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The control of effluent discharges by a Direct Toxicity Assessment (DTA) approach

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EXECUTIVE SUMMARY

This report outlines a procedure for using toxicity measurement to control complex and variable discharges. It focuses on the principles underlying the approach such as how the use of toxicity-based control relates to the traditional chemical-specific approach and the reason for each stage of the protocol.

The first step in the procedure is to identify and prioritise candidate effluents that may be appropriate for toxicity-based control. This involves a desk based appraisal of available data on effluent composition and variability and the dilution capacity of the receiving water. Collection of toxicity data from a battery of rapid and complementary screening tests is also usually required. For effluents considered suitable there follows in-depth testing with the most appropriate screening test and acute higher organism (alga, invertebrate and fish) tests representative of the water that receives the discharge. The data from the most sensitive of the tests are used to derive an acceptable environmental concentration (AEC). The AEC is then compared with the receiving water concentration (RWC) of effluent at the edge of a defined mixing zone to assess whether a toxicity-based discharge consent should be derived or the toxicity of the effluent needs to be reduced.

For effluents appropriate for toxicity-based control, establish whether a correlation exists between the most sensitive higher organism test and the most appropriate screening test. Where a highly significant positive correlation exists a 'calibrated' screening test consent condition can be derived. For discharges where no correlation exists the toxicity-based discharge consent should specify the most sensitive test. The toxicity-based consent can be expressed as an absolute limit or as an effective (EC_{50}) or lethal (LC_{50}) concentration. The variability in the toxicity of an effluent governs the level of testing required to establish the discharge consent and the frequency of monitoring necessary to assess compliance.

KEY WORDS

Toxicity-based consents, effluents, screening tests, discharge consents

1. INTRODUCTION

1.1 The current situation

The traditional chemical-specific approach to discharge control involves measuring the concentrations of substances in an effluent and assessing the effect that constituents may have on the receiving water from relevant toxicological data. Control is achieved by establishing maximum permitted concentrations of the relevant pollutants in the effluent which will satisfy established Environmental Quality Standards (EQSs) or Likely Safe Environmental Concentrations (LSECs). Appropriate sampling and chemical analysis of the effluent are undertaken to ensure the limits are not exceeded and there is compliance with the discharge consent.

This approach is satisfactory for simple effluents of well defined and consistent composition, but it has a number of disadvantages for effluents of a complex nature since:

1. Many effluents contain organic chemicals which are not readily or accurately identifiable or measurable by even the most sophisticated analytical techniques available to laboratories conducting routine monitoring;

2. There may be data on levels of potential pollutants, but toxicological data upon which to set EQSs or LSEC are sparse or unavailable for many thousands of synthetic chemicals. Furthermore, the information that is available may not be directly relevant to indigenous organisms;

3. It is costly to measure accurately all the chemicals present in a complex effluent and these provide no indication of toxicity. There may also be problems in applying EQSs or LSECs, which are derived on the basis of single substance toxicity. This takes no account of interactions between the effluent constituents and between the constituents and the receiving water;

4. The variable composition of many complex effluents, particularly those from plants operating batch processes, may further compound the difficulties.

These difficulties have prompted an interest in recent years in applying Direct Toxicity Assessment (DTA) to control complex and variable effluents and provide greater protection for receiving water communities.

1.2 The need for toxicity-based control

Toxicity-based control of discharges is achieved on the basis of whole effluent toxicity rather than that of individual components. For this approach to be considered useful by regulators, compliance with toxicity-based consent conditions has to ensure that the quality of receiving waters is maintained or improved. Studies in the United States have shown that in both freshwater and marine waters, effluent toxicity is correlated with both toxicity in the receiving water and resulting biological impact as measured by macroinvertebrate surveys (US EPA 1991). Figure 1.1 shows the results of a study by the North Carolina Division of Environmental Management and indicates the high accuracy (88%) of predicting receiving water impacts from whole effluent toxicity tests on 43 point source discharges (Eagleson *et al* 1990). In 5.0 % of instances false negatives occurred where toxicity tests with the water flea *Ceriodaphnia dubio* predicted no in-stream toxicity while macro-invertebrate sampling indicated an effect. In-stream toxicity was predicted from toxicity tests where no impact was noted with biological monitoring (false positives) in 7.0% of instances.

Traditionally the use of a toxicity-based approach in the United Kingdom has been limited to NRA Anglian and Welsh regions and the Clyde River Purification Board (Mackay and Haig 1988, Haig *et al* 1989, Mackay *et al* 1989). However, the potential role for direct toxicity assessment was indicated by recommendation 16 of the NRA report on 'Discharge Control and Compliance Policy' (NRA 1990), which states that:

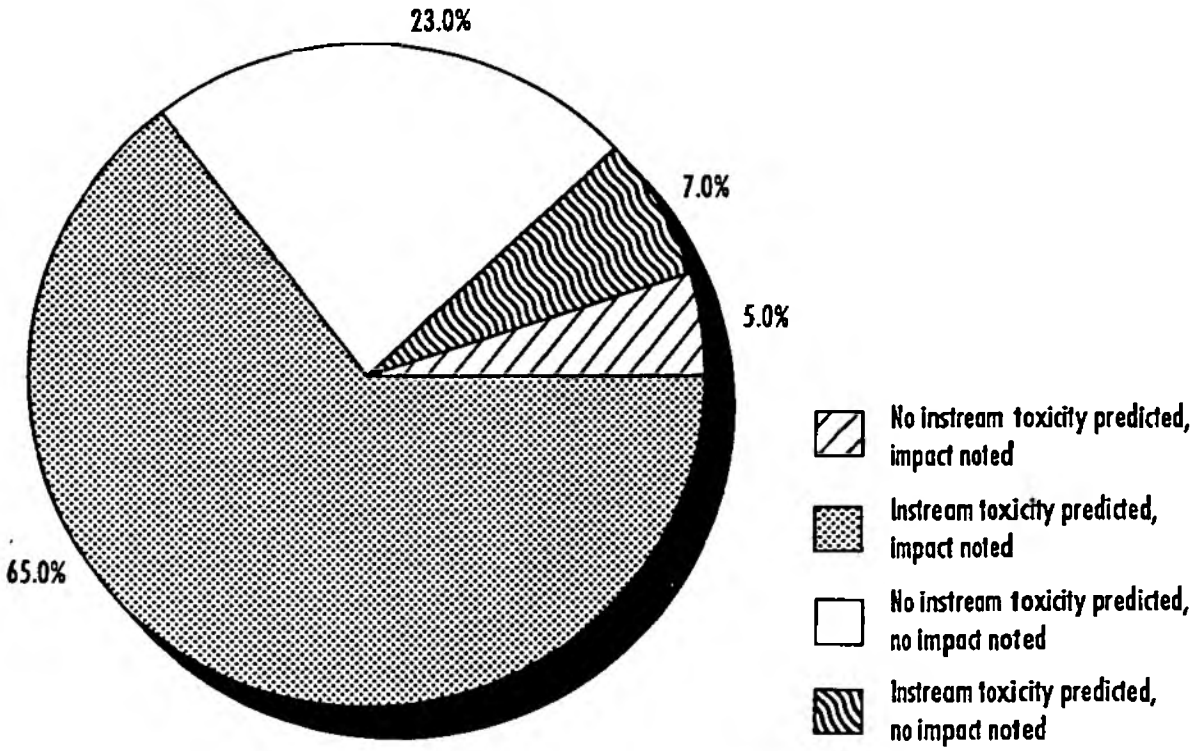


Figure 1.1 Comparison of conclusions drawn on the impact of 43 point source discharges on freshwater receiving systems in North Carolina from effluent toxicity data and macroinvertebrate surveys.

> "For environmentally significant discharges of complex composition where not all important constituents can be individually identified and numerically limited, consents should specify a clearly defined toxicity limit, the appropriate form of toxicity test to be used, and the minimum frequency with which it should be applied."

In describing any approach advocating the use of toxicity-based consents it is important to emphasise that direct toxicity assessment should not be considered to be an alternative to chemical-specific control. Existing or proposed discharges containing substances for which EQSs are established will require numerical concentration limits in the consent. The DTA approach should be viewed as a complementary tool, which allows regulatory agencies to exert greater control over complex and variable discharges and results in more effective protection of receiving water communities. However, it is important that this approach is only applied to those discharges for which toxicity-based control is necessary and appropriate.

The application of a DTA approach in the UK requires:

1. A common validated protocol for consistency;

2. Quality control procedures to ensure the acceptability of results to regulatory agencies, dischargers and the public.

These requirements have been addressed in this research and development programme (A18.049) carried out by WRc, in close collaboration with regulatory authorities throughout the UK. An initial protocol developed by Hunt (1989) has been assessed in a series of case studies and this report outlines the refined procedure which is proposed as the basis for deriving and implementing toxicity-based consents for appropriate discharges. The specific methods and procedures to be used in practice are described in detail in an accompanying draft protocol (Johnson *et al* 1992b).

Butler *et al* (1992a,b) report in detail the case studies on which this reports recommendations are based. This report summarises the points that were considered when preparing the protocol for applying toxicity-based discharge consents and proposes a way forward taking into account the experience gained in the case studies. Practices in other countries, especially the United States, were reviewed (OECD 1987, ECETOC 1990, Crane *et al* 1991) and integrated where they were considered appropriate.

