Toxicokinetic mixture effects of co-formulants and active substances in plant protection products *in vitro*

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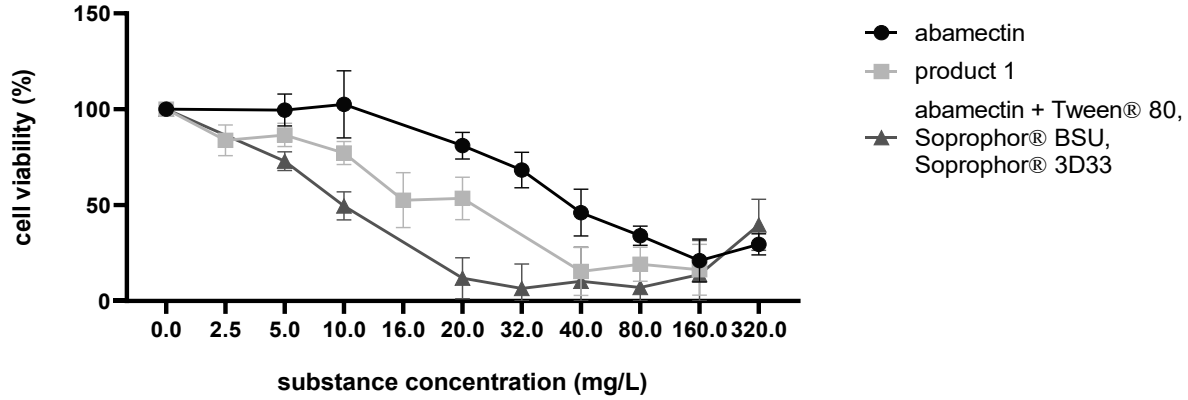
Bloch, D. and Marx-Stoelting, P. contributed equally to this paper

Supplementary Information

a



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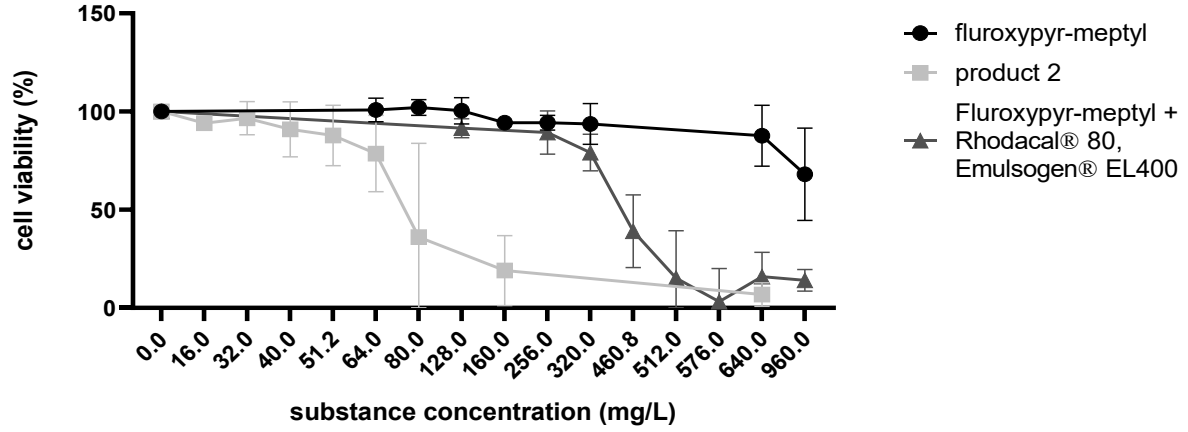

