Results of the validation ring test for the new Algae Growth Inhibition Test on Microplate (Draft DIN 38412-59 „Water quality – Algal growth inhibition test on microplate with unicellular green fresh water algae”) investigating toxic effects of waste water and chemical substances using *Desmodesmus subspicatus and Raphidocelis subcapitata*.

Organization of the ring test

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Dr. Marcus Lukas

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Statistical Evaluation

Dr. Monika Ratte, ToxRat Solutions GmbH & Co. KG
monika.ratte@toxrat.com

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Content

Summary

Between October 2020 and January 2021, the algae growth inhibition test on microplate (Draft DIN 38412-59) was investigated in a validation ring test. Thirteen laboratories participated (eleven from Germany, two from Switzerland). *Desmodesmus subspicatus* and *Raphidocelis subcapitata* were used as test organisms, with *Desmodesmus* being mandatory (twelve labs provided data) and *Raphidocelis* being voluntary (six labs provided data). Two natural waste water samples – one of moderate toxicity (A), one of low toxicity (B) – and two samples of chemical substances (potassium dichromate ($K_2Cr_2O_7$) and 3,5-dichlorophenol (3,5-DCP)) were investigated in a series of eight dilutions or concentrations, each. For each test, two different microplates were used, each with control, blank and positive control (PC). Each laboratory had to perform three repeated measurements per sample, to be performed on different days within a time period of a few weeks. Thereby, a data set was created that – quite unique for ring tests in ecotoxicology – allows to calculate not only inter-laboratory variability, but also intra-laboratory variability.

The objectives of the ring test were
(1) to investigate whether the proposed validity criteria are feasible,
(2) to identify a suitable reference substance and its optimal concentration and
(3) to estimate the performance and precision of the test in terms of repeatability and reproducibility for both waste water samples and samples of chemical substances[1].

For controls and substance samples, growth rates (GR) were calculated with blank corrected fluorescence data. For waste water samples, also an autofluorescence correction was applied. The validity criteria were as follows (and in some cases different from Draft DIN 38412-59):

- GR of the controls after 72 hours is $\geq$ 1.2 d^{-1} (Draft DIN 38412-59: $\geq$ 1.4 d^{-1})
- the coefficient of variation (CV) of the GR of the controls after 72 hours is $\leq$ 7 % (Draft DIN 38412-59 $\leq$ 5 %)
- the mean CV of the sectional-growth-rates (0h-24h, 24h-48h and 48h-72h) in controls is $\leq$ 35 %

Validity was assessed specifically for each microplate. For the test to be valid, both controls need to meet the validity criteria. 3,5-DCP with a nominal concentration of 2.5 mg/L was used as PC. The inhibition of GR in the PC was assessed, but not used as validity criterion, since it was one purpose of the ring test, to identify a suitable reference substance and its optimal concentration.
For 3,5-DCP and $K_2Cr_2O_7$, EC(r)50[2] values for GR were determined as toxicity measures. For waste water samples, LID[3] and EC(r)50 values were determined. The effect threshold for LID was 10% inhibition of GR, thereby, inhibitions were calculated specifically for each microplate, i.e. in relation to the respective control. EC(r)50-values and 95 % confidence limits were determined by nonlinear regression, using the pooled controls. Statistical evaluation of repeatability and reproducibility were performed according to DIN ISO 5725-2: 2002-12. Since EC(r)50s and LIDs are assumed to be log-normal distributed, all calculations according to DIN ISO 5725-2 were performed with the log transformed data. The results were retransformed according the formulas for the log normal distribution.

For *Desmodesmus*, in total, twelve laboratories provided 149 tests. After data consolidation, 130 tests of nine laboratories were considered for validity check. 39 out of 130 tests were invalid, because at least one of the two controls per test failed to meet the validity criteria (30.0 %). Thereby, the main reason for invalidity was a too high coefficient of variation of the sectional growth rate. Thus,

[1] Repeatability and reproducibility standard deviations are given as percentages. I.e. the lower the relative repeatability standard deviation and reproducibility standard deviation, respectively, the better is the repeatability and reproducibility.
[2] EC(r)50 = concentration or volume fraction of test sample which results in a 50% reduction in specific growth rate relative to the controls
[3] Lowest ineffective dilution

maintaining exponential growth (i.e. CV% of sectional growth rate $\leq$ 35 %) proved to be the greatest limitation. In contrast, more than 80 % of the controls achieved the minimum growth rate of 1.2 d^1, the median growth rate was 1.387 d^{-1}. Validity was not a basic problem, but seems to be an issue in certain laboratories: while four laboratories accounted for 33 of all 39 invalid tests (84.6 %), there were also four laboratories with always 100 % valid tests. One EC(r)50 result for 3,5-DCP and $K_2Cr_2O_7$, each, was identified as statistical outlier because of intralaboratory variability, but – according to DIN ISO 5725-2: 2002-12 – were retained as there were no other indications for outliers and to not further decrease the data base. So, depending on sample, the statistical evaluation was based on 20-24 results from nine laboratories with 1-3 test results per laboratory, with "3" being the most frequent number of results per laboratory for all samples.

For waste water A, the mean LID was 4.9 (95 % CI 3.9 - 6.2), with a repeatability of 22.6 % and a reproducibility of 31.1 %. The mean EC(r)50 was 44.9 Vol % (95 % CI 40.3 Vol % - 50.0 Vol %), repeatability was 8.5 %, reproducibility was 14.1 %. For waste water B, all laboratories reported LID values of 2 or 3, resulting in a mean LID of 2.2 (95 % CI 1.9 - 2.5) and repeatability and reproducibility standard deviations of 14.2 % and 17.0 %. Due to low toxicity, no EC(r)50 data could be determined for waste water B.
The mean EC(r)50 for 3,5-DCP was found to be 2.15 mg/L with a 95 % CI of 1.87mg/L - 2.47 mg/L. Repeatability was 7.7 %, reproducibility was 18.1 %. The mean EC(r)50 for $K_2Cr_2O_7$ was found to be 0.90 mg/L with a 95% CI of 0.77 mg/L - 1.06 mg/L. Repeatability was 15.2%, reproducibility was 20.6%.

For *Raphidocelis*, in total six laboratories provided 71 tests. Using *Raphidocelis* was voluntary, i.e. those laboratories that performed tests with *Raphidocelis* expended a considerable amount of additional effort to enable at least some orientation regarding the performance of *Raphidocelis*. Unfortunately, 44 of 71 tests were invalid (62%). It should be emphasized, that the high percentage of invalid tests obviously is not related to *Raphidocelis* as test species, but to laboratory specific performance: among the six laboratories that tested *Raphidocelis* were those four that accounted for 85 % of the invalid tests with *Desmodesmus*.
A minimum growth rate of 1.2 d^{-1} was achieved and even exceeded by far in most cases (namely in 131 out of 142 controls[4] = 92.3 %), the median growth rate was 1.565 d^{-1}. Hence, *Raphidocelis*, on average, showed higher growth rates than *Desmodesmus*. However, also for *Raphidocelis,* maintaining exponential growth (i.e. CV% of sectional growth rate $\leq$ 35 %) proved to be the greatest limitation. The number of laboratories providing valid LID and EC(r)50 results was three to five depending on sample. Therefore, neither outlier testing nor calculation of repeatability and reproducibility was performed, but geometric means for LID and EC(r)50 results and their 95% confidence limits were calculated to get an idea about the sensitivity of *Raphidocelis* compared to *Desmodesmus*.

For waste water A, the mean LID was 5.4 (95 % CI 3.5 - 8.5) and the mean EC(r)50 was 37.1 Vol % (95 % CI 27.9 Vol % - 49.2 Vol %). For waste water B, the mean LID was 2.9 (95 % CI 2.0 - 4.2), the mean EC(r)50 was 69.1 Vol % (95 % CI 49.7 Vol % - 96.2 Vol %).
The mean EC(r)50 for 3,5-DCP was found to be 2.26 mg/L with a 95 % CI of 1.60mg/L - 3.12 mg/L. The mean EC(r)50 for $K_2Cr_2O_7$ was found to be 1.13 mg/L with a 95 % CI of 0.77 mg/L - 1.66 mg/L.

Thus, the LID and EC(r)50 data obtained with *Desmodesmus* and *Raphidocelis* were similar. Moreover, the completely overlapping confidence limits argue against major differences in sensitivity between the EC(r)50 for $K_2Cr_2O_7$ of both algae species. However, due to the limited data base for *Raphidocelis*, this cannot be finally assessed.

The initial objectives can be answered as follows:

[4] Two plates used per test, i.e. 71 tests = 142 controls

(1) When the validity criteria proposed in Draft DIN 38412-59 have been somewhat adjusted (minimum growth rate 1.2 d^{-1} instead of 1.4 d^{-1} and CV % growth rate 7 % instead of 5 %), there were several laboratories which performed 100 % valid tests. Thus, the applied validity criteria proved to be feasible. If validity failed, it was more likely due to laboratory-specific issues than to general issues of the test species. Maintaining exponential growth was found to be the biggest limitation for both algae species. A closer look to practical issues with test conduction seems promising in view of improving the rate of valid tests.

(2) Only in about half of the tests with *Desmodesmus* and in about one fourth of the tests with *Raphidocelis,* the proposed range of 20 % to 80 % inhibition of growth rate in the PC (3,5-DCP) was met. An error in the preparation of the test solution was identified as a possible reason. Moreover, there are indications that the concentration of 2.5 mg/L 3,5-DCP for the PC was chosen somewhat too high: For 3,5-DCP, the mean EC(r)50 was 2.15 mg/L (*Desmodesmus*) and 2.26 mg/L (*Raphidocelis*). $K_2Cr_2O_7$ was not used as PC, but the mean EC(r)50 of $K_2Cr_2O_7$ was found to be 0.90 mg/L for *Desmodesmus* and 1.13 mg/L for *Raphidocelis*, thus a bit higher than the proposed concentration of 0.80 mg/L as reference substance.
The question of a suitable substance to be used as positive control and its optimal concentration cannot yet be completely answered, but there are arguments to keep 0.8 mg/L for $K_2Cr_2O_7$ and to somewhat reduce the proposed concentration of 2.5 mg/L for 3,5-DCP as reference substance.

(3) The data base provided by the present ring test allows to assess the reproducibility of the toxicity measures LID and EC(r)50 including all sources of variability, i.e. including both between-laboratory variance *and* within-laboratory variance (repeatability). For tests using *Desmodesmus*, repeatability standard deviations for LID and EC(r)50 data of four different samples were between 7.7 % and 22.6 %, reproducibility standard deviations were between 14.1 % and 31.1 %. These ranges are considered as quite low, i.e. argue for high precision. This is confirmed by the fact that the ratio between reproducibility standard deviation and repeatability standard deviation (sR/sr) was between 1.2 and 2.4 and thus indicates a high degree of standardization.
For *Raphidocelis*, the data base did not allow to derive precision data.

Overall, the present ring test indicates that the biggest challenge was to perform a valid test, especially to ensure exponential growth. However, there is clear evidence, that this is an issue of practical handling rather than of test species or test principle. When validity was achieved, for *Desmosdesmus subspicatus* used as test species, the results proved to be very reliable in terms of repeatability and reproducibility. This applies to both natural waste water samples of different toxicities and to chemical substances. The ring test did not intend to provide a comprehensive data base also for *Raphidocelis subcapitata*, hence, for *Raphidocelis,* no final conclusions can be drawn regarding performance, precision and sensitivity. The available data suggest that *Raphidocelis* has a higher growth rate, but otherwise has similar characteristics than *Desmodesmus*. This, however, needs to be verified.

1. Participants, samples and prescribed measurements

Between October 2020 and January 2021, a ring test to investigate the performance of the algae growth inhibition test on microplate (Draft DIN 38412-59) was performed. Thirteen laboratories participated (eleven from Germany, two from Switzerland, see Table 1) and were labeled by a random laboratory code between L01 and L13. *Desmodesmus subspicatus* and *Raphidocelis subcapitata* were used as test organisms, with *Desmodesmus* being mandatory (twelve labs provided data) and *Raphidocelis* being voluntary (six labs provided data). Two different waste water samples (in the following named A and B) were investigated in a series of eight dilutions, each. Additionally, two chemical substances were investigated using eight concentrations, each: potassium dichromate ($K_2Cr_2O_7$) and 3,5-dichlorophenol (3,5-DCP). The two latter aimed at identifying the optimal concentration for possible reference substances. 3,5-DCP, one of the reference substances in the draft standard, was used as positive control to be tested in each test with a concentration of 2.5 mg/L.
The waste water samples were pre-examined prior to the ring test to prescribe uniform dilutions to be used by all laboratories. Test concentrations for 3,5-DCP and $K_2Cr_2O_7$ were also specified centrally, i.e. all laboratories used the same concentration series. The tested dilutions and concentrations were as follows:

- Waste water A and B: D 1.25 - 2 - 3 - 4 - 6 - 8 - 12 - 16
- 3,5 DCP: 1.3 - 1.6 - 1.9 - 2.2 - 2.5 - 2.8 - 3.1 - 3.4 mg/L
- $K_2Cr_2O_7$: 0.2 - 0.4 - 0.6 - 0.8 - 1.0 - 1.2 - 1.4 - 1.6 mg/L

For each test, two different microplates were used, each with control, blank and positive control, as prescribed in the draft DIN 38412-59. Each laboratory had to perform three repeated measurements per sample in order to enable the assessment of both, repeatability and reproducibility. The repeated measurements per sample should be performed on different days within a time period of a few weeks. For every waste water sample, each laboratory received three frozen subsamples. For the substance samples and positive control, centrally prepared stock solutions were provided.

Table 1: List of participants. Alphabetical order, not related to laboratory codes.

Bavarian Environment Agency (LfU)	DE
Ecotox Centre for applied ecotoxicology in Switzerland	CH
ECT Oekotoxikologie GmbH	DE
Evonik Operations GmbH	DE
Federal Institute for Hydrology (BfG)	DE
German Environment Agency (UBA)	DE
Hydrotox GmbH	DE
Institute Dr. Nowak	DE
Institute of Environmental Engineering, RWTH Aachen University (ISA RWTH Aachen)	DE
Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency (NLWKN)	DE
State Agency for Nature, Environment and Consumer Protection (LANUV NRW)	DE
State Institute for Environment Baden-Württemberg (LUBW)	DE
University of Applied Sciences and Arts Northwestern Switzerland	CH

2. Statistical Evaluation - Methods

Statistical evaluation includes calculation of growth rates, determining data for validity check, determining toxicity measures, checking for potential outliers and calculating overall means and precision data, such as repeatability and reproducibility.

2.1. Calculation of growth rates

For controls and substance samples, growth rates were calculated with blank corrected fluorescence data. For waste water samples, the fluorescence data were also corrected by autofluorescence as prescribed in the draft DIN 38412-59.

2.2. Validity criteria

The validity criteria were as follows (and in some cases different from Draft DIN 38412-59):

- GR of the controls after 72 hours is $\geq$ 1.2 d^{-1} (Draft DIN 38412-59: $\geq$ 1.4 d^{-1})
- the coefficient of variation (CV) of the GR of the controls after 72 hours is $\leq$ 7 % (Draft DIN 38412-59 $\leq$ 5 %)
- the mean CV of the sectional-growth-rates (0h-24h, 24h-48h and 48h-72h) in controls is $\leq$ 35 %

A minimum growth rate of 1.2 d^{-1} instead of 1.4 d^{-1} was agreed, since OECD 201-2006 gives a growth rate range of 1.2 d^{-1} to 1.5 d^{-1} for *Desmodesmus subspicatus* when cultivated “at light intensity approx. 70 µE m^{-2} s^{-1} and 21 °C” (see OECD 201-2006, Annex 2), i.e. under the same conditions as prescribed in the present ring test. Indeed, for *Desmodesmus subspicatus,* more than 50 % of the controls failed to meet a minimum growth rate of 1.4 d^{-1} (157 out of 298 controls = 52.7 %). In contrast, in 113 out of 298 controls (37.9 %), growth rates between 1.20 d^{-1} and 1.39 d^{-1} were obtained. Hence, in total, 254 out of 298 controls (85.2 %) showed growth rates $\geq$ 1.2 d^{1}. A minimum growth rate of 1.2 d^{-1} as criterion for validity therefore seems to be justified.
A coefficient of variation (CV) of the GR of the controls after 72 hours of $\leq$ 5 % was fulfilled by 268 out of 298 controls (89.9 %) and thus was not critical. However, by setting the limit to 7 %, four more tests with CV% between 5 % and 7 % could be judged as valid, which would otherwise have been evaluated as invalid. A maximum coefficient of variation of 7 % is also in line with OECD 201 (2006).
A limit of 35 % for the mean CV of the sectional-growth-rates (0h-24h, 24h-48h and 48h-72h), as prescribed in the Draft DIN 38412-59, was maintained to ensure exponential growth in controls. This is also in line with OECD 201-2006.

For *Raphidocelis*, the obtained growth rates were basically substantially higher than those for *Desmedesmus*: 131 out of 142 controls (92.3 %) showed growth rates $\geq$ 1.4 d^{-1} and only in 7 out of 142 controls (4.9 %) the growth rates were below 1.2 d^{-1}. So, for *Raphidocelis*, growth rate was no limiting factor at all for validity and the same validity criteria as for *Desmodesmus* was applied.

Validity was assessed specifically for each microplate. For the test to be valid, both controls need to meet the validity criteria.

3,5-DCP with a nominal concentration of 2.5 mg/L was used as positive control (PC). The inhibition of growth rates in the positive controls were assessed, but not used as validity criterion, since it was one purpose of the ring test, to identify a suitable reference substance and its optimal concentration.

2.3. Toxicity measures

For 3,5-DCP and K_2Cr_2O7, EC(r)50[5] values for growth rate were determined as toxicity measures. For waste water samples, LID[6] and EC(r)50 values were determined. The effect threshold for LID was 10 % inhibition of growth rate. Inhibitions were calculated specifically for each plate, i.e. in relation to the respective control. EC(r)50-values and 95 % confidence limits were determined by nonlinear regression using the software ToxRat Professional 3.3.0. Since nonlinear regression analysis is based on original growth rates, rather than on inhibitions, the measured growth rates in both controls from the two microplates need to be pooled for regression.

