Environment Agency

Evidence

Review of urban pollution management standards against WFD requirements

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This report is the result of research commissioned and funded by the Environment Agency.

The purpose of the report is to provide a set of standards for intermittent discharges for consideration by Water Framework Directive UK TAG for implementation as measures for achieving WFD objectives.

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Miranda Kavanagh

Director of Evidence

Executive summary

Intermittent wet weather discharges from urban wastewater systems represent a potentially significant source of release of pollutants to the aquatic environment. Therefore, the effective control of the environmental impacts of these discharges, alongside continuous discharges from point sources, is vital if environmental quality objectives and standards are to be met. For the past 13 years, water quality standards in the UPM 2 Manual (*Urban Pollution Management Manual*) have been used by regulators and dischargers in preparing permit applications and designing improvement works address unsatisfactory intermittent wet weather problematic discharges.

With the implementation of the Water Framework Directive (2000/60/EC) (WFD) and its new water quality and ecological standards, the UPM 2 standards (fundamental intermittent standards and 99 percentiles) need to be reviewed. This report describes a project carried out in a series of steps that reviewed the UPM2 standards currently used to protect inland water quality and ecology. The programme of work comprised four tasks.

- Task A –Carry out a literature review/data collection.
- Task B – Establish an effects matrix.
- Task C – Compare the effects matrix against existing standards.
- Task D – Propose UPM standards for WFD.

This report describes the results of Tasks A to D. The key findings are summarised below.

Task A - Literature review/data collection

The review of new data generated the following key information:

- Additional data on the relative sensitivities of aquatic organisms to dissolved oxygen and/or unionised ammonia – Maltby (1995), Mummert *et al.* (2003), Turnpenny *et al.* (2004), Wang *et al.*(2007) and Gomułka *et al*. (2011).
- Confirmation of the effects of repeated exposure to pulses of unionised ammonia and the influence of the return period – Diamond *et al.* (2006).

An assessment of available data (including post-1992 information) indicated that:

- Based on an evaluation of toxicity data including post-1992 information and comparison with other standards for short-term exposure, the fundamental intermittent standards (UPM2 FIS) are generally “fit for purpose”.
- The available toxicity data indicates that the magnitude of the standards for different exposure periods (one, six and 24 hours) are generally appropriate. For dissolved oxygen, this finding is largely based on a direct comparison of the 24-hour toxicity data (and to a lesser extent the six-hour data) with the corresponding UPM2 FIS for a one-year return period. For unionised ammonia, a probabilistic approach using species sensitivity distribution (SSD) modelling was used to derive a *salmonid threshold* and a *cyprinid threshold* for short-term mortality which were compared with six- and 24-hour UPM2 FIS for a one-year return period.
- The available toxicity data (in terms of the ratios of one-hour to six-hour LC_{50}(lethal concentration) values and six- hour to 24-hour LC_{50} values) are, in some instances, consistent with the ratios of the standards. However, in other

cases, the toxicity ratios are slightly smaller than those of the standards. There is greater certainty for the unionised ammonia standards due to the larger dataset.

- Smaller ratios of toxicity values (e.g. LC_{50} values) than those of UPM2 FIS mean that the shorter term (one- and six-hour) standards should account for long-term sub-lethal effects as well as short-term mortality. In contrast, higher ratios mean that the shorter term standards may not account for short-term mortality and long-term sub-lethal effects.
- In summary, for sustainable salmonid and sustainable cyprinid fishery ecosystem types, the UPM2 FIS provide protection against both short-term mortality and longer term effects on the physiology, growth and reproduction of the fish. For dissolved oxygen, the standards are also generally protective against macroinvertebrate drift.

An initial comparison of the roles of the UPM2 FIS and 99 percentiles has been conducted.

Task B - Establish effects matrix

Initial effects matrices were developed for dissolved oxygen and unionised ammonia using all the available, relevant lethal and sub-lethal data. The effects matrix was developed taking into account the requirement of biological classification under the WFD.

Based on the effects matrix, data for each substance *estimated threshold limits* were derived for the protection of sustainable salmonid and cyprinid fisheries.

For dissolved oxygen, the *estimated threshold limit* for salmonid fisheries is 4.0 mg/l whilst the corresponding value for cyprinid fisheries is 3.0 mg/l. These values should ensure protection of salmonid and cyprinid fisheries respectively from short-term mortality and macroinvertebrate drift, irrespective of the duration of exposure.

Based on the complete dataset for unionised ammonia and applying the SSD approach, the following *estimated threshold limits* should be protective of salmonid fisheries and ensure that no behavioural, sub-lethal or lethal effects occur in exposed salmonids and sensitive macroinvertebrates for return periods of more than 2.6 days (that is, a frequency of three events per week):

Exposure duration	*Estimated threshold limits for return periods from 1 month to 1 year*
One hour	0.247 mg/l (as NH_3-N)
Six hours	0.134 mg/l (as NH_3-N)
24 hours	0.044 mg/l (as NH_3-N)

The following *estimated threshold limits* should be protective of cyprinid fisheries and ensure that no behavioural, sub-lethal or lethal effects occur in exposed cyprinids and macroinvertebrates for return periods of one month to one year:

Exposure duration	*Estimated threshold limits for return periods from 1 month to 1 year*
One hour	0.541 mg/l (as NH_3-N)
Six hours	0.204 mg/l (as NH_3-N)
24 hours	0.063 mg/l (as NH_3-N)

Since the UPM2 FIS for marginal cyprinid fisheries are not currently applied and given the small datasets, no *estimated threshold limits* were derived for this ecosystem.

Task C - Compare effects matrix against existing standards

Comparing the UPM2 FIS with the *estimated threshold limits* derived from the effects matrix showed that, for most concentration/duration/frequency combinations, the standards for dissolved oxygen and unionised ammonia provide a margin of safety for salmonid and cyprinid fisheries. These provide protection against potential effects of reduced dissolved oxygen and elevated unionised ammonia concentrations for which measured data is not available. This approach is consistent with the process used to derive environmental quality standards, where the assessment (safety) factor applied would in part account for untested species and endpoints. Overall, the UPM2 FIS are “fit for purpose” and, based on the available data, provide an adequate degree of protection.

Meeting the UPM2 FIS standards should ensure that for typical wet weather events, the existing good quality status of a water body is not compromised by these intermittent discharges.

Task D - Proposed UPM standards for WFD

Comparing the UPM2 FIS with the *estimated thresholds* derived from the effects matrix indicated that the UPM2 FIS are “fit for purpose” and no modifications are required. For most concentration/duration/frequency combinations, the standards for both dissolved oxygen and unionised ammonia provide a margin of safety for salmonid and cyprinid fisheries. They should also provide a degree of protection against potential effects of reduced dissolved oxygen and elevated unionised ammonia concentrations for which toxicity data is not currently available. Therefore, when a fishery meets the relevant standards, no long-term behavioural and physiological effects and no short-term fish and macroinvertebrate mortality should result from irregular wet weather pollution events with at least an annual return period. Therefore, meeting the UPM2 FIS should ensure that the good quality status of a water body is not compromised.

To date, the vast majority of the practical experience of implementing the UPM procedure in the UK has been obtained in rivers. However, the UPM2 FIS for cyprinid standards should also be applicable to still waters such as lakes.

The *99 percentiles* developed for biological oxygen demand (BOD) and total ammonia by the Environment Agency (see Section 2.3.2) for WFD ecological statuses were established by interpolating the corresponding 90 percentile values of each River Ecosystem Class. Meeting these standards should protect freshwater aquatic life from short duration intermittent urban wet weather events and also ensure that the existing good quality status of a water body is not compromised by intermittent wet weather discharges.

Given that the 99 percentiles were originally developed from the RE Classes and have now been produced for Water Framework River Typology classes they are specifically applicable to rivers rather than still waters such as lakes.

Recommendations

- The fundamental intermittent standards (FIS),as reviewed in this report, should be translated into the revised version of the *Urban Pollution Management Manual*.
- The FIS should continue to be used by regulators and dischargers in preparing permit applications and in designing improvement works to address unsatisfactory intermittent wet weather discharges from urban wastewater networks.

- The outcome of the review regarding the ongoing use of FIS and 99 percentiles should be presented to the United Kingdom Technical Advisory Group to confirm their suitability for use under the Water Framework Directive.

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Contents

1. Introduction

1.1 Background

Intermittent wet weather discharges from urban wastewater systems represent a potentially significant source of release of pollutants to the aquatic environment. Therefore the effective control the potential environmental impacts of these discharges, alongside continuous discharges from point sources, is vital if environmental standards are to be met. Such wet weather events may affect river water quality for relatively short time periods, but the high pollutant concentrations can have a disproportionate impact on the ecosystem.

For the past 13 years, water quality standards in the UPM 2 Manual (*Urban Pollution Management Manual*) have been used by regulators and dischargers in preparing permit applications and in designing improvement works to address unsatisfactory intermittent wet weather discharges from urban wastewater networks.

With the implementation of the Water Framework Directive (2000/60/EC) (WFD) and its new water quality and ecological standards the UPM 2 standards needed to be reviewed. This project outlines a series of steps that reviewed the UPM2 standards currently used to protect inland water quality and ecology. Standards are already in place for assessing the impact of intermittent discharges against the requirements of the current and revised Bathing Waters Directives and the Shellfish Waters Directive.

A separate exercise is being undertaken in parallel with this project, to review the guidance in the UPM2 manual to make it fit for purpose in complying with the Water Framework Directive (WFD) and other developments, such as climate change that have taken place since 1998, when the manual was published.

1.2 Key assumptions

The work programme assumed that the nature of the UPM standards would not change, that is:

- The standards would still be based on levels of water quality appropriate for protecting ecology from intermittent wet weather discharges for urban wastewater networks.
- The standards would still be framed as concentration-duration thresholds with an allowable return period (RP) or frequency and as high (99) percentiles.
- The standards reviewed would be the standards based on general water quality indicators, that is, dissolved oxygen and ammonia. The project did not aim to derive new standards for priority or priority hazardous substances. If necessary, these would be dealt with through the UK Technical Advisory Group (UKTAG).

1.3 Scope of the work

The work programme comprised four tasks, which are summarised below.

Task A - Literature review/data collection

An important first step was to review the research used to derive the original UPM standards, as described in Report 123 by Milne *et al*. (1992). In deriving the standards, some moderate invertebrate drifting was allowed. Would such drifting compromise achievement of WFD objectives? Also, when applying correction factors and calculating unionised ammonia, practitioners have adopted a pH “capping” of 8.0. Confirmation of the validity of this approach would be useful.

A second step was to review any new ecotoxicological work that might be relevant to the UPM2 fundamental intermittent standards (FIS). In 1994, a preliminary Aqualine search for any relevant papers since 1991 identified about 120 papers, 30 of which could have been relevant.

The final step for this task was to revisit the previous review of the relationship between the different types of UPM standards. A set of 99 percentile standards that would be obtained for WFD by direct interpolation of the existing 99 percentiles was developed by the Environment Agency. The project attempted to establish how these percentiles align with the current and any recommended new duration/frequency standards.

Task B - Establish effects matrix

For this task an “effects profile” for ammonia (total and/or unionised) and dissolved oxygen was developed based on the previous research and more recent work. This was displayed as a matrix showing the likely *ecological* effects for a range of concentrations at different return periods, extending beyond the one-year return period if possible. Ecological effects need to consider all the relevant elements of WFD classification. The ecological basis for ammonia and dissolved oxygen standards for intermittent pollution and any shortfalls were discussed.

Task C - Compare effects matrix against existing standards

For this task the effects matrix was compared to the existing UPM FIS and any differences were highlighted.

Task D - Propose UPM standards for WFD

This task generated proposals for a set of UPM standards to meet WFD objectives. Consideration, in particular, was given to the following:

- How the current UPM2 FIS compared to WFD objectives and if the existing standards needed to be changed at all.
- How the current and interpolated 99 percentiles compared to WFD objectives.
- If a set of longer return period UPM2 FIS was necessary for the protection of aquatic life.
- Situations where use of the standards would not be appropriate, for example, in still waters.

In the report, unionised ammonia refers to NH_3-N whilst ionised ammonium refers to NH_4-N.

This report describes the results of Tasks A to D which were designed to provide the technical data to assess the UPM2 standards against Water Framework Directive objectives.

1.4 Current application of the standards

The Environment Agency *Guidance on Water Discharge and Groundwater Activity Permits* (EPR 7.01) assigns three levels of significance to intermittent discharges that are assessed using UPM methods and provides guidance on the level of modelling and types of standards that should be applied in each case. It operates on the assumption that simple models and standards produce conservative solutions. That is to say, Formula A calculations produce a more protective (conservative) solution than 99 percentiles using simple models, which in turn produce a more protective solution than FIS using complex modelling. The guidance recognises that these assumptions are not valid in all circumstances. It states that:

"Where FIS are used, the minimum environmental standards for freshwater ecosystems will be those given in the Fundamental Intermittent Standards Tables (Table 2.2 and Table 2.3) of the second edition of the UPM Manual in order to achieve an ecosystem suitable for a sustainable cyprinid fishery.

The sewerage undertaker must also demonstrate that the proposed scheme will allow the receiving water to achieve the required water quality at any other percentile standards relevant to its classification or designation as well as any other relevant objectives and uses. The FIS for sustainable salmonid fishery ecosystem will only be applied to discharges affecting established salmonid (designated and un-designated) fisheries and salmonid spawning grounds".

In Scotland, Scottish Environmental Protection Agency Regulatory Method WAT-RM-07 Version 2 (SEPA 2009) states that:

"Demonstration of compliance with 99 percentile standards for the target river class will be required as a minimum where impact modelling is carried out. If a discharger uses Fundamental Intermittent Standards (FIS) then the salmonid FIS should be used. Cyprinid FIS may be appropriate in a limited number of circumstances for some lowland slow-flowing rivers. Where meeting the 99 percentile standards compared to the FIS produces a significantly greater cost for the scheme, SEPA will consider the approach based on the FIS but will need high confidence in the result and detailed sensitivity analysis of the model".

2. Review of FIS and 99 percentiles for dissolved oxygen and ammonia

2.1 Task A1 – Review of the original research

The requirements of this task as stated in the tender specification were:

"An important first step is to review the original research as described in the R&D Report (Milne et al., 1992). In deriving the standards some moderate invertebrate drifting was allowed. Would such drifting compromise achievement of WFD objectives? Also, when applying correction factors and calculating unionised ammonia practitioners have adopted a pH "capping" of 8.0. Confirmation of the validity of this approach will be useful."

2.1.1 Background information on the effects of dissolved oxygen and ammonia on aquatic organisms

Dissolved oxygen

Major sources of dissolved oxygen in water are the atmosphere and photosynthesis by aquatic vegetation. The amount of oxygen available for aquatic life, however, depends on factors that affect its solubility (such as water temperature and salinity). The saturation concentration of dissolved oxygen is quickly achieved at the air-water interface, and in shallow moving water, will be relatively consistent throughout the water column. In large and deep freshwater systems, oxygenation depends on circulation by winds, currents and inflows to move aerated water away from the surface.

The dissolved oxygen content in water is largely controlled by the balance between inputs of oxygen and consumptive metabolism of the oxidisable matter in the water column and associated with the bed sediment.

Ammonia

Ammonia is highly soluble in water and its speciation is affected by a wide variety of environmental parameters including pH, temperature and ionic strength. In aqueous solutions, equilibrium exists between unionised (NH_3-N) and ionised (NH_4-N) ammonia species.

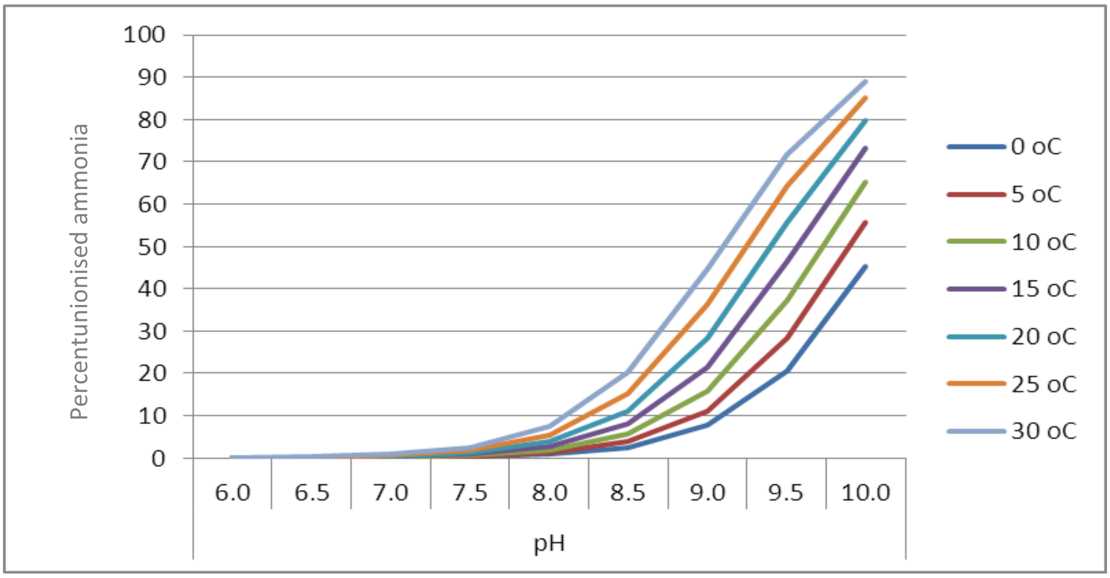
Several factors are known to affect the toxicity of ammonia in freshwaters. These factors may have an effect on the concentrations of unionised ammonia in water or impact directly on the exposed organism making it more or less susceptible to ammonia. Factors shown to affect ammonia toxicity include pH, temperature, dissolved oxygen concentration, ionic strength, previous acclimatization to ammonia, fluctuating or intermittent exposure, and the presence of other toxic substances. Of these, pH is thought to be the most important factor influencing ammonia toxicity in freshwaters (BC

MoE 1997). Salinity is also a factor in determining ammonia speciation in transitional and coastal waters.

The speciation of ammonia is very important to understanding ammonia toxicity. As unionised ammonia is known to be more toxic than the ammonium ion, the influence of pH and temperature on the relative proportion of ionised and unionised ammonia, in particular, is important. Unionised ammonia is thought to be more toxic to aquatic organisms because it is a neutral molecule and is, therefore, able to diffuse across biological membranes more readily than other forms of ammonia (USEPA 1998).

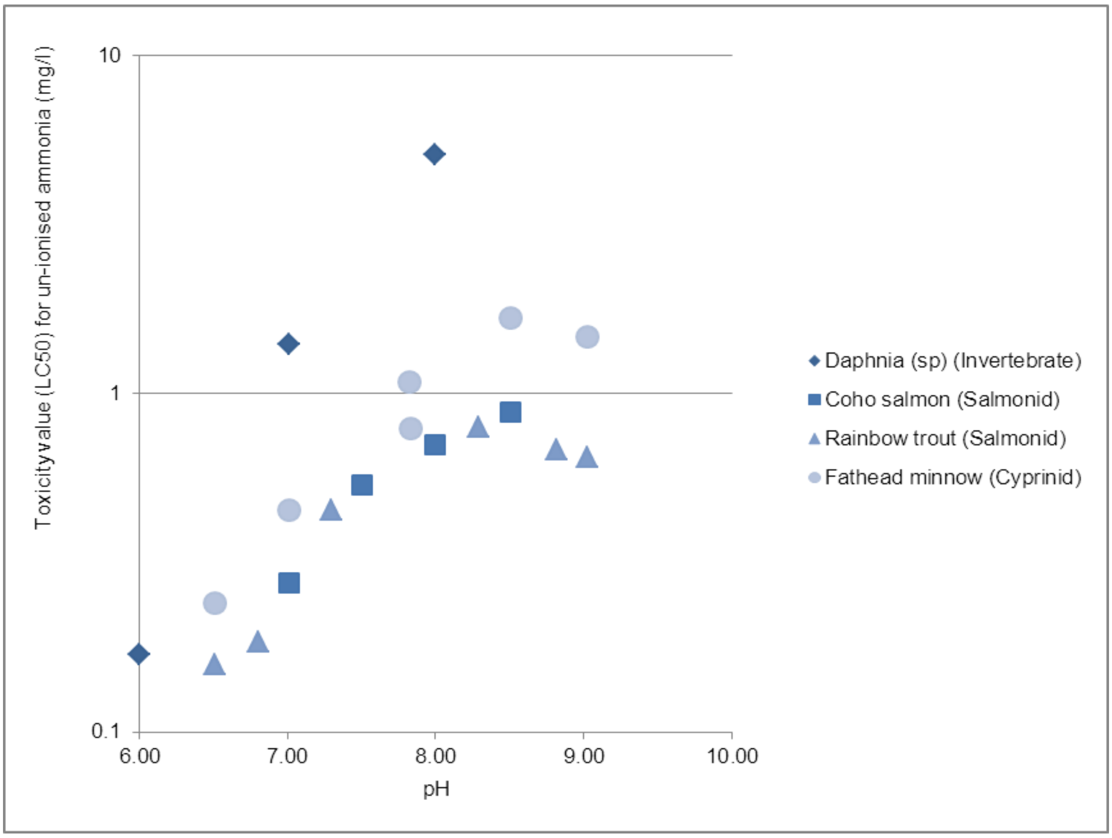
In freshwaters, the proportion of unionised ammonia of a total ammonia concentration increases with increasing temperature and pH. At pH 8.5, the proportion of unionised ammonia is approximately 10 times that at pH 7.5 and by pH 9.0 the vast majority of ammonia is in the form of unionised ammonia. For every 9°C rise in temperature, the proportion of unionised ammonia approximately doubles (see Figure 2.1).

Figure 2.1 Relationship between percent unionised ammonia in solution and pH and temperature



As pH increases, the acute toxicity of ammonia increases due to the increasing relative proportion of unionised ammonia up to pH 8.0. As water pH levels increase above pH 8.0, then the toxicity values for a given species either stabilise or decline. This point is illustrated in Figure 2.2 which shows the available data for the freshwater crustacean *Daphnia* and three fish species (the salmonids coho salmon and rainbow trout and the cyprinid fathead minnow) as reported by the US Environmental Protection Agency (USEPA 1985).

Figure 2.2 Relationship between the acute toxicity of unionised ammonia to freshwater crustaceans and fish and water pH



This data has been used to cap the pH at 8.0 when applying correction factors and calculating unionised ammonia concentrations from total ammonia levels.

In order to review the original data on the effects of dissolved oxygen and ammonia on aquatic organisms following pulsed exposure, it was necessary to review the factors that affect the toxicity of these substances following single exposure. Table 2.1 summarises the available data on the key factors affecting the toxicity of dissolved oxygen and unionised ammonia.

