Supporting Information

Improving risk assessment by predicting the survival of field gammarids exposed to dynamic pesticide mixtures

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Keywords: Toxicokinetics-toxicodynamics, survival, pesticides, GUTS, invertebrate, environmental quality standards

Summary

Number of pages: 23

Number of tables: 9

Number of figures: 6

Table S1. a) GUTS-RED-SD and b) GUTS-RED-IT calibrated parameters. The background mortality (h_b) was pre-fitted to the control data and hence the h_b for SD and IT are the same. Note that in openGUTS, the option to parameterize h_b with other parameters is also available (and in this case, the h_b values for SD and IT may change). Only ME, NRMSE and AIC are shown here, but the "survival probability prediction error" (SPPE) are found in Table S2. EFSA recommends an NRMSE of <50%. Lower AICs and higher MEs are considered more optimal.

		a) Calibrated Parameters - GUTS-RED-SD with their 95% CI in brackets				Dependent Parameter	Calibration Efficiency			
Compound	**Raw Survival Data Source**	**mw_{SD} (ng/L)**	**bw, (L/ng-d)**	**h_{bSD} (1/d) [d]**	**kd (1/d)**	**DRT95 (d)**	**ME[b]**	**NRMSE**	**AIC**	**LL**
Chlorpyrifos	Ashauer, et al. [1] Exp. A	42.57 (26.87,48.03)	3.08×10^{-3} (1.93×10^{-3},4.20×10^{-3})	0.011	0.22 (0.12,0.31)	13.70 (9.68,25.50)	0.92	10.88%	932.14	463.07
Chlorpyrifos	Rubach, et al. [2]	0.57 (3.42×10^{-3}*,9.33)	2.65×10^{-3} (1.3×10^{-3},0.62)	1.00×10^{-6}, [c]	0.49 (1.64×10^{-3}*, 2.38)	6.08 (1.26,1.83 $\times 10^{3}$)	0.76	20.34%	190.36	92.18
Chlorpyrifos	Combined Ashauer, et al. [1] and Rubach, et al. [2]	44.06 (1.04,49.17)	2.71×10^{-3} (8.72×10^{-4},4.19×10^{-3})	0.011	0.32 (0.20,0.91)	9.51 (3.31-14.70)	0.78	15.86%	1151.45	572.72
Diazinon	Ashauer, et al. [3]	198.30 (0.40*,698.60)	3.57×10^{-5} (2.0×10^{-5},9.73×10^{-5})	0.025	0.08 (0.03,0.15)	39.50 (19.90,106.00)	0.87	18.00%	1431.86	714.93
Propiconazole (constant)	Nyman, et al. [4]	5.79 $\times 10^{6}$ (5.38×10^{6},6.01×10^{6})	3.82×10^{-7} (2.52×10^{-7},5.59×10^{-7})	0.013	2.19 (1.63,3.35)	1.37 (0.89,1.84)	0.98	7.95%	257.63	125.82
Propiconazole (pulsed)	Nyman, et al. [4]	1.04 $\times 10^{3}$ (0.43,7.83 $\times 10^{3}$)	2.72×10^{-5} (1.67×10^{-5},2.7×10^{-3})	0.011	4.90 (1.45,14.80)	0.61 (0.20,2.07)	0.89	7.25%	682.47	338.23
Imidacloprid (immob data)	Roessink, et al. [5]	0.274 (0.27*,566.50)	5.30×10^{-5} (4.14×10^{-5},6.74×10^{-5})	0.057	143.80 (9.232,143.80*)	0.02 (0.02,0.32)	0.85	38.85%	411.41	202.71
Azoxystrobin[a]	Rösch [6] (PhD Thesis)	2.41 $\times 10^{4}$ (1.01 $\times 10^{4}$,3.51 $\times 10^{4}$)	1.977×10^{-5} (1.13×10^{-5} ,5.09×10^{-5})	1.00×10^{-6}, [c]	1.23 (0.35, 3.08)	2.43 (0.97, 8.56)	0.88	37.86%	193.58	93.79

		b) Calibrated Parameters - GUTS-RED-IT with their 95% CI in brackets				Dependent Parameters		Calibration Efficiency			
Compound	**Raw Survival Data Source**	**mw_{IT} (ng/L)**	**Fs (-)**	**$h_{b,IT}$ (1/d)**	**kd (1/d)**	**DRT95 (d)[e]**	**β[f] (-)**	**ME[b]**	**NRMSE**	**AIC**	**LL**
Chlorpyrifos	Ashauer, et al. [1] Exp. A	2.32 (1.98,3.83)	4.52 (3.10,7.46)	0.011	1.64×10^{-3} (1.64×10^{-3}*,2.60×10^{-3})	1.83 $\times 10^{3}$ (1.15 $\times 10^{3}$,1.83 $\times 10^{3}$)	2.43 (1.82,3.25)	0.87	14.10%	1054.91	524.45
Chlorpyrifos	Rubach, et al. [2]	0.95 (0.73,44.03)	9.11 (5.52,18.82)	1.0×10^{-6}, [c]	1.64×10^{-3} (1.64×10^{-3}*,8.34×10^{-2})	1.83 $\times 10^{3}$ (35.80, 1.83 $\times 10^{3}$)	1.66 (1.25,2.14)	0.74	21.62%	204.44	99.22
Chlorpyrifos	Combined Ashauer, et al. [1] and Rubach, et al. [2]	1.93 (1.67,3.04)	5.57 (3.89,8.82)	0.011	1.64×10^{-3} (1.64×10^{-3}*,2.52×10^{-3})	1.83 $\times 10^{3}$ (1.19 $\times 10^{3}$,1.83 $\times 10^{3}$)	2.13 (1.68,2.70)	0.79	12.59%	1274.88	633.94
Diazinon	Ashauer, et al. [3]	1.60 $\times 10^{4}$ (122.10,1.92 $\times 10^{4}$)	1.27 (1.11,2.77)	0.025	0.57 (1.64×10^{-3}*, 0.85)	5.22 (3.52, 1.83 $\times 10^{3}$)	15.50 (3.60,36.10)	0.50	35.72%	1492.40	743.20
Propiconazole (constant)	Nyman, et al. [4]	6.07 $\times 10^{6}$ (5.24 $\times 10^{6}$,6.86 $\times 10^{6}$)	1.71 (1.51,2.05)	0.013	0.73 (0.55,0.94)	4.11 (3.20, 5.49)	6.80 (5.11,8.88)	0.96	11.67%	261.92	127.96
Propiconazole (pulsed)	Nyman, et al. [4]	3.74 $\times 10^{3}$ (58.11,6.66 $\times 10^{3}$)	9.23 (3.92,20.00*)	0.011	0.17 (1.64×10^{-3}*, 0.34)	1.65 (1.22, 2.68)	1.65 (1.22,2.68)	0.81	9.29%	690.22	342.11
Imidacloprid (immob data)	Roessink, et al. [5]	1.60 $\times 10^{3}$ (796.00,2.61 $\times 10^{3}$)	14.00 (7.84,20.00*)	0.057	0.37 (0.16,0.65)	7.99 (4.63, 18.2)	1.39 (1.22,1.78)	0.94	24.03%	355.88	174.94
Azoxystrobin[a]	Rösch [6] (PhD Thesis)	217.90 (187.90, 2.67 $\times 10^{4}$	3.2 (2.53, 4.43)	1.0×10^{-6}, [c]	1.64×10^{-3} (1.64×10^{-3}*,2.3×10^{-1})	1.83 $\times 10^{3}$ (13.0, 1.83 $\times 10^{3}$)	3.15 (2.46, 3.95)	0.89	35.86%	179.64	86.82

