Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary information for

Release and stability of two tebuconazole nanoformulations in different aquatic media

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LC-MS/MS analysis

HPLC analysis was performed using an Agilent 1200 chromatographic system (Agilent, Santa Clara, CA, USA) equipped with a vacuum degasser, binary pump, autosampler, and a column thermostat, which was connected online to an ESI/QqQ mass spectrometer Agilent Triple Quad 6410 (Agilent, Santa Clara, CA, USA). The chromatographic/mass spectrometric system was controlled by Mass Hunter software. The HPLC conditions were as follows: ACE 3 C18 chromatographic column 150 mm in length x 2.2 mm internal diameter and 3 µm particle size, with integrated guard column 20 mm in length x 2.2 mm internal diameter and 3 µm particle size (ACE, Scotland, UK). The column temperature was maintained at 30 °C. The mobile phase comprised 0.1% acid formic (98% Sigma-Aldrich, Germany) in water and acetonitrile. The mobile phase gradient was as follows: 0–1 min 10% B, 1–7 min from 10% B to 98% B, and 98% B held to 12 min, 12–13 min from 98% B to 10% B with a subsequent equilibration step of 10% B to 25 min. The flow of the mobile phase was 0.3 mL/min during all the analyses.

The injection volume was 5 µL of the samples and 5 µl of the instrumental standard solution (D-metolachlor in acetonitrile, 50 ng mL^{-1}; Dr. Ehrenstorfer).

The mass spectrometer was operated in the ESI-positive SRM mode (gas temperature 350 °C, gas flow 9 L min^{-1}, nebulizer gas 40 psi, capillary voltage 4.0 kV). The following ion transitions were monitored: tebuconazole, *m/z* 308.2→70.0 with a collision energy of 20 eV (and for confirmation, *m/z* 308.2→151.0, 20 eV); terbuthylazine, *m/z*, 230.0→174.0, 20 eV (*m/z* 230.0 →104.0, 20 eV); D-metolachlor, *m/z* 290.1→258.1, 10 eV (*m/z* 290.1→182.1, 10 eV).

Quantitation of analytes was performed using an internal standard calibration method. Internal standards (D-metolachlor) were added to all samples for these purposes. Good linearity of calibration curves was obtained across the whole concentration range (from 1 to 1000 ng mL^{-1}).