Table 2.1Data on the key factors affecting the toxicity of dissolved oxygen and unionised ammonia (from Environment Agency 2007 and USEPA 2009)

Factor	Data on the toxicity of dissolved oxygen	Data on the toxicity of unionised ammonia
Duration of exposure	**Macroinvertebrates and fish** The concentrations of dissolved oxygen that cause toxicity to macroinvertebrates and fish depend on the length of exposure. Lower concentrations may not kill or adversely affect organisms over short periods of time, but the same concentrations could kill or impair aquatic life under longer time frames. A number of animals have behavioural strategies to survive periodic exposure to less dissolved oxygen. These include avoidance by mobile animals such as macro-crustaceans and fish, shell closure and reduced metabolic rate in bivalve molluscs and decreased burrowing depth or emergence from burrows for sediment dwelling annelids, crustaceans and molluscs.	**Macroinvertebrates and fish** The concentrations of unionised ammonia that cause toxicity to macroinvertebrates and fish depend on the length of exposure. Lower concentrations may not kill or adversely affect organisms over short periods of time, but the same concentrations could kill or impair aquatic life under longer time frames.
Toxicity and life stage	**Macroinvertebrates** Animals with high metabolic rates are typically less tolerant of reduced oxygen than less active forms (such as early life-stages or behaviourally inactive forms). **Fish** Susceptibility to dissolved oxygen acute toxicity varies by life stage of the fish. Younger fish tend to be more sensitive than older fish. However, different life stage patterns may be found for particular species.	**Macroinvertebrates** Susceptibility to unionised ammonia acute toxicity typically varies by life stage of the macroinvertebrates, with early life-stages showing greater sensitivity. **Fish** Susceptibility to unionised ammonia acute toxicity varies by life stage of the fish. Susceptibility to unionised ammonia generally decreases as fish develop from sac fry to juveniles and increases with age of the fish after the juvenile stage. However, different life stage patterns may be found for particular species.
Relative taxonomic sensitivity	Generally, macroinvertebrates are more sensitive to low levels of dissolved oxygen than fish or plants.	Generally, fish are considered to be more sensitive to unionised ammonia than macroinvertebrates or plants. However, recent data from the USEPA has indicated that larval and juvenile freshwater unionid mussels may be more sensitive than fish. In North America, more than 70% of freshwater mussel populations are listed as endangered, threatened or of special concern.

2.1.2 What data was used to derive the FIS?

Introduction

From the mid-1980s to 1992,WRc carried out research as part of the UK Urban Pollution Management (UPM) programme, to assess the impact of combined sewer overflow (CSO) discharges on receiving water quality and to develop water quality standards for the protection of aquatic life from intermittent pollution caused by CSO. The development and validation of fundamental intermittent standards (FIS) was carried out between 1987 and 1992 for the National Rivers Authority (NRA) as part of its R&D programme, in conjunction with the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER). The results of the work are described in NRA Report 123 (Milne *et al.,* 1992) and were subsequently reported in Milne *et al.* (2000) and Seager *et al.,* (2000).

A laboratory-based ecotoxicological programme investigated the relative importance of concentration, duration and frequency during exposure of fish to short-term pulses of low dissolved oxygen and elevated unionised ammonia. For the experimental conditions used, exposure concentration and duration were found to be important in determining pulse toxicity for both dissolved oxygen and unionised ammonia, whereas frequency of exposure was important for unionised ammonia only.

A review of these, and other ecotoxicological data generated information that was used along with established water quality standards to develop a three-dimensional approach for intermittent-exposure standards, which took into account event magnitude, duration and frequency. Standards were developed for dissolved oxygen and unionised ammonia to protect against intermittent exposure.

In conjunction with the laboratory study a combined sewer overflow impact assessment was carried out at Pendle Water, a tributary of the River Calder, that is one of the major tributaries in the Ribble Catchment of North-East Lancashire. Little research had been carried out on the impact of CSO discharges when the problem of urban storm pollution started to be addressed. One of the objectives of the study was to investigate CSO impact on receiving water chemistry and biology using a range of ecotoxicological methods (including *in situ* bioassays and the WRc Fish Monitor) and biological survey techniques, while assessing the practicability and usefulness of the techniques themselves. Further details of this element of the work programme are given in NRA Report 123 (Milne *et al.,* 1992).

Subsequently, a field-validation exercise for the proposed water quality standards was carried out on the upper reaches of the River Trent (Staffordshire) near Stoke on Trent to relate biological impact to water chemistry. At this site, a range of monitoring techniques was combined to attempt to collect data that could be used for the field-validation exercise. CSO discharge monitoring equipment, continuous chemical monitors and event-triggered water samplers were installed. Quantitative invertebrate community samples were taken monthly and caged invertebrate and fish bioassays were employed in an attempt to relate water chemistry to biological effect.

The most sensitive measure of biological impact appeared to be invertebrate community status. Dissolved oxygen and unionised ammonia concentrations observed during CSO discharges did not approach the water quality standards. Thus, although the standards could not be validated conclusively, there was no reason to believe they were not appropriate.

Laboratory-based ecotoxicological programme

In a series of laboratory experiments, rainbow trout (*Oncorhynchus mykiss*[1]), brown trout (*Salmo trutta*) and roach (*Rutilus rutilus*) were exposed to a range of concentrations of low dissolved oxygen and elevated unionised ammonia for different durations and frequencies(Milne *et al.,* 1992).

Two series of experiments were carried out to investigate duration and frequency respectively:

- In the first series, one, six and 24-hour exposure durations were used for a range of concentrations of dissolved oxygen (rainbow trout and roach) and unionised ammonia (rainbow trout) and lethal responses were observed.
- In the second series, 24-hour dissolved oxygen or six-hour unionized ammonia exposure was repeated over a period of several weeks (using brown trout in both cases) at different frequencies and the resulting effects on growth and physiology were observed.

Table 2.2 summarises the test procedures carried out (in terms of the exposure regimes adopted) and the results obtained.

[1] Previously named *Salmo gairdneri*

Table 2.2 Test procedures (exposure regimes) and results from laboratory ecotoxicological studies

Determinand	Type of parameter evaluated	Exposure conditions		Summary of the results
		Test species	Exposure conditions	
Dissolved oxygen	Exposure duration	Ten-month-old rainbow trout (*Oncoryhnchus mykiss*)	One-hour exposure – four concentrations(3.5, 2.5, 1.5 and 0.7 mg/l) Six-hour exposure – four concentrations(3.2, 2.7, 1.6 and 1.2 mg/l) 24 hour exposure – four concentrations(4.5, 3.5, 2.5 and 1.6 mg/l) Groundwater control Ten fish per test vessel Temperature range: 12.1-13.5°C pH range: 7.64-8.09	• Mortalities generally occurred during the exposure periods only and there was no evidence of significant post-exposure mortality. • In each test there was a clear separation between higher dissolved oxygen concentrations at which most or all fish survived and lower concentrations at which most, or all, fish died. • The threshold concentration separating low and high mortality was dependent on exposure duration but in each case occurred between concentrations about 1 mg/l apart. • For the one-hour exposure duration all fish died at 0.7 mg/l and more than 90 per cent survived at 1.5 mg/l (88 per cent survived at 1.5 mg/l after the post-exposure period). • For the six-hour exposure period all fish died at 1.16 mg/l, 96 per cent died at 1.6 mg/l and more than 90 per cent survived at 2.69 mg/l for six hours (84 per cent survived at 2.69 mg/l after the post-exposure period). • For the 24-hour exposure period all fish died at 1.6 mg/l, 64 per cent survived at 2.5 mg/l and more than 90 per cent survived at 3.5 mg/l. The following results were obtained from the study: • One-hour LC_{50} = 1.06 mg/l (95% confidence intervals = 1.05-1.07 mg/l) • Six-hour LC_{50} = 2.35 mg/l (95% confidence intervals = 2.26-2.45 mg/l) • 24 hour LC_{50} = 2.66 mg/l (95% confidence intervals = 2.51-2.80 mg/l)
		Fry of roach (*Rutilus*	One-hour exposure – three	• During the one-hour exposure test the only mortality

Determinand	Type of parameter evaluated	Exposure conditions		Summary of the results
		Test species	Exposure conditions	
		rutilus)	concentrations (2.43, 1.64 and 0.42 mg/l) Six-hour exposure – three concentrations (2.52, 1.53 and 0.41 mg/l) 24 hour exposure – three concentrations(2.18, 1.33 and 0.33 mg/l) Groundwater control 20 fish per test vessel Temperature range: 13.0-15.0°C pH range: 7.85-8.15	occurred at 0.42 mg/l (target = 0.5 mg/l) where one fish died during the exposure period. • During the six-hour test there were no mortalities. The lowest concentration tested was 0.41 mg/l (target = 0.5 mg/l). • Mortality during the 24-hour exposure test occurred only at the lowest concentration tested, the mean for which was 0.33 mg/l (range 0.1-1.5 mg/l). No mortality was seen at a mean concentration of 1.33 mg/l, which was the next lowest dissolved oxygen concentration tested, or at any concentration above this. Mortality first occurred after 7.5 hours of exposure and continued for a further 12.5 hours by which time 16 fish out of 20 (80 per cent) had died. Following the exposure period, no further mortality occured. • In all three tests, sub-lethal effects were noted when the concentration fell to 0.3 mg/l or less. At 0.5 mg/l the fish appeared to be behaving normally.
Dissolved oxygen	Exposure frequency	Juvenile brown trout (*Salmo trutta*)	24 hour exposure - concentrations (nominal) 4.0 and 5.3 mg/l, exposure frequencies one or two times per week (10 or 20 times over a 75-day period) Groundwater control Sixteen fish per test vessel Temperature range: 12.87-12.95°C pH range: 7.98-8.05	• No mortality was seen during the test. All fish appeared to be in good condition at the end of the test, with no abnormalities, lesions or other visible signs of distress. • There were no statistically significant differences between the mean weights of fish in the different groups. • In each case two-way analysis of variance indicated a significant effect of dissolved oxygen concentration but not of frequency of exposure. Haemoglobin, haematocrit and spleen weight ratio tended to be lower in the experimental groups, while kidney weight ratio tended to be lower with liver weight ratio showing no clear pattern. However, the differences were small with few trends across treatments. Most notable was the increase in haemoglobin in all the exposed groups and increase in spleen weight ratio in the

Determinand	Type of parameter evaluated	Exposure conditions		Summary of the results
		Test species	Exposure conditions	
				two lower dissolved oxygen (4.0 mg/l) groups. • Examination of gill tissues revealed a number of histo-pathological conditions, including hyperplasia, hypertrophy and necrosis but there was no evidence of any concentration-response effects; the most severe conditions were seen in control fish.
Unionised ammonia	Exposure duration	Ten-month old rainbow trout (*Oncoryhnchus mykiss*)	One-hour exposure – four concentrations(0.24, 0.40, 0.85 and 1.30 mg/l) Six-hour exposure – three concentrations(0.20, 0.42, 0.75 mg/l) 24 hour exposure – three concentrations(0.22, 0.43, 0.82 mg/l) Groundwater control Ten fish per test vessel Temperature range: 14.0-15.0°C pH range:7.8-8.0	• All fish survived the lowest exposure concentration (0.20 to 0.24 mg NH_3-N/l) and at the next concentration (0.40 to 0.43 mg NH_3-N/l) only one fish died (after six hours) in the 24-hour exposure. • At 0.75 to 0.77 mg NH_3-N/l all fish died in the six- and 24-hour exposures but in the repeat one-hour test the fish were largely able to survive the pulse at 0.85 mg NH_3-N/l with 80 per cent surviving after seven days (in the first test 70 per cent survived 0.87 mg NH_3-N/l). Mortality occurred in the period immediately after dosing had ceased. • Most fish died rapidly at 1.3 mg NH_3-N/l in the one-hour exposure, with mortality commencing near the end of the exposure and continuing for about 20 minutes after dosing had ceased. • No post-exposure mortality was observed in the six- and 24-hour exposure test vessels. The following results were obtained from the study: • One-hour LC_{50} = 1.05 mg/l (95% confidence intervals = 1.03-1.07 mg/l) • Six-hour LC_{50} = 0.57 mg/l (95% confidence intervals = 0.55-0.59 mg/l) • 24h LC_{50} = 0.53 mg/l (95% confidence intervals = 0.47-0.61 mg/l)

Determinand	Type of parameter evaluated	Exposure conditions		Summary of the results
		Test species	Exposure conditions	
Unionised ammonia	Exposure frequency	Juvenile brown trout (*Salmo trutta*)	***First test*** (53 days duration) Six-hour exposure - concentrations (nominal) 0.2 and 0.3 mg/l, exposure frequencies one or three times per week (eight or 24 times over 53 day period) Groundwater control Ten fish per test vessel Temperature range: 12.79-12.99°C pH range: 7.83-7.84	• No mortalities occurred in either of the tests. • Fish weight was significantly lower than the control group for the three exposures per week groups, from week two to six for the lower concentration and at week four only for the higher concentration. However, no significant differences between any experimental group and the control group were observed at week eight. • The fish were too small for sufficient blood to be extracted for measurements other than haemoglobin, which showed no significant differences between groups. • There were no differences between experimental and control groups for spleen and kidney to body weight ratios. Liver to body weight ratio was significantly reduced for the fish exposed three times per week at the higher concentration. • Gill condition showed slight damage and very severe damage at the lowest concentration (0.2 mg/l) at exposure frequencies of one and three times per week. At the higher concentration (0.3 mg/l) there was no damage and very severe damage at exposure frequencies of one and three times per week.
			Second test Six-hour exposure - concentrations (nominal) 0.3 and 0.4 mg/l, exposure frequencies one or three times per week (eight or 24 times over a 53-day period) Groundwater control Ten fish per test vessel	• No mortalities occurred in either of the tests. • Fish weight was significantly lower than the control group for three exposures per week at both concentrations, from week two for the lower concentration and week four for the higher concentration, through to week eight. A lower weight was also observed at the lower concentration applied once per week from weeks six to eight. • No significant differences between control and experimental groups were observed for haemoglobin concentrations or blood cell counts. There were, however, significant increases in total packed cell volume and red

Determinand	Type of parameter evaluated	Exposure conditions		Summary of the results
		Test species	Exposure conditions	
			Temperature range: 12.86-12.95°C pH range: 7.80-7.83	component packed cell volume in test vessels two, three and five, suggesting erythrocyte swelling. • There were no differences between experimental and control groups for spleen and kidney to body weight ratios. Liver to body weight ratio was significantly reduced for the fish exposed three times per week at both concentrations. • Gill condition showed moderate damage and very severe damage at the lowest concentration (0.3 mg/l) at exposure frequencies of one and three times per week. At the higher concentration (0.4 mg/l) there was slight damage and severe damage at exposure frequencies of one and three times per week.

Key sources of literature data used to develop the FIS

To derive the UPM2 fundamental intermittent standards (FIS), a literature review was conducted to identify relevant data on the toxicity of dissolved oxygen and unionised ammonia to freshwater invertebrates and fish. Data was only included if it was relevant to the types of impacts that occur from intermittent discharges, that is, impacts of a relatively short duration (less than 24 hours). On this basis, for example, toxicity data derived from 96-hour tests were deliberately not included.

Key sources of data were:

- Alabaster, J.S. and Lloyd, R. (1980) *Water Quality Criteria for Freshwater Fish*. Second edition, Butterworth Scientific, London.
- Davis, J.C. (1975) Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: A Review. *Journal of the Fisheries Research Board of Canada*, 32(12), 2295-2332.
- Doudoroff, P. and Shumway, D.L. (1970) Dissolved oxygen requirements of freshwater fish. Food Agricultural Organization of the United Nations, FAO Technical Paper Nº 86, Rome, Italy.
- Environmental Protection Agency (1985) *Ambient water quality criteria for ammonia - 1984.* US EPA 440/5-85-001.
- Environmental Protection Agency (1986) *Ambient water quality criteria for dissolved oxygen.* US EPA 440/5-86-003.

Summary of results and conclusions for dissolved oxygen

The followingkey points were evident from the review of data on dissolved oxygen.

- In the exposure duration studies roach (cyprinids) were more resistant than rainbow trout (salmonids) to short-term exposure to low dissolved oxygen (see Figure 2.3). For both species, a narrow, duration-dependent concentration threshold separated survival from mortality. There was no evidence of post-exposure mortality; fish that survived the pulse made a full recovery (Seager *et al.,* 2000).
- In the exposure frequency studies, the dissolved oxygen concentration rather than frequency of exposure was the important factor in terms of effects on fish. There were no effects on growth rate or condition, but there were effects on the physiological parameters haemoglobin, haematocrit and organ weights. There were few clear patterns and the long-term effects of the observed changes are unclear. Overall, the experimental regimes probably had little deleterious effect on the fish (Seager *et al.,* 2000).
- For macroinvertebrates, WRc-supported work at University of Wales College of Cardiff on invertebrate drifting during low dissolved oxygen (DO) pulses. This work showed that a substantial proportion of the invertebrate community may enter drift at DO concentrations that would not be lethal to fish. In high water quality, low order streams, the DO threshold below which drift increased rapidly was around 4 mg/l for most species, and about 50 per cent of all drifting had occurred when the DO concentration had reached 3 mg/l. The minimum concentration reached was 1 mg/l and the total drift resulted in a decrease in invertebrate abundance of around 80 per cent. Full recovery occurred within

about six weeks. *Gammarus pulex*, one of the species studied, showed a typical drifting pattern, starting to drift at around 4 mg/l dissolved oxygen and showing 50 per cent maximum drifting by around 2.5 mg/l. Another study of macroinvertebrate drift was carried out using three species of mayfly nymphs and one stonefly (Gammeter and Frutiger, 1990). The study found that oxygen concentrations below 2 mg/l increased drift by 20-25 per cent at 1.63 mg/l and 30-35 per cent at 1.19 mg/l in those species where drift is an active behavior (the mayflies *Baetis fuscatus* and *Ephemerella ignita*).

- WRc-supported work at the University of Sheffield showed that *Gammarus* can survive much lower dissolved oxygen concentrations in the laboratory. There was little mortality during 24-hour pulses of 2 mg/l and the 1 mg/l LT_{50} was over 9.5 hours. As with fish, low dissolved oxygen pulses can have sub-lethal effects but there is rapid post-exposure recovery (Maltby and Naylor, 1989).

Summary of results and conclusions for unionised ammonia

The following key points were evident from the review of data on unionised ammonia.

- In the exposure duration studies, the toxicity of unionised ammonia to rainbow trout was dependent on the duration of exposure (see Figure 2.3). There were no mortalities after dosing ceased in the six- and 24-hour exposure tests and while mortality was observed after dosing ceased in both one-hour tests, this was restricted to a period of 30-40 minutes after dosing stopped, before all the ammonia had been flushed out of the test vessels (Milne *et al.,* 2000).

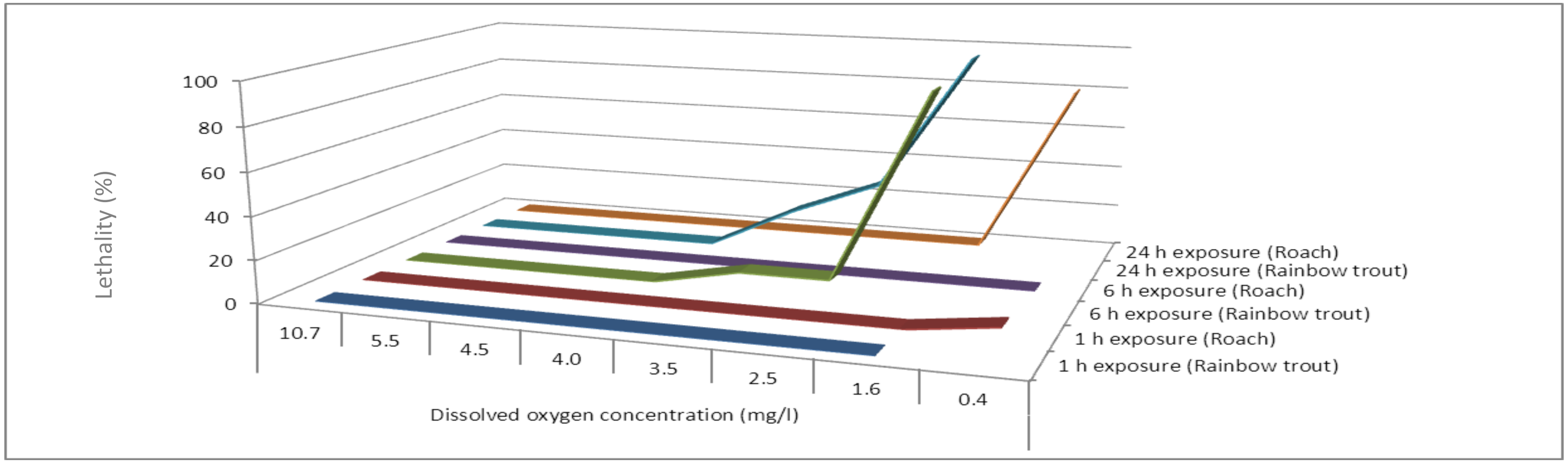
- In the exposure frequency studies, it appeared that salmonids were able to withstand potentially lethal concentrations of unionised ammonia for short periods provided that the time between exposures was sufficient to allow recovery. Although fish may be able to withstand, and recover from, short-term peaks in unionised ammonia concentrations, repeated exposures have a significantly adverse effect on growth (Milne *et al.,* 2000).

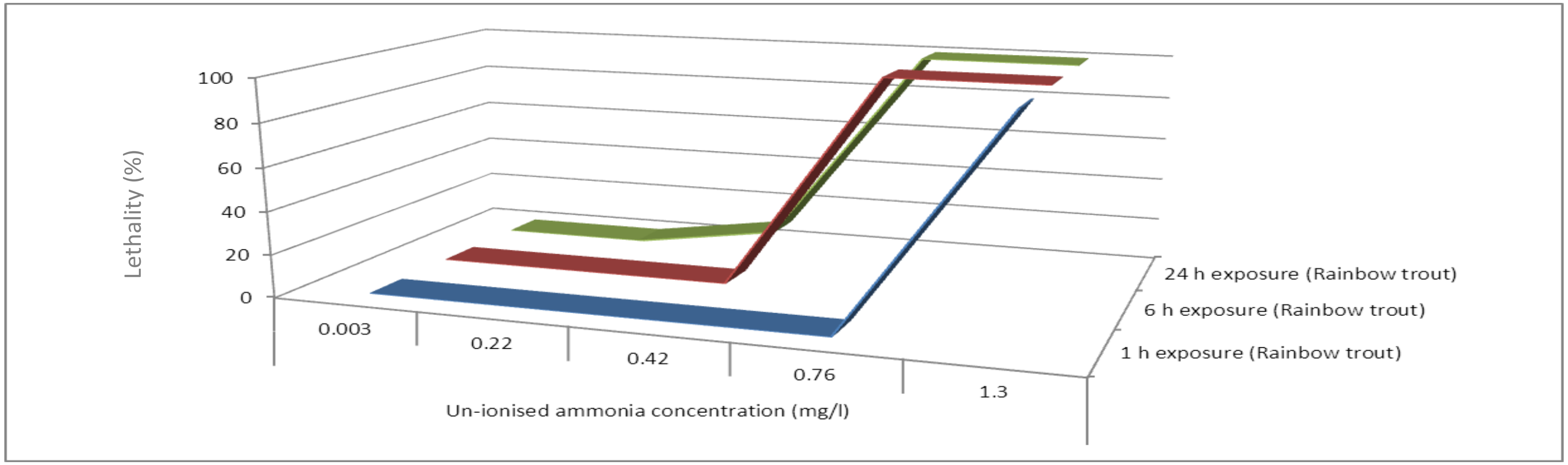
- The most marked histopathological effects on fish were found in the gills. The severity of gill damage appeared to be related more to the frequency of exposure rather than the unionised ammonia concentration. It appears, therefore, that gill condition is a sensitive indicator of unionised ammonia toxicity and a factor likely to be important where low dissolved oxygen is associated with elevated unionised ammonia (Milne *et al.,* 2000).