[a]Since the raw data are not published elsewhere, they are tabulated in Table S2.
[b]Nash–Sutcliffe efficiency as the model efficiency coefficient
[c]Control data show 100% survival during the entre exposure duration, but the GUTS algorithm requires "some" background mortality rate for model to run properly, Therefore, the "optimized" background here is presented as a very low value.
[d]openGUTS was set up to "fix" h_b based on the control data (raw survival input)
[e]DRT95 represents the duration/repair time and in GUTS is estimated as the amount of time it takes for the damage to return to 5% of the value at the end of the pulse (see https://openguts.info/downloads/openguts_interpret.pdf). As proposed in EFSA scientific opinion, DRT95 can be useful to determine the time required for two pulses to be independent and may be employed to design validation exposure studies.
[f]Beta, β, is a parameter related to the damage threshold distribution and is inversely proportional to Fs (see equation 4 in https://openguts.info/downloads/openguts_interpret.pdf)
*edge of 95% parameter CI has run into boundary

Acronym Definitions: SD = stochastic death; IT = individual tolerance; RED = reduced formulation; CI = confidence interval; ME = model efficiency; NRMSE = normalized root mean square error; SD = stochastic death; IT = individual tolerance; m_w = threshold; b_w = killing rate constant; h_b = background mortality; k_d = dominant rate constant; DRT95 = depuration/repair time; Fs = spread factor in the distribution of thresholds; Beta = shape parameter of the distribution of thresholds; AIC = Akaike Information Criterion; LL = log-likelihood can be used to compare between two model simulations.

Table S2. a) GUTS-RED-SD; b) GUTS-RED-IT survival probability prediction error (SPPE) for each treatment described in raw survival data. Please review Figure S2 for parameter space plots and visual prospective posterior check (observed vs. predicted). EFSA recommends a cut-off of ± 50% for SPPE.

Compound	Raw Survival Data Source	SPPE – GUTS-RED-IT	SPPE – GUTS-RED-SD
Chlorpyrifos	Ashauer, et al. [1] Exp. A	Data set treatment value 1 Control +0.15 % 1 T1 -18.30 % 1 T2 -22.70 % 1 T3 -17.80 %	Data set treatment value 1 Control +0.15 % 1 T1 +1.41 % 1 T2 -5.63 % 1 T3 -12.70 %
Chlorpyrifos	Rubach, et al. [2]	Data set treatment value 1 Control $+4.00 \times 10^{-4}$ % 1 T1 -3.88 % 1 T2 -23.80 % 1 T3 +30.10 % 1 T4 -20.90 % 1 T5 -16.60 %	Data set treatment value 1 Control $+4.00 \times 10^{-4}$ % 1 T1 +1.66 % 1 T2 -13.1 % 1 T3 +41.6 % 1 T4 -12.4 % 1 T5 -9.45 %
Chlorpyrifos	Combined Ashauer, et al. [1] and Rubach, et al. [2]	Data set treatment value 1 Control +0.15 % 1 T1 -13.00 % 1 T2 -15.00 % 1 T3 -8.64 % 1 T4 -1.04 % 1 T5 -27.8 % 1 T6 +11.10 % 1 T7 -50.30 % 1 T8 -34.50 %	Data set treatment value 1 Control +0.15 % 1 T1 +1.07 % 1 T2 -3.64 % 1 T3 -5.18 % 1 T4 -1.16 % 1 T5 -28.9 % 1 T6 +9.50 % 1 T7 -39.00 % 1 T8 -23.50 %
Diazinon	Ashauer, et al. [3]	Data set treatment value 1 Control +1.69 % 1 A -19.00 % 1 B -36.00 % 1 C -28.60 %	Data set treatment value 1 Control +1.69 % 1 A -0.568 % 1 B -11.2 % 1 C +8.00 %
Propiconazole (constant)	Nyman, et al. [4]	Data set treatment value 1 Control +0.09 % 1 T1 +0.40 % 1 T2 -5.77 % 1 T3 -4.40 % 1 T4 +21.1 % 1 T5 -9.25 % 1 T6 -4.71 % 1 T7 -1.12 %	Data set treatment value 1 Control +0.09 % 1 T1 +0.09 % 1 T2 -9.91 % 1 T3 -14.90 % 1 T4 +3.79 % 1 T5 -0.56 % 1 T6 -0.69 % 1 T7 -0.03 %
Propiconazole (pulsed)	Nyman, et al. [4]	Data set treatment value 1 Control $+3.55 \times 10^{-3}$ % 1 T1 -9.16 % 1 T2 -14.6 % 1 T3 +0.47 %	Data set treatment value 1 Control $+3.55 \times 10^{-3}$ % 1 T1 -7.27 % 1 T2 -6.60 % 1 T3 -1.10 %
Imidacloprid (immob data)	Roessink, et al. [5]	Data set treatment value 1 Control +3.36 % 1 T1 +4.35 % 1 T2 -2.64 % 1 T3 +1.84 % 1 T4 +2.22 % 1 T5 -0.21 %	Data set treatment value 1 Control +3.36 % 1 T1 +7.39 % 1 T2 +1.56 % 1 T3 +6.13 % 1 T4 +3.33 % 1 T5 -1.14×10^{-19} %
Azoxystrobin	Rösch [6] (PhD Thesis)	Data set treatment value 1 Control $+2.00 \times 10^{-4}$ % 1 T1 -31.00 % 1 T2 +3.32 % 1 T3 +22.8 % 1 T4 -3.03 % 1 T5 -1.52 % 1 T6 -0.86 % 1 T7 +4.47 %	Data set treatment value 1 Control $+4.00 \times 10^{-4}$ % 1 T1 -26.90 % 1 T2 +4.41 % 1 T3 +23.90 % 1 T4 -1.76 % 1 T5 -0.512 % 1 T6 -0.15 % 1 T7 +4.96 %

Table S3. LP_{10} and LP_{50} values predicted by GUTS-RED. SD = stochastic death; IT = individual tolerance; RED = reduced formulation. 95% CI=confidence interval.

		GUTS-RED-SD		GUTS-RED-IT	
Compound	**Raw Survival Data Source**	**LP_{10} (CI)**	**$LP5_0$ (CI)**	**LP_{10} (CI)**	**$LP5_0$ (CI)**
Chlorpyrifos	Ashauer, et al. [1] Exp. A	13.35 (10.77 - 14.43)	22.43 (20.67 - 24.78)	13.84 (10.38 - 17.74)	34.18 (29.07 - 42.09)
Chlorpyrifos	Rubach, et al. [2]	1.20 (0.08 - 3.34)	6.55 (0.39 - 13.18)	3.72 (2.26 - 6.14)	14.01 (10.55 - 21.42)
Chlorpyrifos	Combined Ashauer, et al. [1] and Rubach, et al. [2]	11.89 (2.82 - 13.76)	21.07 (16.74 - 23.09)	10.12 (7.59 - 12.88)	28.33 (24.45 - 33.62)
Diazinon	Ashauer, et al. [3]	7.89 (1.13 - 20.27)	20.66 (7.176 - 31.60)	75.70 (26.47 - 86.25)	87.27 (48.18 - 126.9)
Diazinon	Using chlorpyrifos combined parameters	17.54 (2.46 - 20.08)	25.59 (14.37 - 27.56)	8.48 (6.36 - 10.79)	23.73 (20.48 - 28.17)
Propiconazole (constant)	Nyman, et al. [4]	4.14x10^6 (3.85x10^6 - 4.39 x10^6)	4.97 x10^6 (4.63 x10^6- 5.28 x10^6)	4.12 x10^6 (3.65 x10^6 - 4.52 x10^6)	5.69 x10^6 (5.34 x10^6 - 6.12 x10^6)
Propiconazole (pulsed)	Nyman, et al. [4]	1,491 (600 – 5,223)	4,511 (3,658 – 5,768)	1,874 (742 – 2,636)	7,123 (4,442 – 12,050)
Imidacloprid (immob data)	Roessink, et al. [5]	6.69 (5.27 - 62.05)	43.46 (34.15 - 171.50)	31.78 (18.01 - 54.33)	154.70 (107.10 - 218.30)
Azoxystrobin	Rösch [6] (PhD Thesis)	22.35 (11.60 - 28.58)	30.45 (14.83 - 38.33)	2.10 (1.56 - 19.21)	4.22 (3.61 - 37.80)
Mixture Toxicity					
Group	**Reference chemical**	**LP_{10} (CI)**	**$LP5_0$ (CI)**	**LP_{10} (CI)**	**$LP5_0$ (CI)**
Organophosphates	Chlorpyrifos	2.31 (0.49 - 2.87)	4.81 (2.78 - 5.29)	1.67 (1.25 - 2.13)	4.68 (4.04 - 5.55)
Neonicotinoids	Imidacloprid	1.56 (1.23 - 11.66)	10.10 (7.94 - 26.26)	5.88 (2.86 - 11.30)	28.62 (17.26 - 44.73)

