Supporting Information

Can toxicokinetic and toxicodynamic modelling be used to understand and predict synergistic interactions between chemicals?

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CONTENT:

SI A: SUMMARY OF EXPERIMENTS USED FOR MODEL CALIBRATION AND VALIDATION

Toxicokinetic experiments The experiments and measurements of internal α-cypermethrin concentrations are described in Kretschmann et al. (2016)[1]. In short, 4-5 days old daphnids were exposed to a 7-h pulse of 0.72 nM α-cypermethrin (nominal concentration in the exposure medium) and subsequently transferred into clean medium for up to 54 h (elimination phase). This treatment was performed with and without simultaneous exposure to 1.5 µM propiconazole. The propiconazole treated daphnids were pre- exposed for 15–18 h, to obtain stable internal concentrations[2], and the propiconazole concentrations were maintained throughout the pulse exposure and recovery phase. At different time points during the α-cypermethrin pulse and elimination phase, daphnids were snap-frozen in liquid nitrogen and stored at −80 °C. Each sample consisted of 20 daphnids. Samples of the media were analysed for α-cypermethrin and propiconazole at the start and the end of the pulse exposure and propiconazole was measured additionally at the end of the recovery phase. Extracts were prepared and analysed as described in Kretschmann et al. (2016)[1]. The experiment was conducted twice.

The toxicokinetics of the two azoles, propiconazole and prochloraz, determined in Dalhoff et al (2016)[2], followed first order kinetics. The TK parameters of the azoles in Dalhoff et al. (2016)[2] predict a slightly lower bioconcentration factor (15.6) than the one found by Kretschmann et al. (2016)[1] of 18-24. We choose to use the values of Dalhoff et al. (2016)[2], as these studies included the elimination kinetics. The parameters are given in Table 1.

Toxicodynamic experiments The toxicodynamic experiment using one fixed concentration of the azoles known to induce synergy is described in detail in Kretschmann et al. (2015)[3]. As for the toxicokinetic (TK) experiment, 4-5 days old daphnids were exposed to a 7 h pulse of α-cypermethin in the presence and absence of 1.5 µM propiconazole (or prochloraz), with the propiconazole (or prochloraz) treated daphnids being pre-exposed for approximately 18 h. Contrary to the TK experiment, daphnids were exposed to a range of α-cypermethrin concentrations known to elicit 0-100% immobility in control experiments. The concentrations were: 0.12, 0.42, 1.5, 5.1, and 18 nM. Immobility of the daphnids was monitored during the pulse and the subsequent recovery phase (observation times: 0, 6.0, 10.2, 21.8, 29.6, 44.6, 51.5, 70.7, and 94.6 h). During pre-exposure and recovery phase, daphnids were fed approx. 2.8×10^4 cells of the algae *Pseudokirchneriella subcapitata* per daphnid per day. No feeding was performed during the pulse. The concentrations of α-cypermethrin, propiconazole, and prochloraz were confirmed at the beginning and end of the pulse for one α-cypermethrin treatment. Further details can be found in Kretschmann et al. (2015)[3].

The toxicodynamic experiment testing the synergy potential of a range of lower azole concentrations is described in detail in Bjergager et al. (2017)[4]. In short, tests were performed in 100 mL glass beakers using a constant α-cypermethrin concentration tested in combination with varying concentrations of the azoles propiconazole and prochloraz at nominal concentrations of 0.001, 0.003, 0.013, 0.027, 0.133, 0.265 and 1.327 µM. α -cypermethrin was applied at three different nominal concentrations: 0.024, 0.12 or 0.24 nM, of which only the highest concentration was expected to have an adverse effect when tested alone. The experiments included four replicates of each treatment, twelve controls and four solvent controls. Furthermore, the two highest azole concentrations were tested individually, to test for toxic effects of the azoles. Exposure media were prepared by spiking 5 µL of azole and pyrethroid stock solutions or acetone (≤ 0.01% v/v) for solvent control to 50 mL M7-medium in 100 mL glass beakers. Five neonates were then carefully transferred to each beaker and immobility was observed on day 1, 2, 3, 5 and 7. Daphnids were fed approx. 2.8×10^4 cells of the algae *Pseudokirschneriella subcapitata* per daphnid per day, starting on day two.

Cytochrome P450 (ECOD) assay To test whether the P450 dependant biotransformation rate per daphnid biomass changed with the age of the daphnids, cytochrome P450 activity of *D. magna* was measured over time as described by Gottardi et al. (2016)[5]. Briefly, organisms (n = 4 replicates, N = 40 organisms for each replicate at ages 1 and 2 days, N = 20 at ages 3 to 8 days) were incubated with the substrate 7-ethoxycoumarin (0.01 mM) in M7 medium (2 mL) for 3 hours. Every 30 min, 100 uL of medium was sampled and transferred directly to a black 96-wells microwell plate (BRANDplates® pureGradeTM, Brand, Germany). The fluorescence due to product formation (7-hydroxycoumarin) (excitation: 380 nm, emission: 480 nm) was measured with a Multi-mode microplate reader (SpectraMax i3 Microplate Reader, Molecular Devices, U.S.) at room temperature (22 – 25 ˚C). The measured fluorescence was converted into amount of product produced by a conversion factor equal to $6.86\ 10^5 \pm 6\ 10^3$ RFU $pmol^{-1}$ which was obtained with a standard curve made with the commercially available product 7-hydroxycoumarin.

The inhibitory potential of propiconazole and prochloraz on *in vivo* P450 activity was performed using the same method, but on 5 days old daphnids which had been pre-exposed to fixed azole concentrations 18h prior to the ECOD-activity measurements, as described in Dalhoff et al. (2016)[2].

SI B: IT-IMPLEMENTATION

For GUTS-IT, the individual threshold is described by a cumulative log-logistic distribution:

$$f(t) = \frac{1}{1+(maxD_{pyr}{}^{*}(t)/\alpha)^{-\beta}} \quad \text{(S1)}$$

In this equation, α denotes the mean of the threshold distribution, while β is proportional to the steepness of the cumulative curve, while the "max" function selects the largest value of D_{pyr}* at time t. The resultant survival probability for GUTS-IT is then:

$$S_{pyr}(t) = (1 - f(t)) * e^{-h_b(t)} \quad \text{(S2)}$$

The two extreme cases of the GUTS model assuming either stochastic death (GUTS-SD) or individual tolerance (GUTS-IT) were used to describe data on all the individual compounds. The models used measured internal concentrations described by the TK model of the present paper for α-cypermethrin and the TK model described by Dalhoff et al (2016) for the two azoles. They were then parameterised on the dataset of immobility of daphnids over time exposed to pulses of a range of α-cypermethrin, propiconazole and prochloraz alone described in Kretschmann et al. (2015)[3] and in Dalhoff et al. (2016)[2] (SI Table S2, S4, S5). The TK parameters used are given in Table 1. As the GUTS-SD model described data significantly better than the GUTS-IT model for both α-cypermethrin and the azoles alone (Log-likelihood: -110, -74, 79 AIC: -225, 151, 162 for α-cypermethrin, propiconazole and prochloraz for GUTS-SD, respectively, versus Log-likelihood: -118, -173, -107 AIC: 239, 351 and 218 for GUTS-IT, all Relative likelihood test, $p < 0.001$), it was decided to use GUTS-SD as the default TD model. The main reason for GUTS-IT not describing data well was that immobility continued to increase long after the cessation of the pulse. As mortality is directly related to maximal damage under the IT assumption, individuals cannot continue to die after a pulse has finished and the internal concentrations and damage starts to decrease. Furthermore, as the increase in mortality long after pulse exposures seems to be an inherent property of pyrethroid toxicity[37], the SD model seems the best one to use for this study.

