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Supplementary Material

Nanoformulations can significantly affect pesticide degradation and uptake by earthworms and plants

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Synthesis and characterization of the nanopesticides

Polymeric nanocapsules (NF1) were prepared according to the method of interfacial deposition of a preformed polymer described by Grillo et al. (2012). This method involves a mixture of two phases: an organic containing 100 mg of polymer (poly-ε-caprolactone), 30 mL of organic solvent (acetone), 200 mg of oil (triglycerides of capric and caprylic acids), 40 mg of sorbitan monostearate surfactant (Span 60), and 5 mg of AI (tebuconazole or chlorpyrifos). The aqueous phase was composed of a solution containing polysorbate 80 surfactant (Tween 80). After the dissolution of the components of both phases, the organic phase was poured into the aqueous phase under stirring. The nanoformulation was evaporated with a rotary evaporator to 20 mL, and the AI concentration was 50 μg ml^{-1}.

Nanostructured lipid carriers (NF2) were prepared according to the method of emulsification with solvent evaporation, described by de Oliveira et al. (2015) with modifications. The lipid phase was prepared from 5 mL of chloroform, 250 mg of glycerol tripalmitate, 75 mg of triglycerides of capric and caprylic acids and 5 mg of AI (tebuconazole or chlorpyrifos). After preparation, the lipid phase was inserted into an aqueous phase composed of PVA and the mixture was sonicated for 4 min at a power of 60 W. The pre-emulsion formed was then submitted to high-speed Ultraturrax homogenization at 14000 rpm for 7 min. The nanoformulation was evaporated with a rotary evaporator to 20 mL, and the AI concentration was 50 μg ml^{-1}.

The size of the particles and polydispersity index was measured with dynamic light scattering (DLS; Zetasizer Nano, Malvern) at 25 °C after 1:100 dilution with deionized water using diffusion coefficient of polystyrene. The microelectrophoresis technique (Zetasizer, Malvern) was used to determine ζ potential. Results were expressed as the average of 2-3 independent determinations. Encapsulation efficiency was determined using the ultrafiltration/centrifugation method employing Microcon 30 kDa regenerated cellulose ultrafiltration units (GE Healthcare, Brazil) and was 96–99%. Concentration of particles was determined by nanoparticle tracking analysis (NTA; NanoSight, Malvern).

References

de Oliveira, J.L.; Campos, E.V.; Goncalves da Silva, C.M.; Pasquoto, T.; Lima, R.; Fraceto, L.F. (2015): Solid lipid nanoparticles co-loaded with simazine and atrazine: Preparation, characterization, and evaluation of herbicidal activity. Journal of Agricultural and Food Chemistry 63: 422–432.

Grillo, R.; Pereira dos Santos, N.Z.; Maruyama, C.R.; Rosa, A.H.; de Lima, R.; Fraceto, L.F. (2012): Poly(ε-caprolactone) nanocapsules as carrier systems for herbicides: Physico-chemical characterization and genotoxicity evaluation. Journal of Hazardous Materials 231: 1–9.

LC-MS/MS analysis

Chromatographic separation was performed using an Agilent 1200 chromatographic system (Agilent, Santa Clara, CA, USA) equipped with a vacuum degasser, binary pump, autosampler and the column thermostat 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. A chromatographic column ACE 3 (C18, 150 mm x 2.1 mm i.d., 3 µm) with integrated guard column ACE 3 (C18, 2.1 mm × 10 mm, 3 µm), (ACE, Scotland, UK) was used for chromatographic separation. Water containing 0.1% formic acid (A; 98%, Sigma-Aldrich, Germany) and acetonitrile (B; LC MS, Biosolve, Netherlands) were used as a mobile phase. The mobile phase gradient was as follows: 0–1 min 10% B, 1–7 min from 10% B to 98% B, held 98% B to 12 min, 12–13 min from 98% B to 10%, held 10% B to 25 min. The flow rate was 0.3 ml min^{-1}. The column temperature was maintained at 30 °C. 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 instrument was operated in the ESI-positive SRM mode. Two MS/MS transitions were used for MS analyses. A fragment chosen for the final quantification and the fragment ion used for confirmation can be found in following table. The optimized instrument conditions were as follows: gas temperature 350 °C, gas flow 9 l min^{-1}, nebuliser gas 40 psi, capillary voltage 4.0 kV. MS/MS parameters are summarized in following table:

Precursor and product ions and collision energies of SRM transitions used for pesticides

Analyte	Rt (min)	Transition monitored	Collision energy (eV)	Fragmentor voltage (V)
Tebuconazole (Pestanal®)	9.98	308.2-151	20	120
		308.2-70	20	120
Metolachlor (Pestanal®)	10.20	284.3-252.1	10	120
		284.3-176.1	10	120
D-metolachlor (Dr. Ehrenstorfer)	10.17	290.1-258.1	10	120
		290.1-152.1	10	120
Chlorpyrifos (Pestanal®)	11.67	350-97.0	15	125
		350-197,9	15	125

QA/QC - Solvent blanks and standard solutions were analysed in sequence with each set of analysed samples in order to control for background contamination. In order to obtain high selectivity and sensitivity of LC/MS analyses, selected reaction monitoring (SRM) was chosen as a data acquisition mode. Two identification ions (one precursor ion and one products ions) were used for each analyte. The relative response between two SRM transitions together with retention times were used as a criterion for the identification of the compounds. Agilent Mass Hunter quantitation software was used for these purposes. Quantitation of analytes was performed using an internal standard calibration method. Internal standards (deuterated pesticide) were added to all samples for these purposes. Good linearity of calibration curves was obtained in the whole concentration range (1 - 1000 pg ml^{-1}).
Accuracy of the method used for the LC/MS/MS determination of pesticides samples involving QuEChERS procedure was 95 ± 3.2 % and the precision expressed as RSD was 3.5 %.

