

Electronic Supplementary Information

Toxic equivalent concentrations for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples

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Inhibition of bioluminescence in the marine bacterium *Vibrio fischeri*

The concentration-effect curves of the baseline toxicants are shown in Figure ESI 1.

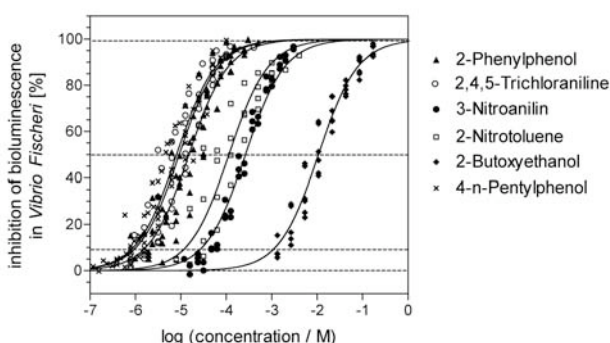


Fig. ESI 1 Concentration-effect curves of the baseline toxicants in the bioluminescence inhibition test with *Vibrio fischeri*

The combined algae test with *Pseudokirchneriella subcapitata*

The concentration-effect curves of the baseline toxicants are shown in Figure ESI 2A-C for the three different endpoints of the combined algae test.

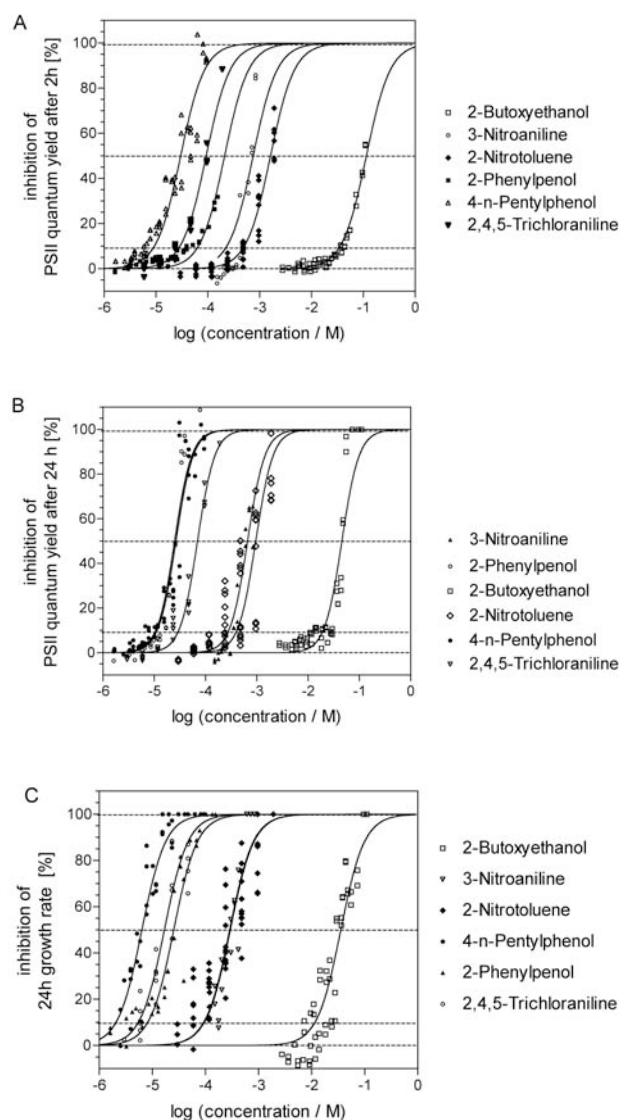


Fig. ESI 2 Concentration-effect curves of the baseline toxicants in the algae test with *Pseudokirchneriella subcapitata*. A. endpoint 2h IPAM, B. endpoint 24h IPAM, C. endpoint 24h growth rate. For the growth rate endpoint, all data of high concentrations where the algae were disintegrating (corresponding to growth rates lower than 0 and inhibition values above 100%), were set to 100% inhibition.

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Validation of the combined algae test

In Figure ESI 3, the EC₅₀ values of diuron and the baseline toxicants are compared between the 24h Maxi Imaging PAM (IPAM) endpoint of the combined algae test and the earlier developed 24h ToxY-PAM test ¹.