SI C: ADDING AZOLE DAMAGE TO THE DAMAGE DONE BY α-CYPERMETHRIN

A one compartment TKTD model was parameterized using the propiconazole data of Dalhoff et al. (2016)[2], and damage as a function of time was calculated for the constant exposure scenario of 1.4 µM propiconazole (Figure S6). As the daphnids had been pre-exposed to propiconazole for 18 hours before being exposed to the α-cypermethrin pulse, damage from t = 18 to t=113 was added to the pyrethroid damage:

$$\frac{dH(t)}{dt} = k_k * max(0,(D_{pyr}(t) + yD_{az}(t) - z) \quad \text{(S3)}$$

The damage threshold, z, was set to zero as for the α-cypermethrin model, and a scaling factor for the propiconazole damage, y, was fitted to the data (y: 11.28±1.68(*10^{-3})) (Figure S7). The fit was not significantly better than the fit to all data without adding the azole damage (Table S9) (Relative likelihood test: p = 0.06 and 0.15 for the α-cypermethrin and α-cypermethrin + propiconazole dataset, respectively).

SI D: FITTING *s* TO DATA BASED ON CO-EXPOSURE TO DIFFERENT AZOLE CONCENTRATIONS

For these datasets, α-cypermethrin exposure was set to an initial concentration of 80% of nominal concentrations letting the concentration decay exponentially with a rate of -0.092, which fits the measured data of Kretschmann et al, 2015[3] at time 7h. First, the entire dataset was fitted with individual *s* parameters for each azole concentration. For propiconazole, the two lowest propiconazole treatments were left out, as there was no mortality at any of the α-cypermethrin concentrations. Also, for four of the propiconazole treatments, the intermediate α-cypermethrin treatment was left out due to control mortality >0.9 in the batch of daphnids used for these treatments. In total 15 treatments were fitted simultaneously (Figure S8). For the prochloraz study, the lowest α-cypermethrin treatment was left out for all prochloraz treatments except the highest, as there were no mortality in neither of the two lowerst α-cypermethrin treatments for these prochloraz treatments. In total, 16 treatments were fitted simultaneously (Figure S9). The TD parameters of these fits are shown in Table S9 and the *s* values are given in figure 5 together with the *in vivo* P450 inhibition data published in Dalhoff et al. (2016)[2]. The *s* values were fitted to a two parameter log-logistic model using the *R*-software and the *drc* package, *b* and *e* parameters derived from the fit were used as starting values in the model with *s* described as a function of *b* and *e* (Equation 5). The fitted parameters are given in Table S10.

TABLES

Table S1 Raw data for the toxicokinetics of α-cypermethrin in *Daphnia magna* shown in Figure 2A. All times are given in hours. Data are originally published in Kretschmann et al. 2016[1].

External concentrations (nM)		Internal + sorbed concentrations (µmol g^{-1} Wet Weight)			
		α-cyp		α-cyp+propiconazole	
Time	C_{w_pyr} (nM)	Time	C_{in_pyr}	Time	C_{in_pyr}
0.0	0.536	0.0	0.0	0.0	0.0
7.5	0.273	0.0	0.0	0.0	0.0
7.6	0.000	0.9	165.6	0.8	193.9
55.0	0.000	1.2	152.1	1.2	121.2
		2.1	155.6	2.1	184.2
		2.3	222.0	2.2	246.5
		3.1	194.5	3.1	268.0
		3.5	225.3	3.4	199.3
		6.6	245.1	4.8	272.7
		6.6	246.9	4.8	228.8
		7.4	114.0	6.4	274.5
		7.5	183.2	6.4	325.9
		7.5	177.9	7.5	171.4
		8.6	64.8	7.5	206.0
		9.5	66.7	7.5	173.1
		9.9	90.7	8.6	159.8
		12.3	37.1	9.7	191.0
		12.7	43.4	9.9	103.2
		20.9	19.3	12.3	134.5
		22.5	23.6	12.8	113.9
		29.3	11.2	20.9	103.6
		30.6	9.1	22.7	98.5
		51.2	16.2	29.2	92.5
		51.6	0.0	30.8	99.9
				51.4	69.8
				51.7	61.8

Table S2 Raw data for the toxicodynamics of α-cypermethrin in *Daphnia magna* shown in Figure 3A and C. All times are given in hours. Treatments are denoted C0-C5, with the lowest treatment concentration having the lowest number. The same annotation is used in the model code. Data are originally published in Kretschmann et al. 2015[3].

External α-cypermethrin concentrations (nM)						
	C0	C1	C2	C3	C4	C5
Time	0nM	0.10nM	0.34nM	1.20nM	4.08nM	14.4nM
0.0	0	0.096	0.336	1.200	4.080	14.40
7.2	0	0.048	0.168	0.600	2.040	7.20
7.3	0	0.000	0.000	0.000	0.000	0.000
95.0	0	0.000	0.000	0.000	0.000	0.000
	Proportion mobile daphnids (*n*=24)					
0.0	1	1	1	1	1	1
6.0	1	1	1	1	0.88	0.38[a]
10.2	1	1	1	0.96	0.88	0.75
21.8	1	1	1	0.96	0.88	0.67
29.6	1	1	1	0.96	0.88	0.63
44.6	1	1	1	0.96	0.88	0.46
51.5	1	1	1	0.95	0.88	0.42
70.7	1	1	1	0.95	0.71	0.33
94.7	1	1	1	0.95	0.46	0.13

[a] Data not included in the fit

Table S3 Raw data for the toxicodynamics of α-cypermethrin together with propiconazole in *Daphnia magna* shown in Figure 3B, D. All times are given in hours. Treatments are denoted C1-C5, with the lowest treatment concentration having the lowest number. The same annotation is used in the model code. Data are originally published in Kretschmann et al. 2015[3].

