EXPERT OPINION ON THE DERIVATION OF A PNEC SILVER FOR FRESHWATER SEDIMENTS

INTERNAL DISTRIBUTION ONLY

15-09-2017

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1. INTRODUCTION

Assessing risks from metals for the sediment compartment is quite often hampered by the fact that no clear relationship has been established between measured total concentrations of metals in sediments and their potential to provoke toxic effects on aquatic life. As a result comparing environmental concentrations expressed on a dry or wet weight basis with an established safety level has the potential to result in an under or overestimation of the associated risk. The observed toxic effects of silver and metals in general for the sediment compartment is governed by (1) the choice of the metal salt used for testing, (2) sediment type and composition and (3) test design.

Different stakeholders have derived a PNEC sediment for silver based on information available in the public domain and/or information from newly generated toxicity data. Table 1 summarizes the different PNECs derived.

Table 1: overview Ag sediment PNEC for the freshwater sediment compartment as derived by the different stakeholders.

Stakeholder	PNEC sed	Comments
UK environment agency (UK EA)	1.2 mg/kg dry wt.	Based on 10d test with *Hyalella azteca* and AF of 10
Precious Metal Consortium (PMC)	438 mg/kg dry wt.	Based on 10d test with *Hyalella azteca* and AF of 10 but PNEC normalized to a sediment with a 5% organic carbon content
European Silver Task Force (ESTF)	9.58 µg/kg wet wt.	Based on 28d test with *Lumbriculus variegatus* and AF of 10. Is in fact calculated as PNEC suspended solids

A large discrepancy is observed between the different PNECs (order of magnitude) even when the starting point was the same study. The present expert opinion aims to critically review the data quality of the key ecotoxicity studies underpinning the PNEC and assessess the validity of the approaches/assumptions being used.

2. CRITICAL REVIEW DIFFERENT APPROACHES

2.1. PNEC SEDIMENT DERIVED BY UK-EA

Used toxicity data

The UK-EA PNEC sediment was based on a 10 day freshwater sediment toxicity test with the amphipod *H. azteca* (Call et al, 2006). Silver nitrate was spiked into two natural lake sediments. Both representative of low binding capacity sediments (TOC < 1 % and AVS < 1.1 µmol/g dry wt.). A flow-through test design was used. Equilibration time was 7 days. Growth was the most sensitive endpoint yielding a NOEC of 12 mg Ag/kg dry wt. (nominal value). Applying an assessment factor (AF) of 10 results in a $PNEC_{sed}$ of 1.2 mg Ag/kg dry wt.

Comments

- The 10 days test with *H. azteca* is labelled by UK EA as a long term toxicity test while actually 10 days is considered to be an acute exposure. For *H. azteca* long-term tests are 28-42 days.
- Sediment concentrations were not measured and hence nominal values have been used.
- As the exposure duration is acute this test should not be used for deriving a long term PNEC sediment.

2.2. PNEC SEDIMENT DERIVED BY PMC

Used toxicity data

The PMC PNEC sediment was based on a 10 day freshwater sediment toxicity test with the amphipod *H. azteca* as described in the paper of Call et al. (2006). Silver nitrate was spiked into two natural lake sediments. Both representative of low binding capacity sediments (Toc < 1 % and AVS < 1.1 µmol/g dry wt.). A flow-through test design was used. Growth was the most sensitive endpoint yielding a NOEC of 12 mg Ag/kg dry wt. (nominal value). Applying an assessment factor (AF) of 10 results in a PNEC of 1.2 mg Ag/kg dry wt. for a sediment with an OC content of 0.29 %.

Although the same key study was used by the UK EA the derived PNEC was further normalized to a reference sediment with 5 % OC. The normalization was based on the observation that the response of *H. azteca* and also *Chironomus tentans* demonstrated a clear relationship between OC and NOEC. Since the $\text{PNEC}_{\text{sed,ref}}$ is based on the effects seen in *H. azteca* the normalization equation was restricted to the *H. azteca* data only as reported in Call et al (2006) and Hirsch (1998):

$\text{PNEC}_{\text{sed,OC}}$ (mg/kg dry wt) = (955.14 XOC – 394.2)/10

As the default OC concentration used in EUSES is by default 5 % this value has been used to set the final PNEC resulting in a PNEC_{sed} (5 % OC) of 438.13 mg kg dry wt.

