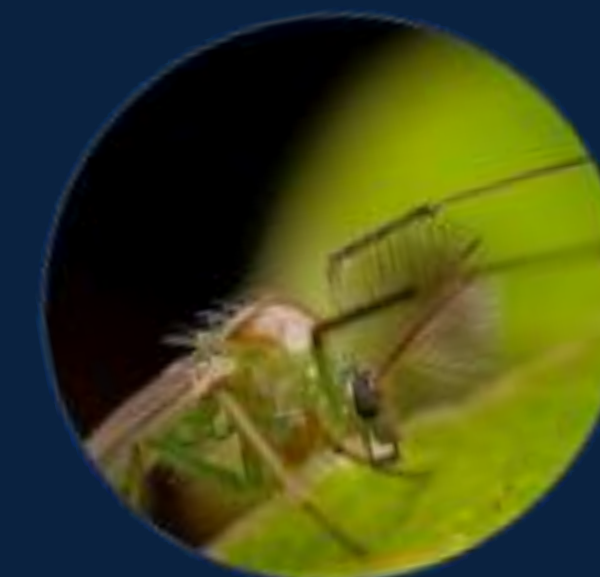


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Abstract

EMA Environmental Risk Assessment requirement guideline (2024), indicates that tests should be conducted at a stable pH consistent with the most bio-available form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). However, what if the required pH is close to or outside the recommended pH values for the test species? How far should this be taken before the performance of the test is potentially compromised? And, how practical is this with multiple compartment test systems?



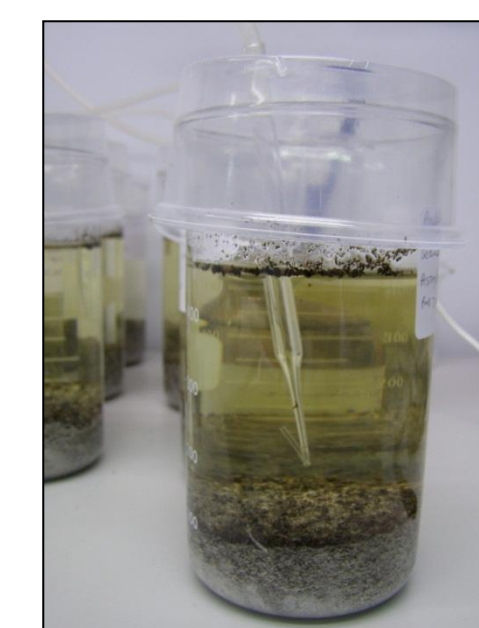
Introduction

Aquatic ecotoxicology testing guidelines generally state a pH range that should be used. For tests which employ an aqueous phase only, pH adjustment is relatively simple especially for tests conducted using static or semi-static dosing methods. Continuous flow-through systems are more problematic but possible. However, can this also be achieved and maintained for tests using multiple compartments, for example, the OECD 218 which consists of both sediment and aqueous phases?

An initial test was conducted to investigate the effect of using a pH at the lower end of the guideline recommended range. The pH of the medium was adjusted to pH 6.3. The sediment was not adjusted outside the guideline recommended pH but was determined to be pH 6.5. After a 48-hour settlement period (without aeration), pH of overlying water was 7.5 indicating that pH adjustment of the medium only was not sufficient to maintain an overlying water pH of approximately 6. Trials were therefore conducted to investigate how a stable pH of approximately 6 could be maintained and the potential effect on organisms.

Standard OECD 218 guideline requirements

- Sediment pH – 7.0 ± 0.5 (adjusted with CaCO_3 as required).
- Sediment organic carbon (OC) content – $2 \pm 0.5\%$ (adjusted with peat/sand whilst remaining within the required 4-5% peat and 75-76% sand constituent ratio)
- Overlying water pH – 6 – 9 throughout the duration of the test
- Final midge emergence in each control replicate should be $\geq 70\%$



pH adjustment for multiple compartment exposure systems is possible but may adversely affect organism performance and require deviation from guideline requirements

Materials And Methods

Trial 1

- Given that an increase in 1 pH unit was observed after 48 hours when only the media pH was adjusted, the pH of both the sediment and medium was adjusted
- pH of <6 was used to investigate pH shift during the 48-hour settlement period
- pH of the medium and sediment was adjusted to approximately pH 5 (4.99 for medium, 5.19 for sediment)
- Three vessels were prepared as for a standard OECD 218
- Maintained under test conditions without aeration to minimize potential pH increase
- pH of overlying water and sediment was measured after 24 and 48 hours

Trial 2

- The sediment and medium were adjusted to approximately pH 6 (medium: 6.00, sediment: 6.07)
- Four vessels were set up as per OECD 218 and left to settle for 48-hours
- After the settlement period, 20 first instar *Chironomus riparius* larvae, from egg ropes cultured in pH adjusted media, were added to each vessel
- Midge emergence was monitored over a 28-day period
- Organism performance was compared to historical control data (HCD) from five most recently conducted OECD 218 studies

Results

Trial 1

Vessel number	Time point (hour)	Overlying water pH	Sediment pH
NA	0	4.99	5.19
1	24	5.03	5.04
2	24	5.06	NA
3	24	5.03	NA
2	48	5.06	5.00
3	48	5.05	5.02

Table 1: Overlying water and sediment pH values over a 48-hour period.

