

1 **SUPPLEMENTARY DATA**

2 **Title:** Variation of tolerance to isothiazolinones among *Daphnia pulex* clones

3

4 *Study clones: populations of origin*

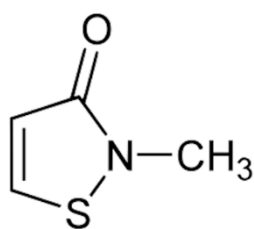
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6 **Table S1.** Locations of the *D. pulex* study populations of origin of the study's clonal lineages

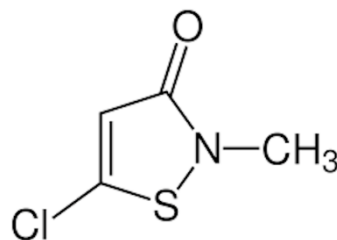
Clonal line		Population origin			Isolation date
Name	Code	Type	Location		
Séné 2	SE2	Natural population	Séné	47.616636, -2.713638	10/2019
Séné 5	SE5	Natural population	Séné	47.616636, -2.713638	10/2019
Pearl 7	PE7	Aquaculture tanks	Rennes	48.112800, -1.709124	10/2019
Pearl 16	P16	Aquaculture tanks	Rennes	48.112800, -1.709124	10/2019
Rennes	RE0	Aquaculture tanks	Rennes	48.112800, -1.709124	12/2016
Goven 6	GO6	Natural population	Goven	47.994867, -1.859119	10/2019
Lassalle	LA0	Natural population	Saint-Judoce	48.359723, -1.951767	11/2019
