



Aquatic Toxicology: A New Element in the Safety Testing of Surfactants?

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ABSTRACT

Aquatic toxicology is becoming increasingly important in assessing the risks from environmental contamination by surfactants. Recent work by the Water Research Centre and others is discussed to show that: (a) there is a need for flexibility in approach even for preliminary screening test procedures and for toxicity tests to be relevant to field situations; (b) where linear chain surfactants pass through a normal biological treatment process these are readily biodegraded and reduced in toxicity, and in these circumstances toxicity tests should be made using biodegraded materials; (c) methods of analysis currently used overestimate the acute toxicity of surfactants to fish.

INTRODUCTION

The aquatic toxicology of surfactants has been studied for many years and has been reviewed recently by Abel (1). While most attention has been given to the survival, growth, and reproduction of fish in aqueous concentrations, and there have been advances in the actual testing procedures themselves, attention has turned more recently to the uptake, metabolism, and excretion of surfactants by fish, e.g., by Tovell et al. (2). There has also been an increase in our understanding of some of the interactions between surfactants and the aquatic ecosystem, for example, from the work of Painter et al. (3) and Matcham et al. (4). Now more explicit consideration is being increasingly given to the most effective ways of using such knowledge to evaluate the risks from environmental contaminants, for example by Government Departments such as the U.K. Ministry of Agriculture, Fisheries and Food (5), industry (6), and international bodies (7,8).

In developing an approach to this problem, two essential interrelated processes are involved. The first is the identification of the different situations and circumstances in which aquatic ecosystems or particular target organisms within the systems are potentially at risk. This involves an estimation of likely aqueous concentrations and their duration bearing in mind relevant physical, chemical, and biological factors. This requires quantitative information on spatial and temporal patterns of usage, disposal, contamination, and persistence in the environment. The second is the choice of tests, or sequences of tests, appropriate to each particular situation. Such a choice should generally be flexible but would be influenced by socioeconomic constraints as well as toxicological considerations. It is also markedly influenced by the precise purpose that is in mind, whether it is the development of water quality criteria, or the setting of standards, or monitoring.

These two processes should be carried out in parallel and also in such depth and in as many stages as appear to be necessary as the overall evaluation proceeds. Essential questions relate to the speed and completeness of, and the conditions required for, biodegradation, as well as the degree of toxicity of the original and of the degraded product to biological treatment processes and aquatic life.

One can envisage a series of tests that might be carried out ranging from the simplest respirometric measurements to full scale trials involving treatment plants and waters receiving effluent discharges. This paper, however, will be confined to discussion of just a few recent laboratory studies by the Water Research Centre (WRC) and others on toxicity and biodegradability.

FLEXIBILITY VS. STANDARDIZATION

It is recognized that some purposes, such as the legal enforcement of a regulation prescribing toxicity or biodegradability, certainly demand some degree of standardization of test procedures, but in general most advances are likely to be achieved with a flexible approach using techniques adapted to the particular circumstances.

Initial Screening Tests for Acute Toxicity to Fish

The European Inland Fisheries Advisory Commission (EIFAC) has considered (9) the extent to which standardization is necessary for toxicity testing, and although it concluded that there was a case for standardized test procedures for preliminary screening of chemicals, as well as for legal tests, it rightly concentrated its attention upon defining the principles that need to be adhered to rather than upon drawing up detailed specifications. It also concluded that, in the present state of knowledge, there was no commonly accepted alternative to the use of the response of the whole living fish to a toxicant or to choosing death as the principal response, although the future possibility of using cultured tissues and sublethal responses was not ruled out.

The International Standards Organisation, however, has drafted a specification for a standard screening procedure using fish to measure acute toxicity, and trial tests are being made on several substances to compare results obtained by workers in different countries. However, surfactants are not included among the test materials. Although in the early 1970s the European Economic Community (EEC) explored the possibility of introducing a standard screening toxicity test (using fish) for raw surfactants, its existing proposals that all such materials marketed should be 90% biodegradable make tests on the biodegraded products generally much more relevant, and not surprisingly the Commission did not pursue the matter further. United Kingdom and German Government Committees concerned with surfactants agree that there is no need for such a statutory test (10).

