in the Mongstad area from study of the rocky sea shore fauna to more comprehensive programs covering numerous monitoring analysis and methods, and sampling undertaken in different environmental compartments have been beneficial for the refinery. The current analysis and methods conducted in the "regular monitoring" surveys provide an enhanced possibility for identification and follow-up of different planned discharges and spill incidences, reflecting the different activities at Mongstad refinery. For instance, identification of potential impacts from small accidental crude oil spills at shore, oil spill from ships at the harbour area, discharge from sea water scrubber outlet as well as planned discharge of process water to the marine recipient from the refinery demand for surveys including a combination of different monitoring methods and analysis.

In a post-spill situation an integrated approach is favourable, combining analytical chemistry with benthic community analysis and effect responses as well as analysis of all the possible affected environmental compartments (soft bottom sediment, hard bottom seashore, water column etc.). Selection from a suite of methods/analysis to be deployed dependent of the incidence and the investigations that are to be undertaken gives the refinery flexibility with respect to design of monitoring programs. The regular monitoring may in some cases be replaced and limited to a "follow-up" study one year later if it is found necessary to focus on the results of a specific finding within a short time frame.

Investigation of the hard bottom sea shore community and blue mussel tissues analysis of aromatic hydrocarbons (Botnen et al, 1998) revealed results that identified the oil spill incidence in 1997 at the sea shore in Mongstadvågen. The oil spill incidence was documented and followed up in a post-investigation conducted in 1998 (Hjohlman, 1999). Results from tissue analysis of blue mussels for oil hydrocarbons in the regular monitoring survey in 1995 (Botnen et al, 1995) showed incidences of small oil spills at the harbour area of Mongstad oil terminal and was followed up with similar monitoring methods in 1996 (Botnen et al, 1996). Similarly, a monitoring program was designed including a suite of monitoring methods in order to study the influence of a sea water scrubber outlet on the marine recipient. The sampling program included hydrographical measurements (temperature, salinity, and oxygen), and sediment analysis of grain size distribution, organic content, percentage clay and silt as well as the content of heavy metals and sulphate. Additionally, species diversity of the soft sediment bottom fauna was included in the program (Botnen et al, 1993b).

The introduction of NDP and their alkyl homologues and fluoranthenes plus pyrenes in analysis of oil hydrocarbons in sediments (and biota) in the regular monitoring studies allowed a more direct link to oil spills as a source of contamination instead of the analysis of content of THC. The chemical complexity of crude oil and discharge form refineries are large. However, chemical analysis techniques have been continuously improved and optimized over the past decades and some methods allow quantitative measurements at low level sensitivities.

A challenge with the blue mussel analysis has been to identify a true non-contaminated reference station. The reference station used around Mongstad is

located at Håvarden, and is relatively close to the refinery. Enhanced levels of oil hydrocarbons were measured in tissue samples from the monitoring survey in 2000 and 2003. At this location, native blue mussels are found and monitored, but also collected for deployment in cages at other stations.

Since monitoring surveys started in the Mongstad area in 1972, the concern has mainly been on monitoring of the seashore and the sediment compartment (analysis of contaminants sediment and benthic biota, soft sediment fauna analysis) and with toxicity as the primary end point parameter. In future monitoring programs potential impacts on water column organisms as well as validation of model predictions of exposure and concentrations of effluent constituents possibly will be more addressed. Additionally, validation of model prediction of exposure to non-stressors, such as inorganic nitrogen (ammonia), increase in temperature and change in O_2 level due to biodegradation of chemicals in the receiving water will be evaluated introduced in future environmental monitoring programs.

Appendix 1

Year	Survey	Hydro-graphy	Sediment analysis		Contaminants in			Soft seabed fauna	Rocky sea shore fauna
			Grain size	Organic content	Sediment	Water	Biota		
1972-1979	Baseline study monitoring survey (BP studies)	No	No	No	No	No	No	No	*Community analysis:* [kI] - Vertical distribution and abundance of intertidal species - Zonation of key species - Shell characteristics (*Nucella lapillus*) - Limpet recruitment - Level analysis
1985 and 1987	Baseline study I and II	Yes[aI]	Yes [bI]	Yes [bI]	THC [dI]	THC [eI] and phenols [eI]	Oil hydrocarbons (NPD) in blue mussels [fI]	*Abundance:* [jI] - No. individuals - No. species *Analysis:* [jI] - Distribution of species in geometric classes - Diversity index (H') - Evenness (J) - E (S100) - Hurlbert species richness curve - Cluster analysis (Bray-Curtis similarity) - Ordination (MDS-analysis)	*Community analysis* *Littoral zone:* [kII] - Square analysis - No. species - Photographic documentation - Wave exposure index - Vertical zonation *In sub-littoral zone:* [kII] - Square analysis - Photographic documentation - Transect analysis - Wave exposure index - No. species *Analysis:* [kII] - Cluster analysis (Bray-Curtis similarity) - Ordination (MDS-analysis) - Diversity indes (Shannon-Wiener) - Evenness (J) - H'max *Censuses of seabirds*
1990-2006	Monitoring survey (regular)	Yes [aII/aIII]	Yes [bII-IV]	Yes [bII-IV]	-THC [dV-VII] -Oil hydrocarbons [dII-VII] -Metals [cI-VI] -PCB [iI]	No	-Hydrocarbons [fII-V] and metals [gI-III] in blue mussels -Oil hydrocarbons in egg wrack [hI] -PCB in blue mussels [iII]	*Abundance:* [jII-VII] - No. individuals - No. species *Analysis:* [jII-VII] - Distribution of species in geometric classes - Diversity index (H') - Evenness (J) - Hurlbert index (EX N=100) - Hurlbert species richness curve - Cluster analysis (Bray-Curtis similarity) - Ordination (MDS-analysis)	*Community analysis* *In littoral zone:* - Square analysis [kIII-XI] - Level analysis [kIII-V] - No. species [kIII-XI] - Length measurement (*patella vulgata*) [kIV-V] - Vertical zonation [kIII-VII] - Coverage of groups of species [kIX-XI] - Coverage of *Pelvetia canaliculata* [kIV-XI] - Photographic documentation [kIX,kXII] *In sub-littoral zone:* [kIV] - Square analysis - Photographic documentation - Video documentation - Transect analysis - Distribution of kelp and sea urchin *Analysis:* [kIII-XI] - Bray-Curtis similarity (Cluster analysis) - Ordination (MDS-analysis)
1989-2004**	Monitoring studies (comple-mentary)	Yes [aIV]	Yes [bV]	Yes [bV]	-Metals [cV]	No	-Oil Hydrocarbons [fVI-VII] and metals [gIV] in blue mussels	*Abundance:* [jII-VII] - No. individuals - No. species *Analysis:* [jII-VII] - Distribution of species in geometric classes - Diversity index (H') - Evenness (J) - H'max - Cluster analysis (Bray-Curtis similarity) - Ordination (MDS-analysis)	*Community analysis* *Littoral zone:* [kXII-kXIII] - Square analysis - No. species - Coverage of sauetang (*Pelvetia canaliculata*) - Photographic documentation *Analysis:* [kXII-kXIII] - Cluster analysis (Bray-Curtis similarity) - Ordination (MDS-analysis)

*) **Regular monitoring surveys 1990-2006:** 1990, 1991, 1992, 1993, 1994, 1995, 1997, 2000, 2003 and 2006
) **Follow-up monitoring studies 1989-2004: 1989, 1990, 1991, 1993, 1994, 1996, 1997, 1998 and 2004.

a) **Hydrography analysis (temperature, salinity and oxygen level):**
I   Baseline survey 1985 and 1987 at four stations (surface and at 5 m intervals down to 40 m) in Fensfjorden: W1 (Mo 51), W2 (Mo 62), W3 and W4.
II   Surveys 1990, 1992, 1993, 1994, 1995, 1997, 2000, 2003 and 2006: at one permanent station in Fensfjorden: Mo 61.
III   Surveys 1991: at the permanent station (Mo 61) plus 2 additional stations: Mo 51 (at Håvarden) and Mo 41 (at Fonnes).
IV   Study 1989, 1993 and 1994: at one station (Vif 3).

b) **Sediment analysis (sediment grain size distribution and organic content):**
I   Baseline survey 1985 and 1987: in 8 areas including 16 stations (Mo 11, Mo 12, Mo 21, Mo 22, Mo 23, Mo 31, Mo 32, Mo 41, Mo 42, Mo 51, Mo 52, Mo 61, Mo 62, Mo 71, Mo 72, Mo 81, Mo 82).
II   Survey 1990, 1992 and 1993: performed at 4 stations: Mo 51 and Mo 52 (at Håvarden), Mo 53 and Mo 61(Fensfjorden).
III   Survey 1991: at 4 stations (Mo 51, Mo 52, Mo 53 and Mo 61) as 1990 plus 3 stations included in the baseline survey: Mo 31 (Sandøy), Mo 41(Fonnes) and Mo 62 (Fensfjorden) plus + 5 stations in shallow water in Håvardsviken (Y4, Z1,Ø2, Ø4 and Å1).
IV   Survey 1994, 1995, 1997, 2000, 2003 and 2006: at 4 stations: Mo 52, Mo 53, Mo 61 and station Mo 55 close to the security basin (Mo 55) replacing Mo 51 from 1990-93.
V   Study 1989-1994 (1989, 1990, 1991, 1993 and 1994): at three stations (Vif 1, Vif 2 and Vif 3).

c) **Sediment analysis of heavy metals contents**
I   Survey 1990: Hg, Pb, Zn. Cd, Cr, Cu, Fe, Ni and V at 4 stations (Mo 51, Mo 52, Mo 53 and Mo 61).
II   Survey 1991: Pb, Ni and V: 4 stations (as for 1990) plus 3 additional stations (Mo 31, Mo 41 and Mo 62).
III   Survey 1992 and 1993: Pb, Ni and V: 4 stations (as for 1990).
IV   Survey 1994: Hg, Pb, Zn. Cd, Cr, Cu, Fe, Ni, V, Co, As, Mo at station Mo 55 (close to the security basin).
V   Study 1990-1994 (1990, 1991, 1993 and 1994): Hg, Pb, Zn. Cd, Cr, Cu, Fe, Ni, V, Co, As at three stations (Vif 1, Vif 2 and Vif 3).

d) **Sediment analysis of oil hydrocarbons (naphthalene, phenanthene, dibenzothiophene and their alkyl homologues, and fluoranthenes+pyrens)**
I   Baseline survey 1985 and 1987: THC analysed at 16 stations. Two of the samples were subjected to NPD analysis (with lower detection limit).
II   Surveys 1990, 1992: at 4 stations (Mo 51, Mo 52, Mo 53 and Mo 61).
III   Surveys 1991: at 4 stations (as for 1990) plus 3 additional stations (Mo 31, Mo 41 and Mo 62).
IV   Surveys 1993: at 4 stations (Mo 51, Mo 52, Mo 53 and Mo 61).
V   Survey 1994: at 3 stations (Mo 52, Mo 53 and Mo 55). Additional analysis of THC and normal alkanes (n-C31).
VI   Survey 1995: at 3 stations (Mo 52, Mo 53 and Mo 55). Additional analysis of THC.
VII   Survey 1997, 2000, 2003 and 2006: at 4 stations (Mo 52, Mo 53, Mo 55 and Mo 61- as 1990). Additional analysis of THC.

e) Water analysis (oil hydrocarbons and phenols)

I   Baseline survey 1985 and 1987: THC and phenols analysed at several depths down to 40 m at four stations.

f) Oil hydrocarbons in blue mussels (naphthalene, phenanthene, dibenzothiophene and their alkyl homologues, and fluoranthenes+pyrens):

I   Baseline survey 1985: Native blue mussels collected from 3 different locations analysed for NPD (naphthalene, phenanthene, dibenzothiophene) only.

II   Survey 1990-91: Native blue mussels collected at Håvarden.

III Survey 1992: Both native (at Håvarden) and deployed in cages in the refinery area (hard bottom station 3R, 6R, 8R, and 16R). Additional analysis of normal aliphatic hydrocarbons (n-C31).

IV   Survey 1994: Both native (at Håvarden) and deployed in cages at the refinery area (hard bottom station 3R, 6R, M5.1, M6.1 and 16R).

V   Survey 1995, 1996, 1997, 2000, 2003 and 2006: Both native (at Håvarden) and deployed in cages at the refinery area (hard bottom station 3R, 6R, M5.1, M6.1, M3.1 and 16R).

VI   Study 1996 and 2004: Both native (at Håvarden) and deployed in cages at the refinery area (hard bottom station 3R, 6R, M5.1, M6.1, M3.1 and 16R).

VII Study 1998: Seashore survey after an oil spill in Mongstadvågen in 1997. Both native (reference station at Håvarden) and deployed mussels in cages (station 16R).

g) Metals in blue mussels

I   Survey 1994: deployed in cages at 2 stations in the refinery area close to the security basin (16R and M5.1): Hg, Pb, Zn. Cd, Cr, Cu, Ni, V, Co, As.

II   Survey 1995: Both native (Håvarden) and deployed in cages at 2 stations in the refinery area close to the security basin (16R and M5.1): Hg, Pb, Zn. Cd, Cr, Cu, Ni, V, Co, As.

III Survey 1997, 2000, 2003 and 2006: Both native (reference station Håvarden) and deployed in cages at the refinery area (hard bottom station 6R, M6.1and 16R): Hg, Pb, Zn. Cd, Cr, Cu, Ni, V, Co, As. In 2006 was station M6.1 lost and was replaced by station M6.4.

IV   Study 1996: Both native (Håvarden) and deployed in cages at the refinery area (hard bottom station M5.1, M6.1and 16R): Hg, Pb, Zn. Cd, Cr, Cu, Ni, Co, As.

h) Oil hydrocarbons in egg wrack (naphthalene, phenanthene, dibenzothiophene and their alkyl homologues, and fluoranthenes+pyrens):

I   Survey 1994 and 1995: station, M5.1, M6.1, M3.1 and 16R.

i) PCB in sediment and blue mussels:

I   Survey 1994: Sum PCB in the sediment at one station close to the security basin (Mo 55).

II   Survey 1994: Sum PCB in blue mussels deployed in cages at 2 stations in the refinery area close to the security basin (16R and M5.1).

j) Soft sediment seabed fauna analysis (abundance): number of individuals and species, diversity index (H`), evenness (J), H'max, cluster analysis (Bray Curtis) and ordination (MDS-analysis):

I   Baseline survey 1985 and 1987: 16 stations (M1.1, M1.2, M2.1, M2.2, M3.1, M3.2, M4.1, M4.2, M5.1, M5.2, M6.1, M6.2, M7.1, M7.2, M8.1 and M8.2).

II   Surveys 1990, 1992 and 1993: at 4 stations: (Mo 51, Mo 52, Mo 53 and Mo 61). Hurlbert index (ES N=100) was also included in the 1993 survey.

III Survey 1991: (littoral and bottom community) at 12 stations including 4 stations (Mo 51, Mo 52, Mo 53 and Mo 61) plus 3 stations included in the baseline survey: (Mo 31, Mo 41 and Mo 62) + 5 stations in shallow water in Håvardsviken (Y4, Z1, Ø2, Ø4 and Å1).

IV   Survey 1994: at 3 stations: Mo 52, Mo 53 and Mo 55. Hurlbert index (ES N=100).

V Survey 1995: at 3 stations: Mo 52, Mo 53 and Mo 55. Hurlbert index (ES N=100) and distribution of species in geometric classes also included. MDS-analysis not included.

VI Survey 1997, 2000, 2003 and 2006: at 4 stations (Mo 52, Mo 53, Mo 55 and Mo 61). Distribution of species in geometric classes also included. MDS-analysis not included.

VII Study 1989-1994 (1989, 1990, 1991, 1993 and 1994): at three stations (Vif 1, Vif 2 and Vif 3). Distribution of species in geometric classes also included.

k) Rocky (hard bottom) sea shore fauna:

I Survey 1971-79: Vertical distribution and abundance of intertidal species, zonation of key species, shell characteristics (Nucella lapillus) and limpet recruitment.

II Baseline survey 1985 and 1987: Sublittoral zones separated in 16 monitoring stations: M1.1, M1.2, M2.1, M2.2, M3.1, M3.2, M4.1, M4.2, M5.1, M5.2, M6.1, M6.2, M7.1, M7.2, M8.1 and M8.2 and 8 reference sites.

III Survey 1990: at four stations 3, 6R, 8R, 10R (BP stations) at the terminal area and station 17 (new station) at Dyrøy (littoral zone). Two methods applied: Level analysis (Dalby et al., 1978, Hartley et al., 1986) and square analysis also used in the baseline study in 1985 (Johannessen and Høisæter, 1986).

IV Survey 1991: 11 stations including BP-stations (3R, 6R, 8R, 10R, 16R and reference station at Lerøy) and stations from the 1985 baseline survey (M3.1, M4.1, M5.1, M6.1) and station 17R established in 1990. Station 3R and 17R is identical to station 3 and 17 from 1990. In 1991 both littoral and sub-littoral zone were investigated.

V Survey 1992: stations as for 1991. Include only survey in the littoral zone.

VI Survey 1994: stations both in the littoral and sub-littoral zone: 3R, 6R, 16R/17R, M5.1, M3.1 (ref.station), M6.1 (ref.station). Station 3R, 6R and 16R is located in the refinery area.

VII Survey 1995: stations in the littoral zone: 3R, 6R, 16R, M5.1, M3.1 (ref.station), M6.1 (ref.station).

VIII Survey 1997: stations in the littoral zone: 3R, 6R, 16R, M5.1, M3.1 (ref.station), M6.1 (ref.station).

IX Survey 2000: stations in the littoral zone: 3R, 6R, 16R, 19 (new station in the refinery area), M5.1 and M6.1 (ref.station).

X Survey 2002: stations in the littoral zone: 3R, 6R, 16R, 19, M5.1 and M6.2 replacing the ref. station M6.1 from previous surveys.

XI Survey 2003 and 2006: stations in the littoral zone: 3R, 6R, 16R, 19, M5.1 and M6.2.

XII Study 1998: Seashore survey in 1997 after an oil spill in Mongstadvågen including station 16R and two new stations (18 and 19).

XIII Study 1991-1997 (1991, 1992, 1994, 1995 and 1997): Egg wrack monitored at 4 stations (16R, M3.1, M5.1 and M6.1).

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CS-4. CASE STUDY 4: A STUDY ON THE ECOTOXICITY OF MOL DANUBE REFINERY EFFLUENTS

CS-4.1. SUMMARY

A study was undertaken to assess the plant streams and the effluents of the MOL Danube refinery. The approaches described have potential for identifying the key streams that contribute to toxicity in a refinery effluent. While effluents with high COD from the residual oil processing (delayed coker, bitumen plant) and distillation were the most toxic samples, refinery wastewater treatment resulted in significant toxicity mitigation mainly due to the dissolved organic removal and natural attenuation in reservoirs. The test organisms used in short term toxicity tests had different sensitivity to the streams, with microorganisms and daphnids affected to a greater degree than zebra-fish. Furthermore, strong relationships were observed between COD and bacterial luminescence inhibition as well as COD and alga growth inhibition. The correlation of chemical parameters and ecotoxicity data obtained from tests using bacteria and algae is higher than that of fish acute toxicity test. However, the correlation of phenols and sulphide content and ecotoxicity data is poor, but joint ecotoxicity developed by individual contaminants can be predicted by group parameters (e.g. COD, DOC).

In this case study, the toxicity has also been expressed as Toxic Units (TU). Normally this would be calculated by dividing 100 by the percent of the effluent causing 50% toxicity. However, in the tables below, the units are expressed as *ml/L* in which case the TU is calculated as 1000 divided the amount in *ml* causing 50% toxicity.

CS-4.2. THE WASTEWATER TREATING SYSTEM OF MOL DANUBE REFINERY (PRE-2004)

Approximately 2000 m^3/hr of waste water was treated by the refinery, of which 80 m^3/hr with a high chemical concentration was biologically treated. The remainder was sent to a non-biological treatment plant as described below. The two effluents were then separately discharged to the river Danube.

CS-4.3. THE BIOLOGICAL WASTEWATER TREATMENT PLANT

The refinery wastewater treating system is designed to annually remove from 1200 to 1800 t COD equivalent, 20 to 80 t phenol and 30 to 60 t sulphide. The plant receives the streams as shown in Figure 1.

Figure 1   Biological wastewater treatment plant
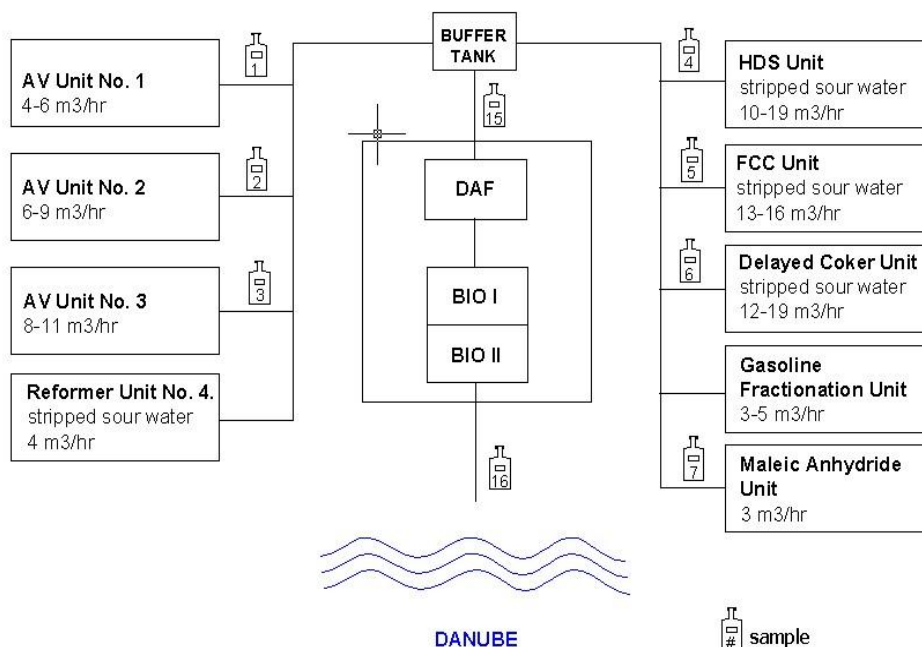
CS-4.4.   NON BIOLOGICAL TREATMENT PLANTS

CS-4.4.1.   Upper Plant Wastewater Treating Plant

Part of the process wastewater streams, containing hydrocarbons, is routed via four sewage systems to the so called Upper Wastewater Treating Plant see Figure 2. Downstream of the sand trap the wastewater, flows in both cases, into two longitudinal flow API settling basins. The wastewaters cleaned in the first mechanical stage are fed via a combination unit into a common collection channel to the Lower Water treatment Plant, where these are further subjected to the second mechanical cleaning stage before being discharged to River Danube.

CS-4.4.2.   The Lower Plant Wastewater Treating Plant

The Lower Treating Plant performs the second stage mechanical cleaning for the oil contaminated plant wastewater generated in the refinery process units, and for the oily precipitation water. During normal operation, the wastewater from the safety divider, flows via the inlet units to the two settling ponds, with 100,000 m^3 capacity each, connected in series. The water discharged from the ponds, flows to the collection unit through a controlled weir. The treated water is discharged to River Danube through a sampling pit, where it is possible to take average samples (based on flow and time) or at specified frequencies.

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Figure 2 Non-biological wastewater treatment plant

DANUBE
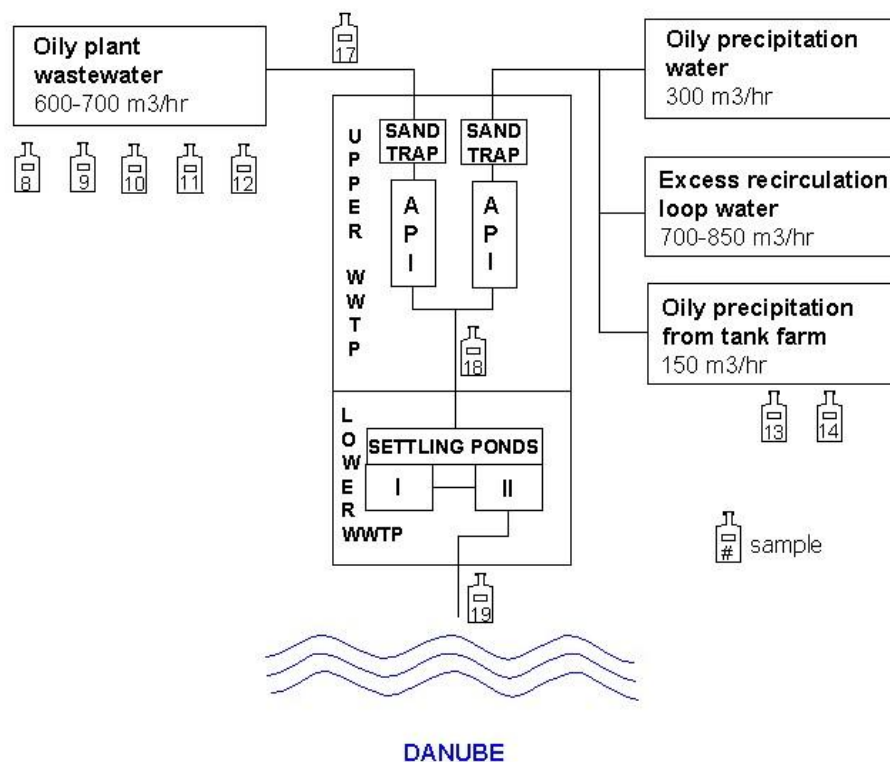
CS-4.4.3. Methods

Aquatic toxicity tests undertaken in this study are outlined in Table 1

Table 1a Aquatic toxicity tests used to address MOL streams and effluents

Test (organism)	Protocol	Testing period	Endpoint	Results expressed as	
Bacterium inhibition *(Vibrio fischeri)*	**ISO 11348-3:1998**	**15 min.**	**luminescence inhibition**	**EC50**	**Toxicity unit (TU)**
Green alga growth inhibition *(Selenastrum capricornutum)*	**OECD 201**	**72 h**	**growth rate**	**EC50**	**TU**
Daphnid acute immobilisation *(Daphnia magna)*	**OECD 202**	**48 h**	**Lethality**	**LC50**	
Fish, acute toxicity *(Brachydanio rerio)*	**OECD 203**	**96 h**	**Lethality**	**LC50**	**TU**

Chemical analyses
Chemical oxygen demand (COD)
Oil and Grease
Phenol
S^{2-}

Analyses were made using spectrophotometric cuvette tests (Kathalin Nohse) with the exception of oil and grease determination (FT-IR).

