What drives alga toxicity of primary fatty amines?

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Introduction

Primary fatty amines (PFAs) are high production chemicals classified as "difficult-to-test-substances" according to OECD Guidance Document 23[1]. At environmental pH levels, PFAs exist as cationic surfactants, making it challenging to maintain stable concentrations in environmental studies due to micelle formation and adsorption to test vessels and biological materials like algal cells. While standard testing typically relies on dissolved concentrations for evaluation, this approach may not be suitable for positively charged surfactants. The adsorption of PFAs to test organisms likely increases local concentrations rather than decreases them, contrary to what dissolved fraction measurements suggest. To understand the actual distribution of PFAs, an alga toxicity study using radiolabeled primary fatty amine was performed.

Method

A growth inhibition test was conducted with the green alga *Raphidocelis subcapitata* following OECD Test Guideline 201[2]. The experimental setup included parallel testing of replicates with and without algae. Radioactivity was analytically determined in the water phase, at the glass vessel walls and after centrifugation at the centrifugation tube walls, in the supernatant and in the algal pellet. Despite testing a mono-constituent substance here, water accommodated fractions (WAFs) were prepared to align with existing UVCB studies.

Test Design	
Substance	Octadecylamine [1-14C] hydrochloride, radiochemical and chemical purity: 99%
Test vessels	Erlenmeyer flasks, filled with 100 mL
Lighting	5938 Lx , continuously
Shaking	70 rpm, continuously
Replicates	3 per loading rate, 6 per control group

WAF Preparation	
Application	On inner glass walls
Volume	2 L
Stirring time	24 h
Speed	60 rpm
Settling time	1 h
Loading rates	25.7 – 53.9 – 113 – 238 – 500 µg/L

Analysis	
Detection	Liquid scintillation counting
System suitability	Daily performance test
Limit of quantification	6.05 Bq
Counting terminator	Until 2 x standard deviation of the counted disintegrations is <0.5%, but max. 20 min.

Results

- Share of substance in WAF very low compared to initial amount (< 10%).

Recovery in Test Medium

- In replicates with algae (Fig. 1):
  - Total substance amount (dissolved + algae-bound) remained stable over time and loadings.
  - Initial decrease observed due to vessel adsorption.
  - As algal density increased, substance transferred from glass surfaces to algae.
- In algae-free replicates (Fig. 2):
  - Continuous decrease in dissolved substance concentration.

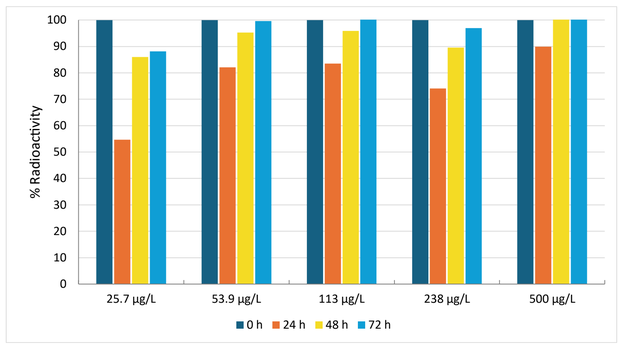


Fig. 1: Substance recovery in test medium – with algae

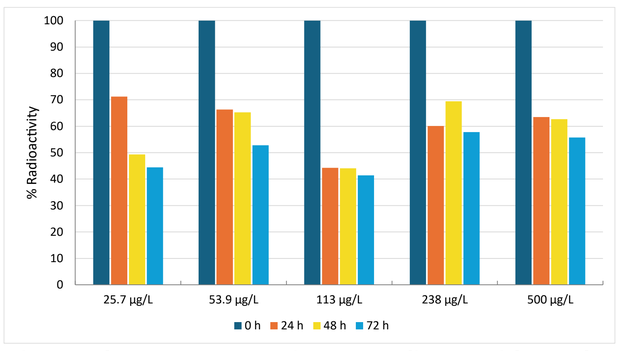
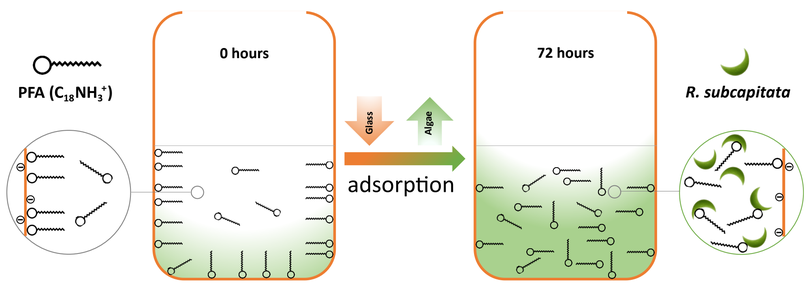


Fig. 2: Substance recovery in test medium – without algae

What happens during exposure?



