

TOXICITY-BASED CONSENTS PILOT STUDY

I Johnson (WRc), J Wharfe, D Tinsley, R Boumphrey and D Forrow (Environment Agency)

**Research Contractor:
WRc plc
Henley Rd Medmenham
Marlow
SL7 2HD**

**Environment Agency
Rio House Waterside Drive Aztec West
Almondsbury Bristol BS12 4UD**

Environment Agency
Rio House Waterside Drive Aztec West
Almondsbury Bristol BS12 4UD

Tel: 01454 624400
Fax: 01454 624409

© Environment Agency 1996

All rights reserved. No part of this document may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior permission of the Environment Agency. This report is the result of work jointly funded by the Environment Agency and the Scotland and Northern Ireland Forum for Environmental Research.

Dissemination status

Internal: Release to Regions
External: Public Domain

Statement of Use

This report presents results of a study to investigate the toxicity of a range of discharges and develop a direct toxicity assessment protocol for their management. The draft procedure developed will assist the Environment Agency's refinement of wastewater discharge control.

Research contractor

This document was produced under R&D Contract 493 by:

WRc plc
Henley Rd Medmenham
Marlow
SL7 2HD

Tel: 01491 571531
Fax: 01491 579094

WRc Report N^o EA 4198/1/7372-1

Environment Agency Project Leader

The Environment Agency's Project Leader for R&D Contract 493:

Dr J Wharfe - Southern Region

Additional copies

Environment Agency staff wishing to obtain further copies should contact their Regional R&D Co-ordinator.

Further copies of this report are available from:

Foundation for Water Research, Allen House, The Listons, Liston Road, Marlow, Bucks SL7 1FD. Tel: 01628 891589, Fax: 01628 472711

CONTENTS	Page
LIST OF TABLES	ii
LIST OF FIGURES	ii
EXECUTIVE SUMMARY	1
KEY WORDS	2
1. INTRODUCTION	3
1.1 Background	3
1.2 The Pilot Study	3
2. THE PROTOCOL	5
2.1 Discharge prioritisation (Stage 1)	5
2.2 Discharge characterisation (Stage 2)	6
2.3 Toxicity reduction and licensing (Stage 3)	9
2.4 Compliance monitoring (Stage 4)	13
3. APPLICATION OF THE PROTOCOL	31
4. FUTURE DEVELOPMENTS	33
4.1 Consultation process	33
4.2 Additional R&D	33
REFERENCES	35

LIST OF TABLES

Table 2.1	Effluents selected as case studies for testing in the pilot study	6
Table 2.2	Comparison of PNEC values derived for case study effluents using NOEC and EC(LC) ₁₀ values	8
Table 2.3	Comparison of PNEC values for the case study effluents derived from testing and using a statistical approach	9
Table 2.4	Assessment of the potential toxicological impact of the case study effluents	10
Table 2.5	Regression relationships between screening tests and the OEL test for case study effluents 7 and 50	12
Table 2.6	Summary of the results of compliance monitoring of effluent 50	14

LIST OF FIGURES

Figure 1.1	Protocol for setting toxicity-based conditions for discharge licences	4
Figure 2.1	Comparison of Microtox and <i>D. magna</i> immobilisation test data for discharges to freshwaters (A) and Microtox and OEL data for discharges to estuarine/marine waters (B)	15
Figure 2.2	NOEC values measured in algal lethality, <i>D. magna</i> immobilisation and fish lethality tests of effluent 8	16
Figure 2.3	NOEC values measured in algal lethality, <i>D. magna</i> immobilisation and fish lethality tests of effluent 12	17
Figure 2.4	NOEC values measured in algal lethality, <i>D. magna</i> immobilisation and fish lethality tests of effluent 14	18
Figure 2.5	NOEC values measured in algal lethality, <i>D. magna</i> immobilisation and fish lethality tests of effluent 60	19
Figure 2.6	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 1	20
Figure 2.7	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 5	21
Figure 2.8	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 7	22
Figure 2.9	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 19	23

Figure 2.10	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 31	24
Figure 2.11	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 38	25
Figure 2.12	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 47	26
Figure 2.13	NOEC values measured in algal lethality, oyster embryo-larval development, <i>A. tonsa</i> lethality and fish lethality tests of effluent 50	27
Figure 2.14	Regression relationship between the ECLOX and OEL tests for effluent 7	28
Figure 2.15	Statistical approach for assessing discharge compliance (after Dhaliwal <i>et al.</i> 1995)	29

EXECUTIVE SUMMARY

This Project Report summarises the main findings of the Toxicity-Based Consents Pilot Study project (493), which was a Direct Toxicity Assessment (DTA) National Centre driven programme managed by a project board comprising Environment Agency and the Scotland and Northern Ireland Forum For Environmental Research (SNIFFER) membership. The aim of the project was to finalise the procedure for using direct toxicity assessment as a tool for consenting appropriate effluents. The pilot study involved testing a draft protocol developed in a previous project (R&D Contract 049) in a series of case studies and making amendments, as necessary, to provide a robust and thoroughly tested procedure which could be used nationally.