Table S3 Continued. Categorization of risk of individual and the mixture of compounds at Eschelisbach.

	GUTS-RED-SD*		GUTS-RED-IT*	
Individual Compound	**Acute (LP_{50}<100)**	**Chronic (LP_{50}<10)**	**Acute (LP_{50}<100)**	**Chronic (LP_{50}<10)**
Chlorpyrifos (Ashauer)	Yes	No	Yes	No
Chlorpyrifos (Rubach)	Yes	Yes	Yes	No
Chlorpyrifos (Combined)	Yes	No	Yes	No
Diazinon	Yes	No	Yes	No
Propiconazole (constant)	No	No	No	No
Propiconazole (pulsed)	No	No	No	No
Imidacloprid (immob data)	Yes	No	No	No
Azoxystrobin	Yes	No	Yes	Yes
Mixture				
Organophosphates	Yes	Yes	Yes	Yes
Neonicotinoids	Yes	No (but CI includes it)	Yes	No

*In Tier-2C_1 acute risk assessment, LP_{50} for all relevant test species must be ≥100 for risks to be considered low. For chronic risks to be acceptable, LP_{50} ≥10. LP_{10} are included here for comparison.

Table S4. Additional model comments/documentation on openGUTS model calibration

Compound	Additional Comments
Azoxystrobin	• Raw survival data only has 3 time points (0h, 24h, 48h) but consists of 7 concentration levels. • SD parameters are better constrained than IT parameters (see Figure S2)
Chlorpyrifos	• Best parameter fit observed for combined data (Ashauer et al 2007a + Rubach et al. 2011) under IT representation, see Figure S2 • For IT representation, the lower boundary is the optimal parameter value for the dominant rate constant, k_d.
Diazinon	• Survival data represents pulsed exposure • Segmented parameter space • SD appears better, but internal threshold not constrained well
Imidacloprid	• Immobility data were employed here • IT model has parameters that were constrained better than SD.
Propiconazole	• For constant exposure, either SD or IT works when representing survival • For pulsed exposure, parameter space is segmented so the calibration may not have been the most optimal. • Note that constant + pulsed exposure was not combined as they came from the same study (compared to chlorpyrifos where survival data were not from the same study). The readers are advised to review Nyman et al[4] for explanations regarding combined datasets.

SD = stochastic death model; IT = individual tolerance model

Table S5. Summary statistics related to the acute EC50 values for organophosphates and neonicotinoids included in this study.

	Organophosphates[b]			Neonicotinoids[b]	
Parameter (µg/L)	Chlorpyrifos[c]	Chlorpyrifos-methyl	Diazinon	Imidacloprid[c]	Thiacloprid
Mean	0.87	1.40	93	33876	19331
Geometric mean	0.37	0.41	11	564	1289
Median	0.27	0.68	86	442	3390
Standard deviation	1.3	1.65	107	83437	31825
Number of data	14	6	4	28	14
Equivalency factor[a]	1	0.92	0.03	1	0.44

Note: Acute EC50s were obtained from the database generated by De Zwart [16] which contains information on the toxicity test endpoints, toxic mode of action, and general descriptors of toxicity tests of >80,000 chemicals. Data specific to *Gammarus spp* were selected, except for chlorpyrifos-methyl, imidacloprid, and thiacloprid where no gammarid-specific data were available. For these compounds, acute EC50s reported for arthropods were used instead. The data were not separated based on their exposure duration, hence we used the geometric means for calculating an average value. See Table S6 for acute EC50s separated based on duration.

[a]Relative toxicity (highlighted) was calculated using the ratio of the geometric mean of the reference compound relative to the compound of interest (same toxic mode of action). This is referred to as equivalency factor (EF) in the manuscript.
[b] Gammarids-specific only considered for the calculation except for chlorpyrifos-methyl, imidacloprid, and thiacloprid where data for arthropods were used due to the lack of data for gammarids.
[c]Chlorpyrifos and imidacloprid were used as the reference compound for organophosphates and neonicotinoids respectively.

Table S6. Comparison of acute EC50 from the database generated by De Zwart [7] and LCx,t provided by GUTS after calibration. Since the measured EC50 were separated based on exposure duration, calculating the mean was sufficient (instead of geometric mean presented in Eqn. 1 of the manuscript). Note also that the number of data points is slightly lower compared to that from Table S5 as we were limited by the availability of the references that can be traced back for data on exposure duration. CI = confidence interval. Here, the acute EC50 by De Zwart [7] database was considered similar to GUTS LC50 calculations.

Raw Acute EC50 values specific to Gammarus spp.			
Compound	Duration (h)	Value (ug/L	Reference
Chlorpyrifos	24	3.1	Rubach, et al. [8]
	24	3.1	Rubach, et al. [8]
	48	0.43	Rubach, et al. [8]
	48	3.4	Ashauer, et al. [1]
	48	0.38	Rubach, et al. [8]
	48	0.3	Van Wijngaarden, et al. [9]
	72	0.23	Rubach, et al. [8]
	72	0.24	Rubach, et al. [8]
	96	0.23	Rubach, et al. [8]
	96	0.07	Van Wijngaarden, et al. [10]
Diazinon	96	0.2	Johnson and Finley [11]
	96	2.04	US EPA [12]
	96	16.82	US EPA [12]

Comparison of reported values from the literature and GUTS LC50 predictions				
Chlorpyrifos (using parameters from pooled dataset (Ashauer et al 2007 + Rubach et al 2011)				
Duration	Average Reported Acute EC50 (ug/L)	No. of data	GUTS-IT (ng/L) Value (CI)	GUTS-SD (ng/L) Value (CI)
24h	3.10	2	1.17 (1.01-1.39)	2.1 (1.76-3.03)
48h	1.13	4	0.59 (0.51-0.70)	0.66 (0.57-0.90)
72h	0.24	2	0.39 (0.34-0.47)	0.34 (0.32-0.47)
96h	0.15	2	0.29 (0.25-0.35)	0.24 (0.22-0.31)
Diazinon				
96h	6.35	3	36.8 (27.6-53.4)	17.8 (14.8-19.9)
Relative Potency of diazinon relative to chlorpyrifos (96h LC50)				
Measured	0.02 = (0.15/6.35)	**Predicted**	0.01 = (0.29/36.8)	0.01 = (0.24/17.8)

Table S7. Survival data for azoxystrobin[a] used to calibrate the GUTS parameters.

