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Importance of Speciation in Aquatic Toxicity Tests with Aluminum

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It is accepted that the form of a metal can have an important influence on its toxicity, fate and behaviour. In water, aluminium is complexed by both inorganic and organic ligands. Below pH 6, organic complexes and the hydrated free ion tend to be the principal dissolved forms. Potts *et al* (1985) report that complexation occurs with humic substances mainly in the pH range 3 to 6. The main feature of aluminium speciation at higher pH values is that dissolved species are likely to constitute only a small proportion of the total. For water pH values in the range 6 to 9, a variety of hydroxy-complexes are predicted to predominate, but the key issue is the proportion of these that is actually dissolved. Particulate forms, ranging from colloidal species containing less than ten aluminium atoms to aluminium-containing mineral particles, constitute the major fraction of the total. At pH values between 5.5 and 9, there is very little aluminium in true solution. This is illustrated by the predicted (Schecher and McAvoy, 1998) dissolved aluminium concentrations shown in Figure 1.

The calculated dissolved concentration, shown in Figure 1, is considerably lower than concentrations measured in natural waters as shown for a modelled/spiked concentration of 100 µg/l at pH 7. The probable reasons for this are: (i) in practice, it is very difficult to determine concentrations of truly dissolved species - low molecular weight colloidal species are invariably included in the dissolved fraction when dissolved and particulate forms are separated, by filtration; (ii) real waters may contain other inorganic and organic ligands, which tend to stabilise the dissolved concentration at a higher level than that predicted by calculation; and (iii) the calculations used to derive dissolved aluminium concentrations are based on the attainment of thermodynamic equilibrium. In real waters, the speed of reactions in dilute solution may be so slow that equilibrium is not reached.

The relatively vague distinction between reactive and unreactive dissolved and undissolved forms of aluminium has led to the development of various analytical techniques designed to determine the reactive species of the metal. The species determined are referred to as "labile" or "labile monomeric" forms and are considered to constitute the most biologically and environmentally significant fraction. It is important to recognise that, in principle, each analytical technique

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for labile aluminium or each variant of a given approach is empirical - the technique defines the fraction determined. In practice, comparisons amongst several of the most common approaches have revealed a moderately good degree of agreement (Clarke *et al*, 1996 and Lazerte, 1984). The procedure described by Barnes (1975) for the determination of “fast reactive” aluminium has been the basis of that used by Dixon and Gardner (1998) for studies of aluminium speciation in UK surface waters and in the toxicity study described below.

MATERIALS AND METHODS

Ecotoxicity studies carried out at the authors’ laboratory on the toxicity of aluminium to spawn and early life stages (ELS) of the common toad, *Bufo bufo,* illustrate the importance of speciation. These tests were undertaken as part of work intended to establish environmental quality criteria for aluminium in surface waters. A review of published toxicity data for aluminium indicates that els of amphibians are amongst the most sensitive taxa to aluminium. Birge (1987) and Clark and LaZerte (1985) reported important effects on the hatching and development of North American amphibians for aluminium concentrations in the range 10 to 50 μg/l. However, in neither study was it clear if aluminium concentrations referred to total values, spiked concentrations or reactive forms. The present tests were undertaken to provide information on toxicity for els of an amphibian species indigenous to the UK, for the range of aluminium concentrations under consideration in the standard setting process.

The tests, designed to assess aluminium toxicity to spawn and tadpoles of the common toad (*Bufo bufo*) at two pH values, 6.0 and 7.5, were conducted over a 13-day exposure period, concluding at a point approximately 96h post hatching. Spawn of age between 0 and 48h was collected from a local pond (water quality: pH 7.5, saturation of dissolved oxygen 85%, total hardness 87 mg/l as $CaCO_3$, alkalinity 98 mg/l as $CaCO_3$). The test medium was water prepared by dilution of a groundwater diluted 1:5 with deionised water to produce a water of hardness 50mg/l as $CaCO_3$. This water was adjusted to the test pH values of aliquots of 6 and 7.5 by addition of 0.1M and 0.01M hydrochloric acid and sodium hydroxide, respectively. The pH value of the lower pH water was stabilised by purging the excess free carbon dioxide with air, so that after aeration overnight the pH value stabilised at 6.0.