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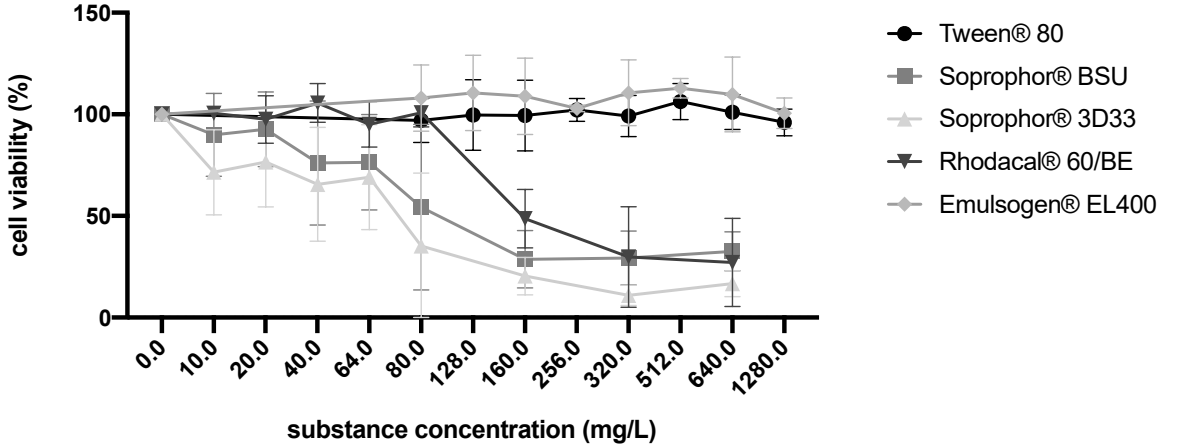

Fig. S1 Results of the NRU cytotoxicity assay in Caco-2 cells after 24 h exposure to increasing concentrations of the sinlge active ingredients and investigated co-formulants, their respective mixtures in the same ratio as in the PPPs and the PPPs. Results are shown as percentage of the viability of the solvent control containing 0.4% DMSO. Triton-X served as a positive control. Concentrations of the mixtures and the PPPs are related to the concentration of the contained active ingredient. Mean values ± SD of n = 3 biological replicates, each performed with six technical replicates, are given.

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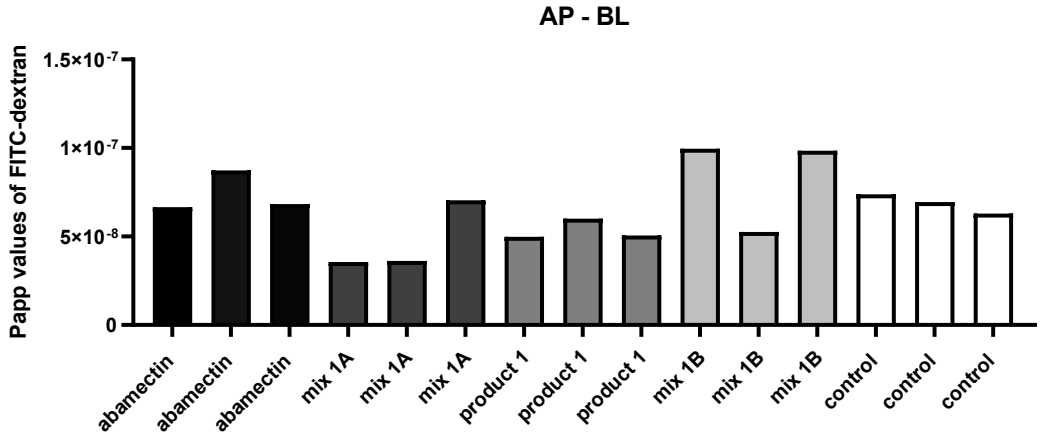

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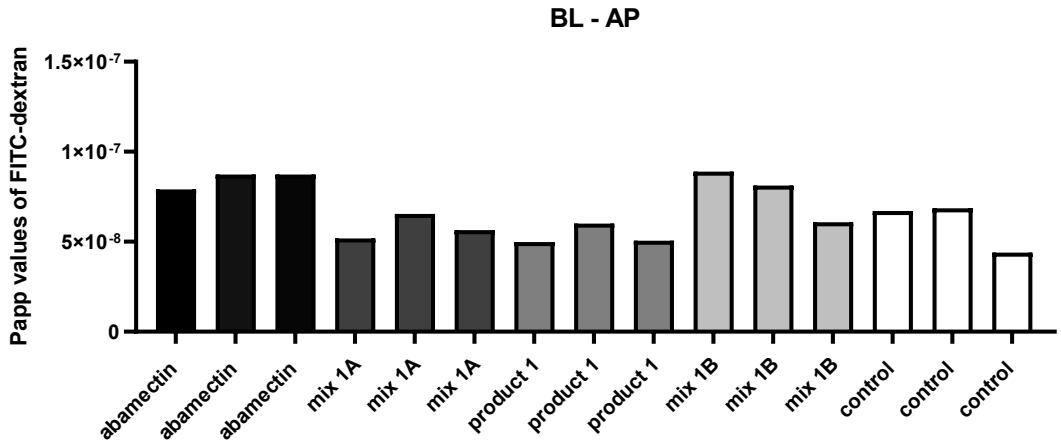