Sixty four discharges were submitted by the Environment Agency (the National Rivers Authority and Her Majesty's Inspectorate of Pollution as was) and SNIFFER as candidates for inclusion in the pilot study. For each discharge, chemical, biological and toxicological information was obtained from the local regulator. Where toxicological data were unavailable experimental screening of the discharge using the Microtox test and either the 24 h *Daphnia magna* immobilisation tests or 24 h oyster embryo-larval development (OEL) tests were carried out on two occasions. This information was used in a desk based appraisal to select 12 appropriate industrial and sewage treatment works discharges to fresh (4) and estuarine/marine (8) waters as case studies. The selection criteria included: toxicity of the effluent, impact on the receiving water, compliance with current chemical-specific consent limits and the level of chemical monitoring of the discharge. During the initial screening programme some discharges showed no toxicity (that is concentrations causing 50% inhibition or 50% effect >100% effluent).

The toxicity of the 12 case study effluents was characterised using both rapid tests (Microtox and ECLOX) and established acute higher organism tests. The latter methods included 72 h algal growth inhibition and lethality tests, 48 h *Daphnia magna* immobilisation tests, 48 h *Acartia tonsa* lethality tests, 24 h oyster embryo-larval development tests and 96 h juvenile fish lethality tests. The case study effluents were generally acutely lethal to higher organisms at concentrations of <5% effluent, with dilutions of down to 1000x being needed to prevent acute lethal effects of some discharges. In some instances these discharges are known to cause demonstrable toxic impacts on the receiving water community.

For each effluent, the data was used to identify the most sensitive higher organism tested and determine the lowest effluent concentration causing no lethal effects, relative to the control, using either No Observed Effect Concentrations (NOEC) or EC(LC)₁₀ values (concentrations causing a 10% effect). This value represented the predicted no effect concentration for lethality (PNEC) which was then compared to the predicted environmental concentration (PEC) at a point beyond which protection is to be achieved. The comparison was used to determine whether or not a toxicity-based limit could be derived or whether toxicity reduction was required before a limit could be set. The designated zone of impact is case specific and takes account of the sensitivity of the receiving water and the minimum available dilution under worst case conditions. Most of the case study discharges were acutely lethal beyond a designated zone of impact in the receiving water and would require toxicity reduction (that is the PEC > PNEC).

The case study data was also used to investigate evidence of correlations between the rapid tests and the most sensitive higher organism test for each discharge. Two case study effluents (7 and 50) showed statistically significant correlations between a rapid test (ECLOX and Microtox) and the most sensitive higher organism (OEL in both cases). The absence of correlations for other case study effluents may have been due to the limited variability in toxicity which meant data values were not available over a sufficiently large concentration range to establish significant correlations.

Overall the protocol can be used to assess the acute lethal toxicity of all major industrial discharges and sewage treatment works effluents, although further development and application of rapid tests would greatly enhance the application. Chronic invertebrate and fish methods with sub-lethal endpoints are being developed to include in the protocol. This will allow these more subtle, but potentially more damaging, effects to be evaluated.

KEY WORDS

Toxicity-based consent, discharge prioritisation desk-based appraisal, screening, discharge characterisation, consent setting, correlation, toxicity reduction, compliance monitoring.

1. INTRODUCTION

1.1 Background

The Environment Agency (the NRA, in collaboration with Her Majesty's Inspectorate of Pollution) and organisations within the Scotland and Northern Ireland Forum For Environmental Research (SNIFFER), instigated a pilot study to test the procedure for using direct toxicity assessment for consenting appropriate effluents. The pilot study has been guided by staff of the Environment Agency DTA National Centre and involves testing a draft protocol in a series of case studies and making amendments as necessary to provide a robust and thoroughly tested procedure which can be used nationally.