Comments

- As indicated previously the 10d test result should be considered as an acute value and should not be used to derive a long-term PNEC for the sediment compartment.
- The scientific justification for the organic carbon normalization equation is only based on three data points, 2 from the study of Call et al. (2006): one with an OC content of 0.29 % and one with an OC content of 2.5 %. Both sediments were spiked with silver nitrate. The NOEC for the lowest OC was 12 mg Ag/kg dry wt. The NOEC of the sediment with the higher OC content was 2150 mg Ag/kg dry wt. PMC explains the large difference due to the difference in OC content but other sediment parameters such as clay content, Fe content and Acid Volatile sulphide (AVS) concentrations (known to form insoluble silver sulfide complexes) are also substantially greater in the sediment with the highest NOEC value. From the results in fact no single factor could be determined for the partitoning of Ag from the sediment to the water. The third point used for the organic carbon normalization was taken from a study by Hirsch (1998). The sediment toxicity tests resulted in an unbounded NOEC value of > 753.3 mg Ag/kg dry wt. for an OC content of 1.63 %. However this result should not be used in the equation as the sediment was not spiked with silver nitrate but with silver sulphide. This is a speciation form that is very insoluble and as a result Ag is almost not

bioavailable. This result should therefore not be used to demonstrate the importance of organic carbon in mitigating silver toxicity in sediments. So only two data points are left which is insufficient to make a scientific justification for the use of an organic carbon normalized PNEC. In fact, as Berry et al. (1999) showed for the marine amphipod *Ampelisca abdita* AVS predominantly controlled silver toxicity in sediments. The use of the SEM-AVS[1] model has already been utilized in several EU-Risk assessments (e.g. copper, cadmium, lead, nickel and zinc) to address the inherent fundamental deficiencies of dry- or wet weight assessment approaches. As silver has a very high affinity to bind with sulphides this approach warrants further exploration to be used in a risk assessment context of silver in sediments.

2.3. PNEC SEDIMENT DERIVED BY ESTF

Used toxicity data

In the draft CAR a sediment PNEC of 9.58 µg Ag/kg ww has been reported. This result was based on a 28 d *L. variegatus* study performed on an artificial OECD sediment with no AVS and an OC content of approximately 2 % (not measured). spiked with silver sulphate and applying an AF of 10. The test sediment containing the test item was prepared in each test vessel separately. Stock solutions prepared in deionised water were added to the sediment directly and mixed. After 48 h equilibration time, tests were conducted with the oligochaete *L. variegatus*. Test organisms were fed contaminated food (Urtica) during the test and overlying water was not renewed (static test design).

Comments

- In the test report provided to ARCHE Consulting a NOEC of 0.332 mg/kg wet wt. (0.441 mg/kg dry wt.) has been reported for the growth endpoint. Applying an AF of 10 would yield a PNEC of 0.033 mg/kg wet wt or 0.044 mg/kg dry wt. However, in the CAR the PEC is calculated for freshly deposited particulate matter. As a consequence the NOEC derived from tests with settled sediments has been transformed in the CAR document to reflect freshly deposited matter with lower density and lower fraction of solids. This results in a theoretical derived $\text{PNEC}_{\text{suspended-solids}}$ of 9.6 µg Ag/kg dry wt. which is much lower than the actual PNEC values when derived on the NOEC expressed as dry wt. This $\text{PNEC}_{\text{suspended-solids}}$ should not be used for a comparison with truly measured silver concentrations in sediments. Only with modelled data such as $\text{PEC}_{\text{suspended-solids}}$.
- Concerns can be raised with regard to the spiking method and more in particular the short equilibration time used (48h) which could lead to unrealistic high metal concentrations in the pore water and overlying water. Reported Ag concentrations in the overlying water ranged by the end of the test (28 days) between 4.4-12.7 µg/L. Clearly it cannot be ruled out that toxicity was caused via the overlying water. Such high concentrations will not be found under real field conditions where the flowing overlying water column has a much larger dilution effect preventing built-up of silver. These technical problems associated with spiking sediments with metals have been discussed by several authors. Incomplete equilibrium between spiked Ni and NiS for example lead to unrealistic toxicity thresholds for the Ni in sediment to oligochaetes as overlying water accumulated toxic Ni