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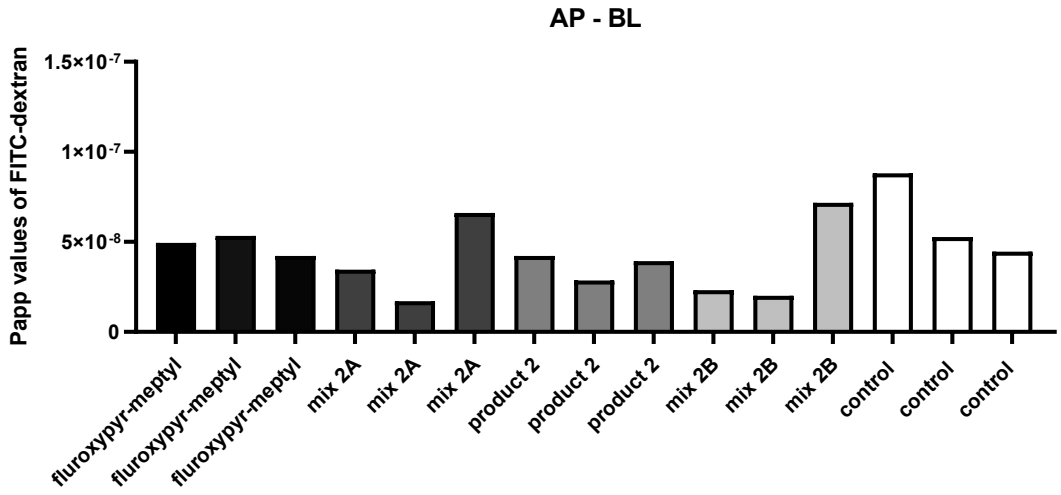

