Algal and Cyanobacterial Toxicity of Four Antibiotics for Human Clinical Use against Gram-Positive Bacteria

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Introduction

In environmental waters, the primary producers are potentially being affected by low concentrations of antibiotics, but show significant species-specific differences in sensitivity. This makes it difficult to select the best representative organisms for the regulatory Environmental Risk Assessment (ERA) testing. Due to the large interspecies differences, the European Medicines Agency (EMA) guidance draft of 2018 foresees newly a tailored risk assessment testing course for antibiotics consisting of growth inhibition tests (OECD Testing Guideline (TG) 201, 2011) with two cyanobacterial species (*Anabaena flos-aquae* and *Synechococcus leopoliensis*) and one green algal species (*Raphidocelis subcapitata*). Also a daphnid reproduction test (OECD TG 211, 2012) is still foreseen, while experimental data from the fish are not any more considered necessary.
A literature survey performed by the authors did not retrieve full datasets in agreement with the new guidance draft for the majority of antibiotic substances, selected by their use volume. As such overview would be particularly interesting in borderline antibiotic cases, where due to the clinical spectrum no indication for (gram-negative) cyanobacterial toxicity is given, the lacking endpoints of four such antibiotics, i.e. Clindamycin, Flucloxacillin, Linezolid and Metronidazole, were determined experimentally.

Material and Methods

The clinical spectrum defined for the medical practice of antibiotic treatments distinguishes between the two major groups of bacteria, i.e. gram-positive and gram-negative ones, depending on the structure of their cell envelope and indicated by the outcome of the gram staining method. As cyanobacteria are gram-negative, strong effects of antibiotics clinically recommended for the treatment of infections caused by gram-negative bacteria seem likely (Wess et al. 2020). Consequently, two out of the tree suggested species in the new tailored assessment approach for antibiotics in the EU (EMA 2018) are cyanobacteria.
In order to test the appropriateness of the suggestions in the new guideline, we selected four antibiotics, clinically not recommended for infections usually caused by gram-negative bacteria (Clindamycin, Flucloxacillin, Linezolid and Metronidazole). For those indication for testing specific (gram-negative) cyanobacterial toxicity is not self-evident. Therefore, the intention was to see whether the new scheme is appropriate also in such a case (Wess et al. 2020).
No full data set as foreseen in the guideline update (EMA 2018) could be compiled based on literature data. Therefore new experiments were performed by Loetscher (2019) to provide lacking data points for two antibiotics (clindamycin and linezolid) and two completely new data sets for another two antibiotics (flucloxacillin and metronidazole).
The Literature data used were evaluated suitable for ERA according to the schemes of Klimisch et al. (1997) and Küster et al. (2009). The tests of Loetscher (2019) were conducted in compliance with OECD TG 201 (OECD 2011):
- Test species: *Raphidocelis subcapitata* (green algae), *Anabaena flos-aquae* and *Synechococcus leopoliensis* (cyanobacteria)
- Abiotic conditions: AAP medium, 23 23°C, 123 123±2 rpm, CO_2 input, light intensity 60-80 µE/(m²·s)
- 6 replicates per control, 3 per test concentration
- Duration: 72 h
- Initial cell density deviation: $4x10^5$ cells/ml for *S. leopoliensis*
- Biomass monitoring by fluorescence (fluorimeter SpectraMax i3x)
- ErC10 determined by regression analysis, with ToxRat® Professional software v. 3.3.0)
- pH variation, test medium appearance and validity criteria according to OECD requirements

Using these literature data and the complementary new test results, we present four full data sets in agreement with the guideline draft requirements (EMA 2018) to enable a discussion of the new approach.

Results

Table 1. Threshold Toxicity Data Sets of Antibiotics According to the New Guideline Draft, Based on Growth Rate Effects

Antibiotic	Test species and their 72 h ErC_{10} [mg API/L]		
	Green algae	Cyanobacteria	
	Raphidocelis subcapitata	*Anabaena flos-aquae*	*Synechococcus leopoliensis*
Clindamycin	**0.00056**[Loetscher 2019; IES#20190190]	0.010[Baumann et al. 2014]	0.0024[Loetscher 2019; IES#20190121]
Flucloxacillin	>95[Loetscher 2019; IES#20190122]	**0.11**[Loetscher 2019; IES#20190123*]	7.7[Loetscher 2019; IES#20190124]
Linezolid	**0.18**[Coors et al. 2017]**	0.73[Coors et al. 2017]	0.34[Loetscher 2019; IES#20190125]
Metronidazole	13[Loetscher 2019; IES#20190179]	20[Loetscher 2019; IES#20190126]	**6.9**[Loetscher 2019; IES#20190127]

The internal IES study numbers are given in superscript (after the reference) for easier identification.
The values printed in bold indicate the lowest threshold concentration and thus the most sensitive species.
* Based on results of the range-finding test (0.1, 1.0, 10, 100 mg/L and control, in three replicates).
** The reference states “Pseudokirchneriella subcapitata”, the former name of the species.

Discussion

Interestingly each of the three species to be tested according to the guideline draft (EMA 2018) represents in at least one of the tested cases the most sensitive species. Such a finding in a rather small data set of antibiotics can be seen as a hint that the species selection made by the authors of the EMA draft guideline is appropriate, especially as the tested antibiotics have been designed to work against gram-positive bacteria, to which cyanobacteria do not belong to, and as green algae are typically not most sensitive to antibiotics. The green algae showed two times the highest sensitivity, however represented by only one of the three test species, which is a slight overrepresentation. Nonetheless our data do not support the hypothesis that the clinical spectrum of antibiotics may justify a differentiation in the environmental safety testing (Wess et al. 2020).

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