The proposed protocol outlined in this report should be regarded as an initial framework on which regulators can build. Clearly it will benefit from use by pollution control officers who can apply their extensive practical experience to the subject and refine the procedure in the light of practical and pragmatic difficulties that may arise.

2. PROTOCOL FOR DIRECT TOXICITY ASSESSMENT

2.1 Introduction

Figure 2.1 summarises the initial DTA protocol proposed by Hunt (1989) for deriving toxicity-based consents. It has elements of the techniques developed by the US Environmental Protection Agency (EPA), adapted to satisfy the requirements of the United Kingdom. Figure 2.2 outlines the protocol described in this report, which is the initial protocol amended in the light of the results obtained from case studies of discharges to fresh and marine waters. These formed an integral part of this project to produce a robust, cost-effective, legally enforceable and easily implemented procedure.

The proposed protocol advocates the use of simple well established short-term toxicity tests. All the toxicity tests to be used in the protocol have been thoroughly validated and have standard operating procedures to which appropriate quality control procedures can be applied. However, this should not restrict the use of more sophisticated methods in situations identified from the application of the protocol.

The approach described can be used for existing discharges and for proposed discharges providing a pilot plant can produce an effluent representative of the actual discharge. Direct toxicity assessment can be used to control both single and multiple discharges to receiving waters. The three stage strategy for identifying and controlling appropriate discharges by toxicity-based consents involves:

1. Selecting and prioritising appropriate discharges;

2. In-depth testing to assess the toxicity of an effluent relevant to the available dilution and determine whether immediate toxicity reduction of the effluent is needed;

3. Deriving an appropriate discharge consent.

There follows a discussion of the requirements of, and rationale for, each stage.

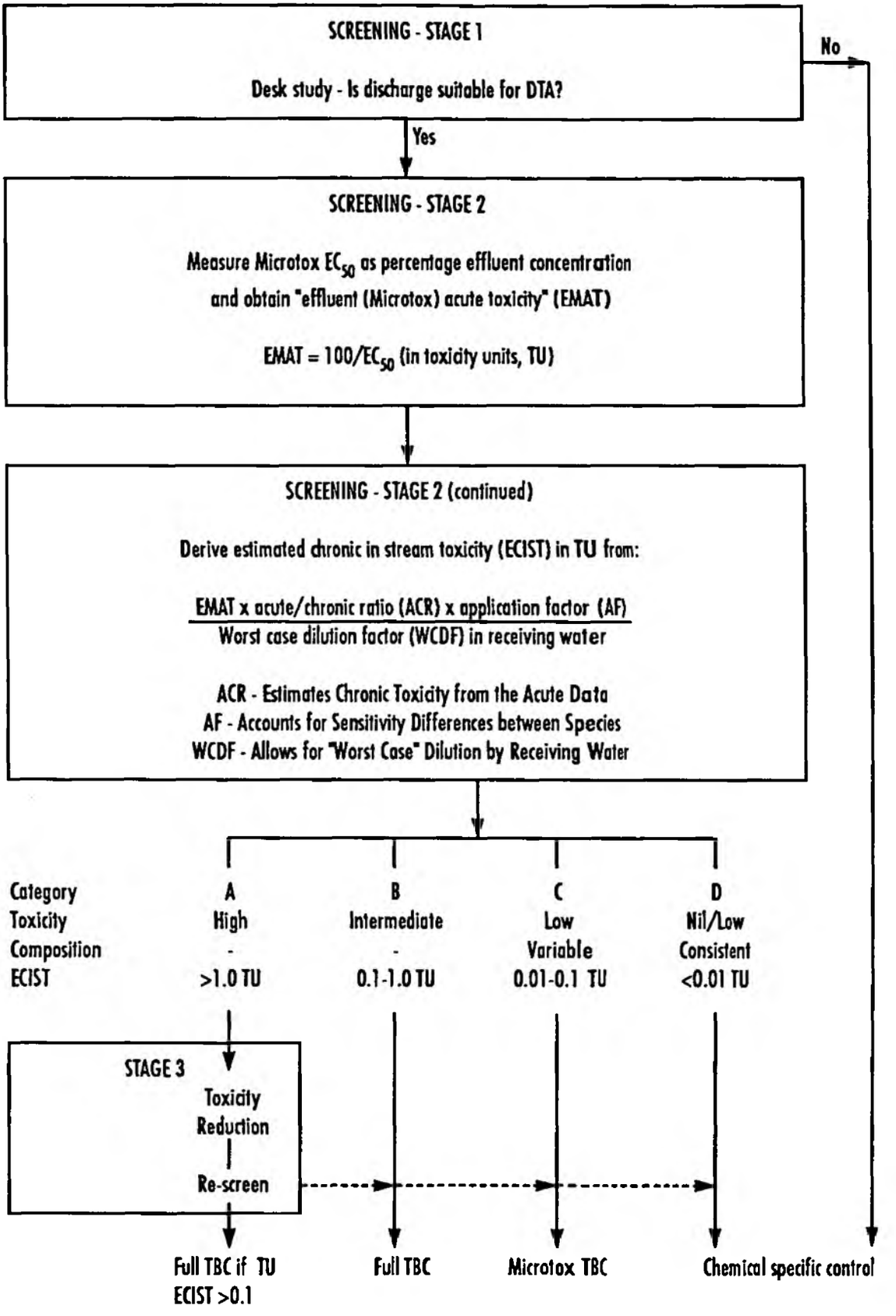


Figure 2.1 The initial Direct Toxicity Assessment (DTA) protocol. (After Hunt 1989)

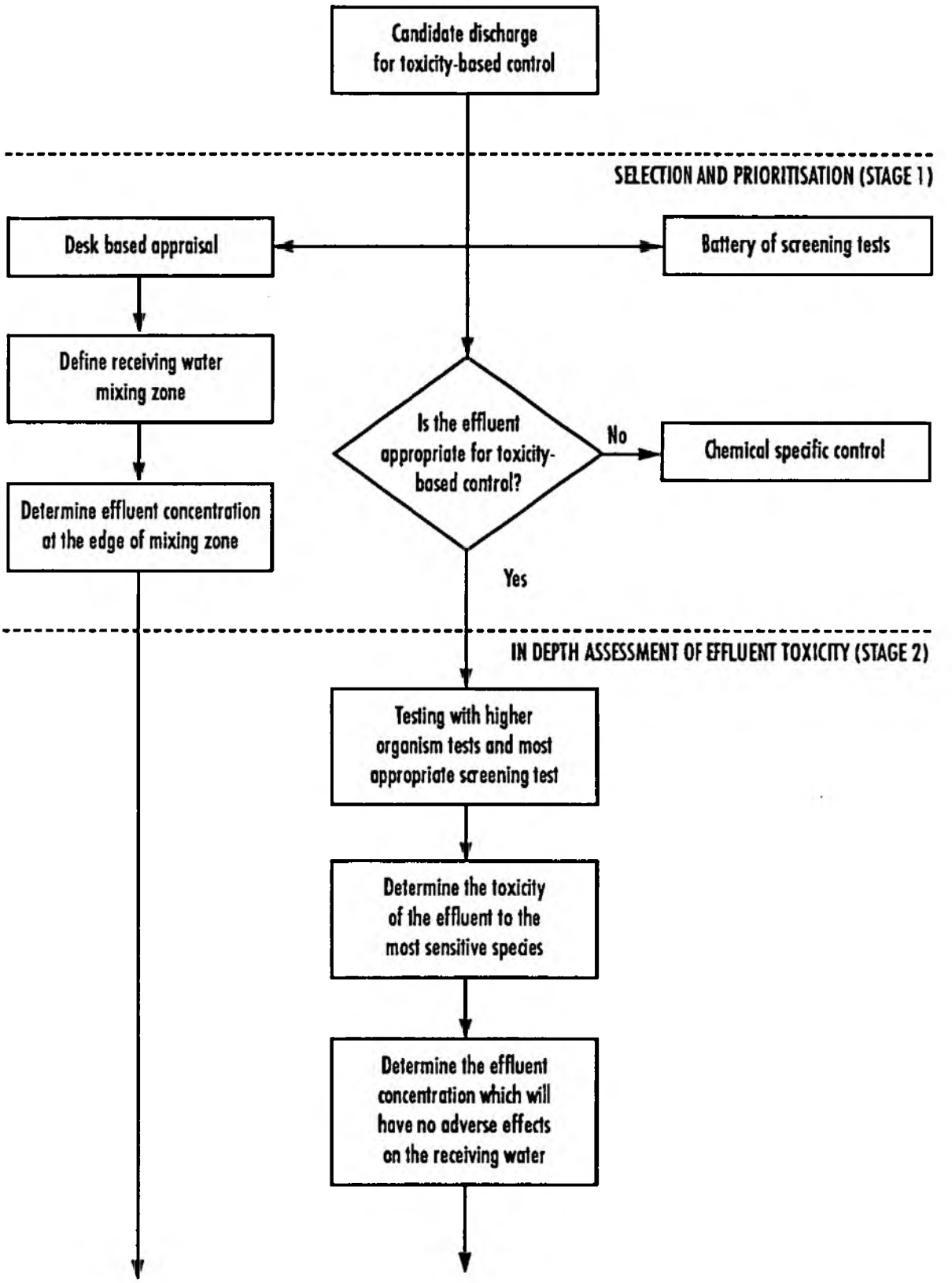


Figure 2.2 Flow diagram of the procedure for selecting appropriate discharges for toxicity-based control, assessing whether a consent should be derived and determining which test should be specified in the consent.