Results of the soil, earthworms and lettuce controls

The non-spiked soils LUFA 2.1 and LUFA 2.4, the earthworms taken from the culture and exposed for 84 days to non-spiked soils, and the lettuce grown for 84 days in non-spiked soils did not contain any detectable CLP or TBZ. This means that the concentrations were below a LOQ of 1 ng ml^{-1}, which corresponds (taking ito account the sample weights, recoveries, extraction ratios, dilutions etc.) 0.002 µg g_{sdw}^{-1} in soils, 0.03 µg g_{edw}^{-1} in earthworms, 0.02 µg g_{ldw}^{-1} in lettuce leaves, and 0.035 µg g_{rdw}^{-1} in lettuce roots. Therefore, there was no need to correct the lettuce results for this.
During the experiment, in 3 out of 36 samples of earthworms exposed to CLP-added soils also TBZ was detected, in 15 out of 38 samples of earthworms exposed to TBZ-added soils also CLP was detected, and in 14 out of 31 samples of lettuce roots exposed to TBZ-added soils also CLP was detected. This might be caused by cross-contamination or unknown errors, or possibly by magnification of compounds from < LOQ soil values (see above that the soil background levels were below about 0.002 µg g_{sdw}^{-1}) to > LOQ levels in biota. However, in these cases the concentration was always (with the exception of 1 sample of earthworms and 2 samples of roots) found up to 1 µg g_{edw}^{-1} and 0.5 µg g_{rdw}^{-1}, which is about 3 × LOQ and 1.5 × LOQ, for earthworms and roots, respectively. This was within inter-replicate variability and reached only the lowest concentrations in the biota exposed in compound-added soils. For leaves and for TBZ in roots, not such cross-contamination was found.

Matrix-SPME problems – controls, blanks and "cross-contamination"

Unfortunately, frequent cross-contamination was found in the SPME fiber extracts: CLP was detected in 13 out of 46 extracts of fibers exposed to TBZ-added soils, TBZ was detected in every extract of fibers exposed to CLP-added soils. Comparison of these results to the un-used fiber blanks (extracts of un-exposed clean fibers) and the extraction blanks (all extraction steps without fibers) identified the cross-contamination levels are comparable to blanks and probably not originating in fiber exposure to soils. Comparing the un-used fiber blanks to the extraction blanks further revealed that the impurity originated in extraction steps not in the SPME fibers before their use (the levels in both variants were comparable). Therefore, all concentrations in the fiber extracts were corrected for this background by subtracting the blank mean values for CLP or TBZ, respectively (19.6 ng $ml_{extract}^{-1}$ and 11.6 ng $ml_{extract}^{-1}$, respectively). Then such corrected concentrations were recalculated to the SPME fiber weight etc. Although correction was done, the interpretation of the SPME results should be careful and with some level of uncertainty kept in mind.

Figure S1: Distribution of the particle sizes of the studied nanopesticides measured with dynamic light scattering (DLS; Zetasizer, Malvern) after 1:100 dilution with deionized water using diffusion coefficient of polystyrene. Results are expressed as the average of 2-3 independent determinations.

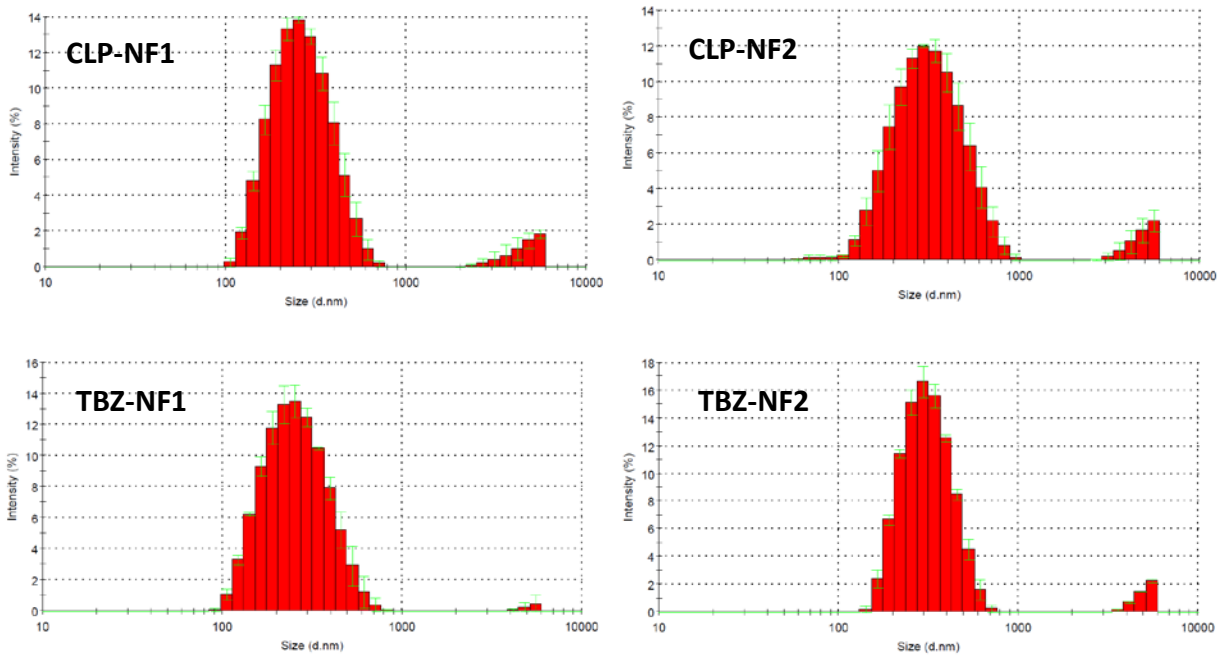

Figure S2: The photos from the experiment. 1) mixing the spiking solutions (CLP-CF); 2) spiking the soil; 3) establishing the microcosms; 4) addition of the earthworms; 5) addition of the lettuce seeds; 6) the SPME fibers cleaned; 7) start in the greenhouse; 8) the detailed view on the microcosm; 9) the microcosms with the grown lettuce; 10)-12) sampling at the time point

Figure S3: The number of the earthworms collected.