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CS-4.4.4. Results

Table 1b   Acute toxicity of MOL Danube Refinery final effluent from non-biological treatment plant

Testing period: 03/2000 – 09/2000

Test (organism)	Number of test (n)	Toxicity unit (TU)	
		mean	range
Bacterium inhibition *(Vibrio fischeri)*	**46**	**16.4**	**<1–23.8**
Green alga growth inhibition *(Selenastrum capricornutum)*	**6**	**<1**	**0.4 (<1)– 2.0**
Daphnid acute immobilisation *(Daphnia magna)*	*8*	**132**	**20–200**
Fish, acute toxicity *(Brachydanio rerio)*	**10**	**<1**	**<1–10**

Table 2   Chemical characteristics of MOL Danube Refinery wastewaters sampled in different units

Sampling period: 03/2000 – 09/2000

No.	Sampling point	COD mg·L^{-1}	BOD$_5$ mg·L^{-1}	BOD/COD ratio	TPH (IR) mg·L^{-1}	Phenols mg·L^{-1}	S^{2-} mg·L^{-1}
1	AV-1 distiller,	1302	822	0.63	95	15.5	1.1
2	AV-2 coalescer reflux	267-500	207-228	0.46-0.78	20-58	0.004-0.11	2.8-17.1
3	AV-3	295-602	100-162	0.27-0.34	114-149	5.5	0.8-1.2
14	HDS stripped sour water	84-112	18-54	0.21-0.48	0.2-1.4	2.2-3.7	0.2-0.46
5	FCC stripped sour water	389-517	235-317	0.60-0.61	2-37	1.4 -80	0.06-0.18
6	Delayed coker stripped sour water	1345-1410	472-617	0.35-0.44	16-81	166-214	0.5-6.5
7	Maleic anhydride and fumaric acid producing, drum washing	12500-16430	5230-6745	0.41-0.42	125-212	0.3-0.8	–
8	AV-2 desalination	447-2340	270-418	0.18-0.60	42-165	0.07-0.78	6.2-9.5
9	FCC pump washing, run off	49-59	35-45	0.71-0.76	13-19	0.01-0.06	0.03-0.04
10	Bitumen blowing	1260-2388	650-1124	0.47-0.52	159-460	1.10-4.5	0.23-0.64
11	Xylene isomerization	161-360	113-250	0.69-0.70	3 -9	0.01-0.68	0.20
12	Reformer-2	1017-1420	614-692	0.49-0.60	7-524	0.03-0.30	0.7-29.2
13	Sloptank dewatering	970-1420	-	-	98-428	0.02-0.28	3.9-5.33
14	Reformer-4, tank dewat.	4990-5485	912-1964	0.18-0.36	402-979	8.6-14.6	–

Table 2b   Toxicity of MOL Danube Refinery wastewaters sampled in different units

No.	Sampling point	Average flow	Bacterial enzyme lum. inhibition		Alga growth inhibition		Fish acute toxicity	
			EC50 ml·L⁻¹	Toxicity units	E,C50 ml·L⁻¹	Toxicity units	LC50 ml·L⁻¹	Toxicity units
		m³/hr						
1	AV-1	4-6	24.5-40	25-41	167	6	–	-
2	AV-2 coalescer reflux	6-9	11.5-22.5	44-87	65-243	4.1-15.4	120-180	5.6-8.3
3	AV-3	8-11	82-145	6.9-12.2	306	3.3	445-720	1.4-2.2
4	HDS stripped sour water	10-19	32-77	13-31	166-205	4.9-6	100-180	5.6-10
5	FCC stripped sour water	13-16	12.5-32	31-80	113-188	5.3-8.8	400-550	1.8-2.5
6	Delayed coker stripped sour water	12-19	12.2-21	48-82	30-39	26-33	80-96	10.4-12.5
7	Maleic anhydride and fumaric acid producing, drum washing	3	0.6-2.2	455-1667	2.8	357	5.4-12.5	80-185
8	AV-2 desalination	3-5	12.5-16.5	61-80	15.5-19.5	51-65	50-80	12.5-20
9	Sloptank dewatering	3-5	10-14.5	69-100	12.5-22	45-80	200-250	4-5
10	Tank dewat., Reformer-4	1-2	1.2-1.8	556-833	2.3	435	24-86	11.6-42
11	Xylene isomerization	1-1.5	545-730	1.4-1.9	319-408	2.5-3.1	350-480	2.1-2.9
12	Reformer-2	2.5-4	625-895	1.1-1.6	307-505	2-3.3	620-780	1.3-1.6
13	FCC pump washing, run off	2-3	585-620	1.6-1.7	254-357	2.8-3.9	>1000	<1
14	Bitumen blowing	15-25	13-20	50-77	68-104.5	9.6-14.7	120-250	4-8.3

Green highlight represents biological treatment

Table 3a   Chemical characteristics of MOL Danube Refinery wastewaters sampled at different points of the two WWTP

Sampling period: 03/2000 – 09/2000

Sampling point	COD mg·L^{-1}	BOD$_5$ mg·L^{-1}	BOD/COD ratio	TPH (IR) mg·L^{-1}	Phenols mg·L^{-1}	S^{2-} mg·L^{-1}
Biological Wastewater Treatment Plant (80 m^3·hr-1)						
No. 15 Inlet before DAF	2150-3210	1340-2100	0.62-0.65	65-127	15.6-44.0	0.51-10.3
No. 16 Treated effluent	55-148	20-94	0.36-0.63	0.1-2.8	0.003	0.005-0.03
Non biological Treatment Plant (1750-2000 m^3·hr^{-1})						
Upper WWTP						
No. 17 Oily process water inlet	320-440*	214-296*	0.67-0.69	165-230	0.8-17.1	0.1-1.0
No. 18 Oil separation (API) effluent	258-289	205-263	0.79-0.80	34-55	0.005-0.010	0.30-0.40
Lower WWTP						
No. 19 Settling ponds effluent discharged to Danube	103-155	30-107	0.29-0.69	8.0-10.1	0.01-0.28	0.22-1.20

*samples not shaken

Table 3b   Toxicity of MOL Danube Refinery wastewaters sampled at different points of the two WWTP

Sampling point	Bacterial enzyme lum. inhibition		Alga growth inhibition		Fish acute toxicity	
	EC50 ml·L^{-1}	Toxicity units	E$_r$C50 ml·L^{-1}	Toxicity units	LC50 ml·L^{-1}	Toxicity units
Biological Wastewater treatment Plant (80 m^3·hr-1)						
No. 15 Inlet before DAF	**30-75**	**13.3-33**	**50-120**	**8.3-20**	**68-125**	**8-14.7**
No. 16 Treated effluent	**350-500**	**2-2.9**	**540-755**	**1.3-1.9**	**>1000**	**<1**
Non biological Treatment Plant (1750-2000 m^3·hr^{-1})						
Upper WWTP						
No. 17 Oily process water inlet						
No. 18 Oil separation (API) effluent	**80-125**	**8-12.5**	**100-500**	**2-10**	**125-200**	**5-8**
Lower WWTP	**100-167**	**6-10**	**300-333**	**3-3.3**	**500-900**	**1.1-2**
No. 19 Settling ponds effluent discharge	**250-415**	**2.4-4**	**500-1000**	**1-2**	**>1000**	**<1**

Data evaluation of chemical and acute toxicological testing of MOL Danube Refinery process wastewaters from different units and points of the two WWTP

Table 4   Regression coefficients showing chemical characteristics and acute toxicity relationship

Parameters	Bacterial enzyme lum. inhibition	Alga growth inhibition	Fish acute toxicity
COD	0.779	0.817	0.402
TPH	0.539	0.521	0.224
Phenols	0.234	0.021	0.080
Sulphide	0.134	0.318	0.002

COD and alga growth inhibition

Oil an grease content and alga growth inhibition

COD and bacterial luminescence inhibition

Phenol content and bacterial luminescence inhibition
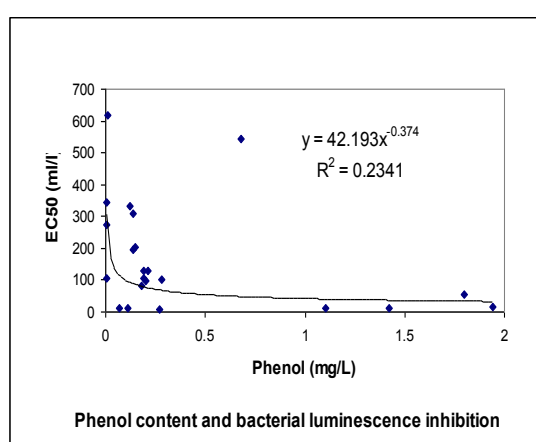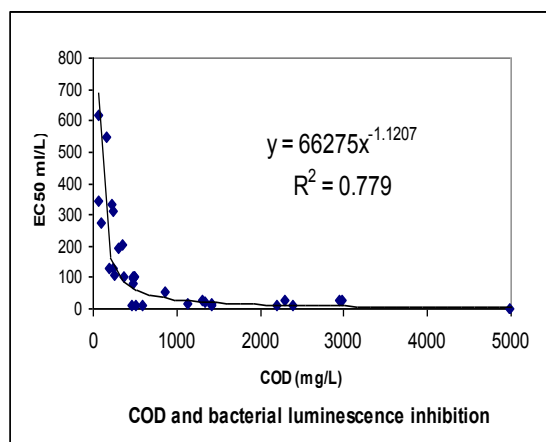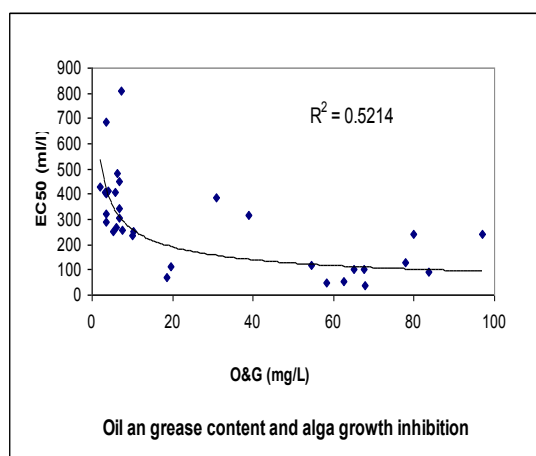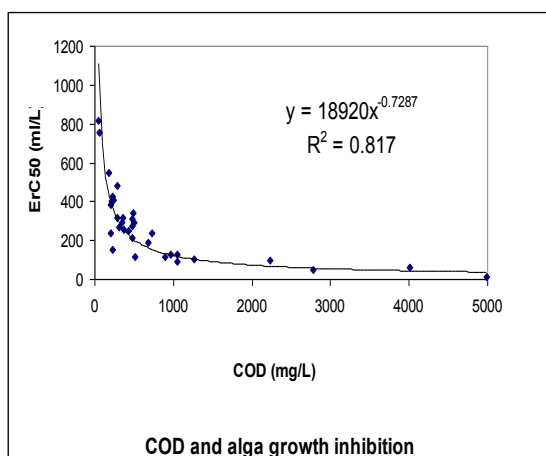
CS-5. CASE STUDY 5: PREDICTING THE EFFECT OF REFINERY EFFLUENTS

CS-5.1. SUMMARY AND KEY LEARNINGS

A tiered environmental assessment of facility effluents in Europe is described. At the first tier of this analysis, the extent of secondary treatment and effluent dilution is determined. This analysis identifies the facilities that exhibit the highest exposure potential for further investigation. In the second tier, SPME techniques are applied to those sites of highest priority. In addition, the described study characterized additional parameters (pH, conductivity, ammonia, metals).

The study highlighted two key learnings;

- The design of the sampling regime must be set up to take into account the potential for variability both at the effluent and at the measurement stages.

- There is a need to be very careful
  - in sampling effluents
  - about the origin and cleanliness of sampling systems

CS-5.2. INTRODUCTION

This case study is based on the experiences in ExxonMobil over a three year period, 2001 - 2004, during which the European refineries and petrochemical plants were investigated. The programme was aimed at establishing practical application of SPME biomimetic extraction techniques for environmental assessment of complex hydrocarbon mixtures. The results were published by Leslie et al (2005).

The study was conducted over three phases. In the first, information on volume of effluent, dilution factor and treatment facilities were assessed. In the second phase, the facilities that had been identified in the first phase were investigated, and the data examined. This phase is further discussed below. Finally there was a third phase, in which effluents that had been identified as being of concern in phase 2, were re-examined to identify to what extent the values obtained in the second phase were consistent, plus further facilities were also added to extend the database and ensure that the initial assessment of them being of low priority was supported by the test data.

CS-5.2.1. Phase 1

In the first phase, effluents were assessed on the basis of a number of important characteristics;

- Volume of the discharge – high volume discharges (>1000 m^3/day) were considered to be of a higher priority than lower volume. No specific values were used, but this allowed for an initial ranking of the effluents.

- Dilution factor – the dilution factor, taken as a simple ratio of the average flow of the effluent to the average flow of the receiving water, was then used with the volume data (above). These two parameters enable a quick assessment of those effluents that;

  - had the potential for immediate impact (low dilution e.g. less than 10)

- had the potential for longer-term impact (high volume and dilutions of less than 100)
- were of lower priority (high dilutions, e.g. greater than 1000)
- were of very low priority (low volume and high dilutions)

- Treatment – the level or type of treatment that a site had was also factored into this to enable the generation of a final list of candidates for the next stage of assessment.

CS-5.2.2. Phase 2

In the second phase, a number of chemical analytical techniques were used to assess the effluents.

- The determination of potential bioaccumulatable organic compounds (Cfiber concentrations) in effluents from facilities using solid-phase microextraction (SPME)

- Metals (total) - Cu, Cd, Hg, Pb, Zn, Co, Cr, Ni, V

- Free-ammonia, pH and conductivity

The latter two groups of determinants, as well as helping to characterise effluents, are also useful in explaining unexpected (or excess) toxicity when compared with that predicted from the SPME information. In both phase 2 and 3, samples were collected in two sampling periods and sent to the laboratory for assessment.

Industrial effluents were assessed using solid-phase microextraction (SPME) fibers (Leslie et al, 2002, 2005; Parkerton et al, 2000). Extracted organic chemicals were measured by GC-FID. Per effluent, sum parameters for bioavailable, non-ionic organic chemicals were determined. Levels measured ranged from 4 to 31 mmol/l polymer phase of the SPME fiber. In all cases, no lethal effects on organisms due to narcotic toxicity were expected. When taking effluent dilution factors into account, the risk of narcotic toxicity is lower still. Low method selectivity meant a large diversity of organic compounds were accounted for, making this method well-suited for screening purposes in WEA (whole effluent assessment). The ability of the method to differentiate between levels in different effluents is useful in effluent prioritization in WEA. The method can be applied to address two concerns of the European regulators – bioaccumulation and toxicity.

Solid-phase microextraction (SPME) is used in this study to (1) screen the toxicity potential of non-ionic chemicals in site-specific effluent samples that could collectively contribute to narcotic effects in exposed organisms and (2) gain insights into the identity of predominant chemicals in samples.

CS-5.2.3. Phase 3

In phase 3, further sites not previously assessed, due to the prioritization process and sites identified in Phase 2 as needing further characterization of their effluents, were assessed. The programme was the same as in phase 2, except that effluents on which SPME extraction was conducted, could potentially be assessed using GC-MS to begin a process of source identification (see Figure1).

Figure 1   Strategy for addressing refinery effluents
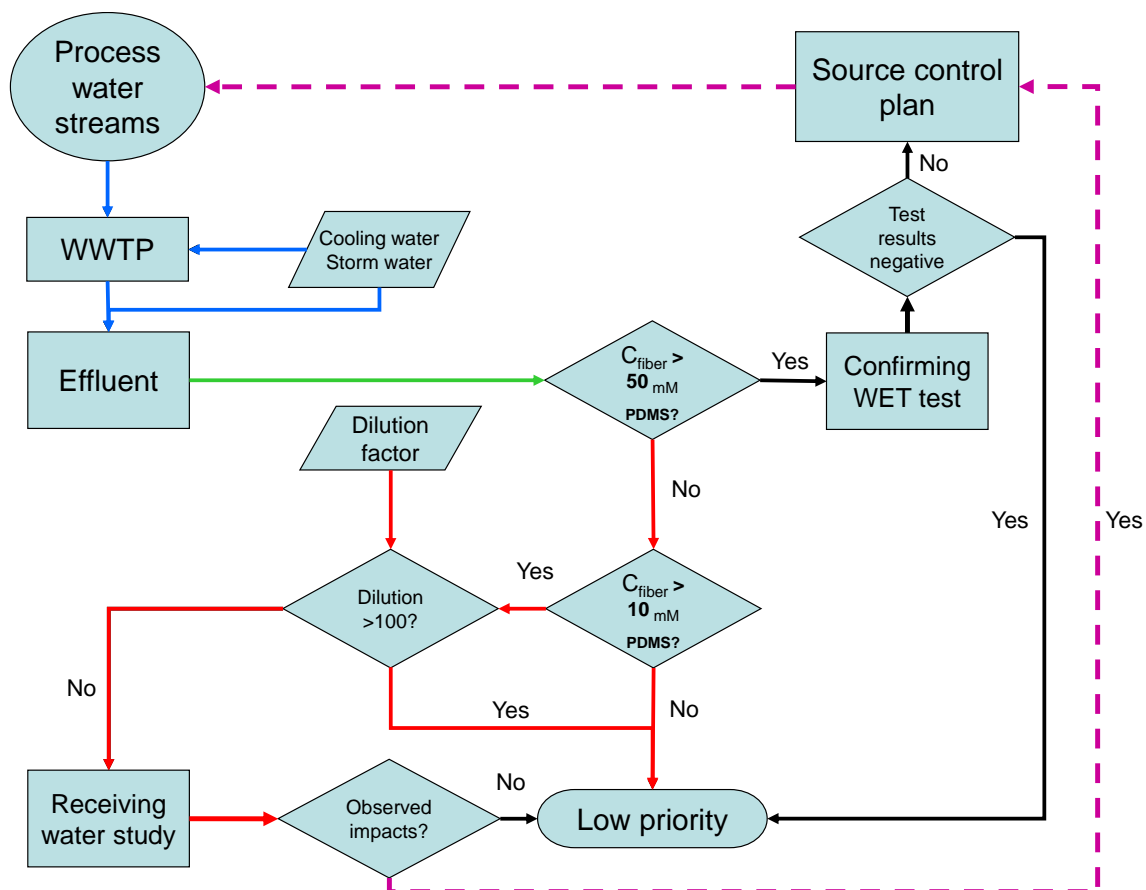
CS-5.3.   METHODOLOGY

CS-5.3.1.   Sample collection and storage

During each phase, spot samples of the effluents were collected on two separate occasions. Pre-cleaned 1-litre bottles that contained silver nitrate (1 mg per bottle) for conservation were filled to the top, without an air gap, and closed with a stopper with PTFE inlay. As the water was conserved with silver nitrate no other conservation actions were taken. Bottles were sent back as soon as possible by express mail to the contract laboratory and were stored at 4°C.

Table 1   Facilities selected for screening Whole Effluent Quality and Overview of average (n=3) Cfiber concentration (mM) in effluents (Phase 1 and 2)

Phase and Facility #	Facility Type	Effluent Treatment	Receiving Water Dilution Factor	Cfiber (mM)			
				Average first sampling	Average second sampling	RSD[3] (%) first sampling	RSD[3] (%) second sampling
1/1	Polymers	No biological treatment	2	4.4	8.4	38	8
1/2	Polymers	No biological treatment	14	22		39	
1/3	Chemical Intermediates	Aerated lagoon	45		7.6		10
1/4	Basic Chemicals	WWTP biox	721	4.7	7.2	14	20
1/5	Basic Chemicals	No biological treatment	> 1000	41	63	5	19
1/6	Petrochemical Refinery	WWTP biox	472	20		13	
1/7	Chemical Intermediates	No biological treatment[1]	> 1000[2]	2.5		29	
1/8	Chemical Intermediates	WWTP biox	>1000		56		8
1/9	Petrochemical Refinery	No biological treatment	>1000	9.4		32	
2/1	Petrochemical Refinery	WWTP biox	>1000	4.0	6.0	20	4
2/2	Petrochemical Refinery	No biological treatment	>1000	13	6.2	34	16
2/3	Basic Chemicals	No biological treatment	>1000	15	16	15	6
2/4	Polymers	No biological treatment	<100	25	31	56	10
2/5	Petrochemical Refinery	No biological treatment	>1000	6.0	5.3	39	47

[1] *effluent was collected from process unit before entering refinery WWTP biox (biox – biological treatment)*
[2] *after subsequent biological treatment*
[3] *RSD = Relative Standard Deviation*

CS-5.3.2. SPME analyses

CS-5.3.2.1. SPME Extraction and GC-FID screening

SPME extractions were performed according to the RIZA protocol (de Maagd, 2000), Verbruggen et al (2000), and Leslie et al (2005). Briefly, 3 glass 250 ml bottle were washed with the effluent sample. PTFE stir bars were added to each vessel, placed on a magnetic stirrer and the bottles filled to the top, without an air gap, with

the effluent sample and closed with a PTFE septum/stopper. Commercially available SPME fibres with a 100-µm PDMS coating were thermally desorbed directly prior to extraction to ensure they were clean. For the extraction, one SPME fibre was exposed for 24 h to the effluent in each extraction vessel under conditions of continuous, vigorous stirring. The molar amount of the fibre (C_{fibre}) was quantified using an external standard (2,3-dimethylnaphtalene). GC with FID with a DB-1 GC column (L 10 m x ID 0.25 mm, film thickness 0.1 µm) was used for the separation and detection of compounds. The molar response of the external standard, the PDMS fibre volume (0.621 µl) and the peak area of all peaks in the sample were used to calculate the molar concentration in the fibre (C_{fibre}). The diameter of the silica core of the 1 cm-long fibres used was 110 µm. Therefore the volume of PDMS in the 100-µm thick layer surrounding the core is 0.66 µl. This is used to calculate the concentration as total mM PDMS.

CS-5.3.2.2. SPME Screening Analysis by GC-MS

For relevant effluents, screening with GC-MS was conducted; those that matched library spectra with a high degree of certainty were listed. Siloxanes, were not addressed as these would have originated from the PDMS fibre.

CS-5.3.3. Additional parameters

In addition ammonia, pH, conductivity were determined in the 'total' effluent samples. Selected 'Total' Metals (Cd, Cu, Co, Cr, Hg, Pb, Ni, Zn, V) using ICP-AES or ICP-MS were determined, Hg was measured using AAS.

CS-5.4. RESULTS

CS-5.4.1. Biomimetic extraction (SPME extraction)

Table 1 shows an overview of the average concentration (n=3) and RSD for C_{fibre} in mmol/l (mM) of the effluents for the first and second sampling periods and each phase. The data are shown in figures 1 and 2. To put the data into context, and to be able to utilise the values, these should be compared with the data in Table 2, which shows a number of critical C_{fibre} concentrations for fish, algae and zooplankton (Parkerton et al, 2001). This approach relies on the relationship that exists between the log K_{ow} of a chemical and its potential to bioconcentrate/bioaccumulate and the ecotoxicity exerted as baseline toxicity. Previously it has been shown that the partitioning of the chemical onto the SPME fibre is also related to the chemicals log K_{ow}. (Leslie et al, 2002; Parkerton et al, 2000, Ditoro et al, 2000a,b), and is of particular significance where the main source of the chemicals is from petroleum products.

**Table 2**   Critical acute benchmarks of C_{fibre} for trout, algae and zooplankton.

Endpoint	Critical C_{fibre} (mM)	Reference
Acute narcotic effect trout	77	Parkerton et al, 2001
Acute narcotic effect algae	57	Parkerton et al, 2001
Acute narcotic effect *Dapnia magna*	42	Parkerton et al, 2001

The C_{fibre} concentrations measured in the two phases varied between 2.5 and 63 mmol/l (PDMS). Two data points showed rather high concentrations, which based on narcotic toxicity studies, could potentially show acute toxicity if tested, although, in practice the effluents highlighted have dilution factors of >500 and thus the potential toxicity would not be observed in the receiving waters.

The RSD of the total molar concentrations in fibres exposed to effluents 4 to 56%, although the majority were in the 20 – 40% range. In most cases the variability measured, would not have prevented an assessment being made versus the benchmarks used. In one case, Data point 2/4, where the RSD was 56% and the average 25 mmol/l PDMS, there would have been uncertainty in the interpretation and probably the need to resample. In this case the second sample supported the average value (31 mmol/l PDMS)

Figure 1   Total molar concentration of organic chemicals in PDMS (C_{fibre}) after fibre exposure to undiluted effluents from the plants, also indicating median lethal effect concentration for hydrocarbon mixtures (expressed as mM PDMS) for Trout, algae and *Daphnia magna* [Parkerton et al, 2001].
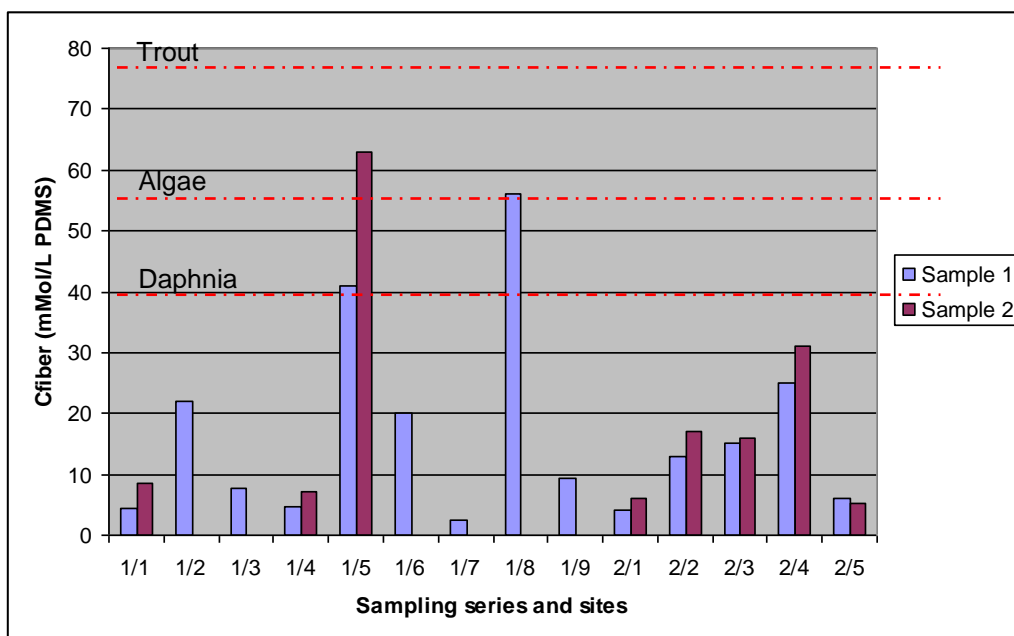
CS-5.4.2. GC and MS analyses

CS-5.4.2.1. Initial GC screening

The peak patterns of the effluents between the facilities showed large differences (Figures 3-6). Not unexpectedly there were large differences between the number of peaks observed depending upon whether the effluent was derived from a petrochemical plant (Figure 3) or a refinery (Figure 4). Normally the peak patterns were consistent between sampling periods, although perhaps again not unsurprisingly, the variation was greater for the petrochemical plants than the refineries.