External α-cypermethrin concentrations (nM)						
	C0	C1	C2	C3	C4	C5
Time	0nM	0.10nM	0.34nM	1.20nM	4.08nM	14.4nM
0.0	0	0.096	0.336	1.200	4.080	14.40
7.2	0	0.048	0.168	0.600	2.040	7.20
7.3	0	0.000	0.000	0.000	0.000	0.000
95.0	0	0.000	0.000	0.000	0.000	0.000
	Proportion mobile daphnids (*n*=24)					
0.0	1	1	1	1	1	1
6.0	1	1	1	0.92	0.79	0.08[a]
10.2	1	1	1	0.88	0.79	0.33[a]
21.8	1	1	0.96	0.83	0.71	0.5
29.6	1	1	0.75	0.58	0.5	0.5
44.6	1	1	0.75	0.5	0.38	0.25
51.5	1	1	0.75	0.38	0.29	0.17
70.7	1	0.92	0.75	0.26	0.17	0.08
94.7	1	0.88	0.58	0	0.04	0

[a] Data not included in the fit

Table S4 Raw data for the toxicodynamics of propiconazole in *Daphnia magna*. All times are given in hours. Treatments are denoted C1-C5, with the lowest treatment concentration having the lowest number. The same annotation is used in the model code. Data are originally published in Dalhoff et al. (2016)[2].

External propiconazole concentrations (μM)						
Time	C0	C1	C2	C3	C4	C5
0	0	5.49708	10.9649	21.9298	43.8596	87.7193
18	0	5.49708	10.9649	21.9298	43.8596	87.7193
18.1	0	0	0	0	0	0
168	0	0	0	0	0	0
Proportion mobile daphnids (*n*=24)						
0	1	1	1	1	1	1
18	1	1	1	1	1	1
23.9	1	1	1	0.96875	0.875	0.75
47.8	1	1	1	0.9375	0.53125	0.25
71.8	1	1	1	0.90625	0.46875	0.03125
95.9	1	1	1	0.90625	0.46875	0
120	1	1	1	0.90625	0.46875	0
143.9	1	1	1	0.90625	0.46875	0
167.9	1	1	1	0.90625	0.46875	0

Table S5 Raw data for the toxicodynamics of prochloraz in *Daphnia magna*. All times are given in hours. Treatments are denoted C1-C5, with the lowest treatment concentration having the lowest number. The same annotation is used in the model code. Data are originally published in Dalhoff et al. (2016)[2].

External prochloraz concentrations (μM)						
Time	C0	C1	C2	C3	C4	C5
0	0	1.67242	3.31829	6.63658	13.2732	26.5463
18	0	1.67242	3.31829	6.63658	13.2732	26.5463
18.1	0	0	0	0	0	0
168	0	0	0	0	0	0
	Proportion mobile daphnids (*n*=24)					
0	1	1	1	1	1	1
18	1	1	1	1	1	1
23.9	1	1	1	0.96875	0.75	0.3125
47.9	1	1	1	0.875	0.71875	0.15625
71.8	1	1	1	0.875	0.59375	0.03125
95.9	1	1	1	0.875	0.5625	0
119.9	1	1	1	0.875	0.53125	0
144	1	1	1	0.875	0.53125	0
167.9	1	1	1	0.875	0.53125	0

Table S6 Raw data for the toxicodynamics of α-cypermethrin together with prochloraz in *Daphnia magna* shown in Figure 4A and B. All times are given in hours. Treatments are denoted C1-C5, with the lowest treatment concentration having the lowest number. The same annotation is used in the model code. Data are originally published in Kretschmann et al. 2015[3].

External α-cypermethrin concentrations (nM)						
	C0	C1	C2	C3	C4	C5
Time	0nM	0.10nM	0.34nM	1.20nM	4.08nM	14.4nM
0.0	0	0.096	0.336	1.200	4.080	9.390
7.2	0	0.048	0.168	0.600	2.040	5.869
7.3	0	0.000	0.000	0.000	0.000	0.000
95.0	0	0.000	0.000	0.000	0.000	0.000
	Proportion mobile daphnids (*n*=24)					
0.0	1.00	1.00	1.00	1.00	1.00	1.00
6.0	1.00	1.00	1.00	1.00	0.67[a]	0.25[a]
10.2	1.00	0.88	0.92	0.92	0.71	0.17[a]
21.8	1.00	0.88	0.79	0.75	0.67	0.33
29.6	1.00	0.83	0.75	0.58	0.42	0.21
44.6	1.00	0.83	0.63	0.50	0.38	0.17
51.5	1.00	0.71	0.50	0.42	0.17	0.08
70.7	1.00	0.67	0.13	0.17	0.04	0.00
94.7	1.00	0.54	0.00	0.00	0.00	0.00

[a] Data not included in the fit

Table S7 Raw data for the toxicodynamics of α-cypermethrin together with propiconazole in *Daphnia magna* shown in Figure S8. All times are given in hours. α-cypermethrin treatments are denoted C1-C3. The fractions of mobile daphnids are given below the exposure concentrations denoting the propiconazole treatment above the rows. Here concentrations are given in mol/L. The treatment annotations in the model code start with the α-cypermethrin concentrations followed by the azole concentration of the co-exposure given in ng/L and µg/L, respectively. The data presented in the grey columns were not included in the modelling, as the almost full mobility did not add information to determine toxicity parameters. Data are originally published in Bjergager et al. (2017)[4].

	External α-cypermethrin concentrations (nM)			
Time	C0	C1	C2	C3
0.000	0.000	0.019	0.096	0.192
1.000	0.000	0.018	0.088	0.175
1.500	0.000	0.017	0.084	0.167
2.250	0.000	0.016	0.078	0.156
3.375	0.000	0.014	0.070	0.141
5.063	0.000	0.012	0.060	0.121
7.594	0.000	0.010	0.048	0.096
11.391	0.000	0.007	0.034	0.067
17.086	0.000	0.004	0.020	0.040
25.629	0.000	0.002	0.009	0.018
38.443	0.000	0.001	0.003	0.006
57.665	0.000	0.000	0.000	0.001
86.498	0.000	0.000	0.000	0.000
129.746	0.000	0.000	0.000	0.000
167.000	0.000	0.000	0.000	0.000
168.000	0.000	0.000	0.000	0.000
336.000	0.000	0.000	0.000	0.000

Propi	**1.33 µM**			**0.27 µM**			**0.133 µM**			**0.027 µM**			**0.013 µM**			**0.0027 µM**			**0.0013 µM**			**0 µM**		
time	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
24	1	1	1	1	1	1	1		1	1		1	1		1	1		1	1		1	1		1
48	1	1	1	1	1	1	1		1	1		1	1		1	1		1	1		1	1		1
120	1	1	0	1	1	0.7	1		0.85	1		0.95	1		0.95	1		1	1		0.95	1		0.9
168	1	0.65	0	1	0.6	0.2	0.95		0.65	1		0.95	1		0.95	1		1	1		0.95	1		0.9
216	0.95	0.5	0	0.95	0.55	0.2	0.9		0.6	1		0.95	0.95		0.95	1		1	1		0.95	1		0.9
288	0.95	0.5	0	0.95	0.55	0.2	0.9		0.5	1		0.95	0.95		0.9	1		1	1		0.95	0.95		0.9
336	0.95	0.5	0	0.95	0.55	0.2	0.9		0.5	1		0.95	0.95		0.9	1		1	1		0.95	0.95		0.9

Table S8 Raw data for the toxicodynamics of α-cypermethrin together with prochloraz in *Daphnia magna* shown in Figure S9. All times are given in hours. α -cypermethrin treatments are denoted C1-C3. The fractions of mobile daphnids are given below the exposure concentrations denoting the propiconazole treatment above the rows. Here concentrations are given in mol/L. The treatment annotations in the model code start with the α-cypermethrin concentrations followed by the azole concentration of the co-exposure given in ng/L and µg/L, respectively. The data presented in the grey columns were not included in the modelling, as the almost full mobility did not add information to determine toxicity parameters. Data are originally published in Bjergager et al. (2017)[4].