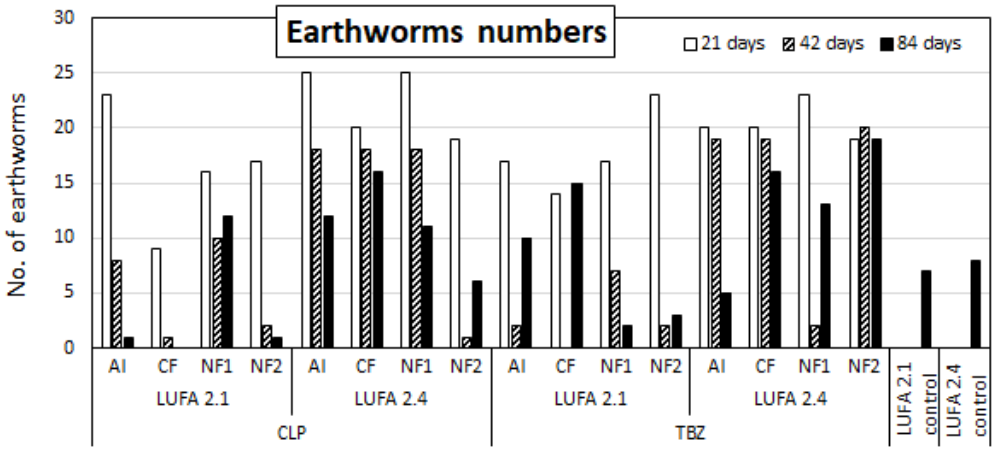

Figure S4: Weight (g_{edw} per 1 individual) of earthworms during the experiment.

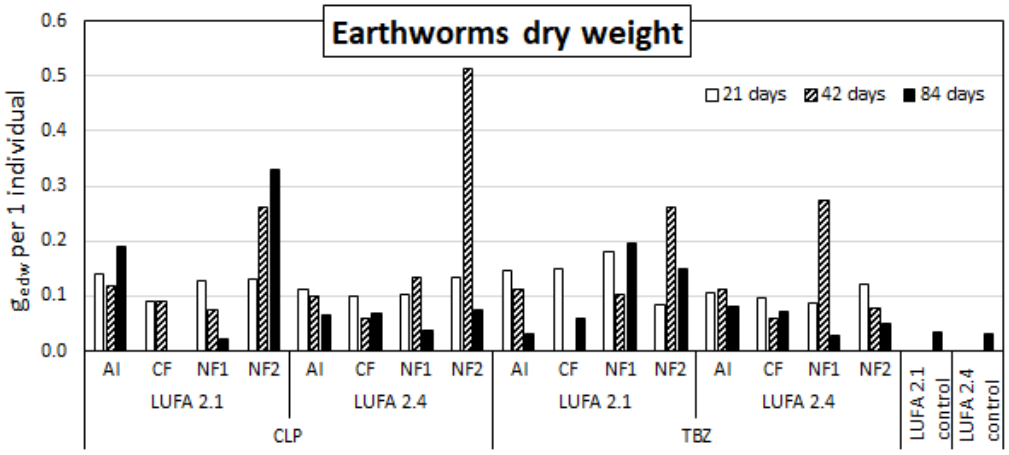

Figure S5: Biomass of lettuce leaves and roots (g_{ldw} and g_{rdw}, respectively) during the experiment. Black and white triangles show the weights in the controls with LUFA 2.1 and 2.4, respectively.

