JOURNAL OF LIMNOLOGY

DOI: 10.4081/jlimnol.2021.2015

SUPPLEMENTARY MATERIAL

Behavioural responses of juvenile *Daphnia magna* to two organophosphorus insecticides

Valeria Di Nica,[1] Cristiana Rizzi,[1]* Antonio Finizio,[1] Lorenzo Ferraro,[2] Sara Villa[1]

[1]Department of Earth and Environmental Sciences, University of Milano Bicocca, Piazza della Scienza 1, 20126 Milano

[2]Department of Materials Science, University of Milano Bicocca, Via Roberto Cozzi 55, 20125 Milano, Italy

***Corresponding author:** cristiana.rizzi@unimib.it

Key words: *Daphnia magna,* behaviour, chlorpyrifos, chlorpyrifos-methyl, video-tracking

a) Paragraph S1 and Table S1: Chemical analysis of test solutions

Exposure water of the higher and lower tested solutions were analysed to check nominal concentrations at the beginning of the test (t = 0) and before renewals (after 24h). Samples were extracted on-site using Oasis HLB cartridges (Waters, 500 mg, 6 mL). The Oasis cartridges were eluted with 15 mL of n-hexane, 10 mL of n-hexane: methylene chloride (30:70), and 6 mL of ethyl acetate. The eluted were evaporated to 0.5 mL and transferred into analysis vials.

Identification and quantification were performed by the MSD 5977B equipped with GC 8860 (Agilent Technologies, CA, USA) in selected ion monitoring (SIM) mode. Separations were achieved by a GC-column HP-MS5, 30 m, 0.25mm, 0.25 µm (Agilent Technologies) with a 1.5 mL/min of carrier gas flow (He). Before injections, the Agilent 7693A Automatic Liquid Sampler added 0.2 µL of internal standard (atrazine-d5) to each calibration level and samples.

Details of the injector and oven program for the analysis are:

Injector: Splitless, temperature 250 °C, pressure 12.9 psi, total flow 22.9 mL/min.

Oven: 100 °C hold for 1 min, 20 °C/min to 150 °C, 5 °C/min to 200 °C, 40 °C/min to 280 °C, hold for 4,5 min. Total run time 20 min.

The MS settings, the linearity of detector response tested in the range from 0.1 ng/mL to 50 ng/mL, LOD and LOQ are reported in Table S1.

The method was developed and validated according to SANTE 11813/2017 guidelines, the recovery of each analyte has been quantified and the average recovery falls within the recommended range of 70-120% .

Table S1. MS settings (m/z of both quantifier and qualifier ions), Retention Time (RT), LOQ, LOD and linearity of each selected compound. The parameter fulfils the SANCO/825/00 rev. 8.1 guideline requirements.

compound	RT	m/z quantifier	m/z qualifier	LOD (ng/mL)	LOQ (ng/mL)	linearity
chlorpyrifos	14.35	314	316	0.25	1.26	0.997
chlorpyrifos-methyl	13.10	286	288	0.1	0.45	0.999

Table S2 A-B. Mean values of the Active time [%], Distance moved [cm], AVG velocity [cm s^{-1}], and AVG Acceleration [cm s^{-2}] measured on *Daphnia magna* after 24 h (A) and 48h (B) of exposure to CTRLs, to ethanol (EtOH) and to different dilutions of CPF

A) CPF		**24h**					
		CTRL	**EtOH**	**0.05TU**	**0.1TU**	**0.25TU**	**0.5TU**
	N. of obs	72	72	68	69	65	70
Active time [%]	**M**	**22.89**	**27.54**	**23.17**	**22.82**	**20.39**	**19.32**
	SD	8.96	7.2	9.53	10.86	8.71	6.77
	SEM	0.73	1.1	1.16	1.15	0.85	0.62
Distance moved [cm]	**M**	**13.86**	**17.82**	**14.08**	**13.95**	**11.52**	**10.85**
	SD	6.76	6.33	7.75	8.73	6.25	4.93
	SEM	0.55	0.97	0.94	0.93	0.62	0.46
AVG Velocity [cm s^{-1}]	**M**	**0.99**	**1.06**	**0.96**	**0.95**	**0.91**	**0.91**
	SD	0.19	0.16	0.20	0.22	0.21	0.16
	SEM	0.02	0.02	0.02	0.02	0.02	0.01
AVG Acceleration [cm s^{-2}]	**M**	**23.00**	**25.00**	**22.28**	**21.94**	**21.03**	**21.53**
	SD	4.27	4.14	4.49	5.45	5.01	3.72
	SEM	0.35	0.63	0.55	0.58	0.50	0.34
B) CPF		**48h**					
		CTRL	**EtOH**	**0.05TU**	**0.1TU**	**0.25TU**	**0.5TU**
	N. of obs	71	70	67	65	63	66
Active time [%]	**M**	**19.69**	**19.89**	**19.28**	**21.44**	**23.7**	**26.17**
	SD	7.36	7.69	9.89	9.43	9.16	8.607
	SEM	0.70	1.3	1.17	1.14	0.718	1.076
Distance moved [cm]	**M**	**11.23**	**10.12**	**9.033**	**11.39**	**12.62**	**14.27**
	SD	5.59	5.58	5.12	7.00	6.64	6.26
	SEM	0.53	0.94	0.63	0.85	0.52	0.78
AVG Velocity [cm s^{-1}]	**M**	**0.91**	**0.80**	**0.82**	**0.83**	**0.84**	**0.88**
	SD	0.18	0.15	0.17	0.17	0.17	0.13
	SEM	0.02	0.02	0.02	0.02	0.01	0.02
AVG Acceleration [cm s^{-2}]	**M**	**21.91**	**19.17**	**19.35**	**19.50**	**20.18**	**20.70**
	SD	4.47	3.28	3.74	3.86	4.00	3.24
	SEM	0.42	0.55	0.44	0.47	0.31	0.40