In the absence of aeration, the pH of the sediment and overlying water remained consistent over the 48-hour settlement period. The results indicated that providing both sediment and media are adjusted to a similar pH, the pH can be maintained over a 48-hour period such that organisms could be exposed to the desired pH on addition.

Trial 2

The pH of the overlying water at organism addition was measured to be 6.14 to 6.18.

pH adjustment of the sediment resulted in an organic carbon content of 1.3% which was below the guideline recommended minimum of 1.5%. Additional peat could not be added as the maximum 5% had been used in the sediment mixture.

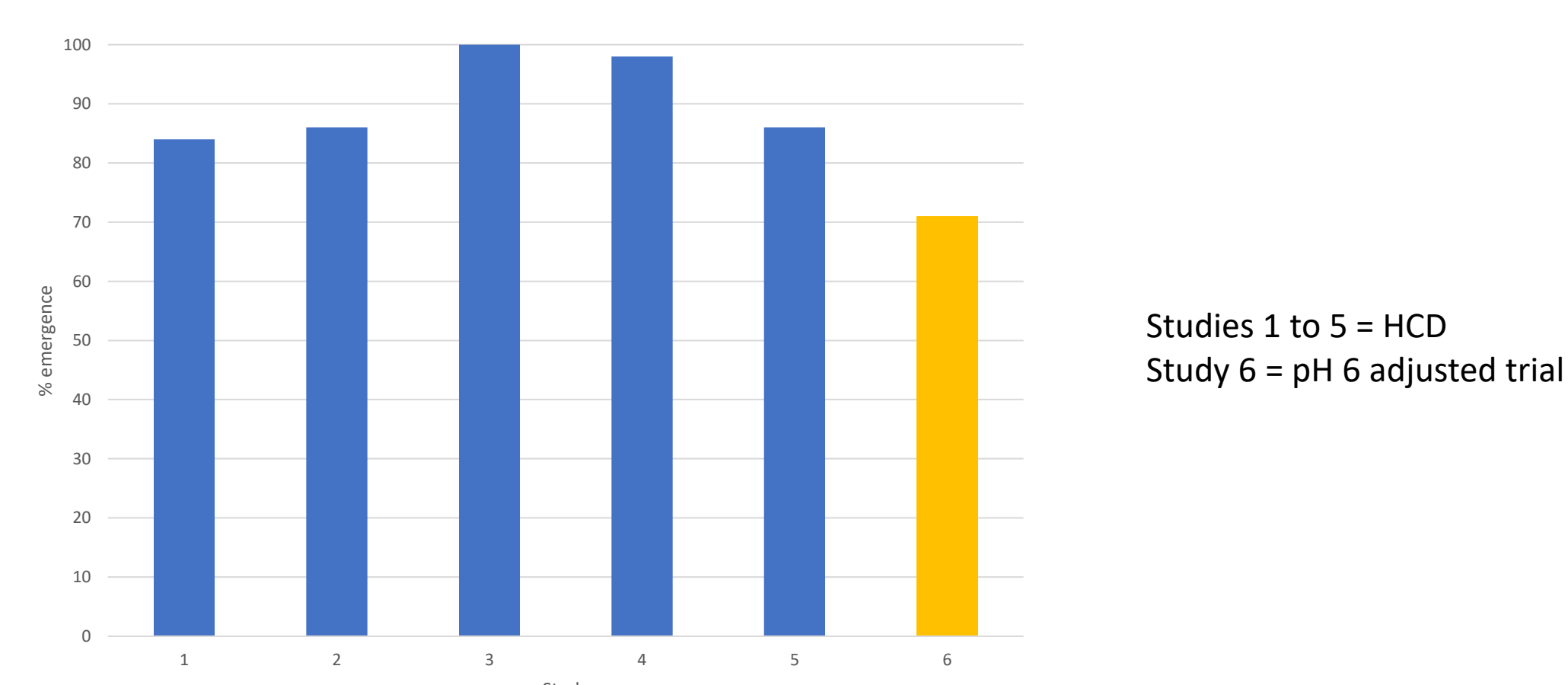


Figure 1: Midge emergence from pH adjustment trial and historical control data.

Mean midge emergence was 71 compared to >80% observed from the HCD. In addition, not all replicates achieved $\geq 70\%$ (60 – 85%) which would fail the OECD 218 validity criterion.

CONCLUSIONS

- pH adjustment of both medium and sediment may be required to achieve and maintain the desired starting pH
- It may be necessary to adjust the pH of the sediment outside the guideline range of 7.0 ± 0.5
- pH adjustment of the sediment may also result in a final organic carbon content outside the range required by the test guideline
- Organism performance may be affected at low pH

FUTURE CONSIDERATIONS

- Is it possible to adjust the pH of the sediment and still remain within the test guideline requirements?
- Will similar issues be observed if testing at a higher pH is required?
- Would it be necessary to culture test organisms in low or high pH environments to maintain performance within guideline requirements?