- For macroinvertebrates,WRc-sponsored work on artificial dosing of streams showed that unionised ammonia causes increased drifting but only at concentrations well above those lethal to fish (>2 mg NH_3-N/l). Similar results were found by Gammeter and Frutiger (1989) with concentrations of unionised ammonia up to 30 mg NH_3-N/l having little, if any, effect on drifting of three species of mayfly nymphs and one stonefly species. However, the exposure concentrations did cause mortality.

- WRc-sponsored work at Sheffield University investigated the effects of short-term exposure to unionised ammonia for *Gammarus pulex* (Maltby, 1989). Animals were exposed to 1.75, 4.36 and 7.01 mg NH_3-N/l for one, six and 24 hours. No mortality was seen for one-hour exposure and LC_{50}(lethal concentrations) for six- and 24-hour exposures were >5.64 and >3.24 mg NH_3-N/l respectively. There was little evidence of post-exposure mortality.

Figure 2.3 Summary of data on short-term fish toxicity tests with dissolved oxygen and unionised ammonia





2.1.3 What fundamental intermittent standards were derived?

Table 2.3 summarises the fundamental intermittent standards (FIS) for dissolved oxygen and unionised ammonia derived, initially, on the basis of the data generated in the R&D Programme (Milne *et al.,* 1992). In the standards, the return period of a particular set of conditions is the average period of time over a sequence of years which elapses between two events when the water body conditions are equal to or worse than the stated conditions.

Dissolved oxygen

For a return period of ***one month***, the derived standards for dissolved oxygen levels were one hour: 3.5 mg/l; six hours: 4.5 mg/l; and 24 hours: 5.0 mg/l. These levels would likely cause some physiological stress (increased ventilation rate and so on), particularly for salmonids, but would not induce strong avoidance reactions, and would avoid most invertebrate drift.

For a ***three-month*** return period, the derived standards were one hour: 3.0 mg/l; six hours: 4.0 mg/l; and 24 hours: 4.5 mg/l. These conditions would likely cause a greater degree of physiological stress, possibly inhibit migrating salmonids and cause avoidance and would cause moderate invertebrate drifting. Invertebrate recolonisation is rapid, particularly where a colonising source is available, so these levels should not cause long-term depletion of invertebrate populations. As noted above, fish recover almost immediately from sub-lethal stress. Rainbow trout can survive 24-hour exposure at 4.0 mg/l twice a week with no apparent effect on growth. Therefore, protecting invertebrate communities may be the more important factor.

For a return period of ***one year***, the derived standards were one hour: 2.5 mg/l; six hours: 3.5 mg/l; and 24 hours: 4.0 mg/l. These concentrations should not lead to fish mortality. Invertebrate drifting would be minor for a 24-hour event, but would be more significant for a one-hour event.

The difference between no lethal effect and a major fish kill may be a relatively small drop in dissolved oxygen (0.5 mg/l or less). Therefore, the standards allow little or no room for exceedance.

Unionised ammonia

For a return period of ***one month***, the one-hour standard for unionised ammonia was judged to allow a considerable margin to avoid harmful effects. Stress would likely be incurred but not long-term effects. The 24-hour standard concentration was less than half that allowing 100 per cent survival of rainbow trout in the laboratory and should therefore provide a good safety margin against long-term effects.

For a ***three-month*** return period, the one-hour standard concentration was likely to cause stress but long-term, harmful effects were unlikely to occur. The 24-hour standard was below concentrations affecting growth in the laboratory and was, therefore, considered unlikely to cause any severe stress.

For a return period of ***one year***, the one-hour standard concentration was likely to cause stress to fish but would not lead to mortality and was thought unlikely to cause permanent damage. It was less than nearly all >24-hour LC_{50} values and well below threshold concentrations for mortality at either one- or six-hour exposure. The 24-hour standard was about half the lowest salmonid LC_{50} values and well below concentrations allowing 100 per cent survival of rainbow and brown trout in the laboratory.

Combined dissolved oxygen and unionised ammonia standards

There is an interaction between the toxic effects of dissolved oxygen and unionised ammonia. Therefore, a correction factor of 0.5 was proposed to adjust unionised ammonia standards for dissolved oxygen concentrations of 3.0-5.0 mg/l and a factor of 0.25 for dissolved oxygen concentrations below 3.0 mg/l.

For dissolved oxygen standards, it was proposed that for unionised ammonia concentrations of 0.04-0.15 mg, NH_3-N/l standards should be increased by 1 mg/l and for unionised ammonia concentrations over 0.15 mg, NH_3-N/l standards should be increased by 2 mg/l.

Table 2.3 Fundamental intermittent standards for dissolved oxygen and unionised ammonia

Return period	Dissolved oxygen concentration (mg/l)		
	One hour	**Six hours**	**24 hours**
One month	3.5	4.5	5.0
Three months	3.0	4.0	4.5
One year	2.5	3.5	4.0

Return period	Unionised ammonia concentration (mg NH_3-N/l)		
	One hour	**Six hours**	**24 hours**
One month	0.175	0.100	0.040
Three months	0.250	0.150	0.060
One year	0.275	0.175	0.075

Overall, the standards should afford protection to all river aquatic life except the most sensitive life-stages of salmonid fish, where modified standards would be applied.

Further consideration of the research and expert discussions led to the current three sets of standards, relating to different levels of protection for intermittent exposure episodes up to a return period of one year. These are:

a) ecosystem suitable for sustainable salmonid fishery;

b) ecosystem suitable for sustainable cyprinid fishery;

c) marginal cyprinid fishery ecosystem.

Tables 2.4 and 2.5 summarise the current UPM2 FIS for dissolved oxygen and unionised ammonia which are the concentration/duration thresholds not to be breached more frequently than shown for each type of fishery. One of the additional sets of standards (the ecosystem suitable for a sustainable salmonid fishery) affords a higher level of protection than the original set (which are designated as ecosystems suitable for a sustainable cyprinid fishery). The other set affords a lower level of protection and is for a marginal cyprinid fishery ecosystem.

For the ecosystems suitable for sustainable salmonid and cyprinid fisheries, the standards provide protection to all life-stages of all aquatic life (plants, invertebrates and fish) associated with the specific ecosystem type. In most instances the standards should protect against invertebrate drift that is known to occur at concentrations below 4 mg/l (see Section 2.1.2). The possible exception is the one-hour standard (with return periods of three months and one year) for ecosystems suitable for a sustainable cyprinid fishery.

For the marginal cyprinid fishery ecosystem, the standards provide a lower level of protection for adult coarse fish, with the possible exception of the most sensitive coarse fish species, and may not afford adequate protection to sensitive life forms of all coarse species. These standards are not currently used in the UK.

Overall, the standards are based on the objective of no long-term detrimental effects on the aquatic ecosystem type and no fish mortality for wet weather pollution episodes of up to a one-year return period.

Table 2.4 Fundamental intermittent standards for dissolved oxygen in the *Urban Pollution Management Manual* (second edition)

Ecosystem suitable for a sustainable salmonid fishery			
Return period	**Dissolved oxygen concentration (mg/l)**		
	One hour	**Six hours**	**24 hours**
One month	5.0	5.5	6.0
Three months	4.5	5.0	5.5
One year	4.0	4.5	5.0
Ecosystem suitable for a sustainable cyprinid fishery			
Return period	**Dissolved oxygen concentration (mg/l)**		
	One hour	**Six hours**	**24 hours**
One month	4.0	5.0	5.5
Three months	3.5	4.5	5.0
One year	3.0	4.0	4.5
Marginal cyprinid fishery ecosystem			
Return period	**Dissolved oxygen concentration (mg/l)**		
	One hour	**Six hours**	**24 hours**
One month	3.0	3.5	4.0
Three months	2.5	3.0	3.5
One year	2.0	2.5	3.0

These limits apply when the concurrent dissolved unionised ammonia concentration is below 0.02 mg/l. The following correction factors apply at higher concurrent unionised ammonia concentrations:
A) 0.02-0.15 mg NH_3-N/l: correction factor = + (0.97 x log (mg NH_3-N/l) + 3.8) mg O_2/l.
B) >0.15 mg NH_3-N/l: correction factor = +2 mg O_2/l.
A correction factor of 3 mg O_2/l is added for salmonid spawning grounds.

Table 2.5 Fundamental intermittent standards for unionised ammonia in the *Urban Pollution Management Manual* (second edition)

Ecosystem suitable for a sustainable salmonid fishery			
Return period	**Unionised ammonia concentration (mg NH_3-N/l)**		
	One hour	**Six hours**	**24 hours**
One month	0.065	0.025	0.018
Three months	0.095	0.035	0.025
One year	0.105	0.040	0.030
Ecosystem suitable for a sustainable cyprinid fishery			
Return period	**Unionised ammonia concentration (mg NH_3-N /l)**		
	One hour	**Six hours**	**24 hours**
One month	0.150	0.075	0.030
Three months	0.225	0.125	0.050
One year	0.250	0.150	0.065
Marginal cyprinid fishery ecosystem			
Return period	**Unionised ammonia concentration (mg NH_3-N /l)**		
	One hour	**Six hours**	**24 hours**
One month	0.175	0.100	0.050
Three months	0.250	0.150	0.080
One year	0.300	0.200	0.140

These limits apply when the concurrent dissolved oxygen concentration is above 5 mg/l. At lower concurrent dissolved oxygen concentrations the following correction factor applies:
<5 mg/l DO, multiplicative correction factor = 0.0126 (mg DO/l)$^{2.72}$
The standards also assume that the concurrent pH is greater than seven and temperature is greater than 5°C. For lower pH and temperatures the following correction factors apply:
pH <7, multiplicative correction factor = 0.0003 (pH) $^{4.17}$.
Temperature <5°C, multiplicative correction factor = 0.5.

2.1.4 On what assumptions were the FIS based?

The key assumptions underlying the current fundamental intermittent standards (UPM2 FIS) are as follows.

Dissolved oxygen

- Fish recover rapidly following sub-lethal exposure to low dissolved oxygen levels, with little evidence of long-term effects, even when the concentration is close to the lethal concentration.
- The threshold concentration separating low and high mortality was dependent on the duration of exposure but in each case occurred between concentrations about 1 mg/l apart.
- The dissolved oxygen concentration and duration of exposure are more important factors than the frequency of exposure.
- Invertebrates can show large-scale drifting response following mild oxygen depletion (below 4 mg/l) but populations recover within several weeks (if a colonising source is available).

Unionised ammonia

- Fish are more sensitive to unionised ammonia than plants or invertebrates, in terms of both lethal and sub-lethal responses. Sub-lethal exposure to unionised ammonia can cause permanent damage to fish, particularly gills, and can reduce growth rates.
- Event concentration, duration and frequency are all important factors in determining toxic effects.
- Frequency appears to become a dominant factor when it is high (greater than once a week).
- The standards are based solely on data on toxicity to fish, with the assumption that this will afford adequate protection for other organisms.

2.1.5 What level of protection should the FIS afford to macroinvertebrates and fish?

The UPM2 FIS are based on the objective of no long -term detrimental effects on the aquatic ecosystem and no fish mortality for wet weather pollution episodes of up to a one-year return period.

The standards for sustainable salmonid and cyprinid fisheries are thought to provide protection to all life-stages of all aquatic life (plants, invertebrates and fish) associated with the specified ecosystem type. In most instances, the standards should protect against invertebrate drift that is known to occur at concentrations below 4 mg/l. The possible exception is the one-hour standard (with return periods of three months and one year) for ecosystems suitable for a sustainable cyprinid fishery.

On the other hand, the marginal cyprinid standards (which are not used in the UK) only provide adequate protection for adult coarse fish, with the possible exception of the most sensitive coarse fish species, and may not afford adequate protection to sensitive life-forms of all coarse fish species. Sensitive macroinvertebrates are also not expected to tolerate the dissolved oxygen conditions allowed by the marginal fishery standards.

2.2 Task A2 – Review of new studies

The requirements of this task as stated in the tender specification were:

“A second step in this stage is to review any new ecotoxicological work that may be relevant to the UPM2 FIS. In 1994 a preliminary Aqualine search for any relevant papers since 1991 identified about 120 papers, 30 of which could have been relevant.”

2.2.1 Approach adopted

A targeted literature review was carried out to identify data generated since 1992 of relevance to the UPM2 FIS.

A literature search was carried out using the following terms:

Dissolved oxygen, unionised ammonia, ammonia, fundamental intermittent standards, intermittent exposure, return period, salmonid fishery, cyprinid fishery, macroinvertebrate drift, lethality, mortality, development, growth, reproduction, Water

Framework Directive, good ecological status, ecological effects, effects matrix, invertebrates, fish, salmonids, cyprinids.

The review identified about 250 papers of potential relevance and the abstracts of these papers were reviewed. A standard template was prepared to summarise the relevant data in the post-1992 key studies and summaries of key papers are given in Appendix A.

2.2.2 Implications of new data

The areas where new data could impact on the UPM2 FIS include the following:

1. Information that revises the relative sensitivities of aquatic organisms to dissolved oxygen and/or unionised ammonia and indicates lower reliable short-term toxicity values for particular taxonomic groups.

2. Information that confirms or changes the effects of repeated exposure to pulses of dissolved oxygen and/or unionised ammonia and the influence of the return period.

Recent data on unionid mussels indicated that early life-stages of this taxonomic group show similar or greater sensitivity to ammonia than early life-stages of salmonid fish (Mummert *et al.,* 2003, Newton and Bartsch, 2007, Wang *et al.,* 2007). This data resulted in the United States Environmental Protection Agency producing a *Draft 2009 update of aquatic life ambient water quality criteria for ammonia – Freshwater* (USEPA 2009) in which revised acute criteria (one-hour average concentration of total ammonia nitrogen that should not be exceeded more than once every three years) were proposed for the water column with and without unionid mussels. In North America, more than 70 per cent of freshwater mussel populations are listed as endangered, threatened, or of special concern (Augspurger *et al.,* 2003). For this report, data on the ammonia concentrations causing toxicity to unionid mussels were converted to unionised ammonia based on the pH and temperature of the exposure solutions.

Maltby (1995) investigated the lethal effects of short-term exposure to hypoxia and ammonia on different life-history stages of the isopod *Asellus aquaticus* and the amphipod *Gammarus pulex*. The key conclusions from the study were that:

- Both *G. pulex* and *A. aquaticus* were able to survive 24-hour exposure to oxygen concentrations down to 2 mg/l, but below this threshold concentration survival decreased rapidly with decreasing DO.

- The lowest observed effect concentrations (LOEC) for unionised ammonia were 2.14 mg/l for *G. pulex* and 6.66 mg/l for *A. aquaticus.* For dissolved oxygen, the 24-hour LC_{50} values were 0.32 mg/l and <0.25 mg/l for adult male and juvenile *A. aquaticus* and 1.63 mg/l and 1.26 mg/l for adult male and juvenile *G. pulex*. The 24-hour LC_{50} values for unionised ammonia were 9.45 mg/l and 12.91 mg/l for adult male and juvenile *A. aquaticus* and 4.32 mg/l and 6.21 mg/l for adult male and juvenile *G. pulex*.

- Based on 24-hour LC_{50} values *A. aquaticus* was five times more resistant to hypoxia and two times more resistant to elevated unionised ammonia concentrations than *G. pulex*.

- For both stressors, most mortality occurred during exposure and post-exposure mortality was only significant for juvenile *G. pulex* exposed to low dissolved oxygen concentrations.

Turnpenny *et al.* (2004) investigated the tolerance of estuarine fish species and life-stages to low dissolved oxygen (DO) concentrations at two laboratories. Estimates of the six- and 24-hour LC_{10} and LC_{50}were obtained for most of the species and life-stages selected, although difficulties in keeping sand-smelt and gobies at the Chiswick laboratory meant that these species were only tested at the Fawley laboratory. The key conclusions from the study were that:

- Salmonids, including the Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) were the most sensitive species, with gobies and flounder being the most robust. Smelt, a species normally associated with high sensitivity to hypoxia, at the sizes tested were unexpectedly tolerant of hypoxia, although it remains possible that the newly hatched fry might be more sensitive, as was found with dace.
- A minimum standard of 1 mg DO/l may not be adequately protective of any of the species examined, although the lack of any recorded history of occurrence of such low levels within the last 12 years makes the risk difficult to assess. A 1.5 mg/l minimum standard, however, appears to create a sustainable outcome.

Diamond *et al*. (2006) investigated sub-acute effects of pulsed copper, zinc and ammonia exposures, including a range of pulse concentrations, durations, frequencies, and recovery times between pulses, using 21-day water flea *Daphnia magna* and short-term chronic fathead minnow *Pimephales promelas tests*. The key conclusions from the study were that:

- For ammonia, under 24-hour pulsed exposures below the acute criterion are unlikely to result in sub-lethal effects and much of the response, if any, would be short-term mortality.
- The results consistently demonstrated that 24hour pulsed exposures, at magnitudes above the species continuous exposure chronic toxicity threshold but well below its acutely toxic level, had little or no measurable effects on survival or sub-lethal indicators for either *Daphnia magna* or *Pimphelas promelas.*
- Recovery time (which ranged between 24 and 192 hours) was not a significant factor affecting either *Daphnia magna* or *Pimphelas promelas* survival following ammonia exposure.
- Sub-lethal effects of pulsed exposures are more likely if the magnitude approaches the species acute threshold (such as the 48-hour LC_{50}) and there are multiple pulses within a short time period (days).

The results of this study were consistent with the findings of the research reported in Milne *et al.* (2000) and Seager *et al.* (2000).

Gomułka *et al*. (2011)investigated the acute toxicity of ammonia in four life-stages of juvenile chub, *Leuciscusce phallus* (cyprinid fish): 1, 10, 20 and 30 days after the first feeding. The fish used for the toxicity test were reared intensively in a closed recirculation system. Each acute toxicity test was run for 96 hours and lethal concentrations LC_1, LC_{50} and LC_{99}were calculated for 24, 48, 72 and 96 hours. The key findings from the study were:

- The susceptibility of chub to acute ammonia toxicity decreased linearly with age and stage of development.
- The LC_{50} (48-hour) ranged from 0.62 mg/l of unionised ammonia for one day after first feeding larvae to 1.73 mg/l for 30 days after first feeding.

- A significant linear relationship between chub larvae susceptibility to ammonia toxicity and both body weight and length was found.
- The critical level of unionised ammonia for chub larvae was suggested as 0.49 mg/l.

Summary

The review of new data generated the following key information.

- Additional data on the relative sensitivities of aquatic organisms to dissolved oxygen and/or unionised ammonia – Maltby (1995), Mummert *et al.*(2003), Turnpenny *et al.* (2004) Wang *et al.*(2007) and Gomułka *et al*. (2011).
- Confirmation of the effects of repeated exposure to pulses of unionised ammonia and the influence of the return period – Diamond *et al.* (2006).
- No indication that the correction factors for combined dissolved oxygen and unionised ammonia standards given in Tables 2.4 and 2.5 should be modified.

2.3 Task A3 – Suitability of FIS and 99 percentile standards

The requirements of this task as stated in the tender specification were:

"A final part of this stage is to revisit the previous review of the relationship between the different types of UPM standards. A set of the 99 percentile standards that would be obtained for WFD by direct interpolation of the existing 99 percentiles has been developed by the Environment Agency. The project should attempt to establish how these percentiles align with the current and any recommended new duration/frequency standards."

2.3.1 Are the current FIS still "fit for purpose"?

After reviewing the data used to derive the FIS (including the assumptions on which they were based) and new data since 1992,we then assessed whether the UPM2 FIS for each ecosystem type remained "fit for purpose".

Within this assessment, the following issues were considered for the dissolved oxygen and unionised ammonia standards.

1. Are the threshold concentrations for each ecosystem type scientifically defensible?
2. Will the threshold concentrations be protective of the resident freshwater life in each ecosystem type?

For each ecosystem type, the dissolved oxygen and unionised ammonia standards were derived for exposure periods of one, six and 24 hours. The ratios between these standards should reflect the time-related differences in toxicity observed for freshwater organisms.

Re-assessment of FIS based on new data

The derivation of the existing FIS, whilst based on a large body of experimental data, involved an element of expert judgement. However, in the years since the UPM2 FIS were generated, new probabilistic statistical procedures for deriving threshold standards have been developed. Therefore, it was considered important that the complete current dataset should be used to derive robust thresholds using an alternative approach to that adopted originally. Acceptable agreement between the current UPM2 FIS and thresholds derived using the probabilistic approach would mean the FIS can be regarded as “fit for purpose”.

Probabilistic methods adopt species sensitivity distribution (SSD) modelling in which all reliable toxicity data (usually chronic no observed effect concentration or NOEC or short-term LC_{50}) are ranked and a model fitted (see Posthuma *et al.,* 2002). From this, the concentration protecting a certain proportion of species (typically 95 per cent) can be estimated (as the hazardous concentration, HC_5). Expert judgement is then used to determine whether an assessment (safety) factor needs to be applied to the $HC_5$to generate a water quality standard. Typically, assessment factors of 1-5 would be applied to the HC_5. To use the SSD modelling approach to set long-term environmental quality standards for the Water Framework Directive, it is recommended that at least 10 NOEC from eight taxonomic groups are used (Lepper, 2005, EU, 2011).

Dissolved oxygen

A review of data on dissolved oxygen indicated that it was not possible to use the SSD approach to generate short-term thresholds, because there was insufficient short-term data on the effects of low dissolved oxygen on a wide range of aquatic invertebrates. Furthermore, there were difficulties with interpreting the data based on the fact that aquatic organisms can show significant tolerance or resistance to low oxygen concentrations.

The specific oxygen requirements of aquatic invertebrates have been studied extensively and the wide range of tolerances identified is predictable for such a diverse group. Davis (1975) reported that organisms which are most able to tolerate low oxygen conditions are capable of some form of anaerobic metabolism. This enables some normally aerobic invertebrates to inhabit low oxygen environments for extended periods without mortality, while others may be able to tolerate only a brief oxygen debt (due to accumulated metabolic products which must be oxidised). Animals with high metabolic rates are typically less tolerant of reduced oxygen than sluggish forms (such as early life-stages or behaviourally inactive forms). Many invertebrates can regulate oxygen uptake over a range of oxygen tensions (organisms are oxygen-independent), while in others, oxygen uptake conforms to availability (organisms are oxygen-dependent).

Reductions in the levels of available oxygen have a marked effect on many physiological, biochemical and behavioural processes in fish. Restriction in the supply of oxygen available for metabolic processes including swimming, migrating and feeding are likely caused by hypoxia. Adequate oxygen levels for such activities are necessary for the survival of healthy fish populations. Low oxygen levels have been shown to influence growth rate, food conversion efficiency, and feeding in some species. Avoidance behaviour has been reported for a number of species in response to low oxygen, although reports of oxygen thresholds to initiate this behaviour vary considerably. Observed behaviour patterns in low oxygen include increased activity, poor feeding behaviour, and altered phototaxis. Avoidance behaviour is likely to be a useful protective mechanism that enhances the survival of some species. Some fish show an ability to acclimate to lowered ambient oxygen. However, the degree of advantage gained is not clear. Acclimation can enhance blood oxygen capacity and oxygen utilisation somewhat, but may not aid active swimming performance. This acclimation phenomenon may be useful to fish subjected to gradual reductions in oxygen levels, but would be of little advantage when encountering reduced oxygen levels for the first time, especially if the oxygen depression was severe.