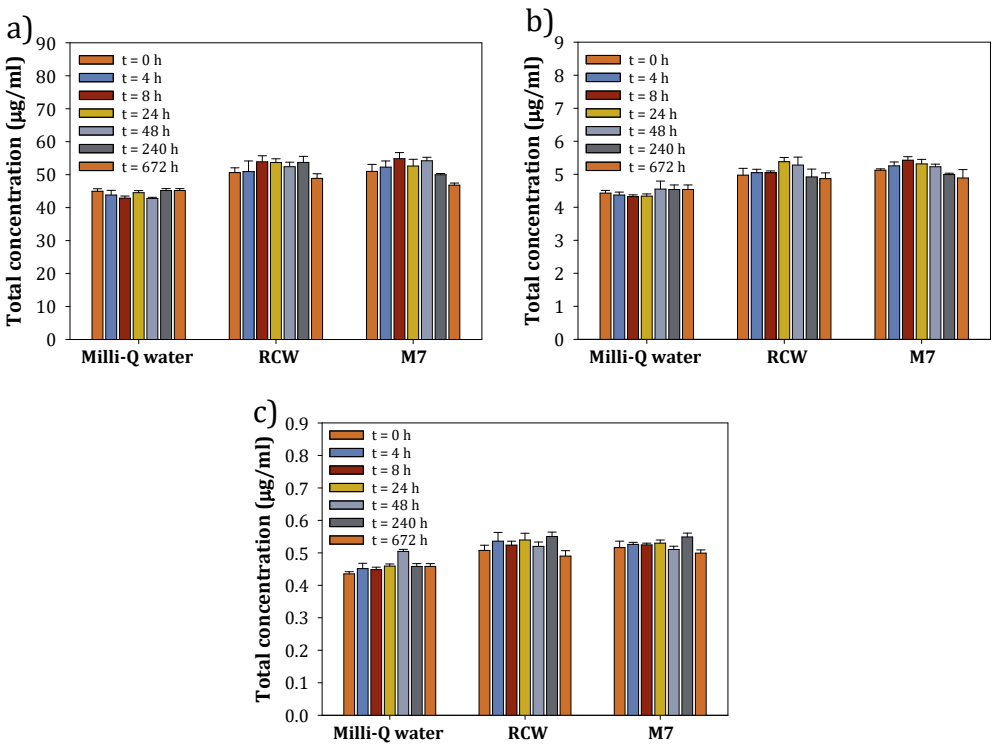

Fig. S1. Total concentration of a) 50 µg/mL, b) 5 µg/mL, and 0.5 µg/mL dilutions of PCL-TBZ over time.

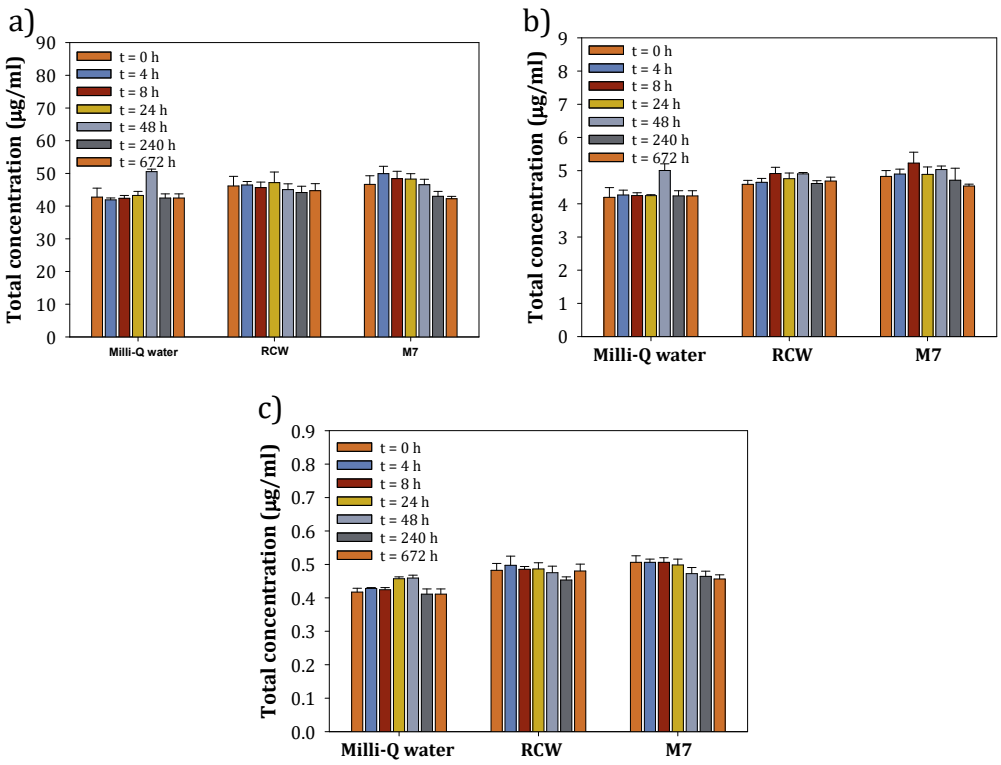

Fig. S2. Total concentration of a) 50 µg/mL, b) 5 µg/mL, and c) 0.5 µg/mL dilutions of NLC-TBZ over time.