CTRL = Control, EtOH = Control plus amount of ethanol contained in groups treated with 0.5TU of pesticide, N. of obs= number of observations, M = Mean values, SD= standard deviation, SEM = standard error of the mean.

Table S3 A-B. Mean values of the Active time [%], Distance moved [cm], AVG velocity [cm s^{-1}], and AVG Acceleration [cm s^{-2}] measured on *Daphnia magna* after 24 h (A) and 48h (B) of exposure to CTRL, to EtOH and to different dilutions of CPF-m

A) CPF-m		**24h**					
		CTRL	**EtOH**	**0.05TU**	**0.1TU**	**0.25TU**	**0.5TU**
	N. of obs	72	72	69	70	67	69
Active time [%]	**M**	**22.89**	**27.54**	**22.91**	**22.04**	**20.77**	**22.55**
	SD	8.96	7.2	6.73	8.74	8.3	5.25
	SEM	0.73	1.1	0.71	0.98	0.81	0.64
Distance moved [cm]	**M**	**13.86**	**17.82**	**13.56**	**13.02**	**12.9**	**12.4**
	SD	6.76	6.33	5.22	6.38	6.99	3.70
	SEM	0.55	0.97	0.55	0.72	0.68	0.46
AVG Velocity [cm s^{-1}]	**M**	**0.99**	**1.06**	**0.94**	**0.96**	**0.97**	**0.92**
	SD	0.19	0.16	0.13	0.18	0.22	0.13
	SEM	0.02	0.02	0.01	0.02	0.02	0.02
AVG Acceleration [cm s^{-2}]	**M**	**23.00**	**25.00**	**22.45**	**22.35**	**23.58**	**21.53**
	SD	4.27	4.14	3.26	3.92	5.71	2.74
	SEM	0.35	0.63	0.36	0.44	0.56	0.34
B) CPF-m		**48h**					
		CTRL	**EtOH**	**0.05TU**	**0.1TU**	**0.25TU**	**0.5TU**
	N. of obs	71	70	67	68	65	66
Active time [%]	**M**	**19.69**	**19.89**	**19.69**	**15.62**	**15.38**	**29.17**
	SD	7.36	7.69	9.49	8.12	7.6	7.64
	SEM	0.70	1.3	1.05	0.86	0.83	0.94
Distance moved [cm]	**M**	**11.23**	**10.12**	**9.58**	**7.53**	**8.23**	**16.16**
	SD	5.59	5.58	5.71	4.85	5.66	6.16
	SEM	0.53	0.94	0.63	0.53	0.62	0.76
AVG Velocity [cm s^{-1}]	**M**	**0.91**	**0.80**	**0.79**	**0.81**	**0.79**	**0.89**
	SD	0.18	0.15	0.16	0.18	0.19	0.14
	SEM	0.02	0.02	0.02	0.02	0.02	0.02
AVG Acceleration [cm s^{-2}]	**M**	**21.91**	**19.17**	**18.78**	**19.39**	**19.25**	**21.50**
	SD	4.47	3.28	3.84	4.22	5.08	3.22
	SEM	0.42	0.55	0.42	0.45	0.56	0.40

CTRL = Control, EtOH = Control plus amount of ethanol contained in groups treated with 0.5TU of pesticide, N. of obs= number of observations, M = Mean values, SD= standard deviation, SEM = standard error of the mean.