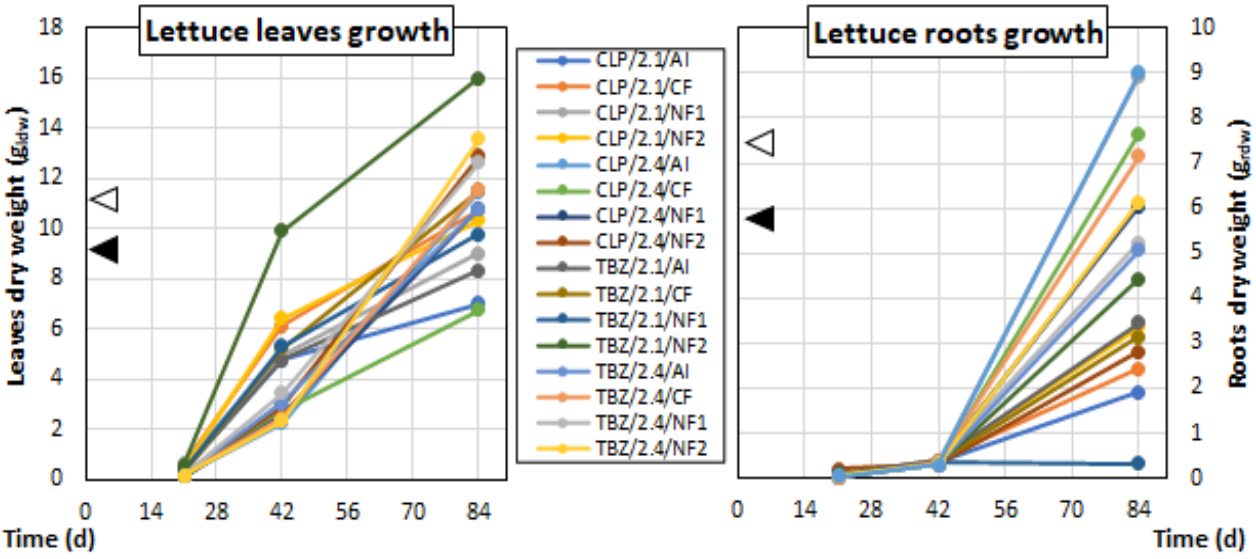

Table S1: Properties of two experimental soils – LUFA 2.1 and LUFA 2.4 – as presented in the protocol attached to the soils when delivered from LUFA Speyer (mean values of different batch analyses ± standard deviation; all values refer to dry matter).

	LUFA 2.1	LUFA 2.4
Organic carbon (% of C)	0.71 ± 0.08	1.99 ± 0.21
Nitrogen (% of N)	0.06 ± 0.01	0.22 ± 0.02
pH (0.01M $CaCl_2$)	4.9 ± 0.3	7.4 ± 0.1
Cation exchange capacity (meq 100 g^{-1})	4.3 ± 0.6	32.9 ± 4.5
WHC (g 100 g^{-1})	32.1 ± 1.7	44.8 ± 2.1
Weight per volume (g 1000 ml^{-1})	1437 ± 41	1250 ± 39
clay (below 0.002 mm; %)	3.1 ± 0.9	26.2 ± 1.4
Sand (above 0.063 mm; %)	84.7 ± 3.3	28.1 ± 3.4

Table S2: Soil concentrations (C_s, µg g_{sdw}^{-1}; mean ± SD; n = 3) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. Because the variants had different initial (0d) concentrations after spiking, it is not possible to compare between the formulations, soils and compounds. The results are shown as mean ± SD (n = 3). For day 0 and day 1, also relative SD (RSD) is shown to document the homogeneity of the soil spiking.

			0 day	0 day RSD	1 day	1 day RSD	21 days	42 days	84 days
CLP	LUFA 2.1	AI	0.482 ± 0.019	4.0%	0.599 ± 0.015	2.4%	0.255 ± 0.009	0.158 ± 0.012	0.117 ± 0.01
		CF	0.504 ± 0.010	1.9%	0.518 ± 0.040	7.7%	0.300 ± 0.008	0.205 ± 0.009	0.124 ± 0.006
		NF1	0.347 ± 0.009	2.7%	0.433 ± 0.015	3.5%	0.371 ± 0.015	0.227 ± 0.011	0.123 ± 0.012
		NF2	0.449 ± 0.008	1.7%	0.453 ± 0.016	3.5%	0.292 ± 0.003	0.204 ± 0.002	0.110 ± 0.007
	LUFA 2.4	AI	0.398 ± 0.011	2.7%	0.383 ± 0.015	4.0%	0.048 ± 0.002	0.028 ± 0.001	0.008 ± 0
		CF	0.483 ± 0.065	13.5%	0.417 ± 0.010	2.4%	0.059 ± 0.001	0.037 ± 0.003	0.010 ± 0
		NF1	0.479 ± 0.005	2.0%	0.372 ± 0.024	6.5%	0.058 ± 0.001	0.033 ± 0.002	0.010 ± 0.001
		NF2	0.393 ± 0.015	3.7%	0.384 ± 0.007	1.7%	0.051 ± 0.002	0.035 ± 0.004	0.010 ± 0.001
TBZ	LUFA 2.1	AI	0.727 ± 0.021	2.9%	0.660 ± 0.020	3.0%	0.368 ± 0.015	0.362 ± 0.033	0.234 ± 0.010
		CF	0.831 ± 0.036	4.4%	0.748 ± 0.042	5.6%	0.516 ± 0.024	0.449 ± 0.007	0.247 ± 0.011
		NF1	0.792 ± 0.008	1.0%	0.654 ± 0.02	3.1%	0.453 ± 0.014	0.434 ± 0.029	0.290 ± 0.010
		NF2	0.917 ± 0.119	20.6%	0.739 ± 0.024	3.2%	0.438 ± 0.026	0.395 ± 0.034	0.319 ± 0.031
	LUFA 2.4	AI	0.826 ± 0.004	0.5%	0.678 ± 0.006	0.9%	0.347 ± 0.026	0.166 ± 0.010	0.068 ± 0.002
		CF	0.890 ± 0.044	4.9%	0.888 ± 0.131	14.8%	0.380 ± 0.017	0.207 ± 0.003	0.084 ± 0.005
		NF1	0.866 ± 0.039	4.5%	0.773 ± 0.022	2.9%	0.488 ± 0.013	0.275 ± 0.032	0.095 ± 0.010
		NF2	1.013 ± 0.023	4.5%	0.864 ± 0.089	10.3%	0.363 ± 0.012	0.227 ± 0.009	0.08 ± 0.003

The explanation of statistics in the Tables S3 – S10

The effect of time for each variant is shown by Greek letters under the results – the same letter indicates that these time-points are not significantly different. The differences between formulations for each time-point are shown by Latin letters to the right of the results – the same letter indicates that these formulations are not significantly different. To simplify the view, where effect of time or formulation was significant, it is also highlighted in green or blue, respectively.
The differences between two soils or two compounds are indicated by < ≈ > signs in the columns „soil effect“ or „comp. effect“, respectively
In the “soil effect” column, the < or > sign shows if the result in LUFA 2.1 was significantly lower or higher, respectively, than in LUFA 2.4 (for given compound, formulation and time-point). The ≈ sign shows the results in two soils were not significantly different.
In the “comp. effect” column, the < or > sign shows if the result for CLP was significantly lower or higher, respectively, than for TBZ (for given soil, formulation and time-point). The ≈ sign shows the results for two compounds were not significantly different.