During the course of the discussions within the EEC, however, eleven surfactants were tested within the U.K. using seven different procedures (11) which embraced a range of water hardness of 20 to 268 mg/l as CaCO₃ and a range of temperature of 15 to 20 C, and which included rates of replacement of test solution from nil to 50 min per volume of test vessel and used five species of fish. There was no great interlaboratory variation in results for each compound, and all results were grouped within the same order of magnitude of concentration. Somewhat similar

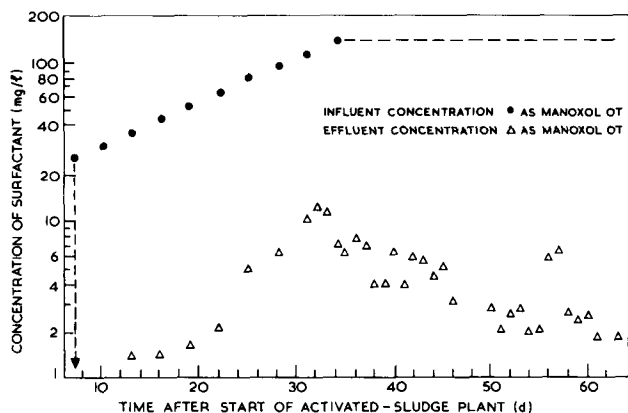


FIG. 1. Concentrations of surfactant in treatment plant influent and effluent.

findings have been recently reported for several other surfactants using five different procedures and three species of fish (12). With results of this kind one questions the need for strict standardization even for a preliminary screening test in which the data obtained are meant to provide only a very rough estimate of the concentration of a substance likely to be lethal to aquatic life in order to rank groups of substances in an approximate order of their toxicity; such tests certainly should not be used alone for the preparation of water quality criteria and for the estimation of concentrations that might *not* be lethal to aquatic life, though they could be used, together with other relevant information, to help select those substances which might or might not warrant further investigation. An initial screening test for acute lethal toxicity might be followed, for example, depending upon the circumstances concerned, by tests on other species or communities or organisms under a variety of environmental conditions, over a longer period and measuring sublethal effects including bioaccumulation, metabolism, and excretion.

A particularly relevant situation, however, for testing of a surfactant as sold would be where it was present in a formulation of a chemical, often a pesticide, which was likely to reach the aquatic environment in its undegraded state, since in increasing the efficiency of the chemical it might also increase the toxicity of the chemical to aquatic life (13).

RELATIONSHIP BETWEEN TOXICITY AND BIODEGRADABILITY

Because surfactants are biodegradable and, weight for weight, may become less toxic in the process, studies of both biodegradability and the accompanying changes in chemistry and toxicity are essential in developing water quality criteria for these substances, especially where a high proportion of the material reaching the aquatic environment would first pass through biological sewage treatment processes.

Recent U.S. Work on Linear Alkylbenzene Sulfonate

Most data available for the toxicity and biodegradation of surfactants relate to linear alkylbenzene sulfonate (LAS) which replaced the poorly degraded branched alkylbenzene sulfonate (ABS), and it will be instructive to mention a few recent studies [bearing in mind that Weaver and Coughlin (14) have pronounced, in the context of verification of the facts about biodegradation of LAS, that "education requires repetition"]. Dolan and Hendrick (15), for example, using an initial concentration of 30 mg/l of a high molecular weight LAS in about 378 liters of tap water con-