Table S 4- A. Results of statistical significance tests (Two-way ANOVA) among CTRL groups; treatment groups vs CTRLs and between congeners of corresponding treatment levels for the swimming parameter Distance moved after 24h of exposure to CPF and CPFm

Table Analyzed					
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.4662	0.4978	ns	No	
Row Factor (treatements)	8.639	< 0.0001	****	Yes	
Column Factor (congeners)	0.03352	0.5752	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	182.8	5	36.56	F (5, 852) = 0.8742	P = 0.4978
Row Factor	3387	5	677.4	F (5, 852) = 16.20	P < 0.0001
Column Factor	13.14	1	13.14	F (1, 852) = 0.3143	P = 0.5752
Residual	35626	852	41.81		
Number of missing values	0				

Within each column, compare rows (simple effects within columns)					
Number of families	2				
Number of comparisons per family	5				
Alpha	0.05				
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Distance moved_24h_CPF					
EtOH vs. CTRL	3.96	1.185 to 6.735	Yes	**	0.0013
0.05TU vs. CTRL	0.22	-2.555 to 2.995	No	ns	0.9999
0.1TU vs. CTRL	0.09	-2.685 to 2.865	No	ns	> 0.9999
0.25TU vs. CTRL	-2.34	-5.115 to 0.4346	No	ns	0.1421
0.5TU vs. ETN	-6.97	-9.745 to -4.195	Yes	****	< 0.0001
Distance moved_24h_CPFm					
EtOH vs. CTRL	3.96	1.185 to 6.735	Yes	**	0.0013
0.05TU vs. CTRL	-0.3	-3.075 to 2.475	No	ns	0.9995
0.1TU vs. CTRL	-0.84	-3.615 to 1.935	No	ns	0.9429
0.25TU vs. CTRL	-0.96	-3.735 to 1.815	No	ns	0.9033
0.5TU vs. ETN	-5.42	-8.195 to -2.645	Yes	****	< 0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF

Distance moved_24h_CPF								
EtOH vs. CTRL	17.82	13.86	3.96	1.078	72	72	3.674	852
0.05TU vs. CTRL	14.08	13.86	0.22	1.078	72	72	0.2041	852
0.1TU vs. CTRL	13.95	13.86	0.09	1.078	72	72	0.08351	852
0.25TU vs. CTRL	11.52	13.86	-2.34	1.078	72	72	2.171	852
0.5TU vs. ETN	10.85	17.82	-6.97	1.078	72	72	6.467	852
Distance moved_24h_CPFm								
EtOH vs. CTRL	17.82	13.86	3.960	1.078	72	72	3.674	852
0.05TU vs. CTRL	13.56	13.86	-0.3000	1.078	72	72	0.2784	852
0.1TU vs. CTRL	13.02	13.86	-0.8400	1.078	72	72	0.7794	852
0.25TU vs. CTRL	12.90	13.86	-0.9600	1.078	72	72	0.8908	852
0.5TU vs. ETN	12.4	17.82	-5.42	1.078	72	72	5.029	852

Compare each cell mean with the other cell mean in that row.								
Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant ?	Summary	Adjusted P Value			
Distance moved_24h_CPFm - Distance moved_24h_CPF								
0.05TU	-0.52	-3.362 to 2.322	No	ns	0.9974			
0.1TU	-0.93	-3.772 to 1.912	No	ns	0.9477			
0.25TU	1.38	-1.462 to 4.222	No	ns	0.7393			
0.5TU	1.55	-1.292 to 4.392	No	ns	0.6248			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Distance moved_24h_CPFm - Distance moved_24h_CPF								
0.05TU	13.56	14.08	-0.52	1.078	72	72	0.4825	852
0.1TU	13.02	13.95	-0.93	1.078	72	72	0.8629	852
0.25TU	12.9	11.52	1.38	1.078	72	72	1.28	852
0.5TU	12.4	10.85	1.55	1.078	72	72	1.438	852

CTRL = Control, EtOH = control plus amount of ethanol contained in groups treated with 0.5TU of pesticide
Groups treated with 0.05TU, 0.1TU and 0.25TU of pesticide were compared with the CTRL; groups treated with 0.5TU of pesticide were compared with the EtOH.
*p-value<0.05; **p-value<0.01; ***p-value<0.001; ****p-value<0.0001.