A review of the available data (see Table B1 in Appendix B) indicates that 24-hour LC_{50} values range from 0.03 mg/l for the isopod *Asellus intermedius*, which can tolerate low oxygen environments, to 4.30 mg/l for the amphipod *Gammarus fasciatus*, which is typically found in well-oxygenated waters (Sprague 1963). The 24-hour LC_{50} values for salmonid fish are in the range 1.20-2.66 mg/l while the reported value for the cyprinid *Rutilus rutilus* is 0.75 mg/l, indicating a greater sensitivity of salmonids to low dissolved oxygen conditions.

Table 2.6 summarises the ratios of the one-hour/six-hour and six-hour/24-hour UPM2 FIS standards for different ecosystem types (see Table B3 in Appendix B for complete dataset). There are no marked differences in the dissolved oxygen concentrations causing six-hour and 24-hour LC_{50} values. Only slightly lower oxygen concentrations resulted in 50 per cent mortality over a six-hour exposure compared to concentrations over a 24-hour period. Therefore, the ratio of the six-hour to 24-hour UPM2 FIS for the sustainable salmonid fishery (see Table 2.6) is consistent (within 10 per cent) with the data for salmonids (mean ratio of six-hour to 24-hour LC_{50}= 0.94 in Table B1). No data is available to compare with the ratio of the one-hour to six-hour standards. The ratio of the six-hour to 24-hour UPM2 FIS for the sustainable cyprinid fishery is lower than the only available data for the cyprinid *Rutilus rutilus* (ratio of six-hour to 24-hour LC_{50} is greater than 1.88 in Table B1). However, additional data for other cyprinids are needed to confirm this single result.

Table 2.6 Summary of ratios of time-based UPM2 FIS (data from Table 2.4 and Table B3)

Ecosystem type	Ratio of one-hour to six-hour standards[a]	Ratio of six-hour to24-hour standards[a]
Sustainable salmonid fishery	0.89-0.91	0.90-0.92
Sustainable cyprinid fishery	0.75-0.80	0.89-0.91
Marginal cyprinid fishery	0.80-0.86	0.83-0.88

[a] Range of values for different return periods

Unionised ammonia

A review of the available data for unionised ammonia indicated that it was possible to apply the SSD approach to generate short-term thresholds for sustainable salmonid and cyprinid fisheries. After the information for salmonids, sensitive cyprinids and sensitive macroinvertebrates was removed from the dataset, there was insufficient data (at least 10 LC_{50} values from eight taxonomic groups) to use the SSD approach to derive a short-term threshold for marginal cyprinid fisheries.

Table 2.7 summarises the 24-hour LC_{50} data for unionised ammonia used to calculate the HC_5. Toxicity (24-hour LC_{50}) values were available for thirty different species, comprising eighteen invertebrate species (including crustaceans, insects, molluscs, rotifers, worms) and eleven fish (cyprinid and salmonid) species. Data for one algal species provided a 24-hour $LC_{50}$of greater than1.00 mg/l (which could not be used in the SSD modelling). The dataset included a number of studies since the derivation of the UPM2 FIS, including that for unionid molluscs (Mummert *et al.,* 2003; Wang *et al.,* 2007). To adopt a precautionary approach, the lowest toxicity value for each species was used to derive the HC_5 values for salmonid and cyprinid fisheries.

Table 2.7 Measured 24-hour $LC_{50}$for different algal, invertebrate and fish species ranked from most to least toxic

Taxa	Species	Type of organism	24-hour LC_{50} (mg NH_3-N/l)	Reference
Fish	*S.salar*	Atlantic salmon	0.12	Alabaster *et al* (1979)
Invertebrate	*V.iris*	Mussel	0.22	Mummert et *al* (2003)
Fish	*O.tshawtscha*	Chinook salmon	0.30	Harader and Allen (1983)
Invertebrate	*L.fascioloa*	Mussel	0.32	Mummert et *al* (2003)
Invertebrate	*A.ligamentina*	Mussel	0.47	Wang *et al* (2007)
Fish	*H.molitrix*	Silver carp	0.48	Xu et *al* (1994)
Fish	*O.mykiss*	Rainbow trout	0.53	Milne *et al* (1992)
Fish	*N.guentheri*	Killifish	0.57	Shedd et *al* (1999)
Invertebrate	*L.siliquoidon*	Mussel	0.60	Wang *et al* (2007)
Fish	*S.trutta*	Brown trout	0.60	Milne *et al* (1992)
Fish	*L.guntea*	Guntea loach	0.61	Sangii and Kanabur (2000)
Fish	*C.carpio*	Common carp	0.68	Xu et *al* (1994)
Invertebrate	*L.rafinesqueana*	Mussel	0.68	Wang *et al* (2007)
Fish	*L.cephalus*	Chub	0.76	Gomulka *et al* (2011)
Algae	*S.costatum*	Diatom	>1.00	Livingston *et al* (2001)
Fish	*P.promelas*	Fathead minnow	1.22	Markle et *al* (2000)
Fish	*P.reticulata*	Guppy	1.35	Rubin and Elmaraghy (1976)
Invertebrate	*M.rectirostris*	Cladoceran	1.61	Gyor and Olah (1980)
Invertebrate	*D.magna*	Cladoceran	1.74	Kaniewska-Prus (1982)
Invertebrate	*L.stagnalis*	Snail	1.92	Williams *et al* (1986)
Invertebrate	*P.tennuis*	Turbellarian	1.95	Williams *et al* (1986)
Invertebrate	*L.intermis*	Insect	2.18	Williams *et al* (1986)
Invertebrate	*P.fontinalis*	Bladder snail	2.20	Williams *et al* (1986)
Invertebrate	*B.rhodini*	Mayfly	2.30	Williams *et al* (1986)
Invertebrate	*L.hoffmeisteri*	Tubificid worm	2.80	Williams *et al* (1986)
Invertebrate	*C.riparius*	Midge larvae	3.00	Williams *et al* (1986)
Invertebrate	*B.rubens*	Rotifer	3.20	Snell and Persoone (1989)
Invertebrate	*G. pulex*	Amphipod	3.20	Williams *et al* (1986)
Invertebrate	*E.ignita*	Damselfly	3.29	Williams *et al* (1986)
Invertebrate	*A. aquaticus*	Isopod	4.04	Williams *et al* (1986)

Shaded values are from studies carried out after 1992 or data not given in the R&D Report 123

RIVM ETX software was used to calculate an $HC_5$from the complete 24-hour dataset with the resulting value being 0.221 mg/l. The LC_{50} values were normally distributed and the Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises tests for normality were all accepted at the 0.01 to 0.05 per cent levels of significance. Since the $HC_5$is derived from LC_{50} values, it is necessary to apply an assessment factor to convert the $LC_{50}$to NOEC for the mortality endpoint. Based on the comparative NOEC and $LC_{50}$for invertebrates given in Table B2 in Appendix B a factor of five is proposed which results in a precautionary 24-hour *salmonid threshold* for short-term mortality of 0.044 mg/l.

The 24-hour *salmonid threshold* of 0.044 mg/l unionised ammonia derived using the complete toxicity dataset in Table 2.7 would protect against short-term (24-hour) mortalities in receiving waters containing certain early life-stages of salmonids. It is slightly higher than the UPM2 24-hour standard for a one-year return period of 0.030 mg/l applied for an ecosystem suitable for a sustainable salmonid fishery. Therefore, the current UPM2 FIS is protective against both short-term (24-hour) mortality and certain long-term effects following acute exposure to unionised ammonia.

A corresponding $HC_5$of 0.314 mg/l for cyprinid fisheries was also derived using all the data in Table 2.7 except that for salmonid species (such as salmon and trout). The LC_{50}were normally distributed and the Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises tests for normality were all accepted at the 0.01 to 0.025 per cent levels of significance. Using an assessment factor of five to convert from LC_{50} to NOEC generated a 24-hour *cyprinid threshold* for short-term mortality of 0.0628 mg/l unionised ammonia. This value is similar to the UPM2 24-hour standard for a one-year return period of 0.065 mg/l for an ecosystem suitable for a sustainable cyprinid fishery. Therefore, the relevant current UPM2 FIS is protective against short-term (24-hour) mortality in cyprinid fisheries, including early life-stages.

Table 2.8 summarises the six-hour LC_{50} data for unionised ammonia used to calculate the HC_5 for sustainable salmonid and cyprinid fisheries. Toxicity (six-hour LC_{50}) values were available for fifteen different species, comprising twelve invertebrate species (including crustaceans, insects, molluscs, rotifers, worms) and three fish (cyprinid and salmonid) species. The dataset included a number of studies generated since the derivation of the UPM2 FIS, including that for unionid molluscs (Mummert et *al.,* 2003; Wang *et al.,* 2007).

Table 2.8 Measured six-hour $LC_{50}$for different algal, invertebrate and fish species ranked from most to least toxic

Taxa	Species	Type of organism	Six-hour LC_{50} (mg NH_3-N/l)	Reference
Fish	*O.mykiss*	Rainbow trout	0.57	Milne *et al* (1992)
Fish	*S.trutta*	Brown trout	0.83	Milne *et al* (1992)
Invertebrate	*L.rafinesqueana*	Mussel	0.68	Wang *et al* (2007)
Invertebrate	*A.ligamentina*	Mussel	0.98	Wang *et al* (2007)
Invertebrate	*L.siliquoidon*	Mussel	1.15	Wang *et al* (2007)
Fish	*C.carpio*	Common carp	2.39	Xu et *al* (1994)
Invertebrate	*B.rhodini*	Mayfly	2.81	Williams *et al* (1986)
Invertebrate	*L.intermis*	Insect	3.04	Williams *et al* (1986)
Invertebrate	*L.stagnalis*	Snail	3.17	Williams *et al* (1986)
Invertebrate	*P.fontinalis*	Bladder snail	3.49	Williams *et al* (1986)
Invertebrate	*L.hoffmeisteri*	Tubificid worm	3.83	Williams *et al* (1986)
Invertebrate	*P.tennuis*	Turbellarian	4.07	Williams *et al* (1986)
Invertebrate	*C.riparius*	Midge larvae	4.16	Williams *et al* (1986)
Invertebrate	*E.ignita*	Damselfly	4.20	Williams *et al* (1986)
Invertebrate	*G. pulex*	Amphipod	4.36	Williams *et al* (1986)

Shaded values are from studies carried out after 1992 or data not given in the R&D Report 123

The HC_5 calculated from the complete six-hour LC_{50} dataset was 0.671 mg/l. Only the Kolmogorov-Smirnov tests for normality were accepted at the 0.01 to 0.025 per cent levels of significance. Since the $HC_5$is derived from LC_{50} values, it is necessary to apply an assessment factor to convert the $LC_{50}$to NOEC. Based on the comparative 24-hour NOEC and LC_{50} for invertebrates given in Table B2 in Appendix B, a factor of five is proposed which results in a precautionary six-hour *salmonid threshold* for short-term mortality of 0.134 mg/l.

The six-hour *salmonid threshold* of 0.134 mg/l unionised ammonia derived using the complete toxicity dataset in Table 2.8 would protect against short-term (six-hour) mortalities in receiving waters containing early life-stages of salmonids. It is significantly higher than the UPM2 six-hour standard for a one-year return period of 0.040 mg/l for an ecosystem suitable for a sustainable salmonid fishery. Therefore, the current relevant UPM2 FIS is protective against both short-term (six-hour) mortality and certain long-term effects following acute exposure to unionised ammonia.

A corresponding $HC_5$of 1.020 mg/l was derived for cyprinid fisheries using all the data in Table 2.8 except that for salmonid species. Only the Kolmogorov-Smirnov tests for normality were accepted at the 0.01 to 0.025 per cent levels of significance. Using an assessment factor of five to convert from LC_{50} to NOEC generated a six-hour *cyprinid threshold* for short-term mortality of 0.204 mg/l unionised ammonia. This value is similar to the UPM2 six-hour standard for a one-year return period of 0.150 mg/l for an ecosystem suitable for a sustainable cyprinid fishery. Therefore, the relevant current UPM2 FIS is protective against short-term (six-hour) mortality in cyprinid fisheries, including early life-stages.

Table 2.9 summarises the results of the SSD modelling using the six-hour and 24-hour toxicity datasets.

Table 2.9 Results of the SSD modelling using the six-hour and 24-hour toxicity data

Parameters	**Salmonid fisheries**		**Cyprinid fisheries**	
	Six–hour exposure	**24-hour exposure**	**Six-hour exposure**	**24-hour exposure**
Hazardous concentration (HC_5)	0.671 mg/l	0.221 mg/l	1.020 mg/l	0.314 mg/l
Lower limit HC_5	0.359 mg/l	0.130 mg/l	0.587 mg/l	0.187 mg/l
Upper limit HC_5	1.002 mg/l	0,326 mg/l	1.434 mg/l	0.455 mg/l
Number of data points	15	29	13	25
Assessment factor	5	5	5	5
Threshold	***0.134 mg/l***	***0.044 mg/l***	***0.204 mg/l***	***0.063 mg/l***

Insufficient data on the toxicity of unionised ammonia to a range of aquatic organisms after one hour of exposure prevented the use of SSD modelling and calculation of HC_5. However, one-hour *threshold* values for salmonid and cyprinid fisheries were derived by extrapolation from the six-hour values using information on the ratio of one-hour to six-hour LC_{50} values for rainbow trout (Milne *et al.,* 1992) and minnows (Danecker, 1964).

For salmonids, the one-hour: six-hour LC_{50} ratio for rainbow trout (*O.mykiss)*was1.84 based on a one-hour $LC_{50}$of 1.05 mg/l (95% confidence intervals = 1.03-1.07 mg/l) and a six-hour $LC_{50}$of 0.57 mg/l (95% confidence intervals = 0.55-0.59 mg/l) (Milne *et al*., 1992). The resulting one-hour *salmonid threshold* derived from the six-hour threshold was 0.247 mg/l.

For cyprinids the one-hour: six-hour LC_{50} ratio for minnows was 2.65 based on a one-hour LC_{50} value of 8.2 mg/l total ammonia and a six-hour LC_{50} value of 3.1 mg/l total ammonia (Danecker,1964). The resulting one-hour *cyprinid threshold* derived from the six-hour value was 0.541 mg/l.

Table 2.10 summarises the ratios of the one-hour to six-hour and six-hour to 24-hour UPM2 FIS for different ecosystem types (see Table B4 in Appendix B for complete dataset). There are robust 24-hour LC_{50} datasets, and to a lesser extent six-hour LC_{50} datasets for freshwater organisms (see Table B2 in Appendix B). However, the one-hour LC_{50} dataset is extremely limited, which increases the uncertainty of the one-hour: six-hour LC_{50} ratio data.

The data shown in Table B2 indicates that the average ratio of the six-hour to 24-hour $LC_{50}$is 1.52 (range of values = 1.08-2.09) for invertebrates and salmonids which is consistent (within 10 per cent) of the ratios in Table 2.8 of the standards for the sustainable salmonid fishery. The comparative data for rainbow trout (*Oncoryhnchus mykiss*) indicate a one-hour: six-hour LC_{50} ratio of 1.84 (see Table 2.2) which is slightly lower than the ratio of the standards for the ecosystem type (see Table 2.10). For invertebrates and cyprinids the average ratio of the six-hour to 24-hour LC_{50} is 1.55 (range of values = 1.22-2.09) which is lower than the ratio for the sustainable cyprinid fishery (see Table 2.8).

Table 2.10 Summary of ratios of different time-based standards (data from Table 2.5 and Table B4)

Ecosystem type	Ratio of one-hour: six-hour standards[a]	Ratio of six-hour:24-hour standards[a]
Sustainable salmonid fishery	2.60-2.71	1.33-1.40
Sustainable cyprinid fishery	1.67-2.00	2.31-2.50
Marginal cyprinid fishery	1.50-1.75	1.43-2.00

[a] Range of values for different return periods

Comparison with other relevant standards

Table 2.11 summarises the current national short-term standards in North America (Canada and the United States) for the protection of freshwater aquatic life from intermittent exposure to dissolved oxygen and unionised ammonia.

For dissolved oxygen, the current 24-hour threshold with a one-year return period for an ecosystem suitable for a sustainable salmonid fishery (5.0 mg/l, see Table 2.4) is the same as the corresponding warm water one-day value of 5.0 mg/l derived by the United States Environmental Protection Agency (USEPA(see Table 2.11). USEPA criteria are designed to protect the more sensitive populations of organisms against potentially damaging production impairment. The one-hour threshold with a one-year return period for an ecosystem suitable for a sustainable salmonid fishery (4.0 mg/l, see Table 2.4) is consistent with the British Columbian Ministry of the Environment instantaneous minimum level of 5.0 mg/l for all life-stages other than buried embryos and alevins (see Table 2.11).

For unionised ammonia, the current one-hour threshold with a one-year return period for an ecosystem suitable for a sustainable salmonid fishery (0.105 mg NH_3-N/l, see Table 2.5) is higher than the corresponding British Columbian Ministry of the Environment instantaneous minimum level (0.054 mg NH_3-N/l, see Table 2.11 which is estimated from total ammonia values). However, it is consistent with the USEPA acute criteria at pH 7.0 and 15-25°C (0.070-0.078 mg NH_3-N/l where unionid mussels are present and 0.117-0.122 mg NH_3-N/l where unionid mussels are absent, see Table 2.11). The USEPA acute criteria describe the one-hour average concentration of total ammonia nitrogen (in mg N/l) that should not be exceeded more than once every three years.

Table 2.11Current national short-term standards for dissolved oxygen and ammonia in North America (Canada and US)

Organisation and country	**Summary of current short-term standards**	
	Dissolved oxygen	**Ammonia**
Environment Canada	In the British Columbian water quality guidelines for dissolved oxygen, the instantaneous minimum level is 5 mg/l for all life-stages other than buried embryos and alevins (BC MoE 1997). The instantaneous minimum should be maintained at all times to protect aquatic life.	In the British Columbian water quality guidelines for ammonia the maximum (acute) concentration of total ammonia at pH 7.0 and 15°C is 19.7 mg N/l (which corresponds to 0.054 mg/l unionised ammonia)[a] (BC MoE 2009). The maximum concentration should not be exceeded to prevent detrimental effects from occurring to a water use, including aquatic life, under specified environmental conditions.
Environmental Protection Agency, United States	In the document *Ambient aquatic life water quality for dissolved oxygen* (US EPA 1986) the following criteria are given. Coldwater criteria: One-day minimum for early life-stages = 8.0 mg/l;one-day minimum for other life-stages = 4.0 mg/l. Warmwater criteria: One-day minimum for early life-stages = 5.0 mg/l;one-day minimum for other life-stages = 3.0 mg/l. The one-day minima are instantaneous concentrations that should be achieved at all times. The criteria are designed to protect the more sensitive populations of organisms against potentially damaging production impairment.	In 2009 the Environmental Protection Agency published a *Draft 2009 update of aquatic life ambient water quality criteria for ammonia – freshwater* (USEPA 2009) which proposed the following acute criteria: • At pH 7.0 and 15°C – 28.4 mg N/l (which corresponds to 0.078 mg/l unionised ammonia)[a] where freshwater unionid mussels are present and 42.9 mg N/l (which corresponds to 0.117 mg/l unionised ammonia)[a] where freshwater unionid mussels are absent. • At pH 7.0 and 25°C – 12.4 mg N/l (which corresponds to 0.070 mg/l unionised ammonia)[a] where freshwater unionid mussels are present and 21.5 mg N/l (which corresponds to 0.122 mg/l unionised ammonia)[a] where freshwater unionid mussels are absent. • At pH 8.0 and 15°C – 6.64 mg N/l (which corresponds to 0.175mg/l unionised ammonia) where freshwater unionid mussels are present and 9.99 mg N/l (which corresponds to 0.264 mg/l unionised ammonia)[b] where freshwater unionid mussels are absent. • At pH 8.0 and 25°C – 2.90 mg N/l (which corresponds to 0.155 mg/l unionised ammonia) where freshwater unionid mussels are present and 5.00 mg N/l (which corresponds to 0.267 mg/l unionised ammonia)[b] where freshwater unionid mussels are absent. These values are proposed to replace the 1999 acute criteria where salmon

Organisation and country	Summary of current short-term standards	
	Dissolved oxygen	Ammonia
		are present. A commenting period ended in April 2010 but no further action has since been taken by the USEPA to adopt the draft ammonia criteria as approved national criteria. The acute criteria are the one-hour average concentrations of total ammonia nitrogen (in mg N/l) that should not be exceeded more than once every three years. If achieved, freshwater aquatic organisms should not be unacceptably affected.

[a] - Based on the unionised concentration being 0.273 per cent of the total ammonia concentration at pH 7.0 and 15°C and 0.566 per cent at pH 7.0 and 25°C

[b] - Based on the unionised concentration being 2.64 per cent of the total ammonia concentration at pH 8.0 and 15°C and 5.34 per cent at pH 8.0 and 25°C

Summary

Based on an evaluation of the available toxicity data including post-1992 information and comparison with other relevant standards for short-term exposure, the UPM2 FIS are generally "fit for purpose" (see Table 2.12).

The available toxicity data indicates that the magnitude of the standards for different exposure periods (one, six and 24 hours) are generally appropriate. For dissolved oxygen, this finding is largely based on a direct comparison of the 24-hour toxicity data (and to a lesser extent, the six-hour data) with the corresponding UPM2 FIS for a one-year return period. For unionised ammonia, a probabilistic approach using SSD modelling was used to derive six- and 24-hour *salmonid* and *cyprinid thresholds* for short-term mortality which were compared with six- and 24-hour UPM2 FIS for a one-year return period.

The available toxicity data (in terms of the ratios of one-hour to six-hour LC_{50} and six-hour to 24-hour LC_{50}) are, in some instances, consistent with the ratios of standards of different time durations. However, in other cases the ratios for the toxicity data are slightly smaller than those for the UPM2 FIS. There is greater certainty for the unionised ammonia standards due to the larger dataset. Smaller ratios of toxicity values mean that the shorter term (one- and six-hour) standards should account for long-term sub-lethal effects as well as short-term mortality. In contrast, higher ratios mean that the shorter term standards may not completely account for short-term mortality and for any long-term sub-lethal effects.

In summary, for the sustainable salmonid fishery and sustainable cyprinid fishery ecosystem types, UPM2 FIS provide protection against short-term mortality and longer term effects on the physiology, growth and reproduction of resident organisms. For dissolved oxygen, they are also generally protective against macroinvertebrate drift.

As indicated in the UPM2 Manual, the standards are not designed to specifically protect salmonid spawning grounds and areas where sensitive macroinvertebrate species (such as pearl mussels) may spawn.

Experience with the application of UPM2 FIS indicates that the six-hour *thresholds* are likely to be more critical than longer durations, which reflects the typical duration of spill events. Crabtree *et al*.(1998) conducted detailed modelling which showed that the one-year return period thresholds were most likely to be critical for dissolved oxygen. If these are achieved, the one-month and three-month return period thresholds should also normally be satisfied. In some studies, the one-month return period has been shown to be the critical threshold for unionised ammonia. The UPM2 FIS should only be used for design purposes and not for compliance assessment.