Survival time [d]	Control	T1	T2	T3	T4	T5	T6	T7
0	20	20	20	20	20	20	20	20
1	20	20	18	13	4	0	0	1
2	20	8	5	6	0	0	0	1
Concentration unit:	ng/L							
Concentration time [d]	Control	T1	T2	T3	T4	T5	T6	T7
0	0	50000	100000	150000	200000	250000	300000	350000

[a]Experiment completed by Rebecka Hischier during the completion of Bachelor Thesis at Eawag in 2017. "Synergistic potential of prochloraz towards the toxicity of azoxystrobin in *Gammarus pulex*". Department of Environmental Systems Sciences, ETH Zurich.

T = treatment

Table S8: Full GUTS-SD model as presented in Jager and Ashauer [13]. These equations were used to illustrate the results of varying exposure duration and magnitude as shown in Figure S5 (chlorpyrifos only). The parameters were taken from Galic, et al. [14] and adjusted to reflect the formulation in the GUTS-SD model (see equations 3.16a-c in Jager and Ashauer [13]).

Process	Mathematical Representation	Parameters	Parameter Value	Parameter Definition
Toxicokinetics	Internal Concentration: $\frac{dC_{int}(t)}{dt} = k_e\left(\frac{K_{iw} \times C_r(t)}{1000} - C_{int}\right)$ Variables: C_{int} = internal concentration [ng/g] C_r = river concentration [ng/L] 1000 is conversion factor for kg to g	K_{iw} [L/kg]	1660	Partition coefficient environment – organism. Calculated as k_u/k_e
		k_u [L/kg-d]	747	Uptake rate constant
		k_e [1/d]	0.5	Elimination rate constant
Toxicodynamics[a]	Scaled Damage: $\frac{dD(t)}{dt} = k_r(C_{int}(t) - D(t))$ Hazard: $h(t) = b_w \times max\,(0, D(t) - z)$ Variables: D = damage [ng/g] h = hazard rate [1/d] MW = molecular weight for conversion (i.e., 350.59 ng/nmol) 1000 is the conversion factor for pg to ng	k_r [1/d]	0.169	Damage repair/recovery constant
		b_w [g/pmol-d]	0.00047	Killing rate constant adjusted to the units as reflected by the scaled damage formulation (see Figure S5 simulation). The value for k_k in our simulations is 1.340 x 10-[4] g/ng-d (referenced to internal concentration). The value by Galic et al was divided by the chlorpyrifos molecular weight.
		z [-]	0.022	In Galic, et al. [14] the threshold parameter is unitless and hence not representative of the formulation in the GUTS model. To illustrate the impacts on hazard rates, this damage threshold was adjusted to 0.5 ng/g. This value (0.5ng/g) had no relation to the original parameter value in Galic et al., but just a mere representation of a threshold that can trigger the hazard linked to external exposure to the test chemical.

[a]The cumulative hazard rate was also calculated to visualize the contribution of each peak, see Figure S6. Corresponding SageMath codes to solve these equations are found at the end of this document. Judging by the magnitudes of the elimination and damage repair constants (Table S8), damage recovery rate constant (0.169 1/d) is lower than the elimination rate constant (0.5 1/d) suggesting that the damage dynamics may be more dominant than toxicokinetics. However, this part of the analysis was for illustration purposes only.

Table S9. Table of quality standards used to compare results of GUTS model (SD/IT). EQS = environmental quality standard; RAC = regulatory acceptable concentration; n.a. = not available

	EQS (ng/L) [a]		RAC (ng/L)	
Target Compound	**Acute**	**Chronic**	**Switzerland[b]**	**Germany[c]**
azoxystrobin	550	220	3300	550
chlorpyrifos	4.4	0.46	n.a.	0.45
chlorpyrifos-methyl	n.a.	n.a.	100	n.a.
diazinon	20	12	n.a.	n.a.
imidacloprid	100	13	n.a.	9
propiconazole	n.a.	n.a.	n.a.	2000
thiacloprid	80	10	n.a.	4

[a]Updated Swiss nationwide Water Protection Ordinance (SR 814.20, Annex 2, No. 11, Par. 3)
[b]Directly taken from Knauer [15]
[c]From Germany's Information System Ecotoxicology and Environmental Quality Targtet (ETOX). http://webetox.uba.de/webETOX/public/basics/literatur/download.do;jsessionid=23528A74579B92B6A6A74BFB6A209B85?id=528. Revision 04 UBA (26.06.2020)/BK

Supplemental Figures

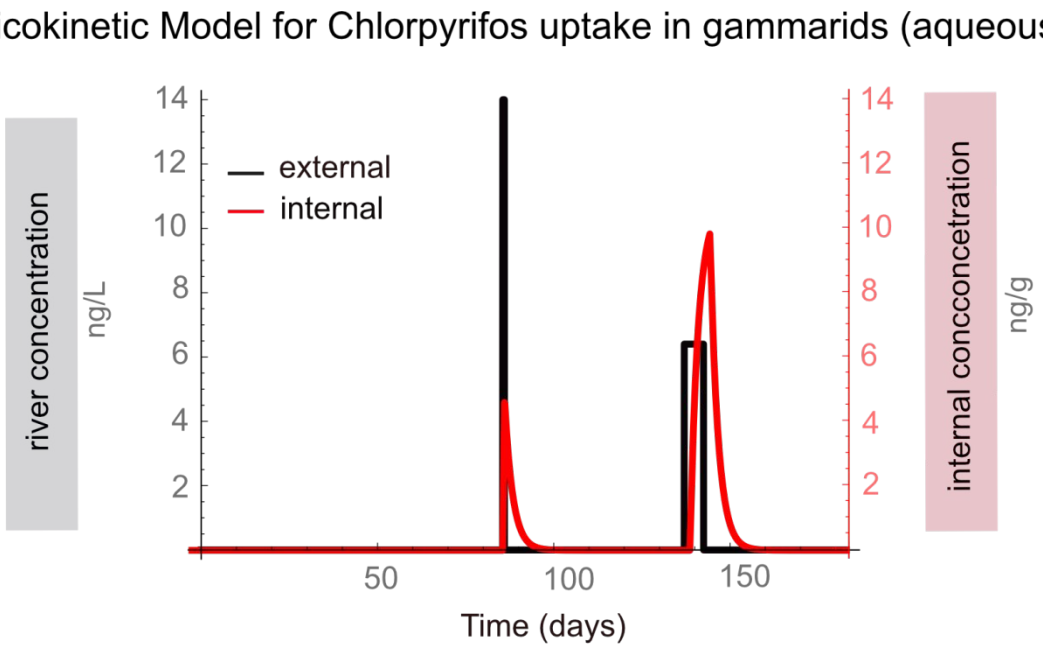


Figure S1. Predicted internal concentrations for chlorpyrifos using the first-order toxicokinetic (TK) model. C_{org} is the internal concentration in gammarids, k_{up} and k_{dep} are uptake and depuration rate constants, respectively (values take from Ashauer, et al. [1]), C_{river} is the river concentration. Based on TK alone, lower but longer duration exposure produced higher whole-body burden in gammarids than higher, short duration conditions. The impact of exposure duration and the magnitude of the environmental concentration is described further in Figure S2.