2.4. Ring test statistics according to DIN ISO 5725-2

To estimate the precision of the Algae growth inhibition test on microplates, the results for EC(r)50 and LID were evaluated according DIN ISO 5725-2: 2002-12, i.e. the intralaboratory variance s^2_r (repeatability, within laboratory variance) and the interlaboratory variance $s^2_{L.}$ (between laboratory variance) were determined. These sum up to estimate the overall variance s^2_R (reproducibility):

$$s_R^2 = s_L^2 + s_r^2$$

The corresponding standard deviations can be derived as square root of the variances. Repeatability and reproducibility standard deviations are given as percentages. I.e. the lower the relative repeatability standard deviation and reproducibility standard deviation, respectively, the better is the repeatability and reproducibility.

DIN ISO 5725-2: 2002-12 basically assumes the same number of test results in each laboratory. In the present evaluation any attempt was made to keep the number of measurements as uniform as possible (see section 3, data base). However, it is obvious that this cannot always be fulfilled in practise.

Since EC(r)50s and LIDs are assumed to be log-normal distributed, all calculations according to DIN ISO 5725-2: 2002-12 were performed with the log transformed data. In a first step, all EC(r)50s and LIDs were transformed to Y = ln (X), then the mean μ and the standard deviations σ [7] for Y were calculated (i.e. σ_r, σ_L and σ_R). Subsequently, the results were retransformed according the formulas for the log normal distribution, to obtain the parameters for the original scale X:

Geometric mean =	EXP (μ)
95 % confidence interval (CI) [8] =	EXP ($\mu \pm \frac{\sigma}{\sqrt{n}}$* t(0.05, n-1)) with t = value of the t-distribution for p = 5 %, two sided)

[5] EC(r)50 = concentration or volume fraction of test sample which results in a 50 % reduction in specific growth rate relative to the controls

[6] LID = lowest ineffective dilution

[7] μ and σ actually are used for theoretical sample populations. Here they are used to clearly identify the mean y_ = μ and the standard deviation $s_y = \sigma$ of the log transformed sample data used to derive all other measures on the original scale.

[8] The 95 % confidence interval (CI) of the mean gives the range which will contain the true mean value with 95 % probability.

95 % and 99 % prediction interval (PI) [9] = EXP ($\mu \pm \sigma * z$)
with z= 1,96 for 95 %; z=2,57 for 99 %

Coefficient of variation (CV%) = $\sqrt{e^{\sigma^2} - 1}$

For calculation of confidence intervals and prediction intervals, the reproducibility standard deviation σ_R was used. The coefficients of variation CV% s_r, CV% s_L and CV% s_R were calculated based on the corresponding standard deviations σ_r, σ_L and σ_R. All calculations were performed with MS Excel™ (2019).

2.5. Outlier tests

DIN ISO 5725-2: 2002-12 prescribes a systematic outlier testing. In total, four different outlier tests are applied. Mandels k-statistic and Cochran test check if there is strikingly high intralaboratory variability, whereas Mandels h-statistic and Grubbs test check for deviations of the individual laboratory means from the overall mean. Mandels h and Mandels k statistic are graphical consistency techniques, whereas Cochran test and Grubbs test are numerical outlier tests.
All outlier tests were performed with the log transformed data using Excel. Critical thresholds were taken from DIN ISO 5725-2: 2002-12 depending on number of laboratories reporting at least one test result (p) and on number of test results per laboratory (n) for a certain sample. If n is varying, i.e. if the number of repeated measurements per laboratory is not uniform, the most frequent number of n was used according to DIN ISO 5725-2: 2002-12. In the present ring test, for all samples the most frequent value for n was three. Data which were significant at the 5 % significance level, were classified as "stragglers". Data, which were significant at the 1 % level, were classified as "statistical outliers". If a straggler or statistical outlier cannot be explained by some technical error, DIN ISO 5725-2: 2002-12 states that "the stragglers are retained as correct items and the statistical outliers are discarded unless there are good reason to decide to retain them".

3. Data base

Twelve laboratories performed tests with *Desmodesmus subspicatus* as test organisms, five of them also used *Raphidocelis subcapitata* (Table 2). It should be noted, that using *Raphidocelis* was voluntary, i.e. those laboratories that performed tests with *Raphidocelis* expended a considerable amount of additional effort. One laboratory only used *Raphidocelis subcapitata.*

The data base for evaluation was specified in several steps. First, a data consolidation was performed and the reported test conditions were checked for conformity with the test protocol. This leads to exclusion of some tests from further evaluation (see section 3.1). Then, for the remaining tests, validity was checked (section 3.2). In a third step, if the data base was at least six valid test results for LID and EC(r)50, outlier tests were performed and the final data base for calculating means, repeatability and reproducibility was defined (section 3.3).

[9] The 95% or 995 prediction interval (PI) gives the range, in which 95% or 99% of the measurements are to be expected.

Table 2: Tests performed with the different test organisms, *Desmodesmus subspicatus* and *Raphidocelis subcapitata.* Using *Raphidocelis* was voluntary.

Laboratory	*Desmodesmus*	*Raphidocelis*
L01	x	
L02	x	x
L03	x	
L04	x	x
L05		x
L06	x	
L07	x	x
L08	x	
L09	x	x
L10	x	
L11	x	
L12	x	x
L13	x	

3.1. Data consolidation

The raw data were checked for completeness and obvious errors. If necessary, data were corrected and supplemented after consultation with the participants.
In the next step, test conditions were summarized and checked for conformity with the test protocol (e.g. storage of samples, test irradiation, test temperature, pH adjustment of waste water samples, date of test start, initial cell number). A complete overview is given in Table A1 - A5 Annex A (*Desmodesmus*) and Table B1 - B5 Annex B (*Raphidocelis*).
In the following, special features of certain tests are listed and consequences for statistical evaluation are explained.

Tests using *Desmodesmus*

- **L11** measured each of the three subsamples of waste water A and waste water B three times by mistake. Hence, each subsample of waste water A and B was thawed and refrozen several times. Therefore, only the very first test with each of the three subsamples was considered for statistical evaluation. As a consequence, for L11, other than specified in the test protocol, all repeated measurements of waste water A and waste water B, respectively, were from the same day.
- **L04, L10 and L13** provided four repeated measurements instead of three for some samples for different reasons. To keep the number of measurements as uniform as possible (see section 2.4), only the first three tests were considered, even if the fourth was also valid[10].
- **L06** reported strong issues with condensation in the microplates in all tests for all samples. Moreover, it turned out, that though all tests were valid, the corresponding LID values obtained in waste water A and B showed irregularities: they were markedly increased (see

[10] For L04 and L13, the fourth tests were invalid at all. For L10, there were three valid tests without the fourth test being considered.

Tables A6 and A7, Annex). However, the decision, to consider or not consider a result for statistical evaluation cannot be made on the basis of whether it "fits" or not, but need to be based on objective criteria and then must be applied regardless of the individual result. In view of the reported issues with condensation, all data of L06 were excluded for statistical evaluation.

- In a few cases, **deviations from the test protocol** occurred, such as wrong storage temperature (L04 for 3,5-DCP), too low irradiation (L03), missing or wrong pH adjustment (L01, L08, L13, waste water B) – for a complete overview see Table A1 - A5, Annex A. However, in order not to reduce the data base excessively and as otherwise no inconsistencies occurred with these tests, subject to validity, they were kept for evaluation.

Table 3 summarizes which tests using *Desmodesmus* were not considered for further evaluation.

Table 3: Overview tests using *Desmodesmus* not considered for statistical evaluation.
L04: Test 4 was performed 4-6 weeks later than Test 1-3; exceeds three repetitions per lab
L06: Laboratory reports issues due to condensation
L10: Test 4 exceeds three repetitions per lab
L13: Test 4 was performed at the same day as test 3; exceeds three repetitions per lab

tests not considered					
	L04	**L06**	**L10**	**L13**	**total**
Waste Water A	Test 4	Test 1, 2,3	Test 4	-	5
Waste Water B	Test 4	Test 1, 2,3	Test 4	Test 4	6
3,5-DCP	-	Test 1, 2,3	Test 4	-	4
$K_2Cr_2O_7$	-	Test 1, 2,3	Test 4	-	4

Tests using *Raphidocelis*

- **L02** reported some exceptionally high numbers for fluorescence for waste water B in test 2. Assuming typing errors, the corresponding replicates were deleted for evaluation.
- **L04** provided data sets that they themselves had already marked as invalid. These were not considered for further evaluations and they were also not included in the numbers of invalid tests presented in this report.
- **L05** reported the start date for preculture two weeks before test starts for all second tests (test start 02 Nov 2021, start date for preculture 19 Oct 2021).
- **L05**, 3,5-DCP, test 1 and 2: start data control reported as “zero” in some replicates, missing data for some replicates in day 3; the corresponding replicates were deleted for evaluation
- **L07** missed to adjust pH in the waste water B for the second test.
- **L12** reported a loss of volume of test solutions in the wells in some tests.

For a complete overview of tests see Table B1 - B5, Annex B. However, in order not to reduce the data base excessively and as otherwise no inconsistencies occurred with these tests, subject to validity, all tests with *Raphidocelis subcapitata* were kept for evaluation.

3.2. Implications of validity check for data base

Desmodesmus subspicatus

Individual results for validity (per test and per microplate) are given in Tables A6 - A9, Annex A. 39 out of 130 considered tests were assessed as invalid, i.e. 30.0 % (Table 6). It turned out, that validity was not a basic problem, but seem to be more an issue in certain laboratories (see Table 4a). In two labs, 92 % and 100 %, respectively, of the tests were invalid, two other labs had percentages of 42 % and 45 %, respectively. Hence, four labs accounted for 33 of all 39 invalid tests (84.6 %). On the other hand, there were four laboratories with always 100 % valid tests (L3, L07, L10, L11). This clearly supports the basic practicability of the algae growth inhibition test using *Desmodesmus subspicatus* in microplates. A closer look at the reasons for invalidity shows that more than 80 % of the controls achieved the minimum growth rate of 1.2 d^1, the median growth rate was 1.387 d^{-1}. In contrast, maintaining exponential growth (i.e. CV% of sectional growth rate $\leq$ 35 %) proved to be the greatest limitation (Table 4a). It should be analysed, whether there are certain technical conditions or any practical issues with handling in the laboratories with high percentages of invalid tests being different from that of other labs.

Table 4a: Occurrence of invalid tests using *Desmodesmus subspicatus* and reasons for invalidity. Data base: laboratories considered (11) and tests considered (130). 1 = growth rate < 1.2 d^{-1}, 2 = CV% growth rate > 7 %, 3 = CV% sectional growth rate > 35 %.

	reasons for invalidity						
	Waste Water A	Waste Water B	3,5-DCP	K2Cr2O7	tests considered	invalid tests	% invalid tests
L01	3	3	-	-	12	2	17 %
L02	1,2,3	1,2,3	1,2,3	1,2,3	12	11	92 %
L04	3	1, 3	1, 3	3	12	5	42 %
L08	-	-	2, 3	2, 3	12	2	17 %
L09	3	3	1, 3	3	11	5	45 %
L12	1, 3	1, 3	1, 3	1, 3	12	12	100 %
L13	-	1, 3	3	-	11	2	18 %

Raphidocelis subcapitata

Individual results for validity (per test and per microplate) are given in Tables B6 - B9, Annex B. 44 out of 71 considered tests were assessed as invalid, i.e. 62 % (Table 4b and 7). Thereby, in four out of six labs testing *Raphidocelis*, 50 % up to 75 % of the tests were invalid; for one laboratory, even 100 % of the tests were found to be invalid. A minimum growth rate of 1.2 d^{-1} was achieved and even exceeded in 92.3 % of the controls, i.e. by far in most cases (Tables B6 - B9, Annex B). The median growth rate was 1.565 d^{-1}, i.e. *Raphidocelis* showed higher growth rates than *Desmodesmus*. However, as for *Desmodesmus*, also for *Raphidocelis* maintaining exponential growth (i.e. CV% of sectional growth rate $\leq$ 35 %) proved to be the greatest limitation (Table 4b).

The high percentage of invalid tests is probably not related to *Raphidocelis* as test species, but to laboratory specific performance: among the six laboratories that tested *Raphidocelis* were the ones that had also problems to meet the validity criteria with *Desmodesmus*, namely L02, L04, L09, L12 (see Table 4a). L05 (100 % invalid tests) did not test *Desmodesmus*, therefore, no comparison can be performed.

Table 4b: Occurrence of invalid tests using *Raphidocelis subcapitata* and reasons for invalidity. Data base: laboratories considered (6) and tests considered (71). 1 = growth rate < 1.2 d^{-1}, 2 = CV% growth rate > 7 %, 3 = CV% sectional growth rate > 35 %.

	reasons for invalidity						
	Waste Water A	**Waste Water B**	**3,5-DCP**	**K2Cr2O7**	**tests considered**	**invalid tests**	**% invalid tests**
L02	1,2,3	1,2,3	3	-	12	7	58 %
L04	3	-	3	3	11	6	55 %
L05	2,3	2,3	2,3	2,3	12	12	100 %
L07	3	-	3	3	12	4	33 %
L09	2	3	3	3	12	6	50 %
L12	3	3	2	3	12	9	75 %

3.3. Implications of outlier analysis for data base

Desmodesmus subspicatus

According to DIN ISO 5725-2: 2002-12, four different methods of outlier analysis were applied (for details, see section 2.4.). The graphical presentations of Mandels-h and Mandels k analysis are shown in Fig A1, Annex A. Table 55 presents the results of all outlier tests.
For waste water B, no outlier testing was performed, since the sample proved to be of low toxicity and thus in 18 out of 22 valid and considered tests, the LID was found to be “2”, in four tests it was found to be “3”. As a consequence, outlier testing with results for LID from waste water B makes no sense due to too many identical numerical values. Moreover, in nine tests with waste water B, determination of EC(r)50 failed and there were only five laboratories left. According to DIN ISO 5725-1:1997-11, the recommended number of laboratories (p) to ensure a sound statistical evaluation, is at least eight[11], therefore, no further evaluations were carried out for parameter EC(r)50 for waste water B.
It should be noted, that Mandels k-statistic and Cochran test check for strikingly high intralaboratory variability, whereas Mandels h-statistic and Grubbs test check for deviations of the individual laboratory means from the overall mean. It turned out, that some results of L03 and L09 were identified as straggler, however, according to DIN ISO 5725-2: 2002-12, “stragglers are retained as correct items”. In contrast, the results of L13 for EC(r)50 of 3,5-DCP and those of L11 for EC(r)50 of $K_2Cr_2O_7$ were identified as “statistical outliers” and thus should be “discarded unless there are good reason to decide to retain them”. In the following, it will be argued, why the data of L11 and L13 were not excluded as outliers for further evaluations.
(1) The findings for L13 and L11 are solely based on Mandels-k-statistics and Cochran test, i.e. on individual intralaboratory variability, rather than on the mean values for the obtained EC(r)50s. Poor intralaboratory repeatability of course is not desirable – but it seems to be realistic in biotesting and thus does not justify to exlude the corresponding laboratory from further statistical evaluation.
(2) Excluding L11 and L13 would probably improve the corresponding overall repeatabilities and reproducibilites. However, it would also mean decreasing the sample size for the corresponding

[11] DIN ISO 5725-1: 1997-11, p 30: „It can be seen that estimates of the repeatability and reproducibility Standard deviations could differ substantially from their true values if only a small number (p « 5) of laboratories take part in a precision experiment, and that increasing the number of the laboratories by 2 or 3 yields only small reductions in the uncertainties of the estimates when p is greater than 20. It is common to choose a value of p between 8 and 15”.

statistical evaluations to eight. In view of the fact, that the sample size is anyway at the lower limit (see section 3.4.), excluding L11 and L13 thus would considerably increase the uncertainty of the obtained precision measures (see Fig A2, Annex).
Thus, for the present evaluation, no data were excluded as outliers.

Table 5: Result of outlier tests; blue* significant at 5 % level = straggler; red ** significant at 1 % level = statistical outlier

outliertest according to DIN ISO 5725-2	**significances of outlier tests, blue* = 5 % level = straggler, red** = 1 % level = outlier**					
	EC(r)50				LID	
	Waste water A	Waste water B	3,5-DCP	$K_2Cr_2O_7$	Waste water A	Waste water B
Mandels k	-	not performed because of too low sample size (n=5)	L13	L09, L11	L03	not performed because of too many ties
Cochran	-		L13	-	-	
Mandels h	-		-	L09	L03	
Grubbs	-		-	-	-	

Raphidocelis subcapitata

According to DIN ISO 5725-1:1997-11, the recommended number of laboratories to ensure a sound statistical evaluation – including outlier analysis – is at least eight[12]. For *Raphidocelis*, the data base of only 3-5 laboratories providing valid tests thus did not allow a reliable outlier analysis.

3.4. Data base for ring test statistics

Desmodesmus subspicatus

In total, twelve laboratories provided 149 tests. The results of some laboratories and tests were not considered for different reasons, the remaining results were further decreased due to invalidity of tests. Finally, the number of laboratories considered for ring test statistics was nine. Although being at the lower limit of the recommended number of participants, this is still a sufficient data base to obtain reliable results[13].
Depending on sample, 20-24 single test results with 1-3 test results per laboratory were available, with “3” being the most frequent number for all samples. Hence, in all cases, repeatabilities and reproducibilities could be calculated with sufficient certainty of about 35 %-40 % (see Fig A2, Annex A).

Raphidocelis subcapitata

In total, six laboratories provided 71 tests, 27 tests were found to be valid. The number of laboratories providing valid LID and EC50 results was three to five depending on sample. Since according to DIN ISO 5725-1:1997-11, the recommended number of laboratories to ensure a sound statistical evaluation, is at least eight, and in view of only one valid repeated measurement per laboratory in most cases, no evaluation of repeatability and reproducibility was carried out for results with Raphidocelis. Geometric means for LID and EC50 results with 95 % confidence limits results

[12] Same as footnote 11
[13] Same as footnote 11

were calculated. Due to the low sample size, the corresponding 95 % confidence intervals became large (Table 7).

