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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 legislatory 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 lead 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 ar· ·ow 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 legislatory authorities.

3. *CRITERIA FOR BIOMONITORING ASSAYS*

3.1. Relevance

The endpoint of the test should be clearly defined. The physiological or behavioural parameters selected for biomonitoring must be reliable indicators of aquatic toxicants.

The sensitivity of the test method should not be influenced by external factors such as test site conditions, seasonal changes or atmospheric conditions. A correlation between the sensitivity of the test system and the possible effects on the receiving environment should be established if the intention is to protect the latter.

3.2. Technical Aspects

As effluents may vary in composition and in degree of toxicity, a case by case approach is recommended for the choice of a bioassay. The choice of the test method, duration and endpoint of the test and test species will depend on the type of effluent, the receiving environment and the potential effects to be monitored. Thus test systems should be sufficiently flexible to take into account possible variations of effluent and receiving water characteristics. The method should be relatively easy to use with a minimum of maintenance. The influence of temperature, oxygen, salt content, pH etc. on the test organism should be known and taken into account.

3.2.1. Test Method. Monitoring effluent of variable composition, or effluents where the act of sampling might alter composition, is undertaken ideally, on a continuous "in line" basis. Where continuous measurement of toxicity is not possible, discrete effluent samples (grab or composite) are taken for static (including static renewal) or flow-through testing in a laboratory. The advantages and limitations of both approaches are described in Table 1. The choice of the most appropriate method of exposure will depend on the

variability in composition of the effluent over time, its stability, the choice of test species and the selected endpoint.

In general a static toxicity test is adequate if grab samples or composite samples are representative for a given effluent and there is evidence that the composition of the sample does not change within the time period from collection until exposure of the test species. For the determination of endpoints such as mortality, immobilisation, acute inhibitory effects, this method is widely used and considered to be adequate.

Static renewal or preferably flow-through methods are recommended for testing effluents containing volatile materials or effluents with frequent changes of composition. Lowering of dissolved oxygen levels in static renewal tests may be overcome by a low loading factor i.e. small test organisms to large volumes of test solution, the latter being renewed frequently if necessary. In some instances it may be necessary to re-aerate test solutions, taking care to minimise any loss of volatile compounds. If there is a requirement to investigate the effects of such effluents on behaviour, reproduction, growth, taint etc. over an extensive period, flow-through tests may be the most appropriate. Not all test organisms are suitable for use in all three test systems (static, static renewal and flow-through).

If the composition of the effluent and the temporal variations are well defined, which may be the case for effluents originating from manufacturing plants producing few products, it may be appropriate to monitor known toxic constituents using only analytical or physical methods. Results are obtained easily and quickly compared with the time taken to generate aquatic toxicity data so enabling plant operators to modify the process. The experience in oil refinery plants in Germany, Canada and USA supports such procedures (Tapp and Williams, 1986).

3.2.2 Test species. Where a national regulatory requirement seeks to control effluent using a specific biomonitoring system, the test organism will be prescribed. Whenever possible, a discharger also seeking to assess the effect of his effluent on the receiving water should use a test species relevant to that situation.

Various possibilities exist for the choice of the test species. The species should be:

i. sensitive and relevant to the local receiving water, e.g. a trout if a trout stream is to be protected. When investigating a particular environmental situation, it may be considered desirable to obtain test organisms from the receiving water itself. Often this is not feasible because of the difficulty of collecting sufficient test organisms of the required age and conditions, lack of knowledge of the maintenance needs and sensitivities of such organisms;

ii. sensitive to the types of chemicals which could potentially occur in the effluent, e.g. pesticides if a pesticide containing effluent is involved;

iii. widely available, amenable to laboratory testing, easily maintained, and with adequate background data such that its sensitivity to toxicants can be related to organisms known to occur in the receiving water. Such a "model" species may be applied to a number of different situations and can be used to compare the toxicities of effluents from similar chemical plants.

For in-line continuous monitoring, only those test organisms can be recommended which possess physiological or behavioural characteristics which can be monitored by automated systems. Such characteristics are for example physiological functions such as heart beat and gill movement in fish and mobility in Daphnia. Automated measurements may require sophisticated computer systems and software to differentiate between significant changes of the monitored

parameter and "background noise". Besides the technical difficulties of maintaining such test systems, natural variability between individuals and groups of individuals needs to be taken into account. The use of such test systems tends to be limited to the monitoring of potable water supplies.

An automated system using bacteria is probably more appropriate for the continuous biomonitoring of an effluent of variable quality. Adverse effects causing changes in respiration rate are easily detected, permitting remedial action to be taken at an early stage. At BASF Ludwigshafen, West Germany, a rapid continuous biological system, based on a mixed bacterial culture is used to monitor the quality of the waste streams flowing to the site effluent treatment plant. When a toxic "slug" is detected it has been claimed that it can be diverted for subsequent treatment (Pagga and Günther, 1981). Bacterial assays, particularly the oxygen consumption inhibition test with activated sludge or Pseudomonas putida are stated to be useful for examining the possible effects of effluent streams in sewage treatment works (Guhl and Gode, 1989). The possibility of bacteria acclimatising to effluents should be considered and additional tests undertaken using a reference substance of known toxicity.

To investigate the relative aquatic toxicities of various in-house manufacturing plant effluent streams, the test species can be chosen from any readily available sensitive organisms known to give a rapid, measurable and reproducible response to toxicants. The selected test organism should be easily maintained in the laboratory and not unduly sensitive to small changes in water quality, such as hardness, salinity, pH and temperature. These parameters should be maintained within narrow limits and measured regularly throughout the test in order to minimise variability.

The choice of test species will also be determined by many practical criteria which are discussed elsewhere in this report.

3.2.3. On-site versus Off-site Testing. The choice of a test system and species also depends on the test location. Testing on site gives ready access to effluent but it must be ensured that the ambient conditions (vibration, noise, chemicals in air, quality of dilution water) do not interfere with the test. Use of a properly established test laboratory at a distant location requires transportation of effluent which may limit sample size and necessitates consideration of holding conditions to minimise changes in sample quality (see Section C 2.4).

3.3. Validation

The chosen test system should have been validated both in terms of the method and its applicability to controlling effluent quality and where appropriate should conform with the requirements of regulatory authorities.

4. *STRATEGIC ASPECTS RELATING TO ON SITE MONITORING*

If the effluent originating from a plant contains constituents of known and low toxicity, control of the levels of summary parameters such as BOD, COD, TOC, pH and conductivity may be appropriate. If the effluent may contain a limited number of well-known toxic constituents it may be appropriate to monitor those constituents using analytical or physical methods rather than biological methods.

In certain situations biomonitoring has little value for routine control purposes. Traditional chemical and physical monitoring permits decisions as to whether further effluent treatment is necessary and the need to design in advance protective measures (e.g. diversion or confinement of the effluent) in those cases of severe plant malfunction.

4.1. Control of Effluent at the Primary Source

When a toxicity problem may arise the monitoring of the effluent should be undertaken as close to the source as possible so that the problem plant can be defined and remedial actions can be taken locally before effluent streams mix.

4.2. Control of Effluent entering the Treatment Plant

Where several effluent streams originating from different manufacturing units mix, biomonitoring of the mix can give some indication about potential adverse effects which might impair the functioning of an effluent treatment plant. When possible toxic effects are observed in such circumstances it is advisable that biomonitoring of the separate incoming effluent streams be performed to detect whether the toxicity of the mix results from one or more specific incoming effluent components and/or if there is a combined action (e.g. synergism).