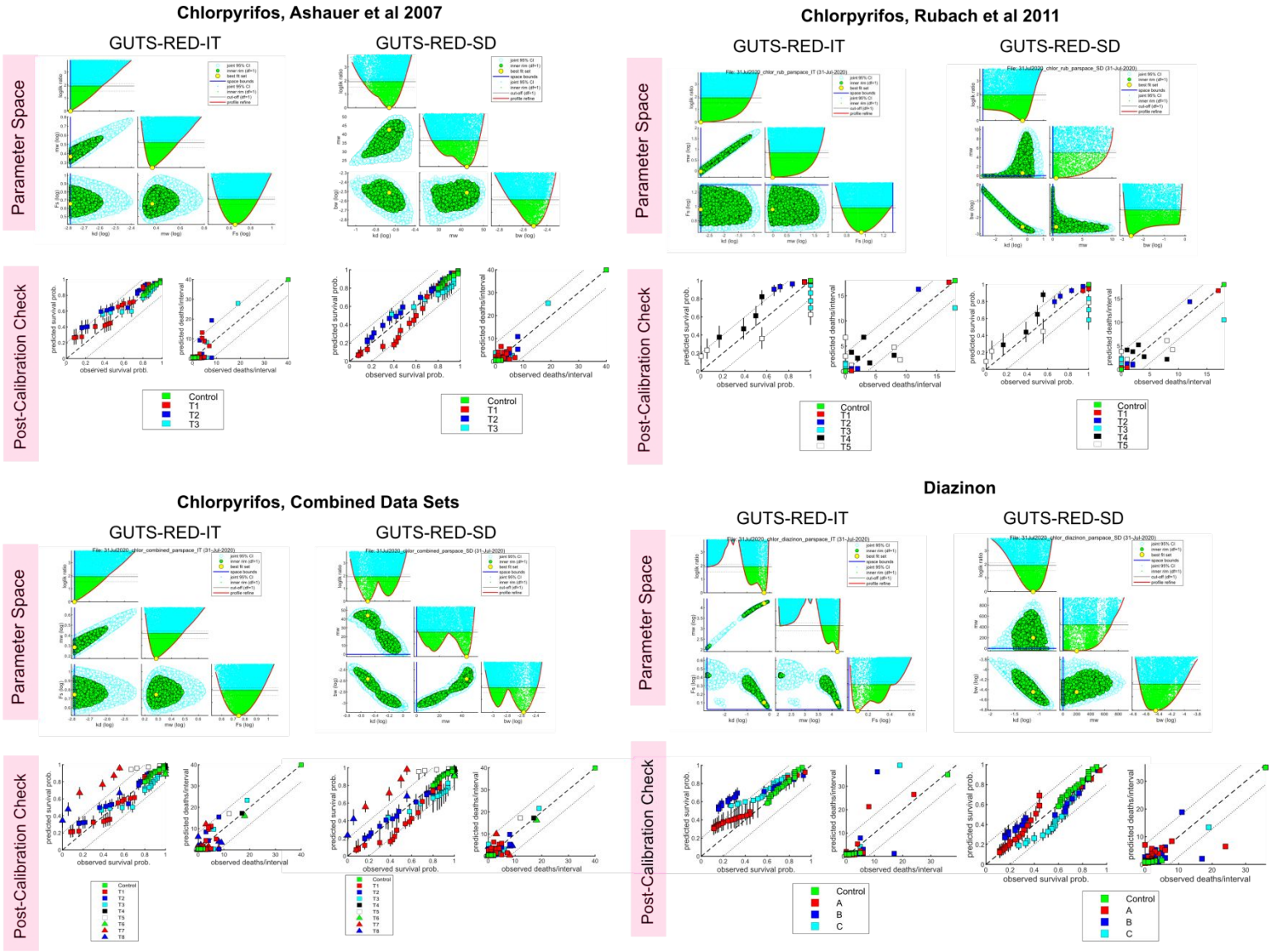


Figure S2. Calibration quality of openGUTS parameters. An optimal calibration has parameters with joint-likelihood profiles that are parabolic (profiles along the diagonal have reached a global minimum). As for the case of chlorpyrifos, the IT parameters seemed to be constrained better than SD but for diazinon, the SD was deemed to represent the survival better. EFSA further recommends that NRMSE should be <50% (Table S1) and SPPE (metric of under/overestimation) should also be within ±50% (See Table S2). There were no changes in our findings based the qualitative checks predicted-observed plot and numerical values of SPPE.

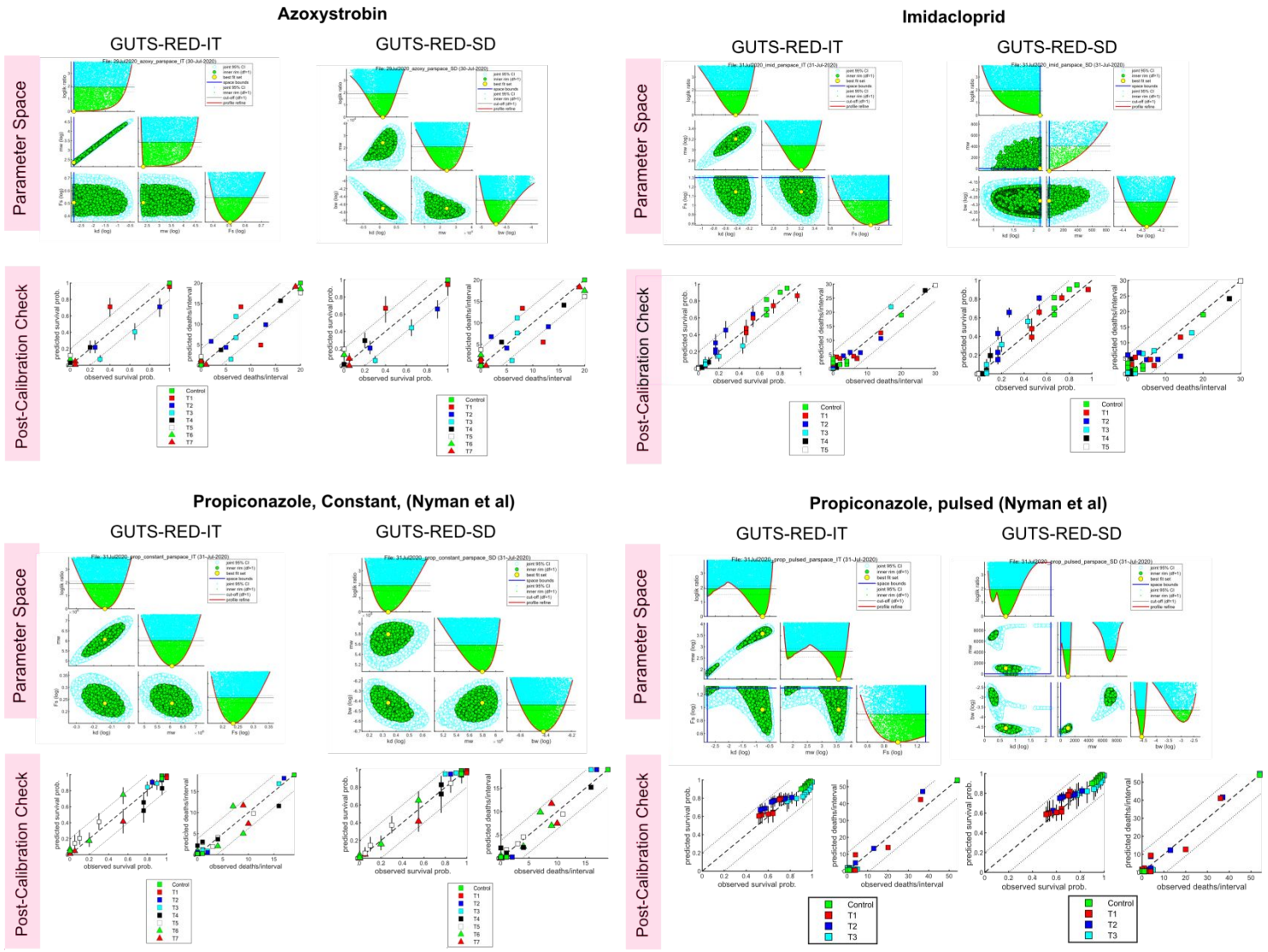


Figure S2 Cont'd. Calibration quality of openGUTS parameters. An optimal calibration has parameters with joint-likelihood profiles that are parabolic (profiles along the diagonal have reached a global minimum). As for the case of azoxystrobin survival better whereas for imidacloprid, IT parameters seemed better constrained than SD. For propiconazole (constant exposure), either models produce a very reasonable explanation of the survival data, and pulsed exposure calibration produced poor quality parameter space profiles EFSA further recommends that NRMSE should be <50% (Table S1) and SPPE (metric of under/overestimation) should also be within ±50% (See Table S2). There were no changes in our findings based the qualitative checks predicted-observed plot and numerical values of SPPE.