4. Statistical Evaluation - Results

In the following, the obtained LIDs and EC(r)50s of waste water samples and their precision data are presented, if available, followed by the EC(r)50 of the chemical substances 3,5-DCP and $K_2Cr_2O_7$. The observed inhibitions in the positive control will be discussed, especially in view of the obtained EC(r)50s for 3,5 DCP and $K_2Cr_2O_7$.
Table 6 and Table 7 summarize the data base for the test species *Desmodesmus* and *Raphidocelis*, respectively, and the results of all statistical evaluations obtained in the present ring test. Laboratory specific results are available in Annex-Tables A6 - A9, Annex A, and B6 - B9, Annex B.
It should be noted, that the mean LIDs and EC(r)50s obtained for tests with *Raphidocelis* should be regarded as preliminary in view of the limited data base. For the same reason, no definitive statement can be made about possible differences in sensitivity of *Desmodesmus* and *Raphidocelis*.

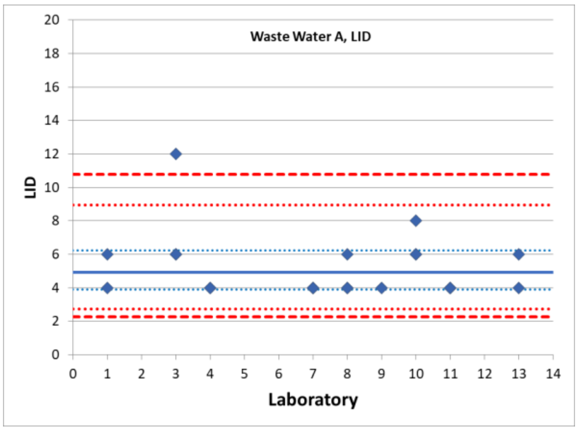
4.1. Results for waste water samples

Desmodesmus subspicatus

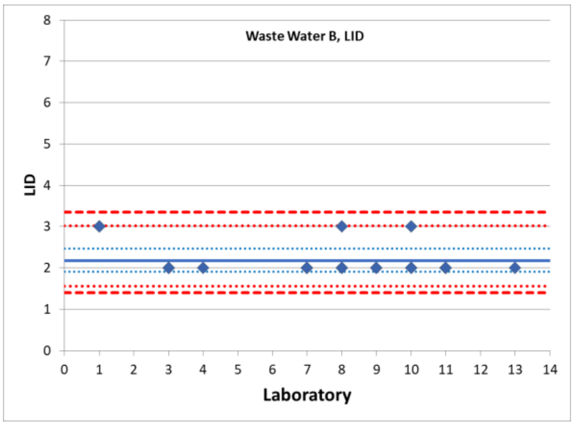
Waste water A was of moderate toxicity. In most tests the LID was 4 or 6, in two tests, LIDs of 8 and 12, respectively, were found. The mean LID was 4.9, with a repeatability of 22.6 % and a reproducibility of 31.1 %. The mean EC(r)50 was 44.9 Vol % with a repeatability of 8.5 % and a reproducibility 14.1 % (Figure 1, Table 6). Due to the discrete character of the LID, its variability was higher than that for EC(r)50.
Waste Water B was of low toxicity. This was confirmed by a nearly uniform LID values: in 18 out of 22 tests the LID was 2, in four tests the LID was 3 (Figure 1). The mean LID was 2.2 with repeatability and reproducibility variances of 14.2 % and 17.0 % (Table 6). Because of the low toxicity of waste water B, EC(r)50 data could not be determined in most of the tests (Table 6).

Raphidocelis subcapitata

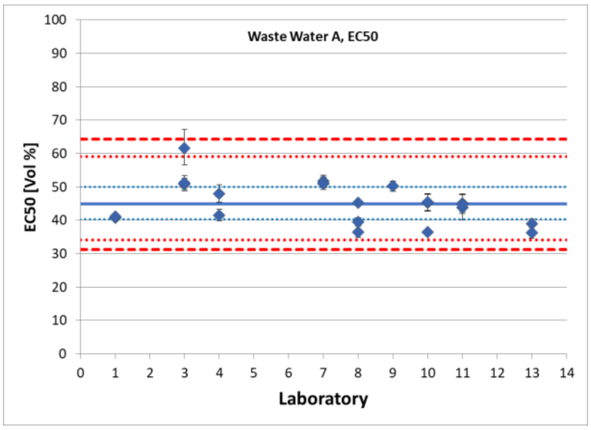
Waste water A was of moderate toxicity. In four valid tests the LID was 4 or 6. The mean LID was 5.4. The mean EC(r)50 was 37.1 Vol % (Fig. 2, Table 7).
Waste Water B was of lower toxicity. In nine valid tests the LID was 2, 3 or 4 (Figure 2). The mean LID was 2.9 (Table 7), the mean EC(r)50 was 69.1 Vol % (Table 7).



Desmodesmus

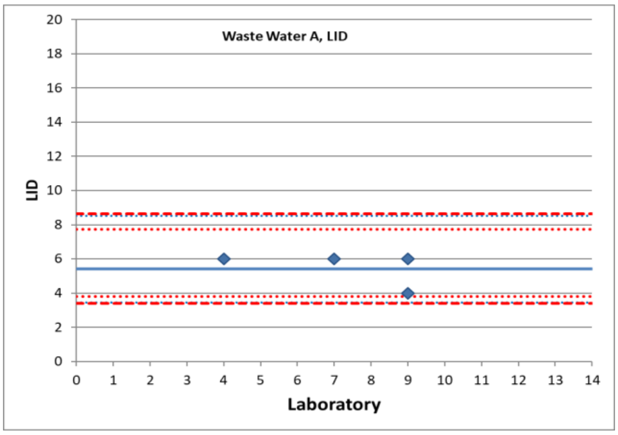


Desmodesmus

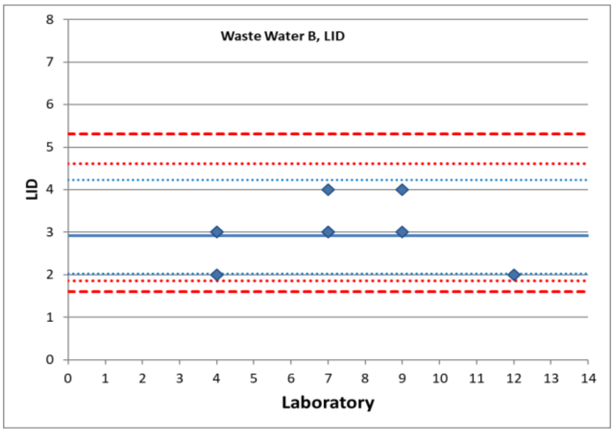


Desmodesmus

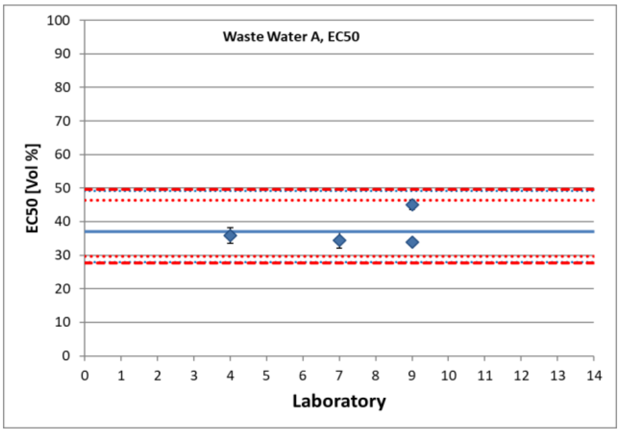
Figure 1: Results for waste water samples A and B using *Desmodesmus subspicatus* as test organism. Blue diamonds: individual laboratory result, one diamond can symbolise several identical or similar values; whisker: 95 % CI of EC(r)50 (sometimes lower than the size of the symbol), blue line: geometric mean and 95 % CI, red dotted line: 95 % prediction interval, red broken line: 99 % prediction interval. Laboratory specific results are available in Annex A, tables A6 and A7.



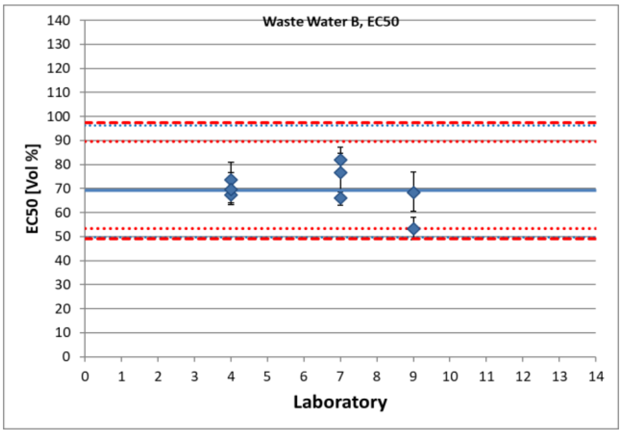
Raphidocelis



Raphidocelis



Raphidocelis



Raphidocelis

Figure 2: Results for waste water samples A and B using *Rhaphidocelis subcapitata* as test organism. Blue diamonds: individual laboratory result, one diamond can symbolise several identical or similar values; whisker: 95 % CI of EC(r)50 (sometimes lower than the size of the symbol), blue line: geometric mean and 95 % CI, red dotted line: 95 % prediction interval, red broken line: 99 % prediction interval. Laboratory specific results are available in Annex B, tables B6 and B7.

4.2. Results for 3,5-DCP and $K_2Cr_2O_7$

Desmodesmus subspicatus

The mean EC(r)50 for 3,5-DCP was found to be 2.15 mg/L with a 95 % confidence range of 1.87 mg/L - 2.47 mg/L. Repeatability (measured in terms of relative repeatability standard deviation) was found to be 7.7 %, reproducibility (measured in terms of relative reproducibility standard deviation) was 18.1 % (Fig. 3, Table 6). The mean EC(r)50 for $K_2Cr_2O_7$ was found to be 0.90 mg/L with a 95 % confidence range of 0.77 mg/L - 1.06 mg/L. Repeatability standard deviation was 15.2 %, reproducibility standard deviation was 20.6 % (Fig. 3, Table 6).

Raphidocelis subcapitata

The mean EC(r)50 for 3,5-DCP was found to be 2.26 mg/L with a 95 % confidence range of 1.60 mg/L - 3.12 mg/L (Fig. 3, Table7). The mean EC(r)50 for $K_2Cr_2O_7$ was found to be 1.13 mg/L with a 95 % confidence range of 0.77 mg/L - 1.66 mg/L (Fig. 3, Table 7).

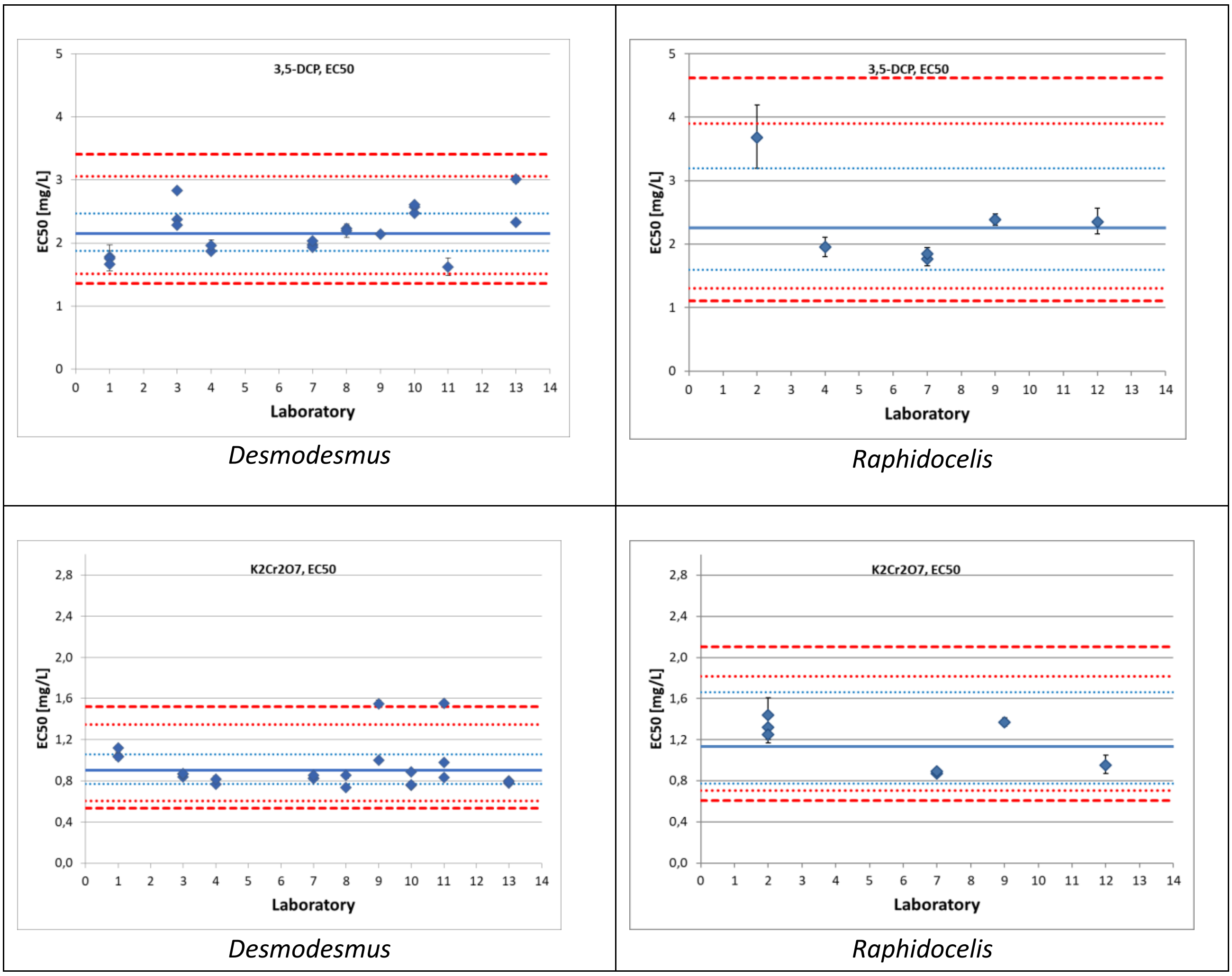


Figure 3: Results for 3,5-DCP and $K_2Cr_2O_7$ samples using *Desmodesmus subspicatus* (left side) and *Raphidocelis subcapitata* (right side) as test organism. Blue diamonds: individual laboratory result, one diamond can symbolise several similar values; whisker: 95 % CI of EC(r)50 (sometimes lower than the size of the symbol), blue line: geometric mean and 95 % CI, red dotted line: 95 % prediction interval, red broken line: 99 % prediction interval. Laboratory specific results are available in Annex-tables A8 and A9 (Annex A) and tables B8 and B9 (Annex B), respectively.

Table 6: Summary of data base and results of all samples and toxicity measures obtained in the ring test using *Desmodesmus subspicatus.*
CI = confidence interval, PI = prediction interval, sr% = relative repeatability standard deviation; sL% = relative between standard deviation; sR% = relative reproducibility-standard deviation; Repeatability- and reproducibility standard deviations are given as percentages. I.e. the lower the relative standard deviation, the better is the repeatability and reproducibility, respectively.

	EC(r)50				LID	
Sample / test substance	**Waste Water A**	**Waste Water B**	**3,5-DCP**	**$K_2Cr_2O_7$**	**Waste Water A**	**Waste Water B**
number of laboratories with tests performed	12	12	12	12	12	12
number of laboratories with tests considered	11	11	11	11	11	11
number of tests performed (total: 149)	36	39	37	37	36	39
number of tests considered (total: 130)	31	33	33	33	31	33
number of valid tests (total: 91)	22	22	23	24	22	22
number of invalid tests (total: 39)	9	11	10	9	9	11
percentage invalid tests	**29 %**	**33 %**	**30 %**	**27 %**	**29 %**	**33 %**
number of labs with results n.d. in one or more tests	0	5	2	0	0	0
number of valid tests with result n.d.	0	9	3	0	0	0
number of laboratories for statistics	**9**	**5**	**9**	**9**	**9**	**9**
number of tests for statistics	**22**	**13**	**20**	**24**	**22**	**22**
repeated measurements for statistics	1x1, 3x2, 5x3	3x2, 2x3	2x1, 3x2, 4x3	3x2, 6x3	1x1, 3x2, 5x3	1x1, 3x2, 5x3
laboratories identified as outlier (not excluded)	0	n.d.	1 (a)	1 (b)	0	n.d.
min / max	36.4 / 61.7	no statistics. too low sample size	1.62 / 3.01	0.73 / 1.55	4 / 12	2 / 3
geometric mean	**44.9 Vol %**		**2.15 mg/L**	**0.90 mg/L**	**4.9**	**2.2**
95 % CI geometric mean	40.3 - 50.0		1.87 - 2.47	0.77 - 1.06	3.9 - 6.2	1.9 - 2.5
sr %	8.5		7.7	15.2	22.6	14.2
sL %	11.2		16.3	13.8	20.7	9.2
sR %	**14.1**		**18.1**	**20.6**	**31.1**	**17.0**
sR / sr	**1.7**		**2.4**	**1.4**	**1.4**	**1.2**

(a) L13, Mandels k + Cochran test significant, points to strikingly high intralaboratory variability

(b) L11, Mandels k test significant, points to strikingly high intralaboratory variability

Table 7: Summary of data base and results of all samples and toxicity measures obtained in the ring test using *Raphidocelis subcapitata*.
CI = confidence interval, PI = prediction interval, sr% = relative repeatability standard deviation; sL% = relative between standard deviation; sR% = relative reproducibility-standard deviation; Repeatability- and reproducibility standard deviations are given as percentages. I.e. the lower the relative standard deviation, the better is the repeatability and reproducibility, respectively.