When biomonitoring shows an effluent to be extremely toxic and presents a hazard to a treatment plant, it may be appropriate to undertake further biomonitoring in association with fractionation of the effluent streams in order to identify the offending component(s) so permitting remedial action to be taken. This process of Toxicity Reduction Evaluation (TRE) is being used in the USA (Faro et al., 1988).

4.3. Control of Effluent at Discharge

After treatment of the effluent it is advisable to have supplementary biomonitoring just before discharge into the receiving water. A similar procedure is recommended to monitor effluent discharges which are "normally" innocuous but which may "occasionally" become contaminated, e.g. cooling water.

5. *INTERPRETATION OF RESULTS*

5.1. Numerical Expression of Results

Where continuous and automated biomonitoring of an effluent is undertaken, interpretation is limited to a decision on the effect level for providing an early warning of adverse conditions. This decision will depend on the aim of the test and any information on the degree of effluent dilution necessary to safeguard the receiving system, be it the sewage treatment plant or environment.

Where a biomonitoring programme simply seeks to compare the relative toxicities of different waste streams on a chemical manufacturing site, range-finding tests can provide quick and relatively inexpensive data as well as indicating whether more extensive (definitive) tests are necessary (cf Chapter D). Results, usually in terms of pass or fail, are based on acute tests which determine lethality or inhibition of movement within short and specified time scales (48 or 96 hours). Results are expressed either as LC50 or EC50 values. This permits comparison of results with other available data, a useful facility where a specific chemical is suspected of being the toxic component of an effluent.

For regulatory purposes the trend in the USA is to reduce the complex data from a long term test to a single number which may be considered statistically significant and is used as the basis for control. Besides eliminating much useful information, this approach assumes that a statistically significant result is also biologically significant which is not always the case.

The results of chronic tests, which establish responses to toxicants over relatively long time periods (lifecycles or reproduction cycles) are reported as:

i) the Maximum Acceptable Toxicant Concentration (MATC) which is

the geometric mean of the lowest exposure concentration that causes a statistically significant adverse effect and the highest exposure concentration where no effect is observed;

ii) an "Effective Concentration" (EC50) which is the concentration causing a measured response in 50 % of the test population;

iii) an estimated "safe level" which, in long term tests, is the highest exposure concentration where no adverse effects are observed. In such tests the safe level is usually the same as the NOEC.

Although biomonitoring of effluents is being used increasingly in the control of receiving water quality, many problems remain to be resolved.

5.2. Interpretation of the Biological Significance of the Results

Extrapolation of laboratory results to the natural environment requires both the generation and interpretation of a variety of laboratory and environmental data. Some particular problems should be considered.

5.2.1. Extrapolation of Laboratory derived Data to Environmental Systems.

The data are usually obtained from acute toxicity tests on a single species and need to be interpreted for complex aquatic ecosystems containing numerous species.

An attempt to resolve the extrapolation of laboratory derived data may be undertaken by obtaining test data on a range of species, e.g. bacteria, algae, crustaceans and fish. Monitoring for control purposes would normally be on the species shown to be the most sensitive. The test organism selected may be relevant only to that particular situation or effluent as species differ in their sensitivity to specific chemicals and effluent.

5.2.2. Prediction of Long Term Effects from Acute Data. One attempt to overcome this problem is by the application of factors to provide some estimate of chronic toxicity from acute toxicity data. These factors tend to be based on multiples of 10, e.g. LC50/10, LC50/100, depending on information about the effluent and the number of species tested, although the rationale for units of multiples of 10 is not clear.

Because of this problem, the US-EPA has developed short term tests (Chapter D) which provide growth or reproductive responses indicative of chronic toxicity over time scales similar to those for assessing acute toxicity. While they represent a step forward, there is no evidence of their validation against full long term tests where chronic effects can result from bioaccumulation or continuous exposure at sublethal doses leading to cumulative toxicity.

5.2.3. Partition and Fate of Effluent Constituents. The partitioning and fate of effluent constituents entering the receiving water is markedly influenced by processes such as adsorption onto sediments, degradation, volatilisation, bioconcentration, bioaccumulation, etc. and would significantly modify the toxic effects. The significance of these processes have not been studied in detail.

6. *APPLICATION OF THE RESULTS*

At a simplistic level to ensure environmental protection, biological control of a discharge can be based on a cut-off value obtained from a single acute toxicity test. For example, an effluent is deemed acceptable for discharge if, based on regular biomonitoring, the results of 48 hour acute toxicity tests show that at least 50 % Daphnia magna survive in a 50 % diluted effluent. Here the tests are usually carried out by the discharger, results being made available to the controlling authority for auditing purposes.

The above is a conservative approach and considering the inherent variability of biological tests it usually requires treatment of wastes to high levels in order to ensure compliance. Nevertheless stricter standards are being proposed (Environment Ontario, 1989) by which the effluent should be non-toxic as it discharges from the end of the pipe. This concept is, however, extremely rigorous and possesses technical problems as the undiluted effluent represents the highest dosage at which a bioassay can be conducted and hence no safety factor can be determined which would allow for variations in effluent treatment efficiency or species sensitivity.

The most practical approach, in current use in the USA, is to make a judgement of the toxic impact of the effluent in the receiving water on the basis of exposure assessment. This is interpreted to mean whether the concentration of the effluent, after an allowed level of dilution in the receiving water, does not exceed the determined no observed effect concentration. Such an approach recognises a mixing zone in the receiving water around the pipeline end. At the zone boundary the effluent is diluted to a determined no effect level. The dimension of the mixing zone to ensure safe levels for acute and/or chronic toxic effects will depend on criteria used for control purposes and the obtained effluent and receiving water toxicities, local ecology, water movement, including minimum water flows in a river situation. The latter includes an understanding of how the effluent mixes with the receiving water both in time and space in order to minimise the magnitude, duration and frequency of any effect.

D. ASSESSMENT OF EXISTING TEST METHODS FOR BIOMONITORING OF EFFLUENTS

1. *INTRODUCTION*

While a range of test protocols exists for determining the acute and chronic aquatic toxicity of chemicals for product control and classification (OECD, 1984 a,b), very few tests are specifically prescribed for use in controlling effluent quality in Europe. The majority of tests for effluent and receiving water control were developed by the US-EPA for regulatory use in the USA. This is a developing area, with many regulatory authorities indicating an intention to use ecotoxicity data to support traditional methods of effluent control. In Sweden (1989) industry will be required to evaluate the potential hazard of effluents using international standard methods. The programme also requires evaluation of the effect (acute or chronic) on the actual receiving waters.

Regulatory effluent biomonitoring assays together with details as to whether they may be used as a static or dynamic method or for continuous monitoring are listed in Appendix 3. Indications are also given on their status of validation, their degree of flexibility, their relevance to other toxicity tests and their convenience for on-site manufacturing plant control of effluents.

