

**Technical Report**

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**Biomonitoring of Industrial Effluents**

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### **BIOMONITORING OF INDUSTRIAL EFFLUENTS**

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## CONTENTS

SUMMARY .....	1
A. INTRODUCTION .....	3
B. BACKGROUND .....	5
C. PRACTICAL PROCEDURE .....	10
1. Introduction .....	10
2. Sampling Programme .....	12
3. Criteria for Biomonitoring Assays.....	15
4. Strategic Aspects relating to On Site Monitoring.....	19
5. Interpretation of Results.....	21
6. Application of Results.....	23
D. ASSESSMENT OF EXISTING TEST METHODOLOGIES FOR BIOMONITORING OF EFFLUENTS .....	25
1. Introduction .....	25
2. Available Bioassays .....	26
E. CONCLUSIONS AND RECOMMENDATIONS .....	37
BIBLIOGRAPHY .....	40
TABLE 1.....	44
APPENDICES .....	45
1. Glossary of Terms .....	45
2. Regulatory Control of Effluent Discharges in Europe and North America indicating the Current and Possible Future Application of Toxicity Tests in Effluent Quality Control .....	48
3. Overview of Current Regulatory Tests for Biomonitoring of Effluents .....	59
4. Members of the Task Force .....	60
5. Members of ECETOC Scientific Committee .....	61

## SUMMARY

Biomonitoring of effluent is the assessment of the integrated ecotoxic potential of an effluent on aquatic organisms. Observations are made according to a defined spatial and temporal programme. Biomonitoring will be used increasingly by authorities for assessing industrial effluents in relation to the control of receiving water quality. Nevertheless there are significant gaps in our knowledge about chemical partitioning, degradation and bioaccumulation which make it difficult to extrapolate laboratory test results to the natural environment. At present the value of the latter extrapolation is limited. The principle of industrial discharge control based on pass/fail criteria using poorly understood test systems is questionable.

Besides the nature of the effluent, the choice of test system and species will depend on other factors including test location and whether the test is prescribed; no single test applies to all situations. Where a choice of test system and species exists, a major consideration is the use to be made of the data generated.

Interpretation and application of results will relate to the study aim. Application of biological tests by industry for internal plant monitoring is relatively straightforward. Results of tests on grab or composite samples are usually expressed as the effluent concentration causing a measured response in 50% of the test population and are used either to compare the toxicity of effluent streams or to follow the effluent quality with time. Combined with effluent fractionation techniques, such tests might identify problem chemicals. Interpretation of continuous and automated biomonitoring based on measurable physiological and biochemical parameters is limited to decisions on the effect level for providing early warning of adverse conditions.

A number of biomonitoring assays are reviewed. Test methods are considered which are specified by regulatory authorities together with some non-regulatory tests known to be in general use for assessing effluent quality.

The majority of static and flow-through tests employed are acute toxicity tests and involve a range of organisms. Tests with particular fish species may be a national requirement which prevents the harmonisation of test species. Tests with bacteria, algae and crustacea, however, may have general application. The bacterial fluorescence (MICROTOX) test is rapid and cheap but the relevance of the results obtained is questionable, particularly for freshwater situations. Concern that acute toxicity data cannot adequately indicate the long term consequences of an effluent discharge has lead the US-EPA to develop "short term chronic" test protocols. These tests require further development and validation.

Used sensibly, biomonitoring techniques can provide the chemical plant manager with the means to investigate effluent toxicity from source to discharge. Biomonitoring is, however, not a substitute for classical physico-chemical and biochemical effluent control methods. In complex effluent situations it may provide a useful adjunct. At present there are a number of technical and administrative problems that require resolution before biomonitoring data can safely be used for legislative control purposes.

As skills and knowledge develop biomonitoring is likely to be used more widely for control of effluent to preserve the receiving aquatic environment. Different tests may be needed for application to various environmental situations. In the present state of development any relevance to the environment must be, simply, to determine trends in the effluent so that corrective action may be taken by the plant.

## A. INTRODUCTION

It has long been recognised that aqueous effluents can have a deleterious effect on natural waters. Early concerns were with the discharge of biodegradable organic material which resulted in oxygen depletion in the receiving water. Efficient biological treatments reducing biochemical oxygen demand demonstrated that benefits can be derived from planned effluent control. Successful implementation of such treatments has, however, revealed other problems associated with effluents. Increased public awareness concerning the environment has led to higher standards for acceptable effluent control leading to further improvement in the quality of natural waters (e.g. Programme Project Rhine 2000, 1987).