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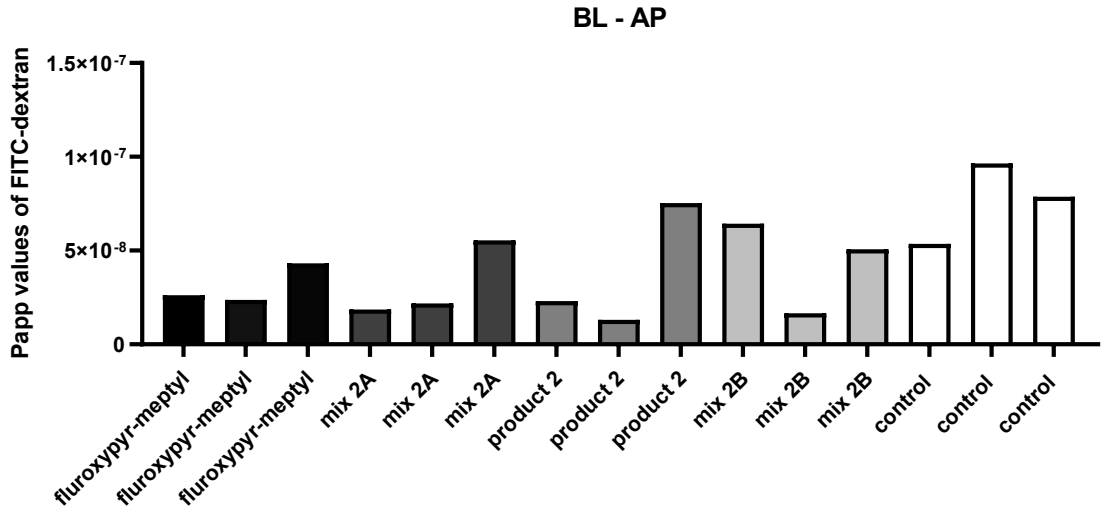