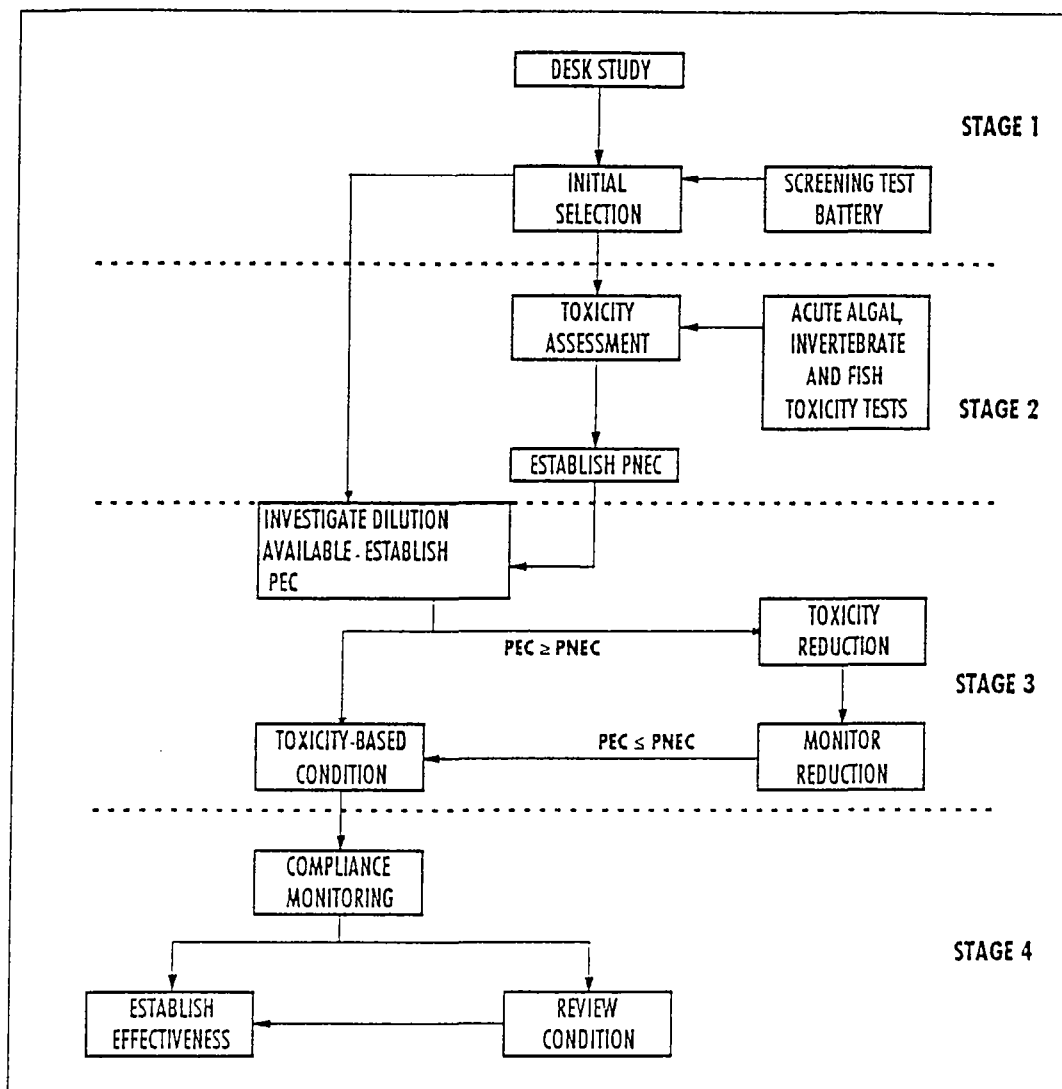
1.2 The Pilot Study

At the outset of the project the Environment Agency and SNIFFER submitted a total of 64 discharges for inclusion in the pilot study as potential candidates for toxicity-based control. This initial list was reduced to 12 case study effluents which were then used to extensively study the draft protocol.

The draft protocol (Figure 1.1) comprises four stages:

1. discharge prioritisation;
2. discharge characterisation;
3. toxicity reduction and licensing;
4. compliance monitoring.

This Project Report describes the main findings from the pilot study in relation to the different components of the draft protocol.



PEC = Predicted Environmental Concentration
 PNEC = Predicted No-Effect Concentration

Figure 1.1 Protocol for setting toxicity-based conditions for discharge licences

2. THE PROTOCOL

The implications of the findings of the pilot study for each stage of the draft protocol are given. These have been used to define the requirements for testing to derive a toxicity-based limit and monitor compliance.

2.1 Discharge prioritisation (Stage 1)

The purpose of discharge prioritisation is to assess whether or not a particular discharge is appropriate for toxicity-based control. In the draft protocol, the selection and prioritisation of discharges involves collating all available chemical, biological and toxicological data on the discharges to evaluate:

- the discharge type and the complexity of the discharge in terms of the number of substances identified by chemical analysis as being present at levels above the limit of detection;
- the toxicity of the effluent in terms of the lowest measured test response from a series of screening tests;
- the impact of the effluent on the receiving water as identified by biological surveys;
- the compliance of the discharge with current chemical-specific limits.

At this stage, a decision to proceed to full discharge characterisation may be taken or further information may be requested to determine the level of toxicity and the extent of environmental impact by biological surveys. The priority in applying a toxicity-based approach to existing discharges is to target those complex effluents which cannot satisfactorily be controlled by a chemical-specific approach. Such discharges would be toxic and could be complying with current chemical-specific consent conditions, subject to permissive monitoring and be causing impact in the receiving water.

Prior to the final selection of discharges in the pilot study, 17 freshwater effluents were screened for toxicity with the Microtox and *D. magna* immobilisation test while 27 effluents discharged to estuarine/marine waters were screened with the Microtox and the oyster embryo-larval development (OEL) test. Toxicity information was available on the other 20 candidate effluents. Discharges were considered to be toxic to Microtox if the IC₅₀ values (concentrations causing 50% inhibition in light production) were $\leq 91\%$ effluent and toxic to the *D. magna* immobilisation and OEL tests if EC₅₀ values (concentrations causing 50% effects) were $\leq 100\%$ effluent. The discharges were generally tested on two occasions.