As it is the animal and plant life in the aquatic environment that has to be protected, it is logical to suggest that directly interpretable biological tests on such species should be considered alongside the traditional physico-chemical and biochemical tests that had been used conventionally to assess effluent quality. Progress and achievements in this direction have proved more difficult than expected.

Effluents are, in general, complex variable mixtures and the assessment of their possible biological effects presents difficulties when compared to the assessment of specific chemicals. The problems of relating the quality of chemical effluents, as defined in laboratory toxicity tests, to likely effects in the waters that receive them are well known (Maki et al., 1986).

Biological studies, ranging from laboratory toxicity determinations to broadly based ecological investigations, may play a role in assessing the acceptability of an aqueous effluent. They are used to:

- i) characterise and establish acceptable limits of toxicity and
- ii) monitor the effluent to ensure that these limits are respected.



A Task Force was set up with the following Terms of Reference :

- *to summarise the role and need for biological monitoring methods for effluents in order to have a biological means for effluent control and to consider the suitability of this approach for controlling receiving water quality;*
- *to assess the currently applied biological monitoring methods;*
- *to develop recommendations for biological monitoring methods.*

It was clear from the start that precise definitions of the terms used are necessary. These are given in Appendix 1.

## B. BACKGROUND

Effluents, even after treatment, may introduce chemicals into receiving waters. Some effluent constituents may be easily detectable and thus controllable, whereas others, often present in trace quantities, are not easily identified but may be harmful to the environment. Continuous efforts are made to maintain or improve the quality of natural waters by minimising the quantities of harmful substances in industrial and sewage effluents. Traditional control of industrial and domestic sewage effluent discharge has been by placing limits on the values of certain generic measures. For industrial and domestic sewage effluent discharge examples of controlled parameters are BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand), TOC (Total Organic Carbon) and suspended solids and physical parameters such as temperature, pH and colour. In addition there may be analysis for certain well known toxic materials (toxicants) such as phenols, ammonia, chlorine, cyanide, etc. In recent years heavy metals and certain groups of organic chemicals have been similarly controlled.

Such control has brought about significant improvement in water quality. Nevertheless some aquatic ecosystems have not improved as anticipated presumably because of the presence of toxic substances in effluent discharges which affect the ecological balance of the receiving water. There are two possible approaches to resolve such toxicological problems and ensure good effluent quality. One is based on a knowledge of all components of an effluent and the other which considers the effluent as a single entity.

A compound by compound chemical analysis approach is unlikely to provide all the information required to regulate complex effluent discharge because :

- i. it is not realistic to analyse a discharge for all chemicals that can be present;
- ii. chemical analysis provides numbers which require translation into

possible biological effects on the basis of available toxicity data and former experience;

- iii. toxicity data may be lacking for some constituents particularly trace metabolites and reaction products;
- iv. the chemical approach cannot account for any additive, synergistic or antagonistic effects that might occur.

To overcome the problems of this first approach and ensure good water quality, a number of countries have begun to use biological tests to complement physico-chemical methods in the control of effluent discharges. These tests have the following advantages in that they:

- i. integrate the effects of all effluent components and permit control of one limiting parameter, namely effluent toxicity;
- ii. may indicate a likely biological response in the environment;
- iii. may be more resource effective than a full detailed chemical analysis.

There are some limitations which are specific to the use of all biological tests, including biomonitoring tests, namely:

- i. the precision and reproducibility of biological systems are variable which may give rise to problems of interpretation and enforcement;
- ii. the time taken to perform some of these biological tests is long and the test may not be useful for the short-term control of effluent quality;
- iii. test results do not provide information on the cause of a toxic effect without additional data;

- iv. extrapolation of results from laboratory biological tests to possible effects in the real environment is at present poorly developed.

Despite these limitations there is an increasing use of bioassays in the regulatory control of effluents.

In Canada and USA fish toxicity tests have been used for a number of years to control oil refinery discharges. Refining industry experience is that although tests have been carried out to meet regulatory requirements they have little value in the day to day control of plant operations because of the time required to produce results (Tapp and Williams, 1986). Nevertheless toxicity limits based on acute toxicity tests are now applied to all discharges in the USA. Further, because the US-EPA does not consider that acute toxicity data are easily extrapolated to the environmental situation, it has developed and is using so-called "short term chronic tests" which give information on mortality, growth and reproduction in aquatic organisms.