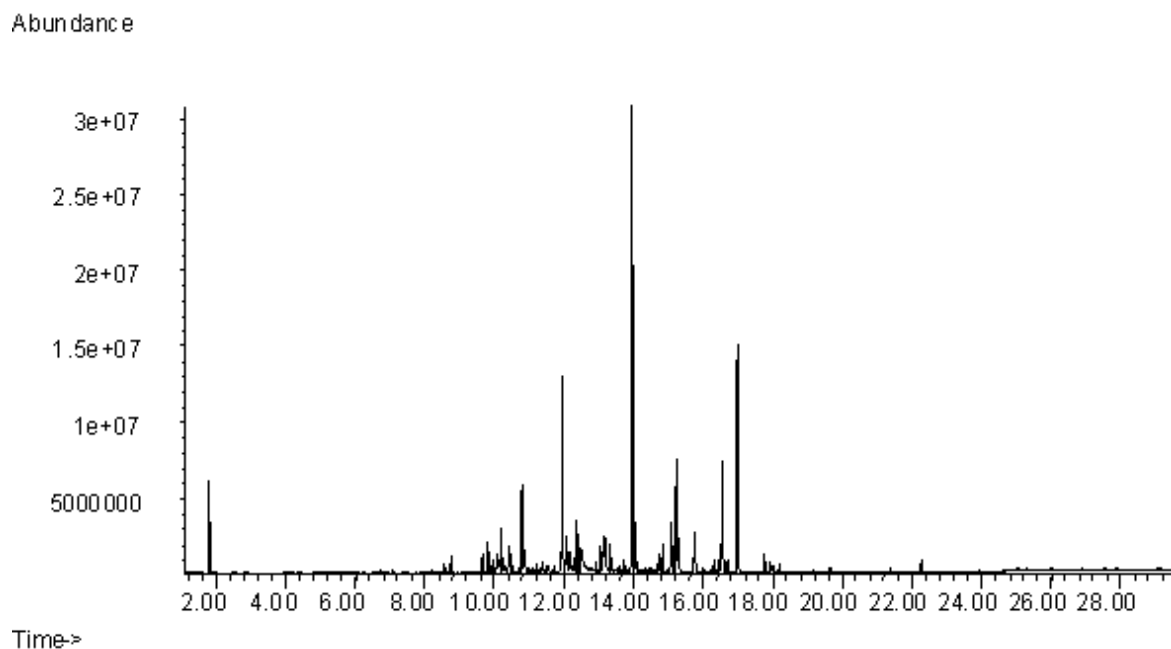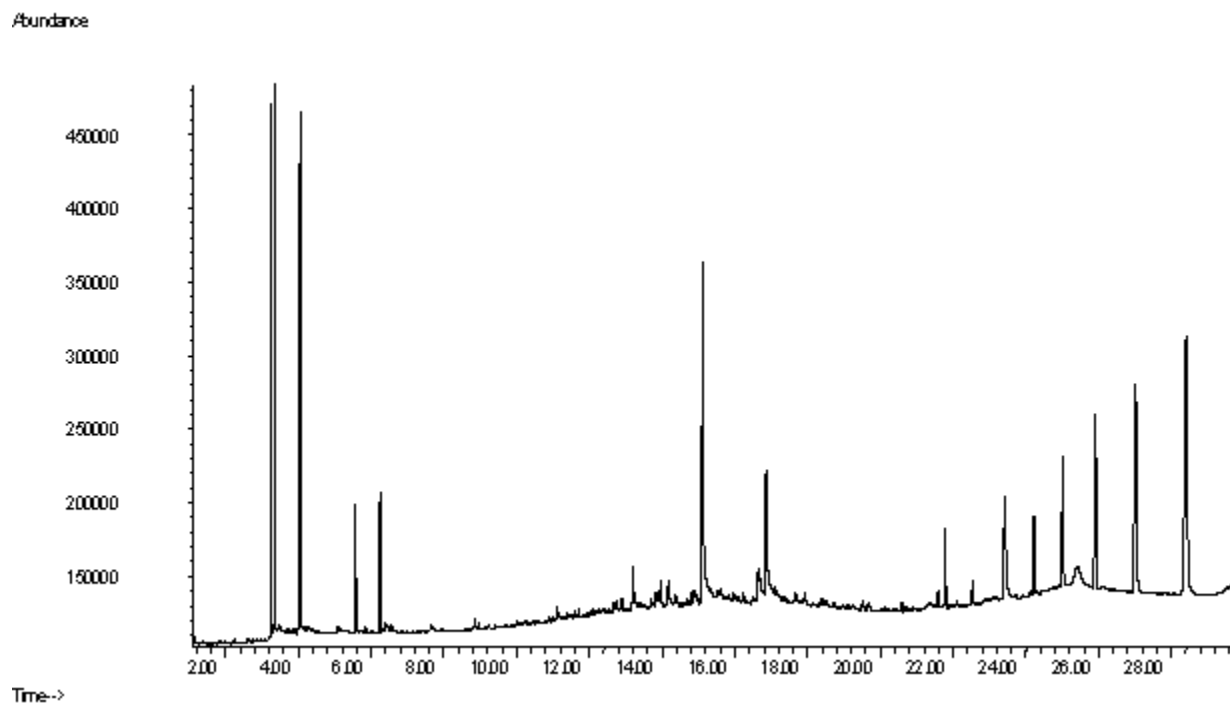**Figure 3**   Sample – Petrochemical plant effluent

Figure 4   Sample – Refinery effluent

CS-5.4.2.2. Supplemental Screening with GC-MS analysis

The supplemental screening with GC-MS was performed on samples from phase three of the study. Candidates for the identified peaks were identified if they had a

minimum of 80% reverse fit and were sorted according to increasing retention time. In several instances, more than one peak had a reverse fit of over 80% for the same chemical. It is not possible in the screening step performed in this study, to be certain of the identification of a given peak. Should detailed information be required about particular chemicals of interest, other standard analytical methods can be applied to measure targeted individual compounds with more precision.

In two samples, rather unexpectedly, tetrachloroethylene was found. This was further investigated, as a chlorinated compound of this nature was not expected in the effluents from these facilities. It was established that the sampling bottles were probably contaminated during the sampling process, by the sampler compositers. The compositers had been used to take previous samples to be analysed for oil in the water, a method which requires the addition of tetrachloroethylene as an extractant. This highlights the need to be careful in sampling effluents and being very careful about the origin and cleanliness of sampling systems.

The most obvious note from the screening with GC-MS was the different effluents were dominated by different hydrocarbons, which would reflect the processes occurring upstream in the facility. Thus some effluents were dominated by alkanes, with some PAHs, and others with aromatic compounds, mainly PAHs, with a few large peaks of hept-2-enes, dicarbonitriles and indene. And still others a complex mixture of aromatic chemicals, benzenes, PAHs, phenols and alcohols, but containing many compounds typical for oil.

CS-5.4.3. Metals

In all the samples the levels of Cd (<0.004 mg/l), Cu (<0.04 mg/l), Hg (<0.0036 mg/l), Pb (<0.068 mg/l) and Zn (<0.4 mg/l) were below the relevant detection limits. Cobalt was found in the two effluents 0.25 and 0.14 mg/l respectively, and chromium was found in four at a concentration between 0.02 and 0.03 mg/l. Nickel was found in the effluents of three sites, 0.03-0.12 mg/l, while vanadium was present in most effluents at concentrations from 0.03 to 2.1 mg/l. In phase 3, those facilities that were re-tested showed similar values to those obtained in phase 2.

The toxicity of metals is dependent on the species and abiotic factors (including bioavailability). The measured aquatic acute toxicity of Cobalt is generally well in excess of the values found in this study, and though chronic toxicity is observed at these and lower values, the dilution in the receiving waters and the likely impact of hardness would indicate that no toxicity would be observed. In the case of Ni and V concentrations, while some sensitive species exhibit mortality after exposure to Ni-levels a factor two lower than those reported here, many 96h-LC50's are higher (Hoang et al, 2004). V toxicity increases with valency (penta-valent is most toxic and the most prevalent over a large range of pH). An example of a regulatory limit is that in the Netherlands, where the maximum permissible concentration in the environment is 0.035 mg/l. The values reported here, would not give rise to concern due to the dilution factors involved.

CS-5.4.4. Ammonia, pH and conductivity

The pH varied between 5.6 to 7.8, and the conductivity between 611 to >1999 μS. Total ammonia varied between 0.09 to 6.4 mg/l. The total ammonia concentration is difficult to related to toxicity; a better parameter is freely available ammonia. However, considering the pH of the effluent samples, the majority of any ammonia would be present as the ammonium ion because the pKa is >9 and the pH of the samples was <9.

CS-5.5. DISCUSSION

CS-5.5.1. Impact of dilution on potential toxicity

In all the cases assessed in these phases of the study, the likelihood that narcotic toxicity would be observed in receiving waters (i.e. after effluent dilution) is very low. Many of the effluents would receive dilutions in excess of 100, and only in one case the dilution factor was lower than 10. In taking the measurements, biomimetic SPME is used in a nondepletive manner (nd-SPME) (Vaes et al, 1996) and can be regarded as a measure of the freely dissolved concentration (activity) of the chemicals as opposed to the total concentration of them. This is a relevant parameter for the interpretation of toxicity studies since the freely dissolved phase is considered bioavailable. The dilution of effluents will act to reduce the total concentration of organic chemicals present.

CS-5.5.2. Assumption of narcosis as a basis for assessing toxicity

The toxicity that is assigned to effluents, when based on SPME measurements, assumes a mode of action, narcosis, and additivity of the individual hydrocarbons. Recently a number of papers have been published about this (Di Toro, 2000a, b and McGrath et al, 2005).

However, in addition, toxicity may arise in part from substances that also have a specific mode of toxic action. For example, the Ni and V in one effluent could also contribute to the overall toxicity in that effluent. If this was of concern, either where the receiving water was of poor quality or the dilution factors were very low, this could be tested by way of bioassays. The contribution to total toxicity due to the narcotic mode of action can be estimated with SPME, and can help to distinguish where measures should be taken to reduce toxicity if necessary.

CS-5.6. SUMMARY AND CONCLUSIONS

Using the approach outlined, it is possible, within the petroleum sector, to assume that effluents exert their toxicity via a common mode of action, narcosis, provided at the same time other factors, e.g. metals, are also measured and assessed. In this way, effluents can be assessed, without the use of animals, in a screening approach, that would identify effluents of concern.

CS-5.7.   REFERENCES

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CS-6. CASE STUDY 6: WHOLE EFFLUENT ASSESSMENTS ON REFINERY EFFLUENTS

CS-6.1. SUMMARY AND KEY LEARNINGS

In a study assessing 9 typical refinery effluents, two studies were performed, the first measuring constituents in the effluents using various techniques and the toxicity of those effluents to aquatic organisms and the second evaluating the impact of biodegradation (using two different methods) on the constituents and the associated aquatic toxicity.

The study showed that using either a ready style (DOC-die away) or an inherent style (Zahn-Wellens) test protocol made little difference to the extent of the biodegradation of the constituents. The actual biodegradation observed was significant and a large percentage of the associated material measured after extraction, and therefore potentially associated with bioaccumulation and toxicity in these refinery/petrochemical effluents was rapidly degradable.

The toxicity that was observed could normally be explained by a narcosis mode of action (excluding confounding factors). The results indicated that biomimetic extraction (SPME) could be a useful tool for determining the aquatic toxicity of refinery/petrochemical effluents.

Finally some confounding effects on the toxicity results were noted, following the addition of the degradation medium (t = 4 hours) on toxicity and bioaccumulation which were not fully understood.

CS-6.2. INTRODUCTION

CONCAWE's aim in this project was to ensure that in developing an approach to the use of WEA by OSPAR, the best science was used in a reasonable and efficient way. Specifically with respect to WEA, this led to a programme that addressed the following three points, with specific application to refineries:

- To understand how different biodegradation methods behave when assessing effluents

- Impact on assessing toxicity

- Potential of the constituents to bioaccumulate

The full data summarised in this case study are described in a reports delivered to CONCAWE, (Leonards and Postma, 2006) and were presented at SETAC, see Comber et al, 2006.

CS-6.3. METHODOLOGY

CS-6.3.1. Overview

Nine representative effluents (fresh water and marine) from refineries throughout Northern Europe were selected. The parameters measured included toxicity (T), bioaccumulation (B) and a number of chemical parameters including salinity, pH and specific metals.

Of the nine, three samples were selected for a degradation approach using two different degradation tests (Ready style and Zahn-Wellens style). After degradation the toxicity and bioaccumulation of the effluents were determined to study persistence. This is summarised in this case study.

For the degradation tests one low (COD) level freshwater, one high (COD) level freshwater and one marine effluent were tested. Both acute and chronic toxicity tests were undertaken using freshwater and marine species following the test methods described in below. Potential for bioaccumulation was assessed using both solid phase micro-extraction (SPME) and liquid-liquid extraction (EGOM LLE). A description of these methods together with an evaluation of their performance, based on a recent inter-laboratory study undertaken for OSPAR, is provided by Leslie (2005a,b).

In figure 1 an overview of the persistence/WEA approach for the three samples is presented. The toxicity and degradation studies were performed by AquaSense (Netherlands) and the bioaccumulation studies by RIVO (Netherlands).

Additionally, six other refinery effluents (five freshwater, and one saltwater) were tested using the WEA approach described to provide information on their toxicity, potential to bioaccumulate and chemical composition. However, although no persistence assessments were undertaken for these six effluents the toxicity and potential for bioaccumulation were measured and are reported.

The samples were also analysed for heavy metals, Cu, Zn, Pb, Cd, Hg, Co, V, Cr, Ni, and other typical parameters, COD, TOC, DOC, K, Na, Ca, Mg, $(H)CO_3$, Cl, SO_4, salinity, NH_3 and pH. Table 2 summarises the general characteristics.

CS-6.3.2. Biodegradation methods

A full description can be read in Leonards and Postma, 2006. In summary a DOC-die Away test (ISO 7827/ OECD 301A), and the Zahn Wellens Test (ISO 9888/ OECD 302B) approaches were used. DOC measurements were taken to assess biodegradation and samples were taken 0, 4 h and 14 d for the DOC-die away test and for the Zahn-Wellens test, samples were taken at 0 and 4 h and 7, 14, 21 and 28 d. The high level DOC sample, CONCAWE 2, was tested at high and low DOC content. In the high DOC sample the effluent was undiluted (100%), while the waste water in the low DOC content test was diluted to 25% with demi-water. The marine sample, CONCAWE 4, had a salinity of 30‰ and the activated sludge was therefore acclimated to this salinity before the biodegradation tests were started.

Figure 1   Overview of sample treatment steps, toxicity, bioaccumulation and degradation tests for the three effluents that were selected for the persistence WEA assessment using two types of degradation approaches (DOC-die away and Zahn-Wellens). CONCAWE 1 a low (COD) level effluent, CONCAWE 2 a high (COD) level, and CONCAWE 4 a marine effluents
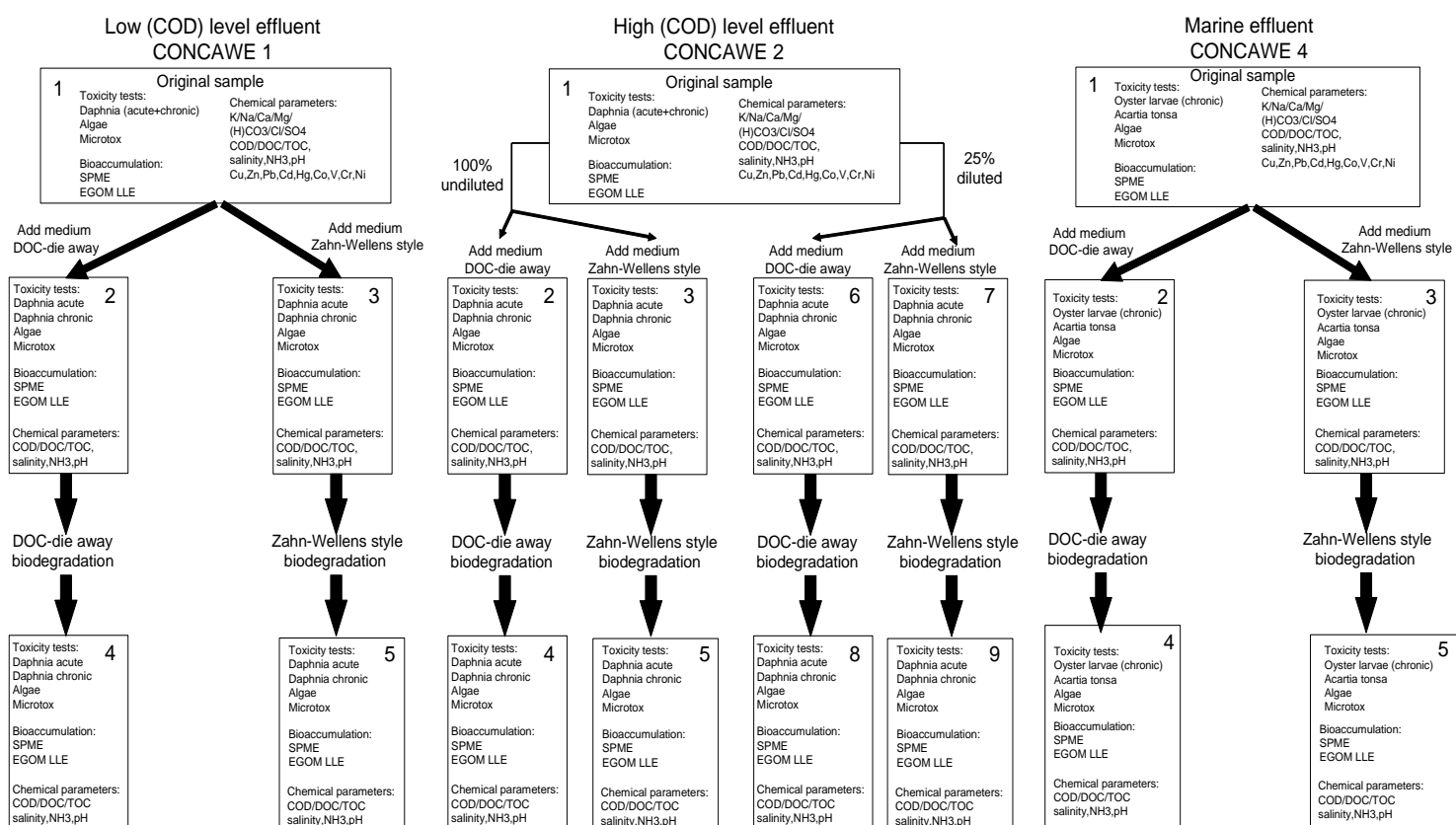
Table 2   General characteristics of the original effluent samples, measured directly after delivery.

Sample code	pH	NH$_4^+$ (mg/l)	Conduct (µS/mm)	Salinity (‰)	TDS[1] (mg/l)	DOC (mg/l)
CONCAWE 1	7.3	10	793	4.9	Ofl.	22.9
CONCAWE 2	7.7	<10	220	1.1	Ofl.	222
CONCAWE 3	7.6	<10	319	1.8	Ofl.	8.2
CONCAWE 4	7.2	<2.5	4200	30.2	Ofl.	7.8
CONCAWE 5	7.7	<10	198	1.0	Ofl.	12.6
CONCAWE 6	7.5	13	278	1.5	Ofl.	10.2
CONCAWE 7	7.3	<10	98	0.3	1054	12.2
CONCAWE 8	7.6	10	94	0.3	1012	12.7
CONCAWE 9	7.1	10	2000	13.4	Ofl.	10.6

[1]: TDS = Total Dissolved Salts. Ofl = Offline, samples should be diluted.

CS-6.3.3. Toxicity

For the freshwater effluents the following bioassays were carried out:

- Chronic toxicity to Daphna magna (16d test)
- Acute toxicity to Daphnia magna
- Acute toxicity to *Pseudokirchneriella subcapitata* (algae)
- Microtox test

For the marine effluents the following bioassays were carried out:

- Oyster larvae test
- Acute toxicity to Acartia tonsa
- Acute toxicity to Phaeodactylum tricornutum (algae)
- Microtox

Full details may be obtained from Leonards and Postma, 2006.

CS-6.3.4. Potentially Bioaccumulating Substances

Potentially bioaccumulating substances (PBS) in the effluents were determined using two different approaches both recently evaluated in an inter-laboratory study for OSPAR (Leslie, 2005a,b). The first method was a partitioning-based methodology using biomimetic solid phase micro-extraction (SPME), (Leslie and Leonards, 2005a), in which exposed SPME fibres were analysed by GC and quantified with 2,3-dimethylnaphthalene. The total peak area of the chromatogram was integrated (between C9 and C38).

The second method was based on liquid-liquid extraction (EGOM LLE), determined according to the protocol used in the OSPAR inter-laboratory study (Leslie and Leonards, 2005b) which was based on the 'EGOM' LLE method that was developed in Sweden by Adolfsson-Erici and Wahlberg (1992) and Hynning (1996) and measure the 'extractable gas-chromatographic organic matter' in an effluent sample. The extracted material is again analysed by GC and the same standard, 2,3 dimethylnaphthalene, used for quantification. The total peak area of the chromatogram was integrated (C9 to C38).

CS-6.4. RESULTS & DISCUSSIONS

CS-6.4.1. Persistence

The Results from the biodegradation tests are shown in Figures 2 and 3. At the same time that samples were taken for DOC measurements, they were also analysed by GC-FID. Figures 4 and 5 show the results of these analyses. Both DOC-die away and Zahn-Wellens style biodegradation tests gave similar results, suggesting that either approach is suitable for studying the persistence of constituents in refinery/petrochemical effluents.

The GC traces are typical for refinery and petrochemical plants, but in this study no attempt was made to identify the specific peaks.