Mass Balance Analysis

- Vessel adsorption showed high variability without clear patterns.
- Inconsistent data prevented reliable evaluation of glass wall adsorption.

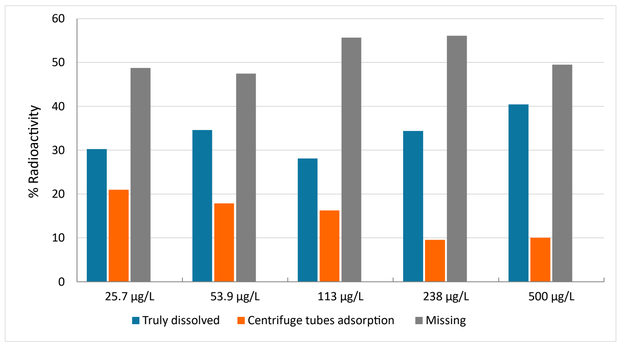


Fig. 3: Distribution of applied radioactivity after 0 hours

Substance Distribution after 72 hours

- Algal adsorption: 17-29% of total substance bound to algae. (Fig. 3, 4, 5)
- Centrifugation tube adsorption: Inversely correlated with algal growth. (Fig. 3, 4, 5)

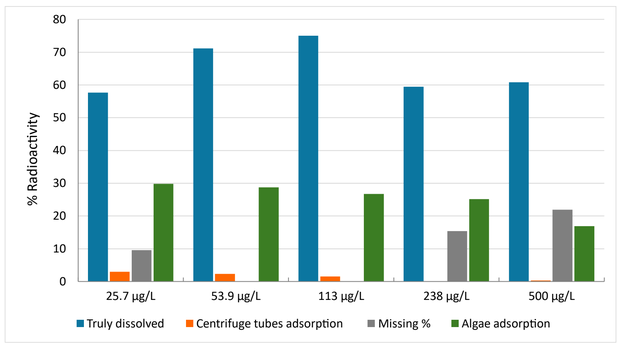


Fig. 4: Distribution of applied radioactivity after 72 hours

Adsorption to Algae

- Strongly increases with cell growth. (Fig. 5)

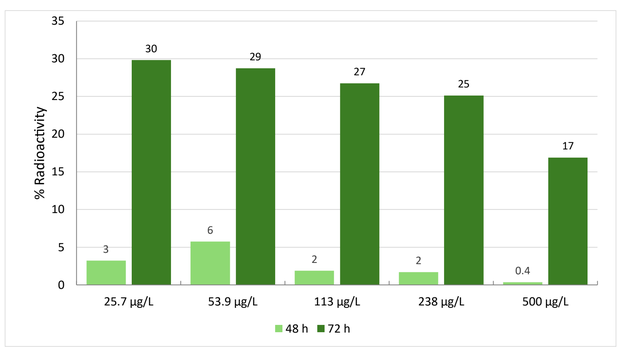


Fig. 5: Radioactivity adsorbed to algae (48 & 72 hours)

Conclusion

The study revealed significant adsorption of primary fatty amines (PFAs) to algal cells and test vessels. Since cationic surfactants exert their toxic effects through cell membrane disruption, the adsorbed portion actively contributes to toxicity. This results in local concentrations at the organism surface must be added to truly dissolved concentrations in the test medium. Therefore, basing toxicity assessments solely on truly dissolved concentrations is inappropriate for cationic surfactants like PFAs.

Our findings demonstrate a dynamic system where increasing algal biomass leads to higher substance attachment to organisms and reduced vessel adsorption. Further research should focus on determining the bioavailability of the adsorbed substance fraction to fully understand the toxicity mechanisms of PFAs.

References

[1] OECD Series on Testing and Assessment No. 23 (second edition): Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Substances, 2019.

[2] OECD Guidelines for the Testing of Chemicals 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, 2006/2011.