Table S 4- B. Results of statistical significance tests (Two-way ANOVA) among CTRL groups; treatment groups vs CTRLs and between congeners of corresponding treatment levels for the swimming parameter Distance moved after 48h of exposure to CPF and CPFm

Table Analyzed	**Distance moved 48h CPF, CPFm**					
Two-way ANOVA	Ordinary					
Alpha	0.05					
Source of Variation	% of total variation	P value	P value summary	Significant?		
Interaction	3.442	< 0.0001	****	Yes		
Row Factor (treatements)	10.26	< 0.0001	****	Yes		
Column Factor (congeners)	0.5983	0.0149	*	Yes		
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value	
Interaction	1167	5	233.4	F (5, 852) = 6.844	P < 0.0001	
Row Factor	3479	5	695.8	F (5, 852) = 20.40	P < 0.0001	
Column Factor	202.9	1	202.9	F (1, 852) = 5.948	P = 0.0149	
Residual	29059	852	34.11			
Number of missing values	0					

Within each column, compare rows (simple effects within columns)					
Number of families	2				
Number of comparisons per family	5				
Alpha	0.05				
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Distance moved_48h_CPF					
EtOH vs. CTRL	-1.11	-3.616 to 1.396	No	ns	0.7696
0.05TU vs. CTRL	-2.197	-4.703 to 0.3089	No	ns	0.1155
0.1TU vs. CTRL	0.16	-2.346 to 2.666	No	ns	> 0.9999
0.25TU vs. CTRL	1.39	-1.116 to 3.896	No	ns	0.5657
0.5TU vs. ETN	4.15	1.644 to 6.656	Yes	***	0.0001
Distance moved_48h_CPFm					
EtOH vs. CTRL	-1.11	-3.616 to 1.396	No	ns	0.7696
0.05TU vs. CTRL	-1.65	-4.156 to 0.8559	No	ns	0.3774
0.1TU vs. CTRL	-3.705	-6.211 to -1.199	Yes	***	0.0008
0.25TU vs. CTRL	-2.997	-5.503 to -0.4911	Yes	*	0.0107
0.5TU vs. ETN	6.04	3.534 to 8.546	Yes	****	< 0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Distance moved_48h_CPF								
ETOHvs. CTRL	10.12	11.23	-1.11	0.9734	72	72	1.14	852

0.05TU vs. CTRL	9.033	11.23	-2.197	0.9734	72	72	2.257	852
0.1TU vs. CTRL	11.39	11.23	0.16	0.9734	72	72	0.1644	852
0.25TU vs. CTRL	12.62	11.23	1.39	0.9734	72	72	1.428	852
0.5TU vs. ETN	14.27	10.12	4.15	0.9734	72	72	4.264	852
Distance moved_48h_CPFm								
EtOH vs. CTRL	10.12	11.23	-1.11	0.9734	72	72	1.14	852
0.05TU vs. CTRL	9.58	11.23	-1.65	0.9734	72	72	1.695	852
0.1TU vs. CTRL	7.525	11.23	-3.705	0.9734	72	72	3.806	852
0.25TU vs. CTRL	8.233	11.23	-2.997	0.9734	72	72	3.079	852
0.5TU vs. ETN	16.16	10.12	6.04	0.9734	72	72	6.205	852

Compare each cell mean with the other cell mean in that row.								
Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value			
Distance moved_48h_CPFm - Distance moved_48h_CPF								
0.05TU	0.547	-2.020 to 3.114	No	ns	0.994			
0.1TU	-3.865	-6.432 to -1.298	Yes	***	0.0005			
0.25TU	-4.387	-6.954 to -1.820	Yes	****	< 0.0001			
0.5TU	1.89	-0.6769 to 4.457	No	ns	0.2764			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Distance moved_48h_CPFm - Distance moved_48h_CPF								
0.05TU	9.58	9.033	0.547	0.9734	72	72	0.562	852
0.1TU	7.525	11.39	-3.865	0.9734	72	72	3.971	852
0.25TU	8.233	12.62	-4.387	0.9734	72	72	4.507	852
0.5TU	16.16	14.27	1.89	0.9734	72	72	1.942	852

CTRL = Control, EtOH = control plus amount of ethanol contained in groups treated with 0.5TU of pesticide
Groups treated with 0.05TU, 0.1TU and 0.25TU of pesticide were compared with the CTRL; groups treated with 0.5TU of pesticide were compared with the EtOH.
*p-value<0.05; **p-value<0.01; ***p-value<0.001; ****p-value<0.0001.

Table S4 – C. Results of statistical significance tests (Two-way ANOVA) among CTRL groups; treatment groups vs CTRLs and between congeners of corresponding treatment levels for the swimming parameter AVG velocity after 24h of exposure to CPF and CPFm

Table Analyzed	**AVG velocity 24h CPF, CPFm**				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.4845	0.4904	ns	No	
Row Factor	6.166	< 0.0001	****	Yes	
Column Factor	0.07259	0.4157	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1437	5	0.02874	F (5, 852) = 0.8852	P = 0.4904
Row Factor	1.829	5	0.3657	F (5, 852) = 11.26	P < 0.0001
Column Factor	0.02153	1	0.02153	F (1, 852) = 0.6631	P = 0.4157
Residual	27.66	852	0.03247		
Number of missing values	0				