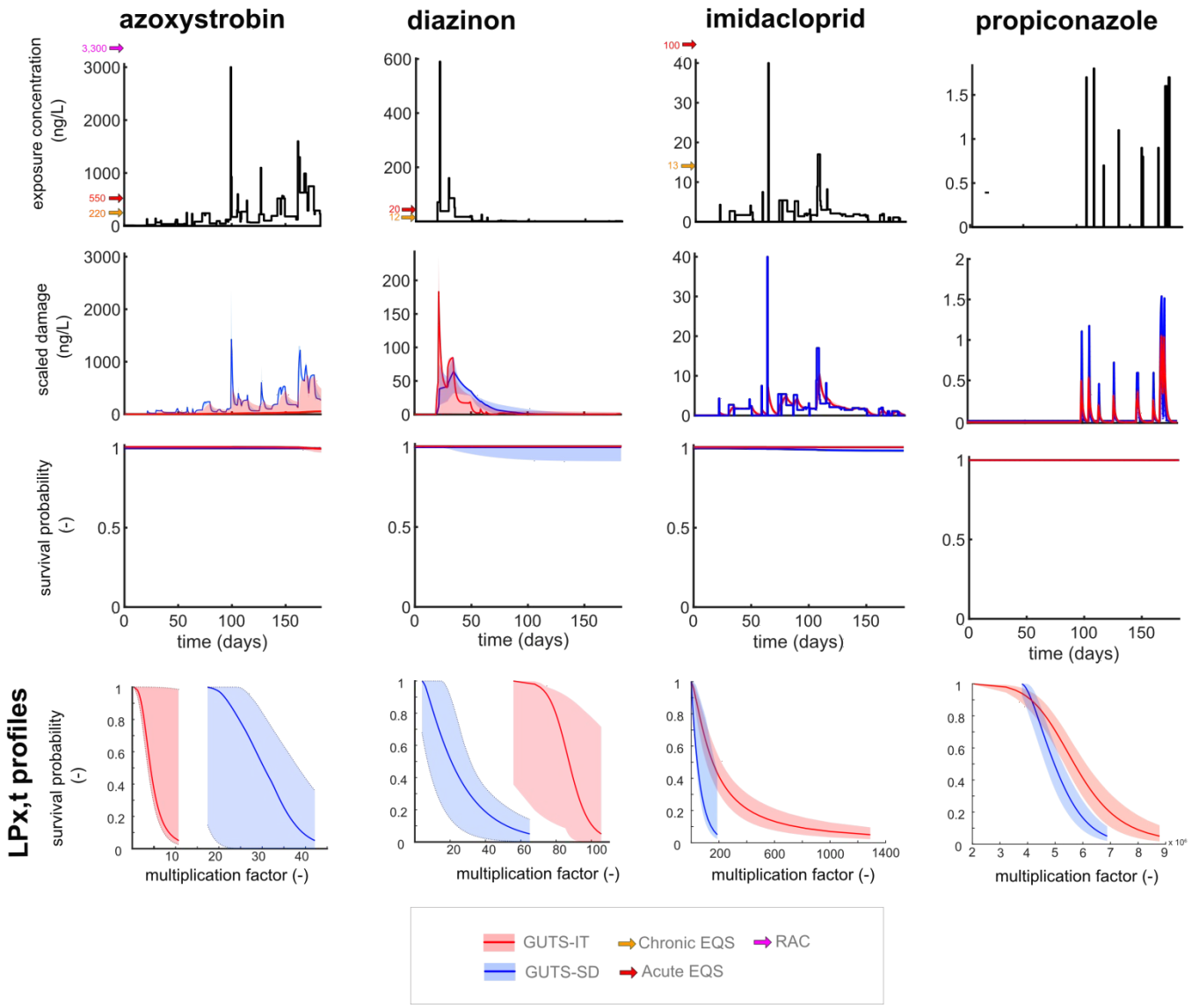


Figure S3. GUTS-RED-SD (blue) and GUTS-RED-IT (red) predictions for azoxystrobin, diazinon, imidacloprid and propiconazole. Survival predictions suggest that survival will be minimally impacted. However, LPx curves may suggest acute risk associated with imidacloprid exposure (under SD only). See Table S3 for the tabulated LP_{50} values.