taining a sludge acclimatized to the LAS, showed that toxicity to fish, which was reduced and finally eliminated, was related to a significant change in the distribution of homologs (from the longer to the shorter C chain lengths) and of isomers (from the 2- and 3-positions to the 4- to 7-positions), the longer and the 2C and 3C isomers being more readily degraded. Kimerle and Swisher (16) demonstrated that pure synthetic models of some of the intermediate biodegradation products were, as expected, less toxic than the intact LAS: using a continuous flow activated sludge unit under stress loading of 20 to 200 mg LAS/l or loaded with a pure homolog of 13 carbon atoms. They also showed that the acute toxicity of the effluent to the water flea (*Daphnia magna*) decreased with increase in biodegradation. Incidentally, reduction in toxicity for the homolog was less than for the commercial LAS because differences in toxicity between isomers of a given chain length are less than those between isomers of different lengths; this would point to the danger of relying solely on data obtained with synthetic model substances when assessing biodegradability of materials actually used.

Water Research Center Results with LAS

The toxicity of LAS and its degradation in the activated sludge process has also been studied at the Water Research Centre using four porous-pot activated-sludge units (17,18) to treat the surfactant at concentrations of 150 mg/l and two further pots to produce "control" effluent.

In one example (19) using a material consisting mainly of C11 to C15 isomers, an influent sewage was made up from excreta and other ingredients, the total organic carbon concentration in the settled sewage being adjusted to 190 mg/l. Initially, the porous aeration vessel was filled with returned activated sludge derived from a plant treating domestic sewage, and the suspended solids concentration was adjusted to 2.5 g/l by dilution with tap water. The solids were maintained at this level by periodical wastage throughout the treatment. The diluted supernatant was pumped through each aeration vessel at a rate of about 1 liter/hr and was continuously aerated with bubbled air to give a dissolved oxygen concentration of 2 mg/l or greater. A temperature of 15 C was maintained throughout the treatment. Surfactant loss (by loss of foam over the edge of the pot) was avoided by smearing silicone grease around the upper end of each pot immediately above the liquid surface.

The performance of the activated sludge units in removing the surfactants is shown in Figure 1. After the full loading of 150 mg/l had been achieved, the concentration of methylene blue active substance (MBAS) (20) in the effluent fell from 12 mg/l, at first rapidly and then more slowly, until an equilibrium level (between 3.3 and 1.8 mg/l) was approached about 20 days after, showing a reduction of about 98% in MBAS readings.

It may be noted that the extremely high levels of surfactant introduced into the influent (150 mg/l compared with about 15 mg/l in normal sewage), to ensure that the acute toxicity of the effluent would be readily measurable, did not appear to impair the efficiency of operation of these units. Reduction in BOD was between 91 and 96%, reduction in COD was 81 to 86%, removal of organic carbon, 74 to 83% and removal of ammonia, 84 to 98%. [The maximum average concentration of ammonia found in the effluent was 2.5 mg/l (as N), a level too low to affect the acute toxicity to fish of the effluent to fish (21) and, actually none of the fish died in tests on effluent from the control units alone.] Between day 53 and day 56 effluent was collected daily to obtain a volume sufficient for the toxicity tests. This bulk effluent was held at 5 C and was found not to change significantly in its concentration of MBAS during the period of holding.