For the discharges to freshwater, there was 87.8% agreement between Microtox and *D. magna* immobilisation data based on toxic/non-toxic designations (see Figure 2.1). There was 68.5% agreement between Microtox and OEL data for discharges to estuarine/marine waters using the same designations (see Figure 2.1). These findings indicate that an appropriate battery of screening tests could identify, in a cost-effective manner, effluents which were toxic to higher organisms.

Overall 68.2% of the effluents screened (30 of 44) showed toxicity based on the toxic/non-toxic designations. The existence of a large number of acutely toxic discharges from the limited initial list indicates that there are probably a considerable number of effluents in the UK which are appropriate for toxicity-based control.

Thirty percent of effluents tested (14 of 44) showed no toxicity to Microtox ($IC_{50} >100\%$ effluent) or the *D. magna* immobilisation and OEL tests ($EC_{50} >100\%$ effluent).

Table 2.1 shows the effluents selected as case studies for stage 2 of the pilot study. These comprised four discharges to freshwaters and eight to estuarine/marine waters. Four discharges were from sewage treatment works receiving industrial inputs and eight were from industrial plants involved in a range of activities including petrochemical works and the manufacture of chemicals and plastics. All the discharges selected were considered to be complex and showed toxicity in the screening tests. Discharges 5, 8, 14, 47 and 60 are known to be causing toxic impacts on the receiving water community based on biological survey data.

Table 2.1 Effluents selected as case studies for testing in the pilot study

Receiving water	Effluent number	Effluent type
Riverine	8	Sewage treatment works with industrial inputs
	12	Chemical manufacturing
	14	Sewage treatment works with industrial inputs
	60	Sewage treatment works with industrial inputs
Estuarine/marine	1	Chemical manufacturing
	5	Chemical manufacturing
	7	Chemical manufacturing
	19	Chemical manufacturing
	31	Sewage treatment works with industrial inputs
	38	Chemical manufacturing
	47	Chemical manufacturing
	50	Petrochemical works

2.2 Discharge characterisation (Stage 2)

In deriving a toxicity-based limit it is necessary to characterise the toxicity and variability of the discharge. In the draft protocol, the initial characterisation is carried out on four samples using a battery of established tests at three trophic levels (that is using algae, macro-invertebrates and fish). The tests use species representative of the receiving water to which the effluent is discharged. The testing is used to identify the most sensitive established test and to determine the predicted no effect concentration (PNEC) at the point of application for protection. The PNEC is derived using the data for the most sensitive species of the batch of established tests.

The extent of the testing carried out during discharge characterisation depends on the variability of the discharge. It is important to analyse as many samples as may be necessary to have a certain statistical probability that the test results obtained are representative of end-of-pipe conditions. Therefore, in the pilot study, most effluents were tested on at least 8 occasions with the battery of established tests. However, each effluent needs to be considered on a case by case basis and testing may identify the most sensitive test after ≤ 4 tests so that the other established tests may no longer need to be carried out.

2.2.1 Derivation of the predicted no effect concentration (PNEC)

Test methods

In the pilot study, invertebrate tests assessing acute lethal toxicity were always as sensitive or more sensitive than the algal or fish lethality tests (see Figures 2.2-2.13). In defining the predicted no effect concentration for acute lethal responses (PNEC), algal lethality would appear to be a sufficiently insensitive endpoint to mean it may not be necessary to include the test in the required battery on all occasions. However, the algal growth inhibition test or *Lemna minor* growth test (for freshwaters) will be needed when assessing the chronic effects of the discharge.

Test endpoints

The PNEC for a discharge could be established using either no observed effect concentration or $EC(LC)_{10}$ data generated in the characterisation stage. Both values can be used to provide an estimate of the no effect concentration, although there is considerable debate about which is the most appropriate (Chapman 1995). Therefore, in the pilot study, both NOEC and $EC(LC)_{10}$ values were derived to ascertain what effect this had on the derivation of the PNEC.

Table 2.2 shows that the PNEC for most effluents was not markedly different depending on whether NOEC values or $EC(LC)_{10}$ values were used. The exceptions were effluents 5, 12 and 50. Consequently, although the NOEC may be preferred, it may be appropriate to use both endpoints during the characterisation stage to ensure that for each discharge the appropriate conclusions are drawn as to whether a toxicity-based limit could be derived or toxicity reduction was required. The Environment Agency is currently considering using a model-based NEC statistic as an alternative to the NOEC or $EC(LC)_{10}$ (Kooijman and Bedaux 1996).

Number of samples

In its Technical Support Document for Water Quality-based Toxics Control the United States Environmental Protection Agency recommends conducting toxicity tests using required species quarterly for 1 year as the minimum requirement for adequately assessing the variability of toxicity observed in effluents (US EPA 1991). However, since the uncertainty regarding whether or not an effluent causes toxic impact is reduced with more data, the US EPA also advocates increasing this frequency of testing where necessary. The test data is used to determine the worst case toxicity for the discharge.