Table S3: Concentrations in earthworms (C_e in µg g_{edw}^{-1}; mean ± SD; n = 1-2) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. To enable comparisons between the formulations, soils and compounds, the results are normalized according to modelled soil initial concentration (C_{si}), because the variants had different initial (0d) concentrations after spiking. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	3.11 ± 0.42	a	>	≈	1.09 ± 0.15	a	>	≈	1.65	na	na	na
		time effect	α				β				αβ			
		CF	3.70 ± 2.03	a	≈	≈	1.44	a	>	na	na	na	na	na
		time effect	α				α				na			
		NF1	8.03 ± 3.34	a	≈	≈	2.15 ± 0.33	a	>	≈	0	na	na	na
		time effect	α				α				α			
		NF2	2.87 ± 0.93	a	≈	≈	0.07	a	≈	≈	2.67	na	na	na
		time effect	α				α				α			
	LUFA 2.4	AI	0.67 ± 0.55	a		≈	0.11 ± 0.05	a		<	0	na		na
		time effect	α				α				α			
		CF	0.19 ± 0.04	a		<	0.08 ± 0.02	a		<	0	na		na
		time effect	α				α				α			
		NF1	0.28 ± 0	a		<	0.28 ± 0.27	a		≈	0	na		na
		time effect	α				α				α			
		NF2	0.25 ± 0.02	a		<	0.11	a		≈	0.09	na		na
		time effect	α				α				α			
TBZ	LUFA 2.1	AI	1.95 ± 0.07	a	>		0.76 ± 0	a	≈		0.09	na	na	
		time effect	α				αβ				β			
		CF	1.88 ± 0.18	a	>		na	na	na		0.24	na	na	
		time effect	α				na				β			
		NF1	2.01 ± 0.43	a	≈		0.97 ± 0.35	a	≈		1.55	na	na	
		time effect	α				α				α			
		NF2	2.05 ± 0.35	a	>		0.60	a	≈		0.11	na	na	
		time effect	α				α				α			
	LUFA 2.4	AI	0.91 ± 0.11	b			0.47 ± 0.04	a			0.11	na		
		time effect	α				αβ				β			
		CF	0.97 ± 0.1	ab			0.50 ± 0.03	a			0.07	na		
		time effect	α				β				β			
		NF1	1.40 ± 0.15	a			1.15	a			1.90	na		
		time effect	α				α				α			
		NF2	0.91 ± 0.1	b			0.44 ± 0.05	a			0.06	na		
		time effect	α				αβ				β			

Table S4: Bioaccumulation in earthworms (BAF in g_{sdw} g_{edw}^{-1}; mean ± SD; n = 3-6) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. BAF were calculated by dividing the concentration in the earthworms C_e (µg g_{edw}^{-1}) by the soil concentration C_s (µg g_{sdw}^{-1}) measured at the same sampling time. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	10.42 ± 1.13	ab	≈	>	5.91 ± 0.74	a	>	>	12.07 ± 1.00	b	>	>
		time effect	α				β				α			
		CF	9.96 ± 4.26	ab	>	>	5.70 ± 0.25	a	>	na	na	na	na	na
		time effect	α				α				na			
		NF1	13.22 ± 4.29	a	>	>	5.79 ± 0.73	a	≈	>	0.00 ± 0.00	c	≈	<
		time effect	α				β				γ			
		NF2	6.99 ± 1.76	b	>	≈	0.24 ± 0.00	b	>	<	17.32 ± 1.13	a	>	>
		time effect	β				γ				α			
	LUFA 2.4	AI	8.92 ± 5.66	a		>	2.60 ± 0.84	ab		<	0.00 ± 0.00	b		<
		time effect	α				β				β			
		CF	2.38 ± 0.37	b		<	1.69 ± 0.32	b		<	0.00 ±0.00	b		<
		time effect	α				β				γ			
		NF1	3.68 ± 0.07	b		≈	6.15 ± 4.67	a		≈	0.00 ± 0.00	b		<
		time effect	β				αβ				α			
		NF2	3.09 ± 0.18	b		<	2.13 ± 0.22	ab		<	5.79 ± 0.45	a		>
		time effect	β				γ				α			
TBZ	LUFA 2.1	AI	5.86 ± 0.28	ab	>		2.34 ± 0.21	a	<		0.430 ± 0.02	c	<	
		time effect	α				β				γ			
		CF	4.52 ± 0.39	c	>		na	na	na		1.18 ± 0.05	b	≈	
		time effect	α				na				β			
		NF1	5.07 ± 0.86	bc	>		2.57 ± 0.73	a	<		6.09 ± 0.21	a	<	
		time effect	α				β				α			
		NF2	6.27 ± 0.89	a	>		2.05 ± 0.17	a	<		0.457 ± 0.04	c	<	
		time effect	α				β				γ			
	LUFA 2.4	AI	3.16 ± 0.36	b			3.43 ± 0.31	b			2.00 ± 0.07	b		
		time effect	α				α				β			
		CF	3.61 ± 0.33	ab			3.42 ± 0.18	b			1.18 ± 0.07	b		
		time effect	α				α				β			
		NF1	3.75 ± 0.32	a			5.46 ± 0.61	a			26.1 ± 2.67	a		
		time effect	β				β				α			
		NF2	3.76 ± 0.34	a			2.92 ± 0.29	b			1.19 ± 0.04	b		
		time effect	α				β				γ			