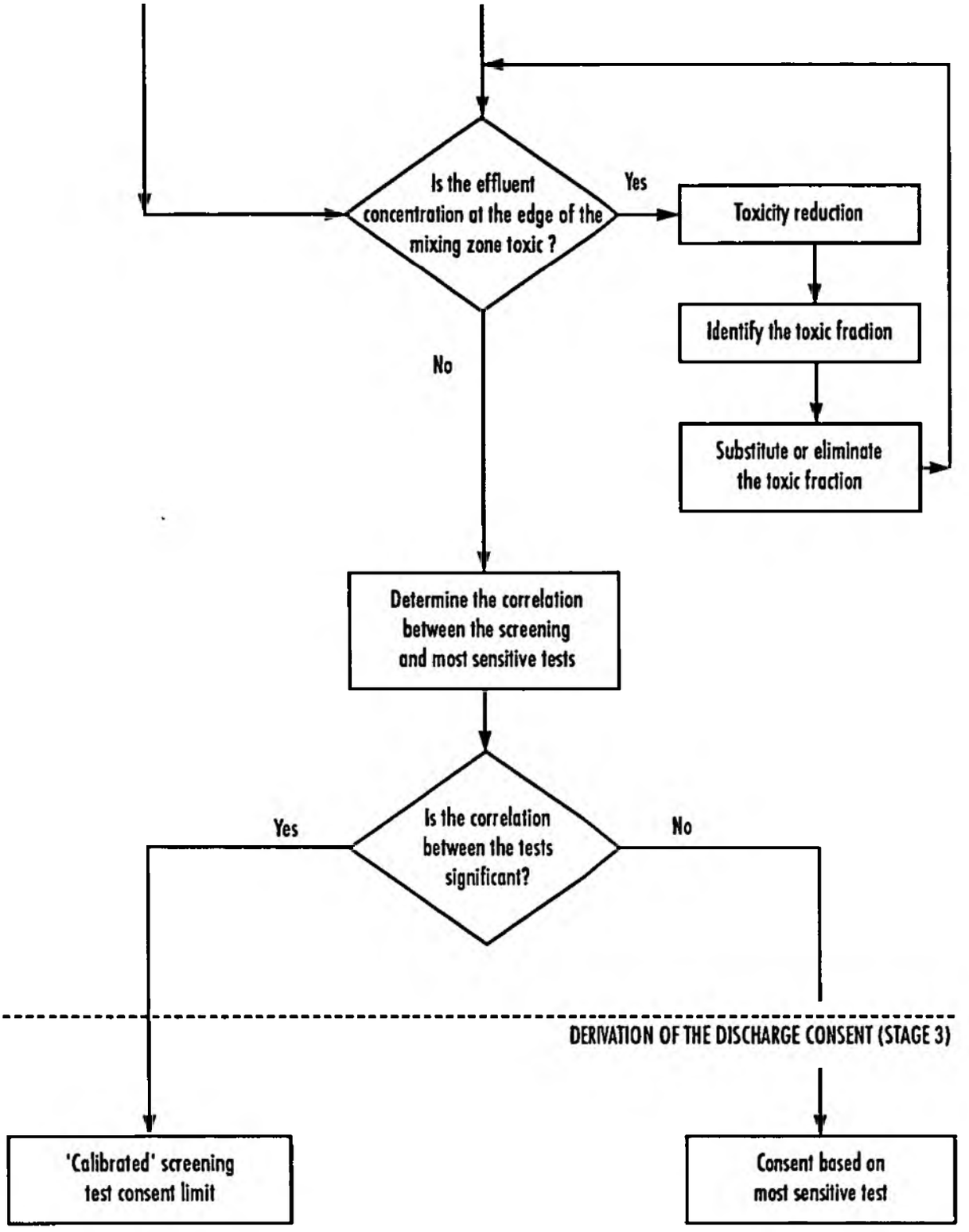


Figure 2.2 (contd.)

2.2 Selection and prioritisation of appropriate discharges

The selection of effluents for which toxicity-based control is appropriate, and their subsequent prioritisation for further toxicity testing, is based on information collated in a desk-based appraisal of the discharge and, if necessary, screening the effluents with a battery of complementary toxicity tests. Candidate effluents should initially be selected where current discharge consents are considered, by pollution control officers, to be inadequate for protecting receiving water communities.

2.2.1 Information required for the desk-based appraisal

Table 2.1 outlines the type of data required from the desk-based appraisal. The information which regulators should request from dischargers is highlighted along with the data which need to be obtained by regulators.

The data collated in the appraisal are needed to:

1. Identify the current or proposed uses of the receiving water and substances which have to satisfy Environmental Quality Standards;

2. Calculate the diluting capacity of the receiving water and the effluent concentration at the edge of the mixing zone;

3. Assess the potential toxicity of the discharge constituents and their propensity for bioaccumulation by comparing chemical concentration data with toxicological information to derive a list of substances of concern, which may require chemical-specific limits.

For existing effluents which already have discharge consents with chemical-specific limits the majority or all of this information will be available and the above procedures will have been conducted. In contrast for proposed discharges most or all of the data will have to be obtained and, in certain instances, may not be immediately available.

Table 2.1 Type of information required for the desk-based appraisal

Information needed	Information obtained From discharger	Information obtained By regulator
Substances present in the effluent	+	
Toxicological data (sub-lethal and lethal toxicity, potential for bioaccumulation) on substances present in the effluent	+	
Current or proposed uses of the receiving water		+
Relevant EQSs which need to be satisfied		+
Volume of effluent discharged to receiving water at peak flow from on-site gauging	+	
Worst case flow in riverine receiving waters and tidal flow/ dispersion in estuarine and coastal waters		+
Chemical monitoring data	+	+
Available toxicity test data on the whole effluent or constituents (including data on the degree of treatment on site)	+	+

Existing discharges

In the case of existing discharges with chemical-specific limits a mixing zone will have been established to calculate the permissible levels of individual substances of concern in the effluent which will satisfy EQSs or LSECs. It may be necessary to review the allowable mixing zone and the derived chemical concentration limits at this stage. The chemical monitoring data should be assessed for indications of likely patterns of temporal variability in effluent toxicity. This can be valuable in planning the sampling regime for the screening tests. However, there may be problems with complex effluents due to

variability in the levels of effluent constituents, each of which contribute differently to the overall toxicity. The identification of a chemical measure which is a realistic surrogate for toxicity would considerably aid interpretation. However, the data from the case studies indicate no general index was apparently suitable for all types of effluents (Butler *et al* 1992a). Table 2.2 shows the correlation coefficients between the chemical measures, biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and toxicity tests (Microtox, algal growth inhibition, oyster embryo-larval development and juvenile turbot lethality) for effluents from one chemical and one pharmaceutical plant discharging to marine waters. The only significant correlations in the limited data set were between BOD and certain toxicity tests for the pharmaceutical effluent.

Table 2.2 Correlations between biochemical oxygen demand (mg l^{-1}) and chemical oxygen demand (mg l^{-1}) and a range of toxicity tests (EC_{50}s as % effluent) for industrial effluents

Toxicity test	Biochemical oxygen demand		Chemical oxygen demand	
	Chemical	Pharmaceutical	Chemical	Pharmaceutical
Microtox	0.727	0.996 *	0.346	0.162
Algal growth inhibition test	0.031	0.928	0.469	0.448
Oyster embryo-larval test	0.067	0.968 *	0.132	0.002
Juvenile turbot lethality test	0.349	0.963 *	0.726	0.345

* indicates significant correlation coefficient ($P<0.05$)

Proposed discharges

For proposed discharges an acceptable mixing zone will have to be defined. Analysis of a representative pilot effluent will also be required to identify

substances which will have to satisfy existing EQSs and other substances of concern which, due to their toxicity or potential for bioaccumulation, may be controlled by establishing maximum permissible limits.

2.2.2 The use of screening tests to assess effluent toxicity

Although the chemical concentration and toxicological information generated in the desk-based appraisal can provide an indication of the likely toxicity of the discharge it is accepted (OECD 1987, ECETOC 1990, US EPA 1991) that only the direct measurement of the toxicity of a complex effluent can provide a realistic and integrated view of the potential problem.

In the United States, EPA protocols specify that the toxicity of effluents is initially assessed using as many as three acute toxicity tests of 24 to 96 hr duration at different trophic levels (US EPA 1991). However, the time and cost involved in such an approach could impede adoption of a DTA approach in the UK, and thereby delay the benefit of improved pollution control. Therefore the proposed UK protocol advocates the use of a battery of complementary screening tests which can rapidly provide data on the toxicity of effluents considered suitable for toxicity-based control.