Within each column, compare rows (simple effects within columns)		**AVG velocity 24h CPF, CPFm**			
Number of families	2				
Number of comparisons per family	5				
Alpha	0.05				
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
AVG velocity 24h_CPF					
EtOH vs. CTRL	0.0723	-0.005015 to 0.1496	No	ns	0.0788
0.05TU vs. CTRL	-0.0264	-0.1037 to 0.05092	No	ns	0.9081
0.1TU vs. CTRL	-0.0403	-0.1176 to 0.03702	No	ns	0.6292
0.25TU vs. CTRL	-0.0791	-0.1564 to -0.001785	Yes	*	0.0422
0.5TU vs. ETN	-0.1452	-0.2225 to -0.06788	Yes	****	< 0.0001
AVG velocity 24h_CPFm					
ETOHvs. CTRL	0.0723	-0.005015 to 0.1496	No	ns	0.0788
0.05TU vs. CTRL	-0.0436	-0.1209 to 0.03372	No	ns	0.5482
0.1TU vs. CTRL	-0.0323	-0.1096 to 0.04502	No	ns	0.8098
0.25TU vs. CTRL	-0.0142	-0.09152 to 0.06312	No	ns	0.9936
0.5TU vs. ETN	-0.141	-0.2183 to -0.06368	Yes	****	< 0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
AVG velocity 24h_CPF								
EtOH vs. CTRL	1.06	0.9877	0.0723	0.03003	72	72	2.407	852
0.05TU vs. CTRL	0.9613	0.9877	-0.0264	0.03003	72	72	0.8791	852
0.1TU vs. CTRL	0.9474	0.9877	-0.0403	0.03003	72	72	1.342	852
0.25TU vs. CTRL	0.9086	0.9877	-0.0791	0.03003	72	72	2.634	852
0.5TU vs. ETN	0.9148	1.06	-0.1452	0.03003	72	72	4.835	852

AVG velocity 24h_CPFm								
ETOH vs. CTRL	1.06	0.9877	0.0723	0.03003	72	72	2.407	852
0.05TU vs. CTRL	0.9441	0.9877	-0.0436	0.03003	72	72	1.452	852
0.1TU vs. CTRL	0.9554	0.9877	-0.0323	0.03003	72	72	1.076	852
0.25TU vs. CTRL	0.9735	0.9877	-0.0142	0.03003	72	72	0.4728	852
0.5TU vs. ETN	0.919	1.06	-0.141	0.03003	72	72	4.695	852

Compare each cell mean with the other cell mean in that row.								
Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant ?	Summary	Adjusted P Value			
AVG velocity 24h_CPFm - AVG velocity 24h_CPF								
0.05TU	-0.0172	-0.09640 to 0.06200	No	ns	0.9934			
0.1TU	0.008	-0.07120 to 0.08720	No	ns	> 0.9999			
0.25TU	0.0649	-0.01430 to 0.1441	No	ns	0.172			
0.5TU	0.0042	-0.07500 to 0.08340	No	ns	> 0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
AVG velocity 24h_CPFm - AVG velocity 24h_CPF								
0.05TU	0.9441	0.9613	-0.0172	0.03003	72	72	0.5727	852
0.1TU	0.9554	0.9474	0.008	0.03003	72	72	0.2664	852
0.25TU	0.9735	0.9086	0.0649	0.03003	72	72	2.161	852
0.5TU	0.919	0.9148	0.0042	0.03003	72	72	0.1399	852

CTRL = Control, EtOH = control plus amount of ethanol contained in groups treated with 0.5TU of pesticide
Groups treated with 0.05TU, 0.1TU and 0.25TU of pesticide were compared with the CTRL; groups treated with 0.5TU of pesticide were compared with the EtOH.
*p-value<0.05; **p-value<0.01; ***p-value<0.001; ****p-value<0.0001.

Table S4 –D. Results of statistical significance tests (Two-way ANOVA) among CTRL groups; treatment groups vs CTRLs and between congeners of corresponding treatment levels for the swimming parameter AVG velocity after 48 h of exposure to CPF and CPFm

Table Analyzed	**AVG velocity 48h CPF, CPFm**				

Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.5065	0.464	ns	No	
Row Factor	5.985	< 0.0001	****	Yes	
Column Factor	0.1967	0.1806	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1263	5	0.02525	F (5, 852) = 0.9249	P = 0.4640
Row Factor	1.492	5	0.2984	F (5, 852) = 10.93	P < 0.0001
Column Factor	0.04903	1	0.04903	F (1, 852) = 1.796	P = 0.1806
Residual	23.26	852	0.0273		
Number of missing values	0				