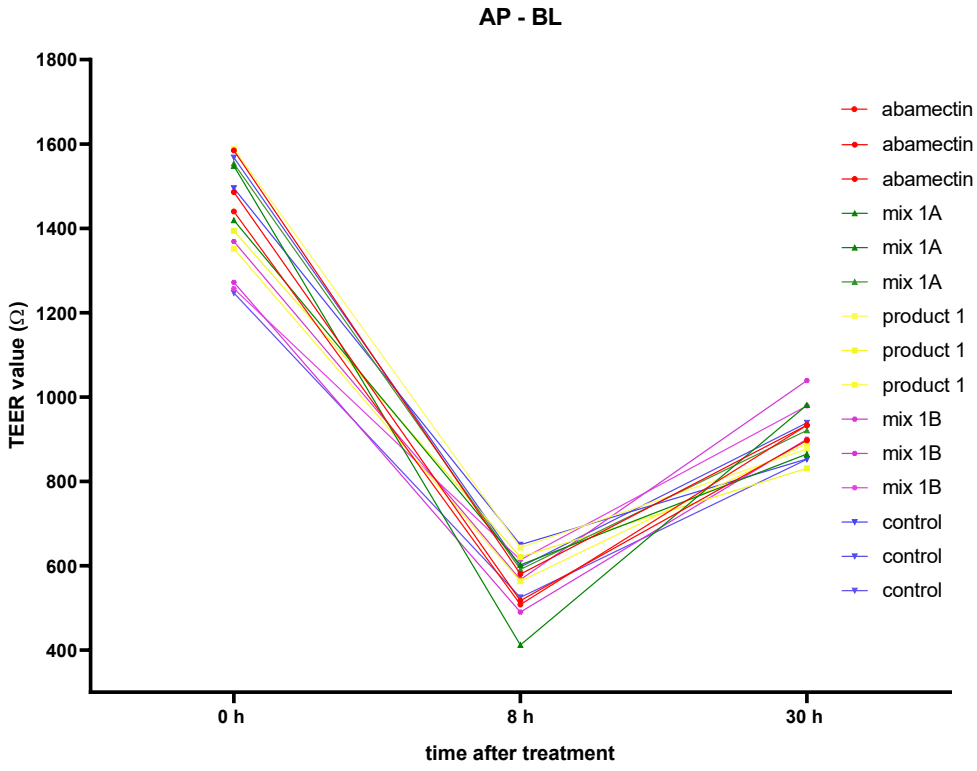
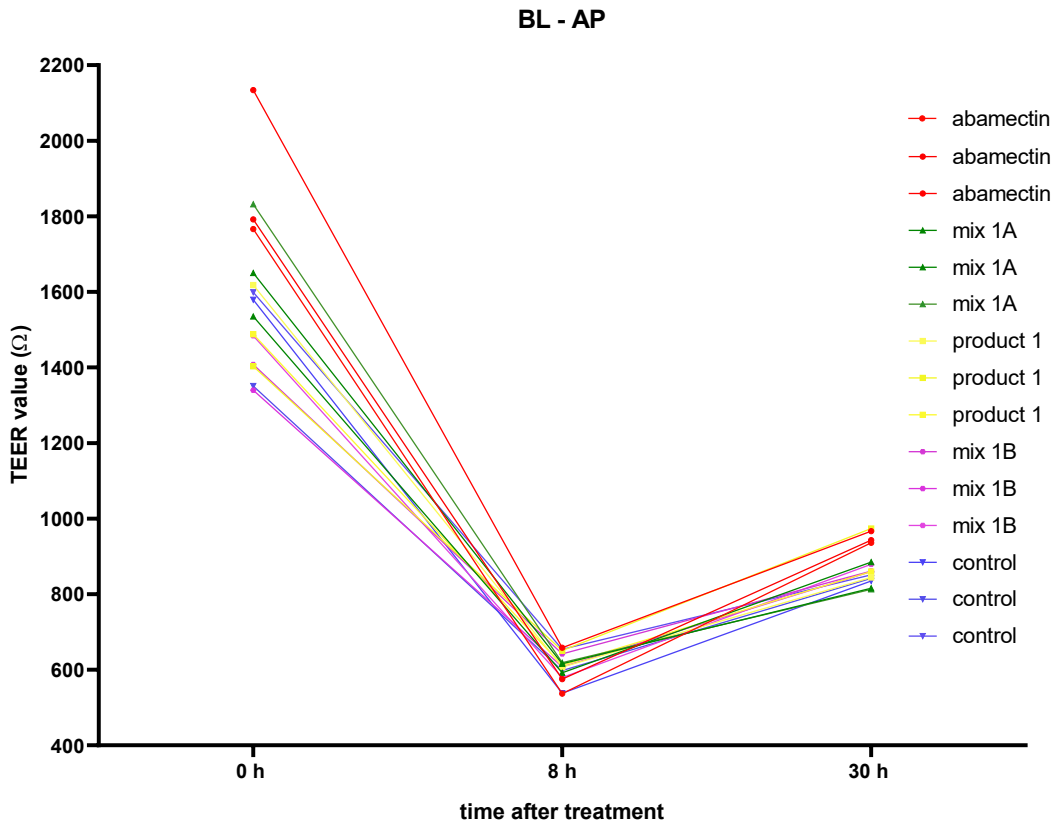