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[1] Acid Volatile Sulfides (AVS) and Simultaneously Extracted Metals (SEM) are operationally defined parameters that are readily extracted by the cold extraction of sediment in 1 M HCL acid.

concentrations during testing (Vandegehuchte et al, 2007). Apparently this problem occurred primarily because Ni concentrations in pore water were elevated (caused by incomplete equilibration and the frequency of overlying water replacements were low) (Brumbaugh et al, 2013). Brumbaugh et al (2013) recommends a 4-10 weeks equilibration time because of slow reaction kinetics with AVS combined with 8 overlying water volume additions and a 7d- pretest equilibration method to allow environmental realistic testing of metals in sediments. Simpson et al (2004) demonstrated also that sediment spiked with Ni required a relatively long time for equilibration up to 70 days. These equilibration times were less for metals that have a higher affinity. But still 15 days were needed for Cu, 40 d for Zn and 45 d for Cd. In the current test for Ag an equilibration time of only 2 days has been used. In the OECD protocol actually an equilibration time of 48 h to 7 days is recommended to minimise degradation of the test chemical (OECD, 2007). However, it is stated that depending on the purpose of the study, e.g. when environmental conditions are to be mimicked, the spiked sediment may be equilibrated or aged for a longer period. As Ag does not biodegrade and for metals in general long equilibration times are needed, there is a concern that the current study does not reflect environmental realistic partitioning of Ag in spiked sediments.

- Recently, another study with the oligochaete *L. variegatus* was performed to test the toxicity of silver nanoparticles and silver nitrate in both artificial and natural sediments (Rajala et al, 2016). The results of the silver nitrate spiked sediments showed that the toxicity of Ag spiked as $AgNO_3$ was more toxic in artificial sediment than in the natural sediments (two lake sediments). While mortality was observed in all tested Ag concentrations with the artificial sediment (lowest measured Ag concentration = 11 mg Ag/kg dw) no mortality was observed in the natural sediments at the highest test concentrations (i.e. on average 400 mg Ag/kg dw), clearly showing the importance of taking bioavailability into account. Reproduction decreased with increasing $AgNO_3$ concentrations in all of the tested sediments but again less outspoken in the natural sediments.

3. CONCLUSIONS

A limited number of whole sediments ecotoxicity tests are available for silver. Most of them are acute tests and cannot be used for deriving a long term PNEC for the sediment compartment. The long term test with *L. variegatus* used by the ESTF is flawed as the applied equilibration period of 48 hours did not allow a proper environmental realistic partitioning of Ag in spiked sediments yielding PNEC values that may be overly conservative due to increased Ag concentrations in the overlying water. The recent study of Rajala et al (2016) where the toxicity of Ag has been compared between natural field sediments and artificial sediments spiked with Ag clearly emphasize the importance of taking bioavailability into account. Therefore, we would reiterate that the SEM-AVS approach is a valid scientifically justified approach to be used for assessing risks of Ag in the sediment compartment. Even more as it is known that Ag is one of the cationic metals with the highest binding affinity for sulfides present in sediments. **It is recommended that well-designed long term whole sediment tests are conducted with different benthic species using natural sediments low in AVS and OC.** Spiking conditions should allow enough equilibration time and overlying water should be frequently renewed to avoid built-up of toxic Ag concentrations in the overlying water.

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