Certain Scandinavian countries which use toxicity tests on a site-specific basis are actively considering the development of national test systems. The Netherlands are also considering toxicity tests but still regard their present control system, based on global physical and chemical parameters, as adequate. Fiscal measures have been introduced by some states as a means of inducing changes. Thus in France an acute aquatic toxicity test is used to levy a pollution tax which is used to sponsor treatment facilities. Germany uses acute toxicity tests both for control and establishment of a wastewater levy. At the international level OECD (1984-a) recommended that member countries adopt the principle of toxicity testing as one factor in decision making to regulate effluent discharge with the added advantage of harmonising pollution control across international boundaries.

The existing regulations relating to the control of effluent quality by chemical, physical and biological means in Europe and North America are summarised in Appendix 2.

Effluent biomonitoring may be used in a number of ways. For example bioassays (usually acute aquatic toxicity tests) generate data for

identifying toxic streams within a chemical complex requiring isolation and treatment. When such toxic streams are identified the wastewater may be broken down into fractions with the aim of identifying the source of toxicity. Information on wastewater toxicity is also required in the planning and design stage of effluent treatment and disposal. Regulatory control requirements seek to apply laboratory derived toxicity data to the environmental situation in order to assess any effect on the latter.

It must be appreciated that information on effluent toxicity in isolation cannot provide a global measure of the hazard that an effluent may present to an aquatic environment. Hazard assessment involves both an evaluation of a toxic potential of a chemical and an exposure assessment which requires consideration of a variety of interacting and complex factors, many of which are poorly understood, for example:

- i. the dilution available in the receiving water and the degree of mixing of the effluent discharge necessitates a consideration of hydrology (rivers/streams) or hydrography (estuaries/coastal waters);
- ii. the choice of the test species will require a consideration of the nature of the effluent and the receiving water;
- iii. the relevance of the few species tested to the wide variety of fauna and flora which may occur in the aquatic environment, and whether laboratory animals mirror the natural fauna in their response to toxins;
- iv. how various effluent constituents might partition in the receiving environment and whether they might persist and bioaccumulate in certain species;
- v. the present inability of laboratory tests with single species measuring lethality, growth and reproduction to provide information on natural environmental factors such as species competition, recruitment and mortality.

In seeking to define, on the basis of laboratory derived toxicity results, a level of effluent dilution which will not cause adverse effects in the aquatic environment, we have to accept that presently this will only be an approximation. Essentially we do not have the means of applying laboratory derived toxicity data determining the effects of effluents on ecosystems with any degree of accuracy.

Biomonitoring of effluents requires standard methods which should be validated and which relate directly to specific characteristics of an effluent, where known; for example, the presence of well defined chemicals such as pesticides and solvents. The tests should provide quantitative information on toxic effects.

Biological monitoring (biomonitoring) can take various forms ranging from laboratory toxicity tests to broadly based quantitative assessments of the ecological status of the receiving environment. This report is concerned with monitoring effluent toxicity to provide a global measure of effect by integrating the toxicities of all constituents in an aqueous effluent. ECETOC therefore defines biomonitoring of an effluent as follows :

*Biomonitoring of an effluent is the assessment of its ecotoxic potential on aquatic organisms. Observations are made according to a defined spatial and temporal programme.*

The regulatory application of toxicity results to the control of receiving water quality may be generally restrictive e.g. no toxicity at pipeline end or maybe judgmental based on a particular situation which permits a degree of dilution in the receiving water.

### C. PRACTICAL PROCEDURE

#### 1. INTRODUCTION

The use of biomonitoring by regulatory authorities for environmental control purposes is still at the developmental stage and only concerns the control of effluent at the point of discharge. Implementation of these techniques on a large scale will depend on the results of further research, particularly with regard to investigation and application of short-term chronic toxicity tests and the use of automatic continuous in-line monitors. It is important that those responsible for regulatory standards are aware of this situation so that they do not attempt to impose controls based on ecotoxicological techniques and interpretations that are presently beyond technical capabilities.

Biomonitoring may be a useful tool for industry to monitor changes in effluent toxicity so that corrective actions can be taken in order to avoid the development of adverse conditions in the receiving water. It can provide a useful adjunct but it is not an alternative to traditional chemical, physical and biochemical methods of effluent control.

There are general criteria to which any biological assay system (toxicity test) must conform. When such a test is to be applied as a monitoring method under a range of situations, often far removed from the controlled environment of the biological laboratory, the technical problems that these demands impose are considerable. Thus it cannot be assumed because a certain biological test method has been successfully developed in the laboratory it can be applied automatically as a monitoring system in an industrial situation.

The choice of a test, or tests, for determining effluent toxicity depends on a variety of factors so that no specific biomonitoring test is applicable to all situations. Biomonitoring should be considered according to its technical and strategic aspects.