Figure 2   DOC-die away test data
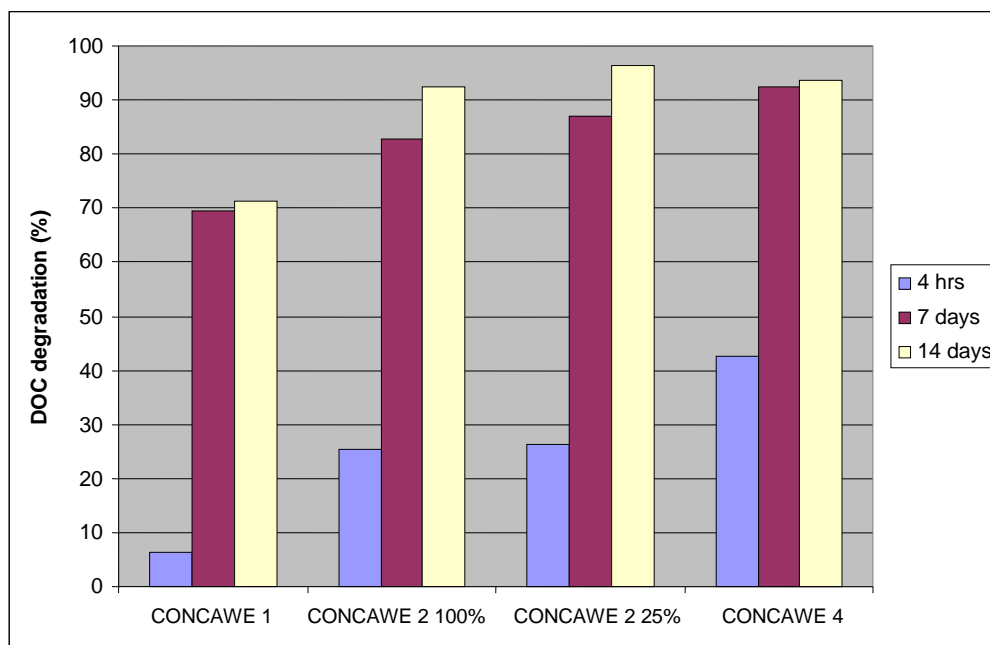
Figure 3   Zahn-Wellens test data
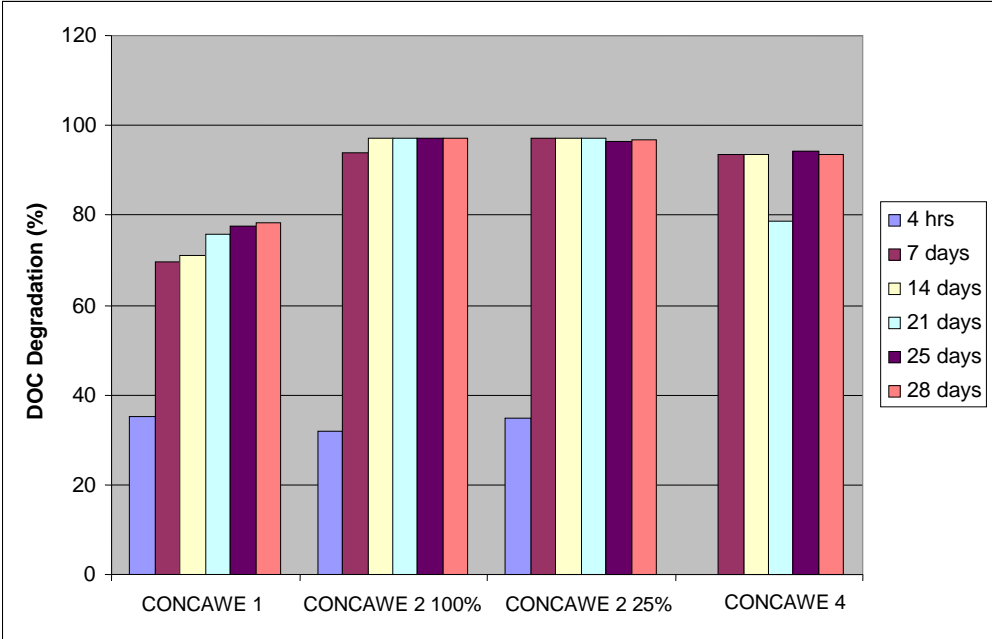
Figure 4   GC FID trace from samples taken before and after the DOC-die away test

DOC-die away
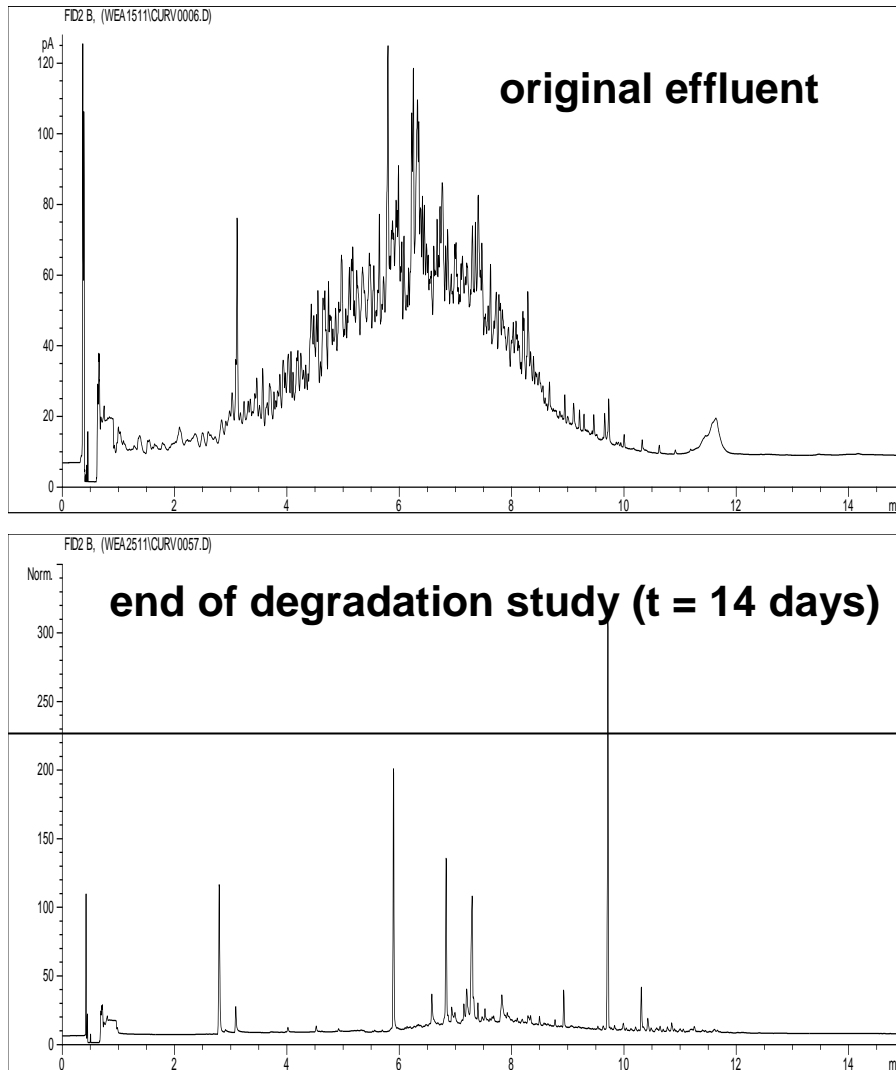
Figure 5   GC FID trace from samples taken before and after the Zahn-Wellens test

Zahn-Wellens

original effluent

end of degradation study (t = 28 days)
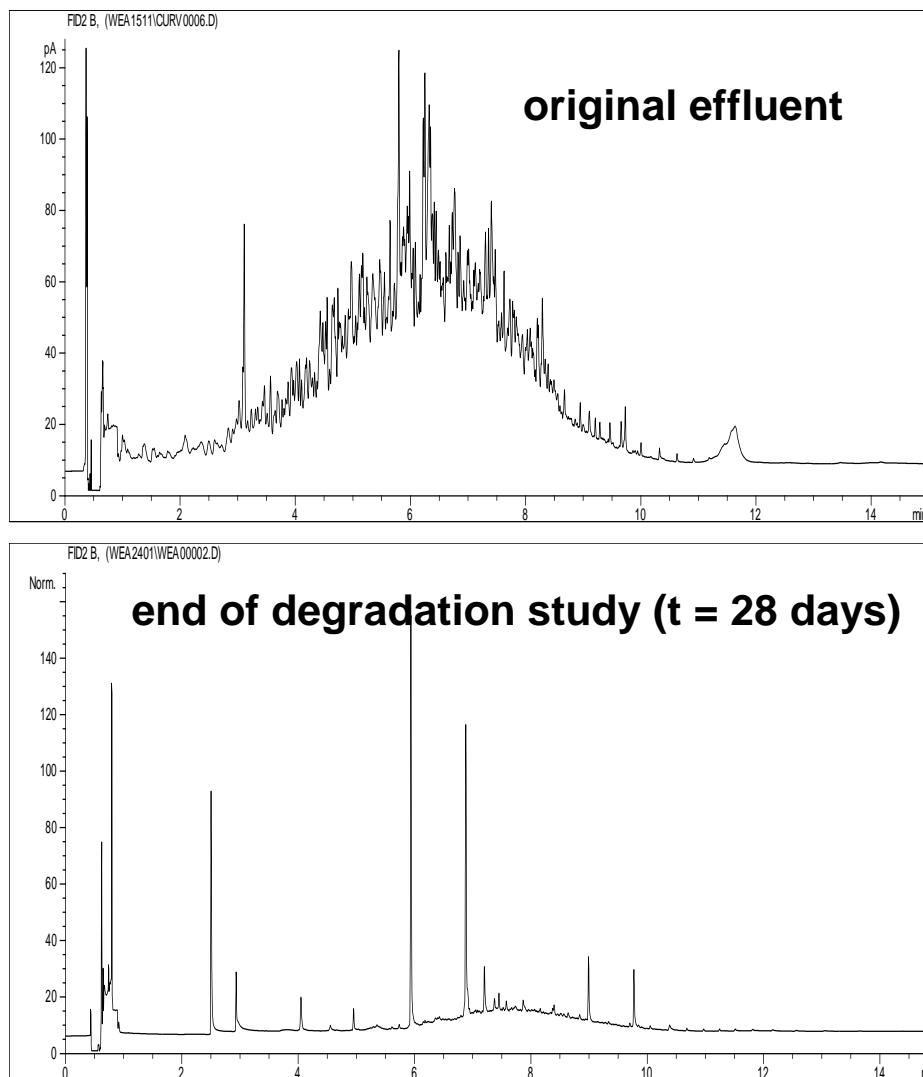
CS-6.4.2. Bioaccumulation

The presence of potentially bioavailable substances was determined on all 9 effluents, using EGOM-LLE and SPME. The data (see Tables 7 and 8) showed a wide variability, but it is important to realise that the very high value (138) is derived from a stream deliberately chosen to address the impact of biodegradation and is not discharged directly to the environment. The impact of conducting degradation tests on three of these effluents and the extractable material is shown in Figures 6 and 7 (see Leonards and Postma, 2006 for full data set).

Table 7   SPME results (mmol/l) in original samples

Sample	#1 mmol/l fibre	#2 mmol/l fibre	#3 mmol/l fibre	AVG mmol/l fibre	SD	RSD (%)
CONCAWE 1	9.3	10.3	10.7	**10.1**	0.7	7
CONCAWE 2	55	48	74	**59**	13.8	23
CONCAWE 3	2.4	1.5	2.4	**2.1**	0.5	24
CONCAWE 4	5.2	4.7	5.8	**5.3**	0.6	11
CONCAWE 5	18.3	22.7	15.9	**19.0**	3.4	18
CONCAWE 6	28.0	33.6		**30.8**	3.9	13
CONCAWE 7	156	118	141	**138**	18.7	14
CONCAWE 8	3.7	7.0	4.4	**5.0**	1.7	34
CONCAWE 9	6.0	6.4	8.3	**6.9**	1.2	17

Table 8   EGOM LLE-results (mg/l) in original samples

Sample	#1 mg/l	#2 mg/l	#3 mg/l	AVG mg/l	SD	RSD (%)
CONCAWE 1	0.00065	0.00064	0.00039	**0.00056**	0.00015	26
CONCAWE 2	0.047	0.023	0.034	**0.035**	0.012	35
CONCAWE 3	<LOD	<LOD	<LOD	**<LOD**		
CONCAWE 4	0.0007	0.0005	0.0006	**0.0006**	0.0001	22
CONCAWE 5	0.0014	0.0019	0.0018	**0.0017**	0.0003	16
CONCAWE 6						
CONCAWE 7	0.037	0.046	0.040	**0.041**	0.0042	10
CONCAWE 8	<LOD	<LOD	<LOD	**<LOD**		
CONCAWE 9	<LOD	<LOD	<LOD	**<LOD**		

Figure 6 DOC Style test : Impact of degradation on SPME data
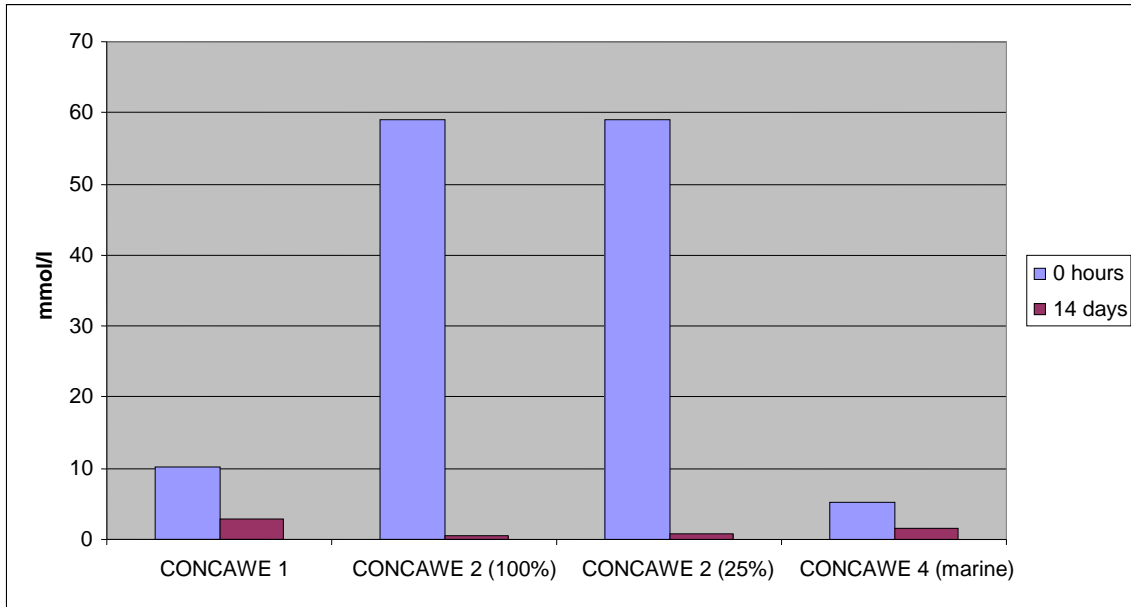
Figure 7 Zahn-Wellens Style test : Impact of degradation on SPME data

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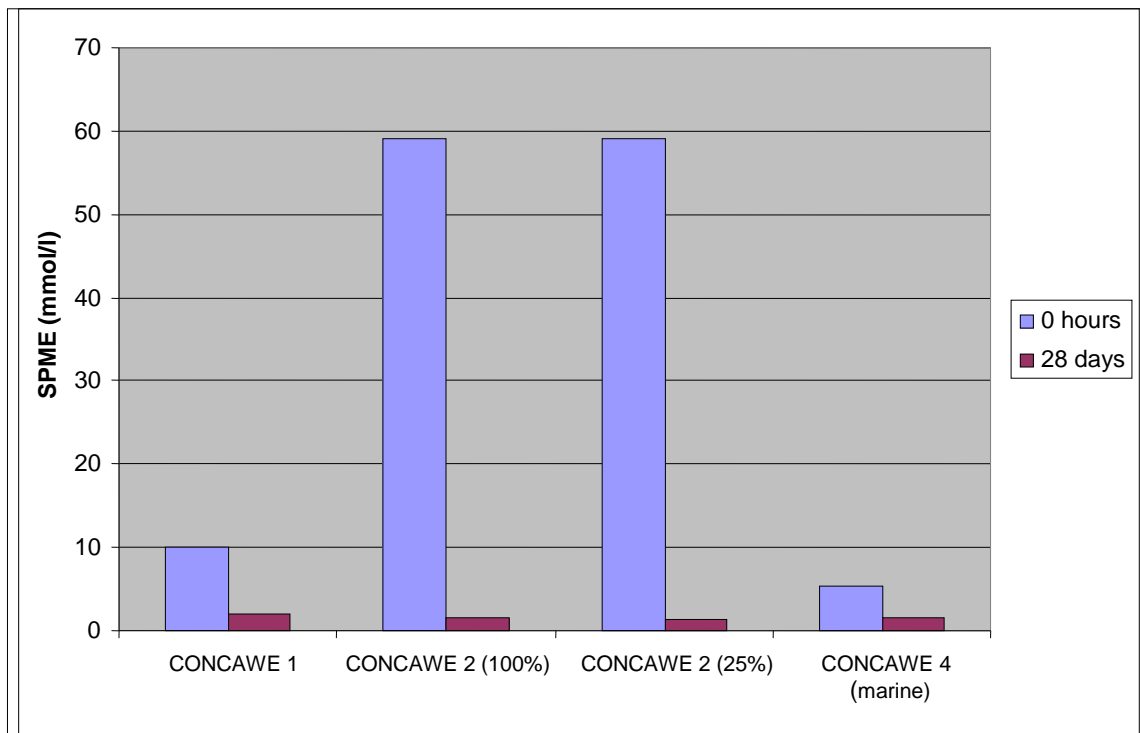
CS-6.4.3. Toxicity

All effluents with PBS concentrations above critical benchmark for acute narcotic effects (determined by Parkerton et al, (2001)) showed a toxic response in the aquatic tests. Figure 8 shows the supporting data for the crustacean test.

The full data sets are given here in Tables 3 – 6.

Other confounding effects were noted, these were caused by high conductivity, reduction in oxygen concentration and the potential for other toxic responses due to metals.

In some biodegradation tests there was an increase in observed toxicity after 4h, in all cases this was reduced or eliminated by end of the test. A large percentage of the PBS and toxicity in these refinery/petrochemical effluents was rapidly degradable.

Figure 8   PBS v Toxicity

Table 3 Overview of the toxicity tests performed with CONCAWE 1 before and after biodegradation.

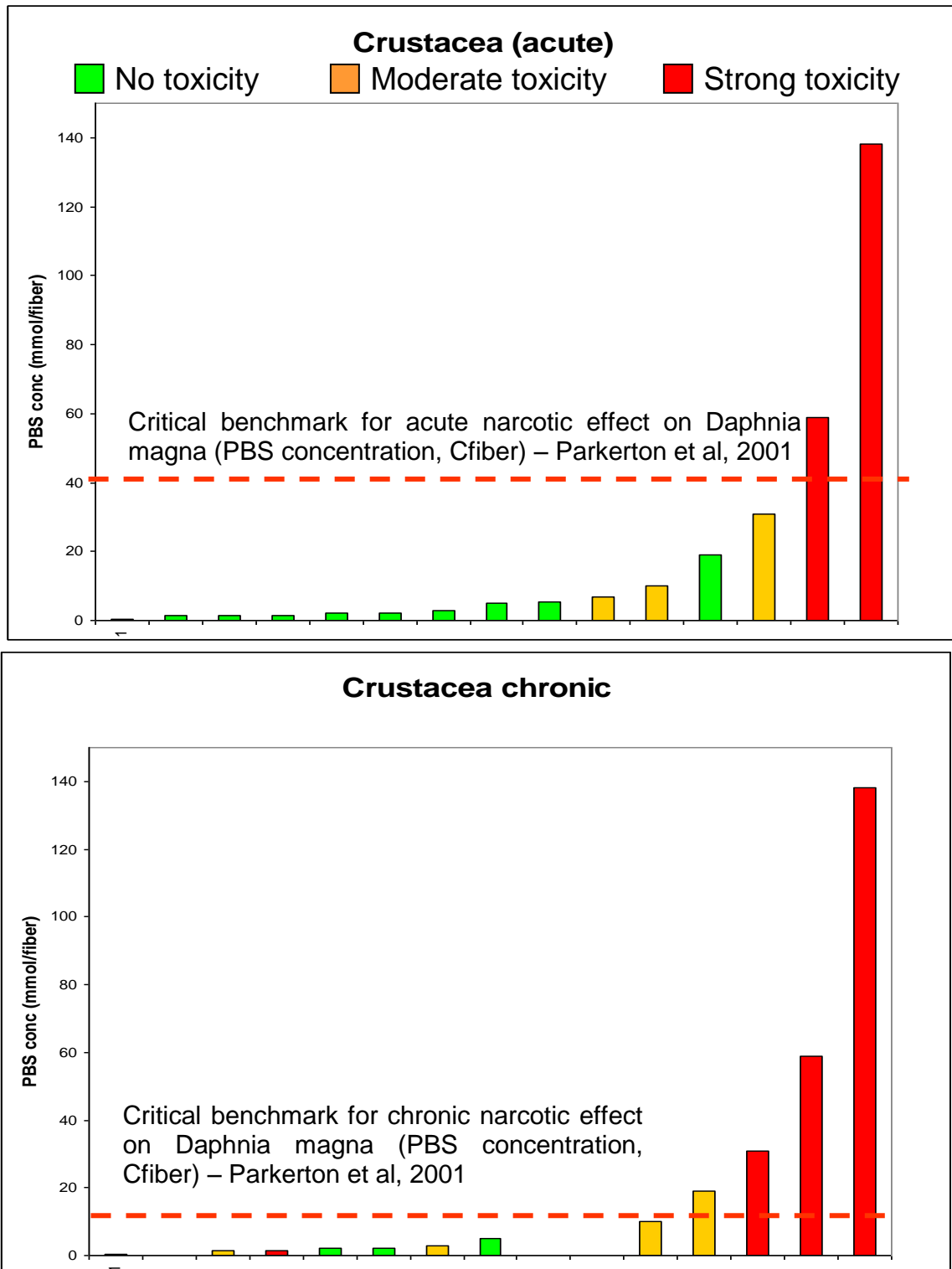| CONCAWE 1		Microtox			Algae			Daphnia - acute		Daphnia - chronic				
										Mortality		Reproduction		
		EC_{20} (vol %)	EC_{50} (vol %)	NOEC (vol %)	EC_{50} (vol %)	Effect in 100 vol%	NOEC (vol %)	LC_{50} (vol %)	Mortality in 100 vol %	NOEC (vol %)	LC_{50} (vol %)	NOEC (vol %)	EC_{50} (vol %)	Reduction R_m in100 vol% (%)
Original sample		15 (14–16)	> 45	49	>98	11	32	63 (55–71)	85	32	> 100	32	> 100	42
DOC-die away	T=4 hr.	10 (9-11)	34 (29-41)	49	>98	46	100	> 100	15	32	> 100	32	71 (57–83)	84
	T=14 days	> 45	> 45	49	>98	49	100	> 100	5	100	>100	32	>100	14
Zahn-Wellens	T=4 hr.	10 (9–11)	37 (34–40)	12	51 (48–55)	96	100	> 100	5	100	> 100	100	>100	5
	T=28 days	> 45	> 45	25	64 (53–100)	73	100	> 100	5	100	> 100	100	>100	0

Table 4 Overview of the toxicity tests performed with CONCAWE 2 (Ecolims nr. 335379) before and after biodegradation.

CONCAWE 2		Microtox			Algae			Daphnia - acute		Daphnia - chronic				
										Mortality		Reproduction		
		EC_{20} (vol %)	EC_{50} (vol %)	NOEC (vol %)	EC_{50} (vol %)	Effect in 100 vol%	NOEC (vol %)	LC_{50} (vol %)	Mortality in 100 vol %	NOEC (vol %)	LC_{50} (vol %)	NOEC (vol %)	EC_{50} (vol %)	Reduction R_m in100 vol% (%)
Original sample		< 5.6	19 (17–20)	<6.1	10[2] (9–11)	75[2]	32[1]	52[1] (46–58)	100	18	24	10	22 (20–23)	100
Without dilution														
DOC-die away	T=4 hr.	17 (14–21)	> 45	12.2	20 (18–23)	78	32	45 (38–53)	100	18	32	18	50 (44-55)	100
	T=14 days	44 (30–65)	> 45	49	93	53	100	>100	15	100	>100	100	>100	0
Zahn-Wellens	T=4 hr.	32 (26–39)	> 45	12.2	29 (26–38)	68	32	71 (50–102)	65	32	51	32	60 (57-63)	100
	T=28 days	> 45	> 45	49	92	95	100	> 100	5	100	>100	18	>100	8
25% dilution (data not corrected for dilution!)														
DOC-die away	T=4 hr.	> 45	> 45	24.5	63 (58–73)	91	100[2]	> 100[2]	0	32	61	32	69 (64-73)	83
	T=14 days	> 45	> 45	49	95	50	100[2]	>100[2]	10	100	>100	32	>100	21
Zahn-Wellens	T=4 hr.	> 45	> 45	24.5	76 (61–91)	75	100[2]	> 100[2]	5	32	77	32	72 (61-91)	78
	T=28 days	> 45	> 45	49	80	96	100[2]	> 100[2]	5	18	26 (23-30)	5.6	29 (27-32)	65

[1]: animals floating on the surface
[2]: unclear dose-effect relation

Table 5 Overview of the toxicity tests performed with CONCAWE 4 (Ecolims nr. 335381) before and after biodegradation.

CONCAWE 4		Microtox			Algae			*Acartia tonsa*		Oyster larvae	
		EC_{20} (vol %)	EC_{50} (vol %)	NOEC (vol %)	EC_{50} (vol %)	Effect in 100 vol%	NOEC (vol %)	LC_{50} (vol %)	Mortality in undiluted sample (%)	NOEC (vol %)	LC_{50} (vol %)
Original sample		> 45	> 45	98	>98	0	100	> 100	5	100[1]	>100
DOC-die away	T=4 hr.	> 45	> 45	25	22	51	100	> 100	13	32	57 (45 – 69)
	T=14 days	> 45	> 45	98	>98	0	100	>100	20	32	56 (56-56)
Zahn-Wellens	T=4 hr.	> 45	> 45	6[1]	>98[1]	0[1]	32	>100	38	10	17 (16 – 18)
	T=28 days	> 45	> 45	49	>98	16	100	> 100	23	10	46 (39-64)

[1]: unclear dose-effect relation, with significant effects at lower test concentrations

Table 6 Overview of the results of the toxicity tests performed with the other samples without the biodegradation study.

		Microtox			Algae		*Acute crustacea*			*Chronic Crustacea*			Oysterlarvae	
Sample	Type water	EC_{20} (vol %)	EC_{50} (vol %)	NOEC (vol %)	EC_{50} (vol %)	Effect in 100 vol%	NOEC (vol %)	LC_{50} (vol %)	NOEC (vol %)	LC_{50} (vol %)	NOEC reproduction (vol %)	EC_{50} reproduction (vol %)	NOEC (vol %)	LC_{50} (vol %)
CONCAWE 3	Fresh	> 45	> 45	98	>98	0	100	> 100	100	>100	100	>100		
CONCAWE 5	Fresh	31 (21 – 45)	> 45	49	>98	19	100	> 100	100	>100	100[2]	>100		
CONCAWE 6	Fresh	11 (10 – 12)	35 (31 – 40)	98	>98	0	100	> 100[3]	18	31	32	62 (54 – 68)		
CONCAWE 7	Fresh	< 5.6	10 (10 – 11)	49	>98	4	< 5.6[1]	< 5.6[1]	10	15	10	15 (14 – 15)		
CONCAWE 8	Fresh	> 45	> 45	49	>98	30	100	> 100	100	>100	100	>100		
CONCAWE 9	Marine	> 50	> 50	25	>98	9	32	56 (38 – 81)					<5.6	16 (8 – 27)

[1]: animals floating on surface
[2]: unclear dose-effect relation, with significant effects at lower test concentrations
[3]: increased (but not statistical significant) mortality in undiluted sample (35%)

CS-6.5. REFERENCES

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4. Hynning, P-Å. (1996) Separation, identification and quantification of components of industrial effluents with bioconcentration potential. *Water Research 30, 5, 1103– 1108*

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CS-7. CASE STUDY 7: A NEW BIOTIC INDEX FOR NON-SPECIALISTS, DEVELOPED BY REPSOL, AS A TOOL FOR WATER QUALITY CONTROL IN SPANISH RIVERS

CS-7.1. SUMMARY AND KEY LEARNINGS

The Repsol refinery and chemical complex sited in Puertollano discharge their treated wastewater into a small river, the Ojailen River. The importance of the industrial effluent waste has meant that any negative changes observed to the river were always attributed to refinery activity. Consequently it was decided that there was a requirement to know the real condition of the river water and to be able to put in place a system that could identify whether any observed changes were caused by the refinery effluent. Due to the fact that the authorities do not use an index, Repsol decided to build their own index based on macro invertebrates. An important reason for developing this index was to make it available for any non-specialists. The index needed to be cheap, easy to maintain and evaluate. The index considers three factors: diversity, abundance and tolerance.

The Indice Biotico del Ojailén-GF, IBO-GF, index has thus been developed is used exclusively for the 45 km stretch of the river that has been assessed. It is important to understand that while the results obtained from one river may be used as a reference for other sites, they are not mathematical models and so require in-situ assessment at other sites.

Over all, the IBO-GF has being able to follow the changes to the river quality and also helped to explain the cause of some of the river incidents. It has also been useful to provide a wider knowledge of the river and its sensitivity to the quality of the refinery effluents, justifying the effort made to purify the waste water.

CS-7.2. INTRODUCTION

The Repsol YPF oil refinery in Puertollano, Ciudad Real (Spain) has a wastewater treatment plant that started up in 1976 (Figure 1). This plant treats the refinery process wastewaters plus those produced at the petrochemical industries located within the same industrial complex.

The treated wastewater is discharged into a small seasonal river called the Ojailén river.

The volume of the industrial effluent waste was such that it was likely that the authorities would always assume that any negative alteration caused to the river would be identified with the refinery activity, regardless of the actual cause. This indicated the need to assess the actual condition of the river water. Thus, a set of sampling and analysis programmes were established along the Ojailén river.