	External α-cypermethrin concentrations (nM)			
Time	C0	C1	C2	C3
0.0	0.000	0.019	0.096	0.192
1.0	0.000	0.018	0.088	0.175
1.5	0.000	0.017	0.084	0.167
2.2	0.000	0.016	0.078	0.156
3.3	0.000	0.014	0.070	0.141
5.0	0.000	0.012	0.060	0.121
7.5	0.000	0.010	0.048	0.096
11.3	0.000	0.007	0.034	0.067
17.0	0.000	0.004	0.020	0.040
25.6	0.000	0.002	0.009	0.018
38.4	0.000	0.001	0.003	0.006
57.6	0.000	0.000	0.000	0.001
86.4	0.000	0.000	0.000	0.000
129	0.000	0.000	0.000	0.000
167	0.000	0.000	0.000	0.000
168	0.000	0.000	0.000	0.000
336.00	0.000	0.000	0.000	0.000

Prochloraz	**1.33 µM**				**0.27 µM**			**0.13 µM**			**0.027 µM**			**0.013 µM**			**0.0027 µM**			**0.0013 µM**			**0 µM**		
time	C0	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
48	1	1	1	0.8	1	1	0.8	1	1	0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
120	1	0.9	0.75	0	1	1	0	1	1	0	1	1	0.1	0.95	1	0.85	1	0.95	0.9	1	1	0.85	1	1	1
168	1	0	0	0	1	1	0	1	0.95	0	1	1	0.05	0.95	1	0.7	1	0.95	0.9	1	0.95	0.85	1	1	1
216	1	0	0	0	1	1	0	1	0.95	0	1	1	0	0.95	1	0.7	1	0.95	0.9	1	0.95	0.85	1	1	1
288	1	0	0	0	1	1	0	1	0.95	0	1	1	0	0.95	1	0.7	1	0.95	0.9	1	0.95	0.85	1	1	1
336	1	0	0	0	1	1	0	1	0.95	0	1	1	0	0.95	1	0.7	1	0.95	0.85	1	0.95	0.85	1	1	1

Table S9 The toxicodynamic model parameters k_k, k_{dr} an z were estimated several times testing different assumptions and hypotheses. For the pulse exposures (Figure 3 and S2, S7) z approached zero and was therefore fixed to that value. Below are the different estimates, the calculated log-Likelihoods (ln(L)) and AIC keeping the TK parameters for α-cypermethrin constant and including reference to the figures where the fits are shown.

Fit conditions of TD parameters	**Dataset**	k_k (*10^{-4})	k_{dr}	z	*Ln(L)*	*AIC*	*Figure*
Fit on α-cypermethrin data only	Table S2	6.64	0.0014	-	-110	224.9	3A
	Table S3				-285	573.3	3B
Fit on all data	Table S2	7.30	0.0018	-	-129	261.6	S2
	Table S3				-280	564.3	S2
Fit on all data and assuming uptake rates decrease when daphnids become immobilized	Table S2	4.56	0.0063	-	-130	265.7	3C
	Table S3				-259	523.7	3D
Fit on all data including propiconazole damage	Table S2	7.45	0.0018	-	-129	263.6	S7
	Table S3				-286	566.2	S7
Fit on α-cypermethrin and variable propiconazole concentrations, fitting *s* for each propiconazole treatment	Table S7	29.3	0.0021	3.08	-127	266	S5
Fit on α-cypermethrin and variable propiconazole concentrations, fitting *s* as a variable (Eq. 5)	Table S8	2.36	0.0249	32.5	-128	268	
Fit on α-cypermethrin and variable prochloraz concentrations, fitting *s* for each prochloraz treatment	Table S7	137	0.0019	1.35	-481	981	S6
Fit on α-cypermethrin and variable prochloraz concentrations, fitting *s* as a variable (Eq. 5)	Table S8	7.09	0.1219	0.46	-368	747	

Table S10 The slope parameter, *b*, and the azole concentration of inhibition of cytochrome P450 activity by 50%, *e*, of the two parameter log-logistic concentration response curves of equation 5. The curve was fitted to metabolic activity measured in *D. magna* either as *in vivo* ECOD activity or as the *s* parameter of the TD fits (Figure 6). Data are given as the parameter ± Stdev

Azole	Measurement	*b*	*e* (*μM azole*)
Propiconazole	ECOD	0.87±0.18	4.87±1.12
	Fitted *s*	2.24±0.70	0.10±0.02
Prochloraz	ECOD	1.45±0.32	0.011±0.002
	Fitted *s*	0.57±0.10	0.031±0.009

FIGURES

Figure S1 Best fitting one compartment TK model (Equation 1) of the measured α-cypermethrin concentrations in *Daphnia magna* exposed to a 7.5 h pulse of α-cypermethrin in the presence (black symbols and line) or absence (grey symbols and line) of 1.4 µM propiconazole. The circles and triangles represent data from two independent experiments. The simple first order model was not able to capture the large increase in measured α -cypermethin concentrations in the daphnids and the subsequent initial quick decrease which then changed to a slower decrease.