National regulatory schemes, where they exist to control effluent and receiving water quality, may require industry to carry out prescribed aquatic toxicity tests. In non-regulatory situations, industry may wish to use toxicity tests for:

- i. internal plant assessment and control of effluent streams;
- ii. the consideration of possible effects of controlled or accidental release of effluent into a receiving water.

Toxicity testing may be required within the plant to:

- i. monitor for changes in effluent quality;
- ii. identify toxic streams and monitor the results of any remedial action; in this respect toxicity tests in combination with effluent fractionation techniques are used to identify the toxic constituent(s) of an effluent (Parkhurst, 1982);
- iii. determine aquatic toxicity for application to specific purposes such as the engineering of a diffuser section which can ensure good dilution of the waste discharge.

When choosing an appropriate test to meet the specific objective of biomonitoring it is also important to consider how the effluent should be sampled. Chemical plant effluents can vary considerably in quality and quantity either randomly or regularly with time depending on the processes (e.g. continuous or batch) involved and the layout of the effluent streams.

Samples taken for evaluation of a toxic effect should account for any variations in quality and quantity and so be representative of the chemical and physical characteristics of the effluent as a whole. These variations and characteristics will also be relevant to a consideration of test materials and procedures.



## 2. SAMPLING PROGRAMME

Samples can be taken before (i.e. directly after production plant) and/or after effluent treatment (i.e. before discharge) into a river. A sampling programme should be based on available knowledge of the operations of plants contributing to the effluent, particularly their schedules of discharge.

In terms of quality and quantity, effluents may be classified as:

- i. non-variable effluents with little or no variation in composition and flow rate with time;
- ii. variable effluents varying in composition and/or flow rate. They may be subdivided into those varying on a regular and predictable basis and those where variations are irregular and unpredictable.

The sampling programme should be based on a consideration of how best to allocate sampling frequency and techniques in relation to effluent variability and the testing contemplated. It may be appropriate to consult a statistician to define sampling frequency.

### 2.1. Sampling Position

The position from which the effluent sample is collected should relate to the sampling aim. Plants usually have a facility where the overall effluent can be sampled either manually or mechanically. Choice of position for sampling individual effluent streams in chemical plants should be based on a knowledge of plant processes and site drainage and accessibility in relation to operator safety. It is preferable that sample collection and flow measurement be made at the same position.

## 2.2. Sampling Techniques

Effluent sampling techniques range from the use of automatic equipment for collecting a sample (composite or continuous) usually related to volume flow rate to the manual collection of a single grab sample. Sampling equipment (material, composition, design) should be considered in order to avoid any reaction with the effluent that might result in anomalous samples caused by chemical or physical changes to the effluent. Variation in chemical and physical composition with depth and width of effluent streams should be considered in order to obtain a representative sample.

- 2.2.1. Continuous Sample. A small volume of an effluent stream continuously fed to a test system gives a profile of the parameter (e.g. toxicity) being monitored. This is particularly useful in the absence of knowledge about effluent variation. Such continuous flow-through toxicity monitoring may be practical only for limited periods or special situations, as it is expensive in manpower, equipment and maintenance.
- 2.2.2. Composite sample. When a sample is obtained by mixing together a number of grab samples, compositing should be limited to periods of 24 hours or less in order to avoid changes in the sample and to minimise effects due to ageing differences between the first and last aliquots.

Composite samples tend to be collected on the basis of time, flow or time and flow intervals and used in compliance monitoring to provide daily or operational averages for specific pollutants. Because of the averaging effect, this type of sample cannot describe changes in effluent quality over time, e.g. the detection of toxicity peaks is not possible.

2.2.3. Grab sample. A single, discrete sample collected at one point in time reflects the characteristics of the effluent only at the time of sampling and is used for effluents of relatively constant composition.

### 2.3. Sample Volume

The volume should be sufficient for the range of tests to be performed and for a sample to be stored for analysis and reference.

### 2.4. Sample Holding and Storage

As its physical-chemical and biological characteristics will tend to change with time, the sample should be stored in an inert, nearly filled container (minimal head space) and held in a manner that minimises transformations (e.g. low temperature). Samples which are either strongly alkaline or acid are usually relatively stable. Samples of effluent treatment plant discharge should, however, be considered as unstable. The samples kept for reference purposes should be stored under conditions which will maximise stability. These constraints apply to the samples collected and transported to the testing facility which may be some distance away. Consideration should be given to possible adsorption of effluent constituents onto the sample container surface and reaction with residual oxygen resulting from the presence of a small air space.

### 2.5. Sample Preparation

After sampling it may be appropriate to modify the pH of the sample in order to undertake the appropriate test prescribed by some legislative authorities.