Subsequently, in order to understand the impact of discharges into the receiving water it was decided that a biotic index was needed. In the absence of an index available from the local authorities, the most frequently used indices in other countries were assessed, and it was subsequently decided to develop a REPSOL specific index, based on macro-invertebrates. After several *"in situ"*, laboratory and office studies, the IBO-GF index (Indice Biotico del Ojailén-GF), was defined and it has been operative since 1986.

The IBO-GF index is used exclusively for the 45 km stretch of the river that has being assessed, but the technology employed in its definition, sampling and evaluation, is applicable to any river at any location. An important purpose on developing this index was to enable its use by non-specialists.

CS-7.3. BIOLOGIC INDEX INTEREST

If macro-invertebrates are measured or assessed, then several other (non-biological) variables are being also addressed; (some of them, probably not even considered): light, temperature, organic matter, nutrients, etc. The effect of these parameters on the macro-invertebrates phase of metamorphosis, their food and predators impacts the presence or absence of the macro-invertebrates.

The biological information measures the disturbance suffered, whereas chemical analysis can only provide the concentration of substances, some of which may be responsible for the observed disturbance. Further, it has frequently been observed that the best approach for control is one that combines physical and chemical determinations together with the biological determinations.

All the biologic indexes attempt to take into account the ecological changes induced by the human activity concerning macro-invertebrates with an indicator value:

- Apparition or extinction of individual species within the community.

- Decrease of the number of species or taxa present in the community.

- Changes within the population of individual species (usually decreases).

- Changes in the proportion of species that compose the community.

The more aspects are included within the index, the more representative this will be.

The use of biotic indexes will have limitations which include:

- They require specialists, expert in the considered fauna.

- There are not enough keys to identify all the Iberian fauna (not all the rivers are identical).

- Nature does not follow a mathematical model.

- It is not possible to foresee under all the circumstances the period that invertebrate metamorphosis takes them from the aquatic into the aerial phase.

As a consequence, the conclusion is that no biological index can be considered universal. The results obtained from one river can be used as a reference, but they are not mathematical models to be automatically applied at another river.

CS-7.4. THE IBO-GF INDEX

As mentioned above, the Ojailén River receives effluents from the petrochemical complex of Puertollano, but also from the nearby industries (energy installations, mining exploitation mainly coal, agricultural and farming) and from the Puertollano population (50.000 inhabitants). Furthermore, it is a highly seasonal river, so a few months per year the river comprises entirely of the wastewater effluents. As consequence, its life is subject to the stress of these flow changes and water qualities. This type of river life is obviously sustained thanks to the wastewater

treatment plants. In fact, before any of these plants were erected, there was no macro invertebrate life in the river, according to a saprobiological study released between 1971 and 1972 by *Berridge Co*, of the United Kingdom. Consequently it was impossible to create a baseline since there were no organisms prior to the 1970's.

CS-7.5. PREVIOUS WORK TO THE BIOTIC INDEX DEFINITION

Five sample points were selected along 45 km stretch of the river. These were chosen to be the most representative of the area as possible; one site was approximately 200 m before the wastewater treatment plant discharge point, another 400 m downstream from the discharge point. The other three were selected along the river further downstream to assess recovery periods (Figure 2).

For each sample point, two zones were selected: one in a running water zone and the other in a more static water zone, due to the differences between the fauna, flora and even the physical properties such as temperature or oxygen concentration.

During the study period, the river was impacted by channelling of the urban discharges to the extent that sometimes the river was dry. For this reason it was not always possible to sample at point No 5.

In order to collect the samples a hand aquatic-net was built, with a thin nylon mesh, in a metallic frame and provided with a scratching material welded into the lower side (Figure 3). The objective was to scrape the bottom river bed, where there are stones and pebbles.

Before taking the sample, ambient temperature, water temperature, and dissolved oxygen were measured in all the sample points and a field file (Figure 4) was filled with the relevant information of each site (river water appearance, water smell, vegetable or animal life etc.). It was very important that these details were captured as it helped to explain anomalous results. For example, with heavy rains, it was not always possible to take the sample and/or, the samples might be altered because of the type of macro-invertebrates swept into or out of the sampling zone by the current.

Once the exact sampling point had been selected, the open net frame side was set into a upstream position in such a way that allowed the sample to be collected, scraping the river bed into a surface roughly of 0,5 m^2, trying to always sample the same surface. The stream current then swept the sediments into the net. In those places where there were stones or pebbles, these were introduced carefully into the net.

To minimize on-site manipulations and reduce potential loss of organisms or contamination, a textile cover was designed for the net frame that was attached to the nylon mesh by hooks (Figure 3). In this way, only one tool and ten meshes (five for the running water zone samples and five for the static water zone ones) were needed. Once the sample had been collected, each net was then placed into a marked flask and sealed for the journey to the laboratory.

Once in the laboratory, the samples were cleaned and the content was put into a translucent tray with an illuminated bottom. The different specimens were identified and counted, with the help of a stereoscopic magnifying glass (Figure 5). As the observations were done within a few hours (usually within 24 hours of sampling) of the collection no preservatives were used.

Up to this point, the work could be carried out by a non-specialist, providing they have the proper training and follow appropriate protocols. However, at the point of identification of the organisms it becomes more difficult for a non-specialist as there is a need to distinguish the different taxa used into the different indexes.

The Phylum, Class and Order identification did not involve too much difficulty as there are clear differences between the different groups. Thus, to develop our index, each morphological group was designated by the taxonomic group that had been previously clearly defined and by means of a distinguishing letter: Diptera A, Odonate K, Annelid A, and so on. In this way it became an easier task to handle "taxonomy" for non-specialist staff. This approach was possible as taxonomic identification was not the aim of the study but to understand the water quality on the basis of the presence or absence of the taxonomic groups.

In practice, cards that depicted the taxonomic characteristics were used for each group found into the river; wherein sometimes morphological aspects were emphasized to help to distinguish the different groups (Figure 6). To-date approximately 60 different morphological groups have been distinguished in our studies.

CS-7.6.   THE IBO-GF DEFINITION

In general terms, mathematical models of biotic indexes are based on numerical data that assign different values to the organisms according to three parameters:

- Diversity: number of species or taxonomic groups into the sample (for this index we used taxonomic groups with different morphology)

- Abundance: Number of individual species contained in a sample (for this index we used individual species that belong to an identified group)

- Tolerance: Species or a morphologic group ability to stand specific contamination conditions.

The models reviewed did not necessarily consider all three parameters, the major difference being the way in which abundance was assessed. As the most stable natural conditions favor increments of some of the species population, and the objective of our study was to not only estimate the water quality but also know that this quality was stable and maintained at each of the sample points, it was became paramount that the abundance of species had to be considered.

An abundance numerical factors table was developed, where intervals were chosen based on bibliographic data (see Table I). A tolerance factors table was then developed based on the experience in this study and using observation and comparison of the groups frequently found at different sample points and at the surrounding area, including the Ojailen tributaries. Morphological groups from cleaner waters and apparently with higher contamination intolerance achieved the maximum table values, and groups with more tolerance or clearly adapted to contamination received minimum values (Table II). The values used were estimates based on the values available at the time of the study. These have subsequently been adjusted as more information became available during the course of the study. In this way the actual river condition has been used to calibrate the IBO-GF index.

After several formulations and trials the following expression was developed:

$$IBO = \sum_{i=1}^{i=n} T_i A_i$$

Where:

T_i is the tolerance table morphological group factor

A_i is the abundance

The index was calculated for a running water zone and for a more static water zone, and for each of the sample points after adding the specimens collected in both zones.

CS-7.7. VALUE OF IBO-GF

The application of this index has allowed us to compare the quality of the river, along the river, at similar times, and over a period of years. The index showed quite accurately the circumstances that had disturbed the water quality over time (Figure 7a, 7b and 8).

The river water quality has been slowly improving due to the positive effect of the waste water treatment plant. In 1986 it had been ten years since that WWT plant started up. In 1986 the biological river quality was recovering after the Fluid Catalytic Cracking Plant startup in 1983. In 1990 a new Coker plant became operational.

Since 1992, due to a drought, a high percentage of the treated waste water is recycled, and this may have an effect on the receiving water and thus the aquatic life. There is good evidence to show that even when legal control parameters have been met, the salinity concentration has increased and it is likely that other chemicals will also have varied concentrations.

From Figure 9, it can be seen that IBO-GF values for sample point 4 follow the same tendency as other points sampled further away from this point, up to 1995 when these values change (and more in 1999-2000) coinciding with the revamping of the styrene, mono-propylene oxide and polyol petrochemical plants, the waste water from which, are processed in the refinery waste water treatment plant. This situation holds up to 2001 when the ratio between the sample points 3 and 4 starts to decrease, a situation that is still seen in recent surveys and that it must be related to a better chemical effluents control. In table IV, the chemical analysis shows the water quality at the different samples points.

Also from Figure 9 a sort of "biological buffering" can be appreciated. The IBO-GF values obtained for sample points 1, 2 and 3, which were set further away than the others, evolved in a quite parallel way, subjected to a combination of natural factors (beyond the temperature and flow, as it was verified) rather than affected by the IBO-GF oscillations in sample points 4 and 5, which are closely dependent on the urban and industrial discharges. This may have been caused by accumulation of physical, chemical and biological circumstances that defined the ability of river

waters to auto purify at stations 1, 2 and 3, or it could have been related to a macro-invertebrate life downstream shift when the water quality upstream got worse.

The IBO-GF index also helped to explain the cause of some of the river incidents, from fish death due to an oxygen default (as a result of an algae bloom into the "tablas de agua" zone), to infection by the water mould, "saprolegnia", coinciding with a high drop of the waters temperature, and consequently reducing fish defenses. The river also suffered some episodes of specific problems of excessive oxygen demand that did not affect the animal life, but produced changes to the benthic level.

Over all, the IBO-GF has been useful to justify the effort made to purify the waste water, providing a wider knowledge of the medium and its sensitivity to the effluents quality. On the other hand, being able to follow the life evolution into the river quality has justified the high investment made (and still being made) on the waste water treatment plant. There are no doubts about the index usefulness, and we recommend to any staff responsible for waste water treatment installations the implementation of a self-index, developed as the IBO-GF (similar models have been developed for applications to sea effluents and it can be assured that with a small effort it could end up being very useful).

CS-7.8.   OUTSTANDING TASKS

It is an outstanding task to define the circumstances under which the metamorphosis phase of organisms occurs. This becomes important when the absence of an organism is due not to water quality changes but because the organism has moved to the aerial phase.

If these circumstances are identified, correction factors can be applied that will allow comparing the changes over short time periods. Currently it is only possible to compare between different points during the same sampling period, or the value of all of them within the same season but over different years, see Figures 7a, 7b, 8 and 9. It is also possible to compare the yearly mean values of each of the sample points, when it can be assumed that the seasonal variations and other changes are caused by similar events across the sampling points over the longer periods of time being assessed (Table III).

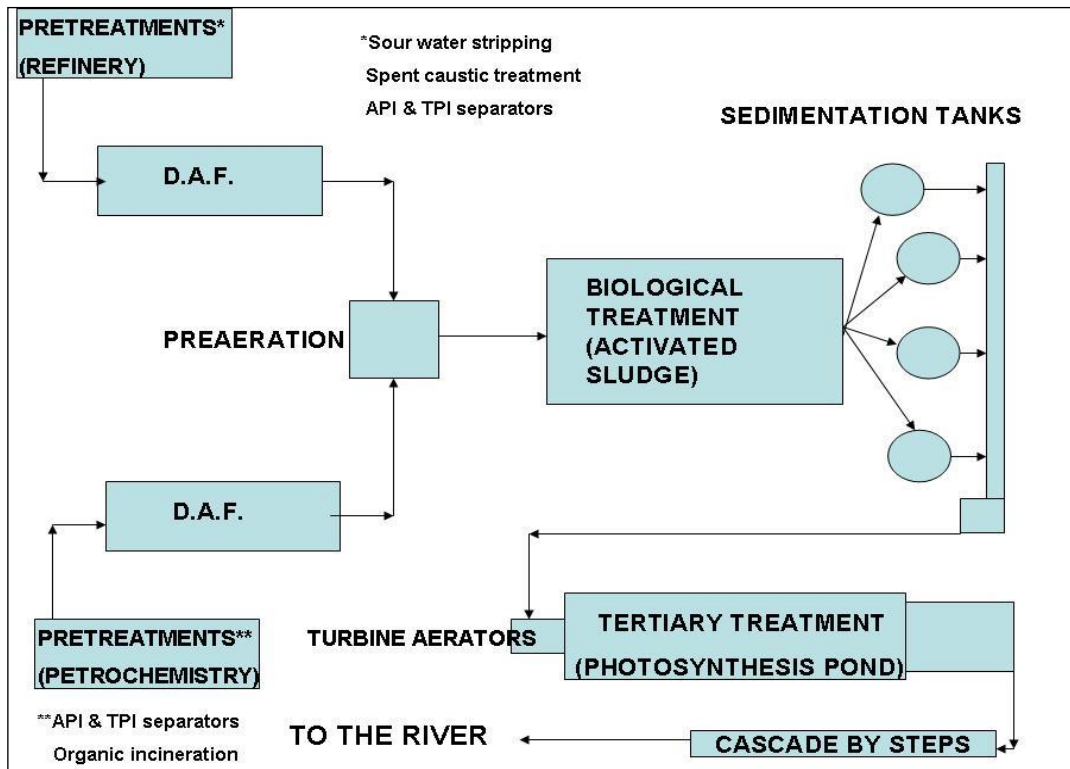**Figure 1** Puertollano Refinery Wastewater treatment plant

Figure 2 Sampling points at the Ojailén river and macro-invertebrates usually find in 1986
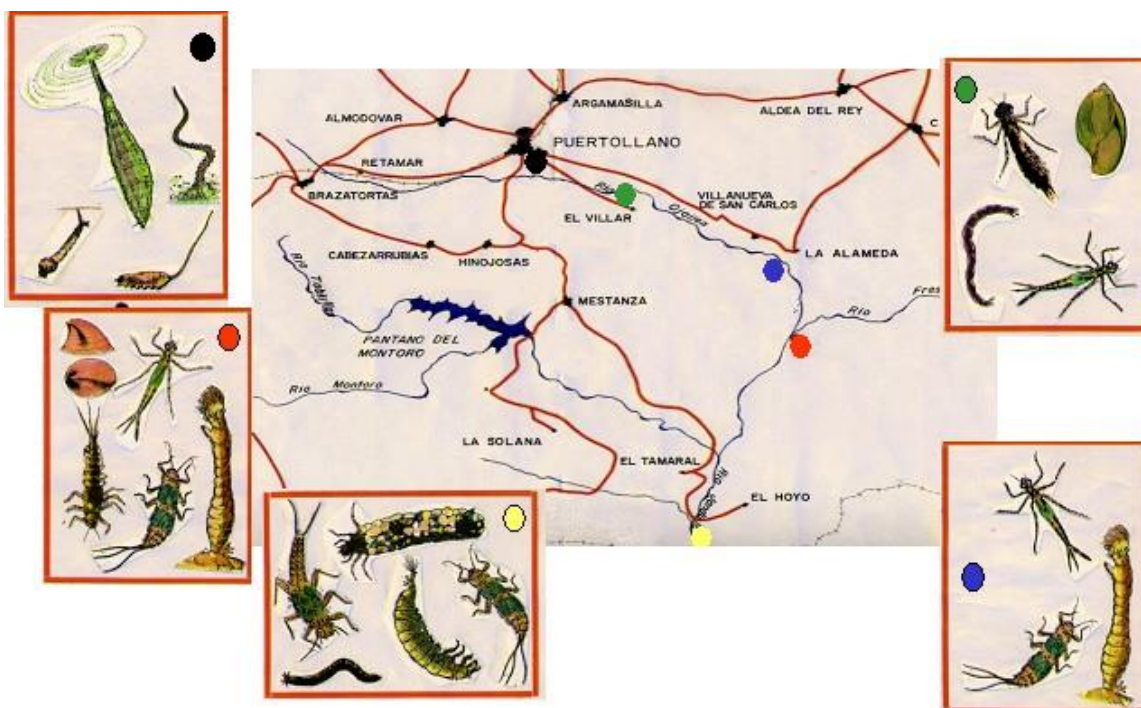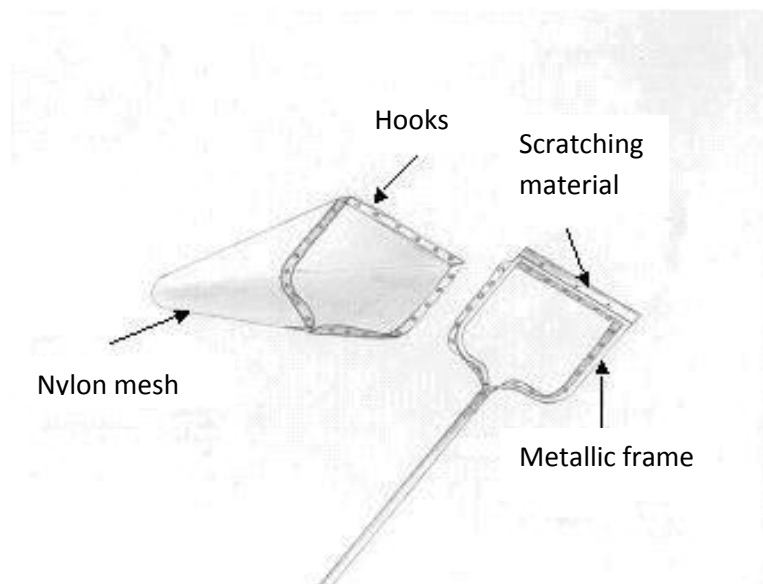
Figure 3   Hand aquatic-net

Figure 4   Field file

IBO-GF			
Field File			
Date/hour (solar)**: - - /**			
Approximate flow (m3/h):			
Temperature		**Dissolved Oxygen**	
Water:	Air:	mg/l:	% saturation:
Colour:			
Cloudy			
None:	Medium:		High:
Foams			
None:	Somme:	High:	
Odour			
Spill C.I.:	Spill EDAR:	Other (describe):	Non odour:
Fauna Description:			
Flora description (aquatic and bed):			
Substrate/sediments:			
Other observations:			

Figure 5   Macroinvertebrate observation

Figure 6   Macro-invertebrates identification files

Figure 7a   IBO-GF at the different simple points between August 1986 and 2005
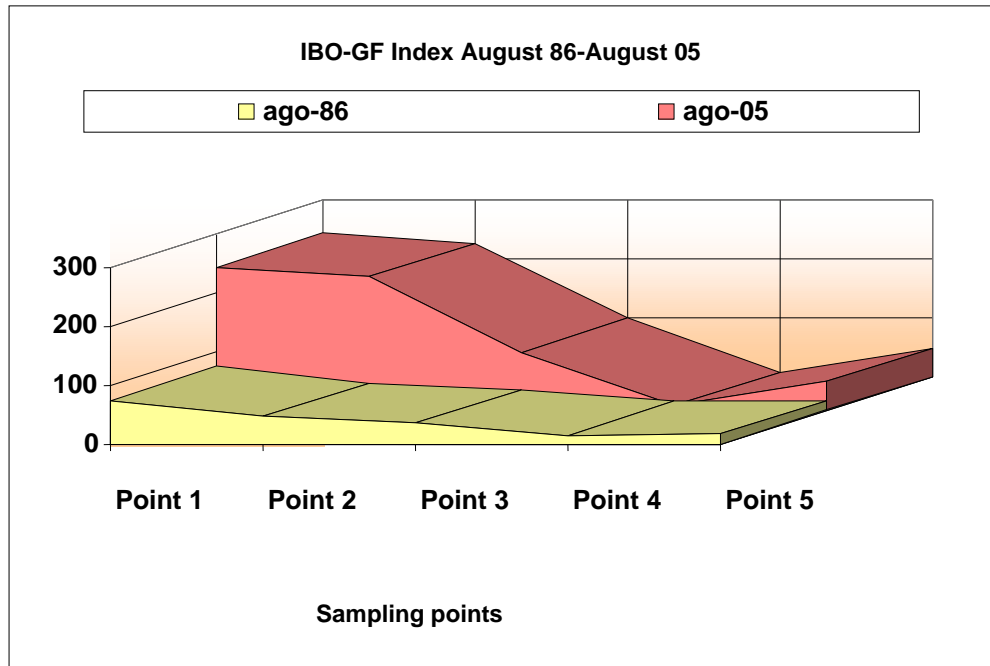

Figure 7b   IBO-GF at the different simple points between November 1986 and 2005
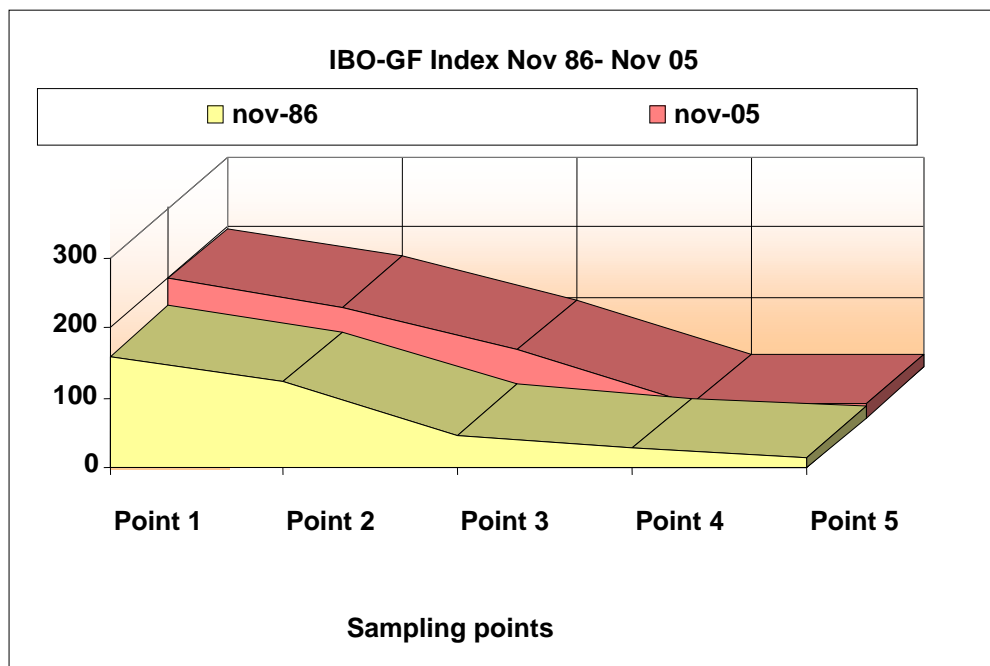
Figure 8   Mean monthly values evolution along the years 1986 and 2005
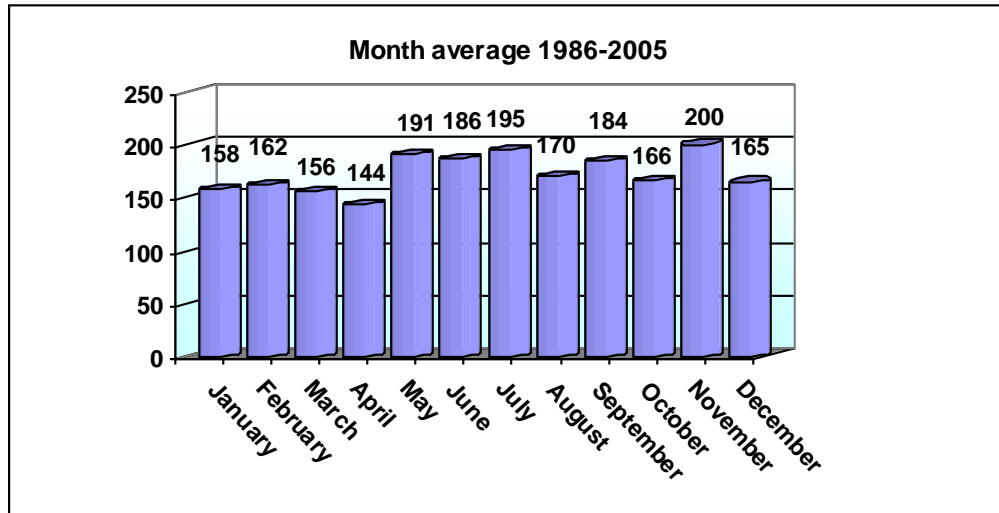

Figure 9   Accumulated mean of the year for each sampling point since 1986-2005
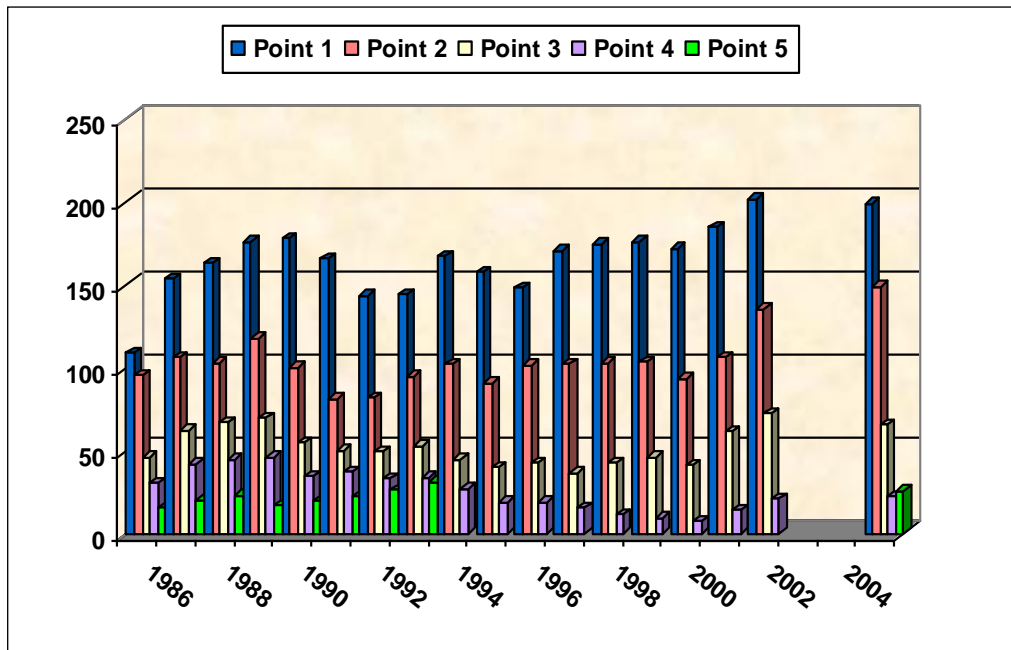
Table 1   Abundance factors (A)

Number of individual species	Factor
1 – 8	1
9 – 20	2
21 – 40	3
41 – 75	4
76 – 100	5
> 100	6

Table 2   Tolerance Factors (T)

Morphological Groups	Factor
Oligochaeta A	2
Mollusc A, B, C, D y E	5
diptera A, B, C, D, G, K, Ll, M, N, X e Y	4
Diptera F, H, P, W y Z	1
Diptera J	3
Coleoptera A, C, E, F, H, J y K	5
Odonata A, B, C, D, E, F, G, H, I y K	5
Hemiptera A y B	5
Ephemeropteran A, B, C, D, E y F	10
Megaloptera A	10
Tricoptera A, B, C, D, E y F	20
Plecoptera A	45
Others.	-

Table 3   IBO-GF year averages, 1986-2005

Year (months)	Sample point				
	1	2	3	4	5
1986 (7)	108,85	95,60	45,85	30,60	15,95
1987 (12)	197,40	116,90	78,20	53,60	25,10
1988 (10)	172,30	98,80	71,80	47,70	25,60
1989 (10)	188,30	132,40	73,10	47,50	11,60
1990 (5)	180,20	82,20	39,80	23,40	22,00
1991 (12)	153,80	62,80	45,00	39,10	25,80
1992 (12)	120,80	81,50	48,30	28,75	29,75
1993 (11)	144,80	108,20	56,20	34,60	35,00
1994 (12)	189,80	109,00	36,40	20,80	-
1995 (11)	147,50	79,10	34,90	10,50	-
1996 (10)	139,10	111,90	46,30	19,10	-
1997 (12)	192,25	103,10	30,40	12,90	-
1998 (9)	177,90	103,40	49,30	7,40	-
1999 (6)	176,70	103,70	49,00	7,67	-
2000 (10)	168,00	83,50	35,70	5,70	-
2001 (4)	197,25	119,50	81,50	21,50	-
2002 (6)	217,70	163,30	83,20	27,80	-
2005(9)	196,30	162,80	59,20	24,10	25,60
Total Average	170,50	106,50	53,60	25.70	
Average (9 years)	162,53	104,58	57,49	36,59	24,04

Table 4   Chemical water analysis – average for 2005

	Sample point				
	Point 1	Repsol Effluent	Point 3	Point 4	Point 5
pH	8,7	7,7	7,95	7,75	7,45
Ammonia (ppm)	0,17	3,6	0,22	7,84	37,8
BOD5 (ppm)	8	23	25	27	35
COD (ppm)	25	79	74	83	102
Phenols (ppm)	<0,1	<0,1	<0,1	<0,1	<0,01
Cyanides (ppm)	<0,02	<0,02	<0,02	<0,02	<0,02
Nitrate (ppm)	1,3	5,2	15,9	7,09	37,8
Fluoride (ppm)	0,61	0,6	0,75	0,53	0,34
Phosphorous (ppm)	0,26	1,12	0,48	0.75	2,9
Oil (ppm)	<2	<2	<2	<2	<2

CS-8. CASE STUDY 8: METHODOLOGY FOR MEASURING THE IMPACT OF TREATED WASTE WATER DISCHARGED IN AN ESTUARY

CS-8.1. SUMMARY & KEY LEARNINGS

The process was tiered and sampling stations selected by modelling, but more pre-evaluation of these sites should have been made before full sampling occurred. Examples of the type of problems experienced were:

- The mussels cages needed to submerged for 24h every day,

- Fish sampling could only be done in navigation channels and at sites large enough to provide a suitable habitat,

- Assumptions made about organism presence/survival need to be checked.