Table 2.2 Comparison of PNEC values derived for case study effluents using NOEC and EC(LC)₁₀ values

Effluent number	Most sensitive higher organism test	PNEC based on test endpoint	
		NOEC	EC(LC) ₁₀
8	<i>D. magna</i> immobilisation test	10 (10x)	11 (9.1x)
12	“	46 (2.2x)	82 (1.2x)
14	“	22 (4.5x)	22 (4.5x)
60	“	4.6 (21.7x)	4.6 (21.7x)
1	Oyster embryo-larval development test	<0.1 (>1000x)	<0.1 (>1000x)
5	“	0.22 (455x)	<0.1 (>1000x)
7	“	0.1 (1000x)	0.14 (714x)
19	“	<0.1(>1000x)	<0.1 (>1000x)
31	“	<0.1 (>1000x)	<0.1 (>1000x)
38	“	<0.1 (>1000x)	0.12 (833x)
47	“	<0.1 (>1000x)	<0.1 (>1000x)
50	“	0.46 (217x)	0.1 (1000x)

Note: Values in parentheses are the dilutions required for no acute lethal effects

To characterize the effects of effluent variability, and reduce uncertainty in the process of deriving effluent limits where data are limited, the US EPA has developed a statistical approach which uses a limited data set to predict the maximum toxicity which could be expected for an effluent if additional testing was carried out. This involves deriving a multiplication factor using a probability approach. This factor is then used to translate the lowest measured toxicity value from the limited data set to the worst case expected value.

Table 2.3 compares the lowest measured PNEC value for each effluent from all the testing in the pilot study with the lowest PNEC predicted from the toxicity data after 4 tests. It is evident that for five of the effluents the application of the US EPA recommended multiplication factor of 2.6 (for a 95% probability basis) would overestimate the PNEC value. Only effluent 7 had a measured PNEC value after 8 tests which was lower than the value predicted from the data after 4 tests. As is the case when using the chemical-specific approach, it is almost impossible to define prescriptive rules for the number of samples to be analysed in the effluent characterisation stage, since this depends on the inherent variability in toxicity of the final effluent. Modification of the protocol may require fewer trophic level tests and more tests with the most sensitive species.

Table 2.3 Comparison of PNEC values for the case study effluents derived from testing and using a statistical approach

Effluent number	PNEC after 4 tests	PNEC after 8 tests	PNEC derived using multiplication factor
8	10	10	3.8
14	22	22	8.5
60	4.6	4.6	1.8
1	0.1	<0.1	0.04
5	0.22	0.22	0.08
7	0.46	0.1	0.18
19	0.1	<0.1	0.04
31	<0.1	<0.1	<0.04
38	<0.1	<0.1	<0.04
47	0.22	<0.1	0.08
50	0.46	0.46	0.18

2.3 Toxicity reduction and licensing (Stage 3)

2.3.1 Derivation of the predicted environmental concentration (PEC)

Obtaining a realistic estimate of the available dilution in the receiving water at the point of protection is a key element of the protocol. This may require dischargers to conduct dye tracing studies and/or modelling of the dispersion pattern of the effluent on discharge to the receiving water.

In each case, it is envisaged that area/regional Environment Agency staff will consider whether a zone of deterioration will deleteriously affect the ecology of the river or estuary, the migration of fish and general water usage. The point of protection is set at the edge of this zone outside of which there should be no acute (or chronic) toxicity. In all instances, the designated zone of impact will be site specific and will take account of the sensitivity of the receiving water and the minimum available dilution under worst case conditions.

2.3.2 Ecotoxicological significance of the data

In the draft protocol, the data generated on the predicted no effect concentration (PNEC) and the predicted environmental concentration (PEC) is used to determine whether or not a toxicity-based limit can be set or whether toxicity reduction of the discharge is necessary before this can be achieved. A toxicity-based limit can be set where the $PEC \leq PNEC$ (that is the available dilution in the receiving water is greater than that needed to result in no acute lethal effects). Toxicity reduction is required where the $PEC > PNEC$ (that is the available

dilution in the receiving water is less than that needed to result in no acute lethal effects). Where possible the relationship of the PEC to the PNEC should be compared with the biological impact noted in the receiving water around the discharge.

Table 2.4 shows the PNEC, dilution required to prevent acute lethal effects, PEC and available dilution in the receiving water for the case study effluents. For all the discharges, except effluents 12 and 50, there was insufficient dilution available at the point of protection to prevent acute lethal effects and toxicity reduction would be required (that is the $PEC > PNEC$). For effluents 12 and 50, the available dilution at the point of protection was sufficient to prevent acute lethal effects (that is the $PEC \leq PNEC$) and a toxicity-limit could be derived.