The present bioassays for assessing effluent quality can be subdivided according to the following characteristics:

i) Species. The species used range from bacteria to fish.

ii) Duration. The acute toxicity test, which is of short duration (1-4 days) usually with death as the endpoint, is a widely used bioassay. Experience shows that the acute toxicity test is the least time-consuming and cheapest method, but is considered of limited value for predicting effects in the environment. Chronic bioassays

may be more relevant as organisms are exposed to low concentrations of effluents over their entire (or partial) lifecycle.

iii) Endpoint. The test endpoint may be death but sublethal effects such as growth, development and impairment of reproduction are also measured. Tests determining sub-lethal effects tend to be complicated as feeding has to be incorporated into the method.

Behavioural and physiological/biochemical bioassays are used in a broad category of methods which seek to measure subtle sublethal effects such as avoidance, locomotor activity, bioaccumulation, respiration and heart function. These methods are non-routine, relatively expensive and provide results which may be complex and difficult to interpret.

iv) Possibility for automation. Aquatic organisms integrate and respond to polluting stress to which they are exposed. The interfacing of these responses with minicomputers has led to the development of automated and continuous monitoring systems which measure such parameters as fish respiration and activity. Although some automatic systems exist, their relevance has not been assessed sufficiently to be acceptable for routine effluent monitoring systems.

2. *AVAILABLE BIOASSAYS*

The following review of biomonitoring methods considers only those test methods specified by regulatory authorities, together with some non-regulatory tests known to be in general use for assessing effluent quality (cf. Appendix 3). The tests have various advantages and limitations which should be taken into account when considered for application to a particular circumstance. The bioassays are classified according to the test species used.

2.1. Bacterial Tests

Bacteria are important test organisms because they are part of the natural biotransformation cycle as well as being used in biotreatment processes. They are handled easily in a laboratory, possess a short lifecycle and are useful for a rapid screening of pollutants. Three bacterial test types are described. At present no bacterial tests are used for the regulatory control of effluents.

2.1.1. Cell Multiplication Inhibition Test with *Pseudomonas putida*.

Inhibition of cell multiplication (at 10% and 50% levels) resulting from addition of effluent to the defined test medium is determined after 16 hours by comparison with a control. The test is acceptable if the control inoculum multiplies at least two times within the test period (Slabbert, 1986). It is recommended for the toxicity screening of metal-containing industrial effluents. There are some technical limitations to this test; effluents containing insoluble ingredients or volatiles require a modification to the protocol, the reaction of effluent constituents with the nutrient solution and coloured effluents may cause interferences.

2.1.2. Respiration Inhibition Test With Activated Sludge.

Inhibition of respiration of activated sludge in the presence of normally five concentrations of effluent is compared with that of two controls fed with a standard amount of synthetic feed (DIN, 1987-b; EEC, 1988). Measurement of oxygen uptake is made after a contact time of 30 minutes or 3 hours or both and the inhibitory effect of the effluent at a particular concentration is expressed as a percentage of the mean respiration rate of the two controls. An EC50 is determined. The test is valid if the two control respiration rates are within 15 % of each other and the EC50 of a reference compound (3,5 - dichlorophenol) is in the accepted range of 5-30 mg/l. As activated sludge is relatively resistant, the test is likely to be less sensitive than the cell multiplication inhibition test and the phosphorescence inhibition test discussed below.

The respiration inhibition test with activated sludge is a rapid screening test whereby substances or effluent constituents which may affect adversely aerobic microbial treatment plants can be identified (Reynolds et al., 1987). It is most readily applied to substances which, due to water solubility and low volatility are likely to remain in the aquatic environment. The present test can be automated and is used for the monitoring of the feed to and from effluent treatment plant. A disadvantage of this test is the variation in the composition of sewage sludge bacteria. For this reason reproducibility of the results is not as good as for other bacterial toxicity tests. The results are highly relevant for a specific sewage treatment plant.

2.1.3. The Respiration Inhibition Test with *Pseudomonas putida*. This test can be used for the toxicity control of untreated effluents but is preferred for the use of testing of new chemicals (Robra, 1976). It is very similar to the respiration inhibition test with activated sludge. Instead of sludge a defined strain of Pseudomonas putida is used as test organism, and is precultured under controlled conditions.

The reproducibility of this test is in the same range as for the growth inhibition test, but for many chemicals the sensitivity is lower than for the latter test. Its relevance to the sensitivity of the bacteria in the sewage treatment plant is not the same as for the test with activated sludge, but Guhl and Gode (1989) claimed that the toxic limits in this test were comparable to the tolerance limits obtained with laboratory activated sludge models.

2.1.4. Light Inhibition Test with *Photobacterium phosphoreum*. Light output by this bioluminescent marine bacterium decreases when it is exposed to some chemicals (Jeffers and Taylor, 1977; Taylor and Jeffers, 1977; Bulich, 1979, 1986). This is the basis for a commercial test system "Microtox" now available in many countries (Beckman Instruments Inc., 1984). Results expressed as the effluent concentration causing 50 % reduction in light intensity (IC50) can be

measured within 30 minutes. Test "sensitivity" is claimed to correlate to that of established fish and invertebrate toxicity tests (Dutka and Kwan, 1981; Vasseur et al. 1984-a, b; Nacci et al., 1986; Ribo and Kaiser, 1985, 1987; Tarkpea et al., 1986; Bazin et al., 1987; Sanchez et al., 1988). Temperature and salinity control are critical as they affect bioluminescence (Ribo and Kaiser, 1987) whilst interference due to turbidity caused by suspended particles in the effluent is possible.

Despite concerns about its relevance "Microtox" is being used increasingly for assessing aquatic toxicity, evaluating the quality of the aquatic environment and monitoring industrial and domestic plant effluents. It is argued by its promoters that it is cheap, quick and easy to perform and provides in some cases a useful screening test. Nevertheless its correlation with more ecologically relevant tests should be proven in each case. The test is not suited as an indicator of long term toxic effect. It can be used on discrete samples with an appropriate sampling regime, to monitor variations in effluent quality (Vasseur et al., 1986).

2.2. Algal Tests

Algae are the basis of the aquatic food web, a factor that is recognised in product registration as data on the aquatic toxicity of chemicals to algae are required. For product control OECD (1984-b) has published a test guideline. Recently the US-EPA (1985-a) has also applied algal tests to effluent control and their introduction is proposed in Germany.

2.2.1. *Selenastrum capricornutum*. The effect of effluent on this freshwater monocellular alga is determined over 96 hours in a static test (US-EPA, 1985-a). Response is measured either as change in cell density, biomass, chlorophyll content or absorbance and expressed as NOEC/LOEC values or as EC50 in OECD (1984-b).

As the test is newly developed, its practical application and sensitivity are not established. A problem of algal tests is that algae require a growth medium and the observed effects may be due to the test substance reacting with the growth medium or to absorption of light from coloured effluents rather than direct effects on the algae.

2.2.2. *Champia parvula*. Male and female branches of this marine multicellular alga are exposed to effluent in a static system for 48 hours and allowed to recover for 5-7 days in a clean medium. If fertilisation has occurred cystocarps (fruiting bodies) develop. Test results are expressed as the effluent concentration which cause a statistically significant reduction in cystocarp numbers (US-EPA, 1987).

Information on the use of this test is lacking. Provision of sufficient test organism requires a considerable pre-test manpower resource to maintain the culture viability sufficient to promote sexual reproduction. The ability to differentiate between male and female sexual branches and also to recognise immature as well as mature cystocarps requires experience.