Alsace	AL0	Commercial strain (Aqualiment©)	-	-	bought in 2016

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8



Methylisothiazolinone



Chloromethylisothiazolinone

10
11 **Figure S1.** Formulas of 2-méthylisothiazol-3(2H)-one (CAS n°2682-20-4) and 5-Chloro-2-methyl-1,2-
12 thiazol-3(2H)-one (CAS n°26172-55-4)

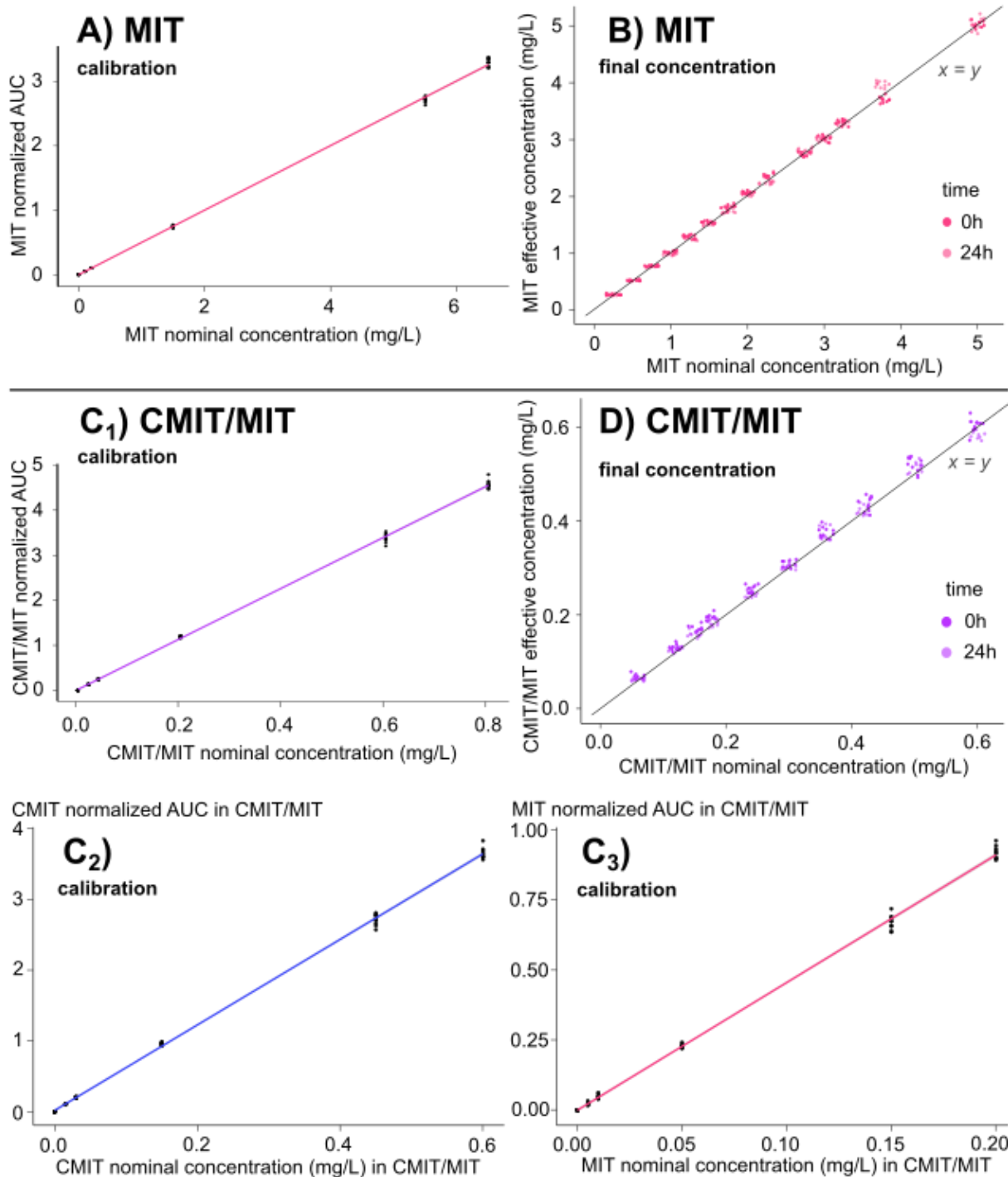
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14 Calibration curves were obtained from standards at the following concentrations: (1) 0.02, 0.04, 0.2,
15 0.6 and 0.8 mg/L and 0.2 mg/L of the internal standard solution for the mixture of CMIT and MIT and
16 at (2) 0.1, 0.2, 1.5, 5.5 and 6.5 mg/L and 1.5 mg/L of the internal standard solution for the MIT alone.
17 The concentrations reported for the mixture are the cumulated concentrations of both CMIT and MIT
18 (3:1 ratio). Each solution and blanks were injected ten times.