Table S5: Concentrations in lettuce leaves (C_l in µg g_{ldw}^{-1}; mean ± SD; n = 1-3) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. To enable comparisons between the formulations, soils and compounds, the results are normalized according to modelled soil initial concentration (C_{si}), because the variants had different initial (0d) concentrations after spiking. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	0.77	na	na	na	0.07 ± 0	a	>	<	0 ± 0	a	≈	<
		time effect	α				β				γ			
		CF	0.46	na	na	na	0.06 ± 0.06	a	≈	<	0 ± 0	a	≈	≈
		time effect	α				β				β			
		NF1	1.16	na	na	na	0.11 ± 0.05	a	>	<	0.01 ± 0.02	b	≈	<
		time effect	α				β				β			
		NF2	0.8	na	na	na	0.07 ± 0.01	a	>	<	0 ± 0	a	≈	<
		time effect	α				β				γ			
	LUFA 2.4	AI	0	na	/	na	0 ± 0	na	/	<	0 ± 0	na	/	≈
		time effect	na				na				na			
		CF	0	na	/	na	0 ± 0	na	/	<	0 ± 0	na	/	<
		time effect	na				na				na			
		NF1	0	na	/	na	0 ± 0	na	/	<	0 ± 0	na	/	<
		time effect	na				na				na			
		NF2	0	na	/	na	0 ± 0	na	/	<	0 ± 0	na	/	<
		time effect	na				na				na			
TBZ	LUFA 2.1	AI	0.87	na	na	/	0.39 ± 0.05	a	>	/	0.13 ± 0.01	a	>	/
		time effect	α				β				γ			
		CF	1.41	na	na	/	0.39 ± 0.09	a	≈	/	0.18 ± 0.14	a	≈	/
		time effect	α				β				β			
		NF1	1.13	na	na	/	0.51 ± 0.03	a	>	/	0.17 ± 0.02	a	>	/
		time effect	α				β				γ			
		NF2	0.69	na	na	/	0.21 ± 0.03	b	≈	/	0.22 ± 0.06	a	>	/
		time effect	α				β				β			
	LUFA 2.4	AI	0.69	na	/	/	0.24 ± 0.05	b	/	/	0.03 ± 0.03	b	/	/
		time effect	α				β				γ			
		CF	0.68	na	/	/	0.27 ± 0.01	ab	/	/	0.08 ± 0.01	ab	/	/
		time effect	α				β				γ			
		NF1	0.95	na	/	/	0.37 ± 0.04	a	/	/	0.10 ± 0.01	a	/	/
		time effect					β				γ			
		NF2	0.82	na	/	/	0.27 ± 0.05	ab	/	/	0.09 ± 0.04	ab	/	/
		time effect	α				β				γ			

Table S6: Bioconcentration factors in lettuce leaves (BCF_l in g_{sdw} g_{ldw}^{-1}; mean ± SD; n = 3-9) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. BCF_l were calculated by dividing the concentration in the leaves C_l (µg g_{ldw}^{-1}) by the soil concentration C_s (µg g_{sdw}^{-1}) measured at the same sampling time. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	2.59 ± 0.09	a	>	≈	0.37 ± 0.03	a	>	<	0 ± 0	b	≈	<
		time effect	α				β				γ			
		CF	1.23 ± 0.04	c	>	<	0.25 ± 0.20	a	>	<	0 ± 0	b	≈	<
		time effect	α				β				γ			
		NF1	1.90 ± 0.08	b	>	<	0.30 ± 0.13	a	>	<	0.07 ± 0.10	a	≈	<
		time effect	α				β				γ			
		NF2	1.94 ± 0.02	b	>	≈	0.25 ± 0.04	a	>	<	0 ± 0	b	≈	<
		time effect	α				β				γ			
	LUFA 2.4	AI	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	a		<
		time effect	α				α				α			
		CF	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	a		<
		time effect	α				α				α			
		NF1	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	a		<
		time effect	α				α				α			
		NF2	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	a		<
		time effect	α				α				α			
TBZ	LUFA 2.1	AI	2.60 ± 0.11	b	≈		1.19 ± 0.16	ab	<		0.60 ± 0.06	a	≈	
		time effect	α				β				γ			
		CF	3.38 ± 0.16	a	>		1.08 ± 0.21	b	<		0.90 ± 0.60	a	≈	
		time effect	α				β				β			
		NF1	2.84 ± 0.09	b	>		1.35 ± 0.10	a	<		0.66 ± 0.08	a	<	
		time effect	α				β				γ			
		NF2	2.12 ± 0.12	c	<		0.72 ± 0.10	c	<		0.91 ± 0.22	a	<	
		time effect	α				β				β			
	LUFA 2.4	AI	2.42 ± 0.18	b			1.77 ± 0.28	a			0.53 ± 0.41	b		
		time effect	α				β				γ			
		CF	2.53 ± 0.11	b			1.86 ± 0.08	a			1.32 ± 0.22	a		
		time effect	α				β				γ			
		NF1	2.53 ± 0.07	b			1.78 ± 0.24	a			1.42 ± 0.19	a		
		time effect	α				β				γ			
		NF2	3.39 ± 0.11	a			1.78 ± 0.30	a			1.75 ± 0.73	a		
		time effect	α				β				β			

