COPPER DEFICIENCY IN AQUATIC ORGANISMS: HOW SHOULD THIS BE CONSIDERED AS PART OF THE PPP RISK ASSESSMENT?

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Background and Aims

The EU regulation for authorising plant protection products (Regulation (EC) 1107/2009) uses a tiered risk assessment approach for aquatic organisms. Under this approach, if an active substance fails the lower-tier, more conservative risk assessment, then intermediate or higher tier approaches can be used to demonstrate low risks to aquatic organisms. Higher tier refinements can include the derivation of toxicity endpoints from more realistic test systems, such as mesocosm studies. Assessment factors (AFs) are then applied to those toxicity endpoints to derive a regulatory acceptable concentration (RAC) for use in the risk assessment. There is some flexibility in terms of the AF value applied by regulators to derive the RAC, which can increase or decrease the conservatism of the risk assessment.

Copper is widely used in a variety of industrial applications as well as for plant protection products and it has been documented that Copper is toxic at high concentrations (worst case). However, Copper is also an essential nutrient that is required at low concentrations and if these levels are too low this may cause adverse effects (deficiency). In regulatory risk assessments the focus is usually based on toxicity at these high levels but there is little or no consideration for the impacts of deficiency at lower levels. For example, would the AF assigned by regulators drop the RAC below the required level for normal growth and development and should deficiency be considered by regulators for nutrients such as copper? This issue of essential metals having a U-shaped dose-response curve is mentioned in the EFSA statement on transition metals (EFSA 2021[1], an example is presented in Figure 1, Oort 2019[2]), but not in the context of AF-setting.

Based on this, a literature search was conducted to investigate the evidence available for copper deficiency, with particular focus on the critical taxa of aquatic invertebrates and fish. This poster discusses if this could then be used to support use of a reduced assessment factor in the aquatic risk assessment for an essential nutrient such as copper.

U-shaped curve

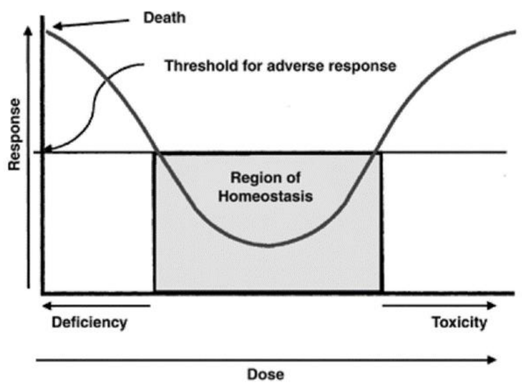


Figure 1. Deficiency and toxicity regions for essential metals

Literature review

- In total, 224 references were screened for relevance in determining the threshold of effects of copper deficiency in freshwater aquatic organisms.
- Of the references screened, only 8 (4%) were considered relevant enough to prepare full evaluations. 151 (67%) were not considered at all relevant for the literature review and 65 (29%) were only partially relevant and therefore only useful information was extracted for general discussion.

Key findings on copper deficiency

- Decreases in reproduction, energy reserves and filtration rates in *Daphnia magna* (cladoceran, Bossuyt and Janssen [3]) at levels of 0.5 µg Cu/L
- Decreased biomass, reduction in growth rate in *Pseudokirchneriella subcapitata* (green algae, Bossuyt and Janssen [3]) and *Thalassiosira oceanica* (oceanic diatom, Figure 5, Kong and Price 2022[4]) at levels of 0.5 µg Cu/L and < 2.5 nM, respectively.
- Reduced growth rate in an aquatic macrophyte species (*Ceratophyllum demersum,* Thomas et al 2016[5]) at levels below 0.3 nM
- Optimal copper concentrations of between 1 and 35 µg Cu/L for *D. magna* and *P. subcapitata* (Table 1 and Figure 2, Bossuyt and Janssen [3])
- Delayed reproduction and increased mortality (Figures 3 and 4, Isaac and Wang [6]) at levels of 0 g Cu/L
- Reduced body weight, weight gain and specific growth rate and increased feed conversion rate in yellow catfish (Chen et al 2015[7]) at levels of 0.76 mg Cu/kg diet

Table 1. The chronic 50% and 10% effective concentration (21-d EC50, 21-d EC10) based on net reproduction with 95% confidence intervals for first-generation (F1) and sixth-generation (F6) *Daphnia magna* acclimated to six different background copper concentrations

Acclimation concn. (µg Cu L^{-1})	21-d EC50 (µg Cu L^{-1})		21-d EC10 (µg Cu L^{-1})	
	F1	F6	F1	F6
0.5	68.5 (63.9–73.3)	48.0 (47.9–48.0)	56.1 (42.7–73.8)	46.5 (46.5–46.6)
1	70.2 (69.7–70.8)	56.4 (52.6–60.3)	57.1 (56.1–58.1)	50.2 (45.7–55.3)
5	72.7 (72.6–72.7)	67.6 (66.3–68.9)	70.5 (70.3–70.7)	56.2 (49.9–63.2)
12	65.2 (59.5–71.5)	55.6 (52.4–59.3)	45.9 (35.4–59.5)	38.7 (34.4–43.5)
35	70.5 (66.1–75.2)	70.7 (53.0–94.3)	51.9 (44.2–61.0)	45.6 (20.5–101.4)
100	74.6 (65.4–85.0)	78.8 (66.3–93.6)	68.2 (44.9–103.4)	51.1 (33.4–78.0)