19 Using quantification transitions, CMIT and MIT areas were normalized by the I.S. area for each
20 injection (calibration and bioassay samples). Calibration curves (Figure 1) were generated with R
21 (version 4.0.0) as linear regression with generalized least squares to correct for heteroscedasticity of
22 residuals.

23
24 **Table S2.** Mass transitions and retention times for the target compounds in UPLC-MS/MS. I.S.,
25 internal standard.

Compound	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy (eV)	Retention time (min)
MIT	116.0170	100.9935	28	0.3 – 0.4
CMIT	149.9790	134.9546	28	1.1 – 1.2
MIT-D3 (I.S.)	119.0360	100.9935	28	0.3 – 0.4

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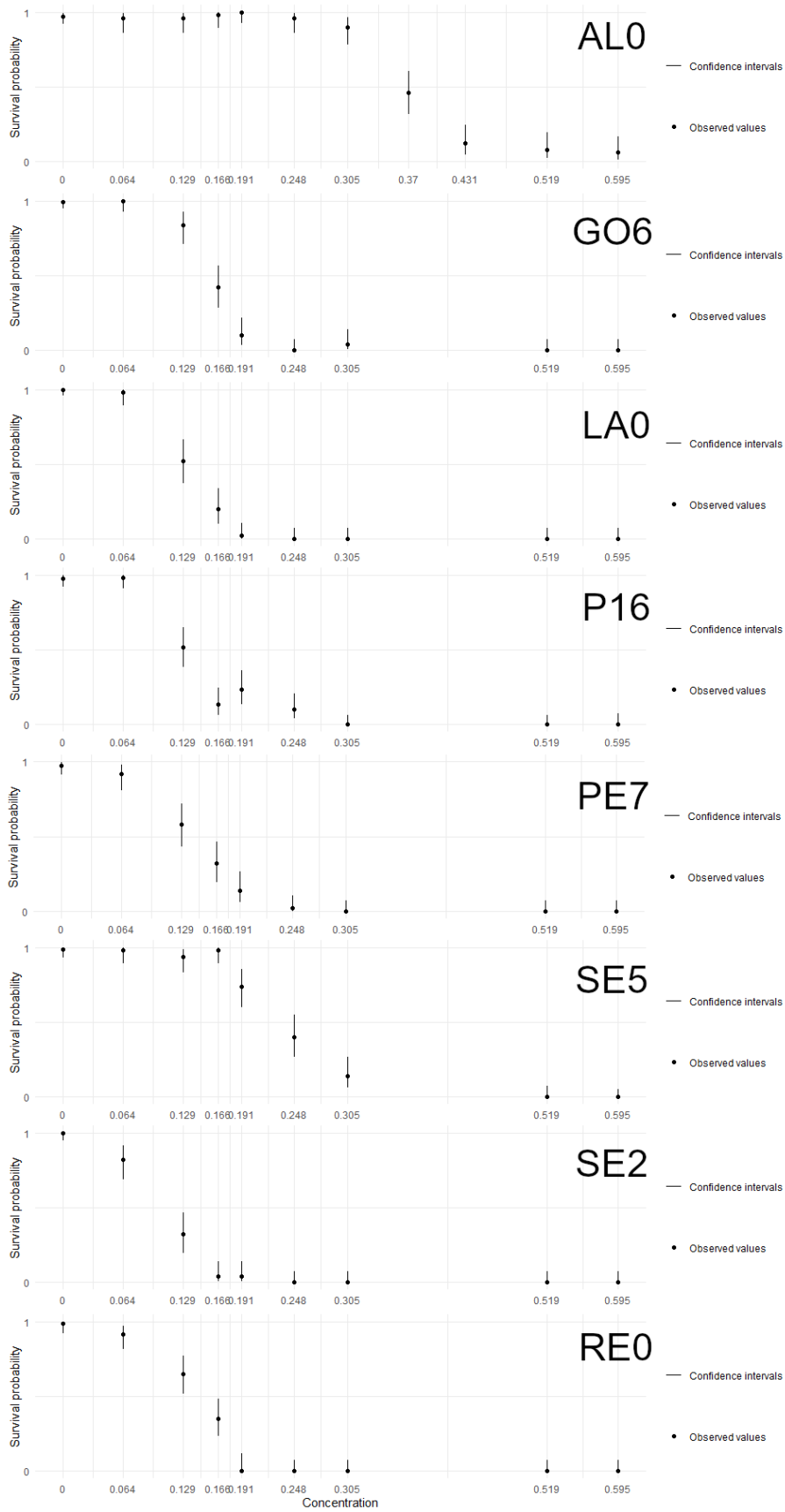
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 28 **Figure S2.** Measures of isothiazolinones concentration in water contaminated with MIT (A and B) or
 29 CMIT/MIT (C₁₋₂₋₃ and D), with 10 technical replicates. In calibration curves (A and C), the areas under
 30 the curve (AUC) are normalized by the internal standard area. For CMIT/MIT samples, calibration
 31 curves show CMIT (C₂, adjusted R² = 0.999, p-value < 0.001) and MIT (C₃, adjusted R² = 0.998, p-value
 32 < 0.001) AUC separately, and the mixture CMIT/MIT (C₁, adjusted R² = 0.999, p-value < 0.001) as
 33 derived from the single molecules' AUC. Based on calibration curves, the bioassay samples' effective
 34 concentrations are plotted against nominal concentrations for MIT samples (B) and CMIT/MIT
 35 samples (D), showing strong linear relationship in both cases (MIT: R² = 0.998, p-value < 0.001;
 36 CMIT/MIT: R² = 0.995, p-value < 0.001). Effective concentrations in samples are displayed at 0 and
 37 24h after contamination and are reported in supplemental material (table S3).
 38

39 **Table S3.** CMIT and MIT concentrations measured with analysis by UPLC-MS/MS (mg/L) and
 40 degradation over 24h. CMIT+MIT concentration was computed by adding CMIT and MIT measures in
 41 mixture.

