PROJECT 034

Interim Guidelines on the Ranges of Enteric Organism Decay Rates (T_{90s}) under Different Marine Conditions

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INTERIM GUIDELINES ON THE RANGES OF ENTERIC ORGANISM DECAY RATES (T90S) UNDER DIFFERENT MARINE CONDITIONS

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SUMMARY

An important consideration in the design of long sea sewage outfalls is the rate at which faecal bacteria and viruses decay once discharged into the marine aquatic environment. A quantitative measure of the mortality rate typically used as a design parameter is the T90 value, which is the time taken for 90% of the organisms to be inactivated.

Unfortunately, the T90 value for a particular bacterium or virus in a marine environment is influenced by a multitude of factors which include the intensity of solar radiation, the amount of sediment suspended in the water, the temperature, and the levels of nutrients and predators. Furthermore, the method used to perform the mortality experiment also affects the T90 value. Thus, it is difficult to assign an exact T90 value to a particular organism in a marine environment.

In this report the ranges of T90 values obtained from analysis of mortality experiments reported in the literature are presented. Various indicator and pathogenic bacteria and enteric viruses in different marine conditions are covered.

This report will therefore be of value to NRA staff and equivalents in Scotland and Northern Ireland who will need to interpret the criteria upon which long sea outfalls are designed and their performance reviewed.

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SECTION 1 - INTRODUCTION

At a meeting with the NRA on 2 May 1990, the urgent needs for Interim Guidelines on the likely ranges of bacterial and viral decay rates under a variety of sea conditions were stressed. Decay rates for bacteria and viruses measured from mortality experiments are usually expressed in terms of the T90 value, which is the time taken for a 90% decrease in the number of bacteria or viruses. The T90 value for a particular enteric microorganism in a marine environment is an important design parameter in the modelling of sewage discharge from long sea outfalls. In this report, T90 ranges are presented for a variety of enteric organisms in different sea conditions eg in the dark, in sunlight and in the presence of sediments.

SECTION 2 - SURVIVAL RATES FOR ENTERIC BACTERIA IN MARINE WATERS

The ranges exhibited by T90 values determined from some of the publications on survival of indicator bacteria (total coliforms, faecal coliforms and faecal streptococci) in marine environments are presented in Table 1. Table 2 demonstrates the effect of the suspended solid content on the T90 values measured for total coliforms in seawater.

Survival data for pathogenic bacteria in marine environments are more scarce than for indicator bacteria. However, some *in vitro* studies have been performed and the T90 values that were determined are presented in Table 3.

It should be noted that in seawater environments under *in vitro* conditions the pathogenic bacteria, Salmonella spp. and *Vibrio cholerae*, survive longer than *E. coli* indicator bacteria (Evison 1988, Hood and Ness 1982).

In marine environments, both *in situ* and *in vitro* experiments have implicated solar radiation to be the major factor influencing bacterial mortality. The T90 values of both indicator and pathogenic bacteria are

Table 1 - Interim guidelines for range of T90 values (hours) for bacterial indicators of faecal pollution under differing marine water conditions.

Organism	daylight	dark or no sunlight	seawater with suspended solids (600 mg/l)
Faecal Coliforms	1.0 - 9.6[a]	21 - 48[c]	--
Total Coliforms	0.57 - 9.0[b]	34 - 89[d]	39•
Faecal Streptococci	2.6 - 23.0[f]	36 - 183[g]	43•

[a] Includes T90 values from eight *in situ* experiments (Gameson 1986).

[b] Includes T90 values from *in situ* experiments (Gameson 1986, Gunnerson *et al* 1972, Harremoes 1970) and also paddling pool experiments (Irving 1977).

[c] *In vitro* experiments performed at 24 °C in the absence of sunlight but under overhead fluorescent lighting for 10 - 12 h/day (Fujioka *et al* 1981).

[d] *In vitro* experiments performed at 20 °C in the dark (Gameson 1984).

• T90 value determined from paddling pool experiment performed outside (Irving 1977). Since the T90 value is longer than the day length, the experiment was continued overnight, during which little mortality occurs. Thus, the T90 value presented is longer than if the experiments were performed under continuous light.

[f] *In situ* experiments performed by Gameson (1986). Some experiments demonstrated either negligible mortality (T90 2420 hours) or even growth.

[g] *In vitro* experiments performed at 24 °C (Fujioka *et al* 1981) in the absence of sunlight but under overhead fluorescent lighting for 10 - 12 h/day and in complete darkness at 25 °C (Evison 1988).

SECTION 3 - SURVIVAL RATES FOR ENTERIC VIRUSES IN MARINE WATERS

The ranges exhibited by T90 values determined from some of the publications on enteric virus survival in marine environments are presented in Table 4.

The two most important physical factors in reducing virus survival are light and temperature. However, in the majority of *in vitro* survival experiments reported to date, the light conditions are not specified although it is presumed that the incubation mixture is exposed to regular room light or maintained in an incubator ie in the dark. Virus survival is enhanced at lower water temperatures. Thus as shown by the *in situ* studies (Table 4) virus survival in seawater is significantly longer during the winter months. Most of the work on virus survival in seawater has been performed in the laboratory at temperatures around 25 °C. T90 values are approximately 13-30 days at 3-5 °C, 0.8-3 days at 22-25 °C and always below 1.6 days at 37 °C (cited from Bitton 1978). Viruses are not inactivated at very low temperatures and are commonly stored in laboratories at -70 °C (Bitton 1980).

The presence of silts and sediments markedly enhances survival of both enteroviruses and rotaviruses in marine waters. T90 values measured in seawater *in vitro* may therefore be shorter than in the sea itself, where circulation of bottom sediments may contribute to extending survival.

At the salinity levels of marine and estuarine waters, changes in salinity appear to have little effect on enteric virus survival. Thus, *in vitro* survival studies performed by Hurst and Gerba (1980) showed the T90 values for rotavirus SA-11, poliovirus 1, echovirus 7 and coxsackievirus B3 to be little affected by changes in the salinity of estuarine water over the range of 12 to 28 g/kg at 20 °C.

Virucidal activity is widespread and a general property of seawater. Some studies have shown that removing microorganisms by autoclaving the seawater enhances virus survival.

A major problem with attributing a particular T90 value to an area of coastal water in modelling long sea outfalls is that the magnitude of the virucidal activity of sea water varies considerably depending on the source of the water sample. Indeed Akin et al (1976) report that "the viral inactivating capacity of Gulf water (and probably all sea water) was highly variable and unpredictable". Magnusson et al (1966) found that the poliovirus 3 inactivating capacity of 24 samples of Baltic and North sea waters varied by over 4-fold at 23 °C.

SECTION 4 - CONCLUSIONS

An area of coastal water will contain a multitude of microcosms each presenting virucidal and bactericidal activities of different magnitudes. Thus in each microcosm a particular species of virus or bacterium will exhibit a different T90 value. The absolute T90 value will vary depending on the light intensity, the amount of sediment suspended in the water, the temperature of the water and the number of predatory micro-organisms in the water. For example, T90 values measured in situ for total coliforms range from 34 minutes to 9 hours. In the presence of suspended solids (600 mg/l) the T90 value for total coliforms is 39 hours. T90 values for total coliforms measured in the dark at 20 °C range from 34 to 89 hours. Interim guidelines for ranges of bacterial T90 values in different marine water conditions are presented in Table 1. T90 values for enteric viruses in marine waters are presented in Table 4.

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