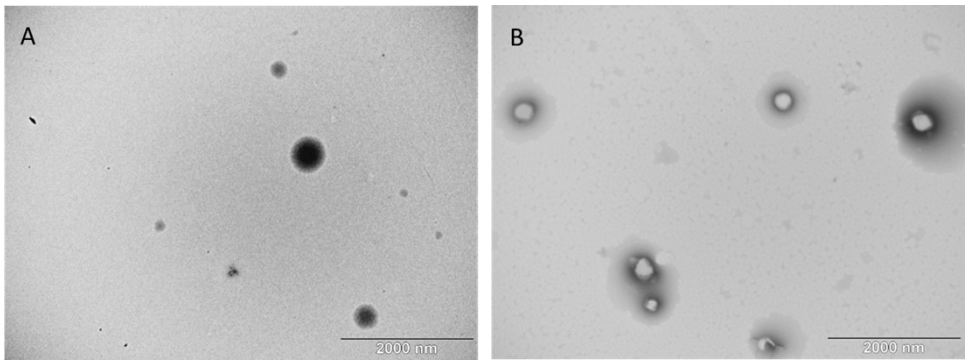

Fig. S3. Transmission electron microscopy micrographs of nanoparticles, A) poly-ε-caprolactone loading tebuconazole (PCL- TBZ), B) nanostructure lipid carriers loading tebuconazole (NLC- TBZ) nanoparticles.

Table S1. Reconstituted test water.

Stock solutions (single substance)		To prepare reconstituted water, add the following volume of stock solution to 1 L water*
Substance	Amount added to 1 L water	
$CaCl_2.2H_2O$	11.76 g	25.00 mL
$MgSO_4.7H_2O$	4.93 g	25.00 mL
$NaHCO_3$	2.59 g	25.00 mL
KCl	0.23 g	25.00 mL

* Water of suitable purity, for example deionized, distilled or reverse osmosis with conductivity preferably not exceeding 10 $\mu S.cm^{-1}$.

Table S2. Elendt M7 medium. Separate stock solutions (I) of individual trace elements are first prepared in water of suitable purity, for example, deionized, distilled or reverse osmosis. From these different stock solutions (I) a second single stock solution (II) is prepared, which contains all trace elements (combined solution), i.e.:

Stock solution(s) I (single substance)	Amount added to water (mg/L)	To prepare the combined stock solution II, add the following amount of stock solution I to water (ml/L)
		M7
H_3BO_3	57 190	0.25
$MnCl_2$. $4H_2O$	7 210	0.25
LiCl	6 120	0.25
RbCl	1 420	0.25
$SrCl_2$. $6H_2O$	3 040	0.25
NaBr	320	0.25
Na_2MoO_4. $2H_2O$	1 230	0.25
$CuCl_2$. $2H_2O$	335	0.25
$ZnCl_2$	260	1.00
$CoCl_2$. $6H_2O$	200	1.00
KI	65	1.00
Na_2SeO_3	43.8	1.00
NH_4VO_3	11.5	1.00
Na_2EDTA. $2H_2O$	5 000	-
$FeSO_4$. $7H_2O$	1991	-
Both Na_2EDTA and $FeSO_4$ solutions are prepared singly, poured together, and autoclaved immediately. This gives:		
21 Fe-EDTA		5

M7 medium is prepared using stock solution II, the macro-nutrients and vitamin as follows:

	Amount added to water (mg/L)	Amount of stock solution II added to prepare medium (ml/L)
		M7
Stock solution II (combined trace elements)		50
Macro nutrient stock solutions (single substance)		
CaCl2. 2H2O	293 800	1.00
MgSO4. 7H2O	246 600	0.50
KCl	58 000	0.10
NaHCO3	64 800	1.00
Na2SiO3. 9H2O	50 000	0.20
NaNO3	2 740	0.10
KH2PO4	1 430	0.10
K2HPO4	1 840	0.10
Combined Vitamin stock		0.10
The combined vitamin stock solution is prepared by adding the 3 vitamins to 1 L water, as shown below:		
Thiamine hydrochloride	750	
Cyanocobalamine (B12)	10	
Biotine	7.5	