2.3. Crustacean Tests

Small crustaceans, an important food for fish, are established as test organisms in regulatory schemes which assess the toxicity of chemical products. Tests with crustaceans are now being applied to the regulatory control of effluents mainly in the USA and in France. Other European countries are also considering the introduction of such a test for effluent control.

2.3.1. Acute Bioassays

2.3.1.1. *Daphnia*. Inhibition of mobility of these freshwater crustaceans resulting from exposure to effluent is determined in static tests using D. magna (AFNOR, 1983; DIN, 1987-a) or D. magna and D. pulex

(EEC, 1984; US-EPA, 1985-a). The test involves a 24 hours preliminary screen followed by the definitive assay (24 or 48 hours). The effluent concentration causing 0, 50 and 100 % immobilisation in a specified time is reported (EC0, EC50, EC100).

Daphnia species are stated to be easy to culture and transport. Being small they require relatively little space but considerable manpower for maintenance. The procedure is relatively simple as the Daphnia are not fed during the test. Some workers have reported difficulties in keeping organisms over the longer term. The reasons are not obvious but may be due to ignorance of the biological needs of Daphnia species. Problems [illegible]ing relate to the provision of large numbers of the requ[illegible] size and thus sensitivity prior to commencing a test, and handling and observation especially in coloured effluents. The assessment of death and immobility can also present problems. There is some evidence that genetic variations between cultures are associated with varying resistance to toxicants.

The use of Daphnia in continuous tests ("Dynamic Daphnia test") was developed for monitoring river waters and effluents (Knie, 1978). In this test Daphnia mobility can be affected by toxic components present in the water tested. In a critical assessment (Caspers, 1988) this test system was assessed as having conceptual and methodological weaknesses.

The American Petroleum Industry (API, 1981) is critical of the EPA test and its application to effluent control.

2.3.1.2. *Mysidopsis bahia*. The test with this marine mysid is basically similar to the EPA Daphnia test, although the definitive test may be either static (48 hours) or flow-through (48 or 96 hours) (US-EPA, 1985-a).

The difficulties of using this species are described below (D 2.3.2.2).

2.3.2. Chronic bioassays

2.3.2.1. *Ceriodaphnia dubia*. Survival and reproduction of this freshwater crustacean are determined during exposure to effluent over seven days in a semi-static test (US-EPA, 1985-b). Reproduction in this species is rapid and the timing of the tests endpoint is critical. NOEC/LOEC values are acceptable if the control organisms produce three broods during the test period.

The US-EPA (1985-b) considers Ceriodaphnia easy to culture, its short lifecycle permitting both acute and chronic tests to be carried out inexpensively and with small volumes of test medium. Nevertheless, regular users of the test system experience problems both in culture and testing (Kraus and Kornder, 1987; Hall and Borton, 1987). Water quality and food requirements of this crustacean are poorly understood and cultures die or suffer reduced reproductive efficiency for no obvious reason. Besides the usual problems of dealing with very small test organisms, taxonomic identification is difficult as is provision of sufficient individuals of the correct size for testing. Manpower involvement is reported to be high.

2.3.2.2. *Mysidopsis bahia*. Juveniles of this marine crustacean are exposed to effluent over seven days in a semi-static test. Survival, growth (dry weight gain) and fertility (percentage of females with eggs) are monitored, results being expressed as NOEC/LOEC values. The test is acceptable if there is 80% survival, 90% of females produce eggs and the average weight is at least 0.3 mg per organism in the controls (US-EPA, 1987).

Advantages which are attributed by the EPA to this organism are its ease of culture on a continuous basis while its small size and relatively short lifecycle permits determination of chronic effects inexpensively in small volumes of test medium. Practical experience has revealed culturing to be expensive both in labour and space. Besides the problem of small size, this organism is

delicate to handle and the test procedure requires considerable manipulative skill. The provision of live Artemia as food during the test is an additional complicating factor.

2.4. Tests with Echinodermata (Sea Urchins) - *Arbacia punctata*

Although adult echinoderms have not been used for toxicity testing, the basis for a test is the well-understood fertilisation process in these animals. The chronic test with this sea urchin is based on the ability of its sperm to fertilise eggs following short term exposure (1 hour) to effluent. Results are expressed as the concentration of effluent causing a statistically significant reduction in fertility compared with a control. The assay is acceptable if the specified sperm-egg ratio results in fertilisation of 70 % of control eggs (US-EPA, 1987).

As the test is newly developed, information on sensitivity and practicality is limited. Although the test is simple to perform and results are obtained within a short time, pre-test maintenance of adults and their stimulation to produce gametes as required may involve considerable effort. Difficulties have been encountered in gamete concentration estimates and in generating tests that meet the acceptable criteria for fertilisation (Boraczek and Rue, 1988).

2.5. Fish Tests

Fish are of commercial and recreational importance and have long been used for acute toxicity testing of chemicals as well as effluents. They are readily available, easy to handle and to maintain in the laboratory. Extensive data bases exist on the effects of chemicals to a variety of fish species. The use of fish in chronic toxicity tests involving partial or complete lifecycles is limited to small warm water species which have a relatively short lifecycle. Nevertheless, large fish are used in automatic monitoring and physiological studies.

2.5.1. Acute bioassays

2.5.1.1. *Salmo gairdneri*. The Canadian test (EPS-Canada, 1980) requires a 4 day exposure of fingerling rainbow trout to undiluted effluent in a static, semi-static or flow-through test, deaths being recorded at specified times. The effluent is acceptable if fish survival exceeds 50%.

Juvenile rainbow trout are used in Ireland (Bolens, 1980), Switzerland (Verordnung, 1975) and Italy (Norme, 1976) to assess effluent quality. In the latter two countries the tests are essentially 24 hours screens using neat effluent (Italy) or a maximum 5-fold dilution of effluent (Switzerland). The Irish test (96 hours) is much more flexible and pragmatic and takes account of any knowledge of the nature of the effluent in reaching a decision on the type of test system to be used.

Rainbow trout are widely used in simple tests as they are easily obtained and maintained in the laboratory. Fish size and loading usually necessitates a large system except the Canadian test which only involves one tank. As the rainbow trout is a cold water species refrigerated water may be necessary. Rainbow trout can acclimatise to moderately high salinities, giving it flexibility as a tests species for brackish and salt water.

2.5.1.2. *Leuciscus idus*. The test determines the mixing ratio of neutralised effluent and dilution water to reach a LCO value for this freshwater golden orfe in a static system. The draft DIN test proposes only 3 fish per effluent dilution and is intended to provide only a yes/no (toxic/non-toxic) answer (DIN, 1980, 1987-c).

The test is simple to operate but requires moderately large equipment. The main problems are limited availability and the variable condition of this fish at certain times of the year.

2.5.1.3. *Pimephales promelas*. One to 90 day old fish are exposed to effluent in a static screen for 24 hours, to establish the range of concentrations for the definitive test, which may be either static (48 hours) or flow-through (48 - 96 hours) (US-EPA, 1985-a).

The test procedure is complicated by the specification for lighting conditions and replicate test concentrations. The flowthrough test requires considerable space and moderate volumes of test material. The comparability of results from static and flow-through tests must be questioned unless it is based on a knowledge of effluent content. The age range of the test fish is surprising considering that sensitivity to toxicants tends to be age/size related.