Fig. S2 Results of FITC-dextran flux (Papp values) representing cell layer integrity in transport studies of (a-b) 1 mg/L abamectin, mix 1A (1 mg/L abamectin + 4,2 mg/L Tween® 80, 1 mg/L Soprophor® BSU, 1 mg/L Soprophor® 3D33), product 1 (containing 1 mg/L abamectin) and mix 1B (1 mg/L abamectin + 80 mg/L Tween® 80, 40 mg/L Soprophor® BSU, 40 mg/L Soprophor® 3D33) and (b-c) 1 mg/L fluroxypyr-meptyl, mix 2A (1 mg/L fluroxypyr-meptyl + 0.1 mg/L Rhodacal® 60/BE, 0.2 mg/L Emulsogen® EL400), product 2 (containing 1 mg/L fluroxypyr-meptyl) and mix 2B (1 mg/L abamectin + 120 mg/L Rhodacal® 60/BE, 120 mg/L Emulsogen® EL400, 160 mg/L Solgad® 150 ULN).

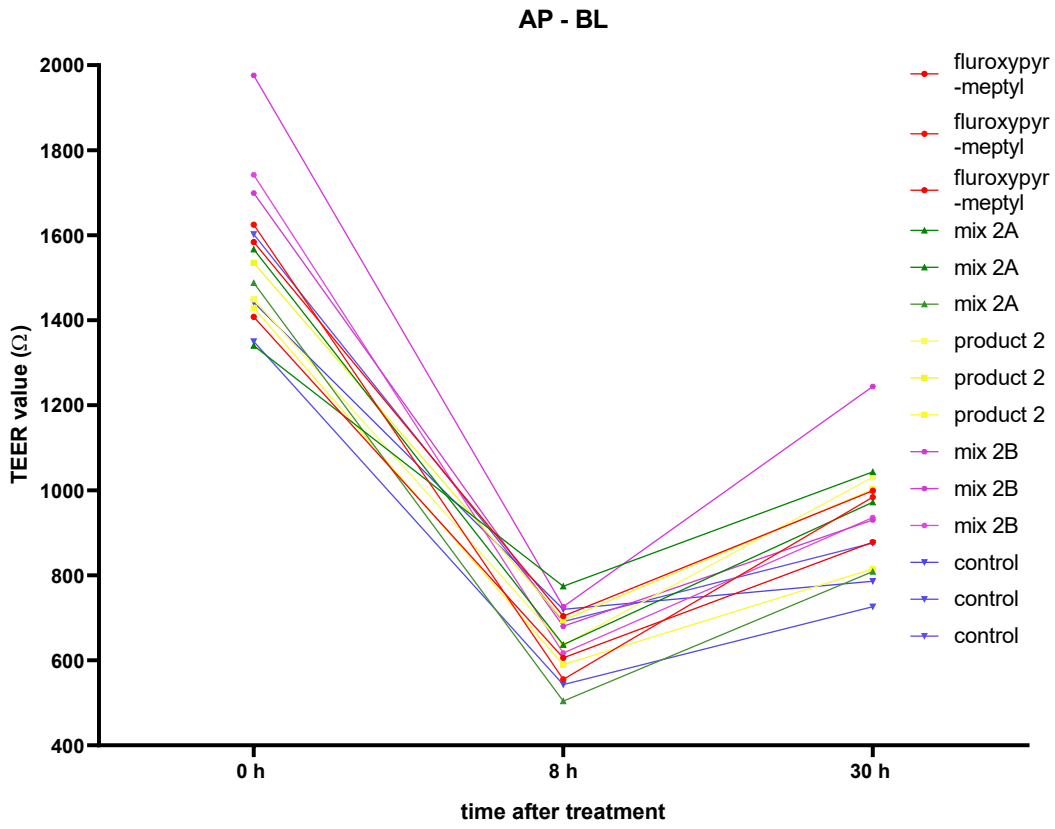
a


AP - BL
TEER value (Ω)
1800
1600
1400
1200
1000
800
600
400
0 h
8 h
30 h
time after treatment
abamectin
abamectin
abamectin
mix 1A
mix 1A
mix 1A
product 1
product 1
product 1
mix 1B
mix 1B
mix 1B
control
control
control

b


BL - AP
TEER value (Ω)
2200
2000
1800
1600
1400
1200
1000
800
600
400
0 h
8 h
30 h
time after treatment
abamectin
abamectin
abamectin
mix 1A
mix 1A
mix 1A
product 1
product 1
product 1
mix 1B
mix 1B
mix 1B
control
control
control

c


AP - BL
TEER value (Ω)
2000
1800
1600
1400
1200
1000
800
600
400
0 h
8 h
30 h
time after treatment
fluroxypyr -meptyl
fluroxypyr -meptyl
fluroxypyr -meptyl
mix 2A
mix 2A
mix 2A
product 2
product 2
product 2
mix 2B
mix 2B
mix 2B
control
control
control