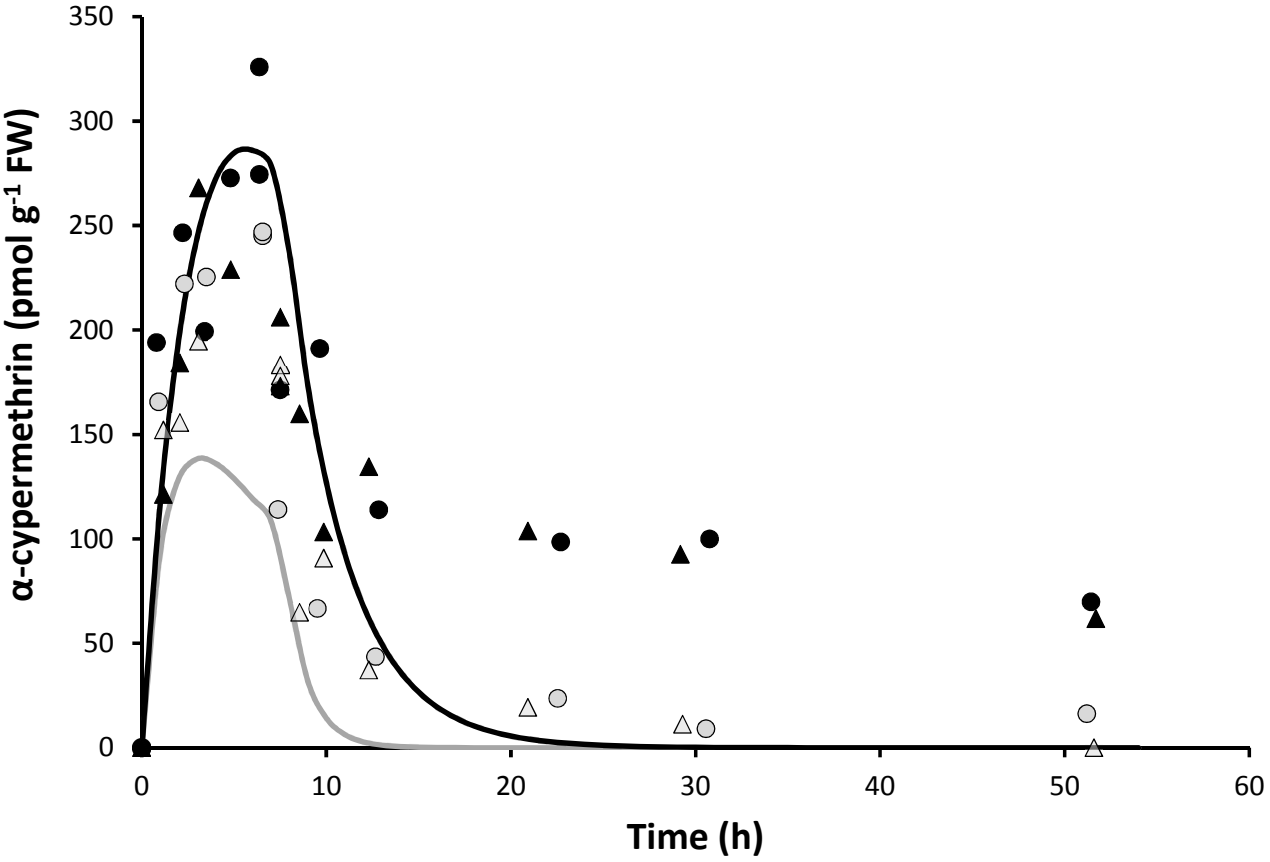


Figure S2 To test whether model parameters could be better estimated using more data, the model was fitted to all the data from figure 3 (Table S2 and S3) of the manuscript, rather than only to α-cypermethrin. Model parameters are given in Table S9 and models are compared in the main text. The figure shows the fraction of mobile daphnids (n = 24) as a function of time for daphnids being exposed to increasing pulse concentrations of α-cypermethrin. The pulse exposure duration is marked with grey. Treatments and time points where daphnids are immobilised during the pulse, but recovering mobility are marked with an asterisk and are not included in the fits.

α-cypermethrin alone **α-cypermethrin with 1.4 µM propiconazole**

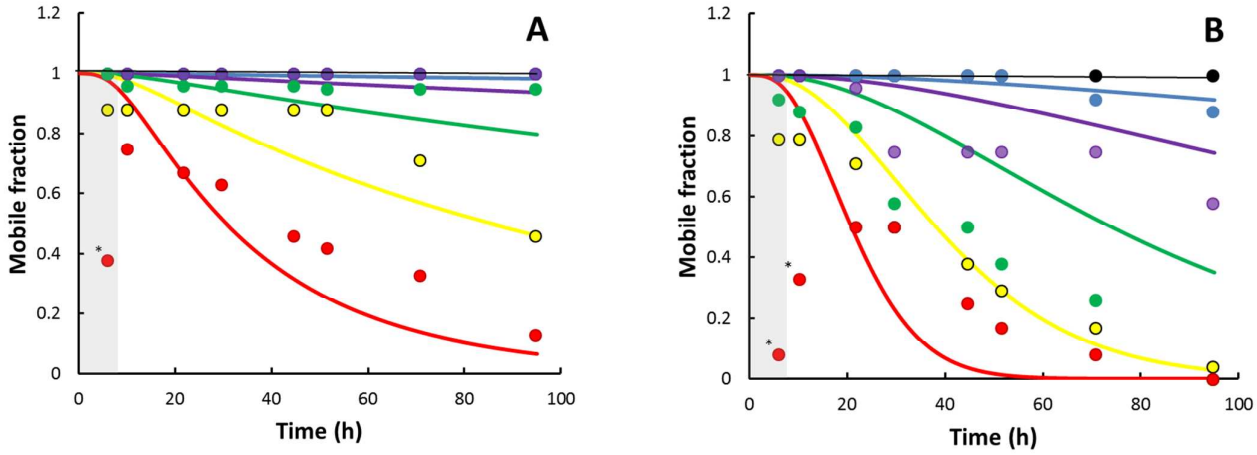


Figure S3 To test the assumption that metabolic activity per daphnid biomass is the same through the early life stages, as we do using a constant k_{m_pyr}, *in vivo* cytochrome P450 monooxygenase enzyme activity was measured as 7-ethoxycoumarin oxidation by *D. magna* of 1, to 8 days of age as a function of their bodyweight. There was a linear correlation between enzyme activity and bodyweight for *D. magna* less than 6 days old (R^2 = 0.97). At day 6, initiation of egg production could be observed, thereby decoupling the correlation between enzyme activity and bodyweight. The decoupling most likely happens as enzyme activity are different for eggs than for living organism tissue. Data are shown as mean ± stdev of four replicates of each 20-40 daphnids, depending on size.