2.5.2. Chronic Bioassays

2.5.2.1. *Pimephales promelas*. Survival and weight increase of the larvae of fathead minnows exposed to effluent for 7 days are determined in a semi-static test. The results are expressed as NOEC or LOEC values. The test is "valid if control survival exceeds 80 % except where survival in any test concentration is 80 % or better" (USA-EPA, 1985-b).

EPA considers that this important North American forage fish is easy to culture and provides embryos, larvae and juveniles for testing in small volumes of media. Kraus and Kornder (1987) and Hall and Borton (1987) reported problems in trying to operate the test. Achieving significant growth within the test period is a major problem as the newly hatched larvae are too small to take live food (brine shrimps). Coloured effluent exacerbates this as the fish is a sight feeder. The dry weight endpoint is critical and the determination of growth requires very careful measurement.

Norberg and Mount (1985) have reported successful application and validation of the fathead minnow test for effluent control but they

are aware of the need to develop a better understanding of the biological requirements of the fish.

2.5.2.2. *Cyprinodon variegatus*. Survival and weight increase of the newly hatched larvae of the marine sheepshead minnow exposed to effluent over 7 days are determined in a semi-static test. The NOEC/LOEC results are acceptable if control larval survival is at least 80 % and their dry weight is greater than 0.6 mg per organism (0.5 mg if preserved) (US-EPA, 1987).

The test is compact and requires minimum operator effort. Practical experience indicates that the larval fish feed well over the exposure period. Nevertheless, to supply sufficient newly hatched larvae to start a test requires the holding and maintenance of a large number of brood stock. The volume of test medium required is small but this necessitates careful feeding with brine shrimp to avoid low dissolved oxygen levels in the test solutions. Determination of larval weight at the end of the test is critical. The test is newly developed and sensitivity is not known.

E. CONCLUSIONS AND RECOMMENDATIONS

Biomonitoring permits the assessment of the combined effects of the chemical and physical characteristics of an effluent in terms of its toxicity. It cannot identify the particular cause of a response unless appropriate chemical analysis are also incorporated into the biomonitoring study design. Under appropriate circumstances it can be a useful adjunct to, but cannot replace, the classical chemical and physical determinations traditionally used to investigate, monitor and control effluent quality.

Biomonitoring for control of effluent to preserve receiving water quality is finding favour with regulatory authorities in a number of countries and its use is supported by OECD. Some tests were developed specifically in the USA for this purpose although the methods and their application lack adequate validation.

There are problems of extrapolating laboratory test results to the environmental situation. It is due particularly to our poor understanding of the fate (e.g. partitioning, degradability and bioaccumulation) of effluent constituents in the receiving water. As a consequence any definition of a safe effluent discharge based on toxicity can only be an approximation. The principle of controlling industrial discharges on a pass/fail basis using poorly understood test systems is questionable. 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 the necessary degree of dilution by the receiving water.

In the current state of development the main purpose of effluent biomonitoring should be on-site manufacturing plant control. Used sensibly, biomonitoring techniques can provide the chemical plant manager with a tool for investigation of effluent quality, in terms of its toxicity, from its source of origin prior to treatment to its discharge. This may identify trends in effluent quality so that, where appropriate, corrective action may be taken before the onset of unfavorable conditions in the receiving water.

The majority of tests employed to determine effluent quality are acute toxicity tests and involve a range of organisms. A number of other acute test methods including various ISO, OECD, EEC, AFNOR and DIN test methods could also be applied either as they stand or after modification to reduce the number of test organisms, test duration and frequency of parameter monitoring.

There is a concern that acute toxicity data cannot adequately indicate the long term consequences of an effluent discharge into the receiving water. The US-EPA have developed "short term chronic" test protocols which seek to assess the longer term effects of effluents as part of a control scheme to ensure that there are *"no toxicants in toxic amounts"* in the receiving environment. Problems with certain tests can be traced to a poor understanding of the biology of the tests organisms, particularly their nutritional and water quality requirements. Other problems of test manipulation e.g. culturing, feeding, cleaning and handling and observing small species in coloured effluents indicate the need for fully trained and experienced operators. It is recommended these tests be validated.

Tests with bacteria, algae and crustacea may have general application. The use of a particular fish species is, however, likely to remain a national requirement, consequently harmonisation of effluent control testing based on these species is unlikely in a near future. There is no doubt that the bacterial fluorescence (MICROTOX) test is attractive because it is rapid and cheap. Nevertheless its relevance to results in terms of toxic effects exhibited by other test species, including waste water bacteria, must be questioned.

The prime consideration in deciding the choice of a test should be the defined objective of the study. Within industry this may relate to routine monitoring, site investigations, dispersion studies and quality control. In addition the regulatory authority may seek to apply toxicity tests both for the control of effluent quality and receiving water quality. The latter is more complex and results obtained with the above described tests are not adequate. The various tests in existence have both advantages and

limitations which should be taken into account when being applied to a particular circumstance (Appendix 3).

Clearly the whole question of applying effluent biomonitoring data to the control of receiving water quality requires further research in a number of areas. If biomonitoring is to be used as a regulatory tool, there is a need for more research to ensure that test methods are sufficiently rigorous and validated to ensure reproducibility of results between laboratories. As more skill and knowledge is gained, biomonitoring will be used increasingly for effluent control purposes.

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TABLE 1

AQUATIC TOXICITY TEST SYSTEMS - STATIC AND FLOWTHROUGH

STATIC	FLOW-THROUGH
Divided into: (a) True static - test solution not changed during test; (b) Static renewal (semi static) - test solution changed at regular intervals. The latter is a compromise - it reduces waste products and helps to maintain dissolved oxygen and biodegradable materials.	There are two methods of test solution input in test system: (a) Test solution continuously enters the test chamber; (b) Test solution periodically enters the test chamber.
<u>Advantages:</u> 1 Simple and inexpensive; 2 Small volume of effluent required; 3 Can provide some measure of persistence of toxicity; 4 Limited resources required; 5 Routinely conducted.	<u>Advantages:</u> 1 Provides a more representative evaluation of effluent; 2 Metabolic wastes do not build up; 3 More organisms can be tested in each test chamber (high loading factor); 4 Loss of volatiles or degradable constituents from the test solutions is reduced.
<u>Limitations:</u> 1 Results do not reflect temporal changes in effluent toxicity; 2 Metabolic wastes can build up and may harm test organisms; 3 Degradation or loss of volatiles is possible.	<u>Limitations:</u> 1 Large volumes of effluent dilution water are required; 2 Tests are more complex and expensive; 3 More space is required; 4 More resources are required.

J. APPENDICES

APPENDIX 1

GLOSSARY OF TERMS

- BIOACCUMULATION/BIOCONCENTRATION. The process of uptake and retention of substances by an organism from its surrounding medium and from food.

- BIOLUMINESCENCE. Emission of energy by biological systems in form of a radiation with a wavelength of visible light.

- BIOMONITORING (Effluents). Biomonitoring of an effluent is the assessment of the ecotoxic potential on aquatic organisms. Observations are made according to a defined spatial and temporal programme.

- CRITICAL LIFE STAGE. The period of time in an organism's lifespan in which it is most susceptible to adverse effect caused by exposure to toxicants, usually during early development (egg, embryo, larvae).

- DYNAMIC TEST. See Flowthrough test.