	Nominal concentration (mg/L)	Effective median concentration in mg/L (SD)	Effective median concentration in mg/L (SD) at 0h	Effective median concentration in mg/L (SD) at 24h	Percentage decrease in concentration over 24h
CMIT+MIT in mixture	0.06	0.064 (0.005)	0.067 (0.005)	0.062 (0.002)	7.5 %
	0.12	0.129 (0.006)	0.131 (0.006)	0.126 (0.004)	3.8 %
	0.15	0.166 (0.009)	0.170 (0.006)	0.158 (0.006)	7.1 %
	0.18	0.191 (0.009)	0.195 (0.006)	0.182 (0.007)	6.7 %
	0.24	0.248 (0.009)	0.253 (0.008)	0.244 (0.007)	3.6 %
	0.30	0.305 (0.007)	0.311 (0.007)	0.303 (0.007)	2.6 %
	0.36	0.370 (0.012)	0.376 (0.011)	0.366 (0.014)	2.7 %
	0.42	0.431 (0.015)	0.437 (0.016)	0.423 (0.014)	3.2 %
	0.50	0.519 (0.014)	0.52 (0.016)	0.519 (0.012)	0.2 %
	0.60	0.595 (0.016)	0.608 (0.015)	0.585 (0.012)	3.8 %
CMIT in mixture	0.045	0.047 (0.004)	0.049 (0.005)	0.045 (0.002)	8.2 %
	0.090	0.093 (0.004)	0.094 (0.005)	0.091 (0.004)	3.2 %
	0.112	0.118 (0.006)	0.122 (0.005)	0.114 (0.005)	6.6 %
	0.135	0.137 (0.007)	0.139 (0.005)	0.130 (0.005)	6.5 %
	0.180	0.177 (0.007)	0.180 (0.007)	0.175 (0.006)	2.8 %
	0.225	0.219 (0.007)	0.222 (0.006)	0.217 (0.007)	2.3 %
	0.270	0.265 (0.007)	0.269 (0.01)	0.261 (0.012)	3.0 %
	0.315	0.305 (0.012)	0.308 (0.012)	0.302 (0.012)	1.9 %
	0.375	0.370 (0.011)	0.369 (0.012)	0.371 (0.009)	-0.5 %
	0.450	0.424 (0.013)	0.430 (0.013)	0.416 (0.009)	3.3 %
MIT in mixture	0.015	0.019 (0.001)	0.019 (0.001)	0.018 (0.001)	5.3 %
	0.030	0.037 (0.002)	0.039 (0.002)	0.036 (0.001)	7.7 %
	0.038	0.048 (0.003)	0.051 (0.002)	0.047 (0.002)	7.8 %
	0.045	0.055 (0.003)	0.058 (0.002)	0.054 (0.003)	6.9 %
	0.060	0.075 (0.003)	0.076 (0.002)	0.072 (0.002)	5.3 %
	0.075	0.092 (0.002)	0.092 (0.002)	0.090 (0.002)	2.2 %
	0.090	0.112 (0.003)	0.112 (0.003)	0.112 (0.003)	0.0 %
	0.105	0.129 (0.004)	0.132 (0.004)	0.127 (0.002)	3.8 %
	0.125	0.155 (0.004)	0.156 (0.004)	0.154 (0.004)	1.3 %
	0.150	0.179 (0.006)	0.181 (0.006)	0.176 (0.004)	2.8 %
MIT	0.25	0.258 (0.110)	0.260 (0.011)	0.255 (0.011)	1.9 %
	0.5	0.507 (0.009)	0.505 (0.009)	0.511 (0.007)	-1.2 %
	0.75	0.764 (0.100)	0.767 (0.011)	0.759 (0.009)	1.0 %
	1.0	0.993 (0.027)	1.012 (0.012)	0.969 (0.015)	4.2 %
	1.25	1.255 (0.034)	1.291 (0.018)	1.233 (0.012)	4.5 %
	1.5	1.520 (0.026)	1.531 (0.016)	1.495 (0.025)	2.4 %
	1.75	1.762 (0.055)	1.801 (0.026)	1.707 (0.021)	5.2 %
	2.0	2.038 (0.026)	2.054 (0.022)	2.030 (0.024)	1.2 %
	2.25	2.305 (0.069)	2.341 (0.030)	2.226 (0.031)	4.9 %
	2.75	2.759 (0.048)	2.715 (0.034)	2.780 (0.044)	-2.4 %
	3.0	3.016 (0.041)	2.996 (0.048)	3.021 (0.033)	-0.8 %
	3.25	3.283 (0.040)	3.296 (0.048)	3.278 (0.033)	0.5 %
	3.75	3.827 (0.146)	3.685 (0.050)	3.955 (0.051)	-7.3 %
	5.0	4.996 (0.085)	4.996 (0.049)	4.996 (0.113)	0 %