Table 2.12 Comparison of FIS with toxicity data and other standards for short-term exposure

Substance	Ecosystem type	Are UPM2 FIS consistent with the available toxicity data?	Are standards consistent with short-term standards from other countries?
Dissolved oxygen	Sustainable salmonid fishery	The available toxicity data show that sensitive invertebrate and salmonid species can survive short-term exposure to the concentrations expressed in the standards. The six-hour:24-hour ratios of invertebrate and salmonid LC_{50} toxicity data are consistent with the ratios of six-hour to 24-hour UPM2 FIS. No toxicity data are available to compare with the ratios of one-hour to six-hour UPM2 FIS.	The current 24-hour threshold with a one-year return period for an ecosystem suitable for a sustainable salmonid fishery (5.0 mg/l) is the same as the corresponding warmwater one-day value derived by the USEPA(5.0 mg/l). The one-hour threshold with a one-year return period for an ecosystem suitable for a sustainable salmonid fishery (4.0 mg/l) is consistent with the British Columbian Ministry of the Environment instantaneous minimum of 5.0 mg/l for all life-stages other than buried embryos and alevins.
	Sustainable cyprinid fishery	The available toxicity data show that sensitive invertebrate and cyprinid species can survive short-term exposure to the concentrations expressed in the standards. The six-hour:24-hour ratios of invertebrate and cyprinid LC_{50} toxicity data are consistent with the ratios of six-hour to 24-hour UPM2 FIS. No toxicity data are available to compare with the ratios of one-hour to six-hour UPM2 FIS.	No corresponding standards have been developed by USEPA and British Columbian Ministry of the Environment for cyprinid fisheries.

Substance	**Ecosystem type**	**Are UPM2 FIS consistent with the available toxicity data?**	**Are standards consistent with short-term standards from other countries?**
Unionised ammonia	Sustainable salmonid fishery	The 24-hour *salmonid threshold* of 0.044 mg/l unionised ammonia derived using the complete toxicity dataset is slightly higher than the UPM2 24-hour standard for a one-year return period of 0.030 mg/l applied for the ecosystem. This indicates protection against long-term effects as well as short-term (24-hour) mortality. The six-hour *salmonid threshold* of 0.134 mg/l unionised ammonia is significantly higher than the UPM2 six-hour standard for a one-year return period of 0.040 mg/l for an ecosystem suitable for a sustainable salmonid fishery. This indicates protection against short-term (six-hour) mortality and certain long-term effects following acute exposure to unionised ammonia. The six-hour:24-hour ratios of invertebrate and salmonid $LC_{50}$are consistent with the ratios of six-hour to 24-hour UPM2 FIS. The corresponding one-hour: six-hour ratios are slightly lower than the ratios of one-hour to six-hour UPM2 FIS.	The current one-hour threshold with a one-year return period for an ecosystem suitable for a sustainable salmonid fishery (0.105 mg NH_3-N/l) is higher than the corresponding British Columbian Ministry of the Environment instantaneous minimum level (0.054 mg NH_3-N/l) but consistent with the USEPA acute criteria at pH 7.0 and 15-25°C (0.070-0.078 mg NH_3-N/l where unionid mussels are present and 0.117-0.122 mg NH_3-N/l where unionid mussels are absent).
	Sustainable cyprinid fishery	The 24-hour *cyprinid threshold* of 0.063 mg/l unionised ammonia derived using the complete toxicity dataset is consistent with the UPM224-hour standard for a one-year return period of 0.065 mg/l applied for the ecosystem. The six-hour *cyprinid threshold* of 0.204 mg/l unionised ammonia is similar to the UPM2 six-hour standard for a one-year return period of 0.150 mg/l for an ecosystem suitable for a sustainable cyprinid fishery. This indicates protection against short-term (six-hour) mortality in cyprinid fisheries, including early life-stages. The six-hour:24-hour ratios of invertebrate and salmonid LC_{50} are slightly lower than the ratios of six-hour to 24-hour UPM2 FIS. No toxicity data are available to compare with the ratios of one-hour to six-hour UPM2 FIS.	No corresponding standards have been developed by USEPA and British Columbian Ministry of the Environment for cyprinid fisheries.

2.3.2 Comparison of FIS and 99 percentile standards

WFD 99 percentile standards for biochemical oxygen demand (BOD) and ammonia obtained by interpolation

Wet weather events may affect river water quality for relatively short time periods, but these events can have a disproportionate impact on the ecosystem. Furthermore, the quality of a water body during these events may not be related in a simple way to the more general quality based on continuous discharges. With wet weather intermittent discharges, short duration, high concentration events extend the tail of the pollutant frequency distribution. The shape of this extended tail reflects the size and frequency of the intermittent discharges and the dilution capacity of the river at the time of the discharge and will be unique to a particular river system (UPM2, 1998). Evidence indicates that intermittent discharges will significantly extend the tail of the distribution beyond the 90 percentile or 95 percentile. Hence, there may be a need for wet weather receiving water standards to define an acceptable tail to the frequency distribution. Therefore, an alternative approach was developed for the second edition of the UPM Manual – UPM2, in 1998, using 99 percentiles based on an extrapolation of the Environment Agency River Ecosystem (RE) classes for 90/95 percentile thresholds for BOD, total ammonia and unionised ammonia respectively (DoE, 1994; DETR, 1997). As described in UPM2, the 99 percentiles offer an alternative description of the acceptability of extreme events.

The FIS and the 99 percentile criteria differ in three main aspects:

1. They use different statistics to describe concentration exceedances in the extended tail of the frequency distribution caused by wet weather events.
2. The FIS (which are directly related to the characteristics of events which cause stress in river ecosystems) consider three elements of an exposure scenario (namely concentration, duration and frequency of exposure), whereas the 99 percentile standards (which are used to assess achievement of RE and WFD classes) only consider concentration and duration of exposure implicitly.
3. The FIS allow for correction factors to account for synergistic effects and site-specific environmental conditions that can influence toxicity.

The 99 percentile criteria use BOD and total ammonia to limit organic loading in receiving waters whilst the FIS apply dissolved oxygen (DO) and unionised ammonia, which are considered to be more directly relevant to ecosystem impact. With regard to the relationship between BOD and DO, high BOD levels can be sustained in well-oxygenated rivers with little impact on DO, while in sluggish rivers relatively low BOD levels can cause DO problems.

The relationship between the high percentile and return period statistics was statistically investigated by Gunby *et al.* (1998). The report concluded that:

- A clear and consistent relationship exists between the UPM-type measurements (in the form of the threshold concentrations for fixed return periods and durations) and the upper annual percentiles.
- The relationship is a simple linear one where UPM-type measurements are proportional to annual percentiles.

- For total ammonia, the ratio of UPM thresholds for 12-month return periods and six-hour durations to 99 percentiles is 1.26 under typical conditions and good effluent quality. For BOD, the ratio is 1.16.
- The consistency of the relationship extends across the range of return periods and durations, and across the upper percentiles (although with varying amounts of noise).
- For both ammonia and BOD, the significant factors affecting the relationship between the 12-month return period, six-hour duration thresholds and 99 percentiles are upstream river flow, effluent quality, and the impermeable area of the catchment. The impermeable area has the smallest effect.

The review found “a slight discrepancy” between the two methods of protecting rivers; the river ecosystems classification seems to demand a more stringent river quality than the derived UPM standards (see Tables 2.4 and 2.5). While having a 99th annual percentile which is better than the RE class 2/3 boundary should give a UPM-type measurement within the derived standards (for any river type), having a river of a quality which complies with UPM standards will not ensure that the river is in RE class 2 or above. This is the case even when there is no effluent discharge and only intermittent discharges.

A possible explanation for this is that the RE classes are based on the long-term requirements for maintaining river aquatic life, whilst the derived UPM FIS offer protection from short-term exposure. The river ecosystem classification is tighter because it has to encompass a greater range of considerations (long-term needs) and cover all river types. The UPM standards, on the other hand, are tailored according to the physical nature of the receiving water, and so are more lenient because they allow for possible improvements in quality due to the river 'self-cleaning' further downstream. UPM2 FIS are based on a matrix of thresholds for different return periods and durations of episodes; all of these standards must be met in order to meet the overall standard. It may be that the derived standards by themselves do not offer sufficient protection, but that the fundamental standards do.

The findings for the RE classes clearly have implications for the potential achievement of 99 percentiles and water quality objectives for water bodies of good and moderate status under the WFD.

Morris and Crabtree (2000) suggested that the 99 percentile approach could be used to define the standards required for the majority of simple intermittent discharges. The approach is simpler in terms of compliance testing but there are situations where more cost-effective solutions could be developed using FIS. The choice of method will depend on the characteristics of the receiving water and the environmental problems being addressed by a particular scheme.

Further assessment of the relative effectiveness of the wet weather standards is complicated. Whilst certain modelling/monitoring data associated with CSO improvements are available, no subsequent, systematic review of the available chemical and/or biological monitoring data from water bodies before and after the installation of a UPM solution has been conducted.

Tables 2.11 and 2.12 summarise the proposed WFD 99 percentile standards for BOD and total ammonia for inland rivers proposed by the Environment Agency. These 99 percentile standards were established to protect freshwater aquatic life from short-duration intermittent urban wet weather events. The WFD 99 percentiles for BOD and total ammonia for differing WFD ecological status were established by interpolation of the corresponding 90 percentile values of each River Ecosystem Class. The tables include the original RE class 90 percentiles and UPM 99 percentiles for comparison.

Table 2.13 RE class and WFD ecological status 90 percentile standards and proposed 99 percentile standards for biochemical oxygen demand as provided by the Environment Agency

Type of river (see notes below)	Biochemical oxygen demand (mg/l)	
	90 percentile	99 percentile
RE1 Class	**2.5**	**5.0**
WFD High Status for Types 1,2,4,6 and Salmonid	3.0	7.0
RE2 Class and WFD Good Status for Types 1,2,4 and 6 and Salmonid and High Status for Types 3,5 and 7	4.0	9.0
WFD Good Status for Types 3,5 and 7	5.0	11.0
RE3 Class and WFD Moderate Status for Types 1,2,4 and 6 and Salmonid	**6.0**	**14.0**
WFD Moderate Status for Types 1,3,5 and 7	6.5	14.0
WFD Poor status for Types 1,2,4 and 6 and Salmonid	7.5	16.0
RE4 Class	**8.0**	**19.0**
WFD Poor Status for Types 1,3,5 and 7	9.0	19.0
RE5 Class	**15.0**	**30.0**

Table 2.14 WFD 90 percentile standards and proposed 99 percentile standards for total ammonia (by interpolation) along with corresponding unionised ammonia standards as provided by the Environment Agency

Type of river (see notes below)	Total ammonia (mg/l)		Unionised ammonia (mg/l)
	90 percentile	99 percentile	99 percentile
WFD High Status for Types 1,2,4,6	0.2	0.5	0.04
RE1 Class	**0.25**	**0.6**	**0.04**
WFD Good Status for Types 1,2,4 and 6 and High Status for Types 3,5 and 7	0.3	0.7	0.04
RE2 Class and WFD Good Status for Types 3,5 and 7	**0.6**	**1.5**	**0.04**
WFD Moderate Status for Types 1,2,4 and 6	0.75	1.8	0.04
WFD Moderate Status for Types 1,3,5 and 7 and Poor status for Types 1,2,4 and 6	1.1	2.6	0.04
RE3 Class	**1.3**	**3.0**	**0.04**
RE 4 Class and Poor Status for Types 1,3,5 and 7	2.5	6.0	No value
RE5 Class	**9.0**	**25.0**	No value

Notes on River Typology for Tables 2.11 and 2.12

Criteria for identifying the types of river to which the DO, BOD and ammonia standards apply					
Site altitude	Alkalinity (as mg $CaCO_3$/l)				
	Less than 10	10-50	50-100	100-200	Over 200
Under 80 metres	Type 1	Type 2	Type 3	Type 5	Type 7
Over 80 metres			Type 4	Type 6	

3. Task B - Establish effects matrix

3.1 Introduction

For this task, an "effects profile" for ammonia (total and/or unionised) and dissolved oxygen was developed based on the previous research and any relevant more recent work. This was displayed as a matrix showing the likely ecological effects for a range of concentrations at different return periods, extending beyond the one-year return period if possible. Ecological effects need to consider all the relevant elements of Water Framework Directive classification. The ecological basis for ammonia and dissolved oxygen standards for intermittent pollution and any shortfalls were discussed.

3.2 Background to development of effects matrix

3.2.1 What are the requirements of the effects matrix in relation to the Water Framework Directive (WFD)?

The overall objective of the Water Framework Directive is to achieve "good" status for all waters by December 2015. Annex V of the WFD gives standard definitions for the classification of water bodies into five ecological quality classes: high, good, moderate, poor and bad. The following requirements are defined for high, good and moderate status:

High status – *No or very minor* deviation from an undisturbed (reference) condition.

Good status – *Slight* deviation from the reference condition.

Moderate status – *Moderate* deviation from the reference condition.

A key element of the requirement is that the ecological effects need to consider all the relevant biological elements of the Water Framework Directive classification scheme. This means that the effects matrix needs to address the following biological elements (if relevant): phytoplankton, macrophytes, phytobenthos, benthic invertebrates and fish communities.

In the assessment process, monitoring data for biological parameters for a given surface water body is expressed as ecological quality ratios (EQR) which consist of the observed biological parameter in the water body divided by the same parameter in the reference condition.

3.2.2 What constitutes a slight deviation in terms of wet weather events?

To ensure that good quality status is maintained in receiving waters subject to wet weather events from urban wastewater systems, the quality standards for dissolved oxygen and unionised ammonia (whether as UPM2 FIS or 99 percentiles) need to ensure that the releases do not cause more than "*a slight deterioration from the reference condition*". This requirement means that the structure and function of all biological elements are largely maintained following wet weather events, irrespective of the concentration, duration and frequency of exposure to decreased dissolved oxygen and/or elevated unionised ammonia levels.

For wet weather events, a slight deterioration could be considered to represent a small change in the structure of the aquatic community, through the loss of sensitive algal, macrophyte, invertebrate and/or fish species or a reduction in productivity. The available data indicates that for both dissolved oxygen and unionised ammonia, macroinvertebrates and fish are the most sensitive taxa and protection of these groups will prevent any adverse effects on algae and macrophytes.

For the benthic invertebrates, it is particularly important that the higher scoring taxa in salmonid or cyprinid fisheries which contribute to the River Invertebrate Classification Tool (RICT) are not adversely affected (through direct toxic effects and/or indirect behavioural effects) by exposure to less dissolved oxygen or more unionised ammonia. In the tool, classification is currently based on the number of scoring taxa (NTAXA) and the average score per taxon (ASPT), which is derived from the total score for the resident taxa and the NTAXA value.

The key taxonomic groups contributing to RICT are mayflies[2], stoneflies[3], dragonflies[4], caddis flies[5] and certain crustaceans such as crayfish. If these taxonomic groups are protected, the lower scoring taxa will also be protected. For fish, it is important that the sensitive life-stages of the ecosystem type are protected to ensure the sustainability of the resident community.

3.2.3 What are the possible ecological consequences of short-term wet weather events?

In response to short-term wet weather events, the most obvious and immediate potential effect on community structure would be the mortality of sensitive species in response to reduced dissolved oxygen and/or elevated unionised ammonia levels. However, behavioural effects could also occur such as macroinvertebrate drift or avoidance of the water body by fish species, although these could be transitory in nature and pre-exposure conditions could be restored rapidly after the wet weather events. Repeated short-term exposure can also result in longer-term effects on the development, growth and reproduction of sensitive species that can significantly affect community structure and function.

For *dissolved oxygen* the following points are evident from the data:

- Fish recover rapidly following sub-lethal exposure to low dissolved oxygen levels, with little evidence of long-term effects, even when the concentration is close to the lethal concentration (Seager *et al.,* 2000).
- Invertebrates can show a large-scale drifting response to mild oxygen depletion (below 4 mg/l) but populations can recover within several weeks (if a colonising source is available) (Milne *et al*., 1992).

For *unionised ammonia* the following points are evident from the data.

- Fish are more sensitive to unionised ammonia than plants or invertebrates, in terms of both lethal and sub-lethal responses. Sub-lethal exposure to ammonia

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2 Of families such as Ephemeridae, Heptageniidae, Leptophlebiidae and Siphionuridae
3 Of families such as Capniidae, Chloroperlidae, Leuctridae, Nemouridae, Perlidae, Perlodidae and Taeniopterygidae
4 Of families such as Cordulegasteridae
5 Of families such as Brachycentridae, Lepidostomatidae, Molannidae, Odontoceridae, Philopotamidae, Polycentropidae and Sericostomatidae

can cause permanent damage to fish, particularly gills, and can reduce growth rates, particularly if the exposure concentrations approach those causing mortality (Milne *et al.,* 2000, Diamond *et al.,* 2006).

- Invertebrate drift is not markedly affected by increasing unionised ammonia concentrations in sensitive taxa such as mayflies and stoneflies (Gammeter and Frutiger, 1990; Milne *et al*., 1992).

3.2.4 How should short-term standards be incorporated into the Water Framework Directive (WFD)?

Any short-term standards used as part of the WFD classification scheme should ensure the healthy functioning of the ecosystem when they are targeted towards the most sensitive taxa. However, it is important to understand that both the length and frequency of the impact and the geographical extent are important in interpreting potential adverse effects.

UPM2 FIS are based on the objective of no long-term effects and no fish mortality for irregular wet weather pollution events with at least an annual return period. The standards apply to those areas where there are few refuges for fish to avoid low dissolved oxygen conditions.

3.3 Development of the effects matrix

A number of assumptions/decisions were made in developing the effects matrices for dissolved oxygen and unionised ammonia and these are summarised in Table 3.1.

Table 3.1 Assumptions/decisions underpinning the effects matrices for dissolved oxygen and unionised ammonia

Assumption/decision	Dissolved oxygen	Unionised ammonia
Which taxa are most sensitive to the substance following short-term repeat exposure?	Fish and macroinvertebrates (through drift rather than short-term mortality)	Fish and macroinvertebrates (through the mortality of unionid mussels)
Which life-stages are most sensitive to the substance following short-term repeat exposure?	Early life-stages	Early life-stages
What factor(s) are most important in causing adverse effects following short-term repeat exposure?	Concentration and duration of exposure are more important factors than the frequency of exposure	Event concentration, duration and frequency are all important factors in determining toxic effects Frequency appears to become a dominant factor when it is high (greater than once a week)

For a given ecosystem type, the slight deviation requirement (see Section 3.2.1) would be satisfied where a change in a given parameter (such as short-term lethal or sub-lethal effects or behavioural effects such as macroinvertebrate drift) of the most sensitive taxa (and life-stages) was less than or equal to a small defined change (for example 10 per cent). Increases in mortality, decreases in growth or increased macroinvertebrate drift above this level will reduce the size of the population in the water body and potentially affect long-term sustainability.

The effects matrices are based on short-term exposure data (under 24 hours) and consider the effects of intermittent pulses of varying frequency.

Experience with the application of the UPM2 FIS indicates that the six-hour duration thresholds are likely to be more critical than longer durations, which reflects the typical duration of spill events. Crabtree *et al.* (1998) conducted detailed modelling which showed that the one-year return period thresholds were most likely to be critical for dissolved oxygen. If these are achieved, the one-month and three-month return period thresholds should also normally be satisfied. In some studies, the one-month return period has been shown to be the critical threshold for unionised ammonia.

3.3.1 Effects matrix for dissolved oxygen

The effects matrix for dissolved oxygen (Figure 3.1) is based on data for:

- the effects of differing oxygen concentrations on the lethality of salmonids (based on the available data for rainbow trout), cyprinids (based on the data for roach) and macroinvertebrates;
- the effects of differing oxygen concentrations on the growth of salmonids (based on the available data for brown trout);
- the effects of differing oxygen concentrations on the drift of macroinvertebrates.

3.3.2 Effects matrix for unionised ammonia

The effects matrix for unionised ammonia (Figure 3.2) is based on data for:

- the effects of differing unionised ammonia concentrations on the lethality of salmonids (based on the available data for brown trout), cyprinids and macroinvertebrates (particularly unionid mussels);
- the effects of differing unionised ammonia concentrations on the growth of salmonids (based on the data for brown trout).

Data on macroinvertebrate drift was not included as this parameter is significantly affected by unionised ammonia concentrations considerably higher than those causing other effects.