Within each column, compare rows (simple effects within columns)		**AVG velocity 48h CPF, CPFm**				
Number of families	2					
Number of comparisons per family	5					
Alpha	0.05					
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value	
AVG velocity 48h_CPF						
EtOH vs. CTRL	-0.1065	-0.1774 to -0.03560	Yes	***	0.0006	
0.05TU vs. CTRL	-0.0851	-0.1560 to -0.01420	Yes	*	0.0103	
0.1TU vs. CTRL	-0.0822	-0.1531 to -0.01130	Yes	*	0.0145	
0.25TU vs. CTRL	-0.0639	-0.1348 to 0.007000	No	ns	0.0987	
0.5TU vs. ETN	0.0734	0.002500 to 0.1443	Yes	*	0.0386	
AVG velocity 48h_CPFm						
EtOH vs. CTRL	-0.1065	-0.1774 to -0.03560	Yes	***	0.0006	
0.05TU vs. CTRL	-0.1217	-0.1926 to -0.05080	Yes	****	< 0.0001	
0.1TU vs. CTRL	-0.1012	-0.1721 to -0.03030	Yes	**	0.0013	
0.25TU vs. CTRL	-0.1171	-0.1880 to -0.04620	Yes	***	0.0001	
0.5TU vs. ETN	0.0918	0.02090 to 0.1627	Yes	**	0.0045	

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
AVG velocity 48h_CPF								
ETOHvs. CTRL	0.8022	0.9087	-0.1065	0.02754	72	72	3.867	852
0.05TU vs. CTRL	0.8236	0.9087	-0.0851	0.02754	72	72	3.09	852
0.1TU vs. CTRL	0.8265	0.9087	-0.0822	0.02754	72	72	2.985	852

0.25TU vs. CTRL	0.8448	0.9087	-0.0639	0.02754	72	72	2.32	852
0.5TU vs. ETN	0.8756	0.8022	0.0734	0.02754	72	72	2.665	852
AVG velocity 48h_CPFm								
EtOH vs. CTRL	0.8022	0.9087	-0.1065	0.02754	72	72	3.867	852
0.05TU vs. CTRL	0.787	0.9087	-0.1217	0.02754	72	72	4.419	852
0.1TU vs. CTRL	0.8075	0.9087	-0.1012	0.02754	72	72	3.675	852
0.25TU vs. CTRL	0.7916	0.9087	-0.1171	0.02754	72	72	4.252	852
0.5TU vs. ETN	0.894	0.8022	0.0918	0.02754	72	72	3.333	852

Compare each cell mean with the other cell mean in that row.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value			
AVG velocity 48h_CPFm - AVG velocity 48h_CPF								
0.05TU	-0.0366	-0.1092 to 0.03603	No	ns	0.7052			
0.1TU	-0.019	-0.09163 to 0.05363	No	ns	0.9825			
0.25TU	-0.0532	-0.1258 to 0.01943	No	ns	0.282			
0.5TU	0.0184	-0.05423 to 0.09103	No	ns	0.9852			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
AVG velocity 48h_CPFm - AVG velocity 48h_CPF								
0.05TU	0.787	0.8236	-0.0366	0.02754	72	72	1.329	852
0.1TU	0.8075	0.8265	-0.019	0.02754	72	72	0.6899	852
0.25TU	0.7916	0.8448	-0.0532	0.02754	72	72	1.932	852
0.5TU	0.894	0.8756	0.0184	0.02754	72	72	0.6681	852

CTRL = Control, EtOH = control plus amount of ethanol contained in groups treated with 0.5TU of pesticide
Groups treated with 0.05TU, 0.1TU and 0.25TU of pesticide were compared with the CTRL; groups treated with 0.5TU of pesticide were compared with the EtOH.
*p-value<0.05; **p-value<0.01; ***p-value<0.001; ****p-value<0.0001.

Table S5 – Results of non-parametrical statistical analysis (U Mann-Whitney test) to compare responsiveness between Distance moved and AVG velocity after 24h and 48 h of exposure to CPF and CPFm of corresponding treatment level

24 h				
Distance moved vs AVG velocity				
	CPF		**CPFmethyl**	
TU	**Mann Whitney test, p value**	**P value summary**	**Mann Whitney test, p value**	**P value summary**
0.05	0.6649	ns	0.5766	ns
0.1	0.6724	ns	0.7451	ns
0.25	< 0.0001	****	0.02	*
0.5	< 0.0001	****	< 0.0001	****
48 h				
Distance moved vs AVG velocity				
	CPF		**CPFmethyl**	
TU	**Mann Whitney test, p value**	**P value summary**	**Mann Whitney test, p value**	**P value summary**
0.05	0.012	*	0.1293	ns
0.1	0.8593	ns	< 0.0001	****
0.25	0.0213	*	0.0003	***
0.5	0.0024	**	< 0.0001	****