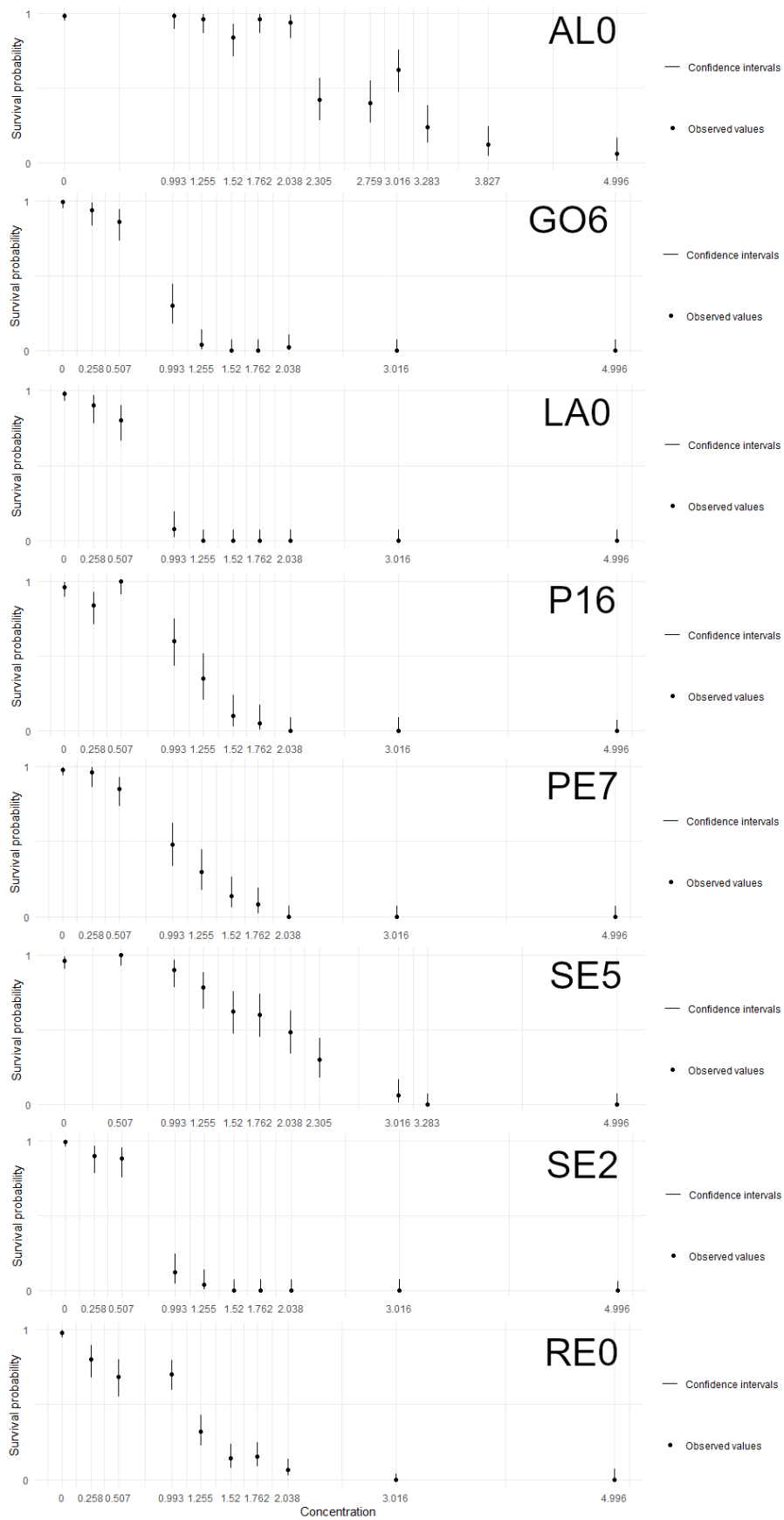
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Figure S3. Survival rate of each clonal line for the CMIT/MIT assay at 48h as a function of concentration, with binomial confidence intervals around the data (n=50).



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49 **Figure S4.** Survival rate of each clonal line for the MIT assay at 48h as a function of concentration,
 50 with binomial confidence intervals around the data (n=50).