2.2.3 The need for a battery of screening tests

The use of a battery of screening tests to assess effluent toxicity is proposed since available data (Qureshi *et al* 1982, Calleja *et al* 1986, Young *et al* 1991, Butler *et al* 1992a,b) indicate that a single screening test could not identify all potential candidate effluents for toxicity-based control. Specificity in the mode of toxic action of certain chemical classes, such as herbicides and insecticides means that tests using non-target species, will often be considerably less sensitive than those using target organisms. A single test is therefore unlikely to be sensitive to all types of effluents and may not show certain types of discharge to be toxic. The use of a battery of complementary tests, which show different sensitivities to various types of effluents, should ensure that the incidence of false negatives is minimised. Effort can then be

directed towards those discharges which are appropriate for toxicity-based control.

2.2.4 Selection criteria for screening tests

The tests used at the screening stage of this draft protocol should ideally be:

1. Rapid, robust and reproducible;

2. Readily available throughout the year;

3. Sensitive to a range of pollutants, with available information on toxic responses to these chemical classes;

4. Fully validated (ring tested) and have a standard recognised method;

It is also useful if these tests only require small test volumes since the availability of effluent may be a limiting factors for proposed discharges where effluent is produced on a pilot plant scale.

2.2.5 Proposed screening tests

At present there are a lack of appropriate validated short-term tests which can rapidly and reproducibly assess the toxicity of effluents. The commercially available Microtox toxicity test, based on the bioluminescent response of *Photobacterium phosphoreum*, is the only test which fulfils the stated criteria and is sufficiently well developed to be used in the screening role. Therefore, at this time, the longer 24 hr *Daphnia magna* (water flea) immobilisation test and 24 hr oyster (*Crassostrea gigas*) embryo larval development tests are advocated as complementary methods to assess the toxicity of effluents discharged to fresh and marine waters respectively. As other appropriate screening tests are developed and validated they can be introduced at this stage of the procedure (see Section 5.1).

The effluents tested in the case studies have confirmed the need for a battery of screening tests to increase the likelihood of detecting all discharges which are suitable for toxicity-based control. Data from the case studies (Butler *et al* 1992a,b) has been analysed to determine the percentage of instances that Microtox and combinations of Microtox and the oyster embryo-larval test would identify toxic discharges that are appropriate candidates for toxicity-based control (Table 2.3). All of the chemical, oil refinery, pharmaceutical and plastics manufacturing effluents tested in the case studies that were toxic to Microtox were also identified as toxic by the oyster embryo larval test. Microtox only identified as toxic 67% of the paper mill effluents which were toxic to oyster embryos.

2.2.6 The need to assess effluent variability

The composition and quality of complex effluents can vary greatly over time due to changes in the quality and quantity of influents and variations in the efficiency of on-site treatment systems. Variations can also be caused by rainfall, runoff and infiltration that are not related to the waste generating process. The time scales over which these fluctuations occur can vary considerably depending on the nature of the processes.

The level of toxicity testing at the screening stage should reflect the inherent variability of effluent and identify whether the toxicity of the discharge:

1. Remains consistent over time;

2. Varies in a definable manner, with a regular pattern of effluent toxicity;

3. Varies in a non-definable manner, with no discernable pattern of effluent toxicity.

Continuous on-line monitoring with rapid screening tests provides the most effective way of assessing and classifying the variability in toxicity of a

Table 2.3 Toxicity of a range of effluent types to Microtox and the oyster embryo-larval test

Effluent type	n	Toxic to Microtox and the oyster embryo-larval test larval test	Toxic to oyster embryo-larval test and non-toxic to Microtox
Chemical	5	100%	-
Oil refinery	9	100%	-
Pharmaceutical	5	100%	-
Plastic manufacturing	3	100%	-
Paper mill	6	67%	33%

n = number of effluents

discharge at the start of a study. At present a standardised and validated system is not available, though there are a number of potentially useful methods under development (see Section 5.4). In the absence of the necessary methods an appropriate sampling regime with rapid tests has to be used to accurately categorise the variability in toxicity of an effluent.

2.2.7 Identification of candidate effluents for toxicity-based control

The initial protocol (Figure 2.1) proposed a screening stage that attempted to classify discharges using Estimated Chronic 'In-stream' Toxicity values. This used information on worst case dilution factors and appropriate safety factors to extrapolate from acute data to chronic sub-lethal effects and account for species sensitivity, and the persistence and capacity for bioaccumulation of effluent constituents. This is considered to be unnecessarily complex at the screening stage. The amended protocol recommends that the identification of candidate effluents, and their prioritisation, should be based primarily on toxicity to the screening test(s) and the extent of the available dilution in the receiving water. Toxicity should be expressed as percentage effluent rather than in toxic units.

Since the conclusions drawn will be specific for an effluent and its discharge site it is difficult to recommend prescriptive rules. In the first instance, attention should be given to discharges which indicate acute toxicity to one or more of the screening tests (that is where a test shows an $EC(LC)_{50} \leq 100\%$). At the screening stage, decisions should be conservative to ensure no potentially toxic discharge is excluded unless there is clear evidence (for example high dilution and no measurable toxicity) that toxicity-based control would not be appropriate.

At this stage the persistence of toxicity of appropriate effluents should also be assessed to determine which dosing regime is needed to accurately measure effluent toxicity in fish tests. The most appropriate screening test is used to measure changes in the toxicity of the effluent in open and closed vessels after given times and determine whether a static, semi-static or flow-through regime should be used.

2.3 In-depth assessment of the toxicity of effluents

2.3.1 Higher organism tests

Effluents identified at the screening stage as appropriate candidates for toxicity-based control are subject to further toxicity testing with the most appropriate screening test and higher organism tests. The higher organism tests used to assess the toxicity of effluents need to satisfy a number of key criteria, such as:

1. The availability of fully validated standard methods describing the holding of animals prior to the tests, the conduct of the test and the calculation of the endpoint;

2. The use of species which are readily available throughout the year either from in-house cultures or commercial suppliers;

3. Recognition of the selected tests within the scientific community as reliable and robust means of assessing effluent toxicity;

4. Easily understandable test endpoints;

5. Relative simplicity with no need for expensive or complicated equipment.

The protocol proposes the use of acute tests with an alga, invertebrate and fish representative of the receiving water to which the effluent is released. The acute (72-96 hr) algal tests determine the effects of effluents on growth, using *Selenastrum capricornutum* (OECD 1984) for discharges to freshwaters and *Phaeodactylum tricornutum* or *Skeletonema costatum* (ISO 1988) for effluents released to marine waters. The invertebrate and fish tests (24-96 hr) usually assess effluent toxicity using lethality as the index. The *Daphnia magna* immobilisation test (OECD 1984) is proposed as the freshwater invertebrate test while the oyster (*Crassostrea gigas*) embryo-larval development test (ICES 1991) is advocated for marine waters. The fish lethality test (OECD 1984) should use brown trout (*Salmo trutta*) or rainbow trout (*Oncorhynchus mykiss*) for

freshwater discharges and plaice (*Pleuronectes platessa*) or turbot (*Scopthalmus maximus*) for marine discharges. Table 2.4 assesses the usefulness of these tests based on a range of criteria, including those given above. All the tests proposed, except the fish lethality tests with *S. trutta* and marine species, have internationally recognised guidelines (OECD 1984, ISO 1988, ICES 1991) and have been subjected to ring tests to maximise the precision of the test. Fish tests with *S. trutta* can be carried out to the OECD guidelines for acute tests with rainbow trout (OECD 1984) while toxicity to marine fish species can be assessed using the United Nations Environmental Programme reference methods for marine pollution studies (UNEP 1989). These methods will become part of the NRA/SNIFFER Ecotoxicology Methods Manual.

In the longer term, sub-lethal tests measuring effluent effects on growth and reproduction in invertebrates and fish should be introduced. However, there are difficulties in using the established sub-lethal tests developed for product testing, such as the *Daphnia* 21 day juvenile production test and the 14-28 day fish growth test. This is due in part to the time and resources required to carry out the tests. Other tests which could fulfil this role require additional development and validation. This issue is discussed more fully in Section 5.2.