- EFFECTIVE CONCENTRATION (EC_x). The concentration which affects X% of a test population after a specified exposure time. The EC_{50} usually relates to effects other than lethality (e.g. loss of equilibrium, paralysis, developmental abnormality or deformity) in 50 % of the test organisms. The effect concentration can involve other percentage such as 10 % and 70 %, e.g. EC_{10} and EC_{70}. An EC_0 can be identified with a No Observable Effect Concentration (NOEC).

- EMBRYO-LARVAL TEST. A chronic test that utilises only the embryo and larval early life stages (usually of fish) as a substitute for full lifecycle testing.

- FLOWTHROUGH TEST. A test in which water is renewed continuously in the test chambers, the test substance being transported with the diluent water in order to renew the test solution.

- HAZARD ASSESSMENT. The estimate of adverse effects which are likely to occur bearing in mind the toxicity of the substances and exposure to those substances.

- MAXIMUM ACCEPTABLE TOXICANT CONCENTRATION (MATC). The geometric mean of the lowest exposure concentration that causes a statistically significant adverse effect and the highest exposure concentration where no effect is observed;

- MEDIAN LETHAL CONCENTRATION (LC_{50} VALUE). A statistically derived concentration which, over a defined period of exposure is expected to cause death in 50 % of the test organisms.

- NO OBSERVED EFFECT CONCENTRATION (NOEC). The highest test concentration at which the test substance has no "statistically significant" effect on the test species.

- SCREEN (LIMIT) TEST. A test in which organisms are exposed to a specific effluent concentration and a control for a short time period (usually not exceeding 24 hours) to quickly and inexpensively determine the potential toxicity of an effluent or toxicant.

- SHORT TERM CHRONIC TOXICITY TEST. Toxicity tests specifically developed to demonstrate any chronic effects of chemicals or effluents over a short period of time (7-10 days). The tests are intended to be a cost-effective approach to the regulatory control of effluent quality.

- STATIC TEST. Toxicity test with no exchange of test solutions or control water over the duration of the test.

- STATIC RENEWAL (SEMI-STATIC) TEST. A toxicity test without flow, but with periodical (usually every 24 hours) batchwise renewal of the test solutions and control water.

- THRESHOLD LEVEL OF OBSERVED EFFECT (LOEC). The lowest test concentration at which the effluent or chemical is observed to have a "statistically significant" effect on test organisms.

- THRESHOLD LEVEL OF LETHAL EFFECT (LEC). The lowest concentration of the effluent or chemical which has a lethal effect.

APPENDIX 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

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
BELGIUM Protection of Surface Water from Pollution Law (1971) (modified by Crown Order, 1976), regulations govern wastewater discharge into surface waters, sewers and stormwater drains. Belgian Institutional Reform Act (1980) - three regional authorities (Flanders, Wallonia, and Brussels) responsible for treatment and control based on physical/chemical limits.	No toxicity test specified.	-	-	Nothing indicated.
CANADA Federal Regulations promulgated under the Canadian Fisheries Act (1970) regulate liquid effluent quality from specific industries. Controls apply uniformly across Canada as national baseline standards on chemical and physical parameters. Provincial agencies may impose more stringent standards depending on local circumstances.	Toxicity tests are specified for refinery liquid effluents, pulp and paper, and meat and poultry products plant.	Refinery - two acute toxicity tests. Routine 24 hrs static bioassay Salmo gairdneri (rainbow trout) at 15°C. Definitive 96 hrs flowthrough Salmo gairdneri at 15°C. Pulp and paper - One test concentration 65% effluent: 35% dilution water and a dilution water control. Fish (unspecified) 96 hrs. Meat/poultry Salmo gairdneri 96 hrs 15°C.	) 50% survival in 100% effluent required. If all control fish survive and <80% of fish in the effluent survive, the latter is regarded as toxic. Percentage mortality established but no indication of how result is applied.	Tests will be used to determine effluent limits and monitor compliance by sector. Prime targets are: petrochemicals, refinery industry, and organics industry. Authorities seek a range of tests with a variety of organisms including some to develop long term effects. Likely to be used to study local problems on a case by case basis.

APPENDIX 2 (cont. 2)

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
DENMARK The National Agency of Environmental Protection (NAEP) develops and coordinates national protection policies to meet requirements of the Environmental Protection Act (1974). NAEP gives guidelines on emission standards and advises regional authorities which control discharges at the local level.	Toxicity data are required but there is no standard test. Routine bioassays are required only in exceptional circumstances. (In one instance a company had to install an "in line" fish monitor.)	No standard test but may use data from literature or laboratory tests (not necessarily carried out in Denmark). Tests which may be used: Marine Mytilus - larval growth rate Artemia - hatching rate Nitocra spinipes - acute toxicity Copepod - sub-lethal test Algae - growth test Fish - growth rates/avoidance Freshwater Daphnia - acute toxicity Algae - growth Guppy - acute	No indication how toxicity data are applied.	Biomonitoring is considered an adjunct to chemical control. Authorities are moving towards a requirement that institutes a "reasonable" programme of tests. These should be: - sensitive - use basal physiological parameters, e.g. growth - relate to field data - reproducible and robust - suitable for use by contract lab staff.
FINLAND Under the Water Act (1962) Water Courts handle applications for permits. Water District Offices are responsible for water pollution control.	Modification to the Water Act (1980) permits use of toxicity tests. The National Board of Waters supervises the use of toxicity tests in water pollution control. Lack of test methods has limited studies on the effects of effluents.	Four tests are now accepted as national standards. Brachydanio rerio 24-96 hrs LC50 test (SFS 3035-SV). Daphnia magna 24-48 hrs LC50 (ISO). Salmo gairdneri. Selenastrum capricornutum (alga). 48-96 hr LC50 (OECD/ISO). Other tests are a 3 hrs short term bacterial test (oxygen electrode method) and a 7 day test measuring toxicity and adaption of heterotrophic microorganisms.	No standard application specified.	Nothing indicated.

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
FRANCE Under Loi sur l'Eau (1964) regional authorities investigate the quality of liquid effluents for control purposes. Controls limit chemical and physical parameters of effluent.	Pollution tax (La Redevance) administered by water financial authorities (Agences de Bassin) is based on acute toxicity test (and certain chemical and physical parameters).	Standard Daphnia test (AFNOR T90-301) on neutralised effluent. Some flexibility - other tests can be substituted in specific instances.	Test results indicate dilution to render the effluent non-toxic. Result is converted to toxic units (Equitox) used to fix tax.	Increasing pressure on chemical industry for more information on effluents. Trend is for more biomonitoring control. Possible additional tests. Salmo gairdneri (rainbow trout) AFNOR T90-305 Brachydanio rerio (zebra fish) AFNOR T90-303 Scenedesmus sp (alga) AFNOR T90-304
GERMANY Under the Water Management Act (1976) (Wasserhaushaltsgesetz) local regional authorities control effluent quality, specifying limits on chemical and physical parameters.	Waste Water Charges Act 1967 (introduced 1978, first used 1981) (Abwasserabgabengesetz) specifies a pollution levy based on an acute fish toxicity test (only half levy is paid if "state of the art" treatment is being applied). *New legislation (to be enacted) 22 Allgemeine Verwaltungsvorschrift ueber Mindestanforderungen an das Einleiten von Abwasser in Gewaesser (Mischabwasser).	Leuciscus idus (golden orfe) 48 hrs test (DIN standard 38412 L20 fish test and DIN draft 38412 L31 fish test) on neutralised effluent. Some regional authorities require acute Daphnia tests (24 hrs test DIN 38412 L30) results being expressed as GD - analogous to GF values. *The new legislation will require bacterial (luminescence inhibition) algal, Daphnia and fish tests.	Test result expressed as dilution factor (GF minimum dilution of the effluent in which all fish survive), e.g. GF2=1 part effluent: 1 part dilution water GF3=1 part effluent: 2 parts dilution water, etc. Tax paid if the GF number is greater than 2 and rises as GF number increases.	Toxicity test only applied to industries which cannot meet a certain acute toxicity level. Pressure from regional authorities for fish test data on new and altered plant, although, until recently there has been a lack of suitable tests. *see other columns.