Table 2.4 Assessment of the potential toxicological impact of the case study effluents

Effluent number	PNEC (% effluent)	Dilution needed to prevent acute lethal effects	PEC (% effluent)	Available dilution	Biological impact	Outcome
8	10	10x	33	3x	Impact measured	Toxicity reduction
12	46	1.8x	2.8	36x	Further data required	Consent
14	22	8.5x	33	3x	Impact measured	Toxicity reduction
60	4.6	21.7x	25	4x	Impact measured	Toxicity reduction
1	<0.1	>1000x	0.1	1000x	Further data required	Possible toxicity reduction
5	0.22	455x	1	100x	Impact measured	Possible toxicity reduction
7	0.1	1000x	2	50x	Further data required	Toxicity reduction
19	<0.1	>1000x	2	50x	Further data required	Toxicity reduction
31	<0.1	>1000x	2	50x	Further data required	Toxicity reduction
38	<0.1	>1000x	2	50x	Further data required	Toxicity reduction
47	<0.1	>1000x	0.2	500x	Impact measured	Toxicity reduction
50	0.46	217x	0.1	1000x	No impact	Consent

The requirement for toxicity reduction will depend on the extent to which the $PEC > PNEC$ and the frequency with which samples exceeded the PEC in the characterisation phase.

2.3.3 Licensing

Only effluents 12 and 50 of the case study discharges showed a $PEC < PNEC$ and would allow a toxicity-based limit to be derived. Effluent 12 showed limited toxicity such that NOEC values could be calculated in the higher organism tests for only one sample. On this basis no further monitoring of the discharge was carried out during the pilot study.

For effluent 50, a toxicity-based condition in a licence could specify that:

‘there should be no significant increase in abnormalities of oyster embryos in a 1000 fold dilution of the effluent, compared to the control, when tested using the OEL method described in the DTA National Centre Ecotoxicology Methods Manual^{1,2}’.

For this effluent, the lack of acute lethal toxicity at the point of protection is consistent with the absence of impact in the receiving water community as measured by biological surveys.

2.3.4 Toxicity reduction

Most of the case study discharges were acutely lethal beyond a designated zone of impact in the receiving water (that is the $PEC > PNEC$) and would require toxicity reduction (see Figures 2.2-2.13). The Environment Agency would require that toxicity was reduced in these effluents to below the acceptable PEC level at the point of protection at the edge of the zone of deterioration. A toxicity reduction plan for each discharge would incorporate measures to be taken to investigate the source of toxicity. Once the source of toxicity is identified, the plan would be modified to incorporate measures to reduce toxicity in the effluent to achieve set toxicity goals. These steps would be to a timescale agreed between the discharger and the Environment Agency. For effluents 5, 8, 14, 47 and 60 the evidence of acute lethal toxicity at the point of protection is consistent with evidence of impact in the receiving water community as measured by biological surveys.

¹ It should be noted that the exact format and wording of the toxicity-based condition written into the licence is to be finalised.

² The toxicity limit set at the point of protection may be based on sub-lethal and chronic toxicity endpoints.

2.3.5 Correlation of screening tests with higher organism tests

In situations where a calibrated rapid test can be shown to be an acceptable surrogate for the most sensitive of the established (algal, invertebrate or fish) tests, the rapid test can be used to set and monitor against a toxicity limit or carry out a toxicity reduction programme. In the context of the draft protocol an acceptable surrogate means:

- the correlation coefficient for the regression equation between the rapid test and the most sensitive of the established tests is significant at the 95% level of probability;
- the data for each test used to derive the regression equation comprises a concentration range of ideally 2 orders of magnitude, but 1 order of magnitude as a minimum;
- the regression equation is not potentially biased by a single value (that is the data is not skewed).

Two case study effluents showed correlations ($P < 0.05$) between a screening test (Microtox and ECLOX) and the most sensitive higher organism (OEL in both cases) (see Table 2.5 and Figure 2.14). For effluent 7, the significant correlation between the rapid ECLOX test and the OEL test means ECLOX could be considered as a surrogate for the OEL test in toxicity reduction (and also compliance and trend monitoring). For effluent 50, although the correlation coefficient between the rapid Microtox test and the OEL test was significant at the 95% level it was skewed due to the distribution of the data points. For effluents 7 and 50 significant relationships between the two tests were evident after 5 samples had been tested.

Table 2.5 Regression relationships between screening tests and the OEL test for case study effluents 7 and 50

Effluent number	Regression relationship	
	Equation	r value (P value)
7	ECLOX $IC_{50} = -0.0015 + 0.032$ OEL NOEC	0.905 ($P < 0.01$)
	ECLOX $IC_{50} = -0.0098 + 0.041$ OEL EC_{10}	0.83 ($P < 0.05$)
50	Microtox $IC_{50} = -4.97 + 2.82$ OEL NOEC	0.815 ($P < 0.05$)
	Microtox $IC_{10} = -0.17 + 0.29$ OEL NOEC	0.806 ($P < 0.05$)

The absence of correlations for other case study effluents may have been due to the limited variability in toxicity which meant data values were not available over a sufficiently large concentration range to establish significant correlations.