2.3.2 The need to assess species sensitivity

The wide variety of organisms present in receiving water communities will have different sensitivities to different effluents. Testing with organisms at different trophic levels provides information on the range of sensitivity likely to be present in the community at the discharge site and the type of organism which is most sensitive to the effluent. Adequate protection for the whole community should be provided by the subsequent derivation of discharge consent limits based on data for the most sensitive species, with an appropriate safety factor to account for the greater range of species sensitivities in the receiving water. This approach considerably reduces the uncertainty which would be associated with extrapolating from a single test. The US EPA considers the use of species at three trophic levels to be

Table 2.4 Overview of the tests proposed for use in the control of complex and variable effluents

Criteria	Test species							
	Bacteria	Algae		Invertebrates		Fish		
	Photobacterium	*Selenastrum*	*Phaeodactylum*	*Daphnia*	*Crassostrea*	*Oncorhynchus*	*Salmo*	*Scopthalmus*
Test Practicality/ Robustness								
Test organisms:								
Size (Defined/Not defined/ Not relevant)	ND	NR	NR	D	ND	D	D	D
Readily available (Y/N)	Y	Y	Y	Y	Y	Y	N	Y
Culture (Easy/Not Relevant/ Moderate	NR	E	E	M	M	E	E	E
Equipment:								
Size (Small/Medium/Large)	S	M	M	S	S	L	L	L
Complex (Yes/No)	N	Y	Y	N	N	N	N	N
On line measurement (Existing/ Possible/Impossible)	P	E	P	P	I	P	P	P
Test specifications:								
Type (Static/Semi-static/ Flow through)	S	S	S	S	S	S/SS/FT	S/SS/FT	S/SS/FT
No of test organisms	NR	NR	NR	10	100	10	10	10
Replicates (Yes/No)	Y	Y	Y	Y	Y	N	N	N
Nature of endpoint	Light	Growth	Growth	Immob	Dev	Death	Death	Death
Expression of endpoint	EC_{50}	EC_{50}	EC_{50}	EC_{50}	EC_{50}	LC_{50}	LC_{50}	LC_{50}
Complexity of endpoint/data (Easy/Complex)	E	C	C	E	C	E	E	E
Maintenance (Low/Medium)	L	L	L	L/M	L/M	M	M	M

Table 2.4 continued

Criteria	Test species							
	Bacteria	Algae		Invertebrates		Fish		
	Photobacterium	*Selenastrum*	*Phaeodactylum*	*Daphnia*	*Crassostrea*	*Oncorhynchus*	*Salmo*	*Scopthalmus*
Flexibility								
Test medium (Fresh/Saline)	S	F	S	F	S	F/S	F/S	S
Validation								
Presence of test protocols (Regulatory/Others)	O	R	O	R	O	R	N	N
Interlaboratory comparison (Yes/No)	Y	Y	Y	Y	Y	Y	N	N
Defined criteria for a valid test (Yes/No)	N	N	N	Y	Y	Y	Y	Y
Convenience for in-house control								
Rapidity to obtain results	Mins	>3d	>4d	1-2d	>1d	2-4d	2-4d	2-4d
Quantity of test material (Small/Medium/Large)	S	S	S	S	S	M/L	M/L	M/L

Light = light reduction, Immob = immobilisation, Dev = development

sufficient to measure any effluents toxicity for the purposes of assessing the receiving water impact and making regulatory decisions (US EPA 1991).

2.3.3 The use of toxicity data from in-depth testing

The in-depth testing generates data on species sensitivity which are needed to:

1. Identify the type of organism (and trophic level) to which the effluent is most toxic (that is the most sensitive test);

2. Determine the acceptable environmental concentration (AEC) using the data from the most sensitive test;

3. Compare the AEC with the receiving water concentration (RWC) of the effluent at the edge of the mixing zone defined in the desk-based appraisal;

4. Determine whether a toxicity-based discharge consent should be derived and whether the current toxicity of the effluent needs to be reduced;

5. Determine the correlation between the screening test and the most sensitive test;

6. Identify which type of test should be specified in the consent.

The procedures involved are outlined in Figures 2.3 and 2.4 and in the following sections. Each stage is described in greater detail in the accompanying draft protocol (Johnson *et al* 1992b).

A. Determining the acceptable environmental concentration (AEC)

Information on the toxicity of the effluent to the most sensitive test is used to derive an acceptable environmental concentration (AEC) which should result in no long-term adverse effects on the receiving water community (Figure 2.3). Initially the most sensitive test is identified as that with the lowest mean

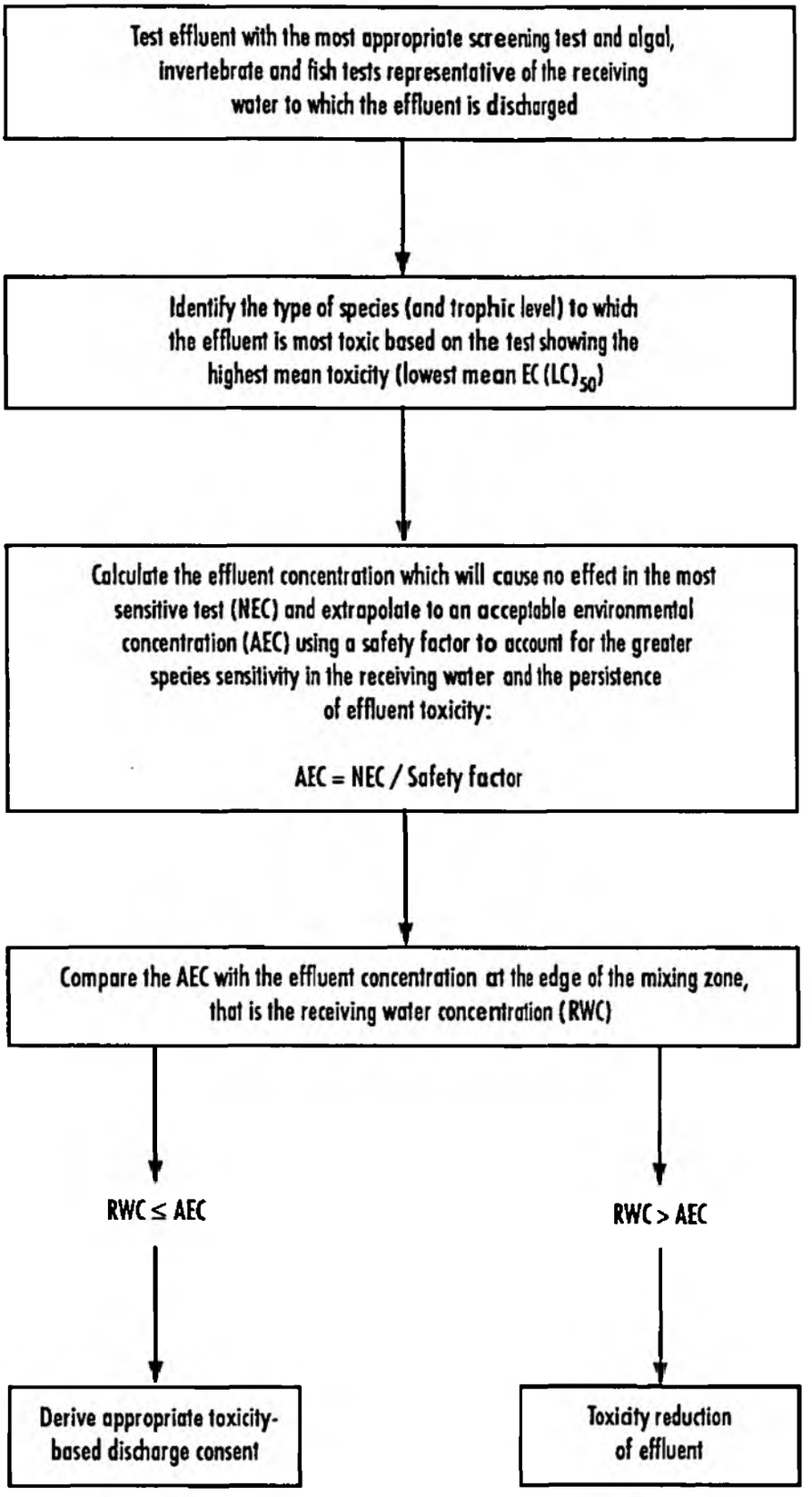


Figure 2.3 The use of data obtained from the in-depth testing of effluents appropriate for toxicity-based control.