Toxicity tests were made with juvenile rainbow trout

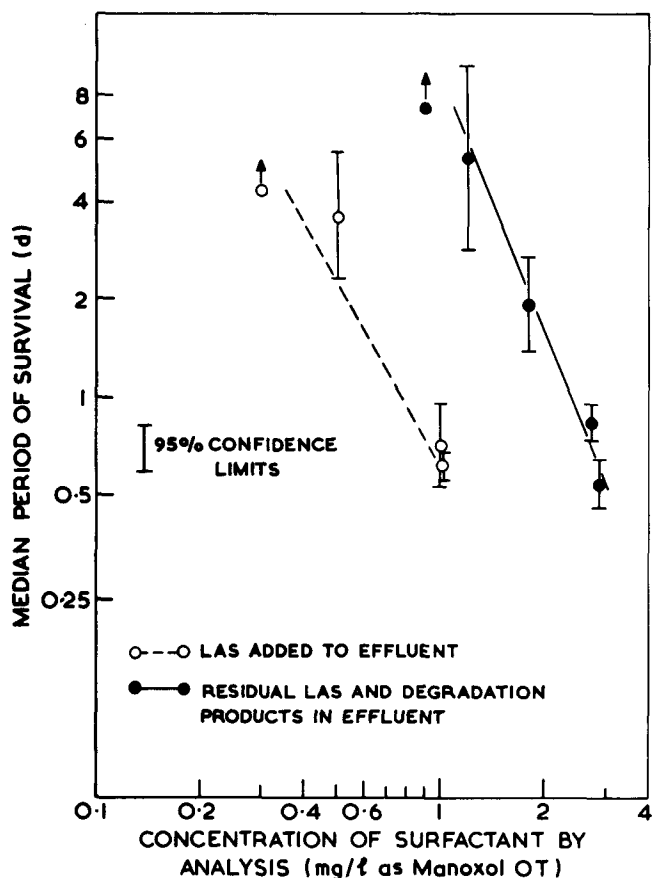


FIG. 2. Comparative toxicities of LAS, when added to control (detergent-free) effluent, and of residual LAS and its degradation products in effluent, in terms of MBAS analysis.

(*Salmo gairdneri*) in 1-liter flasks through each of which a constant flow of solution was maintained automatically (22) at a net rate of about 10 ml/min which was sufficient to maintain the dissolved oxygen concentration above 75% of the air saturation value. All tests were made using a raw natural ground water with a total hardness of about 250 mg/l ($\equiv \text{CaCO}_3$) as diluent.

Surfactant (untreated) added to detergent-free (control) effluent and diluted with hard water had a nominal 4-d LC50 to rainbow trout (i.e., concentration lethal to 50% of the fish in 4 days) of 0.36 mg/l, whereas, after treatment, the corresponding value was 29.5 mg/l expressed in terms of the concentration of surfactant in the influent to the activated sludge treatment units, a difference of about 100-fold.

The observed reduction in toxicity brought about by treatment reflects and confirms the measured reduction of the concentration of MBAS in the treated effluent (Fig. 1). However, when median period of survival is plotted against measured, rather than nominal, concentrations of surfactant (measured as MBAS) as in Figure 2, it should be noted that although the curves obtained under the two test conditions are fairly close, in terms of the measured MBAS concentration, they do not coincide as might be expected if the analysis was measuring compounds with the same toxicological properties. Thus, it is evident in these tests, as in those already referred to, that the MBAS involved are compounds which are toxicologically and therefore, presumably, chemically, different.

General Conclusions on LAS

The results demonstrate, therefore, that this type of surfactant is readily degraded, to a high degree, by a conventional sewage treatment process, and that the MBAS in

the effluent are of low toxicity. The results also highlight the fact that methods that involve measurement of MBAS to define the toxicity of the residual material and degradation products present in aqueous wastes and in river waters after secondary treatment of such surfactants overestimate toxicity and create problems in trying to set meaningful and adequate standards in this way. In this situation the toxicity value used in any water quality standard should obviously reflect the toxicity of degradation products (plus residual surfactant) and not that of the original surfactant.

Where the MBAS in a surface fresh water, originate in the type of material tested here and have reached that water after treatment at a sewage works, then the present results indicate (within the limits of the conditions involved) that concentrations tolerable to rainbow trout of median (or lesser) sensitivity would be some three to five times greater than would be indicated by toxicity data obtained from tests on the undegraded material. Water quality standards are frequently based on data obtained from studies made with rainbow trout and undegraded LAS; when this is the case, then an even greater factor will be involved for the many species more resistant than rainbow trout. In terms of realistic concentrations of these materials in sewage and the degree of treatability shown here, it is evident that these wastes do not directly add significantly to the acute lethal toxicity of sewage effluents. It is worth noting, however, that undegraded surfactants would reach a river in untreated sewage discharges, in which circumstances toxicity would be increased by other substances such as ammonia and by the effects of BOD.