For each pH value, a range of water samples of aluminium concentrations from 0 to 320 μg/l (0, 10, 18, 32, 56 100, 180 and 320 μg/l) was prepared in the test waters by spiking with appropriate portions of a standard solution of aluminium nitrate. These concentration were chosen to bracket those at which effects had been reported previously. Triplicate test vessels were established at each concentration level. “Ropes” of spawn containing 20 eggs were added to 250 ml portions of test water in each vessel. The waters were not aerated, but were replenished using freshly spiked test waters, with minimum disturbance to the spawn, every two days for the 7 day duration of the test. Environmental conditions were controlled at a temperature of 13°C with a photoperiod of 16h light, 8h dark and a 30 min simulation of dawn and dusk. The number of live and dead eggs and tadpoles was recorded daily.

Analytically reactive aluminium was determined (see above) at intervals as a check on the forms of aluminium to which the spawn and tadpoles were exposed. Figures 2 and 3 show the results of these determinations. At pH 6.0, the concentration of monomeric aluminium was found to be substantially less than the total spiked level (Figure 2). Even immediately after spiking, the concentration was much lower than expected; the test solution with a total concentration of 320 μg/l was of a reactive aluminium concentration of less than 35 μg/l. After 24h the monomeric aluminium concentrations of all test solutions were less than 20 μg/l. At the higher pH value of 7.5 (Figure 3), the difference between monomeric aluminium concentrations and the corresponding spiked levels was also substantial (though not so large as at pH 6.0). The 24-h values for monomeric aluminium were similar to those measured initially, indicating greater stability than that observed for samples at pH 6.0. Checks on total aluminium concentrations in fresh and 2-day old water samples confirmed that there were no losses and that the spiked concentrations were consistent with the nominal concentrations.

Of nearly 500 larvae exposed at each pH value less than 2% were observed to be deformed; the mortality rate was also less than 2%. No LC_{50} or LOEC could be predicted. There was no evident relationship between either abnormality or mortality and exposure to aluminium concentration (measured "reactive" or total). These results are not surprising in view of the fact that the actual exposures were low, compared with the intended or nominal exposures.

RESULTS AND DISCUSSION

Although the importance of speciation is accepted widely, it is relatively rare for the determination of key species to form part of toxicity testing, for standard setting or for routine water quality monitoring. There are two principal reasons for this: firstly, the measurement techniques used to determine particular metal species have yet to be developed, refined to the stage where they can be accepted widely and applied on a routine basis; secondly, water quality legislation is usually framed in terms of the total (or dissolved) quantity of a metal, rather than as limit values for individual, important forms. As long as this is the case, there will be a strong incentive to determine only total metal concentrations. The consequence of defining water quality standards in terms of total or dissolved metal is that standards will not necessarily reflect the biologically important forms. This can lead to a given standard being marginally protective for one water in one location, with consequent potential damage to the aquatic ecosystem. Conversely, elsewhere, the "total" standard might be greatly overprotective, leading to unwarranted restrictions on water use or industrial development and possible adverse effects on the local economy.