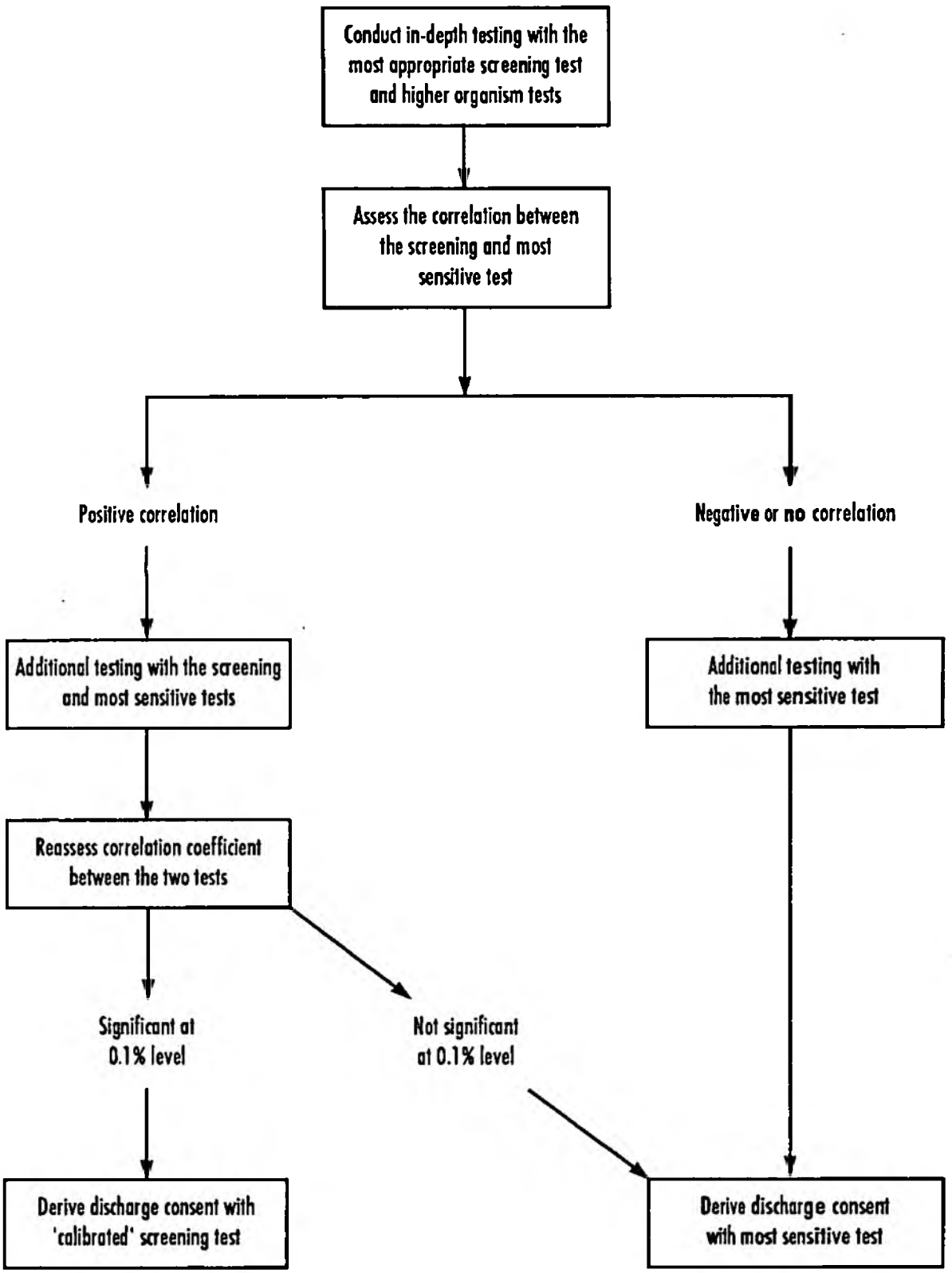


Figure 2.4 Identification of the test which should be specified in the discharge consent.

$EC(LC)_{50}$ and the data are used to determine the no effect concentration (NEC) for that test. For discharges where the algal test, measuring effects on growth, or the oyster embryo-larval test is the most sensitive test the NEC can be calculated directly from the data. In cases where the *Daphnia* or fish test is the most sensitive an acute to chronic ratio (ACR) is used to convert the $EC(LC)_{50}$ value, based on lethality, to a NEC for sub-lethal effects. The no effect concentration is then translated to the AEC using a safety factor which accounts for the greater range of species sensitivity in the receiving water and the persistence of toxicity of the effluent.

A measure of the persistence (or rate of change) of toxicity of an effluent is needed since its properties begin to change as soon as it mixes with the receiving water. After mixing the level of toxicity in the receiving water may remain relatively constant (until further diluted), increase due to transformation or degrade due to fate (photodecomposition, microbial degradation) or compartmentalisation (particulate adsorption and sediment deposition, volatilisation) processes.

Haig *et al* (1989) described approaches for assessing changes in the toxicity of effluents due to sedimentation, chemical degradation, volatilisation and microbial action. These should be carried out with the most sensitive test and the data used to decide on the magnitude of the safety factor for persistence needed in the calculation of the AEC. This involves a judgmental decision and there is a need for a test procedure which provides a numerical value. This will increase the precision of the derived toxicity-based consent. An EPA method (EPA 1989) which could provide the necessary data when carried out with appropriate test species is available and needs to be evaluated (see Section 5.3).

B. Determining whether a discharge consent can be derived

The acceptable environmental concentration (AEC), derived from the toxicity data, is compared with the effluent concentration which will be present at the edge of the defined mixing zone, that is the receiving water concentration (RWC). This comparison is used to decide whether a the toxicity of the effluent is such that immediate toxicity reduction by substitution or elimination of

toxic fractions or treatment is needed, before a discharge consent is derived. A discharge consent can be derived where the RWC is less than or equal to the AEC whereas toxicity reduction is needed where RWC is greater than the AEC.

C. Identifying the type of test to be specified in the consent

After determining whether a consent is appropriate the correlation between the screening and most sensitive test is reviewed and additional testing carried out (Figure 2.4) to identify whether the consent should specify:

1. A 'calibrated' screening test where a positive correlation exists between the two tests after the in-depth testing and the correlation coefficient is statistically significant at the 0.1% level after additional testing;

2. The most sensitive test, where there is no or a negative correlation between the tests after the in-depth testing or the correlation after the additional testing is not significant at the 0.1% level.

Since the screening test is functioning as a surrogate for the most sensitive test a strong correlation is needed between the two tests. This should ensure the screening test accurately represents the toxicity of effluents to the most sensitive test. The requirement for significance at the 0.1% level is stringent and means that the chances of the two tests being correlated by chance is limited, that is only 1 in a 1000. This is obviously considerably less likely than the 1 in 20 rate of chance which would be the case if significance at the 5% level was specified.

The potential for using 'calibrated' screening tests to consent complex and variable discharges has been demonstrated in both the case studies (Butler *et al* 1992a,b) and published studies (Firth and Backman 1990). Positive correlations between the screening test Microtox and the most sensitive oyster embryo test were evident in the case studies for chemical, oil refinery and pharmaceutical effluents after the in-depth testing (Table 2.5). Only the correlation coefficient (r) for the pharmaceutical discharge was significant

after this limited in-depth testing. However, the other discharges may have shown significant r values if the recommended number of tests had been carried out.

Table 2.5 Correlation coefficients between Microtox and the oyster embryo test for effluents tested in the case studies

Effluent type	Number of tests	Correlation coefficient	Critical value at 5% level of significance	Level of significance
Chemical	5	0.756	0.878	NS
Oil refinery	4	0.776	0.950	NS
Pharmaceutical	5	0.950	0.878	P<0.05

NS = not significant (P>0.05)

Correlations between Microtox and higher organism tests have also been found in previous studies. Firth and Backman (1990) showed that for paper mill effluents, the toxicity indicated by Microtox was a realistic surrogate for measurements with 7-day *C. dubio* (water flea) reproduction tests and 96 hr *O. mykiss* (rainbow trout) lethality tests. The absence of a correlation between a screening test and higher organism test may indicate that the effluent exerts its toxic effects by a different, and probably more specific, mode of action to that measured by the screening test.

2.4 Deriving the consent limit

The consent can be derived as an absolute limit for the specified test in terms of either an acceptable effect at a given dilution (for example 50% mortality after 96 hours in effluent diluted a specified number of times) or as a time specific EC_{50} or LC_{50}.

The former approach has been used in toxicity-based consent conditions by NRA Anglian region and the Clyde River Purification Board. The NRA Anglian condition for a discharge from a chemical plant to tidal waters specified that:

> "When the discharge is diluted 5 times with seawater, and tested by the required procedure (see Appendix A) the cumulative mortality of brown shrimps (*Crangon crangon*), within a 96 hour test period shall not be greater than 50%".

The consent condition for a pharmaceutical discharge to marine waters issued by the Clyde River Purification Board stated that

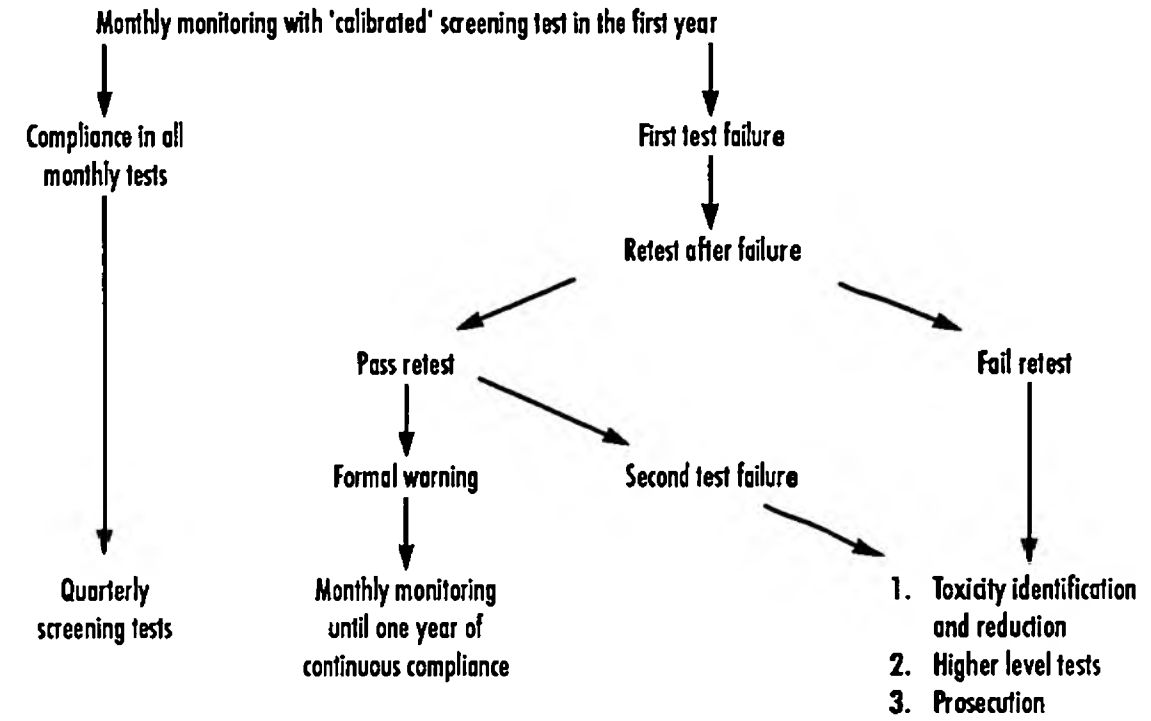
> "The effluent shall be conclusively deemed to comply with the terms of this consent when a sample thereof taken at the sampling point and diluted 125 times with seawater and tested according to the procedure set out in the document headed 'Toxicity Test for Effluent Discharges to Saline Waters' attached to this consent, exhibits a cumulative percentage mortality as hereinafter defined of not greater than 50 percent".