	EC(r) 50				**LID**	
Sample / Test Substance	**Waste Water A**	**Waste Water B**	**3,5-DCP**	**K2Cr2O7**	**Waste Water A**	**Waste Water B**
number of laboratories with tests performed	6	6	6	6	6	6
number of laboratories with tests considered	6	6	6	6	6	6
number of tests performed (total: 71)	17	18	18	18	18	18
number of tests considered (total: 71)	17	18	18	18	17	18
number of valid tests (total: 27)	4	9	7	7	4	9
number of invalid tests (total: 44)	13	9	11	11	13	9
percentage invalid tests	**76 %**	**50 %**	**61 %**	**61 %**	**76 %**	**50 %**
number of labs with results n.d. in one or more tests	0	1	1	0	0	0
number of valid tests with LID or EC50 n.d.	0	1	1	0	0	0
number of laboratories for statistics	**3**	**3**	**5**	**4**	**3**	**4**
number of tests for statistics	**4**	**8**	**6**	**7**	**4**	**9**
repeated measurements for statistics	2x1, 1x2	1x2, 2x3	4x1, 1x2	2x1, 1x2, 1x3	2x1, 1x2	1x1, 1x2, 2x3
laboratories identified as outlier	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
min / max	34.0 / 45,1	53.3 / 81.8	1.77 / 3.68	0.87 / 1.44	4 / 6	2 / 4
geometric mean	**37.1 Vol %**	**69.1 Vol %**	**2.26 mg/L**	**1.13 mg/L**	**5.4**	**2.9**
95 % CI geometric mean	27.9 - 49.2	49.7 - 96.2	1.60 - 3.12	0.77 - 1.66	3.5 - 8.5	2.0 - 4.2
sr %	no statistics, because of poor sample size					
sL %						
sR %						
sR / sr						

4.3. Performance and precision of the test

Regarding general practicability of the test in terms of validity, the ring test provides evidence, that running a valid algae growth inhibition test on microplates seems to be demanding, but feasible. *Raphidocelis* showed on average higher growth rates than *Desmodesmus* (median growth rate *Raphidocelis*: 1.565 d^{-1}, *Desmodesmus* 1.387 d^{-1}), i.e. the minimum growth rate of 1.2 d^{-1} is more easily achieved with *Raphidocelis* (Table A6 - A9, Annex A, and B6 - B9, Annex B). However, regardless of absolute growth rates, maintaining exponential growth (i.e. CV% of sectional growth rate $\leq$ 35 %) proved to be the greatest limitation for both algae species. There is evidence that the test success rate may be related to practical handling and technical issues in the laboratory. It seems promising to focus on what the labs with many valid tests are doing differently than those with a high failure rate.

Desmodesmus subspicatus

With reproducibility standard deviations up to 20 % in most cases and a maximum reproducibility standard deviation of 31 % for LID and EC(r)50 data of four different samples, the algae growth inhibition test on microplates using *Desmodesmus subspicatus* can be regarded as well performing. Moreover, it could be shown, that variance between laboratories is in the same range than variance within laboratories. As a consequence, the ratio between reproducibility and repeatability (sR%/sr%) was between 1.2 and 2.4. The ratio sR/sr is a measure of robustness of the test results and of the degree of standardization of the test. A ratio below 4 is regarded as robust[14], a ratio around or even below 2 thus indicates a high degree of standardization.

Raphidocelis subcapitata

For *Raphidocelis subcapitata*, measures for precision such as reproducibility and repeatability could not be derived due to the limited data base.

4.4. Positive controls and results for potential reference substances

The Draft DIN 38412 L59 prescribes either 3,5 DCP or potassium dichromate as reference substance. In the ring test, 3,5-DCP was used as positive control (PC) in all tests with a nominal concentration of 2.5 mg/L.

Desmodesmus subspicatus

Only in about half of the tests with *Desmodesmus*, the proposed range of 20 % to 80 % inhibition of growth rate in the PC was met. There were four labs which obtained inhibitions higher than 80 % or even higher than 100% in all tests (L01, L04, L11, L13) and thus accounted for 47 out of 71 tests (66 %) with inadequate inhibitions of positive controls. The reasons are unclear, but there are indications of possible factors:

- L01, L04 and L11 showed strikingly low EC(r)50 values for 3,5-DCP (L01: 1.78 mg/L, 1.75 mg/L, 1.67 mg/L; L04: 1.88 mg/L, 1.96 mg/L; L11: 1.62 mg/L), i.e. in most cases even lower than the lower 95 % confidence limit of the overall mean EC(r)50 (1,87 mg/l). Assuming an increased sensitivity of the algae would explain why in the corresponding positive controls with a nominal concentration of 2.5 mg/L inhibitions > 100 % were observed.

[14] Donnevert G, S Uhlig, T Moser. Ring Test Data Evaluation. In: H Moser and J Römbke (eds.),Ecotoxicological Characterization of Waste, DOI: 10.1007/978-0-387-88959-7_4, © Springer Science+Business Media, LLC 2009

- L10 reported an error in the preparation of the mixing ratio for the 3,5-DCP positive control, resulting in significantly increased real 3,5-DCP concentration and thus inhibitions higher than 100 %. After the error had been detected and corrected, L10 measured inhibitions around 50 % in the positive control. The same fault might have occurred in one of the other labs who measured inhibitions > 100 %. Especially for L13, this would be an explanation, since L13 obtained EC(r)50 values of 2.3 mg/L and 3.0 mg/L for 3,5-DCP. Thus, for L13, an increased sensitivity of the algae can be excluded.

The mean EC(r)50 for 3,5-DCP was found to be 2.15 mg/L, with a 95 % confidence limit of 1.87 mg/L to 2.47 mg/L. If a nominal concentration of 2.5 mg/L for the 3,5-DCP positive control and an inhibition range of 20 % to 80 % is maintained, all tests of L01, L04, L11 and L13 and some tests of L07 and L08 of the present ring test will be assessed as not valid because of too high percentage of inhibitions in the PC. In contrast, there were also laboratories which measured quite low inhibitions in the PC with 2.5 mg/L 3,5-DCP and thus could fall below the 20 % inhibition limit if the concentration of the 3,5-DCP-positive control is lowered. However, the corresponding laboratories (L02, L06, L12) experienced problems with test performance at all. So, their results for the PCs might be of lower informative value. All in all, the ring test indicates that the concentration of 3,5-DCP as positive control should possibly be somewhat reduced.

The mean EC(r)50 for $K_2Cr_2O_7$was found to be 0.90 mg/L with a 95 % confidence range of 0.77 mg/L – 1.06 mg/L. This is close to the concentration of 0.8 mg/L currently prescribed for potassium dichromate as reference substance in Draft DIN 37412-59.

Raphidocelis subcapitata

When *Raphidocelis* was used as test organism, about 72 % of the tests failed to meet the prescribed range of 20 %-80 % inhibition in the positive control (see Annex B, Tables B6-B9). As with *Desmodesmus*, with the exception of some tests of L02, generally too high inhibitions were measured. Again, L04 showed a high percentage of tests with positive controls beyond 80 %. However, the same here applies for L07, L09 and L12, which measured suitable inhibitions in positive controls when using *Desmodesmus*. This might point to a generally higher sensitivity of *Raphidocelis* to 3,5-DCP. In this case, the mean EC(r)50 for 3,5-DCP of *Raphidocelis* should be lower than that of *Desmodesmus*. This, however, cannot be confirmed. In contrast, the obtained mean 3,5-DCP EC(r)50 for *Raphidocelis* was 2.26 mg/L (95 % CI 1.60 mg/L - 3.12 mg/L) and thus in the same range than that for *Desmodesmus* with 2.15 mg/L (95 % CI 1.87 mg/L - 2.47 mg/l). This is probably due to the fact, that the mean 3,5-DCP-EC(r)50 for *Raphidocelis* is dominated by an extraordinary high EC(r)50 of L02, namely 3.68 mg/L[15]. If this data is omitted, the mean EC(r)50 of 3,5-DCP for *Raphidocelis* would be 2.05 mg/L (95 % CI 1.63 mg/L to 2.58 mg/L). However, this is also very similar to the mean EC(r)50 of 3,5-DCP for *Desmodesmus.*

The mean EC(r)50 for $K_2Cr_2O_7$ of *Raphidocelis* was found to be 1.13 mg/L with a 95 % confidence range of 0.77 mg/L - 1.66 mg/L. Although this is higher than the result obtained for *Desmodesmus* (0.90 mg/L with a 95 % confidence range of 0.77 mg/L - 1.06 mg/L), the confidence limits are completely overlapping. This argues against major differences between the EC(r)50 for $K_2Cr_2O_7$ of both algae species.

[15] The tests of L02 frequently showed very low inhibitions of the positive controls below 20%, so, there might be an issue with sensitivity. However, due to the poor data base, no reliable outlier testing can be performed.

So, the overall conclusion is, that the proposed concentration of 2.5 mg/L for 3,5-DCP as positive control might be too high for *Desmodesmus subspicatus* and even more for *Raphidocelis subcapitata*. The proposed concentration of 0.8 mg/L for $K_2Cr_2O_7$ as positive control seems to be appropriate or at most slightly too low. These findings provide arguments to keep 0.80 mg/L as concentration for $K_2Cr_2O_7$ as reference substance and to somewhat reduce the proposed concentration of 2.5 mg/L for 3,5-DCP as reference substance.

Generally, it should be noted that the observed inhibitions of positive controls showed some unexplained results both in tests with *Desmodesmus* and with *Raphidocelis* and sometimes even differed between two plates of the same test. So, the question of a suitable substance to be used as positive control and its optimal concentration cannot yet be completely answered.

5. References

Draft German standard methods for the examination of water, waste water and sludge - Test methods using water organisms (group L) - Algal growth inhibition test on microplate with unicellular green fresh water algae (L 59), (E) DIN 38412-59:2020-08

DIN ISO 5725-1: 1997-11 Accuracy (trueness and precision) of measurement methods and results - Part 1 : General principles and definitions (ISO 5725-1 : 1994)

DIN ISO 5725-2: 2002-12 Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a Standard measurement method (ISO 5725-2:1994 including Technical Corrigendum 1:2002).

Donnevert G, S Uhlig, T Moser. Ring Test Data Evaluation. In: H Moser and J Römbke (eds.), Ecotoxicological Characterization of Waste, DOI: 10.1007/978-0-387-88959-7_4, © Springer Science+Business Media, LLC 2009

OECD 201 (2006): OECD GUIDELINES FOR THE TESTING OF CHEMICALS, Freshwater Alga and Cyanobacteria, Growth Inhibition Test

6. Annex A

Overview data base and experimental conditions for tests with *Desmodesmus*, results of stepwise data consolidation, validity check and outlier tests; data base for ring test statistics

Step 1:

- Adjustment file names, completion of laboratory number etc.
- Check whether data templates and protocols are complete and consistent, request for additional information if necessary.
- Overview test conditions (storage of samples, test irradiation, test temperature, pH adjustment of waste water samples, date of test start, initial cell number, any deviations from protocol), clarification of queries, request for additional information if necessary.
- Correction of mistakes in data templates (delete additional sheets / rows / lines, restore cell formulas if they were deleted or overwritten, correct formats (number / text), correct sequence of dilutions, identify missing data).
- Overview storage and test conditions per biotest **(Tab. A1 - A5).**
- Define data base for further evaluations, define tests which are not considered **(Tab. A6 - A9).**

Step 2:

- Results biotests with *Desmodesmus* (validity, LID, EC(r)50) **Tab. A6 - A9**

Step 3:

- Outliertests for valid LID- and EC(r)50-results of tests with *Desmodesmus*; definition data base for further ring test statistics: **Fig A1, Table 4 main part of the report.**

Tab A1: Overview basic test conditions for all samples per laboratory, red: deviation from test protocol. Tests with *Desmodesmus.*

General information		conditions Microplate (MP)				irradiation		flourescence measurement	
Lab	Test	MP Type	cover foil in addition to lid yes/no	taping yes/no	Randomisation on microplate shaker yes/no	lux nominal: 6000-10000	µmol nominal: 60-120 µmol m-2 s-1	wave length	from bottom / top
L01	1 2 3	Falcon, Multiw ellplatte steril mit unbehandelter Oberfläche, FALC351147	no	yes	yes	6300-6500		485 / 670	top
L02	1 2 3	Greiner Cellstar, Art.Nr. 662160	yes, Diversified Biotech, Breathe Easy, Nr. BEM-1	no	no		80-87	458 / 685	bottom
L03	1 2 3	Greiner BioOne, MULTIWELLPLATTE, 24 WELL, Art.-Nr.: 662102	no	yes	yes	4200		485 / 685	bottom
L04	1 2 3 4	Greiner Bio-One, CELLSTAR 24 Well Cell Culture Plate, Art.Nr. 662160	no	yes	no	10747		485 / 685	bottom
L06	1 2 3	VWR, VWR® Tissue Culture Plates 24 wells, surface treated, sterile, 734-2325	no	yes	yes	5970 -6920		430 / 680	top
L07	1 2 3	Greiner, 24-Well Suspensionsplatten, Art.-Nr. 662 102	no	yes	yes		83	435 / 685	bottom
L08	1 2 3	Greiner 24, FlatBottom, Cellstar Suspension Cultur Platte, Cat.No. 662102	no	no	yes	n.d.		465 / 635	top
L09	1 2 3	Falcon 351147	no	yes	yes	7675 lm		485 / 680	top
L10	1 2 3 4	CELLSTAR Greiner Bio-one Nr. 662102_100	no	yes	yes	6200-8450		440 / 690	top
L11	1 2 3	no protocol					85-90 or 107-116 see remarks at each sample	430/670	top
L12	1 2 3	Greiner Bio-One, Cellstar, Cat. Nr. 662102	no	yes "Parafilm without air holes, double wrapped around plate"	yes		101	440 / 680	bottom
L13	1 2 3	Greiner bio-one, cellstar, 24 Well Cell Culture Platte, Art. Nr. 662160	no	yes	no daily 180° rotation, empty plate below each MP to avoid concdesation at lid		102 - 119	465 / 690	top

Tab A2: Overview test conditions for waste water A per laboratory; tests with *Desmodesmus*

Waste Water A					pH defrosted sample, pH adjustment if > 8,5								
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: -18°	sample defrosted on	before	after	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [Z/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01	1	ice core	-18 / -23	14-Dec-20	8,1	-	11-Dec-20	14-Dec-20	22,9-24,9	413600	6500	6500	comment lab: cell densitiy in preculture is initial density
	2			11-Jan-21	8,1	-	08-Jan-21	11-Jan-21	22,0 - 24,1	327300	6500	6500	comment lab: cell densitiy in preculture is initial density
	3			12-Jan-21	8,1	-	08-Jan-21	12-Jan-21	22,8-24,9	562900	6500	6500	comment lab: cell densitiy in preculture is initial density
L02	1	frozen	-18 / -20	11-Jan-21	8,0	-	08-Jan-21	11-Jan-21	23,4 - 23,9	n.d.	n.d.	5200	
	2			12-Jan-21	8,2	-	08-Jan-21	12-Jan-21	23,4 - 23,9	n.d.	n.d.	5000	
	3			18-Jan-21	8,1	-	15-Jan-21	18-Jan-21	23,9 - 23,9	n.d.	n.d.	5400	
L03	1	frozen	-22	16-Nov-20	8,0	-	13-Nov-20	16-Nov-20	22,5 - 23,5	408000	4200	5100	control 2: repl 4-6 at d1 are missing --> repl 4-6 completely omitted; at d3: large differences to repl 1-4
	2			30-Nov-20	7,8	-	27-Nov-20	30-Nov-20	22,5 - 23,5	255600	4500	5200	
	3			11-Jan-21	7,9	-	08-Jan-21	11-Jan-21	22,5 - 23,5	192000	4250	4250	
L04	1	frozen	≤-18	02-Nov-20	8,0	-	29-Oct-20	02-Nov-20	23,4 - 23,6	1,19E+06	1,19E+06	8307	
	2			10-Nov-20	8,0	-	06-Nov-20	10-Nov-20	23,4 - 23,6	732000	732000	9313	
	3			08-Dec-20	8,0	-	04-Dec-20	08-Dec-20	24,5	727871	727871	6614	
	4			12-Jan-21	8,0	-	08-Jan-21	11-Jan-21	23,7 - 23,9	3,09E+06	3,09E+06	4539	
L06	1	frozen	-21 / -22	14-Dec-20	8,2	-	11-Dec-20	14-Dec-20	23,6 - 24,7	2,60E+06	106000	17000	strong condensation
	2			18-Jan-21	8,2	-	15-Jan-21	18-Jan-21	23,3 -24,3	3,10E+06	100000	18000	strong condensation, 3. plate used
	3			25-Jan-21	8,2	-	22-Jan-21	25-Jan-20	23,5 - 24,5	2,50E+06	110000	18000	srong condensation, 3. plate used
L07	1	frozen	-18	07-Dec-20	8,2	-	04-Dec-20	07-Dec-20	22 - 24	532128	92563	8464	
	2			14-Dec-20	8,2	-	11-Dec-20	14-Dec-20	22 - 24	598961	94302	7905	
	3			11-Jan-21	8,0	-	08-Jan-21	12-Jan-21	22 - 24	816290	90638	7843	
L08	1	frozen	≤-18	23-Nov-20	8,1	-	20-Nov-20	24-Nov-20	23,8 - 24,5	1,19E+06	5000	5000	
	2			07-Dec-20	8,2	-	04-Dec-20	08-Dec-20	23,9 - 24,5	1,29E+06	5000	5000	
	3			14-Dec-20	8,1	-	11-Dec-20	15-Dec-20	23,5 - 24,6	1,50E+06	5000	5000	
L09	1	frozen	-18 / -24	17-Nov-20	8,0	-	13-Nov-20	17-Nov-20	21 - 25		5,07E+06	10000	
	2			24-Nov-20	8,1	-	21-Nov-20	24-Nov-20	21 - 25		2,26E+06	10000	
	3			30-Nov-20	8,1	-	27-Nov-20	30-Nov-20	21 - 25		2,68E+06	10000	
L10	1	frozen	-18	02-Nov-20	8,2	-	30-Oct-20	02-Nov-20	23,0 - 24,1	350625	350625	5084	comment lab: error with preparation of positive control: ratio water / solution B mixed up
	2			09-Nov-20	8,1	-	06-Nov-20	09-Nov-20	23,2 - 24,0	762096	762096	5030	
	3			30-Nov-20	8,2	-	27-Nov-20	30-Nov-20	22,5 - 23,2	1,19E+06	1,19E+06	5115	
	4			02/11 + 07/12	8,2	-	27-Nov-20	07-Dec-20	22,3 -22,7	947393	1,19E+06	5021	sample refrosted
L11	1	frozen	-20 / -28	15-Dec-20	8,1	-	11-Dec-20	15-Dec-20	22,2 - 22,9	1,64E+06	1,64E+06	5000	sample A38 Test 1; in PC 1 and treatm 1:1,25 neg numbers after correction at d3--> no GR calculated ; irradiation: 85-90 µmol m-2 s-1, Dec 15 2020
	2			15-Dec-20	8,1	-	11-Dec-20	15-Dec-20	22,2 - 22,9	1,64E+06	1,64E+06	5000	A52 Test 1, A 61 Test 3; both: irradiation: 85-90 µmol m-2 s-1; all tests on Dec 15 2020
	3			15-Dec-20	8,1	-	11-Dec-20	15-Dec-20	22,2 - 22,9	1,64E+06	1,64E+06	5000	
L12	1	frozen	-20	30-Nov-20	8,2	-	26-Nov-20	30-Nov-20	23,5 - 25,1	1,25E+06	1,25E+06	12500	
	2			07-Dec-20	8,0	-	03-Dec-20	07-Dec-20	23,9 - 25,1	2,75E+06	2,75E+06	14000	
	3			07-Dec-20	8,0	-	10-Dec-20	10-Dec-20	24,0	2,92E+06	10000	120000	
L13	1	frozen	-20	07-Dec-20	8,1	-	04-Dec-20	07-Dec-20	23,4 - 23,9	56800	56800	5680	in PC 1 and treatm 1:1,25 neg numbers after correction at d3--> no GR calculated
	2			11-Jan-21	8,2	-	08-Jan-21	11-Jan-21	23,5 - 23,8	55310	55310	5531	
	3												test not performed