2.4 Compliance monitoring (Stage 4)

In the compliance monitoring procedure there exists the possibility of wrongly classifying a sample. For example the sample may be regarded as breaching the toxicity-based limit when, in fact, its true toxicity is actually compliant (Type I error or 'false positive'). Alternatively, the sample might appear to comply with the consent but its true toxicity has been underestimated and it actually breaches the consent (Type II error or 'false negative'). Clearly, both types of mis-classification are to be avoided as far as possible, the first because of possible adverse commercial implications and the second, because of possible environmental impacts which go undetected.

In the pilot study, the statistical framework for compliance assessment proposed by Dhaliwal *et al.* (1995) has been used (see Figure 2.15). This involves the use of limit tests, in which responses in a control group of organisms are compared with the responses of organisms exposed to a single concentration of effluent, rather than using conventional concentration-response tests. Initially, the data are statistically analysed to test the null hypothesis that there is no difference in response between the control and the treatment (that is the effluent diluted to the PEC). In the analysis, a standard t-test is used which does not account for the intra-laboratory variability of the test method. This compliance monitoring approach is consistent with that prescribed under the National Pollutant Discharge Elimination System (NPDES) in the USA. However, in the Dhaliwal *et al.* (1995) approach samples deemed to have failed to comply with a toxicity-based limit on the basis of this initial statistical analysis are then retested using a modified t-test in which account is taken of intra-laboratory test variability for the test method used. In the model, a measure of the intra-laboratory variability of a test method (S_{Δ}) is derived from the results of a series of reference toxicant tests. The critical difference of the treatment response from the control beyond which the original null hypothesis is rejected is termed the *reliable toxicity detection limit* (RTDL):

RTDL = Critical difference between control and treatment responses = $S_{\Delta} \times t$ statistic

In the modified t-test, a difference between the control and treatment which is *less* than the RTDL value is assumed to result from intra-laboratory variability and so cannot confidently be regarded as a breach of the toxicity-based limit. However, if the difference of the treatment from the control is *greater* than the RTDL value then failure to meet the toxicity-based limit is confirmed.

For effluent 50, compliance monitoring was carried out using a limit test with oyster embryos in which the % abnormality in the controls was compared to that at the toxicity limit (0.1% effluent or 1000x dilution). Table 2.6 shows the results from the limit tests and whether or not the samples complied or failed the provisional toxicity-based criteria limit derived for the discharge. In all instances the samples complied with the consent limit.

Table 2.6 Summary of the results of compliance monitoring of effluent 50

Test occasion	Mean % abnormality of oyster embryos		P value for standard	Outcome of modified t-test	Assessment of compliance
	Control	1000 fold effluent dilution	t-test		
1	13	19	0.064	Not required	Compliance
2	15	12	0.244	Not required	“
3	15	19	0.025	D<RTDL	“

Note: D = difference between control and treatment response (=0.053), RTDL = 0.07)

Should samples fail to comply with the toxicity limit, reference would be made to the Agency's enforcement policy which describes a series of retests and investigative action followed by notices requiring toxicity evaluation to ultimately prohibition notices or prosecution should agreement not be reached or persistent failures continue. The approach of retesting an effluent failing its toxicity limit with another limit test and then with a full concentration range test provides greater certainty that remedial action will not be taken on the basis of an initial false positive result.

The use of the RTDL approach allows regulators to define the level of differences between control and effluent treatment groups which they require to be detected as significant. This detection level (for example 10 or 20% difference) then defines the acceptable level of repeatability of test methods which are used in compliance monitoring. Such requirements will become an integral feature of the Register of Approved Laboratories scheme for effluent testing being developed by the Environment Agency.