Figure 3.1 Effects matrix for dissolved oxygen

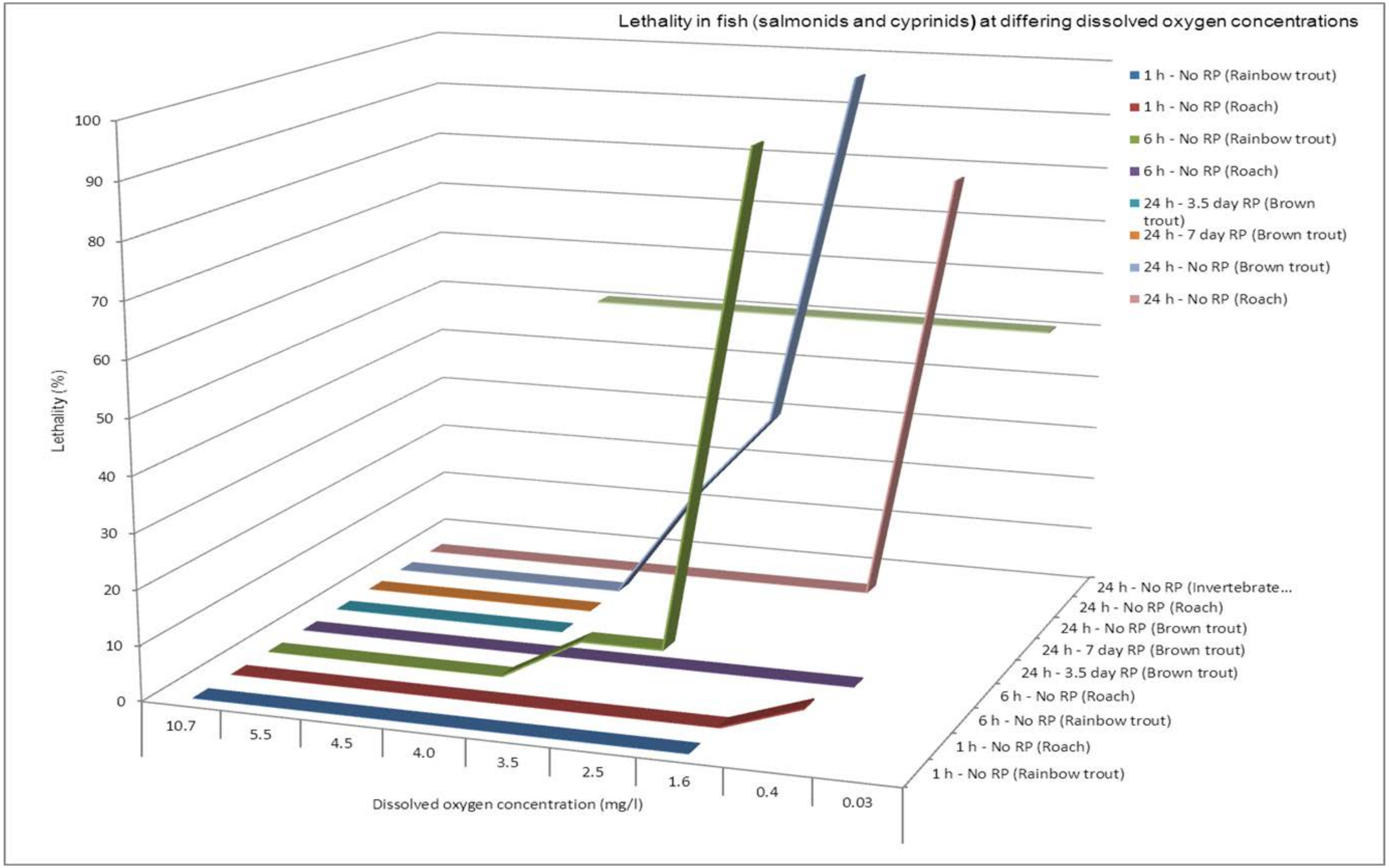
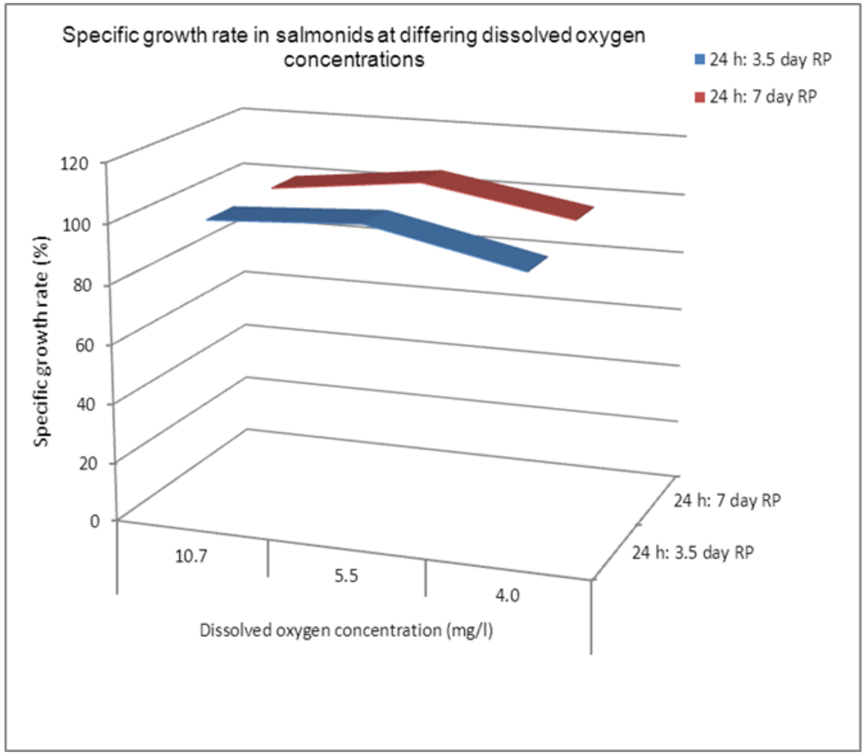


Figure 3.1 Continued



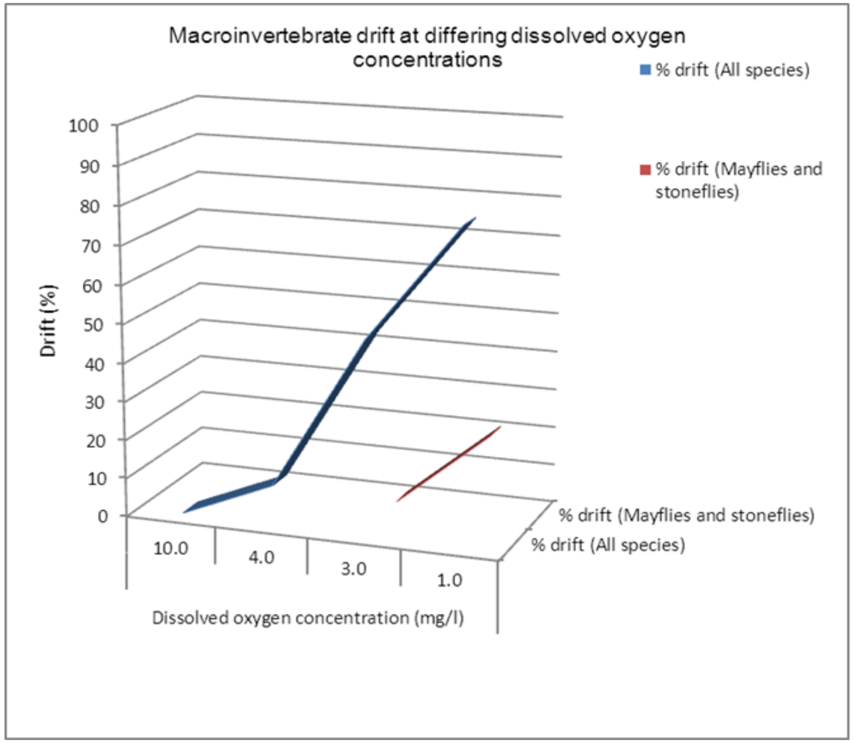


Figure 3.2 Effects matrix for unionised ammonia (as NH_3-N)

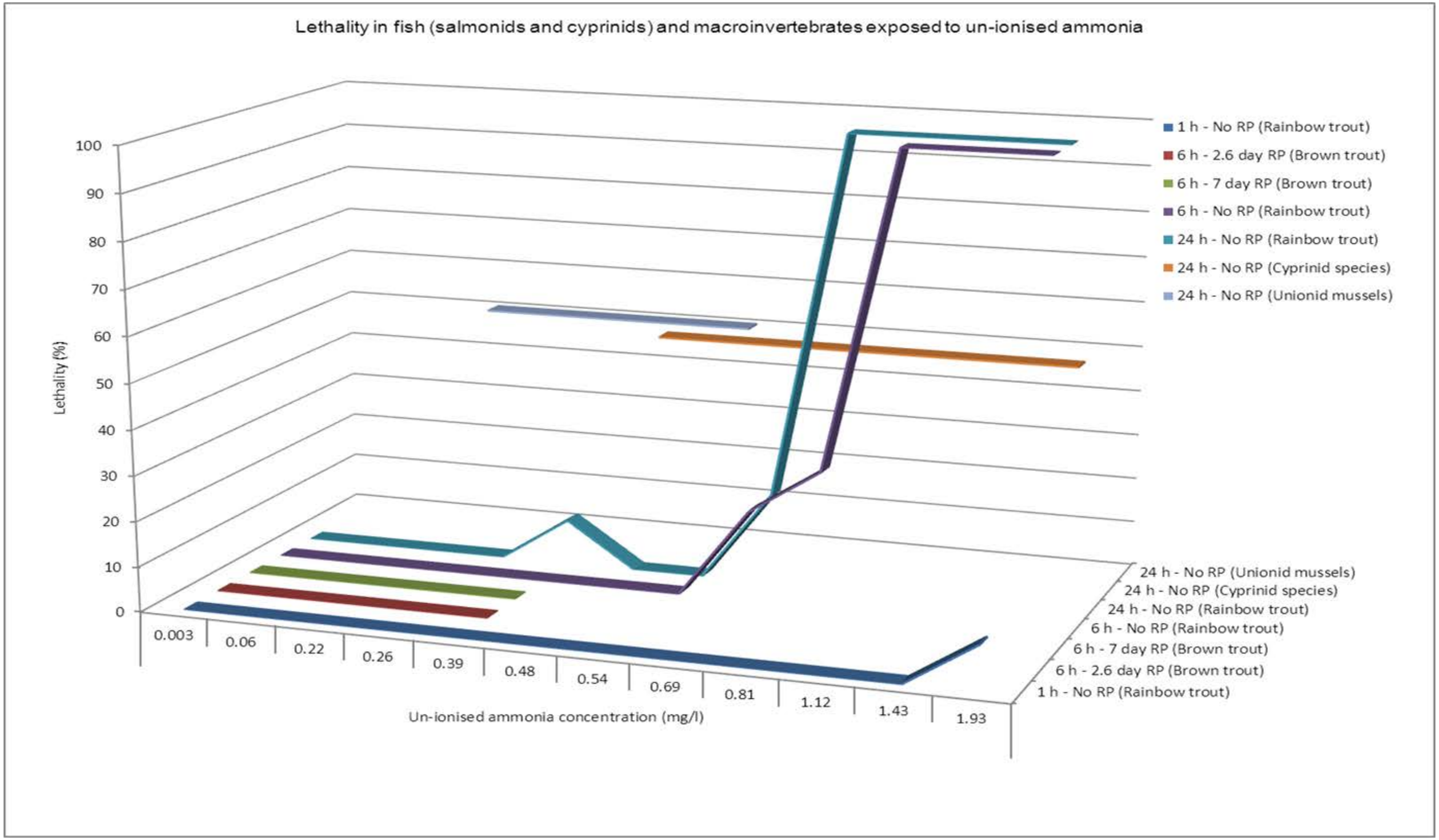
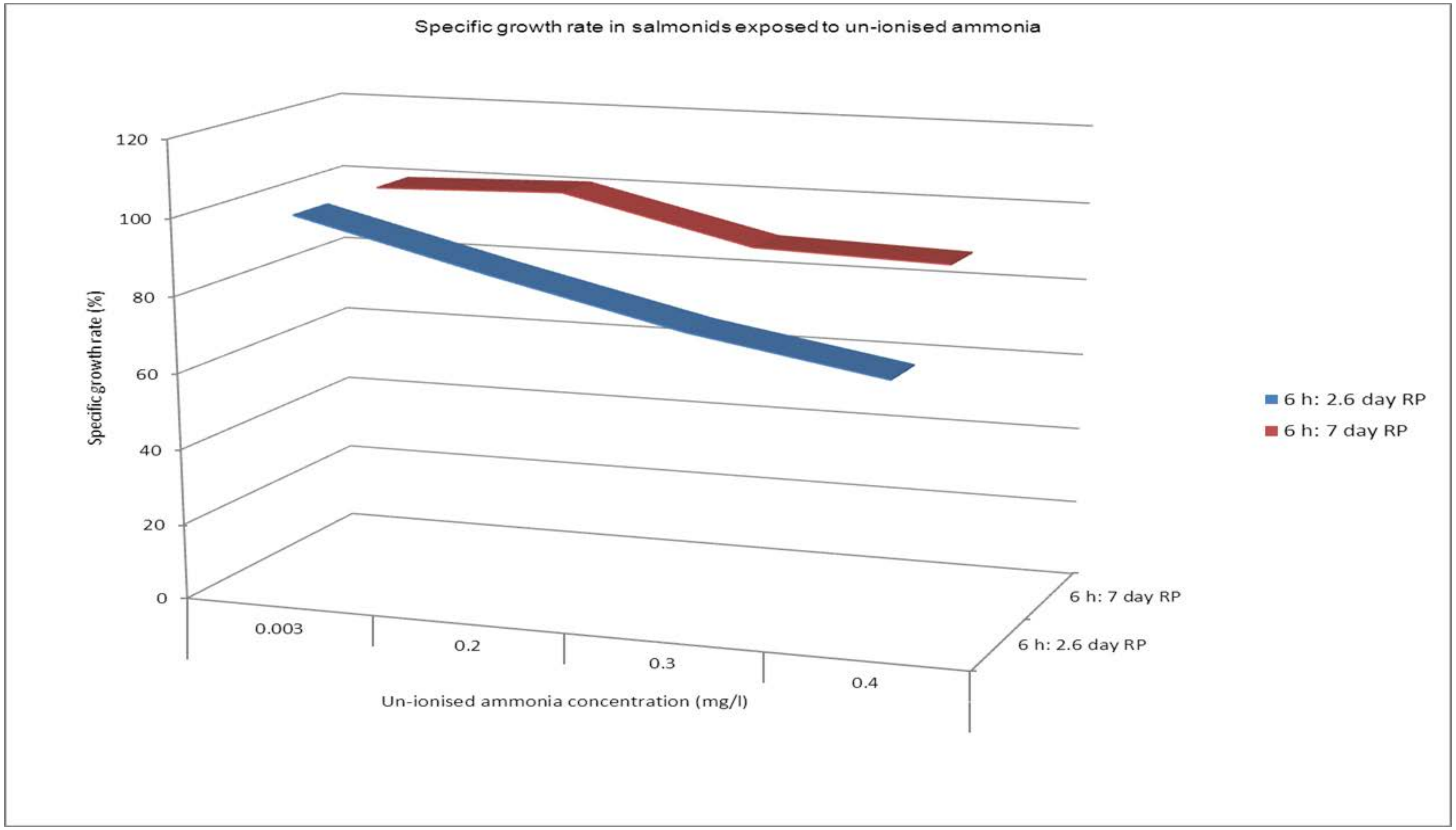


Figure 3.2 Continued



3.4 *Development of estimated threshold limits*

Tables 3.2 and 3.3 summarize the effects matrices for dissolved oxygen and unionised ammonia based on the complete dataset given in Section 2 on the short- and long-term effects of these substances on freshwater organisms present in sustainable salmonid and cyprinid fisheries.

After considering the information given in Figures 3.1 and 3.2 and Tables 3.2 and 3.3, *estimated threshold limits* for salmonid and cyprinid fisheries exposed to lower dissolved oxygen and elevated unionised ammonia levels were derived for the different exposure durations. The *estimated threshold limits* are designed to protect against short-term mortality in macroinvertebrates and fish, drift in macroinvertebrates and sub-lethal effects in fish (such as gill damage and reduced growth).

There was insufficient data to develop *estimated threshold limits* for marginal cyprinid fisheries.

3.4.1 Dissolved oxygen

For dissolved oxygen, as the duration of exposure decreases the dissolved oxygen level required to cause effects also decreases, so lower *estimated threshold limits* are expected for shorter durations of exposure due to the greater tolerance of organisms to reduced oxygen conditions. Furthermore, it needs to be recognised that in some instances, aquatic organisms could be exposed to fluctuating diurnal oxygen conditions in freshwater bodies with high dissolved oxygen levels in the day and reduced levels at night.

Sustainable salmonid fisheries

The effects matrix (see Figure 3.1) shows that dissolved oxygen needs to drop below 4.0 mg/l for a 24-hour exposure period to cause demonstrable short-term mortality in salmonid fish, drift in macroinvertebrates and longer-term growth effects in salmonids. The 4.0 mg/l threshold for effects on salmonids applies when the exposure conditions comprise return periods of 3.5 or seven days (a frequency of exposure of one or two events per week) or occur following a single event. For macroinvertebrates, the available data indicates that the dissolved oxygen threshold below which drift increases rapidly is around 4 mg/l for most species, where about half of all drifting has normally occurred when the dissolved oxygen concentration has reached 3 mg/l.

On the basis of the available data, maintenance of dissolved oxygen concentrations above an *estimated threshold limit* of 4.0 mg/l during wet weather events with a long return period should ensure protection of salmonid fisheries from short-term mortality and macroinvertebrate drift, irrespective of the exposure duration. In defining the *estimated threshold limits,* those for shorter return periods (one to three months) should ensure that the extent of drift in macroinvertebrates is sufficiently small to enable recovery, providing a colonising source of relevant species is available (see Section 2.1.3). Therefore, *estimated threshold limits* of 4.0 mg/l should also be applied for the shorter return periods of one and three months, since concentration and duration of exposure are more important factors for dissolved oxygen than the frequency of exposure (see Table 3.1).

Sustainable cyprinid fisheries

For cyprinids, the effects matrix data (see Figure 3.1) shows that dissolved oxygen concentrations below 1.3 mg/l can cause short-term mortality in roach (for example, 0.42 mg/l for one-hour exposure, 0.41 mg/l for six-hour exposure and 1.33 mg/l for 24-hour exposure). Significant drift in the macroinvertebrates in cyprinid fisheries can also occur at lower dissolved oxygen levels and this is evidently the key parameter in defining the *estimated threshold limits* (see Section 2.1.3). However, sensitive taxa that would be present in salmonid fisheries are generally absent from cyprinid fisheries. Therefore, a lower dissolved oxygen concentration would be required to markedly affect the macroinvertebrate species present in cyprinid fisheries compared to salmonid fisheries. Given the *estimated threshold limit* of 4.0 mg/l for salmonid fisheries, the corresponding value for cyprinid fisheries should be 3.0 mg/l. This value should ensure protection of cyprinid fisheries from short-term mortality and macroinvertebrate drift, irrespective of the duration of exposure.

3.4.2 Unionised ammonia

For unionised ammonia, as the exposure decreases the concentration required to cause effects generally increases; thus, higher *estimated threshold limits* are expected for shorter durations of exposure. For a shorter return period (greater frequency of events), the effects concentration for a given exposure duration would be expected to be lower, as reflected in the current UPM2 FIS.

Sustainable salmonid fisheries

The effects matrix (see Figure 3.2 and Table 3.2) shows that the unionised ammonia concentration needs to rise above 0.2 mg/l (as NH_3-N) for a single event of six-hour duration to result in demonstrable short-term pathological effects and mortality in salmonids. An effect concentration below 0.2 mg/l threshold is evident for six-hour exposures with a return period of 2.6 days (a frequency of three events per week) in terms of demonstrable effects on salmonid gill pathology. When the return period for a six-hour event increases to seven days (a frequency of one event per week), the concentration for demonstrable effects on salmonid gill pathology increases to 0.2 mg/l.

The six-hour and 24-hour *threshold values* of 0.134 mg/l and 0.044 mg/l respectively derived using SSD modelling ensure that no short-term mortality occurs in macroinvertebrates and salmonid fish for events with a long return period (see Section 2.3.1). For exposures of one hour with a long return period, the corresponding *threshold* is 0.247 mg/l, taking into account the expectation of reduced effects at a given concentration as the period of exposure decreases from 6-24 hours to one hour.

Based on the complete dataset, the following *estimated threshold limits* should be protective of salmonid fisheries and ensure that no behavioural, sub-lethal or lethal effects occur in exposed salmonids and sensitive macroinvertebrates for return periods of over2.6 days (a frequency of three events per week):

Exposure duration	*Estimated threshold limits for return periods from 1 month to 1 year*
One hour	0.247 mg/l (as NH_3-N)
Six hours	0.134 mg/l (as NH_3-N)
24 hours	0.044 mg/l (as NH_3-N)

Sustainable cyprinid fisheries

Cyprinids exhibit greater tolerance to unionised ammonia than salmonids following short-term exposures (see Table 3.2 and Table B2 in Appendix B). The reported 24-hour $LC_{50}$for four salmonid species range from 0.12 to 0.6 mg/l whereas those for six cyprinid species range from 0.48 to 1.78 mg/l. For six-hour LC_{50}, the two values for salmonid species are 0.57 to 0.83 mg/l whereas the single value for a cyprinid species is 2.39 mg/l. Therefore, based on the effect matrix for salmonids, higher *estimated threshold limits* for each of the event durations would be expected for cyprinids.

The six-hour and 24-hour *threshold* values of 0.204 mg/l and 0.063 mg/l respectively derived using SSD modelling ensure that no short-term mortality occurs in macroinvertebrates and salmonid fish for events with a long return period (see Section 2.3.1). For the shorter exposure of one hour with a long return period, the corresponding *threshold* value is 0.541 mg/l, taking into account the expectation of reduced effects at a given concentration as the period of exposure decreases from six-24 hours to one hour.

Based on the complete dataset, the following *estimated threshold limits* should be protective of cyprinid fisheries and ensure that no behavioural, sub-lethal or lethal effects occur in exposed cyprinids and macroinvertebrates for return periods of one month to one year:

Exposure duration	*Estimated threshold limit*s *for return periods from 1 month to 1 year*
One hour	0.541 mg/l (as NH_3-N)
Six hours	0.204 mg/l (as NH_3-N)
24 hours	0.063 mg/l (as NH_3-N)

Table 3.2 Summary of the effects matrix data for sustainable salmonid fisheries

Substance	Endpoint	Exposure conditions	Effects at different dissolved oxygen concentrations			
			<4.0 mg/l	4.0 mg/l	5.0 mg/l	6.0 mg/l
Dissolved oxygen	Mortality in salmonids (rainbow trout) after short-term exposure	One-hour exposure and no return period	1.5 mg/l (<10% effect)	-	-	-
		Six-hour exposure and no return period	2.69 mg/l (<10% effect)	-	-	-
		24-hour exposure and no return period	3.5 mg/l (<10% effect)	-	-	-
	Mortality in salmonids (brown trout) after short-term exposure	24-hour exposure with a 3.5-day return period	-	0% effect	-	-
		24-hour exposure with a 7.0-day return period	-	0% effect	-	-
	Growth in salmonids(brown trout) after short-term exposure	24-hour exposure with a 3.5-day return period	-	8% effect	-	-
		24-hour exposure with a 7.0-day return period	-	3% effect	-	-
	Effects on gills of salmonids (brown trout) after short-term exposure	24-hour exposure with a 3.5-day return period	-	No effect	-	-
		24-hour exposure with a 7.0-day return period	-	No effect	-	-
	Drift in macroinvertebrates		-	10% effect	-	-

Substance	Endpoint	Exposure conditions	Effects at different unionised ammonia concentrations (as NH_3-N)				
			<0.2 mg/l	0.2 mg/l	0.3 mg/l	0.4 mg/l	>0.4 mg/l
Unionised ammonia	Mortality in salmonids (rainbow trout) after short-term exposure	One-hour exposure and no return period	-	-	-	-	NOEC = 1.93 mg/l (10% effect)
		Six-hour exposure and no return period	-	-	-	-	NOEC = 0.54 mg/l (0% effect)
		24-hour exposure and no return period	-	-	-	-	NOEC = 0.54 mg/l (0% effect)
	Mortality in salmonids (brown trout) after short-term exposure	Six-hour exposure with a 2.6-day return period	-	-	-	0% effect	-
		Six-hour exposure with a 7.0-day return period	-	-	-	0% effect	-
	Growth in salmonids (brown trout) after short-term exposure	Six-hour exposure with a 2.6-day return period	-	12% effect	-	-	-
		Six-hour exposure with a 7.0-day return period	-	-	10% effect	-	-
	Effects on gills in salmonids (brown trout) after short-term exposure	Six-hour exposure with a 2.6-day return period	Slight damage	Very severe damage	-	-	-

Table 3.3 Summary of the effects matrix data for sustainable cyprinid fisheries

Substance	Endpoint	Exposure conditions	Effects at different dissolved oxygen concentrations			
			<4.0 mg/l	**4.0 mg/l**	**5.0 mg/l**	**6.0 mg/l**
Dissolved oxygen	Mortality in cyprinids (roach) after short-term exposure	One-hour exposure and no return period	0.42 mg/l (5% effect)	-	-	-
		Six-hour exposure and no return period	0.41 mg/l (0% effect)	-	-	-
		24-hour exposure and no return period	1.33 mg/l (0% effect)	-	-	-
	Drift in macroinvertebrates		-	10% effect	-	-
Substance	**Endpoint**	**Exposure conditions**	**Effects at different unionised ammonia concentrations (as NH_3-N)**			
			0.2 mg/l	**0.3 mg/l**	**0.4 mg/l**	**>0.4 mg/l**
Unionised ammonia	Mortality in cyprinids (chub) after short-term exposure	One-hour exposure and no return period	-	-	-	-
		Six-hours exposure and no return period	-	-	-	-
		24-hour exposure and no return period	-	-	-	$\geq$0.49 mg/l (1% effect)
	Drift in macroinvertebrates			-	-	2 mg/l (No effect)

4. Task C - Comparison of effects matrix with existing standards

4.1 Introduction

The requirements of this task as given in the tender specification were:

"During this stage of work, the effects matrix will be compared to the existing UPM2 FIS and any differences will be highlighted. The potential significance of any differences in terms of WFD objectives will be discussed".