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
IRELAND Local Government (Water Pollution) Act 1977 is the basis for protecting water quality for multiple uses. The main control by local authority or sanitary authority is on physical and chemical parameters.	Regulatory control for certain new industrial operations requires a toxicity test. Joint assessment is by the Institute for Industrial Research and Standards and An Foras Forbath (AFF) on behalf of the Industrial Development Authority (IDA).	48 hrs Daphnia LC50 test for diagnosis and preliminary screening of pilot scale effluents. Other species may be used. Where a receiving water contains a commercial fishery, avoidance studies (migratory fish) and tainting potential (fish-shellfish) may be undertaken. Routine monitoring species: Salmo trutta (brown trout), Salmo gairdneri (rainbow trout), Pomatoschistus microps (goby), Crangon, Marinogammarus, Artemia. Monitoring for compliance involves a simple (limit) test.	Toxicity is expressed as toxic units (TU) $TU = \frac{100}{96 \text{ hrs } EC50}$ Guidelines specify limit for certain categories of effluent, e.g. Gp A Chemical & pharms eff. 4% 25 Gp D Agriculture & food eff. 70% 1.4 Mixing at discharge point must be at least 20 times for each toxic unit discharged for (new plant), waived for existing plant if always sufficient dilution to meet TU value.	Toxicity limits reviewed periodically.
ITALY MERLI Law 319 (1976), modified by Law 650 (1979) relate to new plant effluents (Article 13). The law, specifying 51 parameters to be monitored, is implemented by local authorities (communes).	Parameter 48 specifies an acute toxicity test with fish.	24 hrs screening test, fish Salmo gairdneri (rainbow trout) exposed to a 50 % solution of effluent mixed with freshwater. For the marine situation salt water species are required (Method IRSA - not yet available). Liza sp (mullet) and Poecilia reticulata (guppy) are proposed.	Effluent acceptable if 50% fish survive. Failure could result in prosecution and a requirement for further testing. However, many derogations apply.	Priority is being given to developing a standardised toxicity testing scheme using sensitive and relevant species. Political and economic pressure may prevent adoption of testing schemes.

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
NETHERLANDS The Surface Water Pollution Act (1970) specifying water quality objectives, is the main control law. Central government is responsible for major watercourses, but with decentralisation, local Water Boards have responsibility for small watercourses. Control is on physical and chemical parameters. A levy is imposed on those discharging oxygen-consuming substances and some metals.	Biomonitoring considered important in control and monitoring of effluents but use is fragmentary. Routine biomonitoring is restricted to the pesticide industry. Where bioassays are carried out to meet licence conditions, both industry and regulatory authority are involved in testing.		Nothing indicated.	No pressure to include monitoring of biological variables in addition to current chemical control methods. However, Dutch scientists would prefer a tiered approach, looking at both effluent toxicity and the health of populations in the receiving water. There is no concensus on the choice of test species.

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
NORWAY				
Water pollution regulations are based on the Water Quality Act (1982) administered by the Ministry of the Environment. The Norwegian State Pollution Control Authority (Statens-forurensningstilsyn STF) is the competent authority.	No standard test, but STF in conjunction with the Norwegian Institute for Water Research, has reported on the "Use of biological tests on Waste and Receiving Water in Norway". STF has appointed advisers to consider the use of biological tests.	50 different tests for toxicity, biodegradation and bioaccumulation are stated to be available including Algae (freshwater/marine) Chlamydomonas — Growth, photosynthesis Dunaliella — Lethality, cell division Invertebrates Mytilus) Lethality Daphnia) Lethality Artemia) Lethality Marinogammarus) Lethality Mytilus — Activity, growth Sea urchin) Development Balanus) Development Daphnia) Reproduction Sea urchin) Reproduction Fish Frehswater/marine — Lethality, respiration, physiology Bioaccumulative genotoxicity — Mutation studies	There will be no pass or fail standards or comment on response of industry that fails to meet a standard. Data generated will not be confidential and can be used by action groups to bring a prosecution against an industry. The lack of criteria for evaluating the information generated is seen as a weakness. Because of the wide range of species sensitivity control assessment is likely to involve a large number of screening tests rather than determining dose response.	Biological tests are considered of value in the management of industrial pollution. Tests likely to be used on an industry by industry basis and relate to local environmental conditions. Uses: - developing water quality criteria - setting discharge standards - monitoring of effluents and receiving waters - decisions on water treatment.

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
SPAIN Sewage and industrial effluent quality is controlled by ten regional water commissions (Comisaries de Aguas). Relevant control acts promulgated by the Ministry of Public Works, Housing & Town Planning (MOPU) are: Ministerial Act (1959) complemented by a further Act (1962) and Regulations (1961) relating to poisonous and noxious discharges of industrial effluents.	Only the Catalan region has legislation (Law 5/1981) requiring a toxicity test. Relates to application of a Pollution Tax administered by the Department of Political Territories and Public Works.	Standard French Daphnia test (AFNOR T90-301)	Standard formula applied to give toxicity limits on which tax is based (cf. France).	Nothing indicated.
SWEDEN The National Swedish Environmental Protection Board coordinates all tests concerned with environmental safety covered by the Environmental Protection Act (1969). Local councils administer control mechanisms (concession conditions) decided by a National Franchise Board for Environmental Protection. A guidance document for administrative use of tests was issued by NSEPB (1982).	Concession conditions require biological tests but no standards are set.	96 hrs acute toxicity screen with a copepod Nitocra spinipes. Less frequency Brachydanio rerio (zebra fish) and Microtox. Other screening tests that could be used are: - short term toxicity, fish crustaceans - acute/chronic algae, higher plants - bioaccumulation - biodegradation Effluent testing may be complemented by ambient (receiving water) testing.	No specific applications indicated, although test programmes may be applied in a flexible manner depending on type of industry and nature of receiving water. Aim to answer defined questions.	Pressure to use model ecosystems to monitor long term effect of effluents.