Inevitably problems will be encountered with this type of survey, for example when identifying appropriate reference stations. Thus in this study, the salinity of the upstream site was too low for mussel survival, and the data from station 10 appeared to indicate that the downstream reference site was contaminated. This together with a loss of the mussels at the only other site upstream of the effluent made it difficult to attribute biomarker responses at the downstream stations.

Local laboratories were used for immediate fixing and sample preparation prior to sending to specialist laboratories for the taxonomic and biomarker determinations. This thus prevents any deterioration of the samples prior to specialist assessment.

The physical conditions which exist in estuaries, for example the variation of salinity, alternating tides, currents, variation in temperature and granulometry of the sediment on the biological community, are such that they can mask other potential disruptions associated with effluent discharges to the populations. Furthermore, the results showed that the Trophic Level Index could not be used for this type of monitoring because it could not account for the influence of these physical constraints.

In this study no major perturbation was caused by the effluent on the physicochemical state of the estuary and the indicators used indicated no significant impact on the biological community or the mussel biomarkers. Despite the presence of PAH in some sediments, especially in the area where the effluent was discharged, no genotoxic or other irreversible effects were observed in the fish.

To improve estuarine monitoring the methodology used in this study to assess the impact of refinery effluent discharges could include:

- The selection of reference stations of a comparable salinity to those into which the effluent is immediately discharged to aid the interpretation of results.

- The organisms which are naturally present should be given primary consideration as community indicators.

- If it is necessary to keep mussels in cages, duplicate the cages, thus minimising the potential for total loss of control information.

Despite the comments above, it should be noted, that there may be occasions when it is impossible to identify reference stations that meet all the requirements suggested. This will especially be the case where there are upstream industrial discharges.

CS-8.2.   OBJECTIVE

Under the current rules and regulations, the surveillance of the impact of industrial waste disposal in the natural environment represents a major issue. The application of the Water Framework Directive (WFD) imposes that by 2015 the quality of surface water should be good with regards to chemical content and ecological aspect (EC, 2000). All types of surface water are concerned: rivers, lakes, canals, transition zones (estuaries) and coastal areas (one mile from the coast). The regulations to govern this are currently being established in the Member States.

The methodology for measuring the impact of effluents in the estuaries is not known, there is as yet no standard for biological indicators. The purpose of this project was to assess the methodology for measuring the impact of industrial effluents in estuaries and examine their utility under the Water Framework Directive.

CS-8.3.   PRINCIPLES AND METHODOLOGY

The project was undertaken over a period of several years (2003-2005). The key stages are summarised below.

CS-8.3.1. Choosing a site

While the principles can be applied to any effluent and river/estuary, for this project a European estuary was chosen into which a refinery effluent discharged.

CS-8.3.2. Setting up the campaign

The organisation of the campaign was carried out as follows:

2003

- Decision to validate the methodology for measuring the impact of effluent in estuaries
- Consensus on the biological responses that needed to be studied.
- Modelling of the effluent plumes

March 2004

- Preliminary campaign with a selection of samples of sediment and measures of salinity at the different pre-selected stations.
- Confirmation of the stations

Beginning of June 2004

- Placing cages of mussels in all stations

Beginning of July 2004

- Start of the campaign
- Taking of samples
- Preparation of samples on site (using local laboratories)
- Dispatch of samples to the specialist laboratories

October 2004 – October 2005

- Summarising and interpreting the results

CS-8.3.3. Choosing the observation stations

Compared to measuring the impact of effluents in rivers, which is relatively simple because the reference station is upstream of the effluent discharge, and the study station is downstream, the choice of stations is more complicated in estuaries or in the sea, where flows are impacted by tidal variations.

The location of the effluent plumes and the definition of the surrounding area were carried out by modelling.

CS-8.3.3.1. Definition of the surrounding area

A software program (DREAM (Dose Related Risk and Effect Assessment) 2.0 beta software) used by the offshore oil industry was used to model the effluent plumes under study in the estuary. This program is used to forecast the risk of aquatic ecotoxicity in the sea. This software model was developed by Sintef, RF-Akvamiljo and TNO in collaboration with Total, Agip, Hydro and Statoil. This program was not developed for modelling in estuaries, however it was used to help locate the effluent plumes and to position the measuring stations.

CS-8.3.3.2. Stations

Ten stations were positioned according to the saline gradient in the estuary (Figure 1a). The upstream reference station (N° 1) is in a brackish environment. The downstream reference station (N°10) is situated in the sea. The samples of sediment for physicochemical analysis and benthic invertebrates were taken from all 10 stations. The cages of mussels were placed at all 10 stations. The only difference being that station 10 for the positioning of the mussels was slightly different from station 10 for collection of fish. This was because, being nearer to the coast line, the mussel cages remained under water for a month. Benthic fish collection could only be carried out in the navigation channel. Collection was thus only carried out at three stations (stations 3, 6-7 and 10), because the stations had to be large enough to provide a suitable habitat for the fish.

Figure 1a   Positioning of the study stations along the estuary
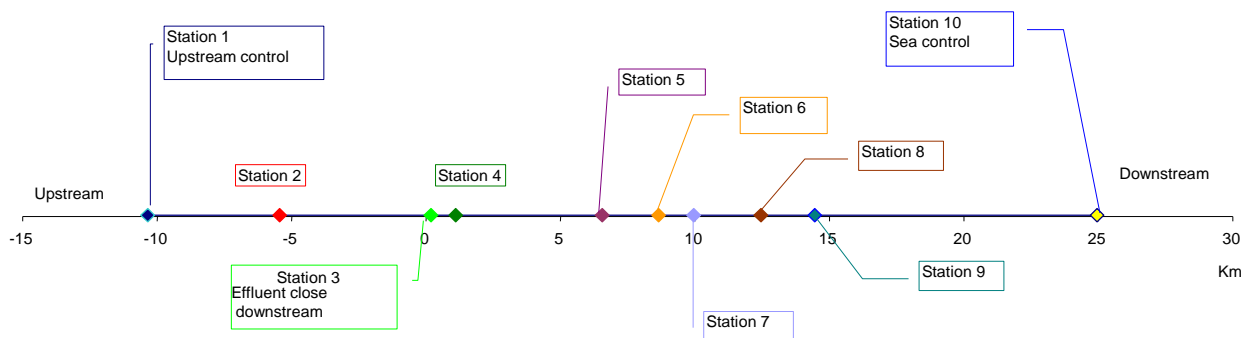
CS-8.3.4. Determinants

CS-8.3.4.1. Physicochemical Measurements

During this campaign, the following were measured in the water;

-    T°, salinity, conductivity, turbidity, nitrates, orthophosphates.

The analysis of the sediment covered;

-    granulometry, dry matter, TOC, Total Nitrogen, Total Phosphorus, Total PAH, Total hydrocarbon

And specific analysis of the sediment for;

-    Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Zn.

CS-8.3.4.2. Biological Measurements

Benthic Invertebrates

The objective of the WFD is to achieve "a good ecological status" for waters, which would need to be measured by ecological indicators. It is therefore important to test such indicators, to ensure that they are capable of detecting modifications in the structure of the community of the ecosystems under consideration. Such assessments would also give information on the biodiversity, and how it changes, in the zones where the effluent discharges.

However, there are no standard ecological indicators for estuaries in Europe. As an alternative, a marine ecological indicator, the Trophic Level Index, TLI, (Word, 1990) based on the invertebrate benthic fauna was chosen. Although this is not standard, it is being used increasingly throughout Europe. The TLI is based on the trophic relationship between different types of invertebrates. Any change in these trophic relationships, compared to the normal situation, is a sign of dysfunction, and therefore provides an indication of the quality of the sediment and the over-lying water. The TLI is especially useful for when the pollution is of organic origin.

After having collecting samples of sediment at the 10 stations, these were sifted, fixed and then the species present identified by experts.

Microbial Loop

The microbial loop is a term coined to describe a trophic pathway in aquatic environments where dissolved organic carbon (DOC) is reintroduced to the food web through the incorporation into bacteria. Bacteria are consumed mostly by protists such as flagellates and ciliates. These protists, in turn, are consumed by larger aquatic organisms (for example small crustaceans like copepods). As microbes are the base of the food web in most aquatic environments, the trophic efficiency of the microbial loop has a profound impact on important aquatic processes. Such processes include the productivity of fisheries and the amount of carbon exported to the ocean floor. Although still in the experimental stage, the assessment of the microbial loop, as a potential indicator of change in the structure of the community, was evaluated. This analysis was carried out on water samples taken when the cages of mussels (see below) were collected. The proportion of each group of the microbial loop was then calculated, without identifying the different species.

Biomarkers in mussels

The biomarker responses, measured in organisms known as field-exposed sentinels, are biochemical or genomic changes in the cells. The observation of these changes, compared with the unexposed control group, gives indications on the exposure of the sentinels to contamination or on the effect of contamination on the organisms in question: exposure biomarkers and effect biomarkers are then used. An exposure biomarker indicates an exposure that is a reversible effect, whereas an effect biomarker indicates an irreversible effect. This approach has still to be standardised and many of the findings are difficult to interpret. One of the key challenges being to link biomarker responses to an effect on the ecosystem, it is important that such data are carefully interpreted.

In this study, mussels were selected as the sentinel organisms. A method for caging mussels was used as indigenous mussels were not present at all the stations, notably the upstream stations, where the salinity is much lower. The weak salinity of the stations in the upper estuary introduced a concern for the viability of the mussels, which was considered to be approximately at their lower tolerance limit of 15 parts per thousand. A preliminary campaign in March was used in making the selection of all the stations that can be found in Figure 1a.

Cages of 500 mussels were used. The cages were maintained in the water column using an anchor and float system. One cage per station was placed between the 7th and the 10th June 2004 and remained under water for one month. To complement this, mussels were obtained from the original supplier of the mussels to constitute a reference station 'R'.

After the cages were collected, the mussels were immediately taken to a local laboratory for dissection to provide tissue samples (digestive glands and/or gills) which were then either frozen in liquid nitrogen, or immediately analysed for exposure and effect biomarker responses.

- Exposure Biomarkers:
    - Index of condition (Crosby and Gale, 1990)

- Inhibition of acetylcholine esterase (AchE) (Ellman et al, 1961): neurotoxicity biomarker;
- Benzo(a) pyrene hydroxylase activity (BHP) (Dehnen et al, 1973): biomarker which is activated in the presence of PAH (polycyclic aromatic hydrocarbons);
- Catalase activity (CAT) (Clairbone, A., 1985): biomarker of oxidizing stress;
- Glutathion S transferase (GST) (Habig et al, 1974): metabolic biomarker;

- Effect Biomarker:
  - Rate of malonedialdhyde (MDA) biomarker of oxidizing stress and of lipidic peroxydation: deconstruction of membranes.
  - Hemolymph biomarkers: cell damage (Neutral Red assay) (Lowe and Pipe, 1994; Grundy et al, 1996): the altered membranes of the lysosomes exude a red colouring: the length of time that this colouring is retained (Neutral Red Retention Time: NRRT) is measured.
  - Biomarker of genotoxicity (Heddel at al, 1983; Seelbach et al, 1993; Kramer, 1998; Zoll-Mereux and Ferrier, 1999): micronuclei - the presence of micronuclei is considered to be a consequence of genomic instability.
  - Immunotoxicity (Hansen et al, 1991): the ability to phagocytose shows the state of health of the organism.

Biomarkers in fish

Fish from the sampling sites were used to measure biomarker responses; only benthic fish were used as they are comparatively stationary and could better reflect the local conditions. The fish were captured in nets from a trawler. Benthic fish such as the flatfish *Solea solea* were obtained from 3 stations: station N°3 (effluent point), station 6-7 and station 10 (sea reference). During this campaign, a decision was made not to choose an upper estuary station because it was thought that the benthic fish in the lower estuary would not be present in fresh water. This was later proved not to be true. The fish that were collected were immediately brought to the local laboratory to take samples for biomarker analysis.

- Exposure biomarkers:
  - BILE FAC (Beyer et al, 1998): metabolite presence of PAH in bile, which shows exposure to PAH.
  - Cytochrome activity P450 EROD (Bucheli and Fent, 1995), which shows the metabolisation (phase I) of PAH ;
  - Catalase activity (CAT) oxidizing stress biomarkers (Clairbone, 1985).

- Effect biomarkers :
  - *Inhibition of the VTG vitellogenin* (Sumpter and Jobling, 1995): reproduction biomarker;
  - Genotoxicity (Dunn et al, 1987): DNA adducts (covalent bond between a substance to the DNA)

CS-8.4. RESULTS

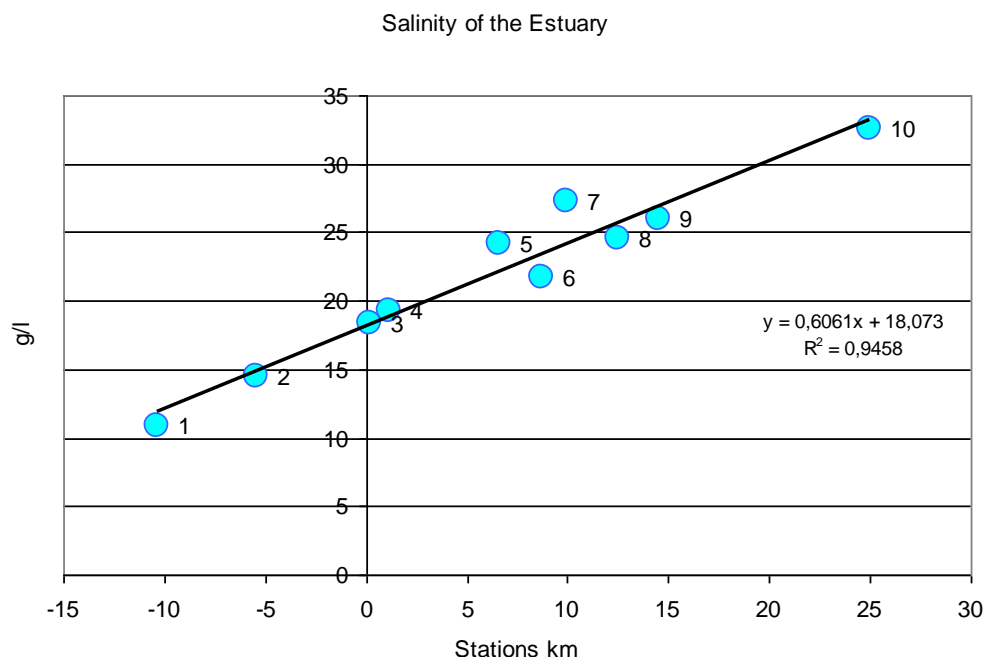### CS-8.4.1. Physicochemical Parameters

Table 1   Physicochemical parameters measured at all the estuary and sea stations
(Nd: non determined)

Stations	pH	Conductivity mS/cm	salinity g/l	T°C	Turbidity FAU	N-NO3 mg/l	P-PO4 mg/l	Distance from the waste discharge (km)
1 (ref upstream)	7,6	18,3	10,8	18,7	105	0,868	0,589	10,3
2	7,6	24,1	14,5	19,1	166	0,644	0,775	5,4
3	7,6	29,9	18,3	19,4	39	0,532	0,279	0,23
4	7,7	31,1	19,2	19,8	116	0,588	0,4526	1,15
5	7,9	32,6	24,1	20,1	170	0,462	0,992	6,6
6	8	29,3	21,7	20	24	Nd	0,248	8,7
7	8	42,8	27,2	17,7	66	0,196	0,31	10
8	8	33,1	24,5	19	Nd	Nd	Nd	12,5
9	7	41,1	26	18,5	80	0,35	0,403	14,5
10 (ref downstream)	8,2	50,4	32,6	17,3	5	0,084	0,031	25

For this case study, the national reference system for the assessment of the quality of water was used (SEQ-EAU v1, 1999; http://sandre.eaufrance.fr/Le-referentiel-de-donnees-sur-l). The comparison of the values obtained for the parameters measured in the estuary shows that the temperature, the pH and the nitrates gave all stations a grade of "very good quality". Based on orthophosphates content, the water is graded "good quality" for all stations except numbers 1, 2 and 6 where the quality was "passable"

Figure 1b shows evidence of the high degree of salinity along the estuary in the area that was explored.

Figure 1b   Salinity according to the distance from effluent discharge (point 3)

Salinity of the Estuary

y = 0,6061x + 18,073
R^2 = 0,9458

The analysis of physicochemical parameters **in the sediments** was carried out for metals and Polycyclic aromatic hydrocarbons (PAH) the data are shown in Annex 1. The amount of total organic carbon, total nitrogen, arsenic, copper, mercury, zinc, chrome, aluminium, nickel, cadmium, lead and total hydrocarbons, along the length of the estuary (that is from stations 1 to 10) remain at levels between low and medium thresholds (N1, N2) (see below). Phosphorus was at a higher level. The PAH levels varied and were high downstream near the point of the effluent discharge (station N°3).

The sediment in the area of the discharge point is made up of almost pure silt in which the proportion of fine sand increases in the direction of the lower estuary near to the sea. The sediment in estuary mouth is poor in carbon and nitrogen but rich in phosphorous, the origin of which can be linked to urban wastewater. The levels of all three nutrients diminish in the direction upper estuary to lower estuary, parallel with the decreasing levels of fine particles.

Likewise, the levels of contamination by metals and hydrocarbons are generally low to medium and reduce from upper estuary to lower estuary. The levels of metal contamination differ slightly according to the element under consideration. The evaluation of these levels (low, medium or high) is carried out firstly in comparison with the geological background levels defined by national research groups and secondly in comparison with the thresholds N1 (<N1: absence of contamination) and N2 (>N2: high contamination) fixed by national regulations on harbour sediments. It should be noted that these levels have nothing to do with the toxicity thresholds. They have been fixed on the basis of background level, where N1 is the background level multiplied by 2, and N2=2xN1. (According to « Arrêté 14 june 2000 », relative to reference thresholds for marine/estuary sediment in natural ecosystem or in harbour)

CS-8.4.2. The responses of the benthic invertebrate communities

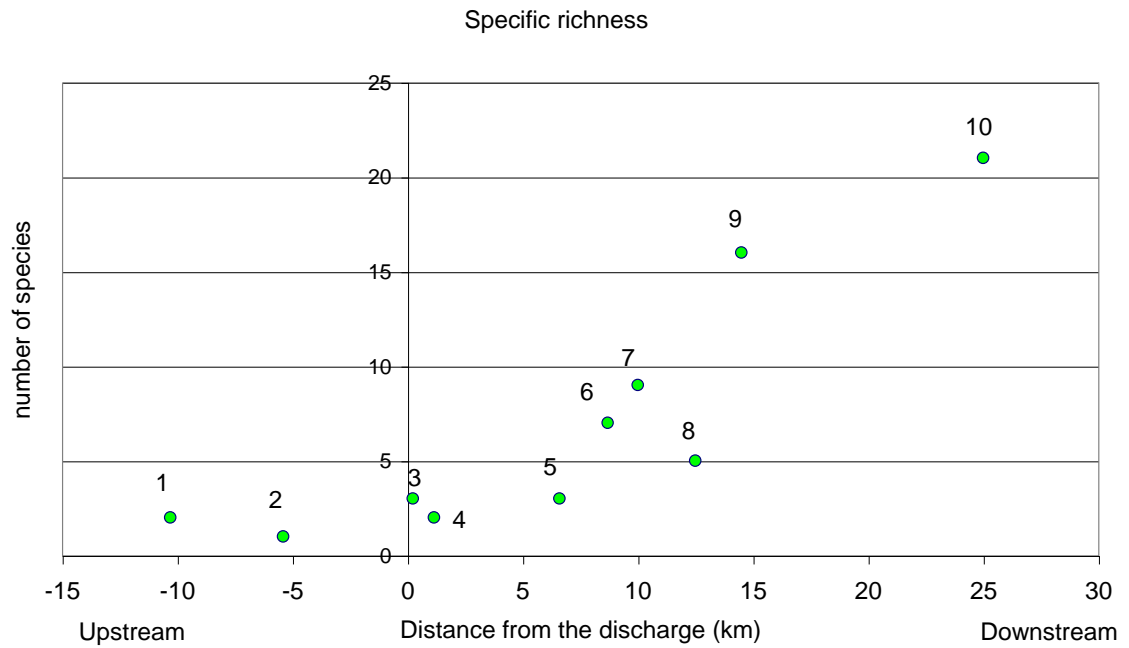*Figure 2*   Spatial evolution of the specific richness of benthic invertebrates

Specific richness

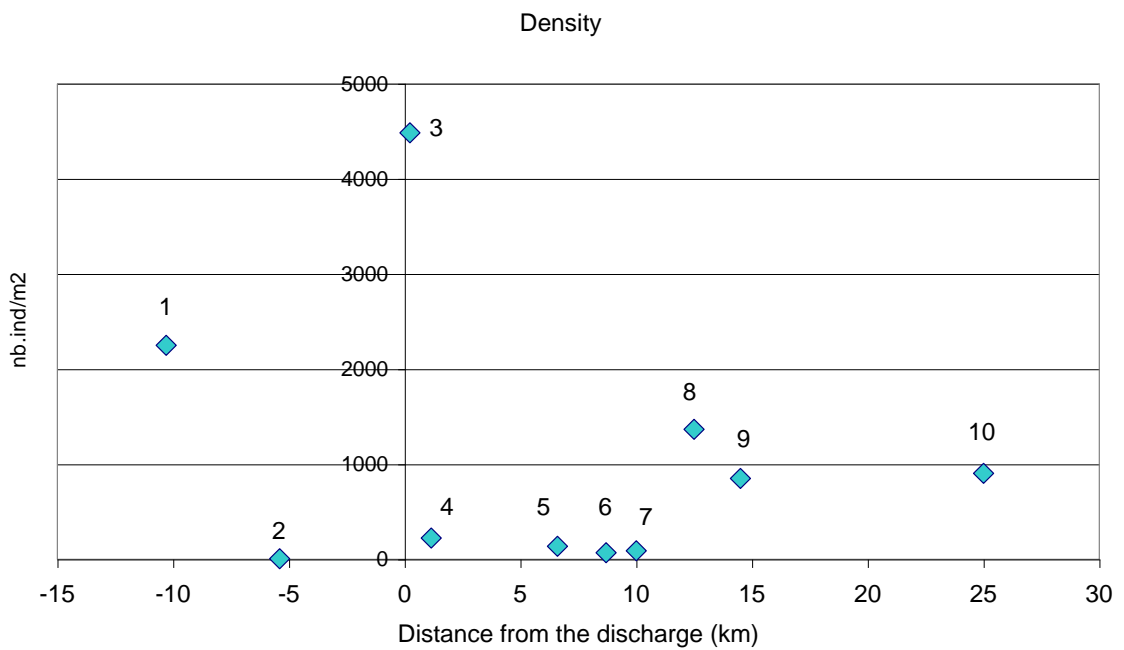*Figure 3*   Spatial evolution of the total density of benthic invertebrates

Density

Figure 4   Spatial evolution of the Shannon Index for the community of benthic invertebrates

Shannon index
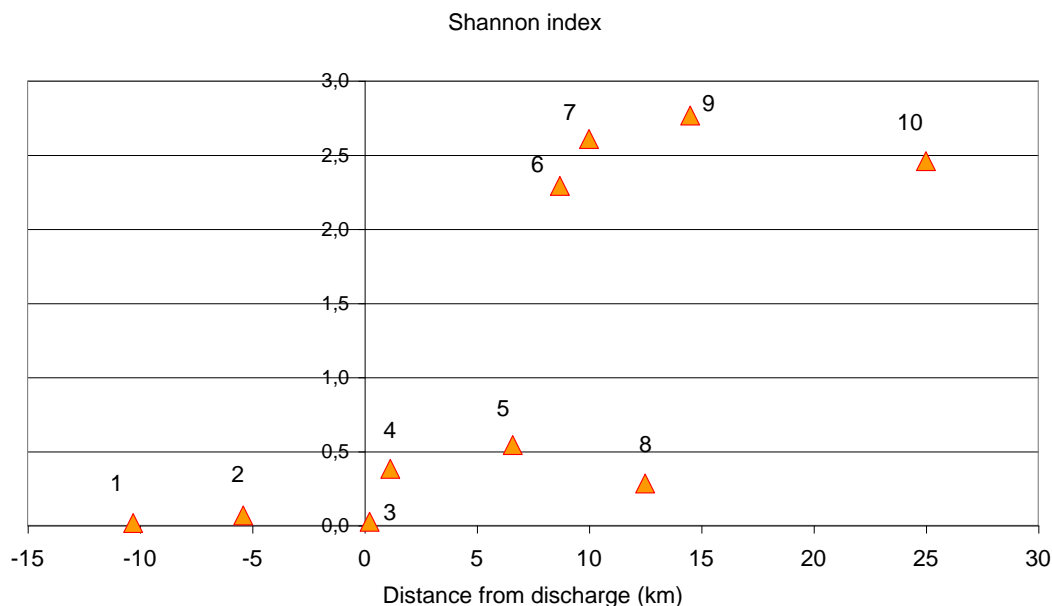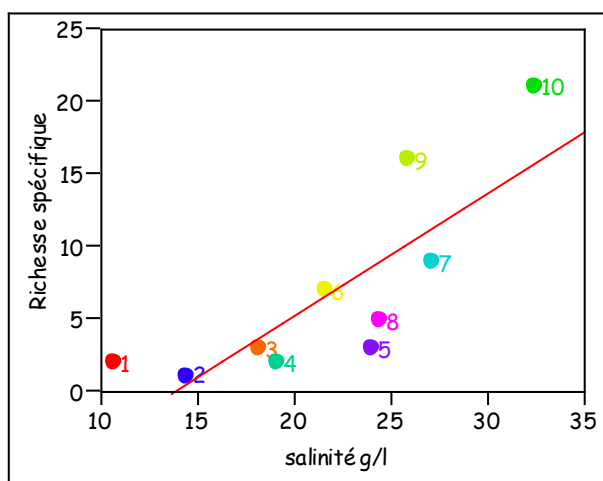

Distance from discharge (km)

Figure 5   Correlation between the specific richness of benthic invertebrates (biodiversity) and salinity along the estuary

Specific richness = -11.54219 + 0.8424938 salinity g/l; $r^2 = 0,65$; $p < 0,0048$

One can observe the presence of 21 species of which there are 9 up to station N°8 (Figure 2). These values correspond to a very weak biodiversity in the upper estuary and to a weak biodiversity in the downstream estuary. An average of local biodiversity is between 30 to 50 species. The specific richness (Figure 5) increases where the level of salinity is higher. The constraints due to the level of salinity and the physical constraints (currents) can explain these low values.