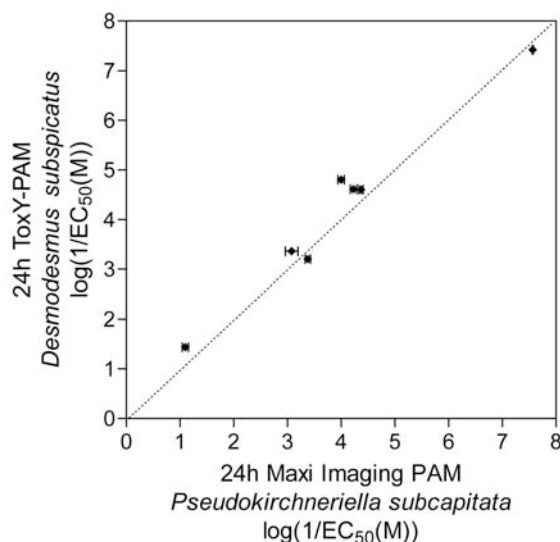


Fig. ESI 3 Validation of the combined algae test with *Pseudokirchneriella subcapitata* by comparison with the earlier developed Tox-PAM test ^{1,2}. The line is not the regression but the 1:1 line.

$$\log(1/EC_{50, \text{Desmodesmus subspicatus, 24h ToxYPAM}} (M)) = (0.95 \pm 0.05) \cdot \log(1/EC_{50, \text{Pseudokirchneriella subcapitata 24hIPAM}} + (0.33 \pm 0.21) \quad (ESI1)$$

$$r^2 = 0.99, n=7, F=426$$

Comparison between the baseline toxicity in *Vibrio Fischeri* and *Pseudokirchneriella subcapitata*

In Figure ESI 4, the EC₅₀ values of diuron and the baseline toxicants are compared between the various endpoints of the combined algae test and the bioluminescence inhibition test with *Vibrio Fischeri*.

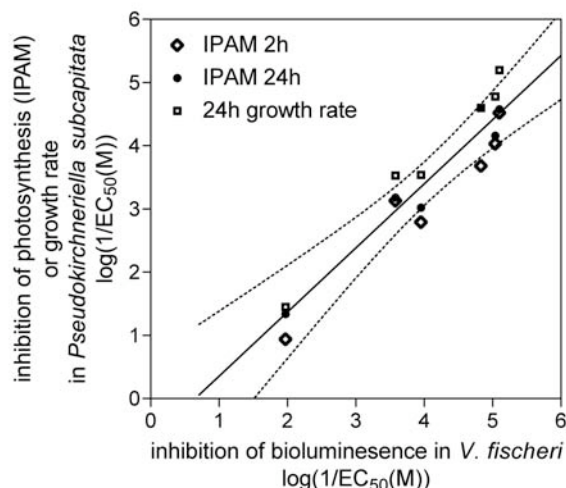


Fig. ESI 4 Comparison of the EC₅₀ values of the baseline toxicants in the bioluminescence inhibition test with *Vibrio Fischeri* and the various endpoints of the combined algae test with *Pseudokirchneriella subcapitata*. The line corresponds to the linear correlation for the IPAM24h endpoint and the dotted curve marks the 95% confidence interval.

Environmental samples tested using the bioassay with *Vibrio fischeri* and the combined algae test species?

In Table ESI1 the TEQ values of the various environmental samples are listed. This table forms the basis of Figure 3 in the main manuscript.

Table ESII Toxic equivalent concentrations

Test organism and endpoint	Toxicity expressed as	STP after primary clarifier (primary effluent)	STP after secondary clarifier (secondary effluent)	STP after sand filtration (final effluent)	River upstream of STP	River downstream of STP	Procedural Blank
<i>Vibrio Fischeri</i>	Baseline-TEQ (mg L ⁻¹) ^a (standard deviation)	5.73 (1.01)	0.45 (0.08)	0.31 (0.07)	0.45 (0.10)	0.35 (0.05)	0.01 (0.00)
<i>P. subcapitata</i> , 24h growth rate	Baseline-TEQ (mg L ⁻¹) (standard deviation)	4.01 (1.76)	0.70 (0.05)	0.43 (0.26)	0.57 (0.01)	0.64 (0.49)	0.08 (0.04)
<i>P. subcapitata</i> , 2h IPAM	DEQ (µg L ⁻¹) (standard deviation)	0.26 (0.06)	0.14 (0.05)	0.18 (0.06)	0.16 (0.10)	0.18 (0.04)	0.00 (0.00)
<i>P. subcapitata</i> , 24h IPAM	DEQ (µg L ⁻¹) (standard deviation)	0.39 (0.03)	0.24 (0.01)	0.28 (0.03)	0.28 (0.10)	0.22 (0.15)	0.02 (0.00)
<i>P. subcapitata</i> , 24h growth rate	DEQ (µg L ⁻¹) (standard deviation)	8.63 (3.80)	1.50 (0.10)	1.08 (0.57)	1.32 (0.16)	1.38 (1.05)	0.18 (0.08)

^a Calculated by eq. 3, using the EC₅₀ values of diuron given in Table 2 and the EC₅₀ of the reference baseline toxicant of 12.2 mg L⁻¹ (see main manuscript for derivation)

References

1. B. I. Escher, N. Bramaz, R. I. L. Eggen and M. Richter, *Environ. Sci. Technol.*, 2005, **39**, 3090-3100.
2. S. M. Bengtson Nash, K. McMahon, G. Eaglesham and J. F. Mueller, *Mar. Poll. Bull.*, 2005, **51**, 351-360.