51 *Modeling*

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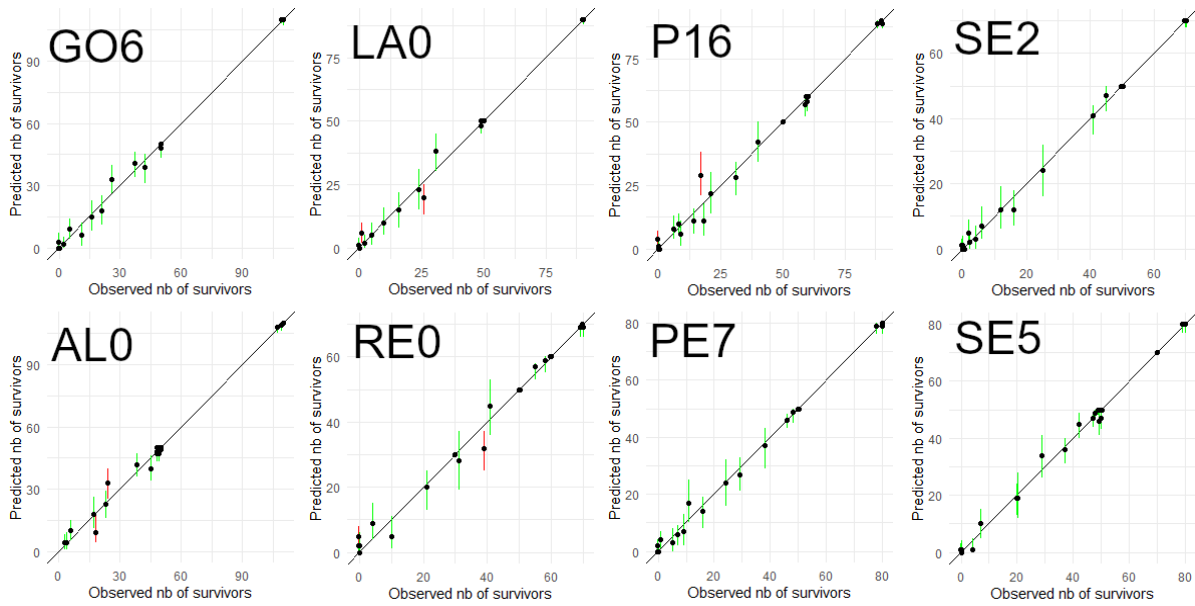
53 **Table S4.** GUTS models parameters estimates for the 8 clonal lineages, median and 95% credible
 54 interval. With general GUTS parameters: h_b as the background mortality, and k_D as the dominant rate
 55 constant, that is to the slowest compensating process dominating the overall dynamics of toxicity.
 56 Parameters specific of IT models are related to the individual threshold concentration which is
 57 described as a log-logistic probability distribution with m_w the median and β the shape of the
 58 distribution

Clone	CMIT + MIT				MIT			
	k_D	h_b (e-02)	m_w	β	k_D	h_b (e-02)	m_w	β
Alsace	1.25 [1.01-1.51]	1.09 [0.49-2.06]	0.34 [0.31-0.37]	6.62 [5.52-7.85]	0.98 [0.72-1.25]	0.81 [0.23-1.92]	2.44 [2.10-2.71]	3.32 [2.77-3.92]
Goven6	0.93 [0.68-1.18]	0.37 [0.05-1.29]	0.13 [0.11-0.15]	5.82 [4.85-6.95]	1.53 [1.12-2.02]	1.22 [0.28-2.97]	0.74 [0.63-0.86]	5.38 [4.24-7.07]
Lassalle	1.31 [0.98-1.67]	0.25 [0.02-1.31]	0.12 [0.10-0.13]	5.17 [4.18-6.36]	0.75 [0.46-1.10]	2.64 [1.00-5.03]	0.52 [0.37-0.66]	5.62 [4.39-7.13]
Rennes	1.82 [1.41-2.28]	1.32 [0.19-3.74]	0.14 [0.12-0.15]	4.89 [3.82-6.33]	0.94 [0.60-1.29]	2.52 [0.81-5.24]	0.81 [0.58-1.02]	2.58 [2.03-3.47]
Sene2	1.23 [0.87-1.63]	0.35 [0.02-2.17]	0.09 [0.07-0.10]	4.22 [3.46-5.19]	0.97 [0.65-1.36]	1.70 [0.67-3.36]	0.63 [0.49-0.76]	6.66 [5.32-8.46]
Sene5	1.69 [1.35-2.07]	0.74 [0.15-2.04]	0.23 [0.21-0.25]	5.71 [4.69-6.92]	1.51 [1.22-1.82]	1.62 [0.60-3.38]	1.75 [1.60-1.89]	4.66 [3.92-5.54]
Pearl16	1.28 [0.97-1.62]	0.76 [0.17-2.21]	0.12 [0.10-0.13]	3.64 [3.05-4.31]	0.66 [0.39-0.94]	3.35 [1.72-5.71]	0.79 [0.54-0.98]	4.86 [3.90-6.00]
Pearl7	1.46 [1.08-1.85]	1.31 [0.28-3.46]	0.13 [0.11-0.14]	4.27 [3.43-5.36]	0.62 [0.36-0.91]	1.15 [0.35-2.64]	0.66 [0.44-0.84]	3.59 [3.01-4.31]

