

Supplementary information for

**EFFECTS OF THREE TEBUCONAZOLE NANOPESTICIDES ON THE SURVIVAL OF
*DAPHNIA MAGNA***

Mahleh Eghbalinejad^a, Rocío López-Cabeza^{ab}, Jan Kotouček^c, Renato Grillo^d, Marek Koutný^e, Zuzana Bílková^a, Jakub Hofman^{a*}

^aRECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, Brno, 625 00, Czech Republic

^bInstituto de Recursos Naturales y Agrobiología de Sevilla, (IRNAS), CSIC, Avenida de Reina Mercedes 10, 41012, Sevilla, Spain

^cDepartment of Pharmacology and Toxicology, Veterinary Research Institute, Brno, Czech Republic

^dDepartment of Physics and Chemistry, School of Engineering, São Paulo State University (UNESP), Ilha Solteira, SP, 15385-000, Brazil.

^eDepartment of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University, Vavrečkova 275, Zlín, 760 01, Czech Republic

* Corresponding author: jakub.hofman@recetox.muni.cz

Table S1: Aachener Daphnien Medium (ADaM).

The medium is prepared by adding synthetic sea salt and analytical grade chemicals to deionized water, as compiled in Table S1. Subsequently, it is aerated for 12 h to guarantee air saturation. Synthetic sea salt contains 7 macronutrients, 60 trace elements, 8 vitamins, 3 amino acids, 1 hormone, and natural colloids. By adding CaCl₂ and NaHCO, the total hardness (2-5 0-2mmol/L), the Ca: Mg ratio (4:1).¹

Compounds	Quantity
Synthetic sea salt (Wimex hw Meersalz Bioelemente)	0.333 g/L
CaCl ₂ solution, 0.8 mol/L ~ (117.6 g L ~ CaCl ₂ . 2H ₂ O)	2.3 ml/L
NaHCO solution, 0.3 mol/ L ~ (25.2 g L ~ NaHCO)	2.2 ml/L
SeO ₂ solution, 0.013 mol/ L ~ (1.4 g/L ~ SeO ₂)	0.1 ml/L

Table S2. The total and free concentration (mg/L) of tebuconazole (TBZ) in poly- ε -caprolactone (PCL), after dilution in ADaM medium versus nominal (theoretical) concentration of TBZ at t=0 and t=48h. The values (in this table) are the mean \pm standard deviation (n=3).

	Nominal concentration (mg/L)	Total concentration (mg/L)		Free concentration (mg/L)	
		t=0 h	t=48 h	t=0 h	t= 48 h
C1	2.25	2.16 \pm 0.07	2.4 \pm 0.2	1.9 \pm 0.03	2.25 \pm 0.10
C2	4.50	4.2 \pm 0.07	4.0 \pm 0.2	3.40 \pm 0.15	3.77 \pm 0.50
C3	9.00	8.10 \pm 0.4	9.2 \pm 0.3	6.12 \pm 0.13	6.3 \pm 0.20
C4	18.00	17.46 \pm 0.2	18.57 \pm 0.8	7.2 \pm 0.64	8.7 \pm 0.20
C5	36.00	33.37 \pm 1.16	35.0 \pm 1.5	9.39 \pm 0.12	11.03 \pm 0.70

Table S3. The total and free concentration (mg/L) of tebuconazole (TBZ) in nanostructured lipid carrier (NLC) after dilution in ADaM medium versus nominal (theoretical) concentration of TBZ at t=0 and t=48h. The values (in this table) are the mean ± standard deviation (n=3).

	Nominal concentration (mg/L)	Total concentration (mg/L)		Free concentration (mg/L)	
		t=0 h	t=48 h	t=0 h	t= 48 h
C1	2.25	2.02±0.02	2.41±0.07	2.05±0.03	2.485±0.05
C2	4.5	3.75±0.06	3.95±0.40	3.845±0.03	4.085±0.03
C3	9	7.94±0.6	8.58±0.40	7.65±0.07	7.855±0.05
C4	18	15.95±0.6	17.93±0.60	12.45±0.62	14.275±0.66
C5	36	30.48±0.6	34.46±1.50	19.85±0.94	20.825±0.41

Table S4. The total and free concentration (mg/L) of tebuconazole (TBZ) in poly(3-hydroxybutyrate) (PHB) after dilution in ADaM medium versus nominal (theoretical) concentration of TBZ. At t=0 and t=48h. The values (in this table) are the mean ± standard deviation (n=2).

	Nominal concentration (mg/L)	Total concentration (mg/L)		Free concentration (mg/L)	
		t=0 h	t=48 h	t=0 h	t= 48 h
C1	2.25	3.23±0.35	2.07±0.20	2.50±0.10	2.07±0.20
C2	4.5	9.31±0.33	4.48±0.14	5.89±0.10	4.30±0.20
C3	9	16.42±1.94	10.42±1.21	10.50±0.70	10.60±0.60
C4	18	34.30±1.98	23.96±6.38	14.70±0.20	19.70±0.70
C5	36	180.93±58.49	62.04±10.39	22.13±0.60	27.10±1.60

Reference

1. Klittgen, B., Dulmer, U., Engels, M., & Ratre, H. T. (1994). Rapid Communication Adam, an Artificial Freshwater for the Culture of Zooplankton. *Science*, 28(3), 743–746.