



Feb 17, 2026

Version 2

Contamination procedure for chemical risk assessment in periphyton V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l6zjpdgqe/v2>



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Protocol Citation: Lin Zi, Mélissa EON, Zahrasadat Alavikakhki, Chloé Bonnineau, Nicolas Creusot 2026. Contamination procedure for chemical risk assessment in periphyton. **protocols.io**

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Protocol status: Working

We use this protocol and it's working

Created: February 17, 2026

Last Modified: February 17, 2026

Protocol Integer ID: 243441

Keywords: Chemical risk assessment, periphyton, contamination, contamination procedure for chemical risk assessment, throughput chemical risk assessment, standardized contamination procedure, chemical risk assessment, using periphyton, contamination procedure, simultaneous evaluation of multiple chemical, multiple chemical, laboratory efficiency, periphyton this document, laboratory

Funders Acknowledgements:

Agence nationale de la recherche

Grant ID: ANR-11-INBS-0010

Agence nationale de la recherche

Grant ID: ANR-21-ESRE-0035

PARC: Partnership for the Assessment of Risks from Chemicals

Grant ID: 101057014

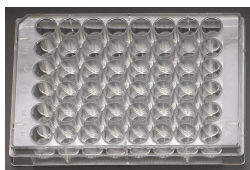
PHARM-ERA

Grant ID: 101119261

Abstract

This document outlines a standardized contamination procedure for high-throughput chemical risk assessment using periphyton in a 48-well plate format. This miniaturized approach allows for the simultaneous evaluation of multiple chemicals and concentration gradients, bridging the gap between field-scale complexity and laboratory efficiency.

Materials



48-well plate



HPLC amber glass vial to prepare chemical stock solution

🔗 periphyton colonized at 9 mm glass discs

Safety warnings

- ⚠️ **Safety:** Wear a lab coat, gloves, and safety glasses; utilize the fume hood and follow chemical safety rules.
- Waste:** Recycle all waste according to quality document EABX_GEN_706.
- Traceability:** Optimize labeling and storage for all samples, reagents, and stock solutions.
- Monitoring:** Perform gravimetric monitoring for all stock solutions.
- Documentation:** Record the weight, volume, and chemical nature of all internal standards in the laboratory notebook.

Before start

Periphyton has already been colonized at 9 mm diameter glass discs which are suitable for 48-well plates. The colonization procedure has been described in protocol "Periphyton growing and sampling in situ or in mesocosm for metabolomic analysis".



Periphyton colonized at 9 mm diameter glass discs

Setting-up discs in 48-well microplates and contaminants preparation

1 Draw up a 48-well plate plan

- 48-well plates allow to have 8 concentrations (including control) in row, and each concentration have 6 replicates in column.
- Define the chemical concentration range: 1–2 environmentally realistic concentrations, 2–3 intermediate concentrations aimed at detecting metabolomic responses, and 2–3 high-range concentrations aimed at inducing functional changes. Algal EC_{50} can be used as a reference for functional disruption.
- Six replicates per concentration is required to account for the inherent biological heterogeneity of periphyton communities.
- In parallel with each batch of experiment, include terbuthylazine (TBA) as a positive control. This facilitates the comparison of results across different experimental batches, accounting for the inherent biological variability of periphyton. TBA was chosen for its well-characterized dose-response relationship, ensuring a reliable reference for validating experimental sensitivity.

Plate 1: TBA 24 h

	1	2	3	4	5	6	7	8
A	C0	C1	C2	C3	C4	C5	C6	C7
B	C0	C1	C2	C3	C4	C5	C6	C7
C	C0	C1	C2	C3	C4	C5	C6	C7
D	C0	C1	C2	C3	C4	C5	C6	C7
E	C0	C1	C2	C3	C4	C5	C6	C7
F	C0	C1	C2	C3	C4	C5	C6	C7

2 Transfer the discs into the 48-wells microplates

- Before transfer the discs into 48-well plate, add 1 mL of 6x diluted DAUTA into each well.

- Transfer the disc into the microplate. Ensure that the colonized periphyton remains face-up throughout handling.
- Acclimate the microplates for 3 days

3 Prepare serial dilution of chemicals

- Prepare chemical stock solution in organic solvent (e.g. 100% Ethanol) or ultra-pure water according to the solubility of the chemical. In case of stock solution in organic solvent, ensure the final content of organic solvent applied to the periphyton is 0.05%.
- Example of prepare the serial dilution of chemicals

1. If you want to achieve the final concentration that applied to the periphyton as below:

A	B	C	D	E	F	G	H
Final concentration of Bisphenols (mg/L) contain 0.05% EtOH	10.00	3.00	1.00	0.30	0.10	0.03	0.01

2. The concentration of stock solution in 100% EtOH would be: $10 \text{ (mg/L)}/0.05\%=20 \text{ (mg/mL)}$. Prepare at least 100 μL of stock solution.

3. Prepare the serial dilution as follows: in this way, after 2000 times dilution, the final concentration of EtOH applied to periphyton is 0.05%, and all the concentrations contain same amount of EtOH. Using the cultivation solution of periphyton in the experiment to prepare the serial dilution (here using 6 x diluted DAUTA)

A	B	C	D	E	F	G	H
Serial dilution in 100% EtOH (mg/mL)	20.00	6.00	2.00	0.60	0.20	0.06	0.02
100 x dilution (mg/L): 10 μL serial 1+990 μL 6x DAUTA	200.00	60.00	20.00	6.00	2.00	0.60	0.20
20 x dilution (mg/L): 400 μL serial 2+7600 μL 6x DAUTA	10.00	3.00	1.00	0.30	0.10	0.03	0.01

- Store the stock solution at -20°C . Prepare the final serial of dilution at earliest 3 days before use.

Apply chemicals to the periphyton: contamination

4 Applied the chemicals well by well to avoid desiccation

- Discard all media of one well
- Gently add 1 mL of contaminants solution as fast as possible
- Duration of exposure: 4 h, 24 h, 72 h, 1 week: to be adapted to the scientific question. According to the duration think if you need to change the solution in the middle.



- For metabolomic analysis, 1 plate for 1 time-point. In case of several time points, start to contaminate for the longer exposure and finish by the shortest in order to be at the same level of growth.

Protocol references

For the periphyton colonization, please refer to the protocol "Periphyton growing and sampling in situ or in mesocosm for metabolomic analysis".