Tab A3: Overview test conditions for waste water B per laboratory; tests with *Desmodesmus.*

Waste Water B					pH defrosted sample, pH adjustment if > 8,5								
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: -18°	sample defrosted on	before	after	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [Z/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01	1	ice core	-18 / -23	14-Dec-20	7,6	-	11-Dec-20	14-Dec-20	22,9 - 24,9	413600	6500	6500	all tests: comment lab: cell densitiy in preculture is intitial density
	2			11-Jan-21	7,8	-	08-Jan-21	11-Jan-21	22,0 - 24,1	327300	6500	6500	
	3			12-Jan-21	9,1	-	08-Jan-21	12-Jan-21	22,8 - 24,9	562900	562900	6500	no pH adjustement
L02	1	frozen	-18 / -20	11-Jan-21	8,2	-	08-Jan-21	11-Jan-21	23,4 - 23,9	n.d.	n.d.	5000	
	2			12-Jan-21	n.d.	-	08-Jan-21	12-Jan-21	23,4 - 23,9	n.d.	n.d.	5800	
	3			18-Jan-21	8,2	-	15-Jan-21	18-Jan-21	23,3 - 23,9	n.d.	n.d.	5400	
L03	1	frozen	-22	16-Nov-20	7,8	-	13-Nov-20	16-Nov-20	22,5 - 23,5	408000	4200	5000	
	2			30-Nov-20	8	-	27-Nov-20	30-Nov-20	22,5 - 23,5	255600	4500	5500	
	3			11-Jan-20	7,8	-	08-Jan-21	11-Jan-21	22,5 - 23,5	192000	4250	4260	
L04	1	frozen	≤-18	02-Nov-20	7,8	-	29-Oct-20	02-Nov-20	23,4 - 23,6	1,19E+06	1,19E+06	8307	
	2			17-Nov-20	8,7	7,1	13-Nov-20	17-Nov-20	23,4 - 23,8	730330	730330	3508	
	3			01-Dec-20	8,3	-	27-Nov-20	01-Dec-20	24,4 - 24,5	465587	465587	4987	
	4			12-Jan-21	9,0	6,9	07-Jan-21	12-Jan-21	23,7 - 23,9	3,09E+06	3,09E+06	4539	
L06	1	frozen	-21 / -22	14-Dec-20	8,6	7	11-Dec-20	14-Dec-20	23,6 - 24,7	2,60E+06	106000	17000	strong condensation
	2			18-Jan-21	8	-	15-Jan-21	18-Jan-21	23,3 - 24,3	3,10E+06	100000	18000	strong condensation
	3			25-Jan-21	8,8	7,1	22-Jan-21	25-Jan-21	23,5 - 24,5	2,50E+06	110000	18000	srong condensation
L07	1	frozen	-18	07-Dec-20	7,5	-	04-Dec-20	07-Dec-20	22,0 - 24,0	532128	92563	8215	
	2			14-Dec-20	8,2	-	11-Dec-20	14-Dec-20	22,0 - 24,0	598961	94302	7594	
	3			11-Jan-21	8,4	-	08-Jan-21	12-Jan-21	22,0 - 24,0	816290	90638	7594	
L08	1	frozen	≤-18	23-Nov-20	8,8	7	20-Nov-20	24-Nov-20	23,8 - 24,5	1,19E+06	5000	5000	
	2			07-Dec-20	8,4	-	04-Dec-20	08-Dec-20	23,9 - 24,5	1,29E+06	5000	5000	
	3			14-Dec-20	9,1	8,4	11-Dec-20	15-Dec-20	23,5 - 24,6	1,50E+06	5000	5000	pH adjustment not correct
L09	1	frozen	-18 / -24	17-Nov-20	8,5	-	13-Nov-20	17-Nov-20	21,0 - 25,0		5,07E+06	10000	
	2			24-Nov-20	7,8	-	21-Nov-20	24-Nov-20	21,0 - 25,0		2,26E+06	10000	
	3			n.d.	8,1	-	21-Nov-20	30-Nov-20	21,0 - 25,0		4,00E+05	10000	
L10	1	frozen	-18	02-Nov-20	8,6	7,2	30-Oct-20	02-Nov-20	23,0 - 24,1	350629	350629	5084	comment lab: error with preparation of positive control: ratio water / solution B mixed up
	2			09-Nov-20	7,7	-	06-Nov-20	09-Nov-20	23,2 - 24,0	762096	762096	5030	
	3			09-Nov-20	8,6	6,8	27-Nov-20	30-Nov-20	22,5 - 23,2	1,19E+06	1,19E+06	5115	
	4			02/11.+07.12	8,6	7,2	04-Dec-20	07-Dec-20	22,3 - 22,7	947393	9,47E+05	5021	sample refrosted
L11	1	frozen	-20 / -28	05-Jan-21	7,7	-	04-Jan-21	05-Jan-21	21,9 - 22,5	1,98E+06	1,98E+06	5000	test 1: B20; test 2: B37, test 3: B70 all: preculture started only 1 day before, 107-116 µmol m-2 s-1; all tests on Jan 05 21
	2			05-Jan-21	8,5	-	04-Jan-21	05-Jan-21	21,9 - 22,5	1,98E+06	1,98E+06	5000	
	3			05-Jan-21	8	-	04-Jan-21	05-Jan-21	21,9 - 22,5	1,98E+06	1,98E+06	5000	
L12	1	frozen	-20	30-Nov-20	8,6	7	26-Nov-20	30-Nov-20	23,5 - 25,1	1,25E+06	1,25E+06	12500	
	2			07-Dec-20	9,0	6,8	03-Dec-20	07-Dec-20	23,9 - 25,1	2,75E+06	2,75E+06	14000	
	3			07-Dec-20	9,0	6,8	10-Dec-20	14-Dec-20	24,0	2,92E+06	10000	120000	
L13	1	frozen	-20	07-Dec-20	8,6	8,3	04-Dec-20	07-Dec-20	n.d.	56800	56800	5680	pH adjustment not correct
	2			11-Jan-21	8	-	08-Jan-21	11-Jan-21	23,5 - 23,6	55310	55310	5531	
	3			18-Jan-21	8,6	7,8	15-Jan-21	19-Jan-21	23,5 - 23,8	56800	56800	5680	pH adjustment not correct
	4			n.d.	8,3	-	15-Jan-21	19-Jan-21	23,5 - 23,8	56800	56800	5680	

Tab A4: Overview test conditions for 3,5-DCP per laboratory; tests with *Desmodesmus.*

DCP										
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: 2-8°	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [cells/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01	1	cooled	1-4 °C	04-Dec-20	07-Dec-20	21,7 - 24,1	479700	6500	6500	
	2			04-Dec-20	08-Dec-20	22,8 - 24,8	646600	6500	6500	
	3			11-Dec-20	14-Dec-20	22,9 - 24,9	413600	6500	6500	control repl 3-6 are missing at d2 --> completely deleted for all days
L02	1	cooled	4-6 °C	04-Dec-20	08-Dec-20	23,4 - 24,1	n.d.	n.d.	5200	
	2			11-Dec-20	14-Dec-20	23,3 - 23,9	n.d.	n.d.	5800	
	3			11-Dec-20	15-Dec-20	23,3 - 23,9	n.d.	n.d.	5000	
L03	1	cooled	2-8 °C	13-Nov-20	16-Nov-20	22,5 - 23,5	408000	4200	4800	
	2			27-Nov-20	30-Nov-20	22,5 - 23,5	256000	4500	5650	
	3			08-Jan-21	11-Jan-21	22,5 - 23,5	192000	4200	4350	
L04	1	cooled	≤ -18	05-Nov-20	09-Nov-20	23,4 - 23,6	1,27E+06	1,27E+06	7840	
	2			12-Nov-20	16-Nov-20	23,5 - 23,7	1608000	1608000	7667	sample storage at -18°, instead 2-8°
	3			19-Nov-20	23-Nov-20	23,5 - 23,8	1108000	1108000	7667	
L06	1	cooled	4-6 °C	11-Dec-20	14-Dec-20	23,6 - 24,7	2,60E+06	106000	17000	strong condensation
	2			03-Jan-21	06-Jan-21	23,4 - 24,5	3,80E+06	94000	14000	strong condensation
	3			15-Jan-21	18-Jan-21	23,3 - 24,3	3,10E+06	110000	18000	strong condensation
L07	1	cooled	4-8 °C	23-Oct-20	27-Oct-20	22,0 - 24,0	732000	94178	7656	
	2			30-Oct-20	03-Nov-20	22,0 - 24,0	619520	96104	8340	
	3			27-Nov-20	30-Nov-20	22,0 - 24,0	591631	95545	7967	
L08	1	cooled	2-8 °C	12-Nov-20	16-Nov-20	23,9 - 24,2	1,04E+06	5000	5000	
	2			19-Nov-20	23-Nov-20	23,8 - 24,5	1,17E+06	5000	5000	
	3			03-Dec-20	07-Dec-20	23,9 - 24,5	1,56E+06	5000	5000	large difference between ctrl 1-3 and ctrl 4-6
L09	1	cooled	2-8 °C	27-Nov-20	01-Dec-20	21,0 - 25,0		2,69E+06	10000	
	2			04-Dec-20	07-Dec-20	21,0 - 25,0		1,18E+06	10000	growth in control on plate 2 stopped
	3			04-Dec-20	08-Dec-20	21,0 - 25,0		3,08E+06	10000	
L10	1	cooled	4°C	30-Oct-20	02-Nov-20	23,0 - 24,1	350625	350625	5084	comment lab: error with preparation of positive control: ratio water / solution B
	2			06-Nov-20	09-Nov-20	23,2 - 24,0	762096	762096	5030	
	3			27-Nov-20	30-Nov-20	22,5 - 23,2	1,19E+06	1,19E+06	5115	
	4			04-Dec-20	07-Dec-20	22,3 - 22,7	947393	9,47E+05	5021	
L11	1	cooled	3-8 °C	04-Dec-20	08-Dec-20	22,1 - 22,8	2,35E+06	2,35E+06	5000	irradiation test 1 (85-90) different from that of test 2 and 3 (107-116)
	2			08-Jan-21	12-Jan-21	22,0 - 22,3	2,10E+06	2,01E+06	5000	
	3			15-Jan-21	19-Jan-21	22,0 - 22,6	2,10E+06	2,10E+06	5000	
L12	1	frozen	4°C	26-Nov-20	30-Nov-20	23,5 - 25,1	1,25E+06	1,25E+06	12500	
	2			03-Dec-20	07-Dec-20	23,9 - 25,1	2,75E+06	2,75E+06	14000	
	3			10-Dec-20	14-Dec-20	24,0	2,92E+06	10000	120000	
L13	1	cooled	2-8 °C	22-Jan-21	25-Jan-21	23,4 - 23,9	56800	56800	5680	
	2			29-Jan-21	01-Feb-21	23,5 - 23,7	58300	58300	5830	
	3			12-Feb-21	15-Feb-21	23,5 - 23,8	58300	58300	5830	

Tab A5: Overview test conditions for $K_2Cr_2O_7$ per laboratory; tests with *Desmodesmus.*

K2Cr2o7										
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: 2-8°	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [cells/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01	1	cooled	1-4 °C	04.12.2020	07.12.2020	21,7 - 24,1	479700	6500	6500	
	2			04.12.2020	08.12.2020	22,8 - 24,8	646600	6500	6500	
	3			11.12.2020	14.12.2020	22,9 - 24,9	413600	6500	6500	
L02	1	cooled	4-6 °C	22.01.2021	25.01.2021	23,9 - 24,1	n.d.	n.d.	6100	
	2			29.01.2021	01.02.2021	23,7 - 23,9	n.d.	n.d.	5800	
	3			29.01.2021	01.02.2021	23,7 - 23,9	n.d.	n.d.	n.d.	
L03	1	cooled	2-8 °C	13.11.2020	16.11.2020	22,5 - 23,5	408000	4200	5000	
	2			27.11.2020	30.11.2020	22,5 - 23,5	255600	4500	5500	
	3			08.01.2021	11.01.2021	22,5 - 23,5	192000	4250	4700	
L04	1	cooled	2-8 °C	05.11.2020	09.11.2020	23,4 - 23,6	1,27E+06	1,27E+06	7840	
	2			12.11.2020	16.11.2020	23,5 - 23,7	1,61E+06	1,61E+06	7660	
	3			19.11.2020	23.11.2020	23,5 - 23,8	1,11E+06	1,11E+06	7667	
L06	1	cooled	4-6 °C	11.12.2020	14.12.2020	23,6 - 24,7	2,60E+06	106000	17000	strong condensation
	2			03.01.2021	06.01.2021	23,4 - 24,5	3,80E+06	94000	14000	strong condensation
	3			15.01.2021	18.01.2021	23,3 - 24,3	3,10E+06	110000	18000	strong condensation
L07	1	cooled	4-8 °C	23.10.2020	27.10.2020	22,0 - 24,0	732000	94178	8215	
	2			30.10.2020	03.11.2020	22,0 - 24,0	619520	96104	8836	
	3			27.11.2020	30.11.2020	22,0 - 24,0	591631	95545	8215	
L08	1	cooled	2-8 °C	12.11.2020	16.11.2020	23,9 - 24,8	1,04E+06	5000	5000	
	2			19.11.2020	23.11.2020	23,8 - 24,5	1,90E+06	5000	5000	
	3			03.12.2020	07.12.2020	23,9 - 24,5	1,53E+06	5000	5000	
L09	1	cooled	2-8 °C	27.11.2020	01.12.2020	21,0 - 25,0		2,69E+06	10000	treatment 1,4 mg/L: initial flourescence only 50% of other treatments --> treatment 1,4 mg/L omitted for evaluation
	2			04.12.2020	07.12.2020	21,0 - 25,0		1,18E+06	10000	
	3			04.12.2020	08.12.2020	21,0 - 25,0		3,08E+06	10000	
L10	1	cooled	4°C	30.10.2020	02.11.2020	23,0 - 24,1	350625	350625	5084	comment lab: error with preparation of positive control: ratio water / solution B
	2			06.11.2020	09.11.2020	23,2 - 24,0	762096	762096	5030	
	3			27.11.2020	30.11.2020	22,5 - 23,2	1,19E+06	1,19E+06	5115	
	4			04.12.2020	07.12.2020	22,3 - 22,7	947393	947393	5021	
L11	1	cooled	3-8 °C	04.12.2020	08.12.2020	22,1 - 22,8	2,35E+06	2,35E+06	5000	irradiation test 1 (85-90) different from that of test 2 and 3 (107-116)
	2			08.12.2020	11.01.2021	22,0 - 22,6	5,45E+05	5,45E+05	5000	
	3			15.01.2021	18.01.2021	22,0 - 22,6	7,45E+05	7,45E+05	5000	
L12	1	frozen	4°C	26.11.2020	30.11.2020	23,5 - 25,1	1,25E+06	1,25E+06	12500	
	2			03.12.2020	07.12.2020	23,9 - 25,1	2,75E+06	2,75E+06	14000	
	3			10.12.2020	14.12.2020	24,0	2,92E+06	10000	120000	
L13	1	cooled	2-8 °C	22.01.2021	25.01.2021	23,4 - 23,9	56800	56800	5680	
	2			29.01.2021	01.02.2021	23,5 - 23,7	58300	58300	5830	
	3			12.02.2021	15.02.2021	23,5 - 23,8	58300	58300	5830	

Tab A6: Waste water A: validity and results for LID and EC(r)x per laboratory; tests with *Desmodesmus.*