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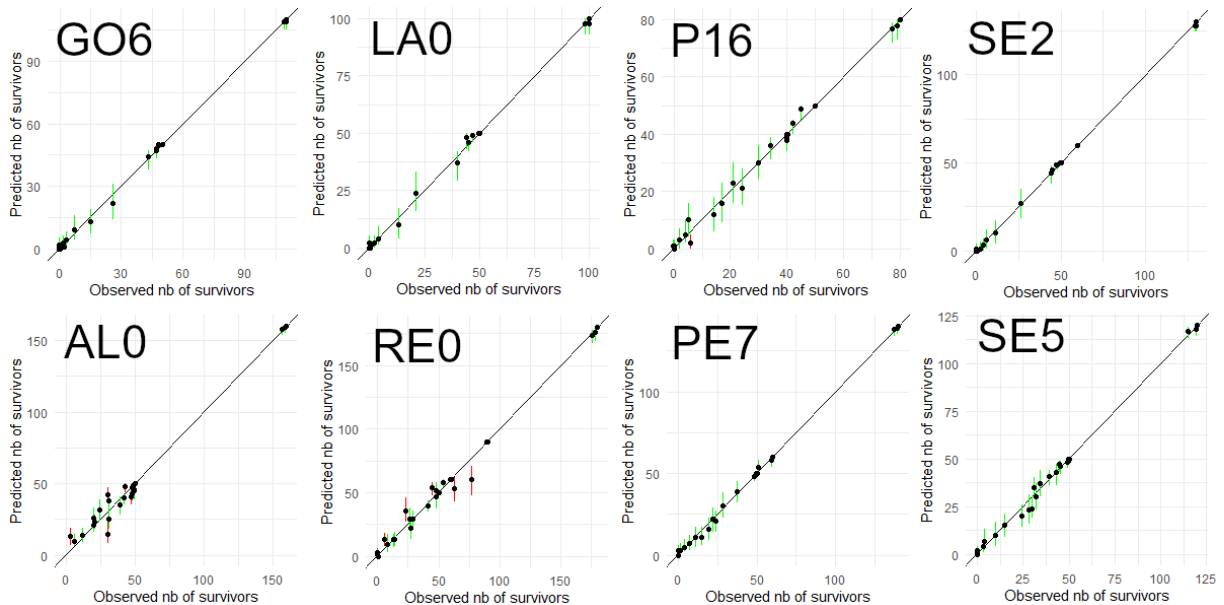
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63 **Figure S5.** Posterior predictive checks of GUTS-RED-IT models for the eight clonal lineages for
64 CMIT/MIT assay. The dots represent the median values of the predicted number of survivors and
65 segments are the 95% credible intervals, and the x-axis correspond to the observed values. The
66 segments are green when the observed value falls in the 95% credible interval and red otherwise.

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69 **Figure S6.** Posterior predictive checks of GUTS-RED-IT models for the eight clonal lineages for MIT
70 assay. The dots represent the median values of the predicted number of survivors and segments are
71 the 95% credible intervals, and the x-axis correspond to the observed values. The segments are green
72 when the observed value falls in the 95% credible interval and red otherwise.

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78 *Time-target model*

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80 Source: morse modelling vignette (<https://CRAN.R-project.org/package=morse>)

81 The number N_t of surviving organisms at time t follows a binomial distribution:

$$N_t \sim B(n_i^{init}, f(c_i))$$

82 With n_i^{init} as the initial number of organisms and c the contaminant level, and f the three parameters
83 log-logistic function:

$$f(c) = \frac{d}{1 + \left(\frac{c}{e}\right)^b}$$

84 Where b relates to the effect intensity of the contaminant and so to the steepness of the slope in the
85 dose-response curve, d is the survival rate in absence of contaminant, and e is the LC_{50}

86

87 **Table S5.** Log-logistic models parameters estimates at 48h for the 8 clonal lineages, median and 95%
88 credible interval. The parameters describe as follow: b relates to the effect intensity of the
89 contaminant and so to the steepness of the slope in the dose-response curve, d is the survival rate in
90 absence of contaminant, and e is the LC_{50} .