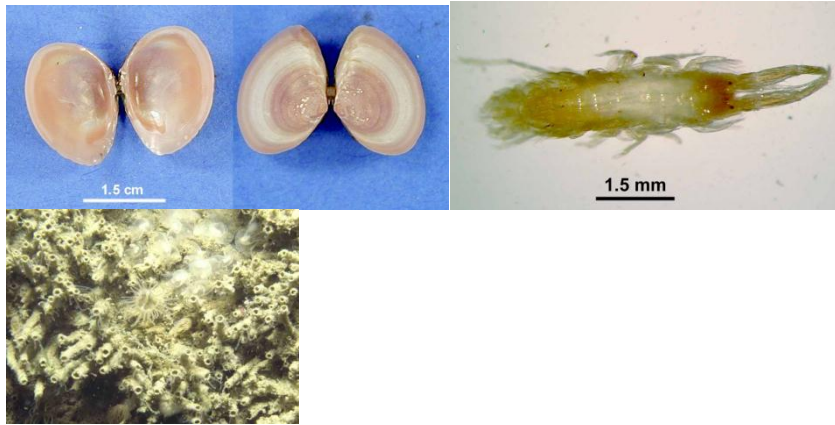Dominant species are illustrated in photos 2 to 4.

Photo 2 - *Macoma balthica* (Baltic Tellin)   Photo 3 - *Corophium volutator*
Photo 4 - *Polydora ciliata*

In terms of density or abundance (Figure 3), discharged wastewater is responsible for a limited increase in abundance because the supply of organic matter is consumed by the fauna which can be found there. This result indicates an increase of organic matter, but no toxicity effect for those organisms.

It can also be noted that a variation in the Shannon Index at station 8 indicates a disturbance in the structure of the community (Figure 4) probably due to the presence of a harbour at this station.

Figure 6   Spatial evolution of the Trophic Index (TI)
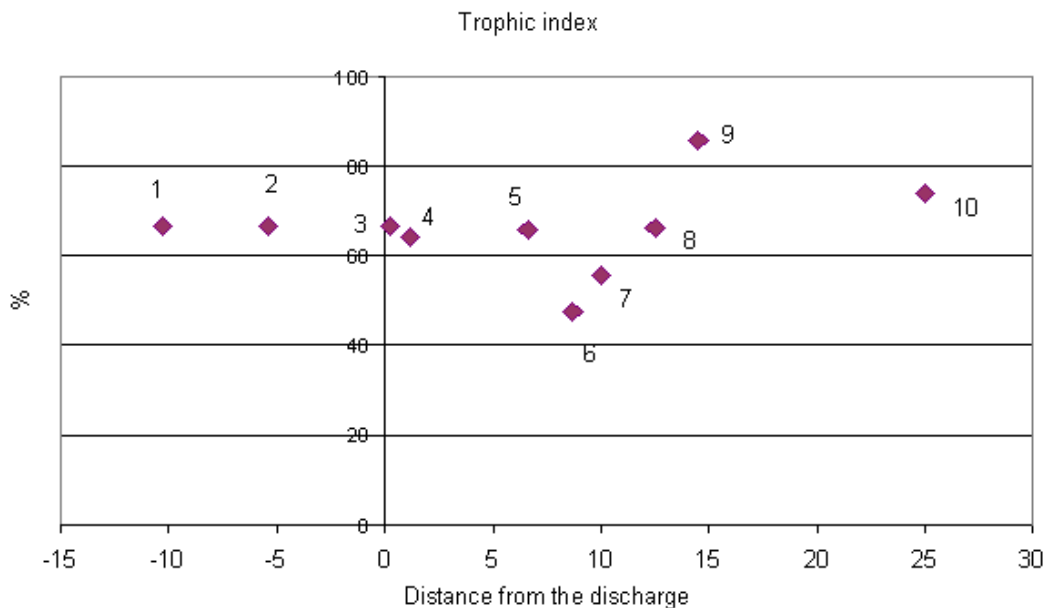
The trophic index was applied to the information collected along the estuary. This index was worked out as described by Word (1990) to evaluate the state of degradation of the ocean depths from the discharge of domestic effluent. This approach is based on species belonging to four trophic groups; which are defined

on the basis of three criteria: the size and type of nutritive elements collected, the compartment in which this material is collected and the trophic strategy set up to collect this material.

Upstream from station N°8, almost all of the present organisms belonged to the trophic group 2. This group is made up of species that feed on very fine particles. Belonging to a unique trophic group excludes the calculation of the index based on the trophic relation between trophic groups: the area where effluent is discharged being, situated upstream from station n°8 therefore, finds itself outside the field of application of the trophic index.

As mentioned above, only the relative values of the stations situated downstream from station N°8 can be used. The spatial distribution of the trophic index does not therefore allow us to give evidence of the effects of effluent on the benthic population of the estuary.

Therefore, for these conditions in the estuary, the trophic index was not applicable. However, a specific index developed for this type of environment with a stressed population experiencing strong physical constraints (Biological potential, (Creocean, 2000)), was applied. This index does not give evidence of the effect of the effluent on the benthic invertebrate fauna, but was developed for euryhaline environments. Consequently, it has proved better adapted to this type of environment because it takes into account the numerous physical constraints. It provided a tool to demonstrate the absence of a negative effect due to aqueous emissions. On the contrary, the organic matter released permits the populations of opportunistic species to form very abundant local benthic populations.

In estuaries as in coastal lagoons, the presence and the dominance of a species depends primarily on its tolerance of the physical conditions of the environment (in estuaries this is due to fluctuations in salinity and large variations in currents and hence the mobility of the sediments). Large and practically mono-specific populations are present at certain stations, whereas at others the benthic organisms are extremely rare. These characteristics of a mosaic population are normal in the transition section of an estuary, since these sections are subject to daily alterations in supplies of fresh water from the river and salt water from the sea. The conditions of wide fluctuations in salinity, added to the instability of the sediment due to the effect of the currents, are a large constraint to the estuarine fauna, usually formed of species of marine origin which are very tolerant of the desalination of the environment. The physical constraints which exist in this type of environment are so strong that they can mask the potential disruptions of the populations related to an excess in the supply of organic matter or micro pollutants. Species which are capable of tolerating this physical stress are effectively also capable of tolerating stress linked to over-enrichment of the environment by organic matter, which is often more of a constraint than physical stress.

The content of nutrients in the sediment does not indicate that the sediment is significantly enriched, nor that the effluent is the source. The concentrations observed for nutrients in the sediment were reasonably constant regardless of the distance to the effluent discharge, and do not cause disturbances in the benthic population, which is naturally very poor.

To conclude, the impact of the emission is weak and has no toxic effect on the benthic population, whereas the supply of organic matter seems to have a local beneficial effect on the population.

CS-8.4.3. Microbial Loop Responses

The analysis of the microbial loop in the waters of the estuary did not show significant changes of one microbial compartment compared to another regardless of the station examined. This parameter does not therefore appear to be relevant for this type of investigation. The analysis of the microbial loop may be more relevant when the observation is carried out on hard substratum, which was not the case for the estuary stations.

CS-8.4.4. Biomarker Responses

CS-8.4.4.1. Mussel biomarkers

The mussel cages were collected at the beginning of the month of July. Certain cages (stations N°2, 8 and 9) had been swept away by a higher than predicted rise in the water level (the length of the chain linking the cage to the mooring was not long enough to deal with such a water level change). The mussels placed at station N°1 did not survive, probably due to the low level of salinity.

The responses of the exposure biomarkers

Benzo(a) pyrene hydroxylase activity (BHP, GST, Catalase, AchE) alter according to the pollutants to which they are exposed: each biomarker has a certain specificity, which is why it is necessary to interpret all the responses of the biomarkers together.

To aid the interpretation of the biomarker responses, the value of each biomarker for each station has been transformed into an index of response known as the Global Biomarker Index (GBI) which is assigned a colour representing the state of pollution (Narbonne et al, 1999).

This method is detailed in the protocol for processing referenced statistics: TS-EIM (Narbonne et al, 1999).

The principle stages are:

- Dosage of biomarkers
- Statistical analysis: Tukey test and discriminant analysis
- Selection of the most discriminant biomarkers: one biomarker per cluster
- Conversion into biomarker indexes
- Calculation of the index of pollution
- Grading and map-making according to Table 2.

Table 2   Level of pollution, multiple grade scale

GBIP	Level of Pollution	Colour
1 to 19	Slightly polluted environment	**Blue**
20 to 29	Moderately polluted environment	**Green**
30 to 39	Significantly polluted environment	**Yellow**
40 to 49	Heavily polluted environment	**Orange**
50 to 59	Highly polluted environment	**Red**

There is value in clearly explaining the choice and the criteria for selecting the biomarkers that were finally retained. For example, the introduction of the NRRT

biomarker, which proved significant in terms of general stress in place of the index of condition, makes the interpretations more refined.

The results of untreated information are given in Table 3.

The data from station 10 appeared to indicate that the marine reference site was contaminated. Although mussel-breeder's station was chosen as control, this, together with the lack of an upstream control (station 1 – mussels did not survive and station 2 the cage was lost), makes it very difficult to attribute the biomarker responses at the other stations. These responses could be due to the impact of the effluent, the salinity gradient, other physical factors and other discharges present in the estuary.

***Table 3***   Contribution of each biomarker to the GBIP

Stations	BI NRRT	BI ACHE	BI GST	BI BPH	BI CAT/TBARs	GBIP □	MPI
3	12	3	4	6	12	37	Yellow
4	12	6	4	12	6	40	Orange
5	12	6	4	3	12	37	Yellow
6	3	12	10	6	3	34	Yellow
7	3	6	4	3	3	19	Blue
R	3	3	4	3	3	16	Blue

BI: Biological Index
GBIP : Global Biological Index of Pollution

In the following sections, comparisons are made using the Dunnets circles. The way these are interpreted is explained in Annex 2.

Cell damage to the membranes of lysosomes (Neutral Red Assay) on hemolymph

Figure 8   Neutral Red retention time by lysosomes from mussels (n=15) and photo N°5 of lymphocytes
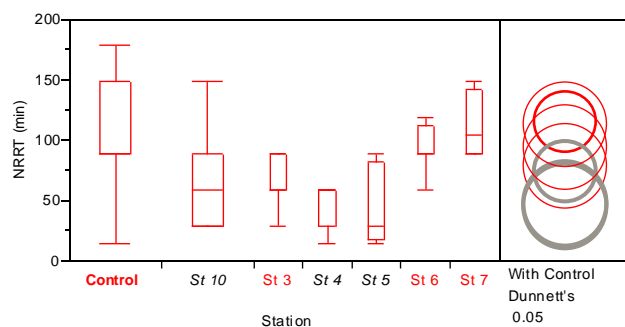
The longer the Neutral Red retention time, the better the state of health of the mussels. Stations 4, 5 and 10 seem to be the most significantly deteriorated, whereas stations 3 (effluent discharge), 6, 7 and the control station (mussel breeder) have not altered (Figure 8).

As already mentioned with the GBIP, it can be noted that station 10, which is supposed to be a reference station, has deteriorated. It can therefore not be considered as a reference station.

At this station the mussel cages were placed in a cove where motor boats come in to anchor. We can therefore suspect that the contamination is due to the presence of these boats.

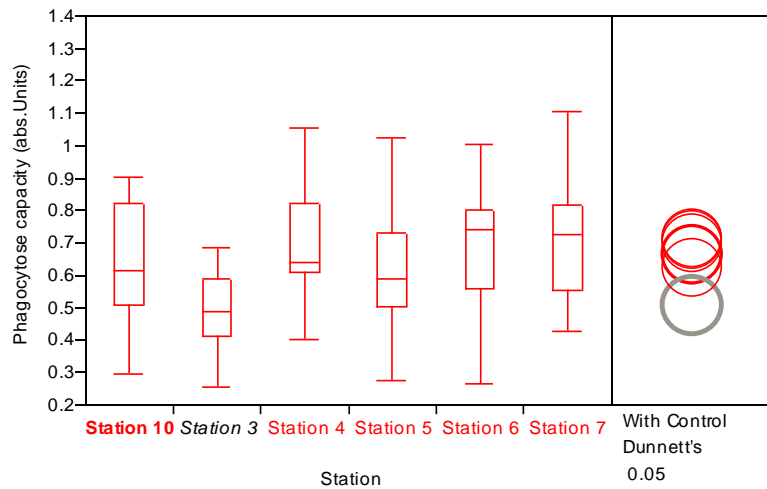### Genotoxicity biomarker: micronuclei

Figure 9   Frequency of the appearance of micronuclei in the hematocytes of mussels (n=70)
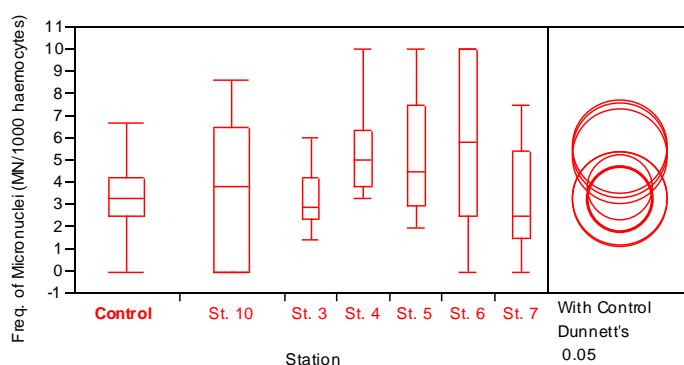
There is no significant effect on the micronuclei, so no genotoxic effect.

Immunotoxicity: the organism's capacity to phagocytose

Figure 10   Phagocytose capacity by unit of volume of hemolymphe in
mussels (n=144), at the different stations

Station 3 is the only station that shows that the capacity to phagocytose has significantly (p=0.05) deteriorated, when compared with the other stations. However as with the other mussel biomarkers, the lack of information upstream of station 3, makes it difficult to assess whether this is an effect due to salinity or pollution. Furthermore, there were no data for the mussel breeder's control stations (R), to assess the significance of the downstream responses.

CS-8.4.4.2. Fish Biomarkers

PAH Metabolites in bile (bile Fac)

Figure 11   PAH Metabolites analysed in the bile of fish caught locally at
stations 3 (downstream close to waste discharge), 6 and 10
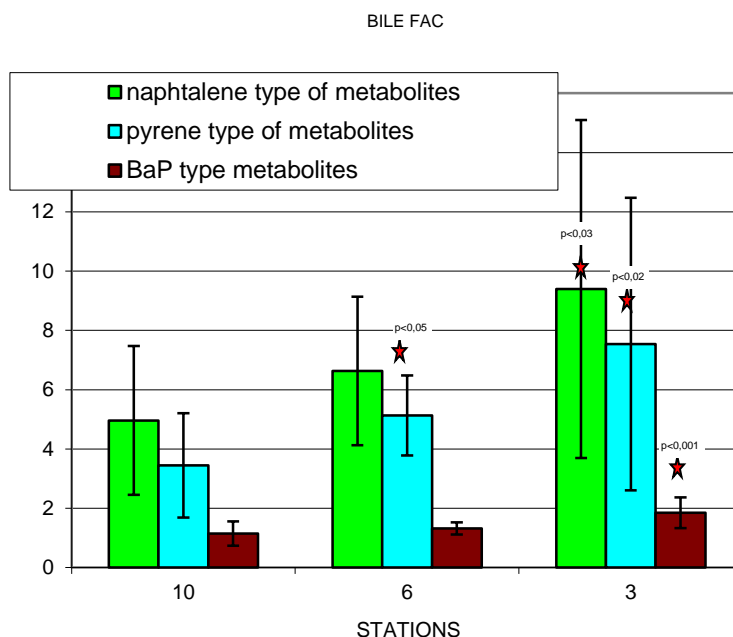
One must note that the fish reference station 10 is slightly different from that of the mussels, due to trawler fishing authorisations, it had to be positioned in the authorised channel. As far as fish are concerned, this station seems to be more significant as a reference station because it is not contaminated, as indicated by the results of the sediment analyses taken there.

The presence of PAH Metabolites can be noted in significantly larger quantities at station 3, with a reduction at station 6 and even lower levels at the reference station 10 (Figure 11). This indicates that the fish caught near where the effluent is discharged have been exposed to PAH and that the metabolisation process (detoxification) was under way. These results are in line with the higher concentration of PAH in the sediment. It should be noted that an upstream station is missing and therefore it is not possible to confirm the source of contamination. On the basis of these results it is not possible to conclude that the fish were exposed to PAH coming from the effluent. Therefore this point must be taken into consideration when establishing the definitive methodology.

EROD Activity

Figure 12   EROD activities in flatfish at the three stations under study
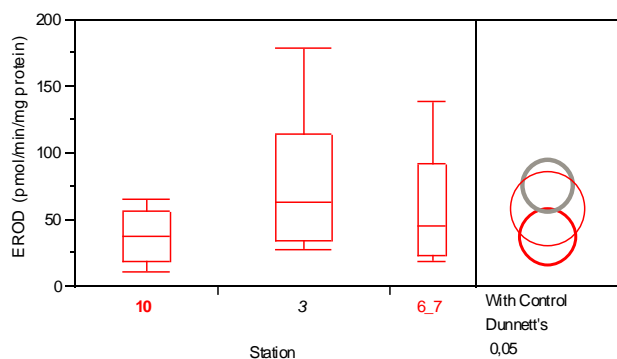
The activation of EROD shows that the process of detoxification of substances such as PAH has been put in place; this biomarker indicates the same tendency as bile FAC. It can be noted here that this activity is significantly higher at station 3, which corroborates the exposure to PAH, which was indicated before (Figure 12).

Catalase Activity

Figure 13   Catalase activity in flatfish at the 3 stations under study
(waste discharge: station 3)
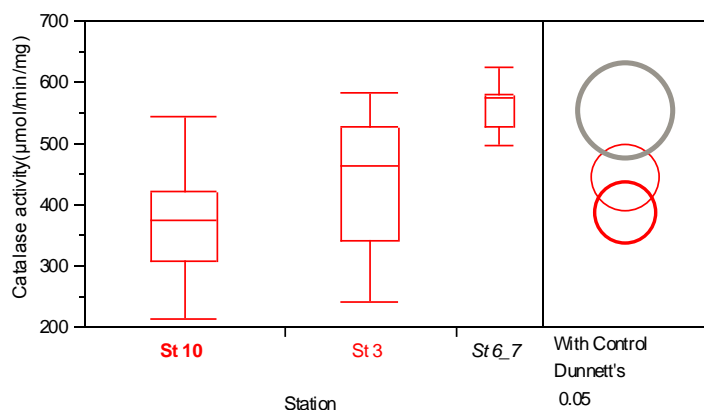
An increase in oxidizing stress was noted at station 6-7 (Figure 13), which is difficult to explain. This change shows that taken as an isolated event, it is difficult to interpret the exposure biomarker responses. Despite the solution of pooling several individuals it is possible to come across situations which from time to time show the activation of one biomarker and not others, which is why it is recommended that one work with a whole range of biomarkers.

Vitellogenin

Figure 14   Level of Vitellogenin in the plasma of female flatfish (a) and males (b) at the 3 stations under study (waste discharge: station 3)
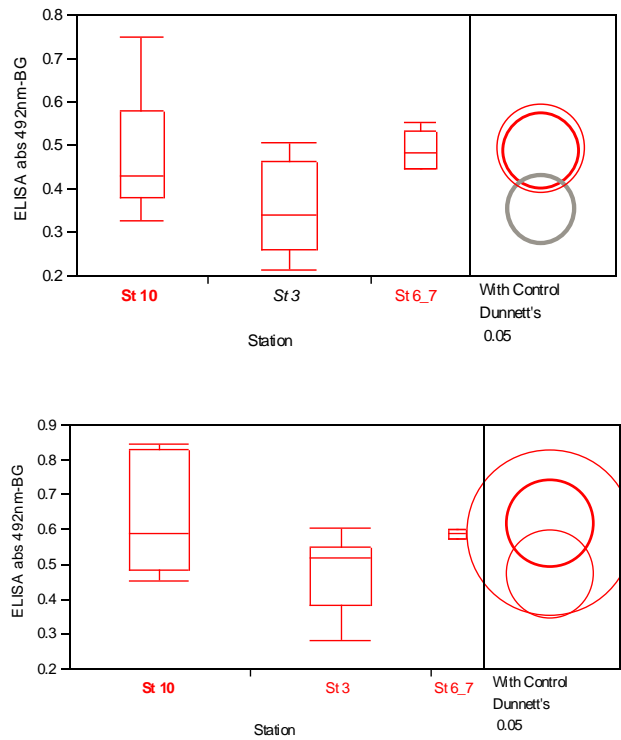
The levels of vitellogenin in the plasma of soles varied according to the stations and sex (Figure 14). Slightly higher levels were observed for male soles, but at all the stations. A higher level for male individuals can show evidence of the presence of estrogen-omimetic substances which cause the formation of vitellogenin in males. The variations noted between stations are difficult to interpret.

DNA Adducts

Figure 15 DNA adducts at stations 3 and 6-7

Station 3   Station 6-7
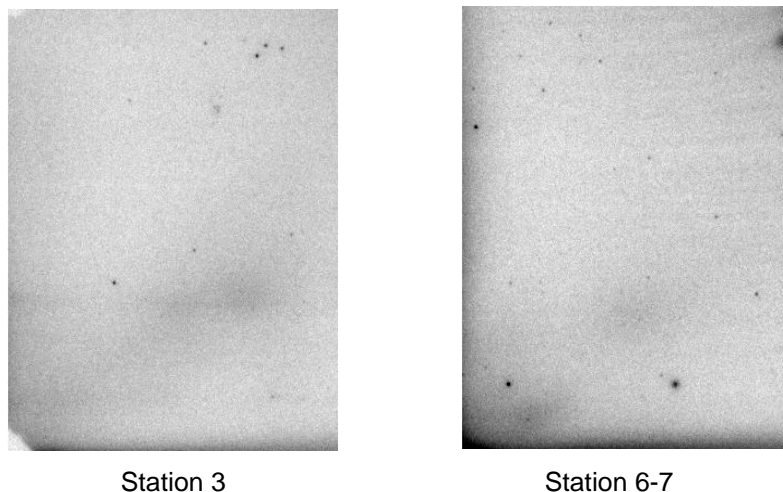
No presence of DNA adducts was observed at the stations under study (Figure 15). When taken with the previous observations, this indicates an exposure to PAHs which were then metabolised without causing genotoxic or other irreversible effects.

CS-8.5. CONCLUSION

After having evaluated the effluent plumes in the estuary using modelling software, 10 observation stations were selected along the gradient of this plume. The upstream stations (stations 1 and 2) and the distant downstream station (station 10) make up the "brackish water" and "sea water" reference stations. The effluent discharge is located at station 3 (See Figure 1).

Several physical-chemical and specific chemicals were determined in the water and in sediment. Other observations included community or biocenotic (benthic invertebrates) in the sediment, biochemical/cellular (biomarkers) responses of caged mussels and benthic fish caught on site.