Waste water A		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?			EC(r)x (non lin reg) (Vol %)				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	LID [≤10% inhib.]	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1	107	104	1.268	1.265	2.9	2.9	35.3	39.5	no	no						
	2	107	106	1.275	1.267	1.9	3.8	5.7	8.2	yes	yes	4	**29.13**	**40.81**	*39.84*	*41.80*	**57.18**
	3	107	106	1.29	1.287	1.5	0.6	5.6	6.1	yes	yes	6	**29.25**	**41.12**	*40.28*	*41.99*	**57.83**
L02	1	15.4	26.3	0.760	0.732	28.5	27.9	186	173	no	no						
	2	20.0	-39.5	0.654	0.68	40.8	34.8	232	176	no	no						
	3	-59.7	-48.9	0.478	0.588	41.1	19	116.1	57.1	no	no						
L03	1	48.2	16.9	1.676	1.753	2.1	0.9	13.6	6.6	yes	yes	12	**34.68**	**61.71**	*56.66*	*67.20*	**109.79**
	2	57.4	57.5	1.683	1.639	0.6	1.1	9.0	13.1	yes	yes	6	**27.87**	**50.76**	*48.89*	*52.70*	**92.47**
	3	56.8	63.9	1.503	1.617	1.3	0.8	10.0	5.9	yes	yes	6	**27.61**	**51.24**	*49.16*	*53.42*	**95.13**
L04	1	102.8	98.7	1.282	1.352	1.6	2.4	29.7	29.4	yes	yes	4	**29.63**	**41.55**	*39.79*	*43.37*	**58.25**
	2	102.1	95.2	1.292	1.471	3.9	2.1	27.4	16.7	yes	yes	4	**34.35**	**47.94**	*45.38*	*50.64*	**66.89**
	3	147.0	125.6	1.030	1.291	1.9	1.7	51.3	35.7	no	no						
	4	*115.9*	*122.4*	*1.163*	*1.274*	*1.8*	*1.7*	*37.1*	*42.6*	*no*	*no*	**not considerd (exceeds three repetitions per lab)**					
L06	1	*34.8*	*27.8*	*1.409*	*1.371*	*2.1*	*4.2*	*11.7*	*14*	yes	yes	*16*	**not considered (issues due to condensation)**				
	2	*23.9*	*20.3*	*1.431*	*1.377*	*1.2*	*4.5*	*17.9*	*13.9*	yes	yes	*24*					
	3	*34.3*	*40*	*1.38*	*1.371*	*4.1*	*2.4*	*16.3*	*13.7*	yes	yes	*12*					
L07	1	74.7	74.2	1.454	1.477	1.1	1.5	11.7	14	yes	yes	4	**35.12**	**50.98**	*49.74*	*52.26*	**74.01**
	2	77.1	75.4	1.382	1.439	1.7	2.9	8.7	8.9	yes	yes	4	**37.27**	**51.66**	*49.77*	*53.62*	**71.60**
	3	70.5	72.1	1.427	1.429	2.8	1.3	15.9	11.3	yes	yes	4	**35.93**	**51.06**	*49.26*	*52.92*	**72.56**
L08	1	71	64.9	1.485	1.304	1.6	6.5	23.2	13.1	yes	yes	4	**35.84**	**45.24**	*44.45*	*46.04*	**57.12**
	2	67.3	70.2	1.37	1.46	1.7	0.8	18.6	16	yes	yes	6	**26.15**	**36.47**	*34.84*	*38.19*	**50.88**
	3	65.7	65.1	1.555	1.561	1	0.2	19.1	18.3	yes	yes	4	**27.69**	**39.49**	*38.28*	*40.69*	**56.26**
L09	1	58.7	57.5	1.33	1.377	5.9	1.4	47.7	51.4	no	no						
	2	no data provided															
	3	44.5	52.9	1.503	1.581	1.4	3.5	14.7	17.2	yes	yes	4	**35.43**	**50.23**	*48.70*	*51.81*	**72.21**
L10	1	138.9	137.2	1.763	1.837	3.2	1.9	7.9	4.6	yes	yes	6	**23.48**	**36.46**	*35.81*	*37.13*	**56.62**
	2	50.7	49.2	1.604	1.800	2.6	2.7	16.0	5.8	yes	yes	8	**31.72**	**45.20**	*42.76*	*47.77*	**64.39**
	3	52.9	50.1	1.731	1.785	1.4	1.7	7.3	8.6	yes	yes	6	**27.62**	**45.39**	*42.89*	*48.05*	**74.59**
	4	*49.6*	*47.1*	*1.786*	*1.783*	*2.8*	*1.5*	*6.6*	*7.9*	yes	yes	*6*	**not considerd (exceeds three repetitions per lab)**				
L11	1	106.5	100	1.406	1.362	2.0	1.1	22.5	26.6	yes	yes	4	**32.57**	**43.86**	*40.14*	*47.93*	**59.06**
	2	105.1	105.5	1.38	1.388	1	1.1	17.7	20.3	yes	yes	4	**31.16**	**45.02**	*42.52*	*47.67*	**65.05**
	3	104.6	103.8	1.403	1.409	1.4	1.5	25.1	21.2	yes	yes	4	**31.02**	**44.77**	*42.11*	*47.60*	**64.63**
L12	1	32.3	33.9	1.225	1.261	1.2	1.5	40.3	36.3	no	no						
	2	24.7	28.4	1.019	1.26	2	4.4	67.3	30.3	no	no						
	3	33.6	25.9	1.118	1.117	2.8	4	45.6	42.3	no	no						
L13	1	92.1	100	1.224	1.379	1.1	0.9	29.1	22.9	yes	yes	4	**31.46**	**39.07**	*37.83*	*40.34*	**45.52**
	2	90.5	97.2	1.274	1.334	1.6	1.3	15.9	22.2	yes	yes	6	**27.35**	**36.38**	*34.63*	*38.21*	**48.38**
	3	test not performed															

Tab A7: Waste water B: validity and results for LID and EC(r)x per laboratory; tests with *Desmodesmus.*

Waste water B		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?			EC(r)x (non lin reg) (Vol %)				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	LID [≤10% inhib.]	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1	107.1	105.1	1.256	1.253	1	1.4	37.3	36.2	no	no						
	2	111.5	105.8	1.294	1.268	1.9	1.7	9.5	4.8	yes	yes	3	**51.02**	**76.20**	*74.07*	*78.39*	**extrapol**
	3	108	98.8	1.298	1.31	2.2	1.8	9.6	12.4	yes	yes	3	**55.47**	**79.45**	*77.71*	*81.24*	**extrapol**
L02	1	26.4	16.1	0.793	0.761	14.7	28.2	227	81	no	no						
	2	18.4	24.9	0.762	0.831	12.9	17.3	179	140	no	no						
	3	-14.8	-9.3	0.689	0.695	8.2	10.5	64.1	87.9	no	no						
L03	1	49.5	-0.5	1.726	1.704	1.3	2.1	7.9	12.9	yes	yes	2	**n.d.**				
	2	57.3	33	1.627	1.648	1.1	1	9	6.6	yes	yes	2	**n.d.**				
	3	57.9	61.4	1.504	1.651	1.6	0.5	17.5	9.5	yes	yes	2	**n.d.**				
L04	1	109.2	107.3	1.248	1.249	2.4	1.2	29.9	32.4	yes	yes	2	**n.d.**				
	2	93.2	93.4	1.125	1.328	4.2	1.2	43.9	20.6	no	no						
	3	128	121	1.196	1.335	1.6	1.7	42.0	28.2	no	no						
	4	*123*	*120*	*1.07*	*1.201*	*1.6*	*1.5*	*48.2*	*41.5*	*no*	*no*		**not considerd (exceeds three repetitions per lab)**				
L06	1	*34.2*	*30.2*	*1.440*	*1.403*	*1.8*	*2.7*	*13.2*	*14.3*	*yes*	yes	*3*	**not considered (issues due to condensation)**				
	2	*35.5*	*31.2*	*1.308*	*1.334*	*4.9*	*3.4*	*19.1*	*14.9*	*yes*	yes	*6*					
	3	*36.1*	*31.9*	*1.353*	*1.354*	*4.7*	*3.5*	*10.1*	*11.3*	*yes*	yes	*3*					
L07	1	69	70.8	1.429	1.443	2.0	2.9	12.7	7.6	yes	yes	2	**n.d.**				
	2	73.5	73.6	1.392	1.411	1.8	1.8	7.9	4.2	yes	yes	2	**n.d.**				
	3	67.2	70.3	1.423	1.395	3.0	2.7	17.8	13.8	yes	yes	2	**n.d.**				
L08	1	77.4	79.2	1.498	1.474	1.1	2.3	22.6	21.3	yes	yes	2	**58.76**	**73.62**	*71.62*	*75.69*	**extrapol**
	2	71.5	74.5	1.386	1.443	5.1	1.9	19.2	16.7	yes	yes	2	**55.29**	**65.65**	*52.74*	*68.69*	**77.94**
	3	69.0	66.5	1.563	1.564	0.3	0.8	20.1	19.4	yes	yes	3	**51.51**	**61.43**	*59.57*	*63.37*	**73.28**
L09	1	60.8	55.1	1.326	1.369	4.3	2.9	47.2	38.5	no	no						
	2	78.2	70.5	1.543	1.523	2.8	1.5	11.7	14.2	yes	yes	2	**55.98**	**73.95**	*72.00*	*75.96*	**extrapol**
	3	28.8	44.8	1.486	1.53	2.4	2.4	13.4	15.3	yes	yes	2	**67.64**	**81.60**	*79.02*	*84.26*	**extrapol**
L10	1	123.5	111.3	1.819	1.782	3.1	1.3	6.4	4.4	yes	yes	2	**62.33**	**83.61**	*81.44*	*85.85*	**extrapol**
	2	48.0	47.6	1.711	1.765	1.3	2.2	10.9	6.8	yes	yes	3	**58.84**	**83.19**	*80.11*	*86.38*	**extrapol**
	3	50.1	52.9	1.660	1.816	2.9	3	9.6	10.4	yes	yes	2	**n.d.**				
	4	*47.5*	*50.6*	*1.679*	*1.805*	*2.7*	*2.2*	*18.3*	*9.6*	*yes*	*yes*	*2*	**not considerd (exceeds three repetitions per lab)**				
L11	1	122.1	128.6	1.446	1.452	1.4	2.5	12.4	18.9	yes	yes	2	**59.10**	**78.51**	*75.49*	*81.65*	**extrapol**
	2	123.8	125.2	1.454	1.445	1.2	1.6	19.3	17	yes	yes	2	**59.83**	**81.53**	*77.78*	*85.45*	**extrapol**
	3	121.9	121.8	1.450	1.467	1.6	3.1	15.8	18.6	yes	yes	2	**56.70**	**78.08**	*74.63*	*81.59*	**extrapol**
L12	1	23.2	33.0	1.210	1.235	2.2	2.3	44.9	40.1	no	no						
	2	24.1	31	1.128	1.217	1.9	1.9	51.7	41.7	no	no						
	3	32.1	32.4	1.088	1.135	1.3	1.8	46.0	46.6	no	no						
L13	1	94.1	86.9	1.155	1.367	1.6	1.6	36.0	12.8	no	yes						
	2	92.5	100.2	1.309	1.349	1.5	1.1	17.8	17.9	yes	yes	2	**n.d.**				
	3	82.8	84.2	1.207	1.244	1.3	0.6	35.3	36.8	no	no						
	4	*81.1*	*88.1*	*1.129*	*1.248*	*2.6*	*1.2*	*27.5*	*37.2*	*no*	*no*		**not considerd (exceeds three repetitions per lab)**				

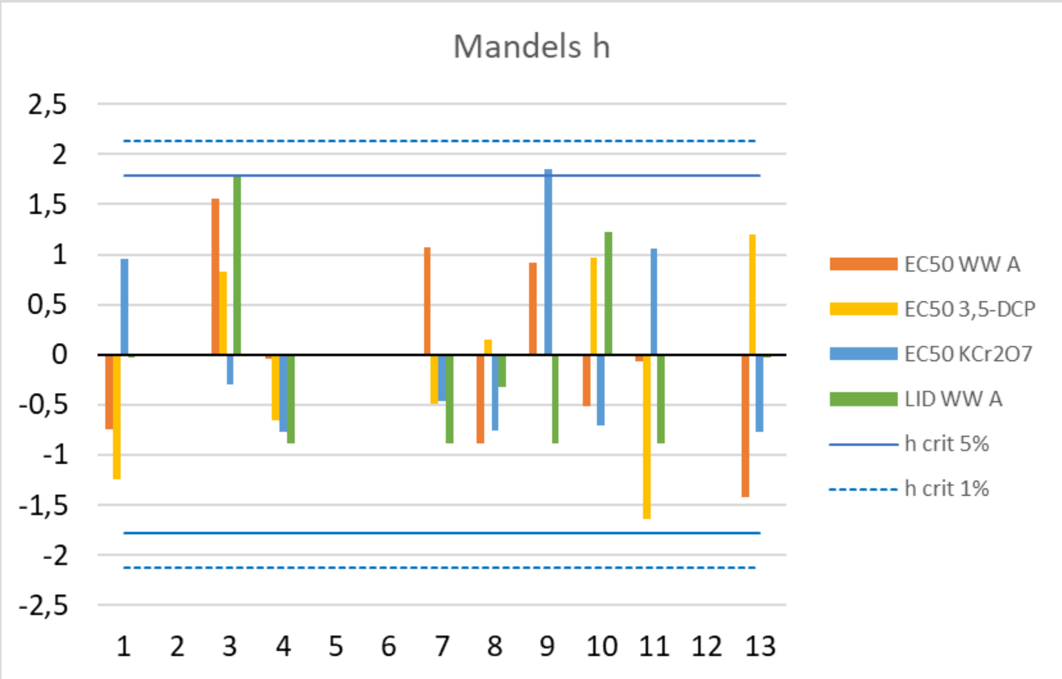
Tab A8: 3,5-DCP: validity and results for ECx per laboratory; tests with *Desmodesmus*.

DCP		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?		EC(r)x (non lin reg) [mg/L]				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1	112.2	111.4	1.352	1.367	1.4	0.7	17.1	12.7	yes	yes	1.44	1.78	1.70	1.88	2.20
	2	103.3	121.1	1.42	1.384	1.6	2	11.5	16.8	yes	yes	1.45	1.75	1.55	1.97	2.12
	3	101.6	104.6	1.267	1.274	0.8	0.3	32.5	33	yes	yes	1.33	1.67	1.56	1.78	2.09
L02	1	28.6	47.1	0.526	0.838	41.0	18.9	111	84.6	no	no					
	2	-5.0	-1.0	1.253	1.321	4.0	2.7	17.2	14.9	yes	yes	n.d. (highest inhibition 10%)				
	3	3.9	-10.0	1.172	1.021	7.4	8	23.5	31.6	no	no					
L03	1	53.5	46.2	1.731	1.689	0.6	1.1	8.8	6.8	yes	yes	1.96	2.83	2.81	2.85	4.08
	2	51.7	61.2	1.661	1.654	0.8	0.3	6.4	11.3	yes	yes	1.62	2.38	2.36	2.39	3.49
	3	64.3	66.4	1.604	1.587	0.8	1.0	6.5	5.2	yes	yes	1.69	2.28	2.27	2.30	3.09
L04	1	107.9	110.0	1.328	1.335	1.0	0.8	27.7	28.9	yes	yes	1.48	1.88	1.86	1.89	2.38
	2	92.6	92.7	1.39	1.414	1.1	2.6	15.5	16.1	yes	yes	1.48	1.96	1.88	2.05	2.61
	3	128.3	132.4	1.192	1.202	1.3	1.9	37	41.2	no	no					
L06	1	30.9	35.2	1.395	1.325	2.9	4.6	8.1	11.7	yes	yes	not considered (issues due to condensation)				
	2	35.5	47.6	1.477	1.329	2.3	2.2	32.6	34.2	yes	yes					
	3	22.8	36.1	1.276	1.068	8.6	34.8	18.2	21.9	yes	no					
L07	1	84	89.1	1.630	1.672	0.6	1.3	12.3	13.5	yes	yes	1.51	1.97	1.96	1.97	2.55
	2	88.6	86.4	1.587	1.603	0.7	0.7	8.5	6.3	yes	yes	1.44	1.94	1.90	1.97	2.59
	3	72.5	71.5	1.481	1.477	1.5	4.2	22.6	7.7	yes	yes	1.52	2.03	2.02	2.04	2.72
L08	1	79.3	78.6	1.535	1.547	1.6	1.0	19.3	19.1	yes	yes	1.86	2.23	2.17	2.29	2.66
	2	82.6	82.6	1.447	1.44	3.0	5.8	29.9	27.2	yes	yes	1.85	2.19	2.09	2.30	2.59
	3	76.2	74.9	1.289	1.23	14.1	18.4	33.3	46.6	no	no					
L09	1	46.6	64.1	1.637	1.585	3.4	1.9	8.3	10.5	yes	yes	1.75	2.14	2.11	2.17	2.62
	2	53.7	39.2	0.815	1.609	3.2	2.6	107.6	19.5	no	yes					
	3	75.4	64.8	1.408	1.432	3.1	3.2	42.2	34	no	yes					
L10	1	138.1	133.1	1.849	1.882	2.7	3.3	5.9	6.2	yes	yes	1.65	2.57	2.56	2.58	4.00
	2	57.2	55.3	1.734	1.82	1.5	1.9	15.3	8.1	yes	yes	1.72	2.47	2.46	2.48	3.55
	3	52.1	50.5	1.672	1.741	1.9	1.2	6.3	10.1	yes	yes	1.78	2.60	2.59	2.62	3.80
	4	48.5	51.4	1.778	1.753	2.0	0.6	5.1	8.4	yes	yes	not considerd (exceeds three repetitions per lab)				
L11	1	115.8	116.1	1.271	1.265	3.6	4.1	8.6	7.8	yes	yes	1.29	1.62	1.48	1.76	2.03
	2	129.9	131.0	1.423	1.448	1.0	1.7	6.9	5.3	yes	yes	n.d. (lowest inhibition 69%)				
	3	121.8	122.3	1.422	1.429	2.3	1.5	16.3	15	yes	yes	n.d. (lowest inhibition 69%)				
L12	1	27.5	34.3	1.130	1.252	2.1	1.2	73.5	39.3	no	no					
	2	30.9	27.4	1.131	1.061	1.8	3	43.2	40.8	no	no					
	3	35.8	30.8	1.189	1.056	4.6	2.7	25.9	48.8	no	no					
L13	1	104.1	106.5	1.389	1.364	0.6	1.4	52.8	21.2	no	yes					
	2	103.9	102.0	1.294	1.294	0.9	1.8	19.2	19.5	yes	yes	1.677	2.333	2.297	2.369	3.245
	3	110.3	114.5	1.200	1.233	1.9	0.9	26.7	33.1	yes	yes	2.325	3.012	2.96	3.065	3.903

Tab A9: $K_2Cr_2O_7$: validity and results for EC(r)x per laboratory; tests with *Desmodesmus.*