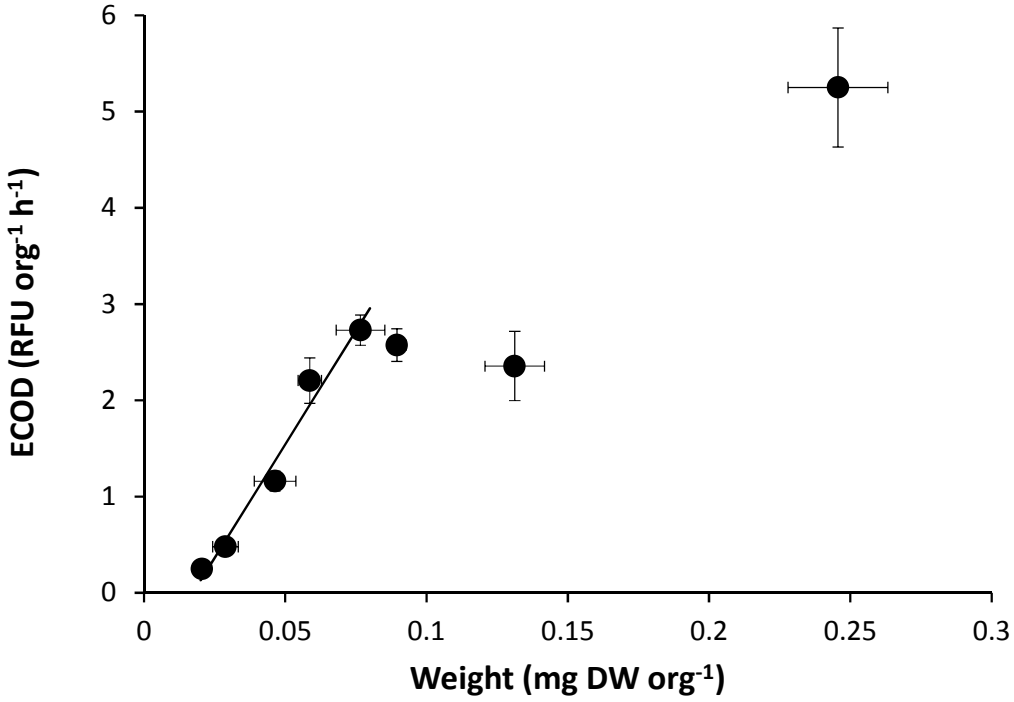


Figure S4 Fraction of immobile daphnids (n = 24) at the end of the α-cypermethrin pulse, either with (black circle) or without (grey circle) propiconazole as a function of modelled internal α-cypermethrin concentrations. The treatments with immobile daphnids could be described with a linear correlation (R^2 = 0.96). Letting the uptake rate decrease with a proportion described by x above the threshold of 213 pmol g^{-1} daphnid FW, decreased the internal concentrations described by the model. Two scenarios for x = 0.1 and x = 0.5 are shown in the figure with white and grey triangles and an associated regression lines. The fit of the data shown in figure 3C and D improved significantly including x, which was estimated to be 0.287±0.023.

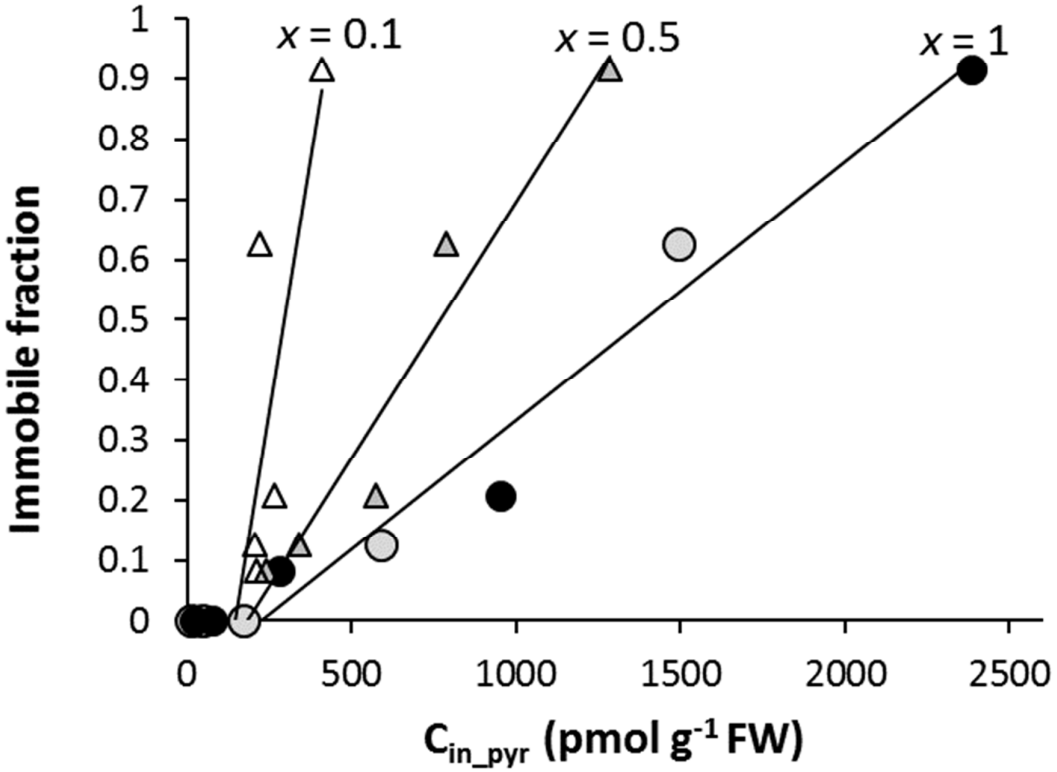


Figure S5 The external concentrations (A), modelled internal concentrations (B and C) and modelled scaled damage (D and E) as a function of time in the two treatments: Pure α-cypermethrin (B and D) and α-cypermethrin + propiconazole (C and E). The model used is the one assuming an internal threshold after which uptake rates are decreased by approximately 30 % (Equation 10 and 11). The mobility of the daphnids is shown together with the survival model in Figure 3C and D.