Table S6 A-B. Pearson correlation analysis results for coupled endpoints (Active time vs Distance moved; AVG velocity vs AVG acceleration) for the CPF (A) and the CPF-m (B) after 24 h and 48 h of exposure

A) **CPF**	**24h**		**48h**	
	Active time vs. Distance moved	**AVG velocity vs. AVG acceleration**	**Active time vs. Distance moved**	**AVG velocity vs. AVG acceleration**
Pearson r				
r	0.9996	0.988	0.9908	0.9986
95% confidence interval	0.9819 to 1.000	0.5339 to 0.9998	0.6231 to 0.9998	0.9309 to 1.000
R square	0.9993	0.9762	0.9818	0.9972
P value	0.0004	0.012	0.0092	0.0014
P value summary	***	*	**	**
Significant? (alpha = 0.05)	Yes	Yes	Yes	Yes
Number of XY Pairs	4	4	4	4
B) **CPF-m**	**24h**		**48h**	
	Active time vs. Distance moved	**AVG velocity vs. AVG acceleration**	**Active time vs. Distance moved**	**AVG velocity vs. AVG acceleration**
Pearson r				
r	0.9109	0.9939	0.9846	0.9989
95% confidence interval	-0.4034 to 0.9982	0.7333 to 0.9999	0.4383 to 0.9997	0.9448 to 1.000
R square	0.8297	0.9879	0.9695	0.9978
P value	0.0445	0.0061	0.0154	0.0011
P value summary	*	**	*	**
Significant? (alpha = 0.05)	Yes	Yes	Yes	Yes
Number of XY Pairs	4	4	4	4

Table S7 - Description of the calculation steps of the CRC values for the Distance moved and AVG velocity after 24 h and 48 h of exposure of daphnids to CPF and CPFm

	24h								
Compound	**TU**	**Distance moved [cm]**	**AVG velocity [cm s-1]**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**
		measured values		**(values normalised on CTRLs) -1**		**(absolute values)*100**		**cumulative values**	
CTRL	**0**	13.86	0.988	0	0				
EtOH	**0**	17.819	1.060	0	0	0	0	0	0
CPF	**0.05**	14.075	0.961	0.016	-0.027	1.554	2.668	1.554	2.668
	0.1	13.950	0.947	0.006	-0.041	0.650	4.084	2.203	6.752
	0.25	11.517	0.909	-0.169	-0.080	16.905	8.010	19.109	14.761
	0.5	10.853	0.915	-0.391	-0.137	39.096	13.684	58.205	28.446
	48h								
	TU	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**
		measured values		**(values normalised on CTRLs) -1**		**(absolute values)*100**		**cumulative values**	
CTRL	**0**	11.230	0.909	0	0				
EtOH	**0**	10.118	0.802	0	0	0	0	0	0
CPF	**0.05**	9.033	0.824	-0.196	-0.094	19.566	9.362	19.566	9.362
	0.1	11.388	0.827	0.014	-0.090	1.410	9.045	20.976	18.407
	0.25	12.617	0.845	0.123	-0.070	12.346	7.031	33.323	25.437
	0.5	14.274	0.876	0.411	0.092	41.072	9.160	74.395	34.597
	24h								
	TU	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**
		measured values		**(values normalised on CTRLs) -1**		**(absolute values)*100**		**cumulative values**	
CTRL	**0**	13.86	0.988	0	0				
EtOH	**0**	17.819	1.060	0	0	0	0	0	0
CPF-methyl	**0.05**	13.564	0.944	-0.021	-0.044	2.133	4.410	2.133	4.410
	0.1	13.022	0.955	-0.060	-0.033	6.044	3.267	8.177	7.677
	0.25	12.899	0.974	-0.069	-0.014	6.931	1.438	15.108	9.115
	0.5	12.396	0.919	-0.304	-0.133	30.433	13.294	45.541	22.409
	48h								
	TU	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**	**Distance moved**	**AVG velocity**
		measured values		**(values normalised on CTRLs) -1**		**(absolute values)*100**		**cumulative values**	
CTRL	**0**	11.230	0.909	0	0				
EtOH	**0**	10.118	0.802	0	0	0	0	0	0
CPF-methyl	**0.05**	9.580	0.787	-0.147	-0.134	14.690	13.388	14.690	13.388
	0.1	7.525	0.807	-0.330	-0.111	32.996	11.137	47.686	24.525
	0.25	8.233	0.792	-0.267	-0.129	26.687	12.887	74.373	37.412
	0.5	16.155	0.894	0.597	0.114	59.667	11.443	134.040	48.855

Table S8 - Statistical parameters of the linear regression models used to fit the CRCs for the Distance moved and the AVG velocity after 24h and 48 of exposure to CPF and CPFm.