d

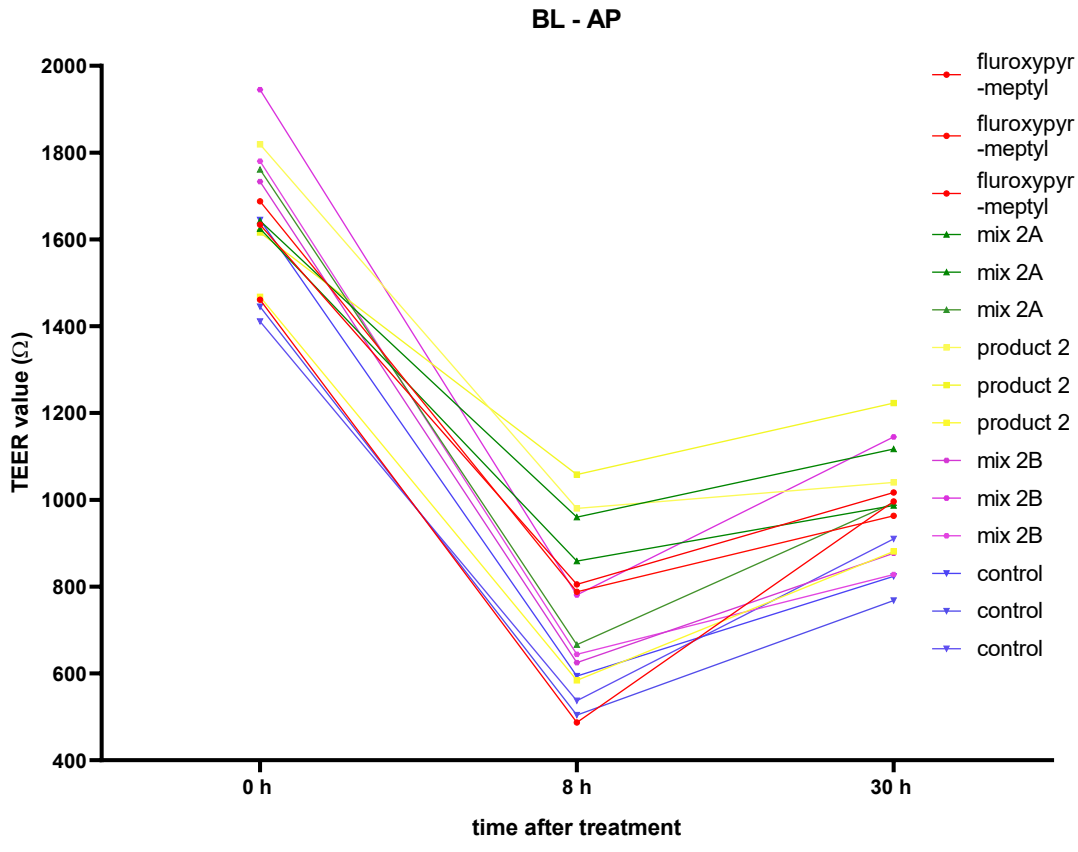
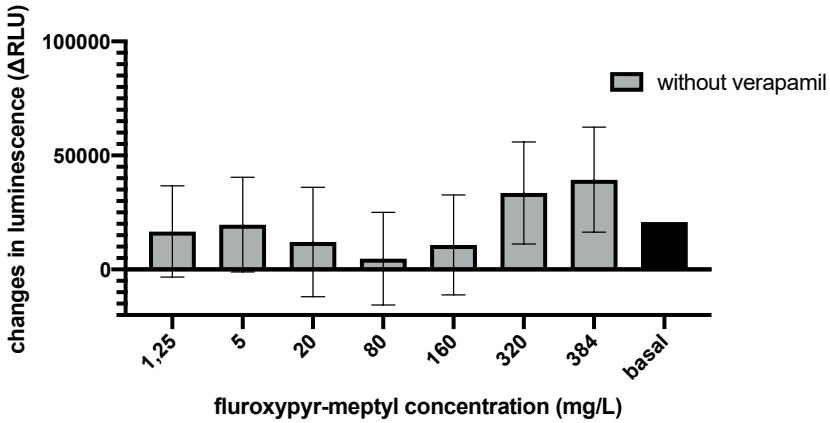

BL - AP
TEER value (Ω)
2000
1800
1600
1400
1200
1000
800
600
400
0 h
8 h
30 h
time after treatment
fluroxypyr -meptyl
fluroxypyr -meptyl
fluroxypyr -meptyl
mix 2A
mix 2A
mix 2A
product 2
product 2
product 2
mix 2B
mix 2B
mix 2B
control
control
control

Fig. S3 Results of transepithelial electrical resistance measurements (TEER values) representing cell layer integrity in transport studies of (a-b) 1 mg/L abamectin, mix 1 (1 mg/L abamectin + 4,2 mg/L Tween® 80, 1 mg/L Soprophor® BSU, 1 mg/L Soprophor® 3D33), product 1 (containing 1 mg/L abamectin) and mix 2 (1 mg/L abamectin + 80 mg/L Tween® 80, 40 mg/L Soprophor® BSU, 40 mg/L Soprophor® 3D33) and (c-d) 1 mg/L fluroxypyr-meptyl, mix 1 (1 mg/L fluroxypyr-meptyl + 0.1 mg/L Rhodacal® 60/BE, 0.2 mg/L Emulsogen® EL400), product 2 (containing 1 mg/L fluroxypyr-meptyl) and mix 2 (1 mg/L abamectin + 120 mg/L Rhodacal® 60/BE, 120 mg/L Emulsogen® EL400, 160 mg/L Solgad® 150 ULN).

