Inter-Laboratory and Inter-Exposure-Duration Endpoint Variability of Antibiotics in OECD TG 201 Studies Using the Same Test Organism

R. Arno Wess
IES Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland

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Introduction

Algal toxicity testing investigates effects on biomass (yield) and the reproduction of generations of the test organisms. Therefore, acclimation and even adaptation may occur during the test period and can accordingly alter test results significantly. Consequently, and despite the precise test protocol prescriptions of the OECD Test Guidance 201, which has been updated several times, there is variability in terms of test results from different experiments. It is a generally understood fact and considered in regulation, that the effects on the growth rate, if available, should be preferred over biomass data. Additionally test ratings are proposed in the literature and used in regulatory practice in order to justify a reliability ranking of the available references. The recent OECD TG 201 states a normal test duration of 72 hours. However, shorter or longer test durations may be used provided that all validity criteria can be met.

Materials and methods

The poster compares example results for antibiotics with test endpoints recorded in the same or different tests after 72 and/or 96 h. The order of magnitude of the inter-laboratory, inter-exposure-duration result variability and their combination is expressed by difference factors. NOEC values depend on the chosen exposure concentrations. Accordingly, the observation of Chapman et al. (1996) that agreement between laboratories was much less for NOECs (difference factors 2.2–9.0) than for the EC_{50}s (difference factors 1.2–2.2), is not surprising. NOECs are not point estimates, do not use most of the data, have no error terms, and can only be compared by their ranges of values (Chapman et al. 1996). Therefore, this poster also compares EC_{10} with NOEC thresholds, as they are sometimes considered exchangeable in regulation.
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°C, 123±2 rpm, CO_2 input, light intensity 60-80 µE/(m²·s)
- 6 replicates per control, 3 per test concentration
- Duration: 72 h, second reading at 96 h (only *Synechococcus leopoliensis*)
- Initial cell density deviation: 4×105 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

Inter-laboratory differences

Data for Metronidazole toxicity to the green alga *Raphidocelis subcapitata* are available from to two journal publications, Fu et al. (2017), Lanzky & Halling-Sørensen (1997) and the measurements of Loetscher (2019). The 72h-ErC_{10} reproduces good with an inter-laboratory difference factor of 1.6, while the ones for the 72h-ErC_{50} are 1.1, 3.1 and 3.5. Table 1 gives an overview of the data.

Table 1. Overview of Metronidazole Literature 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
ErC_{10}	19.9	-	12.5	-
ErC_{50}	40.4	141*	44.8	256#

* Reported as Log $1/ECr_{50}$ [M] 3.48
\# Reported as Log $1/ECr_{50}$ [M] 3.22
** The reference states "*Selenastrum capricornutum*", a former name of the species.

Inter-exposure-duration differences after exposure of 72 h or 96 h from identical test facilities

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 1. The inter-exposure-duration factor of Fu et al. (2017, Table 1) is 1.8.

Likewise, Loetscher (2019) evaluated 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. The inter-exposure-duration factors range from 1.1 to 3.8 and are comparable for ErC_{10} & ErC_{50} (being for ErC_{10} 1.3, 1.7, 2.2 and 3.8 and for ErC_{50} 1.1, 1.2 and 3.0). These data are shown in Table 2.

Table 2. Toxicity Threshold levels to the Cyanobacterium *Synechococcus leopoliensis* After 72 h and After 96 h in the Same Test Run

Test item	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

Combined inter-laboratory and inter-exposure-duration difference factors are more important amounting to 5.7 and 6.3 (Table 1), which is not surprising due to the expectedly larger number of test conduction differences.

Most Significant differences between ErC_{10} and NOErC, even in the Same Test Run

Differences up to a factor of 10 were observed in the same test run. Here we present data from Loetscher (2019). Only data pairs differing by a factor of more then 10 are shown in Table 3.

Table 3. Overview of Metronidazole Data of Toxicity to the Green Alga *Raphidocelis subcapitata* in mg/L

Antibiotic	Species	ErC_{10} [mg/L]	NOErC [mg/L]*	Difference 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

For statistically supported dose-response toxicity thresholds (ErC_{10} and ErC_{50}), the results after 72 and 96 h are similar in most cases (difference factor 1.1 to 3.0), with sometimes higher threshold concentrations at 96 h, but also vice versa. For rankings of different compounds, a difference factor of >3 should therefore be considered. Combined inter-laboratory and inter-exposure-duration difference factors are higher and may reach an order of magnitude relevant for regulatory purposes (classification, risk assessment). For substance rankings, toxicity thresholds should differ by a factor of 5-10 if the exposure duration was not identical.
One more time, the use of the use of the effect-strength-independent NOECs seems questioned, because significant differences depending on statistical power and/or flat dose-response relations rather than toxicity may cause the identified difference factors to EC_{10}s of >>10.

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a.wess@ies-Ltd.ch | www.ies-ltd.ch

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