Nonionic Materials

The nature and toxicity of the biodegradation products of nonionic materials are much less well known than those of LAS but, as with anionic materials, a decrease in the alkyl chain length of nonionic surfactants is associated with a decrease in toxicity to fish. However, a decrease in the ethylene oxide chain length appears to increase toxicity (23,24) so that there would appear to be the possibility that some of the intermediate breakdown products of alkyl-phenol ethoxylates might be more toxic than the starting material.

Reiff (25) has recently measured the acute toxicity of two linear alcohol ethoxylates and one branched alkyl-phenol ethoxylate to rainbow trout and subjected the substances to mild biodegradation conditions at an initial concentration of 20 mg/l in 700 liters of river water with no bacterial seed deliberately added. The reduction in concentration of the three materials was estimated by measuring surface tension, bismuth iodide active substances (BIAS) (26), and time of survival of batches of rainbow trout. The toxicity of the branched chain material was much lower, and the time lag before any measurable reduction in concentration and in toxicity in the river die-away test was much longer (40-50 d) than those of the linear chain materials. The period within which toxicity was no longer measurable was about 10 and 12 weeks, respectively. There was, however, no indication that any of the breakdown products were collectively more toxic than the parent materials.

It may also be noted that during the die-away tests, the three surfactants, and particularly the linear chain materials, were less toxic than expected from the content of BIAS. Possibly this was partly due to some of the surfactant being initially absorbed onto the 300 mg/l of suspended solids originally present in the sample of river water (owing to recent rainfall), although there was a tendency for the discrepancy to increase with time, suggesting that branched chain components, present in the linear chain material, were responsible.

Recently the Water Research Centre made trials (27) with the same materials used by Reiff (25) but at concen-

trations of up to 25 mg/l added to Stevenage domestic sewage (which already contained BIAS at concentrations between 6 and 8 mg/l) and supplied to pilot scale percolating filters. The tests were deliberately done during the winter because of the possibility that biodegradation might have been influenced unfavorably by low temperatures in the filters (5 to 10 C).

At least 95% of the nonionic material already present or added appeared to be removed (as well as 95 to 97% of MBAS present at concentrations of 28 to 30 mg/l) and there appeared to be little or no effect on the performance of the filters. The effluents, although they contained concentrations of 0.74 mg BIAS/l (which was approximately equivalent to the 7-d LC50 for rainbow trout of either of the pure substances) were not lethal to any of the test rainbow trout within a period of 7 days. Although it is not possible to conclude from these tests that the concentrations at which the residues and degradation products of the nonionic surfactants were present in the effluent would have no long term harmful effects on trout, the marked increase in median period of survival that accompanies a small decrease in concentration suggests that survival would at least be very prolonged.

The aquatic toxicology of surfactants may well have been of lesser importance environmentally in the early 1950s than was, say, foaming in sewage treatment plants and on surface waters. And although in those days it certainly was taken into consideration, it has since become of much greater importance (partly because foaming is now much less important as a problem), and it is now taken into account much more explicitly as an essential element in the safety testing of detergents. Considerable attention has been given to the restriction and standardization of testing techniques for screening purposes, but these have only a very limited place in the evaluation of the environmental risks from surfactants, particularly if confined to the raw product rather than the material after it has been degraded biologically.

The few examples of recent laboratory work referred to in this paper illustrate that linear alkylbenzene sulfate surfactants and nonionic materials can be readily and extensively degraded biologically to less toxic materials without adversely affecting the sewage treatment process, and they serve to emphasize the importance of testing the toxicity of degraded rather than undegraded substances. To try to draw more general conclusions from the few data quoted, however, would be to neglect the principles enunciated about the importance of taking into account all relevant factors in assessing the risks from environmental contamination in a particular situation. However, the results clearly demonstrate that measurements of surfactant concentration based upon MBAS and BIAS are quite inadequate in that they overestimate the toxicity of material that has undergone biodegradation.

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