

DOI: [10.4081/ejh.2020.3084](https://doi.org/10.4081/ejh.2020.3084)

Chronic cypermethrin exposure alters mouse embryonic stem cell growth kinetics, induces Phase II detoxification response and affects pluripotency and differentiation gene expression

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Key words: Cypermethrin; embryonic stem cells; cell growth; apoptosis; reactive oxygen species; detoxification response.

Supplementary Table 1. Sequences of the forward and reverse primers used for the gene expression analysis.

Gene	Forward	Reverse	Amplicon length - bp
<i>Cat</i>	5' TCATCAGGGATGCCATATTGT 3'	5' ACTCCAGAAGTCCCAGACCAT 3'	101
<i>Gpx1</i>	5' CTACACCGAGATGAACGATCTG 3'	5' CTTGCCATTCTCCTGGTGTC 3'	100
<i>Gpx4</i>	5' TGTGGAAATGGATGAAAGTCC 3'	5' ACGCAGCCGTTCTTATCAAT 3'	100
<i>Sod1</i>	5' TGTCATTGAAGATCGTGTGA 3'	5' TGCCCAAGTCATCTTGTTTC 3'	94
<i>Sod2</i>	5' CTTACAGATTGCTGCCTGCTC 3'	5' GTAGTAAGCGTGCTCCCACAC 3'	97
<i>Cyp1a1</i>	5' CTGCCTAACTCTTCCTGGAT 3'	5' ATGTTGGCCCTTCTCAAATGTC 3'	107
<i>Cyp1b1</i>	5' CCAGGTGCAAACCTTGAGACA 3'	5' TGCTGCACTAAGGCTGGTG 3'	250
<i>Nqo1</i>	5' TTCTCTGGCCGATTGAGAGT 3'	5' TCTGGTTGTCAGCTGGAATG 3'	306
<i>Gsta1</i>	5' CTCTGACCCCTTCCCTCT 3'	5' GCCAGTATCTGTGGCTCCAT 3'	183
<i>Ugt1a6</i>	5' ATTGCCTCAGACCTCCTCAA 3'	5' GAGACCATGGATCCCAAAGA 3'	236
<i>Oct-4</i>	5' GTGGAGGAAGCCGACAACAATG 3'	5' CACCTCACACGGTTCTCAATGC 3'	107
<i>Nanog</i>	5' CTGCTACTGAGATGCTCTGCAC 3'	5' AGCTTTTGTGGGACTGGTAG 3'	106
<i>Fgf5</i>	5' TGTGTCTCAGGGGATTGTAGG 3'	5' CATCCGTAATTTGGCACTTG 3'	101
<i>Brachyury</i>	5' CTCTAAGGAACCACCGGTCA 3'	5' AGCATGGACAGACAAGCAGA 3'	100
<i>Foxa2</i>	5' AAATGAGAGGCTGAGTGGAGA 3'	5' GGCCCATCTATTTAGGGACAC 3'	110
<i>β2m</i>	5' GAATTCACCCCACTGAGACT 3'	5' TGCTTGATCACATGTCTCGAT 3'	103

Supplementary Table 2. Distribution of control (CTR) and exposed R1 ESCs and 3T3 cells to cypermethrin (CYP) at their respective LD₅₀ dose (0.3 or 0.6 mM) during the cell cycle phases.

	Samples (μ M)	Hours	Frequency of cells (mean \pm SD)		
			G0/G1	S	G2/M
R1	CTR	12	26.9 \pm 4.4	48.9 \pm 2.8	24.4 \pm 1.6
	CYP		29.7 \pm 1.1	47.6 \pm 1.0	22.7 \pm 0.7
	CTR	24	31.6 \pm 2.9	54.2 \pm 3.1	14.2 \pm 0.3
	CYP		31.5 \pm 0.5	53.3 \pm 0.7	15.2 \pm 0.3
	CTR	48	29.1 \pm 2.2	52.8 \pm 1.6	18.1 \pm 1.0
	CYP		25.7 \pm 0.6*	51.6 \pm 0.3	22.7 \pm 0.6*
	CTR	72	30.9 \pm 1.4	54.0 \pm 1.4	15.1 \pm 0.8
	CYP		24.3 \pm 1.3*	54.6 \pm 0.9	21.1 \pm 1.8*
3T3	CTR	8	65.0 \pm 4.0	15.9 \pm 1.9	19.0 \pm 2.7
	CYP		62.3 \pm 1.5	17.1 \pm 0.6	20.6 \pm 2.2
	CTR	24	67.1 \pm 1.7	14.3 \pm 1.1	18.6 \pm 1.4
	CYP		67.9 \pm 3.2	15.1 \pm 1.4	17.0 \pm 1.8
	CTR	48	68.6 \pm 1.4	14.0 \pm 0.6	17.4 \pm 0.8
	CYP		79.2 \pm 1.4*	11.1 \pm 0.8*	9.7 \pm 2.0*
	CTR	72	66.6 \pm 0.7	15.9 \pm 0.4	17.5 \pm 0.9
	CYP		68.1 \pm 1.3	15.2 \pm 0.7	16.6 \pm 1.4