Table S7: Concentrations in lettuce roots (C_r in µg g_{rdw}^{-1}; mean ± SD; n = 1-2) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. To enable comparisons between the formulations, soils and compounds, the results are normalized according to modelled soil initial concentration (C_{si}), because the variants had different initial (0d) concentrations after spiking. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	6.24	na	na	na	6.06	na	na	na	1.24 ± 0.16	ab	>	≈
		time effect	α				α				β			
		CF	10.05	na	na	na	3.39	na	na	na	0.95 ± 0.14	ab	>	≈
		time effect	α				β				β			
		NF1	18.45	na	na	na	5.33	na	na	na	2.33 ± 0.24	a	>	>
		time effect	α				β				β			
		NF2	7.42	na	na	na	3.96	na	na	na	0.49 ± 0.63	b	≈	≈
		time effect	α				α				α			
	LUFA 2.4	AI	3.21	na		na	0.44	na		na	0.03 ± 0.05	a		≈
		time effect	α				β				β			
		CF	1.62	na		na	0.36	na		na	0.03 ± 0.04	a		≈
		time effect	α				β				β			
		NF1	0.48	na		na	0.70	na		na	0.04 ± 0.01	a		<
		time effect	β				α				γ			
		NF2	2.28	na		na	0.3	na		na	0.8 ± 1.13	a		≈
		time effect	α				α				α			
TBZ	LUFA 2.1	AI	1.92	na	na		3.06	na	na		0.80 ± 0.26	a	≈	
		time effect	α				α				α			
		CF	4.60	na	na		2.27	na	na		0.82 ± 0.33	a	≈	
		time effect	α				α				α			
		NF1	5.35	na	na		3.06	na	na		1.32 ± 0.17	a	>	
		time effect	α				α				α			
		NF2	3.05	na	na		1.03	na	na		0.54	a	>	
		time effect	na				na				na			
	LUFA 2.4	AI	1.78	na			0.32	na			0.18 ± 0.04	ab		
		time effect	α				αβ				β			
		CF	1.62	na			0.37	na			0.09 ± 0.01	ab		
		time effect	α				β				β			
		NF1	2.75	na			0.34	na			0.18 ± 0.03	a		
		time effect	α				β				β			
		NF2	0.63	na			0.33	na			0.07 ± 0.01	b		
		time effect	α				αβ				β			

Table S8: Bioconcentration factors in lettuce roots (BCF_r in $g_{sdw}\ g_{rdw}^{-1}$; mean ± SD; n = 3-6) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. BCF_r were calculated by dividing the concentration in the roots C_r (µg g_{rdw}^{-1}) by the soil concentration C_s (µg g_{sdw}^{-1}) measured at the same sampling time. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	20.9 ± 0.7	c	<	>	32.9 ± 2.4	a	>	>	9.1 ± 1.1	ab	>	>
		time effect	β				α				γ			
		CF	27.1 ± 0.8	b	>	>	13.4 ± 0.6	b	>	>	6.2 ± 0.8	b	>	>
		time effect	α				β				γ			
		NF1	30.4 ± 1.2	a	>	>	14.4 ± 0.7	b	≈	>	11.6 ± 1.3	a	>	>
		time effect	α				β				γ			
		NF2	18.1 ± 0.2	d	<	>	13.8 ± 0.1	b	>	>	3.1 ± 3.2	c	≈	≈
		time effect	α				α				β			
	LUFA 2.4	AI	42.8 ± 1.3	a		>	9.9 ± 0.5	b		>	2.5 ± 2.7	b		≈
		time effect	α				β				γ			
		CF	20.4 ± 0.4	c		>	7.3 ± 0.5	c		>	2.3 ± 2.5	b		≈
		time effect	α				β				γ			
		NF1	6.3 ± 0.1	d		<	15.6 ± 0.9	a		>	2.8 ± 0.4	b		≈
		time effect	β				α				γ			
		NF2	28.3 ± 0.8	b		>	5.7 ± 0.6	c		>	53.3 ± 58.6	a		≈
		time effect	α				α				α			
TBZ	LUFA 2.1	AI	5.8 ± 0.2	d	≈		9.4 ± 0.8	a	>		3.8 ± 1	ab	≈	
		time effect	β				α				γ			
		CF	11.1 ± 0.5	b	>		6.3 ± 0.1	b	>		4.1 ± 1.3	ab	>	
		time effect	α				β				γ			
		NF1	13.5 ± 0.4	a	>		8.1 ± 0.5	a	>		5.2 ± 0.5	a	>	
		time effect	α				β				γ			
		NF2	9.3 ± 0.5	c	>		3.5 ± 0.3	c	>		2.3 ± 0.2	b	>	
		time effect	α				β				γ			
	LUFA 2.4	AI	6.2 ± 0.5	b			2.3 ± 0.1	ab			3.1 ± 0.5	a		
		time effect	α				β				β			
		CF	6.0 ± 0.3	b			2.6 ± 0	a			1.6 ± 0.2	c		
		time effect	α				β				γ			
		NF1	7.3 ± 0.2	a			1.6 ± 0.2	c			2.5 ± 0.4	b		
		time effect	α				γ				β			
		NF2	2.6 ± 0.1	c			2.2 ± 0.1	b			1.4 ± 0.2	c		
		time effect	α				β				γ			