K2Cr2O7		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?		EC(r)x (non lin reg) [mg/L]				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
	1	114.1	113.8	1.353	1.365	3.3	1.5	16.7	15	yes	yes	**0.62**	**1.04**	*1.00*	*1.08*	**1.76**
L01	2	109.1	110.9	1.381	1.395	0.8	0.4	16.1	11.6	yes	yes	**0.54**	**1.03**	*1.00*	*1.07*	**1.98**
	3	103.6	102.8	1.271	1.303	1.5	1.7	29.2	25.6	yes	yes	**0.59**	**1.12**	*1.10*	*1.15*	**2.13**
	1	267	240	1.565	1.306	75.0	245.3	484	-67	no	no					
L02	2	13.6	23.5	1.085	1.096	4.1	4.0	38.4	42.7	no	no					
	3	2.4	19.3	1.333	1.503	2.0	14.9	40.9	32.9	no	no					
	1	46.0	39.4	1.661	1.704	1.6	1.0	17.7	8.7	yes	yes	**0.47**	**0.84**	*0.81*	*0.86*	**1.49**
L03	2	58.2	53.7	1.610	1.580	1.7	1.7	6.5	12.9	yes	yes	**0.51**	**0.87**	*0.84*	*0.90*	**1.47**
	3	67.5	61.8	1.531	1.534	1.3	1.9	4.6	7.7	yes	yes	**0.48**	**0.87**	*0.84*	*0.90*	**1.60**
	1	101.7	108.7	1.368	1.358	2.3	1.3	29.1	27.5	yes	yes	**0.42**	**0.77**	*0.75*	*0.79*	**1.39**
L04	2	86.2	86.2	1.402	1.478	2.0	4.2	17.7	10.6	yes	yes	**0.41**	**0.81**	*0.78*	*0.85*	**1.62**
	3	127.3	122.5	1.212	1.26	1.0	1.5	39.3	36.5	no	no					
	1	*27.8*	*32.6*	*1.357*	*1.409*	*1.5*	*3.6*	*12.5*	*14.6*	*yes*	*yes*					
L06	2	*51.2*	*35.0*	*1.332*	*1.461*	*3.1*	*3.1*	*17.8*	*32.9*	*yes*	*yes*	**not considered (issues due to condensation)**				
	3	*19.8*	*25.6*	*1.425*	*1.43*	*2.3*	*2.9*	*17.5*	*17.4*	*yes*	*yes*					
	1	84.1	84.8	1.599	1.628	0.7	0.4	12.4	11.3	yes	yes	**0.56**	**0.83**	*0.81*	*0.84*	**1.21**
L07	2	79.1	79.5	1.521	1.543	1.6	1.0	20.7	11.8	yes	yes	**0.57**	**0.82**	*0.82*	*0.82*	**1.19**
	3	72	71.6	1.472	1.489	0.9	1.8	6.9	10.5	yes	yes	**0.44**	**0.85**	*0.83*	*0.88*	**1.65**
	1	83.9	81.7	1.57	1.549	0.6	0.4	16.4	16.5	yes	yes	**0.35**	**0.73**	*0.71*	*0.76*	**1.55**
L08	2	69.5	77.2	1.135	1.470	15.0	1.8	38.6	26.9	no	yes					
	3	73.7	77.8	1.446	1.440	3.6	6.3	25.2	20.9	yes	yes	**0.46**	**0.86**	*0.82*	*0.89*	**1.58**
	1	37.8	55.9	1.621	1.675	2.3	2.5	7.0	6.0	yes	yes	**0.56**	**1.00**	*0.98*	*1.02*	**1.76**
L09	2	43.7	30.2	1.531	1.526	2.6	2.8	16.9	15.5	yes	yes	**1.08**	**1.55**	*1.51*	*1.59*	**2.02**
	3	67.2	57.8	1.332	1.372	1.5	3.6	44.5	37.3	no	no					
	1	124.7	126.3	1.830	1.813	1.3	2.7	4.1	5.9	yes	yes	**0.40**	**0.76**	*0.74*	*0.78*	**1.45**
L10	2	57.4	54.1	1.868	1.841	4.1	2.6	7.3	4.4	yes	yes	**0.38**	**0.76**	*0.73*	*0.79*	**1.52**
	3	48.3	45.3	1.703	1.743	1.8	0.9	6.1	8.6	yes	yes	**0.49**	**0.89**	*0.86*	*0.92*	**1.61**
	4	*48.6*	*48.4*	*1.744*	*1.78*	*1.4*	*1.9*	*5.9*	*8.7*	*yes*	*yes*	**not considerd (exceeds three repetitions per lab)**				
	1	122.4	118.0	1.254	1.279	3.7	2.7	6.6	7.3	yes	yes	**0.47**	**0.84**	*0.81*	*0.86*	**1.49**
L11	2	129.0	129.4	1.439	1.429	1.3	1.5	15.9	19.5	yes	yes	**0.99**	**1.55**	*1.53*	*1.58*	**2.43**
	3	127.6	126.7	1.363	1.39	2.0	2.7	15.3	15.1	yes	yes	**0.59**	**0.98**	*0.97*	*0.99*	**1.63**
	1	32.0	29.2	1.228	1.273	0.9	0.8	39.5	41.0	no	no					
L12	2	30.5	30.0	1.146	1.17	3.8	3.9	38.1	37.9	no	no					
	3	34.3	33.3	1.091	1.150	4.6	5.5	48.0	26.2	no	no					
	1	104.3	102.6	1.343	1.344	0.3	1.2	18.6	20.4	yes	yes	**0.40**	**0.78**	*0.78*	*0.78*	**1.53**
L13	2	98.6	101.0	1.300	1.323	2.2	2.4	23.2	20.0	yes	yes	**0.41**	**0.80**	*0.78*	*0.82*	**1.64**
	3	99.0	112.1	1.269	1.271	2.0	1.4	29.7	27.9	yes	yes	**0.46**	**0.79**	*0.78*	*0.81*	**1.38**

Fig A 1: Results Mandels-h and Mandels k-statistic (graphical presentation), x-axis: laboratory number. Mandels h investigates laboratory means, Mandels k investigates laboratory specific variability. Tests with *Desmodesmus.*



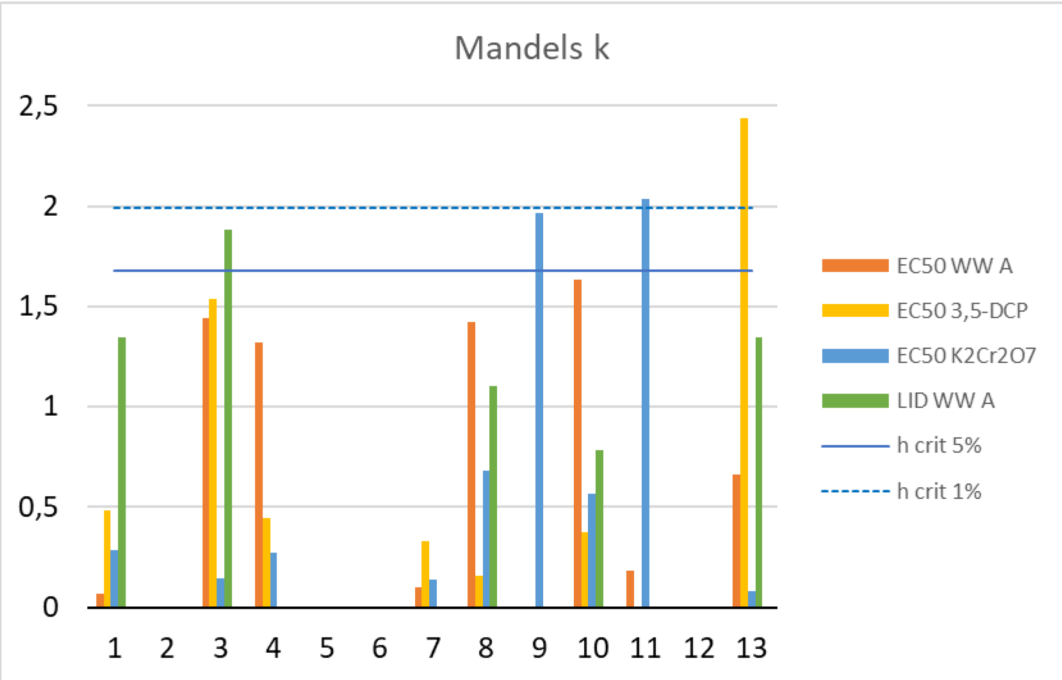
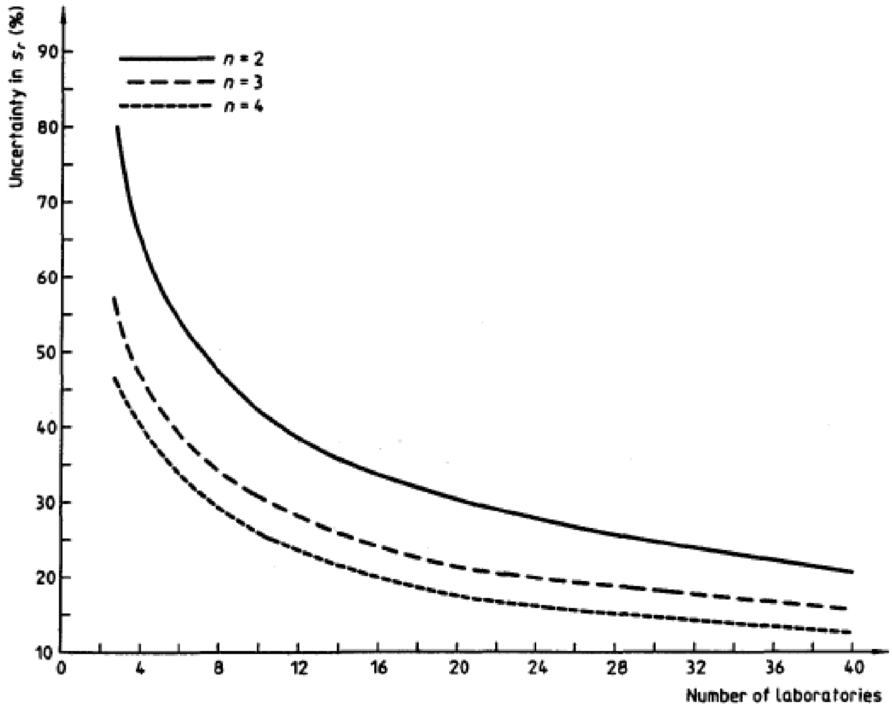
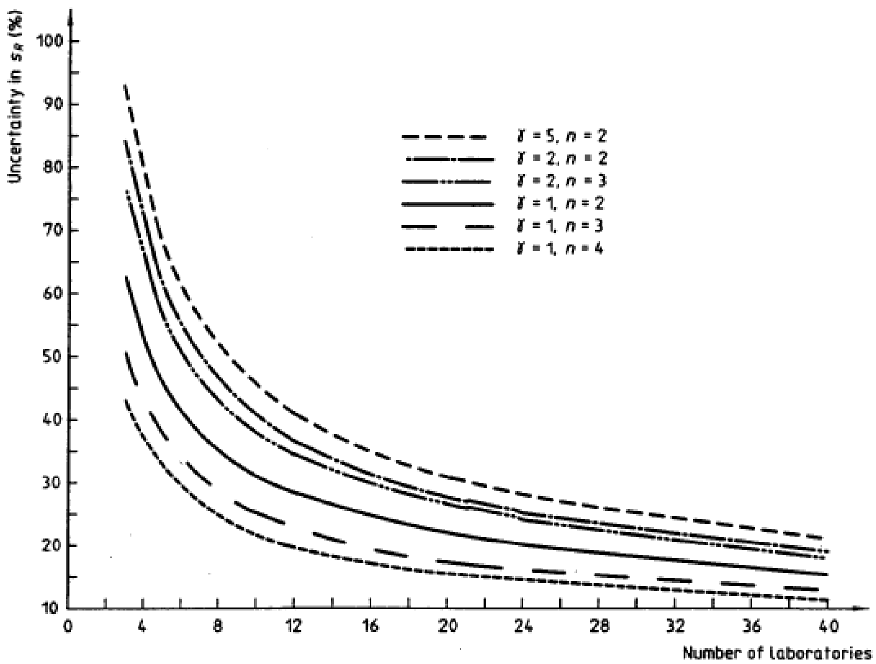


Fig A2: Uncertainty of repeatability (s_r) and reproducibility (s_R) depending on number of laboratories (p), number of repeated measurements per laboratory (n) and ratio s_r/s_R (Υ). From DIN ISO 5725-1:1997-11, Annex B, p 41 and p43



— The amount by which s_r can be expected to differ from the true value within a probability level of 95 %



— The amount by which s_R can be expected to differ from the true value within a probability level of 95 %

7. Annex B

Overview data base and experimental conditions for tests with *Raphidocelis*, results of stepwise data consolidation, validity check and outlier tests; data base for ring test statistics

Step 1:

- Adjustment file names, completion of laboratory number etc.
- Check whether data templates and protocols are complete and consistent, request for additional information if necessary.
- Overview test conditions (storage of samples, test irradiation, test temperature, pH adjustment of waste water samples, date of test start, initial cell number, any deviations from protocol), clarification of queries, request for additional information if necessary.
- Correction of mistakes in data templates (delete additional sheets / rows / lines, restore cell formulas if they were deleted or overwritten, correct formats (number / text), correct sequence of dilutions, identify missing data).
- Overview storage and test conditions per biotest **(Tab. B1-B5).**

Step 2:

- Results biotests with *Raphidocelis* (validity, LID, EC(r)50) **Tab. B6-B9**

Tab B1: Overview basic test conditions for all samples per laboratory. Tests with *Raphidocelis.*

General information		conditions Microplate (MP)				irradiation		flourescence measurement	
Lab	Test	MP Type	cover foil in addition to lid yes/no	taping yes/no	Randomisation on microplate shaker yes/no	lux nominal: 6000-10000	µmol nominal: 60-120 µmol m-2 s-1	wave length	from bottom / top
L01									
L02	1 2 3	Greiner Cellstar, Art.Nr. 662160	yes, Diversified Biotech, Breathe Easy, Nr. BEM-1	no	no		80-87	458 / 685	bottom
L03									
L04*	1 2 3 4	Greiner Bio-One, CELLSTAR 24 Well Cell Culture Plate, Art.Nr. 662160	no	yes	no	10747		485 / 685	bottom
L05	1 2 3	Greiner bio-one; cellstar 24 Well Suspension Culture Plate, Cat.-No. 662 102	no	yes	yes	7000		485 / 685	top
L06									
L07	1 2 3	Greiner, 24-Well Suspensionsplatten, Art.-Nr. 662 102	no	yes	yes		83	435 / 685	bottom
L08									
L09	1 2 3	Falcon 351147	no	yes	yes	7675 lm		485 / 680	top
L10									
L11									
L12**	1 2 3	Greiner Bio-One, Cellstar, Cat. Nr. 662102	no	yes	yes		101	440 / 680	bottom
L13									

* L04. No protocol provided for *Raphidocelis.* Test conditions are assumed to be identical with those for *Desmosdesmus.*
** L12 Laboratory reported problems with loss of volume. Original comment: "Relative Luftfeuchtigkeit im Inkubator HV1: 35.2 %, HV2: 31.4%, HV3: 26.7% RH → beim HV3 hatten wir in einigen Wells deutliche Volumenverluste (z.T. nur noch 750µL statt 2000µL"

Tab B2: Overview test conditions for waste water A per laboratory; tests with *Raphidocelis.*

Waste Water A					pH defrosted sample, pH adjustment if>8,5								
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: -18°	sample defrosted on	before	after	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [Z/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01													
L02	1	frozen	-18 / -20	11-Jan-21	8,0	-	08-Jan-21	11-Jan-21	23,4 - 23,9	n.d.	n.d.	4900	
	2			12-Jan-21	8,2	-	12-Jan-21	12-Jan-21	23,4 - 23,9	n.d.	n.d.	5400	
	3			18-Jan-21	8,1	-	15-Jan-21	18-Jan-21	23,9 - 23,9	n.d.	n.d.	5100	
L03													
L04	1	frozen	<u><</u>-18	10-Nov-20	8,0	-	06-Nov-20	10-Nov-20	23,4 - 23,6	1,78E+06	1,78E+06	8527	
	2			25-Jan-21	8,2	-	21-Jan-21	25-Jan-21	23,8-24,0	3205730	3205730	5186	
	3												
	4												
L05	1	frozen	-20	26-Oct-20	8,04	-	22-Oct-20	26-Oct-20	22,5 - 23,5	3,75E+06	3750000	10000	
	2			02-Nov-20	8,09	-	19-Oct-20	02-Nov-20	22,5 - 23,5	2,50E+06	3000000	10000	
	3			09-Nov-20	8,06	-	02-Nov-20	09-Nov-20	22,5 - 23,5	2,97E+06	3281250	10000	
L06													
L07	1	frozen	-18	07-Dec-20	8,2	-	04-Dec-20	07-Dec-20	22 - 24	211384	73418	7655	
	2			14-Dec-20	8,2	-	11-Dec-20	14-Dec-20	22 - 24	584689	69774	6893	
	3			11-Jan-21	8,0	-	08-Jan-21	12-Jan-21	22 - 24	1233079	66045	6215	
L08													
L09	1	frozen	-18 / -24	11-Jan-21	8,2	-	08-Jan-21	11-Jan-21	21 - 25		7,07E+06	10000	
	2			18-Jan-21	8,1	-	15-Jan-21	18-Jan-21	21 - 25		5,35E+06	10000	
	3			19-Jan-21	8	-	15-Jan-21	19-Jan-21	21 - 25		5,35E+06	10000	
L10													
L11													
L12	1	frozen	-20	09-Nov-20	8,17	-	05-Nov-21	09-Nov-21	22,9 - 25,0	2,36E+06	2,36E+06	10800	
	2			16-Nov-20	8,4	-	12-Nov-20	16-Nov-20	24,2 - 25,1	1,62E+06	1,62E+06	10800	
	3			23-Nov-20	8,1	-	19-Nov-20	23-Nov-20	24,7 -25,2	2,33E+06	2330000	12100	
L13													

Tab B3: Overview test conditions for waste water B per laboratory; tests with *Raphidocelis*..