*P<0.001, when compared to CTR.

Supplementary Table 3. Fold-change values of redox-related, phase I and phase II gene transcripts, relative to CTR, of R1 cells exposed to 0.3 mM cypermethrin for 12, 24, 48 or 72 h.

Groups	Genes	Hours			
		12	24	48	72
Redox-related	<i>Catalase</i>	1.49 ± 0.64	1.05 ± 0.11	4.06 ± 1.69 [#]	2.67 ± 1.71
	<i>Sod1</i>	1.19 ± 0.47	0.67 ± 0.13*	2.24 ± 0.68 [#]	2.02 ± 0.55*
	<i>Sod2</i>	1.42 ± 0.50	0.98 ± 0.11	3.17 ± 0.70 [#]	1.93 ± 0.77*
	<i>Gpx1</i>	1.29 ± 0.20	0.83 ± 0.10	1.19 ± 0.38	1.52 ± 0.41*
	<i>Gpx4</i>	1.15 ± 0.46	1.10 ± 0.09	2.00 ± 0.01 [#]	1.72 ± 0.20*
Phase I	<i>Cyp1a1</i>	0.95 ± 0.37	0.64 ± 0.28*	0.33 ± 0.32*	0.77 ± 0.30
	<i>Cyp1b1</i>	1.36 ± 0.51	0.64 ± 0.28*	0.47 ± 0.21*	3.52 ± 1.42 [#]
Phase II	<i>Nqo1</i>	0.90 ± 0.09	1.18 ± 0.35	1.22 ± 0.36	2.36 ± 0.31 [#]
	<i>Gst1</i>	0.94 ± 0.46	1.20 ± 0.40	2.06 ± 1.11 [#]	6.30 ± 2.61 [#]
	<i>Ugt1a6</i>	0.59 ± 0.03*	2.85 ± 0.81 [#]	2.19 ± 0.01 [#]	5.14 ± 2.47 [#]

*P<0.05; [#]P<0.001.

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Supplementary Table 4. Fold-change values of redox-related, phase I and phase II gene transcripts relative to CTR samples (set at 1) of 3T3 cell line exposed to 0.6 mM cypermethrin for 8, 24, 48 or 72 h.

Groups	Genes	Hours			
		8	24	48	72
Redox-related	<i>Catalase</i>	0.78 ± 0.29	1.19 ± 0.02*	1.08 ± 0.12	2.03 ± 0.51 [#]
	<i>Sod1</i>	0.94 ± 0.08	0.92 ± 0.08	0.89 ± 0.32	0.94 ± 0.44
	<i>Sod2</i>	0.72 ± 0.16	0.83 ± 0.04*	0.72 ± 0.02*	1.26 ± 0.39
	<i>Gpx1</i>	0.93 ± 0.01	0.63 ± 0.03*	0.76 ± 0.09	0.99 ± 0.14
	<i>Gpx4</i>	0.81 ± 0.07	1.01 ± 0.05	0.86 ± 0.31	1.32 ± 0.12*
Phase I	<i>Cyp1a1</i>	0.91 ± 0.07	1.51 ± 0.25*	1.00 ± 0.01	1.00 ± 0.11
	<i>Cyp1b1</i>	1.00 ± 0.23	0.92 ± 0.07	0.85 ± 0.43	1.15 ± 0.70
Phase II	<i>Nqo1</i>	0.97 ± 0.04	0.98 ± 0.04	1.17 ± 0.22	1.14 ± 0.04
	<i>Gst1</i>	0.76 ± 0.10	1.14 ± 0.11	3.80 ± 0.04 [#]	2.43 ± 0.42 [#]
	<i>Ugt1a6</i>	1.22 ± 0.21	2.32 ± 0.22 [#]	4.43 ± 1.23 [#]	3.56 ± 1.63 [#]

*P<0.05; [#]P<0.001.