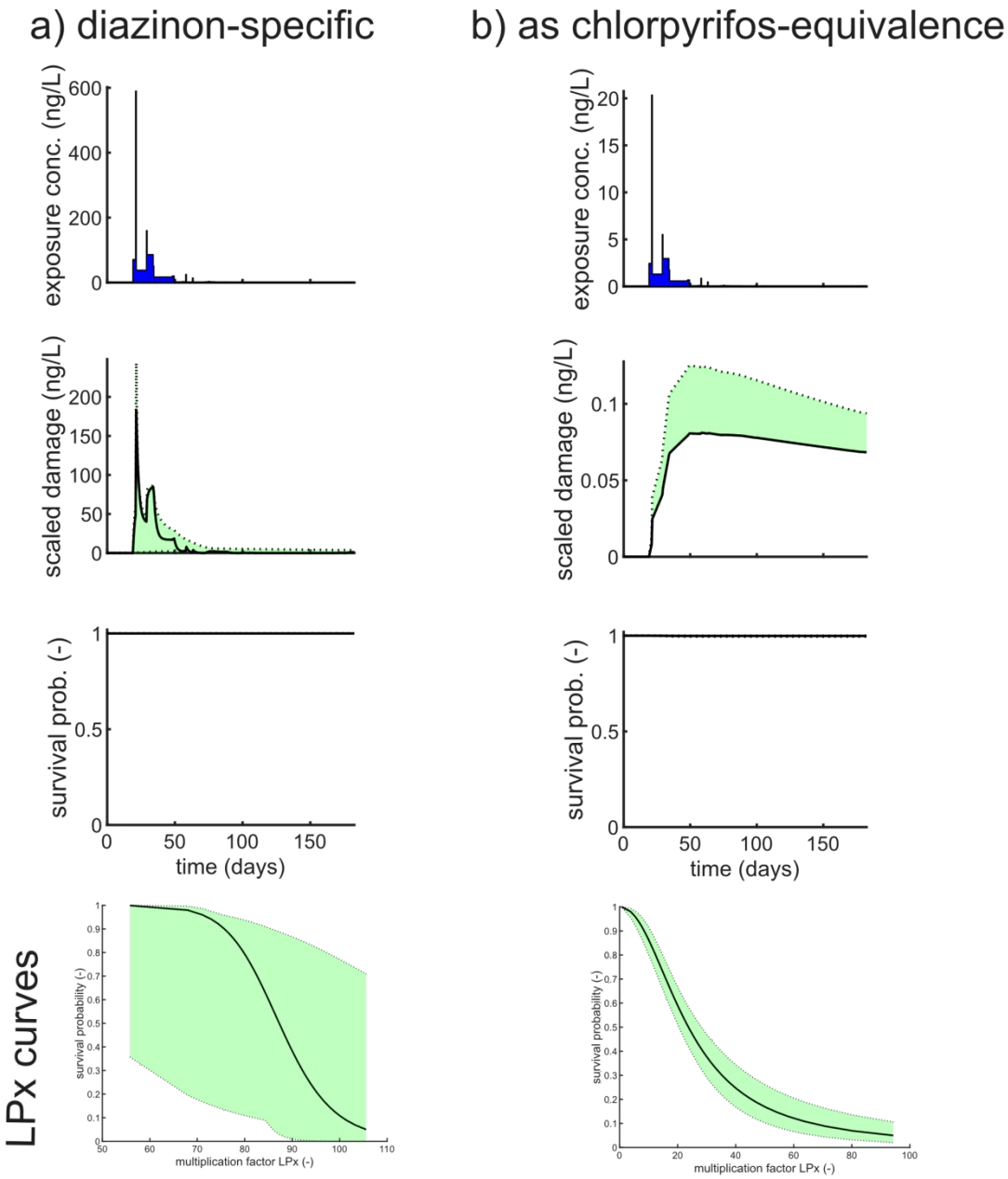


Figure S4. GUTS-RED-IT simulations using a) diazinon-specific parameters or b) when diazinon was expressed as chlorpyrifos-equivalence. The GUTS-RED-SD figure is shown in the main text (Figure 3). The equivalence factor (EF) employed for diazinon to calculate chlorpyrifos-equivalence is found in Table S2. There is a large difference in LP50 values (~4-fold difference). There is evidence in the literature that mortality due to diazinon follows the SD model more than IT [3], so caution must be exercised when interpreting the IT predictions.

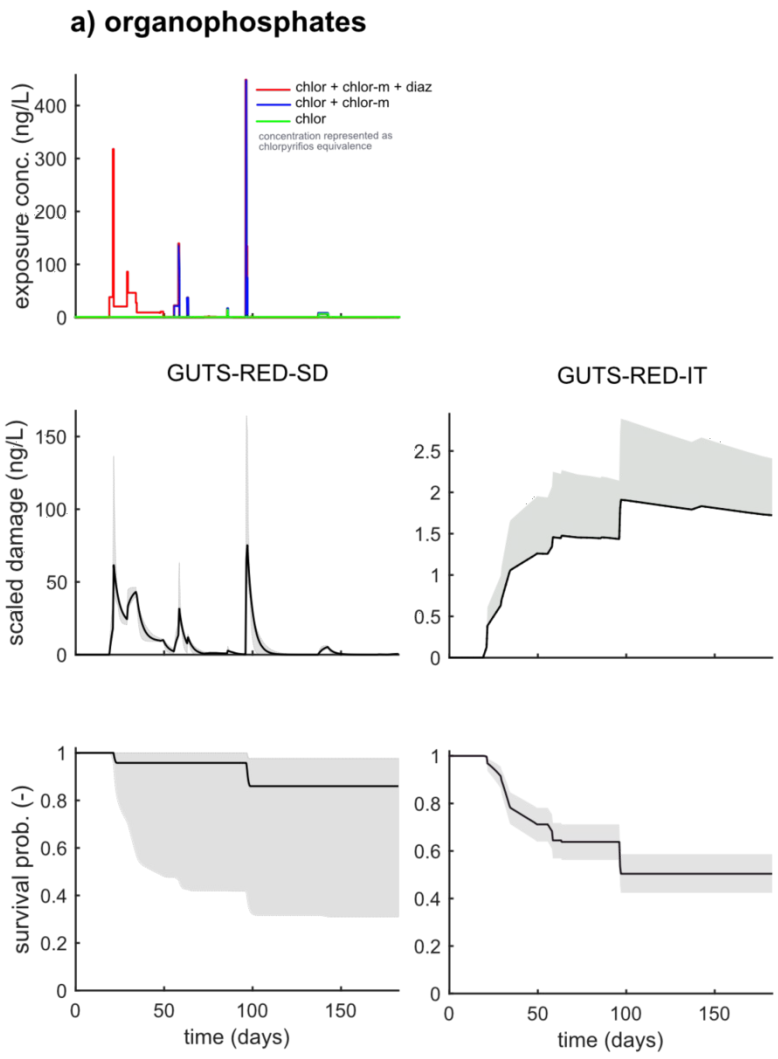


Figure S5. GUTS-RED simulations when the equivalence factors (EFs) were estimated using all arthropod EC50 data found in De Zwart [16]. Note that the survival predictions were more severe than what was illustrated in Figure 4a. See text for more detailed discussion/information.

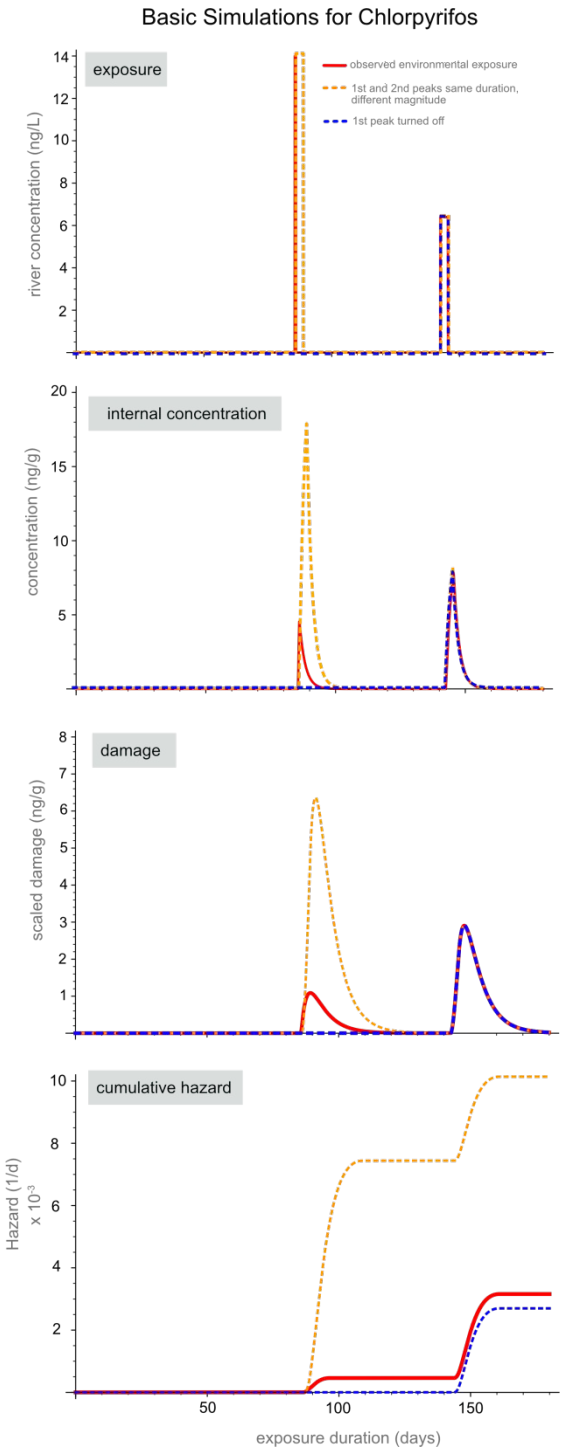


Figure S6. Scenario testing for assessing the impact of exposure duration and the magnitude of the concentrations on the whole-body burden on gammarids (internal concentration), internal damage (scaled), and hazard rate (calculated as cumulative). The curves were generated using the equations described in Table S4 (GUTS-FULL-SD). Note that for illustration purposes, the threshold value (z) used to calculate the hazard rate was changed so that the impact on hazard rate can be easily observed using different combinations of exposure duration and magnitude (threshold here is at 0.5 ng/g). As illustrated in this exercise (**in orange**), if the first peak in river concentration had the same duration as the second peak, then the damage is predicted to be higher in the former than in the latter. This first event also contributes mostly to the total hazard. However, if the first peak occurs at shorter duration than the second peak (**in red**) even though the first peak has higher magnitude), the hazard will be dominated by the second peak. For a proof-of-principle simulation, the absence of first peak will not contribute to the cumulative hazard (**in blue**). SageMath Codes to solve the equations (first case, in red) is provided below.