The large difference between the dilution specified in the two consents is proportionate to the dilution each of the effluents receives in the mixing zone following discharge.

The acceptable acute toxicity limit for the most sensitive test will be the % effluent concentration at which the acceptable environmental concentration is equal to the receiving water concentration. The 'calibrated' screening test limit to be specified in a consent condition should be calculated from the toxicity limit for the most sensitive test using a ratio of sensitivity between the tests derived from the correlation data. In certain instances regulators may consider a consent with toxicity limits for both the most sensitive test and the 'calibrated' screening test to be the most effective means of protecting the receiving water community.

2.5 Compliance monitoring

The frequency of testing needed to monitor compliance will be governed by the variability of the effluent. Proposed monitoring frequencies for effluents of consistent toxicity or definable variability are shown in Figure 2.5, along with proposed implications for compliance and failure. Effluents of undefinable variability will generally require more frequent monitoring to ensure compliance with consent conditions when the effluent discharges is most toxic. Consents with 'calibrated' screening test limits can be monitored more frequently than those for the most sensitive test due to the lower time and cost requirements of the screening tests. The proposed system is based on the requirement for a retest after a failure on a formal sample and the issue of a formal warning if the retest is passed. If the retest is failed or there is a second test failure the discharger may be required to carry out additional higher level tests or a reduction of effluent toxicity, or may be prosecuted.



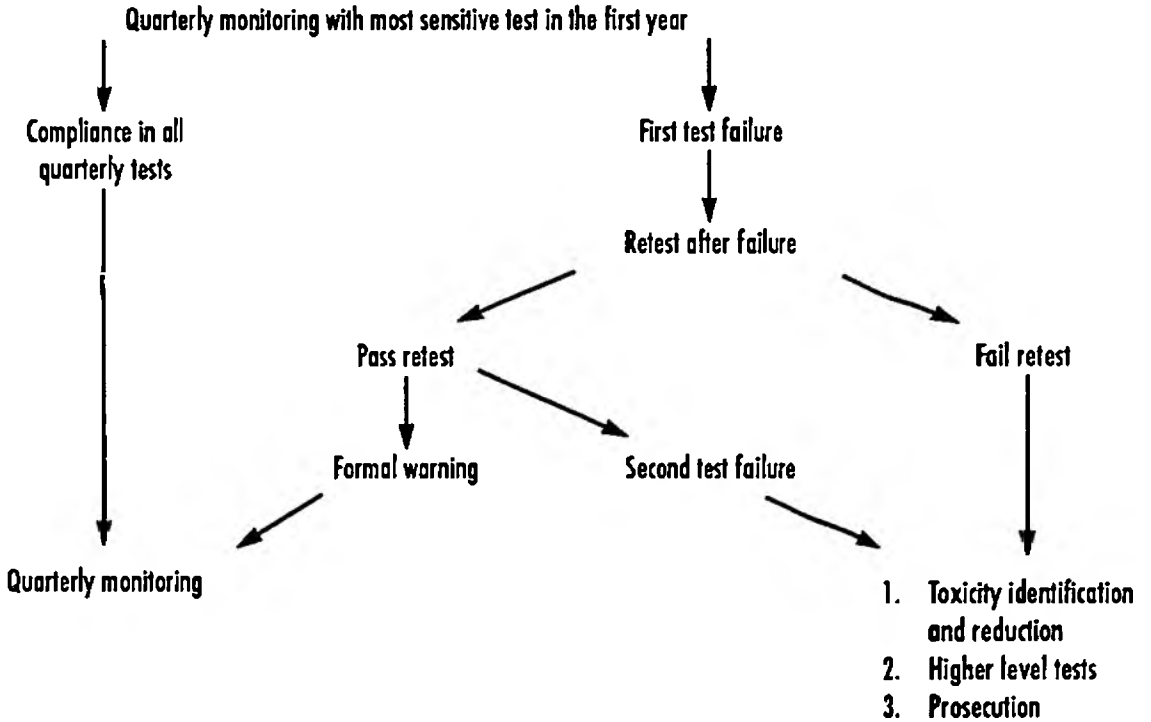


Figure 2.5 Compliance monitoring of effluents with 'calibrated' screening test consent limits or limits derived using the most sensitive tests

3. QUALITY ASSURANCE AND CONTROL PROCEDURES

3.1 Introduction

In the derivation of the consent conditions and subsequent compliance monitoring, appropriate quality control procedures are required to ensure the acceptability of data. This is particularly important if the data are to be presented in a court of law in connection with failure to comply with the consent condition. Areas in which quality assurance and control procedures are required include:

1. Effluent handling (collection, transportation and storage);

2. Routine analysis of dilution water;

3. Controlling toxicity test method precision.

3.2 Effluent handling

Data generated in the case studies showed that changes in effluent composition and toxicity could result from an absence of defined procedures for the collection, transport and storage of effluent samples. Paper mill effluents tested in the case studies (Table 3.1) showed significant changes in toxicity to Microtox after a storage period of 48 hours (Butler *et al* 1992b).

Table 3.1 Microtox 15 minute EC_{50} values for paper mill effluents tested immediately after receipt of the samples and after storage at 4 °C for 48 hours

Effluent	15 minute EC_{50} values (% effluent)		Level of significance
	On receipt	After 48 hours	
A	0.43	0.44	NS
B	6.72	19.30	P<0.05
C	35.7	>100	P<0.05

Therefore the draft protocol proposes that effluent samples should be collected in an appropriate container and tested within 24 hours of collection. Effluents should be stored at 4 °C at all times between collection and testing. Chain of custody forms provide a means of auditing the fate of an effluent from sampling to toxicity testing.

3.3 Routine analysis of dilution water

Routine analysis of fresh and saline dilution water used in the toxicity tests is essential to ensure that no potentially toxic inorganic and organic substances are present which could result in erroneous toxicity values.

3.4 Controlling toxicity test method precision

The implementation of toxicity-based consents nationally will require that the precision of the toxicity test methods used is addressed and sources of intra- and inter-laboratory variability are controlled.

3.4.1 Intra-laboratory variation

The control of intra-laboratory variability is essential to ensure that any temporal differences in effluent toxicity measured reflect changes in the discharge and not those due to the testing procedure. Intra-laboratory variability mainly results from:

1. The influence of the test operators;

2. Temporal variability in the sensitivity of test organisms.

The potential effect of test operators is reduced by having effective training, standard test protocols and working to a scheme such as Good Laboratory Practice (GLP). This approach enhances the reproducibility of tests and allows tests to be audited to identify potential reasons for differences.

Temporal variation in the sensitivity of test organisms can result from phenotypic (environmental) and/or genotypic (genetic) factors. The relative contributions of these factors need to be quantified and controlled where possible to increase the precision and repeatability of test data.

The influence of phenotypic factors can be reduced by defining the environmental conditions, such as handling and feeding of the organisms and water quality parameters (temperature, pH, hardness, salinity) which have to be rigorously adhered to while test organisms are maintained prior to use in the toxicity tests. Genotypic variability can be reduced by using a single standard genotype in the tests, which is an approach advocated by Baird *et al* (1991). However, it has to be accepted that there will always be a residual level of variability in sensitivity. The quality assurance and control procedures adopted in the protocol aim to reduce variability to the lowest possible level.

It is proposed that the level of variability in each type of test conducted at a facility is assessed using appropriate inorganic and organic reference toxicants and control charts. This approach is consistent with that adopted by the US EPA (US EPA 1991) and Environment Canada (Environment Canada 1990). Initially, toxicity tests with appropriate pure substances are carried out to create a control chart which identifies the level of variability which can be expected for a particular test. Reference toxicant tests carried out subsequently on organisms being used for effluent testing can then be compared with the control chart to determine whether the sensitivity of the organisms was acceptable.

In the derivation and monitoring of consents the response of test organisms to a known standard or standards must be shown to be consistent and within predetermined limits. In a court of law it could be argued that without knowing the sensitivity of a particular group of organisms to a reference material, a toxicity-based consent failure cannot be adequately proven.