Waste Water B					pH defrosted sample, pH adjustment if > 8,5								
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: -18°	sample defrosted on	before	after	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [Z/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01													
L02	1	frozen	-18 / -20	11-Jan-21	8,2	-	08-Jan-21	11-Jan-21	23,4 - 23,9	n.d.	n.d.	5700	
	2			12-Jan-21	n.d.	-	08-Jan-21	12-Jan-21	23,4 - 23,9	n.d.	n.d.	5300	
	3			18-Jan-21	8,2	-	15-Jan-21	18-Jan-21	23,3 - 23,9	n.d.	n.d.	5100	
L03													
L04	1	frozen	≤-18	25-Jan-21	9,0	7,1	21-Jan-21	25-Jan-21	23,8 - 24,0	3,21E+06	3,21E+06	5186	
	2			26-Jan-21	7,8	-	22-Jan-21	26-Jan-21	23,8 - 24,0	3465355	3465355	4803	
	3			01-Feb-21	9,1	7,1	28-Jan-21	01-Feb-21	23,8 - 23,9	2956000	2956000	5137	
	4												
L05	1	frozen	-20	26-Oct-20	7,78	-	22-Oct-20	26-Oct-20	22,5 - 23,5	3,75E+06	3750000	10000	
	2			02-Nov-20	8,44	-	19-Oct-20	02-Nov-20	22,5 - 23,5	2,25E+06	3000000	10000	
	3			09-Nov-20	8,6	7	01-Nov-20	09-Nov-20	22,5 - 23,5	2,97E+06	3281250	10000	
L06													
L07	1	frozen	-18	07-Dec-20	7,5	-	04-Dec-20	07-Dec-20	22,0 - 24,0	211384	73418	7740	
	2			07-Dec-20	8,7	-	04-Dec-20	08-Dec-20	22,0 - 24,0	522316	73757	7146	
	3			14-Dec-20	8,2	-	11-Dec-20	14-Dec-20	22,0 - 24,0	584689	69774	6977	
L08													
L09	1	frozen	-18 / -24	11-Jan-21	7,9	-	08-Jan-21	11-Jan-21	21,0 - 25,0		7,07E+06	10000	
	2			18-Jan-21	8,5	-	15-Jan-21	18-Jan-21	21,0 - 25,0		5,35E+04	10000	
	3			19-Jan-21	9	6,9	15-Jan-21	19-Jan-21	21,0 - 25,0		5,35E+06	10000	
L10													
L11													
L12	1	frozen	-20	09-Nov-20	9	6,8	05-Nov-20	09-Nov-20	22,9 -25,0	2,36E+06	2,36E+06	10800	
	2			16-Nov-20	7,7	-	12-Nov-20	16-Nov-20	24,2 -25,1	1,62E+06	1,62E+06	10800	
	3			23-Nov-20	7,4	-	19-Nov-20	23-Nov-20	24,6 - 25,2	2,33E+06	2330000	12100	
L13													

Tab B4: Overview test conditions for 3,5-DCP per laboratory; tests with *Raphidocelis.*

DCP										
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: cooling	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [Z/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01										
L02	1	cooled	4-6	11-Dec-20	14-Dec-20	23,3 - 23,9	n.d.	n.d.	5300	
	2			11-Dec-20	15-Dec-20	23,3 - 23,9	n.d.	n.d.	5300	
	3			15-Jan-21	19-Jan-21	23,0 - 23,7	n.d.	n.d.	4900	
L03										
L04	1	cooled	2-8	07-Jan-21	11-Jan-21	23,07 - 23,9	4,21E+06	4,21E+06	4503	
	2			21-Jan-21	25-Jan-21	23,8 - 24,0	3205730	3205730	5186	
	3			29-Jan-21	02-Feb-21	23,8 - 23,9	4150780	4150780	5128	
L05	1	cooled	4	22-Oct-20	26-Oct-20	22,5 - 23,5	3,75E+06	3750000	10000	
	2			19-Oct-20	02-Nov-20	22,5 - 23,5	2,25E+06	3000000	10000	
	3			02-Nov-20	09-Nov-20	22,5 - 23,5	2,97E+06	3281250	10000	
L06										
L07	1	cooled	4-8	23-Oct-20	27-Oct-20	22,0 - 24,0	872316	74774	7740	
	2			30-Oct-20	03-Nov-20	22,0 - 24,0	636384	74096	7147	
	3			27-Nov-20	01-Dec-20	22,0 - 24,0	948418	74977	7401	
L08										
L09	1	frozen	2-8	22-Jan-21	25-Jan-21	21,0 - 25,0		1,60E+06	10000	
	2			29-Jan-21	01-Feb-21	21,0 - 25,0		7,30E+05	10000	
	3			29-Jan-21	02-Feb-21	21,0 - 25,0		7,30E+05	10000	
L10										
L11										
L12	1	frozen	4	05-Nov-20	09-Nov-20	22,9 - 25,0	2,36E+06	2,36E+06	10800	
	2			12-Nov-20	16-Nov-20	23,9 - 25,1	1,62E+06	1,62E+06	10800	
	3			19-Nov-20	23-Nov-20	24,7	2,33E+06	2330000	12100	
L13										

Tab B5: Overview test conditions for $K_2Cr_2O_7$ per laboratory; tests with *Raphidocelis.*

K2Cr2O7										
Lab	Test	sample condition at arrival frozen / ice-core / cool / warm	Storage at [T°C] nominal: cooling	date preculture	date test start	Test temp nominal: 21-25 °C	cells/ml at end preculture	cells/ml in preculture [Z/ml]	initial cell number at test start [cells/ml] (protocol)	remarks
L01										
	1			04-Dec-20	08-Dec-20	23,4 - 24,1	n.d.	n.d.	5200	
L02	2	cooled	4-6	11-Dec-20	14-Dec-20	23,3 - 23,9	n.d.	n.d.	5900	
	3			11-Dec-20	15-Dec-20	23,3 - 23,9	n.d.	n.d.	6100	
L03										
	1			20-Nov-20	23-Nov-20	23,5 - 23,8	3,11E+06	3,11E+06	7847	
L04	2	cooled	2-8	07-Jan-21	11-Jan-21	23,7 - 23,9	4213335	4213335	4503	
	3			22-Jan-21	26-Jan-21	23,8 - 24,0	3465355	3465355	4808	
	1			22-Oct-20	26-Oct-20	22,5 - 23,5	3,75E+06	3750000	10000	
L05	2	cooled	4	19-Oct-20	02-Nov-20	22,5 - 23,5	2,25E+06	3000000	10000	
	3			02-Nov-20	09-Nov-20	22,5 - 23,5	2,97E+06	3281250	10000	
L06										
	1			23-Oct-20	27-Oct-20	22,0 - 24,0	872316	74774	7655	
L07	2	cooled	4-8	30-Oct-20	03-Nov-20	22,0 - 24,0	636384	74096		
	3			27-Nov-20	30-Nov-20	22,0 - 24,0	582994	78927		
L08										
	1			22-Jan-21	25-Jan-21	21,0 - 25,0		1,60E+06	10000	
L09	2	frozen	2-8	29-Jan-21	01-Feb-21	21,0 - 25,0		7,30E+05	10000	
	3			29-Jan-21	02-Feb-21	21,0 - 25,0		7,30E+05	10000	
L10										
L11										
	1			05-Nov-20	09-Nov-20	22,9 - 25,0	2,36E+08	2,36E+08	10800	
L12	2	frozen	4	12-Nov-20	15-Nov-20	24,2 - 25,1	1,62E+08	1,62E+08	10800	
	3			19-Nov-20	23-Nov-20	24,7 - 25,2	2,33E+08	2,33E+08	12100	
L13										

Tab B6: Waste water A: validity and results for LID and EC(r)x per laboratory; tests with *Raphidocelis.*

Waste water A		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥ 1,2 d-1]		CV% GR control [≤ 7%]		Exponent. growth? [CV% sectional GR ≤ 35%]		Test valid?			EC(r)x (non lin reg) (Vol %)				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	LID [≤ 10% inhib.]	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1																
	2																
	3																
L02	1	28.5	34.8	1.011	1.007	8.2	5.1	59.8	65.7	no	no						
	2	55.7	48.9	1.657	1.785	4	5.3	38.4	37.6	no	no						
	3	26.6	23.5	1.661	1.686	1.5	1.3	48.1	42.7	no	no						
L03	1																
	2																
	3																
L04	1	152.1	131.4	1.419	1.649	3.7	2.5	55.2	49.9	no	no						
	2	141.2	112.7	1.455	1.676	4.4	2.9	20.2	20.9	yes	yes	6	**24.38**	**35.87**	*33.53*	*38.26*	**52.77**
	3																
	4																
L05	1	105	49.5	1.963	1.852	58.3	20.5	39.6	33.5	no	no						
	2	38.7	9.1	1.504	1.643	7.7	7.9	21.2	20	no	no						
	3	no data	105.1	1.614	1.516	14.8	8.5	39.4	35	no	no						
L06	1																
	2																
	3																
L07	1	165.4	180.9	1.471	1.484	1.5	1.4	16.8	17.6	yes	yes	6	**21.88**	**34.39**	*32.07*	*36.77*	**54.06**
	2	176.5	201.6	1.433	1.452	1.4	1.0	31.8	37.1	yes	no						
	3	157.4	162.9	1.500	1.521	0.7	0.9	21.9	36.2	yes	no						
I08	1																
	2																
	3																
L09	1	127.4	131.5	1.693	1.729	2.3	2.6	34.3	29.8	ja	ja	6	**23.14**	**33.95**	*33.25*	*34.67*	**49.80**
	2	135.4	112.9	1.827	1.839	8.3	4.9	34.9	27.5	no	ja						
	3	84.4	68.4	1.942	1.879	4.9	0.6	32.1	30.2	ja	ja	4	**29.22**	**45.05**	*43.64*	*46.48*	**69.45**
L10	1																
	2																
	3																
I11	1																
	2																
	3																
L12	1	84.2	73.9	1.478	1.536	0.6	1.0	39.7	26.3	no	yes						
	2	82.7	56.0	1.396	1.429	1.0	2.1	31.0	41.2	yes	no						
	3	108.2	99.2	1.608	1.607	1.1	1.9	27	38.9	yes	no						
L13	1																
	2																
	3																

Tab B7: Waste water B: validity and results for LID and EC(r)x per laboratory; tests with *Raphidocelis.*

Waste water B		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?			EC(r)x (non lin reg) (Vol %)				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	LID [≤10% inhib.]	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1																
	2																
	3																
L02	1	51.6	33.1	1.174	1.139	1.7	6.4	65.6	36.3	no	no						
	2	21.7	7.9	1.131	1.086	9.8	6.7	51.9	52.9	no	no						
	3	25.6	7.9	1.673	1.086	1.6	6.7	44.6	52.9	no	no						
L03	1																
	2																
	3																
L04	1	118.5	122.3	1.568	1.534	3.8	4.1	24.1	21.3	yes	yes	2	**57.50**	**73.63**	*66.82*	*81.02*	**84.28**
	2	116.3	111.3	1.540	1.615	3.8	1.6	34.6	25.7	yes	yes	3	**48.73**	**67.44**	*63.96*	*71.02*	**93.35**
	3	114.3	113.9	1.539	1.754	2.3	3.5	33.1	28.1	yes	yes	3	**55.34**	**69.57**	*63.42*	*76.60*	**87.47**
	4																
L05	1	54.8	79.6	1.645	1.702	7.5	10.5	37.4	34	no	no						
	2	17.2	39.4	1.641	1.906	11.5	19.4	32.3	32.3	no	no						
	3	92.3	73.6	1.499	1.548	10.7	6.0	44.3	27.8	no	no						
L06	1																
	2																
	3																
L07	1	158.5	172.5	1.414	1.446	0.8	1.8	22.8	19.5	yes	yes	3	**55.31**	**81.83**	*76.40*	*87.33*	**extrapol.**
	2	169.4	162.0	1.503	1.519	1.7	0.9	15.4	14.9	yes	yes	3	**45.50**	**66.12**	*62.97*	*69.33*	**96.08**
	3	200.6	181.2	1.426	1.43	2.6	1.0	33	32.6	yes	yes	4	**45.14**	**76.55**	*68.64*	*84.82*	**extrapol**
I08	1																
	2																
	3																
L09	1	127.5	123.0	1.719	1.72	2.1	2.5	24.3	17.1	yes	yes	4	**31.01**	**53.26**	*48.74*	*58.06*	**91.48**
	2	119.8	116.8	1.907	1.853	4.2	4.2	23.6	37.7	yes	no						
	3	67.2	91.0	1.969	1.865	2.0	7.0	33.8	27.2	yes	yes	3	**43.73**	**68.40**	*60.54*	*76.92*	**106.98**
L10	1																
	2																
	3																
I11	1																
	2																
	3																
L12	1	54.2	51.8	1.503	1.489	0.8	1.6	32.1	29.7	yes	yes	2	**n.d.**	**n.d.**	*n.d.*	*n.d.*	**n.d.**
	2	90.8	82.2	1.435	1.419	3.7	2.4	32.5	39.7	yes	no						
	3	84	101.8	1.561	1.546	0.9	1.7	36.6	33.2	no	yes						
L13	1																
	2																
	3																

Tab B8: 3,5-DCP: validity and results for ECx per laboratory; tests with *Raphidocelis*

DCP		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?		EC(r)x (non lin reg) [mg/]				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1															
	2															
	3															
L02	1	4.8	2.9	1.456	1.476	2.1	1.7	30.9	30.2	yes	yes	**n.d.**	*n.d.*	*n.d.*	*n.d.*	**n.d.**
	2	18.8	17.0	1.747	1.692	1.7	2.3	25.4	25.3	yes	yes	**2.48**	*3.68*	*3.2*	*4.19*	**extrapol.**
	3	25.1	21.6	1.318	1.355	4.7	4	66.6	68.8	no	no					
L03	1															
	2															
	3															
L04	1	113.4	113.3	1.564	1.611	2.9	4.4	47.3	56.4	no	no					
	2	130.3	117.6	1.602	1.62	1.2	2.4	28.7	26.6	yes	yes	**1.855**	*1.96*	*1.807*	*2.11*	**2.07**
	3	111.9	100.2	1.403	1.431	3.5	3.5	39.1	29.8	no	yes					
L05	1	92.9	56.0	1.401	1.571	5.4	12.2	51.9	34	no	no					
	2	51.9	37.4	1.672	1.625	13.5	9.6	24.3	28.6	no	no					
	3	106	104.2	1.698	1.578	13.8	7.7	30.8	30.1	no	no					
L06	1															
	2															
	3															
L07	1	81.8	81.4	1.679	1.692	1.0	0.4	11.3	10.3	yes	yes	**1.31**	*1.77*	*1.66*	*1.89*	**2.400**
	2	82.3	85.8	1.708	1.699	1.2	1.0	12.1	15.4	yes	yes	**1.32**	*1.85*	*1.76*	*1.95*	**2.60**
	3	166.5	163.5	1.439	1.435	0.8	2.1	38.7	36.7	no	no					
l08	1															
	2															
	3															
L09	1	125.2	127.9	1.757	1.819	2.5	1.6	29.8	33.2	yes	yes	**2.25**	*2.39*	*2.3*	*2.48*	**2.53**
	2	109.6	103	1.752	1.863	1.4	1.4	36.2	31.2	no	yes					
	3	326	322	1.713	1.739	3.1	4.5	40.8	27.8	no	yes					
L10	1															
	2															
	3															
l11	1															
	2															
	3															
L12	1	80.1	86.1	1.545	1.557	2.3	1.3	28.4	31.4	yes	yes	**2.34**	*2.35*	*2.16*	*2.57*	**2.470**
	2	80.1	91.6	1.419	1.438	10.6	1.9	39.2	37.8	no	no					
	3	114.7	102.9	1.53	1.503	3.1	0.4	36	46.6	no	no					
L13	1															
	2															
	3															

Tab B9: $K_2Cr_2O_7$: validity and results for EC(r)x per laboratory; tests with *Raphidocelis.*

DCP		PC: 2,5 mg/L 3,5-DCP Inhibition GR [%] nominal 20-80% not relevant for validity		GR Control [≥1,2 d-1]		CV% GR control [≤7%]		Exponent. growth? [CV% sectional GR ≤35%]		Test valid?		EC(r)x (non lin reg) [mg/l]				
Lab	Test	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	plate 2	plate 1	EC(r)20	EC(r)50	EC(r)50 95% CL lower	EC(r)50 95% CL upper	EC(r)80
L01	1															
	2															
	3															
L02	1	4.8	2.9	1.456	1.476	2.1	1.7	30.9	30.2	yes	yes	**n.d.**	*n.d.*	*n.d.*	*n.d.*	**n.d.**
	2	18.8	17.0	1.747	1.692	1.7	2.3	25.4	25.3	yes	yes	**2.48**	*3.68*	*3.2*	*4.19*	**extrapol.**
	3	25.1	21.6	1.318	1.355	4.7	4	66.6	68.8	no	no					
L03	1															
	2															
	3															
L04	1	113.4	113.3	1.564	1.611	2.9	4.4	47.3	56.4	no	no					
	2	130.3	117.6	1.602	1.62	1.2	2.4	28.7	26.6	yes	yes	**1.855**	*1.96*	*1.807*	*2.11*	**2.07**
	3	111.9	100.2	1.403	1.431	3.5	3.5	39.1	29.8	no	yes					
L05	1	92.9	56.0	1.401	1.571	5.4	12.2	51.9	34	no	no					
	2	51.9	37.4	1.672	1.625	13.5	9.6	24.3	28.6	no	no					
	3	106	104.2	1.698	1.578	13.8	7.7	30.8	30.1	no	no					
L06	1															
	2															
	3															
L07	1	81.8	81.4	1.679	1.692	1.0	0.4	11.3	10.3	yes	yes	**1.31**	*1.77*	*1.66*	*1.89*	**2.400**
	2	82.3	85.8	1.708	1.699	1.2	1.0	12.1	15.4	yes	yes	**1.32**	*1.85*	*1.76*	*1.95*	**2.60**
	3	166.5	163.5	1.439	1.435	0.8	2.1	38.7	36.7	no	no					
I08	1															
	2															
	3															
L09	1	125.2	127.9	1.757	1.819	2.5	1.6	29.8	33.2	yes	yes	**2.25**	*2.39*	*2.3*	*2.48*	**2.53**
	2	109.6	103	1.752	1.863	1.4	1.4	36.2	31.2	no	yes					
	3	326	322	1.713	1.739	3.1	4.5	40.8	27.8	no	yes					
L10	1															
	2															
	3															
I11	1															
	2															
	3															
L12	1	80.1	86.1	1.545	1.557	2.3	1.3	28.4	31.4	yes	yes	**2.34**	*2.35*	*2.16*	*2.57*	**2.470**
	2	80.1	91.6	1.419	1.438	10.6	1.9	39.2	37.8	no	no					
	3	114.7	102.9	1.53	1.503	3.1	0.4	36	46.6	no	no					
L13	1															
	2															
	3															