Clone	CMIT + MIT			MIT		
	b	d	e	b	d	e
Alsace	8.69 [6.92-10.83]	0.97 [0.95-0.99]	0.37 [0.36-0.39]	4.71 [3.87-5.74]	0.98 [0.95-0.99]	2.73 [2.59-2.88]
Goven6	8.16 [6.23-10.54]	0.99 [0.97-1.00]	0.157 [0.148-0.164]	5.48 [4.10-8.61]	0.97 [0.93-0.99]	0.79 [0.70-0.92]
Lassalle	7.09 [5.34-9.34]	-	0.13 [0.12-0.14]	6.84 [5.02-16.54]	0.95 [0.90-0.98]	0.66 [0.59-0.86]
Rennes	8.11 [5.72-10.98]	0.95 [0.90-0.98]	0.146 [0.137-0.154]	4.33 [2.79-5.81]	0.90 [0.86-0.95]	1.14 [0.96-1.24]
Sene2	4.45 [3.54-5.51]	-	0.10 [0.09-0.11]	6.63 [4.85-10.93]	0.96 [0.93-0.99]	0.74 [0.65-0.86]
Sene5	7.36 [5.71-9.51]	0.98 [0.95-0.99]	0.24 [0.22-0.25]	4.81 [3.91-6.01]	0.96 [0.92-0.98]	1.86 [1.74-1.98]
Pearl16	4.68 [3.72-5.87]	0.98 [0.94-0.99]	0.13 [0.12-0.14]	6.76 [5.01-8.99]	0.93 [0.88-0.96]	1.12 [1.03-1.20]
Pearl7	5.86 [4.22-7.93]	0.95 [0.91-0.99]	0.14 [0.13-0.15]	4.01 [3.12-5.38]	0.97 [0.93-0.99]	0.96 [0.85-1.07]

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93 **Table S6.** Fixed-effects coefficients and variance estimates of random-effects for survival modelled
 94 as a function of line-specific response to dosage.

CMIT/MIT				
Model Coeff.	Est.	PDI_{0.025}	PDI_{0.975}	pMCMC
β_0 ALS	6.33	5.58	7.17	0.001
β_0 GOV	8.37	6.60	10.11	0.001
β_0 LAS	8.60	6.74	10.53	0.001
β_0 PEA	4.92	2.91	6.98	0.032
β_0 REN	5.82	3.91	7.73	0.386
β_0 SEN2	5.93	3.99	7.88	0.522
β_0 SEN5	6.83	5.03	8.99	0.426
β_0 U3E7	6.62	4.60	8.63	0.674
β_1 ALS	-16.35	-19.35	-13.90	0.001
β_1 GOV	-55.73	-62.00	-49.75	0.001
β_1 LAS	-70.78	-78.60	-62.43	0.001
β_1 PEA	-37.72	-45.93	-30.14	0.001
β_1 REN	-46.30	-53.42	-40.15	0.001
β_1 SEN2	-58.99	-65.60	-52.79	0.001
β_1 SEN5	-29.70	-36.95	-22.72	0.001
β_1 U3E7	-53.48	-60.35	-46.78	0.001
Var. Comp.	Var	Var_{0.025}	Var_{0.975}	
Var{ β_0 Rep Line}	1.7370	1.3870	2.1330	
Var{residual}	0.0592	0.0003	0.2070	
MIT				
Model Coeff.	Est.	PDI_{0.025}	PDI_{0.975}	pMCMC
β_0 ALS	5.41	4.33	6.34	0.001
β_0 GOV	5.07	2.56	7.39	0.612
β_0 LAS	4.13	1.92	6.26	0.046
β_0 PEA	4.41	1.91	6.58	0.122
β_0 REN	4.02	1.78	6.03	0.016
β_0 SEN2	4.20	1.62	6.63	0.150
β_0 SEN5	5.05	2.77	7.19	0.560
β_0 U3E7	4.72	2.48	7.13	0.356
β_1 ALS	-1.90	-2.33	-1.55	0.001
β_1 GOV	-6.51	-8.22	-5.44	0.001
β_1 LAS	-6.50	-8.35	-5.41	0.001
β_1 PEA	-4.58	-5.77	-3.53	0.001
β_1 REN	-3.95	-4.83	-3.09	0.001
β_1 SEN2	-5.88	-8.05	-4.28	0.001
β_1 SEN5	-2.86	-3.86	-2.00	0.004
β_1 U3E7	-4.73	-6.19	-3.80	0.001
Var. Comp.	Var	Var_{0.025}	Var_{0.975}	
Var{ β_0 Rep Line}	2.0370	1.4790	2.7080	
Var{residual}	0.0509	0.0013	0.3011	