Table S9: Concentrations in the SPME fibers (C_f in µg ml_{pdms}^{-1}; mean ± SD; n = 2) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. To enable comparisons between the formulations, soils and compounds, the results are normalized according to modelled soil initial concentration (C_{si}), because the variants had different initial (0d) concentrations after spiking. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	31.3 ± 4.0	b	>	>	21.7 ± 0.3	a	>	>	69 ± 43.3	a	≈	≈
		time effect	α				α				α			
		CF	35.2 ± 6.5	b	>	>	16.7 ± 10.6	a	≈	≈	7.5 ± 10.7	a	≈	≈
		time effect	α				α				α			
		NF1	60.1 ± 7.2	a	>	>	32.7 ± 3.9	a	>	>	0 ± 0	a	<	≈
		time effect	α				β				γ			
		NF2	51.5 ± 3.4	ab	>	>	30.5 ± 13.8	a	≈	≈	0 ± 0	a	≈	≈
		time effect	α				αβ				β			
	LUFA 2.4	AI	0 ± 0	a		≈	0 ± 0	a		≈	0 ± 0	b		≈
		time effect	α				α				α			
		CF	0 ± 0	a		≈	0 ± 0	a		≈	0 ± 0	b		≈
		time effect	α				α				α			
		NF1	0 ± 0	a		<	0 ± 0	a		≈	18.0 ± 3.7	a		>
		time effect	β				β				α			
		NF2	0 ± 0	a		<	0 ± 0	a		≈	0 ± 0	b		≈
		time effect	α				α				α			
TBZ	LUFA 2.1	AI	3.1 ± 1.7	a	≈		2.7 ± 2.7	a	≈		0 ± 0	a	≈	
		time effect	α				α				α			
		CF	4.0 ± 3.1	a	≈		3.6 ± 3.4	a	≈		3.3 ± 4.7	a	≈	
		time effect	α				α				α			
		NF1	4.4 ± 3.1	a	≈		3.4 ± 0.3	a	≈		0 ± 0	a	≈	
		time effect	α				α				α			
		NF2	2.9 ± 0.5	a	≈		1.1 ± 0.4	a	≈		0 ± 0	a	≈	
		time effect	α				α				β			
	LUFA 2.4	AI	1.6 ± 1.1	a			1.4 ± 1.1	a			0 ± 0	a		
		time effect	α				α				α			
		CF	3.7 ± 2.7	a			9.5 ± 13.2	a			0 ± 0	a		
		time effect	α				α				α			
		NF1	1.9 ± 0.3	a			3.7 ± 4.2	a			0	a		
		time effect	α				α				α			
		NF2	1.5 ± 0.2	a			0.4 ± 0.5	a			9.6 ± 13.6	a		
		time effect	α				α				α			

Table S10: Partition coefficients between SPME polydimethylsiloxane (PDMS) and soil ($K_{pdms\text{-}soil}$ in g_{sdw} ml_{pdms}^{-1}; mean ± SD; n = 6) for two pesticides (chlorpyrifos – CLP and tebuconazole – TBZ) added to two different soils (LUFA 2.1 and 2.4) as pure active ingredients (AI), conventional formulations (CF) or nanoformulations with polymeric (NF1) or lipid (NF2) carriers. $K_{pdms\text{-}soil}$ were calculated by dividing the concentration in the fibers C_f (µg ml_{pdms}^{-1}) by the soil concentration C_s (µg g_{sdw}^{-1}) measured at the same sampling time. The statistics is explained on page SI-9.

			21 days	form. effect	soil effect	comp. effect	42 days	form. effect	soil effect	comp. effect	84 days	form. effect	soil effect	comp. effect
CLP	LUFA 2.1	AI	105.1 ± 10.8	b	>	>	117.6 ± 7.7	a	>	>	505.8 ± 249.2	a	>	>
		time effect	β				β				α			
		CF	95.0 ± 13.7	b	>	>	66.2 ± 32.7	b	>	>	49.3 ± 54.1	b	≈	≈
		time effect	α				α				α			
		NF1	98.9 ± 9.9	b	>	>	88.2 ± 9.2	ab	>	>	0 ± 0	b	<	≈
		time effect	α				α				β			
		NF2	125.4 ± 6.5	a	>	>	106.1 ± 37.1	ab	>	>	0 ± 0	b	≈	≈
		time effect	α				α				β			
	LUFA 2.4	AI	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	b		≈
		time effect	α				α				α			
		CF	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	b		≈
		time effect	α				α				α			
		NF1	0 ± 0	a		<	0 ± 0	a		<	1293 ± 216.1	a		>
		time effect	β				β				α			
		NF2	0 ± 0	a		<	0 ± 0	a		<	0 ± 0	b		≈
		time effect	α				α				α			
TBZ	LUFA 2.1	AI	9.2 ± 3.9	a	≈		8.3 ± 6.4	a	≈		0 ± 0	b	≈	
		time effect	α				α				β			
		CF	9.6 ± 5.7	a	≈		9.8 ± 7.3	a	≈		16.7 ± 18.3	a	≈	
		time effect	α				α				α			
		NF1	11.2 ± 6.1	a	>		8.9 ± 0.8	a	≈		0 ± 0	b	≈	
		time effect	α				α				β			
		NF2	8.7 ± 1.4	a	>		3.6 ± 1.1	a	≈		0 ± 0	b	<	
		time effect	α				β				γ			
	LUFA 2.4	AI	5.5 ± 3	b			10.2 ± 6.3	ab			0 ± 0	a		
		time effect	αβ				α				β			
		CF	13.7 ± 7.8	a			64.7 ± 69.7	a			0 ± 0	a		
		time effect	α				α				α			
		NF1	5.1 ± 0.6	b			17.6 ± 15.5	ab			0 ± 0	a		
		time effect	α				α				α			
		NF2	6.2 ± 0.7	b			2.5 ± 2.7	b			181.3 ± 198.8	a		
		time effect	β				β				α			