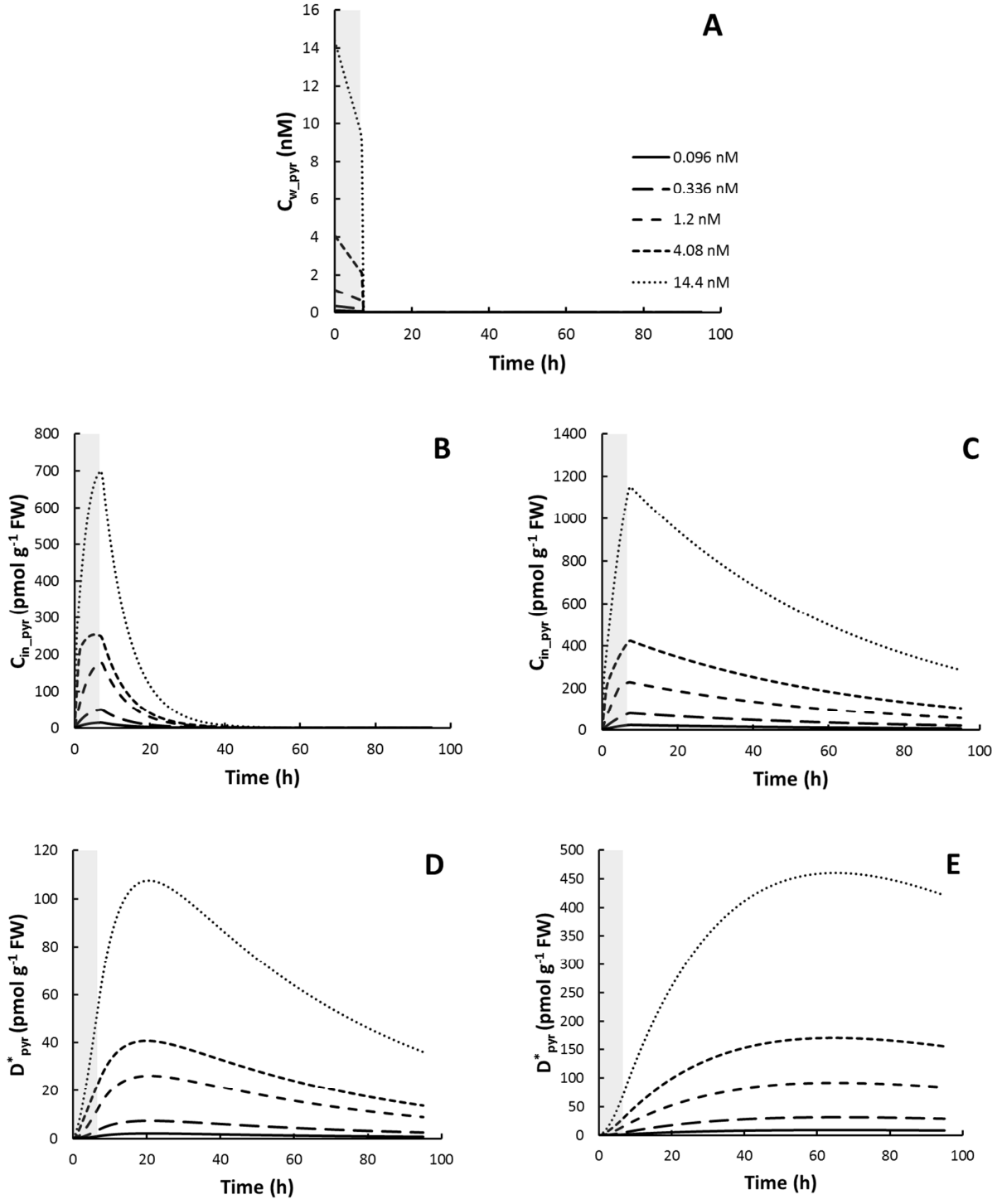


Figure S6 Internal propiconazole concentrations (solid line) and scaled damage (broken line) predicted from a one compartment TKTD model parameterized using the TK parameters of Dalhoff et al. (2016)[2] and the TD parameters of Table 1. The grey area gives the time interval of α-cypermethrin exposure.

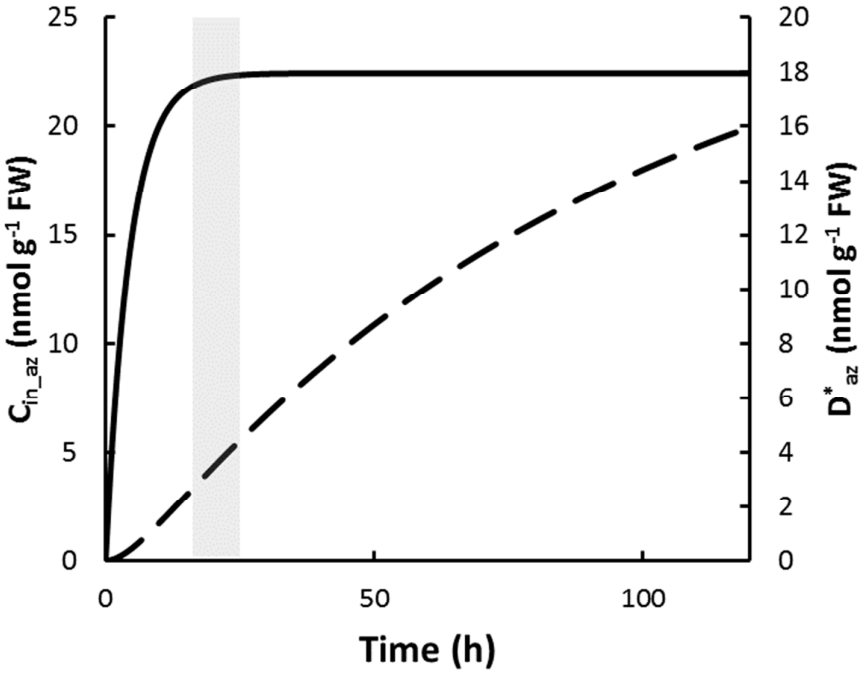


Figure S7 Model fit with addition of relative azole damage of Figure S5 to the α-cypermethrin damage, as described in Equation S3 above and fitting all data. Model parameters are given in Table S9 and models are compared in the main text. The figure shows the fraction of mobile daphnids (n = 24) as a function of time for daphnids being exposed to increasing pulse concentrations of α-cypermethrin. The pulse exposure duration is marked with grey. Treatments and time points where daphnids are immobilised during the pulse, but recovering mobility are marked with an asterisk and are not included in the fits.

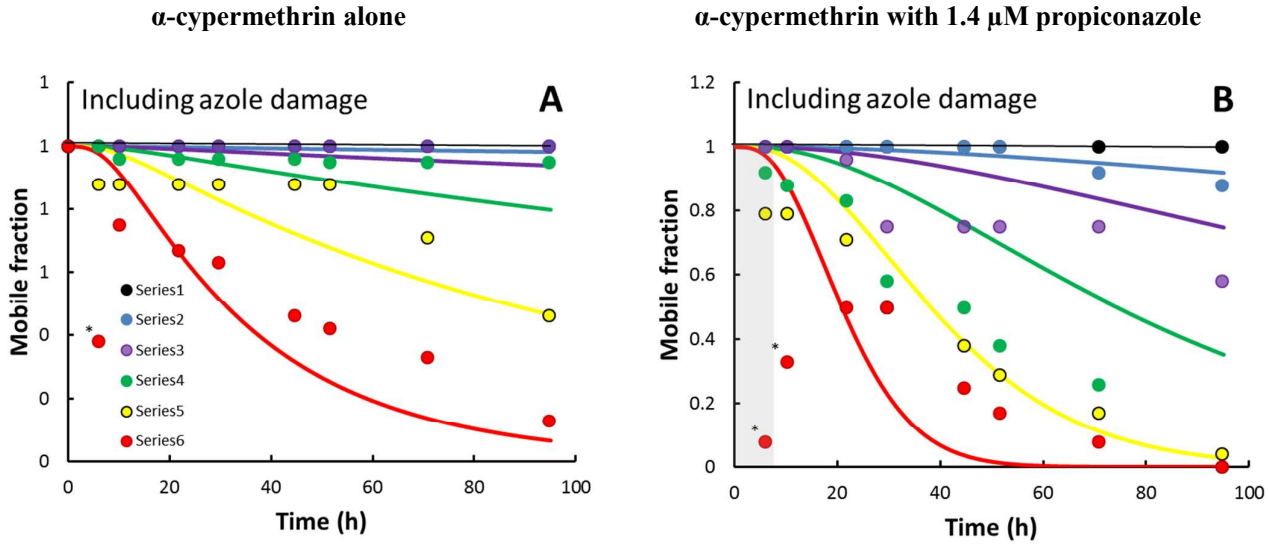


Figure S8 External (A), modelled internal (B) α-cypermethrin concentration and the associated immobility over time (C) of the daphnids treated with 0.02, 0.12 and 0.24 nM α-cypermethrin alone and in the presence of 1.33 µM propiconazole. Figure D-F show the immobility over time in the same α-cypermethrin treatments, but in the presence of increasing concentrations of propiconazole. The majority of the 0.12 nM α-cypermethrin treatments were discarded from this propiconazole dataset due to excess control mortality of that specific daphnid batch. Only data with mobile fractions <0.90 are shown. All control and lower propiconazole concentrations had mobile fraction >0.90. Legends are given at the figures and the lines represent the joint fit to variable *s*-parameters (Table S9).