In the case studies there was greater variability to reference toxicants in the oyster embryo-larval test (where the adults were obtained from stock at 'clean' field sites) than for cultured organisms, such as the Microtox bacterium

Photobacterium phosphoreum and juvenile turbot, which were produced from a small brood stock (Butler *et al* 1992b). Coefficients of variation (CV) in the higher organism tests with cadmium were 63.9% for the oyster embryo test (N=18) and 28.7% for the juvenile turbot lethality test (N=9). This compares with 17.4% and 10.4% for Microtox with the toxicants zinc (N=7) and phenol (N=35). The results probably reflect the lower phenotypic and genotypic variability associated with the Microtox and, to a lesser extent, the juvenile turbot tests compared to the oyster embryo-larval test. However, it may be due in part to problems with the use of cadmium as a reference toxicant. Since variability is apparently greater in animals obtained from field sites, efforts should be made to move towards the culturing of all species required for toxicity testing in-house or at centralised facilities where appropriate quality control procedures are employed.

The US EPA have assessed the variation in response to specific toxicants of cultured test species, which are commonly used in effluent testing programmes. Coefficients of variation are generally in the region of 20-30% (US EPA 1991). This level of variability is considered to be acceptable since it is comparable with that for other analytical procedures (Rue *et al* 1988, Grothe *et al* 1990). These CVs largely reflect genotypic variability as phenotypic differences and those due to the influence of test operators have been controlled.

At present control charts are derived using the responses of organisms to single inorganic or organic reference substances. However, these are not representative of the types of complex effluents which are being tested. It may therefore be appropriate to investigate the use of an inorganic mixture, an organic mixture and an inorganic/organic mixture as reference toxicants. The mixture most closely resembling the effluent being assessed could then be used as the reference toxicant.

3.4.2 Inter-laboratory variability

Inter-laboratory variability has implications for the consistency of approach between laboratories and would be a particular problem when an effluent has failed to comply with a consent and samples were tested at two or more

facilities. In this case it is imperative that causes of variation between laboratories have been addressed and acceptable levels of variability defined.

In the research programme, an inter-laboratory calibration exercise was conducted for Microtox between a number of NRA regional laboratories and regulators in Scotland and Northern Ireland (Butler *et al* 1992b). The variability in the measurements for the reference materials phenol and zinc was 19.2% and 34.6% respectively. Although these differences were not marked, potential causes of the differences were explored. These were found to include factors such as the use of different versions of software for calculating the results. This represents an area where standardisation can occur to minimise potential differences. Irrespective of the way the protocol is implemented it will be vital that ring tests of the recommended acute toxicity tests are carried out between NRA and SNIFFER facilities which will be involved in effluent testing. Tests involving dischargers and commercial test houses will also be needed to ensure the quality of the results produced by these organisations. This approach is consistent with that adopted by regulatory agencies in the United States (Crane *et al* 1991).

4. IMPLEMENTATION OF DIRECT TOXICITY ASSESSMENT

Toxicity testing for consent setting and compliance monitoring could be conducted:

1. In-house by regulators in regional laboratories or in a central test facility;

2. By independent commercial testing houses on behalf of the dischargers;

3. By dischargers own test laboratories.

Although the exact method of implementing the protocol will be a decision for regulatory agencies, the merits and limitations of each approach are shown in Table 4.1.

In-house testing by regulators could require the establishment of suitable testing facilities in most regions, which could involve considerable initial expenditure. The extent of this cost would obviously depend on the available facilities in a region at the time the DTA approach is introduced. A centralised testing facility would be an attractive alternative for the NRA, since the initial costs of establishing this would be shared amongst the regions. However, this might not be a viable solution for SNIFFER organisations. Regulators will always require some in-house biological testing capability which is available for immediate use, such as determining the sources and effects of pollution incidents.

Toxicity testing by dischargers or test houses would free resources in the regulatory agencies for other regional or national problems. Regulators would generally audit the data supplied by a discharger to ensure its accuracy and reliability. However, regulators would be required to periodically analyse effluent samples taken from all discharges to ensure the accuracy of submitted data. They would also need to monitor the capability and the quality of output of dischargers and test houses. This may involve organising ring tests of responses to reference substances to determine differences from values obtained in regulatory facilities. Since the costs of toxicity testing could be passed

on to the discharger, the daily maintenance costs of any facility would be covered. The only potential problem in dischargers testing their effluents in their own facilities was the identification in the NRA report on 'Discharge Control and Compliance Policy' (NRA 1990) of possible legal difficulties in using data provided by dischargers directly to prosecute over consent failures.

Table 4.1 The merits and limitations associated with regulators, dischargers or commercial testing houses carrying out toxicity testing for consent setting and compliance monitoring

Approach	Merits	Limitations
In-house testing by regulators	Builds up a large in-house expertise	Potentially high initial costs to set up.
	Rapid response to compliance failure	Major implications for staffing
		Evidence of failure may be challenged in court by discharger and places onus on NRA to prove the quality of the data
Discharger/ commercial test house	Implications for regulators in terms of necessary staff and facilities are reduced	Slower response to or compliance failure for test house-discharger-regulator chain
	Evidence of failure cannot be challenged in court by discharger, who by sending the data to the regulator tacitly accepts their validity	

In the United States the regulatory agencies responsible for the protection of receiving waters in Connecticut (Department of the Environment Protection), Texas (Water Commission) and Virginia (Water Control Board) all require dischargers to provide the data necessary to derive a toxicity-based consent (Crane *et al* 1991).

5. FUTURE DEVELOPMENTS

5.1 Screening tests

At present there are few rapid repeatable screening tests which can be used to assess the toxicity of effluents. Therefore the potential of other current or new toxicity tests, such as *Daphnia* and *Mysidopsis* IQ tests and ToxKits (using *Artemia* and rotifers) needs to be assessed. The testing should ascertain which could be used in the screening role to supply complementary information to that provided by Microtox. These tests will have to be fully validated before they are used in a screening role. Factors such as sensitivity, reproducibility, transferability and statistical robustness need to be assessed.

5.2 Higher organism tests

At present the acute invertebrate and fish toxicity tests proposed for use in the in-depth study generally assess lethality. Consequently, an acute to chronic ratio (ACR) has to be used in the determination of toxicity-based consent conditions to account for relationships between measured acute effects ($EC(LC)_{50}$) and the chronic no effect level. In order to avoid the use of these factors there is a need for appropriate short-term chronic toxicity tests to determine sub-lethal effects of effluents on parameters such as growth and reproduction.

At present there are certain standard sub-lethal tests with internationally recognised guidelines agreed or under consideration which determine effects on growth and reproduction. However, these tests (for example the *Daphnia* 21 day juvenile production test or the fish growth and early life stage tests) were developed for product testing. The products are usually pure substances and stable exposure concentrations can be maintained for the 21 or more days required to conduct these sub-lethal tests. However, volatilisation and degradation of effluent constituents over these time scales means that toxicity tests cannot be conducted using a single batch of effluent, even with storage at 4 °C. Furthermore the available tests, which are primarily for freshwater

species, are time consuming and expensive to conduct. Consequently there is a need for validated short-term sub-lethal tests analogous to the 7-day sub-chronic EPA tests which assess the effects of effluents on reproduction in water fleas and mysids and growth in fish (US EPA 1991). The *Gammarus* feeding rate test (Crane *et al* 1992) and *Mytilus* feeding rate or scope for growth test (MAFF 1990) could be used in this role and test protocols are available for both methods. Both methods have undergone extensive validation and can be used in the laboratory and in the field. However, although the endpoints measured have been related to growth, they provide an indirect rather than a direct measurement of this parameter. The appropriateness of sub-lethal tests for effluent assessment clearly represents an area where further discussion is needed.

In the future, tests using other appropriate indigenous species may be required. The use of other species, such as aquatic insect larvae may be particularly important for effluents to be discharged to sensitive Class 1 and 2 rivers where representatives of these classes represent the most sensitive receiving water species. Toxicity testing of effluents with these species will require properly validated standard test protocols to satisfy quality control requirements.

5.3 Test for the persistence of toxicity of effluents

In deriving toxicity-based consents, the persistence of effluent toxicity has to be accounted for using a safety factor. The accuracy of the consent would be increased and potential over-stringency avoided by using a test to determine the persistence of toxicity of effluents. The US EPA have developed a laboratory method for assessing the persistence of toxicity of effluents (US EPA 1989) and this needs to be assessed with the test species specified in the protocol. A standard test method can then be developed which will provide a measured numerical value for use in deriving the consent rather than using a safety factor.

5.4 On-line monitoring

In the longer term the most appropriate approach for monitoring discharges, particularly those which are highly variable, would be to use calibrated on-line monitors linked directly to NRA/SNIFFER offices. These would provide an instantaneous indication of excedence of discharge consent conditions. Techniques which could be used in this role need to be identified and introduced following necessary validation.

6. INFORMATION REQUIREMENTS

At the start of a national programme to implement toxicity-based consents for appropriate discharges, regulatory agencies should strongly consider establishing a database similar to the CETIS (Complex Effluent Toxicity Information System) system operated by the US EPA. This database carries information on whole effluent toxicity testing for industrial and sewage treatment works discharges from EPA regional and State discharge permitting programs. A similar database for the UK would mean available data on a specific type of effluent could be accessed when the appropriateness of using toxicity-based controls for a similar discharge was being considered.

It is also imperative that regulators involved in deriving toxicity-based consents have access to toxicological databases which can supply information on the toxicity and potential for bioaccumulation of effluent constituents. The most appropriate system for meeting the needs of the NRA has been addressed in a recent study (A12(91)1) and is currently under consideration.

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