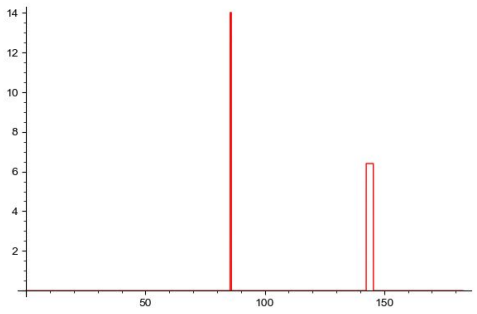
SageMath Codes

(Python-based programming language)

SAGEMATH CODES FOR SOLVING THE DIFFERENTIAL EQUATIONS described in Table S4.

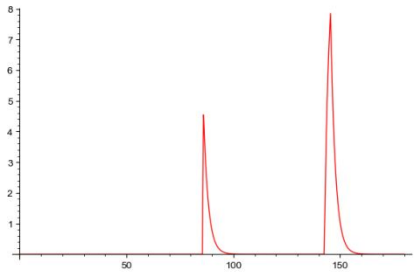
Page 1 of 3 Code (see representation in Figure S5 (in red))

```
#Import required Python-based open source software required for coding in SageMath
import csv
import numpy as np
import datetime
import matplotlib.pyplot as plt

#Plot of river concentation that was fed onto the TKTD model (y,axis ng/L, x-axis, d)
```

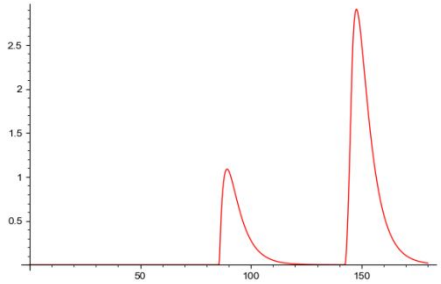



```
#call out the parameters related to the ordinary differential equations

c,D,ch,t=var('c D ch t')#c, internal concentration; D, scaled damage; ch, cumulative hazard; t, time

#parameters referenced from Galic et al ETC 33,2014
SD_param=[0.005,0.169,0.000047,0.022,1]
SD_param_units=['1/d','1/d','g/pmol/d','-','1/d']

############Definition of parameters###############
#kup=uptake rate constant
#kdep=depuration rate constant
#kbg=background mortality rate constant
#kr=damage repair, recovery rate constant (or dominant rate constant)
#kk=killing rate constant
#Z=threshold
#theta = proportionality constant
################################################

#Molecular weight
MW=350.59 #g/mol or ng/nmol

#Toxicokinetic rate constants
kup=747 #L/kg-d
kdep=0.45 #1/d

#define partitioning coefficient
Kiw=kup/kdep  #L/kg

#specify parameters from the SD_Param array
#most parameters have been adapted to the match the units and
#reflect the syntax provided in GUTS model.

#parameter values
kbg=SD_param[0]                                 #[1/d]
kr=SD_param[1]                                  #[1/d]
kk=(SD_param[2]/MW)*1000                        #[g/ng-d] ajusted here using Kiw to reflect the units
                                                #described in the GUTS model
                                                #(see Jager & Ashauer GUTS ebook)
Z=SD_param[3]                                   #[-]
Z_adapted=0.5                                   #[ng/L], adjusted the threshold to observe hazard
                                                #for illustration purposes only, since the value in
                                                #Galic et al. does not represent the GUTS formulation
                                                #i.e. the threshold is unitless
theta=SD_param[4]                               #[1/d] used in Galic et al but not in GUTS formulation
```

```
#Define your set of ODEs for GUTS modeling
#must have a C_RIVER function made up before running this

de1=kdep*((C_RIVER*Kiw*MULT/1000)-(c))
de2=kr*c-kr*D  #solving for scaled damage

#a 3rd differential equation is added here to solve cumulative hazard
#a non-cumulative hazard rate is described simply as: h(t)=kk_adapted*max_symbolic(((D/Kiw)*1000)-(Z_adapted),0)

de3=kk*max_symbolic(D-Z_adapted,0) #solving for cumulative hazard

#calculate system of ODEs using Rungga-Kutta
#simulation starts at 0d then ends after 180 days

solution=desolve_system_rk4([de1,de2,de3],[c,D,cH],ics=[0,0,0,0],ivar=t,end_points=[0,180])


#extract the values from the solution
cint=[ [i,j] for i,j,k,l in solution]
scaled_damage=[ [i,k] for i,j,k,l in solution]
cum_hazard=[ [i,l] for i,j,k,l in solution]

#Visualize Results
# internal concetration

c_int_plot=list_plot(cint,plotjoined=True, aspect_ratio='automatic',color='red')
os.chdir(r'C:\Users\arlos\Documents\Arlos_Maricor\Codes\TKTD\output\plots')
c_int_plot.save('exposure1_intconc.pdf')
c_int_plot
```

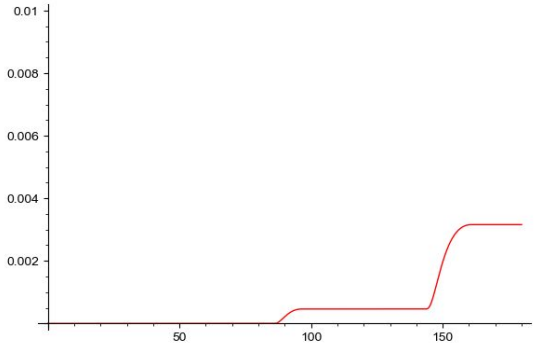



```
#yaxis - ng/g, x-axis, d


#Visualize Results
#scaled damage

scaled_damage_plot=list_plot(scaled_damage,plotjoined=True, aspect_ratio='automatic',color='red')
scaled_damage_plot.save('exposure1_scaled_dam.pdf')
scaled_damage_plot
```



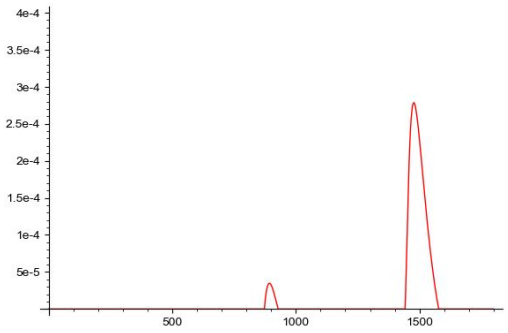

```
#yaxis = ng/g , x-axis = d
```

```
#Visualize Results
#cumulative hazard rate

cum_hazard_plot=list_plot(cum_hazard,plotjoined=True, aspect_ratio='automatic',color='red')
cum_hazard_plot.save('exposure1_cum_hazard.pdf')
cum_hazard_plot
```



y-axis, 1/d; x-axis (days)

```
#non-cumulative hazard rate

scaled_damage_calc=[ k for i,j,k,l in solution]
hazard_rate=np.zeros([len(scaled_damage_calc)],dtype=float)

for i in range (len(scaled_damage_calc)):
    hazard_rate[i]=kk_adapted*max_symbolic((scaled_damage_calc[i]/Kiw)*1000-Z_adapted,0)

hazard_rate_plot=list_plot(hazard_rate,plotjoined=True, aspect_ratio='automatic',color='red',ymin=0,ymax=0.0004)
hazard_rate_plot.save('TG_chlor_hazardrate_galic.pdf')
hazard_rate_plot
```



y-axis, 1/d, x-axis (time)

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