CS-8.5.1. Regarding methodology:

- The results showed that the Trophic Level Index could not be used for this type of monitoring because it does not account for the influence of physical constraints such as the variation of salinity, alternating tides, currents, variation in temperature and granulometry of the sediment on the biological community.

- The use of cages causes technical problems; they get lost or silted up

- The mussels' tolerance of brackish water limited their use to the upper stations, which led to the loss of the estuary control sites, upstream of the effluent discharge.

- The need for a reference station away from the effluent plume at a similar salinity.

CS-8.5.2. Regarding the measurement of impact of the effluent:

- The physical chemistry of the water does not indicate major disruption associated with the effluent discharge. It confirmed the strong variation of salinity typical of an estuary.

- The analysis of sediments showed that the levels of contamination of metals and hydrocarbons were low to medium and decreased going downstream. It was noted however that two stations had higher concentrations of total hydrocarbons (stations 3 and 8) and that above average concentrations of PAH were observed at station 3.

- The community indicators did not give evidence of an impact of the effluent. They appeared to be influenced by the levels of salinity and the presence strong currents in the estuary.

- The results of the biomarkers in the mussels were difficult to interpret.

  - The upstream reference cages were not recoverable after placement: in station 1 the mussels did not survive because the level of salinity was too low; and the cage at station 2 was washed away by strong currents caused by a rise in the water level.
  - Consequently even when mussel's biomarker responses were significantly different from one station to another, the role of salinity could not be determined.

- The biomarker responses in the fish showed that:

  - The Bile FAC type of biomarker, which shows the presence of PAH metabolites in the fish bile, demonstrated a significant exposure at station 3 (downstream near to the effluent discharge) compared with the more distant downstream station 6 and the reference station at sea (station 10). However, the absence of an upstream or reference station do not allow for the confirmation of the source of the PAH.
  - Despite this exposure, the genotoxic effect biomarker (DNA adduct) gave no evidence of effects i.e. there were no adducts observed in fish from station 3. These results indicate that the exposure to PAH did not have an irreversible effect on the fish.

CS-8.6.   REFERENCES

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ANNEX 1 – ANALYTICAL RESULTS

Granulometric analysis results

		Stations									
		1	2	3	4	5	6	7	8	9	10
Muds (<2 µm)	(%)	9,23	5,09	8,57	5,00	4,88	5,77	5,70	7,39	4,62	6,21
Fine silts (2-20 µm)	(%)	49,37	55,11	46,93	57,90	44,42	41,93	34,10	59,41	28,08	45,99
Coarse silts (20-63 µm)	(%)	23,50	24,70	27,20	26,80	21,80	14,80	11,10	24,60	10,10	20,90
Fine sands (63-250 µm)	(%)	13,80	13,00	16,10	9,90	24,70	18,90	44,50	8,60	32,50	23,20
Coarse sands (>250 µm)	(%)	4,10	2,10	1,20	0,40	4,20	18,60	4,60	0,00	24,70	3,70
Fine fraction (<63 µm)	(%)	82,1	84,9	82,7	89,7	71,1	62,5	50,9	91,4	42,8	73,1
Average	(µm)	53,35	37,63	36,92	27,55	60,93	118,60	88,47	22,71	165,50	59,61
Median	(µm)	14,41	15,05	16,14	14,33	20,74	25,41	63,57	12,24	110,00	18,82
Skewness		2,719	3,377	2,398	3,178	2,609	1,761	1,274	2,354	1,441	2,477
Kurtosis		7,420	15,130	6,502	11,950	8,572	2,635	2,669	6,153	1,688	7,180

Physical-chemical analysis in sediment

		Stations									
		1	2	3	4	5	6	7	8	9	10
Density (20°C)	-	1,38	1,21	1,34	1,17	1,35	1,63	1,56	1,22	1,76	1,44
Water content	(%)	37,5	72,7	55,4	76,0	59,0	38,7	40,4	67,8	31,4	54,3
Total organic carbon	(% p.s.)	1,7	3,5	2,0	3,5	1,9	0,9	0,9	3,3	0,7	1,5
Total nitrogen	(mg/kg p.s.)	1900	3900	1900	4000	2000	900	870	3400	620	1400
Total Phosphorus	(mg/kg p.s.)	1000	1100	1100	1100	850	570	690	990	760	660

Metal analysis in sediment (expressed in dry weight)

		Stations									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Aluminium	**(%)**	5,7	6,1	5,4	6,1	5,3	3,7	4,9	5,6	4,2	5,2
Arsenic	**(mg/kg)**	28	28	23	29	18	13	15	26	8	11
Cadmium	**(mg/kg)**	0,36	0,48	0,66	0,48	0,36	0,22	0,22	0,38	0,26	0,24
Chrome	**(mg/kg)**	77	86	79	87	57	37	42	85	37	53
Copper	**(mg/kg)**	15	27	18	28	15	7	7	30	8	12
Mercury	**(mg/kg)**	0,097	0,171	0,142	0,169	0,113	<0,050	<0,050	0,206	0,057	<0,050
Nickel	**(mg/kg)**	32	44	27	34	22	17	17	33	15	30
Lead	**(mg/kg)**	41	56	67	59	37	29	31	56	35	36
Zinc	**(mg/kg)**	120	170	140	180	110	63	74	160	65	82

Hydrocarbon analysis in sediments (expressed in mg/kg of dried weight)

	Station									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Fluoranthene	0,060	0,130	0,370	0,140	0,082	0,015	0,024	0,170	0,160	0,093
Fluorene	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005
Benzo(a)anthracene	0,027	0,046	0,130	0,049	0,038	0,008	0,013	0,045	0,060	0,034
Benzo(ah)anthracene	<0,005	0,012	0,022	0,011	0,005	<0,005	<0,005	0,012	0,010	0,007
Benzo(b)fluoranthene	<0,005	0,033	0,059	0,030	0,025	<0,005	0,007	0,037	0,048	0,023
Benzo(k)fluoranthene	0,023	0,051	0,075	0,051	0,032	0,006	0,008	0,068	0,034	0,024
Benzo(a)pyrene	0,040	0,077	0,160	0,093	0,051	0,013	0,016	0,110	0,062	0,043
Benzo(ghi)perylene	<0,005	0,062	0,120	<0,005	<0,005	<0,005	<0,005	0,062	0,052	<0,005
Indeno(1,2,3-cd)pyrene	<0,005	0,075	0,110	<0,005	<0,005	<0,005	0,017	<0,005	0,059	<0,005
Acenaphthylene	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Acenaphthene	<0,005	<0,005	0,013	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005
Anthracene	0,006	0,018	0,091	0,012	0,018	<0,005	<0,005	0,130	0,017	0,015
Chrysene	0,021	0,058	0,110	0,028	0,041	<0,005	<0,005	0,052	0,057	0,031
Naphtalene	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Phenathrene	<0,005	0,008	0,026	0,008	0,006	<0,005	<0,005	<0,005	0,034	<0,005
Pyrene	0,070	0,140	0,360	0,130	0,081	0,008	0,026	0,084	0,190	0,071
∑ 16 PAH	0,247	0,710	1,646	0,552	0,379	0,050	0,111	0,770	0,783	0,341
Total Hydrocarbons	9	41	80	40	20	25	13	40	72	10

ANNEX 2 – DUNNETS CIRCLES AND SIGNIFICANCE

With Control, Dunnett's whether means are different from the mean of a control group.

Each multiple comparison test begins with a *comparison circles* plot, which is a visual representation of group mean comparisons. The plot follows with a reveal table of means comparisons.

If the intersection angle is close to 90 degrees, you can verify whether the means are significantly. The highlighted circle appears with a thick solid line. Red circles representing means that are not significantly different from the highlighted circle show with thin lines. Circles representing means that are significantly different show with a thick grey pattern
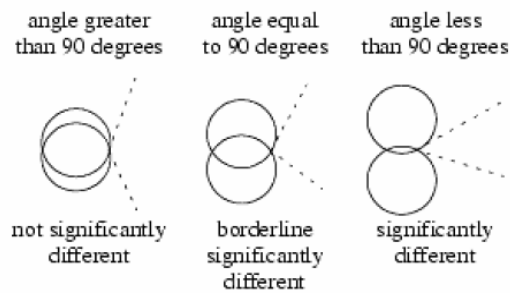
APPENDIX II: FRAMEWORK FOR THE QUALITY ASSESSMENT OF AQUATIC TOXICOLOGY LABORATORIES

SUMMARY AND KEY LEARNINGS

Contracting laboratories usually conduct WET testing for the US refineries in response to the US EPA National Pollutant Discharge Elimination System program (NPDES). The EPA methods for WET testing are considered scientifically defensible and provide reliable results when performed by qualified laboratories. The EPA's WET testing program is important for the Refineries because it provides them with a mean to identify, characterize and eliminate potential toxic effects of point source effluents to discharge waters. However, WET testing has a fair degree of variability since it is a biologically based test system. Thus, 'false positives' are expected and occasionally do occur, especially with the recent implementation of chronic WET testing requirements for many US refinery effluents. The refineries treat WET test failures seriously expending time, energy, personnel, and material resources. For the refinery, a WET testing failure potentially leads to shut down of production units, diverting of staff from normal operations, callouts/overtime, increased immediate sampling, and notifications up to Plant management. If subsequent re-testing does not provide clarification, a labour intensive and costly TRE (Industrial Toxicity Reduction Evaluation), requested by the regulatory agency will follow. The relationship of the refinery with the testing laboratory, which is clearly a partnership, is critical in order to avoid 'some false positive WET results. BP experienced that regardless of the testing laboratory, it is the refinery's responsibility to maintain the quality of the monitoring data, and hence, compliance with the requirements of the regulator. Because the refineries shoulder the responsibility for data quality, the utmost importance is to always select the best available, most competent test laboratory capable of routinely performing aquatic toxicity tests to defined standards. Therefore, BP developed a framework for the assessment of contracting laboratories. The aim was evaluate and select the best available contract laboratory for compliance testing by the refineries.

AII-1.   INTRODUCTION

The US Clean Water Act (CWA) was enacted in 1972 with the objective of restoring the chemical, physical, and biological integrity of US waters. Among the EPA efforts to achieve this objective was implementation of the NPDES program designed to control toxic effluents, implement water quality standards, and restore and maintain "fishable and swimmable" uses in waters of the U.S. Early in this program, effluent quality was controlled by means of technology-based treatment requirements. However, even with technology solutions, many effluents remained toxic and caused water quality problems. Further controls were necessary to achieve compliance with state water quality standards that prohibited the discharge of toxic pollutants in toxic amounts, or otherwise provided for the maintenance and propagation of a balanced population of aquatic life. Therefore, EPA developed a policy to reduce or eliminate toxic effluents. The policy was unique in that it employed both chemical-specific and biological methods for the assessment and reduction of toxic effluents.

Based on the existing regulations of the individual states (which are responsible for NPDES implementation), local regulators of the individual states were responsible for determining whether an effluent has the potential to contribute to or cause exceedances of certain water aquatic standards. In response, the Federal EPA

developed an integrated strategy to assess whether or not discharges to public waters are meeting appropriate standards. This strategy employed three approaches: I.) the chemical specific approach, II.) the WET approach, and III.) the biological assessment approach. The chemical-specific approach uses quality standards, which are especially important for contaminants of known potential for environmental or human health effects. The biological assessment approach addresses one objective of the Clean Water Act, "…the restoration and maintenance of the chemical, physical, and biological integrity of the Nation's waters". Where it is impractical or impossible to protect the biological integrity on a pollutant-by-pollutant basis using chemical quality standards, the biological assessment approach can be used to assess aquatic biological communities. In this approach the biological criteria are based on a designated reference with expected undisturbed biological integrity. The third approach that regulators use to monitor and control the discharge of toxic pollutants is the WET assessment. Two main advantages of WET testing over individual, chemical-specific controls are that 1) they evaluate integrated effects of all chemicals in the effluent sample, and 2) while EPA has established aquatic life criteria for a relatively small number (126) of chemical-specific pollutants, WET tests can measure toxicity caused by other compounds. In 1995, EPA promulgated WET test methods and included them in a list of methods approved under the CWA for use in the NPDES program. These EPA methods have been modified over time, with the most recent revised editions being made available in 2002.

EPA's WET testing program enables the refineries to identify characterize and eliminate toxic effects of point source effluents to open waters. Furthermore, the WET test methods are scientifically defensible and provide reliable results when performed by qualified staff. As the US WET program matured, the number of commercial laboratories offering testing services grew to capitalize on the new regulations. Such laboratories ranged from small entities, where the laboratory focused exclusively on WET testing, to large organizations in which WET testing was a component of a larger capabilities platform.

The goals of this BP case study were to identify the best available contracting laboratory:

1. By describing the important attributes of contracting laboratories for WET testing, and

2. Providing the means by which refineries can judge the proficiency of contracting laboratories by an assessment of the key laboratory attributes.

These attributes cover managerial, operational, and technical elements of laboratory procedures. These are described and discussed further in the following sections.

AII-2.   ELEMENTS OF QUALITY LABORATORY SYSTEMS

Valid laboratory results are essential for surveillance and diagnosis of effluent toxicity. Ideally, testing laboratories address each of the assessment areas described herein such that all work together to form a quality management system. In so doing, the laboratory should be able to demonstrate that it operates a viable quality system and is technically competent and capable of consistently producing technically valid results.

The assessment attributes described below are sufficiently general such that an overall appraisal of a laboratory's capabilities and quality system can be made. In

addition to the essential quality factors described, some specific test methods may have supplemental or other requirements based on factors such as matrix type or test species. It is not within the scope of this document to address issues related to specific tests, as these can be identified in the relevant published test methods for the required test species.

AII-2.1   ORGANIZATION AND PERSONNEL

AII-2.1.1.   Management

A review of a laboratory's management philosophies offers a reflection of their commitment to expertise and quality. For example, a management that is committed to hiring and retaining experienced personnel is likely to provide a higher level of service than one that has filled key positions with individuals lacking relevant training and experience. Experienced analysts are limited in quantity and always in demand, and a good managerial team recognizes that increased knowledge and experience minimizes analyst-induced variability.

AII-2.1.2.   Training and Experience

Based on performance audit inspections of selected laboratories, it is fairly common to find laboratory staffs that are responsible for the WET testing program but have no training in the biological sciences or little practical experience. This lack of experience can be a major source of variability in test results. The experience factor is probably one of the most crucial aspects for successful WET testing. The ability to successfully complete aquatic toxicity tests is a direct function of the training and expertise that technical personnel accumulate over time.

AII-2.1.3.   Number of Personnel

There should be a sufficient number of personnel for the timely and proper conduct of the studies according to the testing guidelines. The laboratory should be open to queries regarding turnaround time (e.g., period between test completion and report delivery), number of laboratory employees directly involved in testing, and approximate number of studies per month, all of which can provide an assessment of laboratory capabilities and workload.

AII-2.1.4.   Quality Assurance Unit

Does the laboratory maintain a quality assurance unit separate from its technical operations? If so this may reflect a commitment to a quality laboratory system. However, the lack of a separate quality assurance unit is not necessarily indicative of a poorly-run laboratory. Not all laboratories are able to justify the expense of separate quality assurance personnel, as this typically is considered an "overhead" expense. The important point here is that there are quality assurance practices that go beyond the minimum technical requirements of acceptable tests (i.e., such as reference toxicant testing, minimum acceptable survival/growth of control organisms, etc.). A person may be designated the quality assurance individual (QA representative) for a test or series of tests for which they have no involvement in the conduct of the tests. This designated QA individual would be responsible for monitoring the study to assure that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the regulations. This person should sign-off on an inspection report that specifies the dates of inspection, and the portions or phases of the test that were inspected with a description of any deviations from test guidelines or permit requirements. It would be the responsibility of those individuals responsible for running the test to make corrective actions and/or explain the impact of any adverse findings.

AII-2.2 FACILITIES

AII-2.2.1. Laboratory Physical Area

The test facilities should be of suitable size and construction to facilitate the proper conduct of studies. Test facilities should also be designed so that there is a degree of separation that would prevent any function or activity from having an adverse effect on another activity. Consideration should be given to partitioning of test species, isolation of individual projects, and availability of appropriate lighting, cooling, and heating controls specific to an area's designated use. The refinery should check whether state or regional guidelines exist regarding minimum requirements for laboratory area or bench space (e.g., linear feet) for aquatic toxicity testing laboratories.

AII-2.2.2. Organism Culture Area

The laboratory should have sufficient holding facilities for the test species. Species should be separated such that the potential for cross-infection of sick organisms is minimized. A separate quarantine area allows organisms to be treated without risks to other, healthy organisms. The laboratory should maintain records of the environmental conditions in the culture area. This ensures that test organisms were maintained under appropriate conditions (e.g., salinity, hardness, dissolved oxygen, pH, etc.) and that no excursions have occurred that may compromise the organism's sensitivity.

AII-2.2.3. Water Source

Depending on the suite of test species offered for testing by the laboratory, various types of water may be required. Besides standard freshwater and saltwater, the laboratory may be required to adjust the level of specific water quality factors to match the characteristics of an effluent-receiving water body (e.g., pH, salinity, hardness). If the laboratory is relying on a natural source, the water should be periodically screened for potential contaminants that may interfere with testing or culturing. If reconstituted water is used, then a means to prepare reagent grade water is necessary (i.e., reagent grade deionized, reverse-osmosis) to which standard salts can be added to achieve a specific water type.

AII-2.2.4. Chemical/Sample Storage

An area for receipt and storage of test samples according to specified environmental conditions is necessary. Refrigeration and ambient temperature storage areas should be available. Storage areas should be secured to prevent sample tampering, and access should be limited to specified individuals. Documentation of personnel use of the area also should be maintained.

AII-2.3 LABORATORY OPERATIONS

Laboratory operations are the practices and procedures implemented by the laboratory in the conduct of performing its testing services. Some practices are specified in test methods or cited in permit requirements.

AII-2.3.1. Standard Operating Procedures (SOPs)

A test facility should have standard operating procedures set forth in writing. SOPs are the guidelines by which management ensures the quality and integrity of the data generated in the course of a study. SOPs should be available to laboratory personnel, and each area of the laboratory should contain SOPs relevant to the

area's designated use. Master copies of SOPs should reside with the QA/QC designee, and any changes to SOPs should be approved by laboratory management. There may be no limit to the number of SOPs that potentially could be created, but at a minimum the laboratory should address the following topics in written SOPs:

- Maintenance and calibration of instruments/equipment.

- Cleaning of test organisms culture/test chambers and glassware used in the preparation of reagents/solutions.

- Sample collection/receiving procedures.

- Chain of custody procedures.

- Test organism culture procedures and health documentation.

- Techniques used in toxicity testing.

- Data analyses. Appropriate statistical tests are typically specified in each method guideline; however, topics such as rounding convention and how laboratories handle unexpected results (e.g., outliers) and their notification to the refinery should be addressed in an SOP.

AII-2.3.2. Training Records of Personnel

There must be some verification that operational personnel are trained to conduct and understand the procedures and practices specified in the SOPs. A quality laboratory will take the time to assure that personnel are provided a formal introduction to and thorough training in equipment and procedures used in the laboratory. This training should be documented in the individual's record of training and should include the signature of the trainer and/or management verifying the individual's competence to perform the procedure. If the employee has received training outside the laboratory, the training should also be described in detail with dates/location of the training and topics covered. If the employee received training at a previous employer, laboratory management must take the responsibility to verify the adequacy of the training and the capabilities of the employee to carry out the procedures.

AII-2.3.3. Reagents and Solutions

All reagents and solutions in the laboratory should be labelled to indicate strength, concentration, storage requirements, and expiration date. Old or outdated solutions should not be used.

AII-2.3.4. Test Organism Care

Animal testing data are produced because of the state specific reasons of regulatory requirements. In the US the permitting agencies show limited concern for the use of fish tests, and alternatives to vertebrate testing are not offered. In general, it is important that the laboratories meet the highest standards for the use of animals in testing, and wherever possible, are also active in the animal number reduction efforts and the identification of alternative test methods. In the EU these concerns are covered by the EU Council Directive 86/609/EEC (EU, 1986). In defining an animal, the directive states:

-     Article 2 - for the purposes of this Directive the following definition shall apply: 'animal', unless otherwise qualified, means any live non-human vertebrate,

including free-living larval and/or reproducing larval forms, but excluding foetal or embryonic forms.

Thus for effluent testing in the EU the use of fish is covered and where possible alternatives, e.g. fish embryo, should be considered. Furthermore, individual countries may have their own specific legislation or requirements e.g. the UK Guidance on the Operation of the Animals (Scientific Procedures) Act (UK, 1986).

The laboratories may culture their own test organisms, purchase them from commercial sources, or collect specimens of wild populations. The preference is for laboratories to maintain self-propagating cultures of organisms, thus giving the laboratory an historical account of the age and health of the organisms. Culturing its own organisms may not be possible, and the laboratory may rely on either test organisms purchased from an aquaculture operation or collections of wild populations. Collections of wild specimens are the least desirable due to a number of concerns regarding their collection and use:

- Supply – Is there an adequate supply of the test organisms to perform the standard and any additional testing on a year-round basis?

- Handling – Is the test organism able to withstand the rigors of capture, shipping, acclimation and taxonomic verification prior to testing? The organisms should be in peak condition at test initiation.

- Life Stage – Can the age of the test organisms be verified, keeping in mind the specifications of the test method (e.g., all organisms must be within a certain age/size of each other?).

- Identification – Taxonomic verification is required, and the process may be difficult or stressful to the organisms.

- Health – The use of wild test organisms includes an important uncertainty regarding their health, sensitivity to contaminants, etc., which potentially can impact the outcome of the test.

- Test Requirements – The specific test procedure may require special conditions or demonstration of success that is not applied to cultured organisms.

Due to the above factors, test organisms gathered from the wild may result in higher test variability, independent of laboratory performance.

If laboratories culture their own organisms, SOPs should be in place for the housing, feeding, handling and care of test organisms. Quality laboratories will have SOPs that address the following aspects of organism culture:

- Source of organisms – For each organism, SOPs detailing specific culture techniques for that organism. The origin of the cultured organisms should be documented including date received, verification of taxonomic classification, and any additional source of organisms added to the culture.

- Source of water – If a natural source is used, the location, treatment, and quality monitoring should be specified. If reconstituted water is used, its recipe should be listed as well as any treatments prior to use. Procedures for screening natural waters for potential contaminants should be specified.

- Hardware – The culture system should be described that would include volume and dimensions of tanks, water delivery systems, aeration, and lighting

specifications in the culture area. Specifications for the cleaning and maintenance of items should be present in the laboratory.

- Organism Feeding – An SOP should describe specified steps taken for test organism feeding. For example, what procedure does the laboratory follow for feeding on weekends/holidays? Records should be maintained on the details of feeding (e.g., time, frequency, amount) with some appraisal of the food's palatability (e.g., "all food consumed within 'x' minutes"). Disinterest in food may provide an early warning signal that the organisms may not be fit for testing.

- Diet – Are the diets prepared in accordance with guideline standards? There should be established expiration dates and storage conditions for the diets. Also, any quality assessments of the diets should be described (e.g., some labs will conduct feeding/growth experiments periodically when new diets are prepared).

AII-2.3.5. QA/QC Practices

A quality toxicity testing laboratory will have a serious commitment to a quality assurance/quality control program that extends beyond basic requirements of compliance testing. Quality laboratories will have prepared QA/QC manuals specifying the practices implemented by the laboratory. Quality QA/QC programs typically include the following:

- Record of performance on reference toxicant tests. Reference toxicant tests indicate the sensitivity of the test organisms being used and demonstrate the laboratory's ability to obtain consistent results with the test method. Therefore, the laboratory must periodically conduct reference toxicant studies on each organism and test method that they perform for compliance monitoring. The frequency of reference toxicant testing is generally given in permit requirements or other specifications of state and/or regional authorities. Endpoints of the test (e.g., EC/LC50 values) typically must fall within a given range for the test to be considered valid. The laboratory should have an acceptance/rejection policy for reference toxicity data which fall outside established criteria boundaries.

- Maintenance of historical control charts. The laboratory should maintain performance charts of historical reference toxicant testing. By doing so, the intra-laboratory precision of the test is determined for each method and species with different batches of organisms. The width of a control chart's limits (e.g., as indicated by 95% confidence intervals of the EC/LC50 values) reflects the laboratory's capability to reproduce the desired endpoints of a reference toxicant test. The width of control chart limits is a function of the reference toxicant, test species, test type, and biological endpoint. These factors must be considered before drawing conclusions regarding laboratory performance.

- Involvement in an effluent monitoring report (DMR) QA program. A DMR-QA study is periodically required by the permitting authority. In such a study, the laboratory is supplied with double-blind samples and performs tests on specified species. The data is then reported to the permitting authority. Such data may be available to the refinery for evaluation and assessment.

- A sample custody tracking system that is always used.

- Attention to facility and equipment maintenance through application of appropriate SOPs.

- Dilution water quality monitoring with acceptance criteria and corrective steps when measurements fall outside the criteria.

- Data checking such that a second individual reviews and attests that laboratory SOPs were followed, mathematical calculations were accurately performed, and quality/acceptability criteria were met.

AII-3. LABORATORY CAPABILITIES

The refinery should consider the full range of capabilities in its evaluation of potential contracting laboratories. The suite of capabilities that the testing laboratory offers should be evaluated independent of any measures of quality, but one laboratory may be favoured over others having similar laboratory quality systems. For example, a laboratory experienced in conducting toxicity identification/reduction evaluations (TIEs and TREs) may be desirable. Performing a TIE/TRE generally entails a team approach involving equipment and personnel from various technical fields (e.g., chemistry, hydrology, etc.) that some laboratories may not maintain.

AII-4. ACCREDITATION

Certain states require compliance testing laboratories to achieve certification to conduct specific tests. The accreditation board is likely to require many of the aspects of quality systems discussed above. The refinery should become familiar with any certification programs as inspection of a vendor's license and performance on certification requirements can be valuable in assisting the selection of a vendor laboratory. For some states, the accrediting board may perform an on-site inspection. The refinery may ask to review the inspection report, as identification of deficiencies and how the laboratory makes corrective actions can provide valuable information to them.

AII-5.   REFERENCES

1.   EU (1986) Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Official Journal of the European Communities No. L358, 18.12.1986

2.   UK (1986) Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, Presented to Parliament by the Secretary of State for the Home Department pursuant to Act Eliz. II 1986, C.14 Section 21, (Animals (Scientific Procedures) Act 1986), Ordered to be printed by the House of Commons, 23 March 2000.

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