OECD TG 201 study results from clindamycin, linezolid, flucloxacillin and metronidazole tests

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Introduction

Pharmaceutical residues in freshwater is the subject of a 2019 issue of the OECD studies on water series. It states that mis- and over-use of antibiotics is an important contributing factor of the emergence and spread of antimicrobial resistance - a global health crisis with the potential for enormous health, food security and economic consequences. Another specific concern is that antibiotic modes of actions potentially affect the photosynthetic activity of primary producers and subsequently primary biomass production and carbon dioxide fixation. In 2018, the European Medicines Agency (EMA) has published a new draft guideline intending to implement a new tailored environmental risk assessment scheme for antibiotics. Documented threshold effect levels to three fixed representative species of green algae (*Raphidocelis subcapitata*) and cyanobacteria (*Anabaena flos-aquae* and *Synechococcus leopoliensis*) will be required. This poster presents OECD TG 201 study results from clindamycin, linezolid, flucloxacillin and metronidazole (Loetscher 2019), which complement the literature data to achieve full datasets as demanded by the EMA draft. We discuss cases of significant differences in published literature data , varying results after exposure of 72 h or 96 h and remarkably differing EC_{10} and NOEC values.

Material and Methods

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 Environmental Risk Assessments 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/(m^2·s)
- 6 replicates per control, 3 per test concentration
- Duration: 72 h, second reading at 96 h (only *Synechococcus leopoliensis*)
- Initial cell density deviation: 4x10^5 cells/ml for *S. leopoliensis*
- Biomass monitoring by fluorescence (fluorimeter SpectraMax i3x)
- ErC_{10} determined by regression analysis, with ToxRat® Professional software v. 3.3.0)
- pH variation, test medium appearance and validity criteria according to OECD requirements

Results

Using literature data and the complementary new test results, four full data sets in agreement with the guideline draft requirements (EMA 2018) are presented in Table 1.

Table 1. Full data sets in agreement with the guideline draft requirements (EMA 2018)

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*", a former name of the species.

Inconsistent Metronidazole Literature Data

Literature data for Metronidazole toxicity to the green alga *Raphidocelis subcapitata* are available from two publications, Fu et al. (2017) and Lanzky & Halling-Sørensen (1997). Table 2 gives an overview of the data from both studies and the measurements of Loetscher (2019). Due to the rather big difference of the ErC_{50} results, the credibility of the available ErC_{10} result seemed uncertain. Therefore, re-measurement has been considered.

Table 2. Overview of Metronidazole Literature and New Measured Data of Toxicity to the Green Alga *Raphidocelis subcapitata* in mg/L

Endpoint	72 h			96 h
	Lanzky & Halling-Sørensen 1997**	Fu et al. 2017	Loetscher 2019	Fu et al. 2017
ErC10	19.9	-	12.48	-
ErC50	40.4	141*	44.81	256#

* Reported as Log 1/ECr50 [M] 3.48
# Reported as Log 1/ECr50 [M] 3.22
** The reference states "*Selenastrum capricornutum*", a former name of the species.

Varying results after exposure of 72 h or 96 h

Loetscher (2019) evaluated the tests with the cyanobacterium *Synechococcus leopoliensis* after 72 h and after 96 h for the ErC_{10} and ErC_{50} of the test items Clindamycin, Flucloxacillin, Linezolid and Metronidazole. These data are shown in Table 3. Likewise the publication of Fu et al. (2017) presents data for Metronidazole after 72 and after 96 h exposure for the green alga *Raphidocelis subcapitata*, which are shown in Table 2, above.

Table 3. Toxicity Threshold levels to the Cyanobacterium *Synechococcus leopoliensis* After 72 h and After 96 h in the Same Test Run

Antibiotic	ErC_{10} [mg/L]		ErC_{50} [mg/L]	
	72 h	96 h	72 h	96 h
Clindamycin	2.39	5.13	42.29	50.94
Flucloxacillin	7.69	10.15	60.14	20.24
Linezolid	0.34	0.09	0.82	>1
Metronidazole	6.88	11.90	79.30	71.91

Significant differences between ErC_{10} and NOErC due to flat dose-response relation or lack of statistical power

Differences up to a factor of 10 may seem high, but fit in the range of inter-laboratory variability of test systems. Here we present data from the same test run. Nonetheless only data pairs differing by a factor of more then 10 are shown in Table 4.

Table 4. Overview of Metronidazole Literature and New Measured Data of Toxicity to the Green Alga *Raphidocelis subcapitata* in mg/L

Antibiotic	Species	ErC_{10} [mg/L]	NOErC [mg/L]*	Δ Factor
Clindamycin	*Raphidocelis subcapitata*	> 95.2	0.95*	>100
Linezolid	*Synechococcus leopoliensis*	0.34	0.032*	11
Metronidazole	*Raphidocelis subcapitata*	12.48	0.32*	39
	Anabaena flos-aquae	19.76	0.32*	62

* According to a Williams' multiple sequential t-test, one-sided smaller, α = 0.05

Discussion

The conflicting literature data on the Metronidazole threshold levels (Δ factor ca. 3) have been resolved in that the Lanzky & Halling-Sørensen (1997) measurement has been confirmed.
The toxicity thresholds after 96 h are similar in most cases, with sometimes higher threshold concentrations at 96 h, but also vice versa.
One more time, the use of the effect strength independent NOEC has been questioned, because significant differences depending on statistical power and/or flat dose-response relations rather than toxicity have been observed.

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