APPENDIX 2 (cont. 8)

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
SWITZERLAND The Sewage Discharge Act (1975) specifying limits on 52 chemical and physical parameters is administered by member cantons.	The regulatory requirement includes an acute toxicity test.	Acute 24 hrs toxicity test with Salmo gairdneri (rainbow trout). Other organisms are considered in certain cases.	Depending on the conditions of the receiving water, an effluent must not be toxic at 0-5 fold dilution.	Pressure to use alternative species fish.
UNITED KINGDOM Historically effluent discharges controlled by Rivers (Prevention of Pollution) Acts 1951 and 1961 now superseded by Control of Pollution Act Pt II 1974 implemented 1984-86. Regional Water Authorities (England & Wales) and River Purification Boards (Scotland) grant consents limiting levels of specified physical and chemical parameters of an effluent.	No national regulatory requirement for a toxicity test. However, under COPA II, regulatory authorities can control discharges by any sensible means. In this context toxicity tests are used on an ad hoc basis.	No standard toxicity tests. Any acute tests use fish or invertebrates relevant to the receiving water.	Where tests have been applied the wording of the consent specifies the number of dilutions of the effluent that will not result in >50% mortality. In one case this dilution must be achieved in the receiving water at the edge of a mixing zone defined by the regulatory authority.	Department of the Environment envisages that tests should be: - available for freshwater and seawater - vary to meet local circumstances - applied broadly with emphasis on certain industries. Use of acute or chronic tests will depend on circumstances and type of exposure with the trend towards long term studies. Industry to selfmonitor with checks by authority. Testing laboratories to be licensed.

Regulations and controlling authority	Requirement for toxicity test	Toxicity test method	Application of test results	Possible future development of biological testing
USA The Federal Water Pollution Control act (1972) introduced a national policy prohibiting the discharge of "toxic pollutants in toxic amounts". Section 101 introduced the National Pollutant Discharge Elimination Scheme (NPDES) to control discharges by issuing permits which limit physical and chemical parameters.	NPDES (2nd round of permitting) required effluent toxicity testing to: - identify environmental problems - establish pollution control priorities - set discharge limits - identify appropriate corrective actions - monitor for unacceptable effluent effects.	Two-tier system 24 hrs Daphnia screen Failure requires a full LC50 test using Daphnia or Promelas pimephales (fathead minnow) over 96 hrs. Other species used by various States include Salmo gairdneri (rainbow trout), mysids, penaeid shrimp, oyster larvae and algae. Most tests are static although some States require static, renewal or flow-through tests.	80% survival in 100% effluent. Application factor of 0.1 applied to 48 hrs EC50 to give acceptable dilution.	US Environmental Protection Agency (EPA) is replacing acute tests by sublethal tests (Short term chronic tests). Freshwater tests - Ceriodaphnia reproduction (7 days) - Pimephales promelas (fathead minnow) growth/survival (7 days) are now being used in NPDES permitting. Marine tests (published Mar 88) - Cyprinodon variegatus (sheepshead minnow) survival - Mysidopsis bahia (mysid) reproduction - Arbacia punctata (sea urchin) reproduction - Champia parvula (red alga) reproduction will be incorporated into the permit scheme.

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APPENDIX 3

OVERVIEW OF CURRENT REGULATORY TESTS FOR BIOMONITORING OF EFFLUENTS

Test Species*	Bacteria			Algae		Crustaceans				Echinoids	Fish				
	Ph ph	Ps p	AS/Ps p	S c	C p	D m	C d	M b	M b	A p	S g	L m	P p	P p	C v
1 Test Practicability/Robustness															
1 1 Test organisms															
Size (Defined/Non Defined/Not Relevant)	ND	NR	NR	NR	NR	D	D	D	D	ND	D	D	D	D	D
Readily available (Yes/No)	Y	Y	Y	Y	Y	Y	Y(US)	Y(US)	Y(US)	Y(US)	Y	M	Y(US)	Y(US)	Y(US)
Culture (Easy/Medium/Difficult/Not Relevant)	E	E	E	M	M	M	(D)	D	D	M	NR	NR	NR	NR	NR
Maintenance (Easy/Medium/Difficult/Not Relevant)	NR	NR	NR	E	M	NR	NR	NR	NR	M	E	E	E	E	E
1 2 Equipment/Personnel															
Size (Small/Medium/Large)	S	M	M	M	S	S	S	S	M	S	L	L	M	M	M
Complex (Yes/No)	N	N	N	Y	Y	N	Y	N	Y	Y	N	N	N	N	N
On line measurement (Existing/Possible/Impossible)	P	E	I	E	I	P	P	I	I	I	P	P	P	I	I
Quality of manpower (High/Medium/Low)	L	L	L	L	M	L	L	L	H	M	L	L	L	M	M
1 3 Test specifications															
Type: Static/Continuous	S	C	S	S	S	S/C	S	S/C	S	S	S/C	S	S/C	C	S
Acute/Chronic/Other	O	O	O	A	C	A	C	A	C	C	A	A	A	C	C
Screening/Definitive	S	D	S?	D	D	S/D	D	S/D	D	D	D	D	S/D	D	D
Control performance (Yes/No/Not Relevant)	NR	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
No of test organisms (Number/Not Relevant)	NR	NR	NR	NR	NR	20	20	10	5	NR	10	5	20	20	(20)
No of concentrations (Number/Not Relevant)	1-10	1-10	1-10	1-10	1-10	1-10	5	1-10	5	>5	1-5	5	5	5	5
Replicates (Yes/No)	Y	Y	N	Y	N	Y	Y	Y	Y	Y	N	N	Y	Y	Y
Reference compounds (Yes/No)	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N
Nature of end point	light	resp	gr	gr	repro	mm	repro	leth	repro	fert	leth/beh	leth	leth	repro	surv/gr
Results expressed as	EC50	EC50	EC50	EC50	EC50	EC50	repro	LC50	repro	fert	LC50	LC50	LC50	NOEL	NOEL
Complexity of end point/data derivation (Easy/Complex)	E	C	E	C	E	E	E	E	C	C	E	E	E	C	C
Maintenance (High/Medium/Low)	L	M	M	L	M	L	M	M	M	H	M	M	M	H	H
2 Flexibility															
Test medium (Salt-, Fresh-, Dilution water)	S	S/F/D	D	S/F/D	S	F/D	F	S	S	S	F/S	F	F	F	S
Known interference (salt, O2, temp., light) (Yes/No)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	?
3 Validation															
Existing regulatory requirement (Country)				US	US/F	US	US	US	US	US	Can/CH/I	D	US	US	US
Presence of test protocols (Regulatory/Others)	O	O	O	R	R/AFN	AF/R	N	R	R	R	R/OECD	DIN	R	R	R
Interlaboratory calibration (Yes/No)	Y	N	N	N	N	Y	N	?	?	?	Y	Y	Y	Y	N
Experience and availability of data (Yes/Limited/No)	Y	N	N	N	N	Y	L	N	N	N	Y	Y	Y	L	L
Defined criteria for a valid test (Yes/No)	N	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4 Convenience for in house control															
Adaptable for on site control (Yes/No)	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N
Rapidity to obtain results (min., hours, days, weeks)	1h	min	5h	>3d	7-9d	1-2d	7d	2-4d	7d	>1-5h	4d	2d	2-4d	(28d)	(7d)
Quantity of test material (Large/Medium/Small)	S	S	S	S	S	S	S	S	S	S	L	L	M	L	M
5 Relevance															
Correlated with other tests (Yes/No)	Y	N	Y	N	N	Y	N	N	N	N	Y	Y	Y	Y	N

*Ph ph - Photobacterium phosphoreum; Ps p - Pseudomonas putida; AS - activated sludge; S c - Selenastrum capricornutum; C p - Chlorella pyrenoidosa; D m - Daphnia magna; C d - Ceriodaphnia dubia; M b - Mysidopsis bahia; A p - Arbacia punctulata; S g - Salmo gairdneri; L m - Leuciscus idus; P p - Pimephales promelas; C v - Cyprinodon variegatus

APPENDIX 4

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