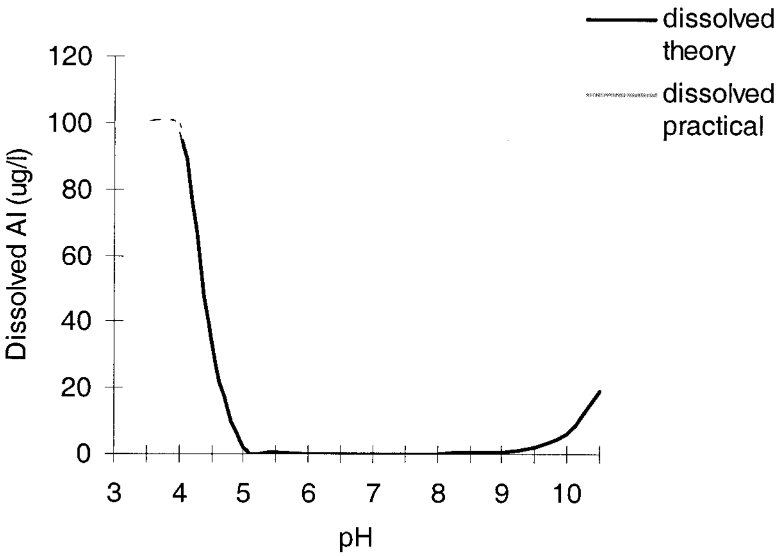

Figure 1. Predicted versus neasured concentration of "dissolved" aluminium for a total concentration of 100 μg/l.

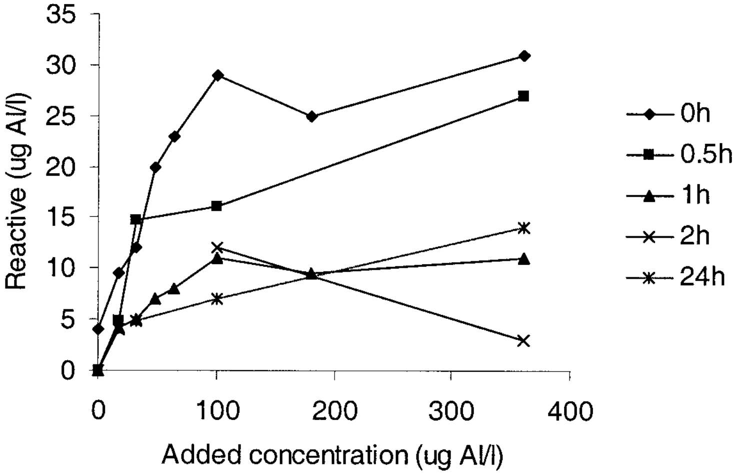

Figure 2. Measured concentrations of reactive aluminium versus added aluminium at pH 6.0

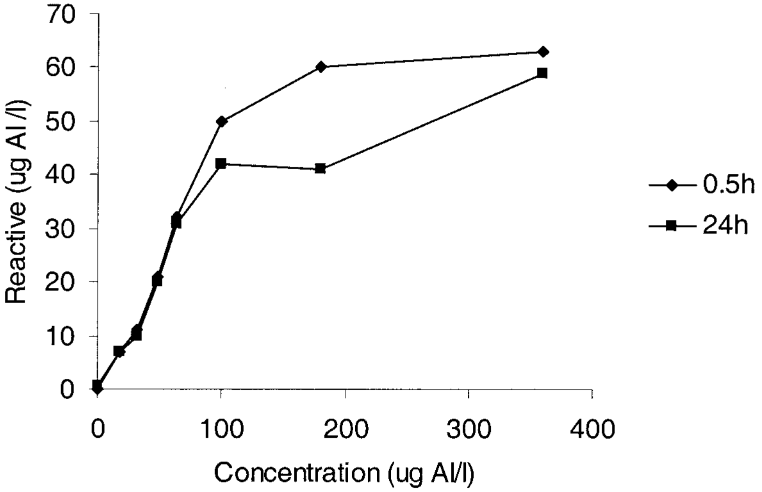

Figure 3. Measured concentrations of reactive aluminium versus added aluminium at pH 7.5

These considerations have led to the definition in the UK of pH-dependent standards, in two pH bands, expressed in terms of reactive aluminium

Speciation needs careful consideration in the design of toxicity tests. In the above example, the marked differences between nominal and reactive aluminium concentrations indicated that the organisms were exposed to a sawtooth regime of concentration of reactive metal. Only on replenishment with freshly spiked samples, and then only for seconds or minutes, did exposure to reactive aluminium approximate to the nominal levels. Care is needed to ensure that the form of metal used in toxicity tests is similar to that present in the waters of interest. This implies: (i) the conditions under which toxicity tests are conducted, including the aluminium species present, and possible ligands which may affect speciation, should be monitored; (ii) the test should be carried out using a water sample of matrix (or matrices) similar to that of the water(s) of interest; and, (iii) the potential for exchange (during the test) between one form and another should be examined.

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