For this task, the *estimated threshold limits* developed from the effects matrix given in Tables 3.2 and 3.3 were compared with the existing UPM2 FIS for dissolved oxygen (see Table 2.4) and unionised ammonia (see Table 2.5). The comparison assessed the extent to which there is a margin of safety between the *estimated threshold limits* and UPM2 FIS which allows for unmeasured behavioural, physiological or toxic effects in sensitive macroinvertebrates and fish and ensures the levels are protective of the relevant fishery. Acceptable agreement between the current UPM2 FIS and the *estimated threshold limits* derived developed from the effects matrix then would mean the FIS can be regarded as "fit for purpose".

4.2 Dissolved oxygen

Figures 4.1 and 4.2 compare UPM2 FIS for the different exposure/frequency combinations with the *estimated threshold limits* of 4.0 mg/l and 3.0 mg/l dissolved oxygen for sustainable salmonid and cyprinid fisheries respectively. Table 4.1 summarises the results of these comparisons.

For sustainable salmonid fisheries (see Figure 4.1), the UPM2 FIS for dissolved oxygen provide a margin of safety of up to 33.3 per cent (0.5 to 2.0 mg/l) compared to the *estimated threshold limits* in all but one case. In that case, the UPM2 FIS is the same as the *estimated threshold limits.*

For sustainable cyprinid fisheries (see Figure 4.2), the UPM2 FIS for dissolved oxygen provide a margin of safety of up to 45.5 per cent (0.5 to 2.5 mg/l) compared to the *estimated threshold limits* in all except two cases. In these cases, UPM2 FIS are within five per cent of the *estimated threshold limits.*

As a result, the UPM2 FIS can be considered protective against potential behavioural, physiological or toxic effects in macroinvertebrates and fish in salmonid and cyprinid fisheries exposed to reduced dissolved oxygen concentrations during intermittent wet weather events.

Figure 4.1Comparison of UPM2 FIS and estimated threshold limit for dissolved oxygen (solid line at 4.0 mg/l) and salmonid fisheries

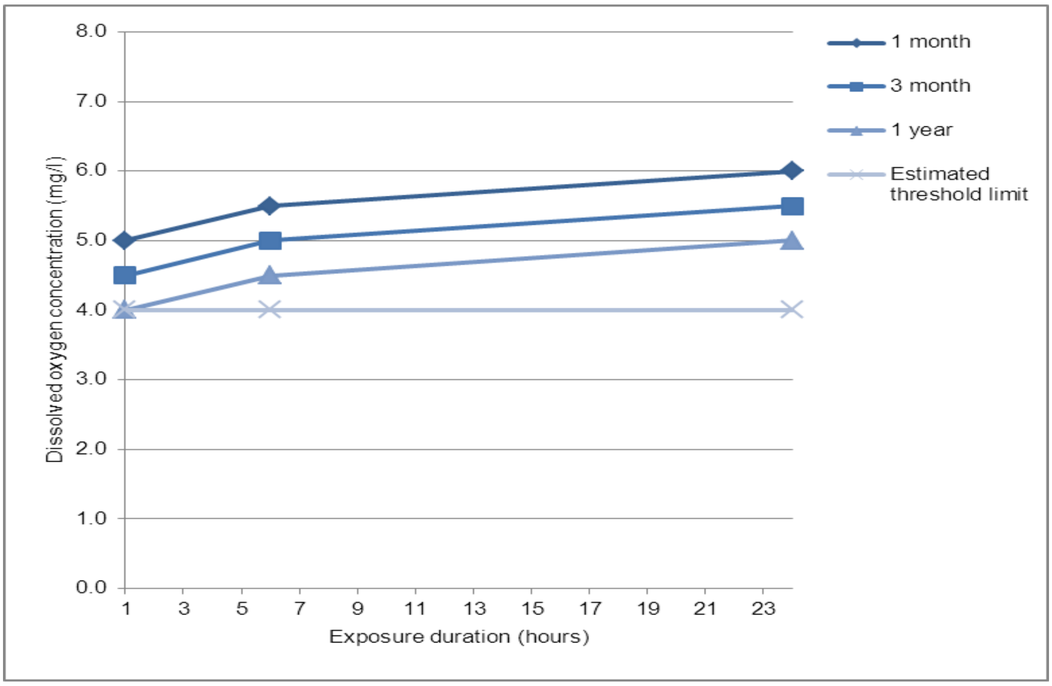
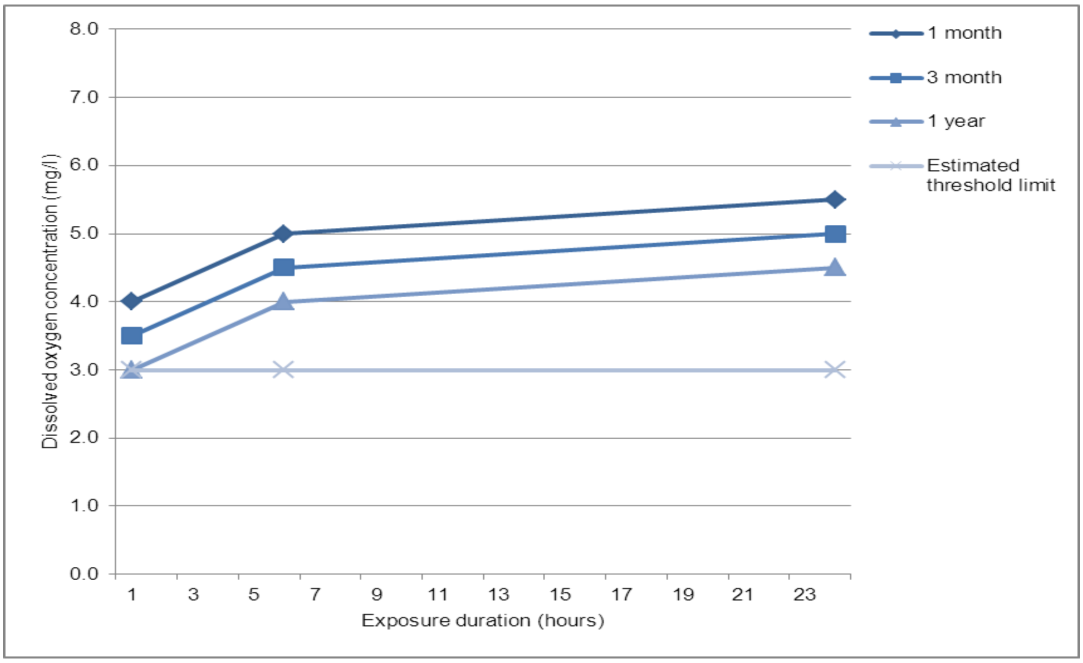


Figure 4.2Comparison of UPM2 FIS and estimated threshold limit for dissolved oxygen (solid line at 3.0 mg/l) and cyprinid fisheries



4.3 Unionised ammonia

Figures 4.3 and 4.4 compare UPM2 FIS for the different exposure/frequency combinations with the *estimated threshold limits* for sustainable salmonid and cyprinid fisheries respectively. Table 4.1 summarises the results of these comparisons.

For sustainable salmonid fisheries (see Figure 4.3), the UPM2 FIS for unionised ammonia in all cases provide a margin of safety of 47-436 per cent (0.014 to 0.182 mg/l) compared to the *estimated threshold limits* and should be protective of the fishery*.* The extent of the margin of safety decreases as the duration of exposure increases from between one and six hours to 24 hours. This is not unexpected given, the more limited data available to derive the one-hour *estimated threshold limit.*

For sustainable cyprinid fisheries (see Figure 4.4), the UPM2 FIS for unionised ammonia in all but one case provide a margin of safety of 26-261 per cent (0.013 to 0.391 mg/l) compared to the *estimated threshold limits* and are protective of the fishery*.* The extent of the margin of safety decreases as the duration of exposure increases from one hour to 24 hours. The greater margin of safety for the UPM2 FIS is consistent with the limited data on the effects of different concentration/duration/frequency combinations on cyprinids.

As a result, the UPM2 FIS can be considered protective against potential behavioural, physiological or toxic effects in macroinvertebrates and fish in salmonid and cyprinid fisheries exposed to elevated unionised ammonia concentrations during intermittent wet weather events.

Figure 4.3Comparison of UPM2 FIS and estimated threshold limits for unionised ammonia and salmonid fisheries

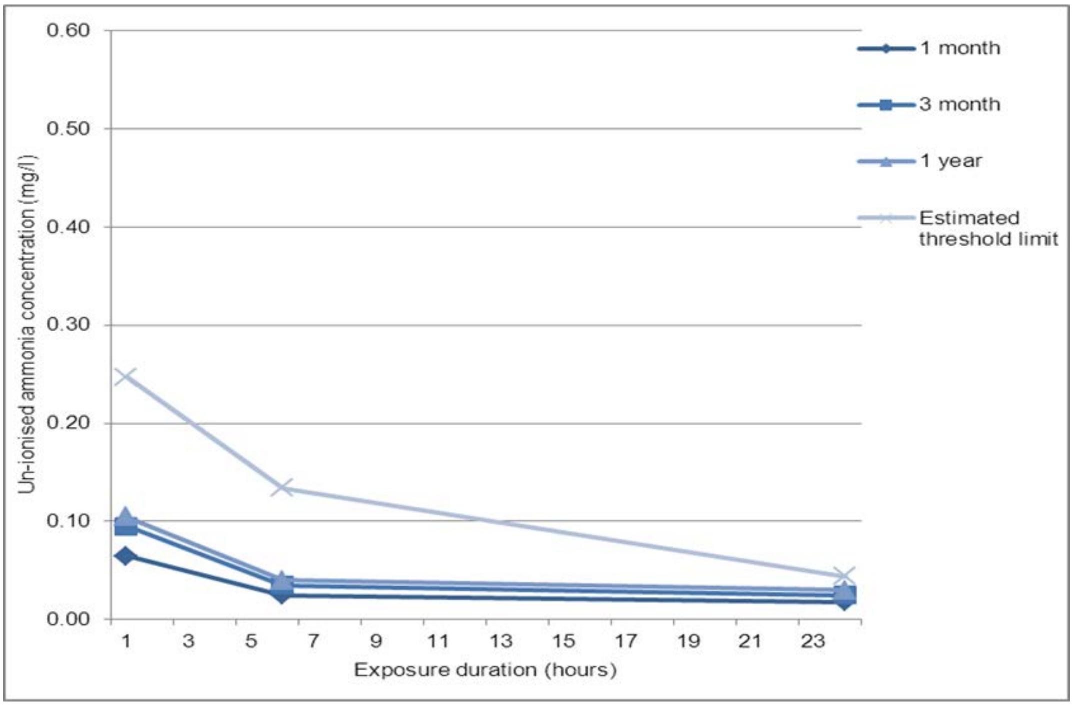


Figure 4.4Comparison of UPM2 FIS and estimated threshold limits for unionised ammonia and cyprinid fisheries

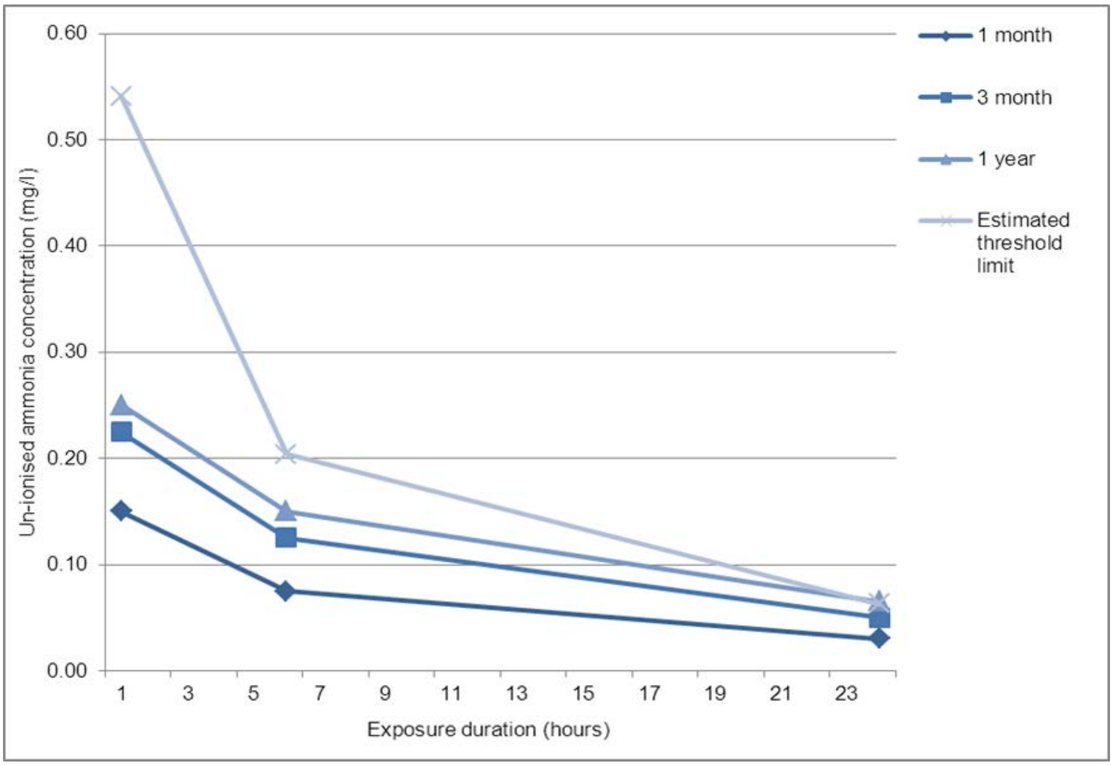


Table 4.1 Summary of comparison of estimated threshold limits developed from the effects matrix against the existing UPM2 FIS for dissolved oxygen and unionised ammonia for salmonid and cyprinid fisheries

Substance	Exposure duration	Return period	Comparison of estimated threshold limit with UPM2 FIS for salmonid fisheries	Comparison of estimated threshold limit with UPM2 FIS for cyprinid fisheries
Dissolved oxygen	1 hour	1 month	UPM2 FIS provides a 20.0% margin of safety (1.0 mg/l difference)	UPM2 FIS provides a 20.0% margin of safety (1.0 mg/l difference)
		3 months	UPM2 FIS provides an 11.1% margin of safety (0.5 mg/l difference)	UPM2 FIS provides an 11.1% margin of safety (0.5 mg/l difference)
		1 year	UPM2 FIS provides no margin of safety (0.0 mg/l difference)	UPM2 FIS provides no margin of safety (0.0 mg/l difference)
	6 hours	1 month	UPM2 FIS provides a 27.3% margin of safety (1.5 mg/l difference)	UPM2 FIS provides a 40.0% margin of safety (2.0 mg/l difference)
		3 months	UPM2 FIS provides a 20.0% margin of safety (1.0 mg/l difference)	UPM2 FIS provides a 33.3% margin of safety (1.5 mg/l difference)
		1 year	UPM2 FIS provides an 11.1% margin of safety (0.5 mg/l difference)	UPM2 FIS provides an 25.0% margin of safety (1.0 mg/l difference)
	24 hours	1 month	UPM2 FIS provides a 33.3% margin of safety (2.0 mg/l difference)	UPM2 FIS provides a 45.5% margin of safety (2.5 mg/l difference)
		3 months	UPM2 FIS provides a 27.3% margin of safety (1.5 mg/l difference)	UPM2 FIS provides a 40.0% margin of safety (2.0 mg/l difference)
		1 year	UPM2 FIS provides a 20.0% margin of safety (1.0 mg/l difference)	UPM2 FIS provides a 33.3% margin of safety (1.5 mg/l difference)
Unionised ammonia	1 hour	1 month	UPM2 FIS provides a 280% margin of safety (0.182 mg/l difference)	UPM2 FIS provides a 261% margin of safety (0.391 mg/l difference)
		3 months	UPM2 FIS provides a 160% margin of safety (0.152 mg/l difference)	UPM2 FIS provides a140% margin of safety (0.316 mg/l difference)
		1 year	UPM2 FIS provides a 135% margin of safety (0.142 mg/l difference)	UPM2 FIS provides a116% margin of safety (0.291 mg/l difference)
	6 hours	1 month	UPM2 FIS provides a 436% margin of safety (0.109 mg/l difference)	UPM2 FIS provides a 172% margin of safety (0.129 mg/l difference)
		3 months	UPM2 FIS provides a 283% margin of safety (0.099 mg/l difference)	UPM2 FIS provides a 63% margin of safety (0.079 mg/l difference)
		1 year	UPM2 FIS provides a 235% margin of safety (0.094 mg/l difference)	UPM2 FIS provides a 36% margin of safety (0.054 mg/l difference)
	24 hours	1 month	UPM2 FIS provides a 144% margin of safety (0.026 mg/l difference)	UPM2 FIS provides a 110% margin of safety (0.033 mg/l difference)
		3 months	UPM2 FIS provides a 76% margin of safety (0.019 mg/l difference)	UPM2 FIS provides a 26% margin of safety (0.013 mg/l difference)
		1 year	UPM2 FIS provides a 47% margin of safety (0.014 mg/l difference)	UPM2 FIS is less than the *estimated threshold limit* by 3.1% (0.002 mg/l difference)

UPM2 FIS provides more than a 5% margin of safety above the *estimated threshold limit*
UPM2 FIS provides less than a 5% margin of safety around the*estimated threshold limit*

4.4 Issues for compliance with Water Framework Directive

The comparison of UPM2 FIS with *estimated threshold limits* derived from the effects matrix data (see Table 4.1) shows that for most concentration/duration/frequency combinations, the standards for both dissolved oxygen and unionised ammonia provide a margin of safety for salmonid and cyprinid fisheries. They provide protection against reduced dissolved oxygen and elevated unionised ammonia for which robust toxicity data is not currently available. Such an approach is consistent with the process to derive environmental quality standards where the assessment (safety) factor applied would in part account for untested species and endpoints. Overall, the UPM2 FIS are "fit for purpose" and, based on the available data, provide an adequate degree of protection.

Achievement of the UPM2 FIS should ensure that for typical wet weather events, the existing good quality status of a water body is not compromised by exposure to these intermittent discharges.

5. Task D - Proposed UPM standards for WFD

5.1 Introduction

The requirements of this task as stated in the tender specification were:

"This stage will comprise of proposals for a set of UPM standards that will meet WFD objectives. Consideration in particular needs to be given to:

- *how the current UPM FIS standards compare to WFD objectives and whether the existing standards need to be changed at all;*
- *how the current and interpolated 99 percentiles compare to WFD objectives;*
- *whether a set of longer return period FIS are necessary for the protection of aquatic life, and*
- *Situations where the application of the standards is not appropriate, i.e. situations where applying UPM water quality standards may not work e.g. still waters".*

5.2 Development of proposed UPM standards for the Water Framework Directive

The overall objective of the Water Framework Directive is to achieve "good" status for all waters by December 2015. Annex V of the WFD gives standard definitions for the classification of water bodies into five ecological quality classes: high, good, moderate, poor and bad. The following requirements have been defined for high, good and moderate status:

High status – *No or very minor* deviation from undisturbed (reference) condition.

Good status – *Slight* deviation from the reference condition.

Moderate status – *Moderate* deviation from the reference condition.

A key element of the requirement is that the ecological effects need to consider all the relevant biological elements of the Water Framework Directive classification scheme. This means that any UPM standards need to address the impacts of the substances of concern on the following biological elements (if relevant): phytoplankton, macrophytes, phytobenthos, benthic invertebrates and fish communities (see Section 3 for further information).

To ensure that good quality status is maintained in receiving waters subject to wet weather events from urban wastewater systems, the quality standards for dissolved oxygen and unionised ammonia (whether as UPM2 FIS or 99 percentiles) need to ensure that the releases do not cause more than "*a slight deterioration from the reference condition*". This means that the structure and function of all biological elements are largely maintained following the events, irrespective of the concentration, duration and frequency.

For wet weather events, a slight deterioration could be considered to represent a small change in the structure of the aquatic community, through the loss of sensitive algal, macrophyte, invertebrate and/or fish species or a reduction in productivity. The available data indicates that for both dissolved oxygen and unionised ammonia, macroinvertebrates and fish are the most sensitive taxa and that protection of these groups will prevent any adverse effects on algae and macrophytes (see Section 3 for further information).

5.2.1 UPM2 FIS

Comparison of the UPM2 FIS with *estimated thresholds indicates* that the UPM2 FIS are "fit for purpose" and no modifications are required with respect to the Water Framework Directive. For most concentration/duration/ frequency combinations, the standards for both dissolved oxygen and unionised ammonia provide a margin of safety for salmonid and cyprinid fisheries. They should also protect against the effects of reduced dissolved oxygen and elevated unionised ammonia for which robust toxicity data is not currently available. Therefore, when a fishery meets the relevant standards, no long-term behavioural and physiological effects and no short-term fish and macroinvertebrate mortality should result for wet weather pollution events with at least an annual return period. Achievement of the UPM2 FIS should ensure that the existing good quality status of a water body is not compromised.

At present, the longest return period addressed by the UPM2 FIS is a one-year return period, but the question has been raised as to whether a set of longer return period (RP) FIS are necessary to protect aquatic life. This issue was considered in the EU-funded programme, *Integrated planning and management of urban drainage, wastewater treatment and receiving water systems*. In *Work Package 3 – Regulatory Issues*, environmental quality standards to protect aquatic life from intermittent discharges (in current regulation or recommended for inclusion in the regulatory framework) were reviewed (Milne *et al*., 1998). The review found that standards had been applied in Denmark, Germany, Ireland, Spain, Switzerland and the United Kingdom. Numerical values for the standards were cited for Denmark, Switzerland and the UK, covering dissolved oxygen and unionised ammonia (UK - both dissolved oxygen and ammonia; Switzerland - unionised ammonia only; Denmark - dissolved oxygen only).

It was concluded that "*the standards cover return periods of up to one year, and it is recommended that this is the most appropriate focus for sewerage system design. Considering events with return periods considerably greater than one year, there comes a point where extremely rare events have catastrophic impacts such as large scale mortality of fish. From the fisheries management viewpoint, it is questionable whether fisheries should be managed to allow for large scale mortalities even if they only occur once every eight to sixteen years (e.g. the Danish standards). However, a cost-benefit consideration would indicate that it is not cost-effective to design to protect against the impact of the most extreme events*".

No additional data was identified to modify the conclusion of the review, thus it was not deemed necessary to derive standards for return periods longer than one year.

To date, the vast majority of practical experience in the UPM procedure in the UK has been obtained in rivers. However, UPM2 FIS for cyprinid and/or salmonid standards should also be applicable to still waters such as lakes.

Additional data has shown that certain species of freshwater mussels are particularly sensitive to ammonia. There is insufficient data to derive new standards for these species, but the standards should be applied with caution to any intermittent discharges into rivers where freshwater mussels are protected.

Regulatory bodies may need to review their approach in deciding when to require discharges to meet salmonid FIS to ensure WFD objectives are met.

5.2.2 99 percentiles

The 99 percentiles developed for BOD and total ammonia by the Environment Agency (see Section 2.3.2) for differing WFD ecological status were established by interpolation of the corresponding 90 percentile values of each River Ecosystem Class. Therefore, achievement of these standards should protect freshwater aquatic life from short duration intermittent urban wet weather events and also ensure that the existing good quality status of a water body is not compromised by intermittent wet weather discharges.

Given that the 99 percentiles were developed from the RE Classes, they are specifically applicable to rivers rather than still waters such as lakes.