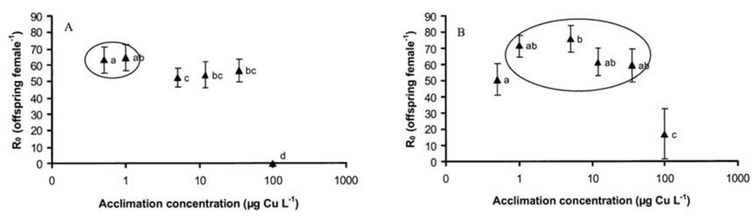


Figure 2. Net reproduction (R_0) of acclimated daphnia in first (A) and sixth (B) generation. Values with same letter are not sig dif. Ovals indicate optimal concentration area.

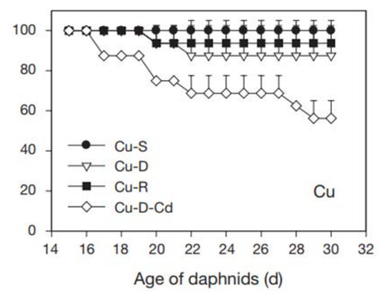


Fig. 3. *Daphnia magna*. Total neonates produced per adult during one single generation. S: sufficient; D: deficient; R: recovery from trace element deficiency; D-Cd: deficient with 1 µg Cd/L added. Mean ± SD (n = 3 replicates, 10–15 ind. per replicate)

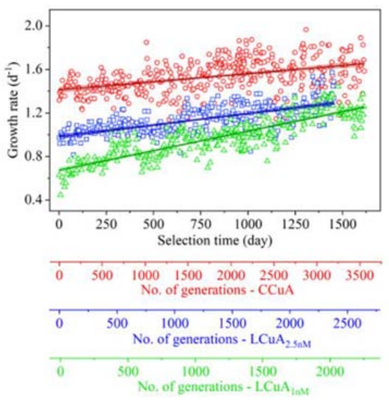


Fig. 5. Specific growth rate (d-1) of *T. oceanica* as a function of selection time in Cu-sufficient (red), Cu-deficient medium containing 2.5 nM (blue) or 1 nM (green) .

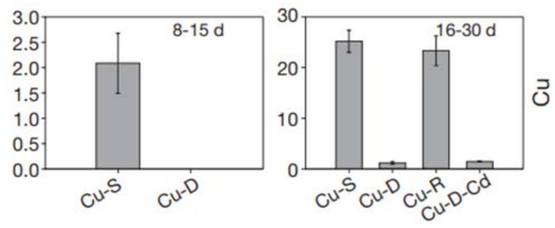
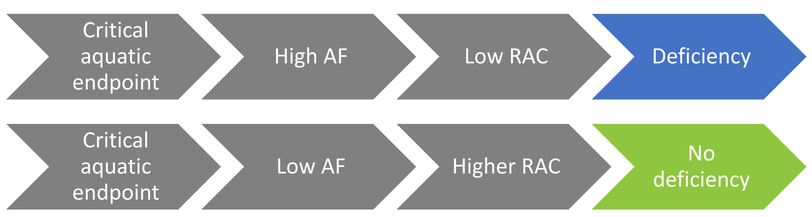


Fig. 4. *Daphnia magna*. Survivorship of daphnids maintained under different trace element conditions. S: sufficient; D: deficient; R: recovery from trace element deficiency; D-Cd: deficient with 1 µg Cd/L added. Mean ± SD (n = 3 replicates, 10–15 ind. per replicate)

Importance of deficiency for AF setting



Conclusions

- It is clear from the literature that adverse effects can occur from insufficient copper being available to aquatic organisms, which is to be expected for an essential metal. Effects of copper deficiency have been studied in aquatic invertebrates, primary producers (algae and macrophytes) and fish. Copper deficiency can result in various effects including reduced growth, reduced reproduction and even increased mortality, indicating the wide role of copper in organism physiological functions and biochemical reactions.
- Experimental designs in the available literature studies are variable in terms of exposure concentrations, analytical methods and exposure durations. Several studies used nominal zero copper as the deficient condition given the difficulty in exclude all copper from the media. This could indicate that deficiency levels may be higher than literature suggest given background levels in water and or food that can not be easily removed.
- Due to the limited number of relevant studies available, it is not currently possible to identify a specific threshold for copper deficiency in aquatic organisms, however, given adverse effects have been observed in studies regarding deficiency, this is an area that requires further research such that this can be considered when defining the RAC for essential metals such as copper.

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