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Supplementary Table 5. Fold-change values of pluripotency gene transcripts of R1 ESCs exposed to 0.3 mM cypermethrin for 12, 24, 48 or 72 h relative to control ESCs (set at 1).

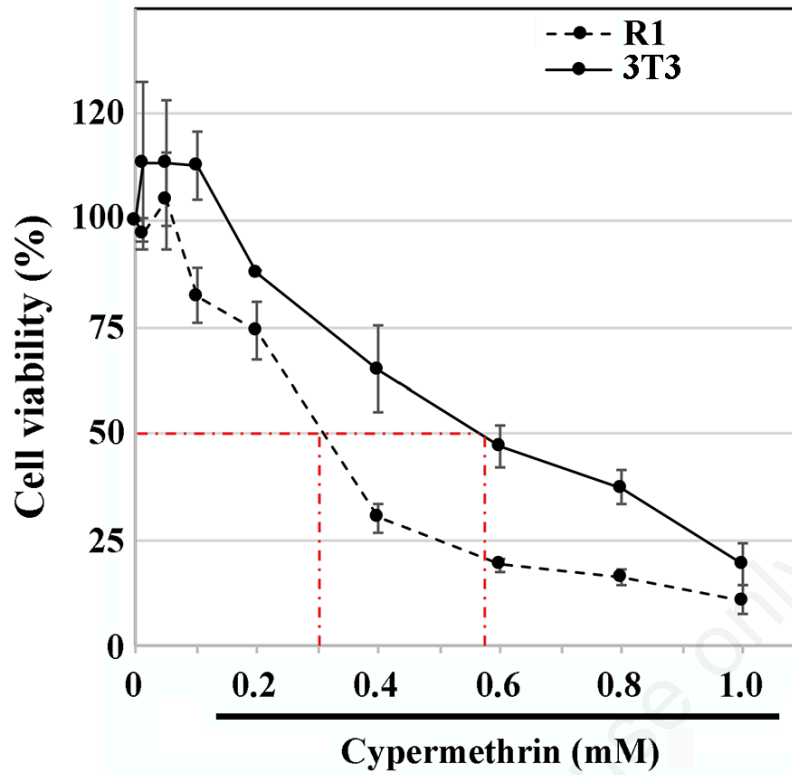
Group	Genes	Hours			
		12	24	48	72
<i>Pluripotency</i>	<i>Oct-4</i>	0.71 ± 0.15*	0.75 ± 0.11*	1.29 ± 0.34*	1.48 ± 0.20*
	<i>Nanog</i>	0.71 ± 0.11*	0.85 ± 0.06*	1.35 ± 0.22*	1.74 ± 0.55*

*P<0.001.

Supplementary Table 6. Fold-change values of early ectoderm, mesoderm and endoderm gene transcripts of EBs differentiated for 5 days from R1 ESCs exposed to 0.3 mM cypermethrin for 12, 24, 48 or 72 h relative to EBs differentiated from control (not exposed) ESCs (set at 1).

Group	Genes	Hours			
		12	24	48	72
<i>Early germ layers</i>	<i>Fgf5</i>	1.00 ± 0.72	0.06 ± 0.01*	0.39 ± 0.08*	0.64 ± 0.34
	<i>Brachyury</i>	0.16 ± 0.05*	1.11 ± 0.04*	1.21 ± 0.26*	0.64 ± 0.06*
	<i>Foxa2</i>	7.28 ± 3.85*	1.82 ± 1.19	7.19 ± 2.69*	3.42 ± 0.12*

*P<0.001.



Supplementary Figure 1.

Dose/response curve to CYP of R1 and 3T3 after 72 h exposure. Data represent the mean \pm SD of 3 independent experiments.