a



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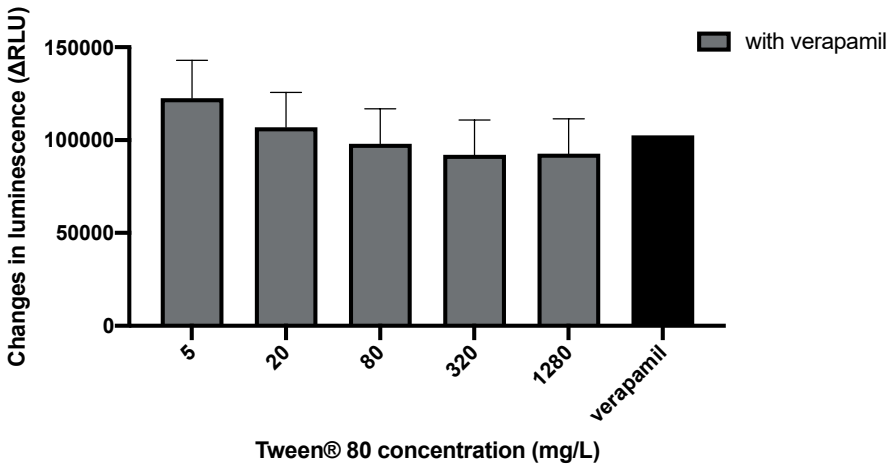

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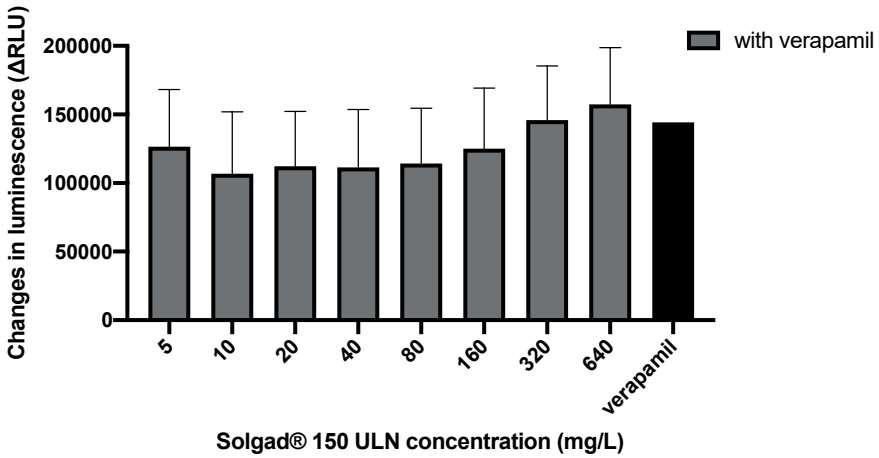

Fig. S4 Pgp ATPase activity presented as changes in luminescence ΔRLU of (a) fluroxypyr-meptyl compared to untreated samples (basal) and (b) Tween® 80 and (c) Solgad® 150 ULN after stimulation with verapamil compared to verapamil control. All measurements were corrected by subtraction of Na_3VO_4 treated signals (non Pgp ATPase activity). Data are presented as mean values of n = 4 independent experiments. Error bars indicate confidence intervals obtained from statistical analysis using an either two-sided (error bars in both direction) or one-sided (error bars in one direction) post-hoc Dunnett-test, with a preceding linear mixed-effects ANOVA test ($\alpha = 0.05$).

Table S1

Gradient conditions for avermectin B1a analysis

Time (min:sec)	% A (0.1% (v/v) formic acid in water)	% B (0.1% (v/v) formic acid in acetonitrile)
0:00	20	80
1:00	5	95
3:00	5	95
3:50	20	80
8:00	20	80

Table S2

Gradient conditions for fluroxypyr-meptyl analysis

Time (min:sec)	% A (0.1% (v/v) formic acid in water)	% B (0.1% (v/v) formic acid in acetonitrile)
0:00	30	70
2:00	30	70
4:00	10	90
10:00	10	90
10:05	30	70
13:00	30	70