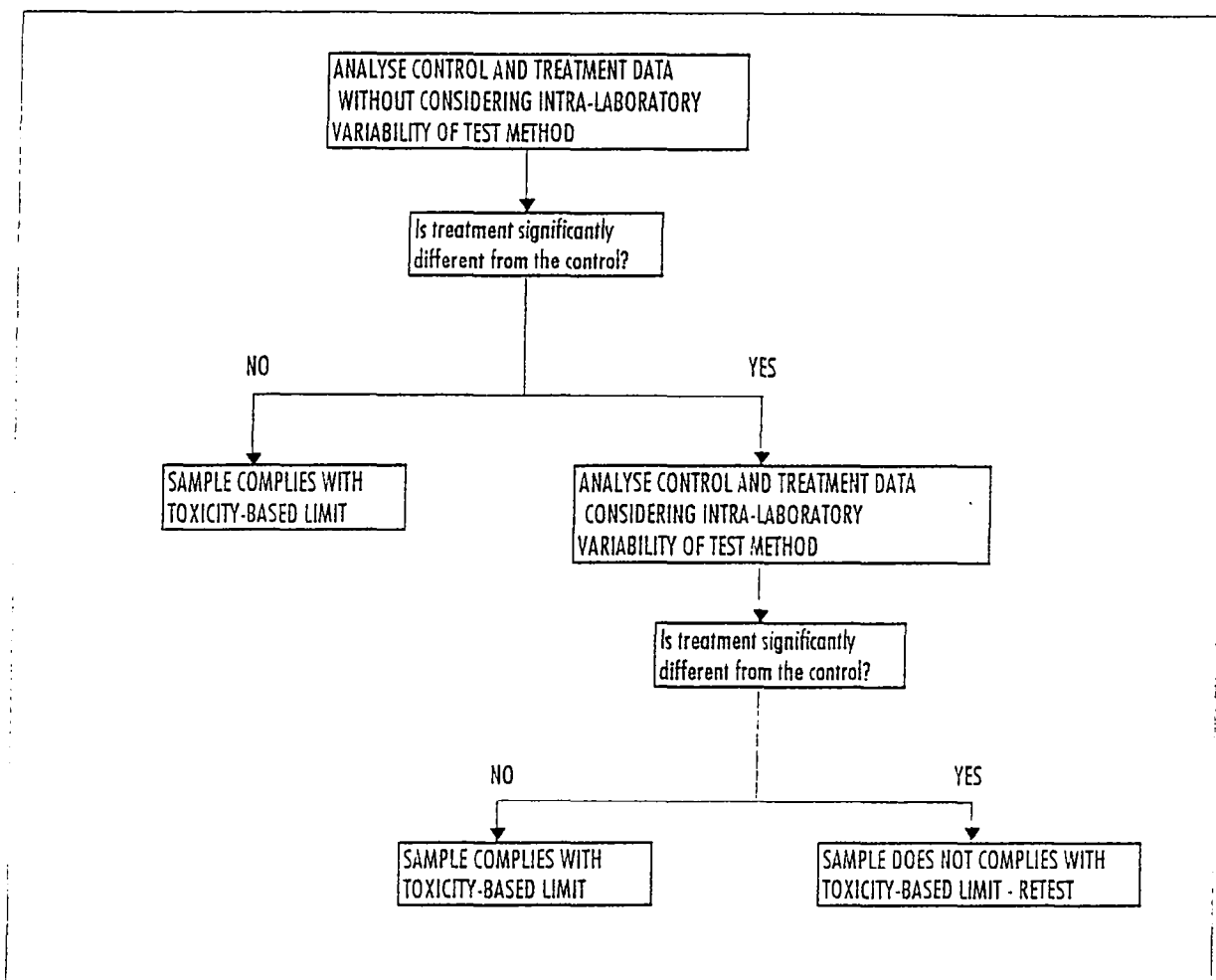


Figure 2.15 Statistical approach for assessing discharge compliance (after Dhaliwal *et al.* 1995)

3. APPLICATION OF THE PROTOCOL

The protocol can be used to assess the acute lethal toxicity of all major industrial discharges and sewage treatment works effluents. The pilot study has shown that there are a considerable number of industrial and sewage treatment discharges which are acutely lethal to higher organisms at concentrations of <5% effluent (see Figures 2.2-2.13). In some instances these discharges are causing toxic impacts on the receiving water community. Discharges with the potential to cause lethality in the receiving water discharges would be more effectively controlled by deriving a toxicity-based limit rather than relying on current chemical-specific limits alone. However, only a limited number of discharges have been tested in the pilot study and a wider screening exercise needs to be carried out to assess the potential scope of application of the approach. Chronic invertebrate and fish tests with sub-lethal endpoints are being developed for use in the protocol and will allow the more subtle biological effects of effluents discharged to the environment to be evaluated.

4. FUTURE DEVELOPMENTS

4.1 Consultation process

The feedback on the proposed scheme for implementing toxicity-based consent conditions received from interested parties during the consultation period (July-October 1996) can be used to revise the strategy where necessary and derive a robust and equitable system which will complement existing Environment Agency consenting policy.

4.2 Additional R&D

Further research is needed to:

1. to develop appropriate methods to control the low level chronic and sub-lethal toxicity of complex discharges;
2. to develop a complete and complimentary battery of rapid and cost-effective rapid tests which can act as surrogates for established acute and chronic lethal and sub-lethal toxicity tests;
3. to apply the developed protocols to a wider range of sewage treatment works and industrial discharges starting with a toxicity screening programme to assess the scope of application of the toxicity-based consenting approach.

REFERENCES

Chapman, P.M., Caldwell, R.S. and Chapman, P.F. (1995) A warning: NOECs are inappropriate for regulatory use. *Environmental Toxicology and Chemistry*, **15**(2), 77-79.

Dhaliwal, B.S., Dolan, R.J. and Smith, R.W. (1995) A proposed method for improving whole effluent toxicity data in regulatory compliance. *Water Environmental Research*, **67**(6), 953-963.

Environment Canada (1990) Guidance document on control of toxicity test precision using reference toxicants. Environment Canada Report EPS 1/RM/12, Environmental Protection Series, Environment Canada, Ottawa.

Kooijman, S.A.L.M. and Bedaux, J.J.M. (1996) The analysis of aquatic toxicity data. VU University Press, Amsterdam, pp 160.

US EPA (1991) Technical Support Document for Water Quality-Based Toxics Control. EPA/505/2-90-001, Office of Water, Washington DC.