Distance moved 24h		
	CPF	**CPF methyl**
Goodness of Fit		
R square	0.9622	0.97
Sy.x	5.554	3.699
Deviation from linearity	Not Significant	Not Significant
Equation	Y = 120.2*X - 5.413	Y = 90.18*X - 2.040
Distance moved 48h		
	CPF	**CPF methyl**
Goodness of Fit		
R square	0.9594	0.9729
Sy.x	6.445	10.11
Deviation from linearity	Not Significant	Not Significant
Equation	Y = 134.5*X + 5.449	Y = 260.0*X + 7.364
AVG velocity 24h		
	CPF	**CPF methyl**
Goodness of Fit		
R square	0.998	0.9496
Sy.x	0.589	2.181
Deviation from linearity	Not Significant	Not Significant
Equation	Y = 56.74*X + 0.3118	Y = 40.62*X + 1.410
AVG velocity 48h		
	CPF	**CPF methyl**
Goodness of Fit		
R square	0.8695	0.8758
Sy.x	5.629	7.837
Deviation from linearity	Not Significant	Not Significant
Equation	Y = 62.34*X + 6.339	Y = 89.26*X + 8.769

Table S9. Statistical significance results from comparison of CRC curves of CPF and CPFm for Distance moved and AVG velocity (multiple t test using the Holm-Sidak method [significance level $p \leq 0.05$])

Distance moved vs AVG velocity								
CPF - 24 h								
TU	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
0.05	no	0.812752	1.554	2.668	-1.114	4.69411	0.237319	142
0.1	no	0.241307	2.203	6.752	-4.549	3.87426	1.17416	286
0.25	no	0.140797	19.109	14.761	4.348	2.9467	1.47555	430
0.5	yes	< 0.0001	58.205	28.446	29.759	2.37265	12.5425	574
CPF - 48 h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	yes	0.000794	19.566	9.362	10.204	2.97615	3.42859	142
0.1	no	0.27454	20.976	18.407	2.569	2.34662	1.09477	286
0.25	yes	0.000566	33.323	25.437	7.886	2.27034	3.47349	430
0.5	yes	< 0.0001	74.395	34.597	39.798	2.01249	19.7755	574
CPFm - 24h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	no	0.472051	2.133	4.41	-2.277	3.1578	0.721072	142
0.1	no	0.847921	8.177	7.677	0.5	2.60491	0.191945	286
0.25	yes	0.008244	15.108	9.115	5.993	2.2579	2.65424	430
0.5	yes	< 0.0001	45.541	22.409	23.132	1.90012	12.1739	574
CPFm - 48h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	no	0.732815	14.69	13.388	1.302	3.8064	0.342055	142
0.1	yes	< 0.0001	47.686	24.525	23.161	2.68251	8.63409	286
0.25	yes	< 0.0001	74.373	37.412	36.961	2.14534	17.2285	430
0.5	yes	< 0.0001	134.04	48.855	85.185	1.97149	43.2085	574
CPF vs CPFm								
Distance moved - 24h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	no	0.902008	1.554	2.133	-0.579	4.69411	0.123346	142
0.1	no	0.124186	2.203	8.177	-5.974	3.87426	1.54197	286
0.25	no	0.175242	19.109	15.108	4.001	2.9467	1.35779	430
0.5	yes	< 0.0001	58.205	45.541	12.664	2.37265	5.33749	574

Distance moved - 48h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	no	0.253577	19.566	14.69	4.876	4.25349	1.14635	142
0.1	yes	< 0.0001	20.976	47.686	-26.71	3.00851	8.87814	286
0.25	yes	< 0.0001	33.323	74.373	-41.05	2.54136	16.1528	430
0.5	yes	< 0.0001	74.395	134.04	-59.645	2.34357	25.4505	574
AVG velocity - 24h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	no	0.417584	2.668	4.41	-1.742	2.1427	0.812992	142
0.1	no	0.586895	6.752	7.677	-0.925	1.7005	0.543957	286
0.25	yes	0.000286	14.761	9.115	5.646	1.54367	3.65751	430
0.5	yes	< 0.0001	28.446	22.409	6.037	1.31451	4.59257	574
AVG velocity - 48h								
	Significant?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
TU								
0.05	no	0.081173	9.362	13.388	-4.026	2.29217	1.75642	142
0.1	yes	0.00152	18.407	24.525	-6.118	1.91084	3.20174	286
0.25	yes	< 0.0001	25.437	37.412	-11.975	1.81615	6.59361	430
0.5	yes	< 0.0001	34.597	48.855	-14.258	1.5635	9.11928	574