6. Recommendations

The following recommendations are made in relation to the Review of urban pollution management standards against WFD requirements:

- The Fundamental Intermittent Standards (FIS), as reviewed in this report, should be translated into the revised version of the *Urban Pollution Management Manual*.
- The FIS should continue to be used by regulators and dischargers as part of the regulatory process for preparing permit applications and in designing improvement works to address unsatisfactory intermittent wet weather discharges from urban wastewater networks.
- The outcome of the review regarding the ongoing use of FIS and 99 percentiles should be presented to the United Kingdom Technical Advisory Group to confirm their suitability for use under the Water Framework Directive.

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Appendix A Review of new studies relevant to the derivation of the fundamental intermittent standards

Reference

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Abstract

Episodic organic pollution results in short-term increases in the ammonia concentration and reductions in the dissolved oxygen (DO) concentration of receiving waters. This study provides information on the lethal effects of 24-hour pulses of high ammonia and low DO on two common freshwater crustaceans. Both inter and intraspecific differences in sensitivity were observed. *Asellus aquaticus* was five times more resistant to hypoxia and two times more resistant to unionized ammonia and *Gammarus pulex*. Moreover, for both stressors, juveniles were less susceptible than adults. Interspecific differences in ventilation rate and blood characteristics (haemocyanin concentration and oxygen affinity) could explain interspecific differences in susceptibility to hypoxia, and possibly ammonia. Intraspecific differences in ventilation rate may partly explain the reduced susceptibility of juvenile *G. pulex* to these stressors.

Key points of relevance to the derivation of FIS

- Both *G. pulex* and *A. aquaticus* were able to survive 24-hour exposure to oxygen concentrations down to 2 mg/l but below this threshold concentration, survival decreased rapidly with decreasing DO.

- The lowest observed effect concentrations (LOEC) for unionised ammonia were 2.14 mg/l for *G. pulex* and 6.66 mg/l for *A. aquaticus.* For dissolved oxygen, the 24-hour LC_{50}values were 0.32 and below 0.25 mg/l for adult male and juvenile *A. aquaticus* and 1.63 and 1.26 mg/l for adult male and juvenile *G. pulex*. The 24-hour LC_{50}values for unionised ammonia were 9.45 and 12.91 mg/l for adult male and juvenile *A. aquaticus* and 4.32 and 6.21 mg/l for adult male and juvenile *G. pulex*.

- Based on 24-hour LC_{50} values, *A. aquaticus* was five times more resistant to hypoxia and two times more resistant to elevated unionised ammonia concentrations than *G. pulex*.

- For both stressors, most mortality occurred during exposure and post-exposure mortality was only significant for juvenile *G. pulex* exposed to low DO concentrations.

Reference
Mummert A K, Neves R J, Newcomb T J and Cherry D S (2003) Sensitivity of juvenile freshwater mussels (*Lampsilis fasciola*, *Villosa iris*) to total and unionised ammonia. *Environmental Toxicology and Chemistry,* 22(11), 2545-2553.
Abstract This study evaluated the sensitivity of juveniles of two freshwater unionid mussel species (*Villosa iris* [Lea] and *Lampsilis fasciola* [Rafinesque]) to unionised and total ammonia. Five concentrations of ammonium chloride were tested using 96-hour static renewal toxicity tests at 12°C and 20°C. Based on their respective mean 96-hour lethal concentrations to 50 per cent (LC_{50}), *V. iris* (0.11 mg/l NH_3-N) was more sensitive than *L. fasciola* (0.26 mg/l NH_3-N). At 96 hours, no significant differences in sensitivity to unionised ammonia between the two temperatures were observed for either species. Comparison of LC_{50} reported for other aquatic organisms to the 96-hour LC_{50} calculated for juvenile *L. fasciola* and *V.iris* shows these two mussel species to be among the most sensitive to unionised ammonia. Based on reported levels of unionised ammonia in the aquatic environment from anthropogenic sources, unionised ammonia may be an important limiting toxicological factor to freshwater mussel populations.
Key points of relevance to the derivation of FIS • In comparing the sensitivity of these two species of juvenile mussels to regulatory standards and environmental ammonia levels, *L. fasciola* and *V. iris* are among the most common species in Virginia, and rarer species may be more sensitive. • Comparison of the USEPA and Virginia Department of Environmental Quality water quality criteria for ammonia with the calculated LC_{50} values for *V.iris* and *L. fasciola* indicates that current US standards should be adequate to protect juveniles of these two mussel species from acute exposure.

Reference
Turnpenny A W H, Clough S C, Holden S D J *et al* (2004) *Thames Tideway Strategy: Experimental studies on the dissolved oxygen requirements of fish*. Babtie Aquatic (FARL) Report FCR 374/04 for Thames Water Utilities Ltd.

Abstract

The water quality and ecological status of the Thames Tideway have improved remarkably since the1960s, from a state of fishlessness to supporting some 121 recorded fish species. However, while water quality conditions generally remain favourable to fish populations, further improvement in ecological status is limited by transient and persistent conditions of hypoxia that affect certain reaches of the estuary. The transient problems occur mainly as a result of storm discharges of untreated sewage that enter the Tideway via combined sewer overflows (CSOs) during the summer months; at this time, temperature (and hence microbial activity) is high and dilution by freshwater runoff is at a minimum. The most affected parts are the upper reaches of the Tideway, from Teddington to Battersea where large fish kills were observed in August 1994 and July 2001. More persistent but less extreme hypoxia occurs in the reaches around the Beckton and Crossness sewage outfalls throughout the summer.

The present study was undertaken to acquire scientific data on the tolerances of estuarine fish species and life-stages to low dissolved oxygen (DO) concentrations. Past estuarine quality standards have been based on poor information, much of it relating to freshwater fish or to estuarine species not found in Britain. An initial literature review revealed very few data pertinent to Tideway fish and therefore an experimental study was commissioned.

Following the literature review, the Project had three main strands of practical work:

1. Laboratory measurement of the acute lethality of low DO to various estuarine life-stages of selected fish ‘indicator’ species.
2. Continuous online field exposure of captive (tank-held) fish to Tideway water over the summer months, in the expectation of them experiencing one or more ‘live’ CSO-related hypoxia events.
3. Laboratory investigations of sub-lethal effects of hypoxia, particularly in relation to the potential avoidance of areas of hypoxic water, but also possible effects on fish growth.

The laboratory lethality work was carried out at two locations. At the first, Fawley Aquatic Research Laboratories(Fawley, Southampton),lethality testing was carried out under controlled, constant temperature conditions using a mixture of bore-hole water and clean sea water. Parallel studies were carried out at a temporary laboratory on the upper Tideway at Chiswick Pier, where tests were conducted in raw Thames water, but without the benefit of temperature control. Chiswick Pier was also the location for monitoring the effects of live CSO events on fish. The experimental work was carried out over two successive summers; the majority of the experiments were completed in 2002, with supplementary laboratory testing and a continuation of live CSO testing in 2003.

Seven fish species were selected as suitable ‘indicators’ for DO tolerance, these being: smelt (*Osmerus eperlanus*), sand smelt (*Atherina presbyter*), flounder (*Platichthys flesus*), common goby (*Pomatoschistus microps*), Atlantic salmon (*Salmo salar*); dace (*Leuciscus leuciscus*) and bass (*Dicentrarchus labrax*). Apart from the salmon, all of these species are known to spawn within the Thames Tideway or live there in the juvenile phase. Salmon, a seasonal migrant through the Tideway, could not easily be procured and managed as an experimental subject within the scope of the study. In the 2002 studies, the smaller but closely related brown trout (*Salmo trutta*) acted as a surrogate for this species. In 2003, young salmon were also used; these were supplied by the Environment Agency after being set aside from the R. Thames annual smolt stocking programme

Testing for lethal do concentrations - Standard OECD ecotoxicological test protocols were adopted throughout for the laboratory exposure of fish to low DO. Parallel tests were conducted at the Fawley Aquatic Research Laboratories and at Chiswick Pier during 2002. In

2003 this testing was carried out solely at Fawley Aquatic Research Laboratories. Measurements were made of six-hour and 24-hour LC_{50} and LC_{10} (lethal threshold values for 50 per cent and 10 per cent of the population respectively).

Monitoring of fish during live CSO events - In the tests at Chiswick Pier, batches of fish were held in tanks, which were fed with water pumped directly from the river. Tanks containing control batches were identical but were continuously aerated.

Sub-lethal effects - Avoidance of hypoxic conditions was tested using a DO-gradient apparatus. After the DO gradient was established, any changes in fish positions were recorded every minute for one hour. Growth effects were considered by comparing weights of fish at the start and end of the Chiswick once-through tests.

The final stage of the project was to review the findings of the experimental studies, within the context of the literature on hypoxia and fish and in relation to proposed DO standards for the Tideway. This included the development of a simple Fish Risk Model to calculate the likely exposure risk to hypoxia, given the seasonal and spatial distributions of different fish life-stages.

Key points of relevance to the derivation of FIS

- Estimates of the six-hour and 24-hour LC_{10} and LC_{50}were obtained for most of the species and life-stages selected, although difficulties in keeping sand-smelt and gobies at Chiswick meant that these species were only tested at Fawley.
- The results showed that the salmonids, including the Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) were the most sensitive species, with gobies and flounder being the most robust. Smelt, a species normally associated with high sensitivity to hypoxia, at the sizes tested were unexpectedly tolerant of hypoxia, although it remains possible that the newly hatched fry may be more sensitive, as was found with dace.
- A minimum standard of 1 mg/l may not be adequately protective of any of the species examined, although the lack of any recorded history of occurrence of such low levels within the last 12 years makes the risk difficult to assess. A 1.5 mg/l minimum standard, however, appears to create a sustainable outcome.

Reference Diamond J M, Klaine S J and Butcher J B (2006) Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits. *Environmental Science and Technology*, 40, 5132-5138
Abstract Sub-acute effects of pulsed copper, zinc, or ammonia exposures were examined, including a range of pulse concentrations, durations, frequencies, and recovery times between pulses, using short-term chronic *Pimephales promelas* and 21-day *Daphnia magna* tests. Sub-lethal effects were rarely observed independent of mortality. Effects were observed only at concentrations near the species continuous exposure 48-hour LC_{50} for each chemical. *Daphnia* often rebounded from temporary reproduction effects, meeting or exceeding control responses by the end of the test. Effects of 24-hour ammonia or copper pulses were diminished soon after the pulse was removed, while 24-hour zinc pulses caused continued effects for several days following removal of the pulse, indicating a slower uptake and/or depuration rate for zinc. *D. magna* exhibited less mortality as copper pulses were spaced further apart, while fish were equally or more affected with longer recovery times between copper pulses, indicative of different adaptation mechanisms between the two species. Responses were not predictable based on average concentration or a combination of duration and concentration. Chronic water quality criteria and effluent permit limits, expressed as a four- or 30-day average concentration, respectively, may not protect against effects of pulsed exposures, depending on the frequency, magnitude and duration of pulses, as well as the recovery period between events.
Key points of relevance to the derivation of FIS • For ammonia, the results suggest that less than 24-hour pulsed exposures below the acute criterion are unlikely to result in sub-lethal effects and much of the response, if any, would be short-term mortality. • The results consistently demonstrated that 24-hour pulsed exposures, at magnitudes above the species continuous exposure chronic toxicity threshold but well below its acutely toxic level, had little or no measurable effects on survival or sub-lethal indicators for either *Daphnia magna* or *Pimphelas promelas.* • Recovery time was not a significant factor for *Daphnia magna* or *Pimphelas promelas* survival following exposure to ammonia. • Sub-lethal effects of pulsed exposures are more likely if the magnitude approaches the species acute threshold value (such as the 48-hour LC_{50}) and there are multiple pulses within a short time period (days). • In general, the 24-hour averaging period used to limit acute exposure in wastewater permits may be appropriately conservative and aquatic life should be protected under most pulsed exposure regimes.

Reference
Wang N*et al* (2007) Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* 26, 2036–2047.
Abstract The objective of the present study was to determine acute toxicity of copper, ammonia or chlorine to larval (glochidia) and juvenile mussels using the recently published American Society for Testing and Materials (ASTM) *Standard guide for conducting laboratory toxicity tests with freshwater mussels.* Toxicity tests were conducted with glochidia (24- to 48-hour exposures) and juveniles (96-hour exposures) of up to 11 mussel species in reconstituted ASTM hard water using copper, ammonia, or chlorine as a toxicant. Copper and ammonia tests also were conducted with five commonly tested species, including cladocerans (*Daphnia magna* and *Ceriodaphnia dubia*; 48-hour exposures), amphipod (*Hyalella azteca*; 48-hour exposures), rainbow trout (*Oncorhynchus mykiss*; 96-hour exposures), and fathead minnow (*Pimephales promelas*; 96-hour exposures). Median effective concentrations (EC_{50}) for commonly tested species were more than 58 µg Cu/l (except 15 µg Cu/l for *C. dubia*) and below 13 mg total ammonia N/l, whereas the $EC_{50}$for mussels in most cases were below 45 µg Cu/l or below 12 mg N/l and were often at or below the final acute values (FAV) used to derive the USEPA 1996 acute water quality criterion (WQC) for copper and 1999 acute WQC for ammonia. However, the chlorine EC_{50} for mussels generally were more than 40 µg/l and above the FAV in the WQC for chlorine. The results indicate that the early life-stages of mussels were generally more sensitive to copper and ammonia than other organisms and that, including mussel toxicity data in a revision to the WQC would lower the WQC for copper or ammonia. Furthermore, including additional mussel data in 2007 WQC for copper based on the biotic ligand model would further lower the WQC.
Key points of relevance to the derivation of FIS • The glochidia and newly transformed juvenile mussels tested in the present study, in most cases, were more sensitive to total ammonia than the commonly tested cladoceran, amphipod, and fish species. • The EC_{50} of total ammonia for the early life-stages of mussels often were at or below the final acute values (FAV) used to derive the USEPA 1999 acute water quality criteria (WQC) for ammonia. • The USEPA 1999 acute WQC for ammonia may not be adequately protective of the mussel species tested.

Reference
Gomułka P, Arski D, Kucharczyk D, Kupren K, Krejszeff S and Targońska K (2011) Acute ammonia toxicity during early ontogeny of chub, *Leuciscusce phalus* (Cypinidae). *Aquatic Living Resources*, 24, 211-217
Abstract Acute toxicity of ammonia was investigated in four life-stages of juvenile chub, *Leuciscus cephalus* (cyprinid fish): one, 10, 20 and 30 days after the first feeding. The fish used for the toxicity test were reared intensively in a closed recirculation system. Each acute toxicity test was run for 96 hours and lethal concentration LC1, LC_{50} and LC_{99} values were calculated for 24, 48, 72 and 96 hours. The susceptibility of chub to acute ammonia toxicity decreased linearly with age and stage of development. The LC_{50} (48-hours) ranged from 0.62 mg l^{-1} of unionized ammonia nitrogen for one day after first feeding larvae to 1.73 mg l^{-1} for 30 days after first feeding ones. A significant linear relationship between chub larvae susceptibility to ammonia toxicity and both body weight and length was found. The critical level of unionized ammonia nitrogen for chub larvae was suggested as 0.49 mg l^{-1}.
Key points of relevance to the derivation of FIS • The susceptibility of chub larval stages to ammonia toxicity decreased with larval development. • The decrease in susceptibility of chub larvae to ammonia toxicity was probably caused by a general incremental change in both physiological efficiency (brain and liver) and muscle capacity for glutamine storage.

Appendix B Summary of the short-term (one-hour to 24-hour LC_{50}) toxicity data for freshwater organisms

Table B.1 Toxicity values for freshwater organisms exposed to dissolved oxygen for different durations

Taxa	Species	Common name	Toxicity values (mg/l)			Reference	Ratio six-hour : 24-hour LC_{50}	Ratio 24-hour NOEC: 24-hour LC_{50}
			six-hour LC_{50}	24-hour NOEC	24-hour LC_{50}			
Invertebrates	*A. aquaticus* (*adult males*)	Isopod	*No data*	1.00	0.32	Maltby (1995)	-	3.13
	A.intermedius (adults)	Isopod	*No data*	*No data*	0.03	Sprague (1963)	-	-
	G.fasciatus (adults)	Amphipod	*No data*	*No data*	4.30	Sprague (1963)	-	-
	G.pseudolimnaeus (adults)	Amphipod	*No data*	*No data*	2.20	Sprague (1963)	-	-
	G. pulex (adult males)	Amphipod	*No data*	5.50	1.63	Maltby (1995)	-	3.37
	H.azteca	Amphipod	*No data*	*No data*	0.70	Sprague (1963)	-	-
Fish	*O.kisutch (underyearling)*	Coho salmon	*No data*	*No data*	1.20	Davison *et al* (1959)	-	-
	O.mykiss (10 months old)	Rainbow trout	2.35	3.50	2.66	Milne *et al* (1992)	0.88	1.32
	O.tshawtscha (juveniles)	Chinook salmon	*No data*	*No data*	1.75	Katz et al (1959)	-	-
	R.rutilus	Roach	<0.4	1.33	0.75	Milne *et al* (1992)	>1.88	1.77
	S.salar (smolts)	Atlantic salmon	2.00	2.40	2.12	Turnpenny *et al* (2004)	0.94	1.13
	S trutta (fry)	Brown trout	1.60	1.92	1.60	Turnpenny *et al* (2004)	1.00	1.20
Mean ratios							**0.94**	**2.03**

Table B.2 Toxicity values for freshwater organisms exposed to unionised ammonia for different durations

Taxa	Species	Common name	Toxicity values (mg/l)			Reference	Ratio six-hour:24-hour LC_{50}	Ratio 24-hour NOEC: 24-hour LC_{50}
			six-hour LC_{50}	24-hour NOEC	24-hour LC_{50}			
Algae	*S.costatum*	Diatom	*No data*	1.00	*No data*	Livingston *et al* (2002)	-	-
Invertebrates	*A. aquaticus(8-10mm)*	Isopod	*No data*	*No data*	4.04	Williams *et al* (1986)	-	-
	A.ligamentina (glochidia)	Mussel	0.98	*No data*	0.47	Wang *et al* (2007)	2.09	-
	B.rhodini (8-10mm)	Mayfly	2.81	*No data*	2.30	Williams *et al* (1986)	1.22	-
	B.rubens	Rotifer	*No data*	0.75	3.20	Snell and Persoone (1989)	-	0.23
	C.riparius (10-12mm)	Midge larvae	4.16	*No data*	3.00	Williams *et al* (1986)	1.39	-
	D.magna (<24hours old)	Crustacean	*No data*	*No data*	1.74	Kaniewska-Prus (1982)	-	-
	E.ignita (8-10mm)	Damselfly	4.20	*No data*	3.29	Williams *et al* (1986)	1.28	-
	G. pulex (8-12mm)	Amphipod	4.36	*No data*	3.20	Williams *et al* (1986)	1.36	-
	L.fasciola (juvenile)	Mussel	*No data*	*No data*	0.32	Mummert et *al* (2003)	-	-
	L.hoffmeisteri (30-40mm)	Tubificid worm	3.83	*No data*	2.80	Williams *et al* (1986)	1.37	-
	L.inermis (6-8mm)	Insect	3.04	*No data*	2.18	Williams *et al* (1986)	1.39	-
	L.rafinesqueana (glochidia)	Mussel	0.98	*No data*	0.68	Wang *et al* (2007)	1.44	-
	L.siliquoidon (glochidia)	Mussel	*1.15*	*No data*	0.60	Wang *et al* (2007)	1.92	-
	L.stagnalis (25-30mm)	Snail	3.17	*No data*	1.92	Williams *et al* (1986)	1.65	-
	M.rectirostris	Crustacean	*No data*	0.28	1.61	Gyor and Olah (1980)	-	0.17
	P.fontinalis (10-12mm)	Bladder snail	3.49	*No data*	2.20	Williams *et al* (1986)	1.59	-
	P.tennuis (10-12mm)	Turbellarian	4.07	*No data*	1.95	Williams *et al* (1986)	2.09	-
	V.iris (juvenile)	Mussel	*No data*	*No data*	0.22	Mummert et *al* (2003)	-	-
Fish	*C.carpio*	Common carp	2.39	*No data*	1.78	Hasan and MacIntosh (1986)	1.34	-
	C.carpio	Common carp	*No data*	*No data*	0.68	Xu et *al* (1994)	-	-
	L.cephalus	Chub (fry)	*No data*	*0.49*	0.76	Gomulka et al (2011)	-	0.65
	L.guntea	Guntea loach	*No data*	*No data*	0.61	Sangli and Kanabur (2000)	-	-
	H.molitrix	Silver carp	*No data*	*No data*	0.48	Xu et *al* (1994)	-	-
	N.guentheri	Killifish	*No data*	*No data*	0.57	Shedd et *al* 1999	-	-
	O.mykiss (10 months old)	Rainbow trout	0.57	0.43	0.53	Milne *et al* (1992)	1.08	0.81
	O.tshawtcsha	Chinook salmon	*No data*	*No data*	0.30	Harader and Allen 1983	-	-
	P.promelas	Fathead minnow	*No data*	*No data*	1.22	Markle et al (2000)	-	-
	P.reticulata	Guppy	*No data*	*No data*	1.35	Rubin and Elmaraghy (1976)	-	-
	S.salar (smolts)	Atlantic salmon	*No data*	*No data*	0.12	Alabaster *et al* (1979)	-	-
	S trutta	Brown trout	0.83	0.50	0.60	Milne *et al* (1992)	1.38	0.83
Mean ratios for invertebrates and salmonids							**1.52**	**0.51**
Mean ratios for invertebrates and cyprinids							**1.55**	**0.35**

Table B.3 Ratios of FIS for dissolved oxygen for different durations of exposure for various ecosystem types

Ecosystem type	Return period	Exposure period (hours)			Ratio one-hour : six-hour standard	Ratio six-hour :24-hour standard
		One	Six	24		
Sustainable salmonid fishery	One month	5.0	5.5	6.0	0.91	0.92
	Three months	4.5	5.0	5.5	0.90	0.91
	One year	4.0	4.5	5.0	0.89	0.90
Sustainable cyprinid fishery	One month	4.0	5.0	5.5	0.80	0.91
	Three months	3.5	4.5	5.0	0.78	0.90
	One year	3.0	4.0	4.5	0.75	0.89
Marginal cyprinid fishery	One month	3.0	3.5	4.0	0.86	0.88
	Three months	2.5	3.0	3.5	0.83	0.86
	One year	2.0	2.5	3.0	0.80	0.83

Table B.4 Ratios of FIS for unionised ammonia for different durations of exposure for various ecosystem types

Ecosystem type	Return period	Exposure period (hours)			Ratio one-hour : six-hour standard	Ratio six-hour :24-hour standard
		One	Six	24		
Sustainable salmonid fishery	One month	0.065	0.025	0.018	2.60	1.39
	Three months	0.095	0.035	0.025	2.71	1.40
	One year	0.105	0.040	0.030	2.63	1.33
Sustainable cyprinid fishery	One month	0.150	0.075	0.030	2.00	2.50
	Three months	0.225	0.125	0.050	1.80	2.50
	One year	0.250	0.150	0.065	1.67	2.31
Marginal cyprinid fishery	One month	0.175	0.100	0.050	1.75	2.00
	Three months	0.250	0.150	0.080	1.67	1.88
	One year	0.300	0.200	0.140	1.50	1.43

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