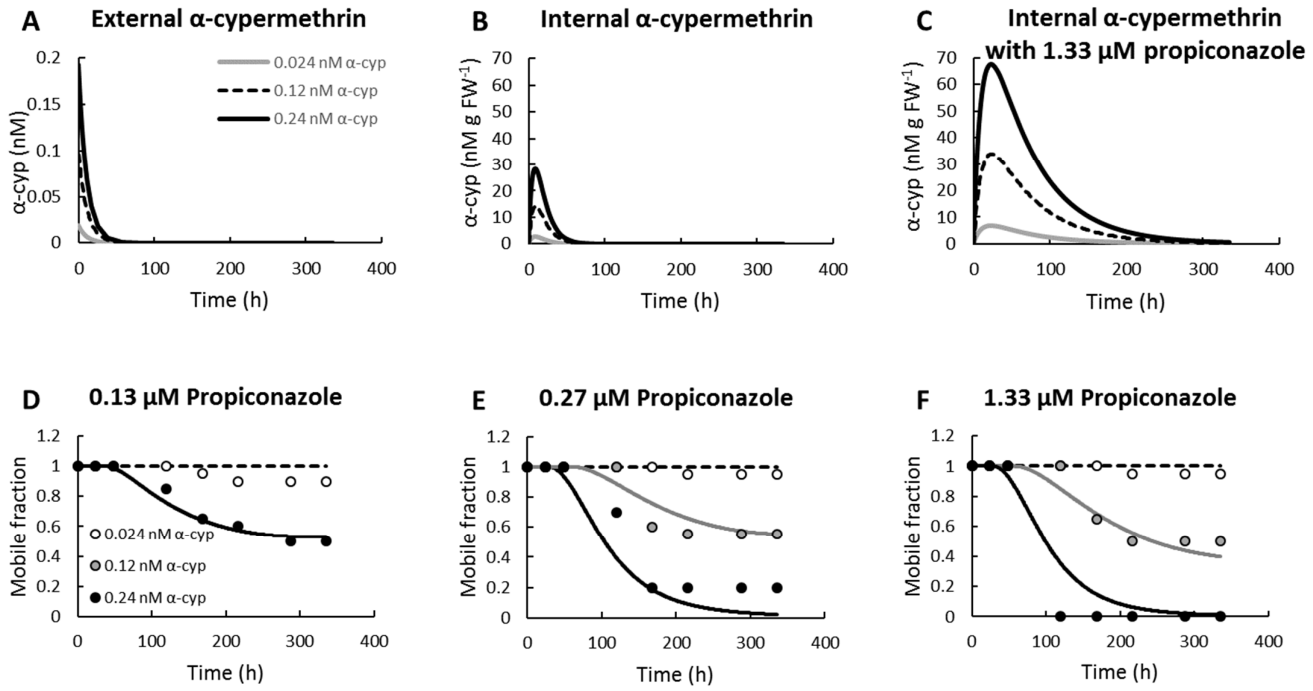
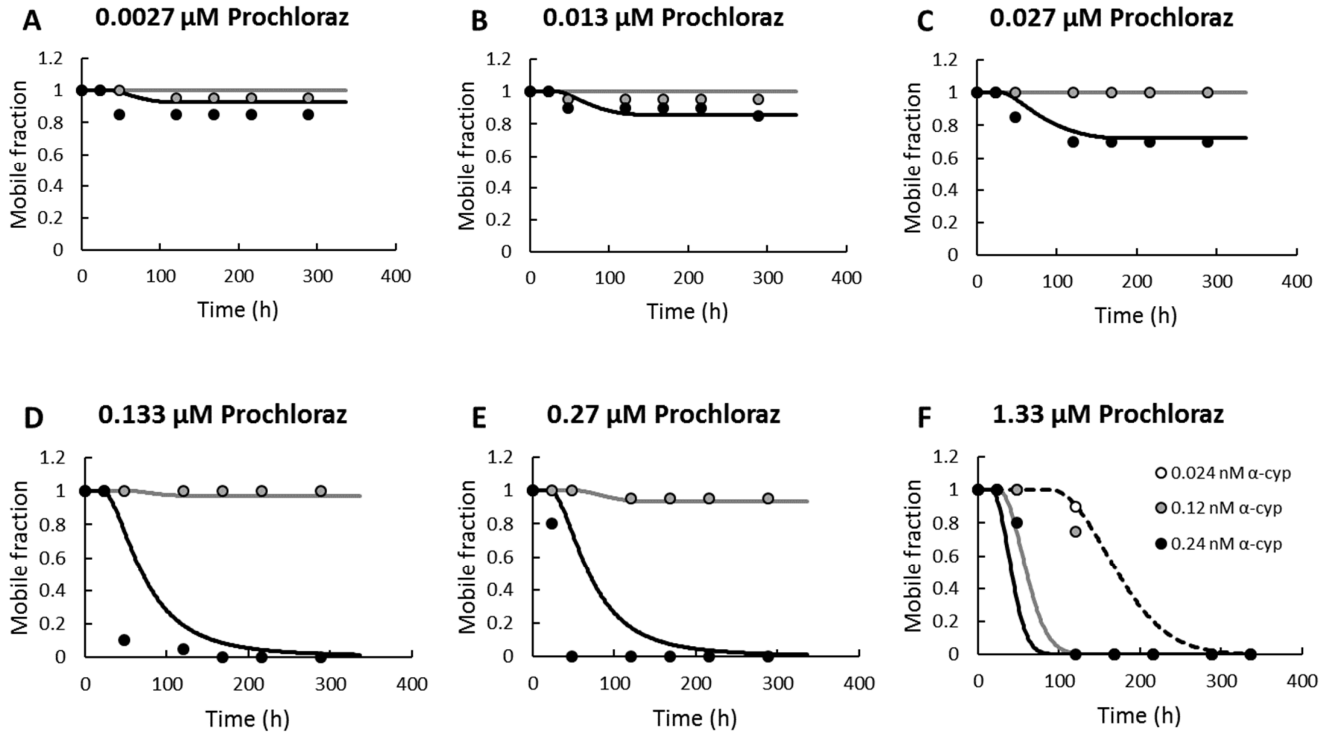


Figure S9 Panels A-H show the immobility over time in the same α-cypermethrin treatments as figure S5, but in the presence of increasing concentrations of prochloraz. Only data with mobile fractions <0.90 are shown. All control and lower prochloraz concentrations had mobile fraction >0.90. Legends are given at the figures and the lines represent the joint fit to variable *s*-parameters (Table S9).



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