Supporting Information for

Disruption of the hormonal network and the enantioselectivity of bifenthrin in trophoblast: Maternal-fetal health risk of chiral pesticides

Meirong Zhao,[†,‡,a] Ying Zhang,[§,⊥,a] Shulin Zhuang,[⊥] Quan Zhang,[†] Chengsheng Lu[‡] and Weiping Liu[⊥,*]

[†]*College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, China;*

[‡]*Department of Environmental Health, Harvard School of Public Health, Landmark Center West, Boston, MA, 02115, USA;*

[§]*School of Ecological and Environmental Sciences, East China Normal University, Shanghai 200241, China;*

[⊥]*College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China;*

[a] The authors make equal contribution to the paper.

Corresponding Author

[*] Phone: 86-571-8898-2341; E-mail: wliu@zju.edu.cn. Address: College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China.

23 **Materials and Methods**

24 **Assessment of cell viability**

25 JEG-3 cells in exponential growth were seeded in 96-well plates at an initial density of
26 1000 cells per well. After attachment for 1 day, the experimental medium was changed to the
27 dosing medium with different concentrations of rac-BF, ranging from 10^{-7} to 10^{-5} mol/L.
28 After 4 days of exposure, thiazolyl blue [MTT, (3-(4,5-dimethylthiazol-2-yl)
29 -2,5-diphenyltetrazolium bromide; Amresco, Solon, OH, USA] solution [5 mg/mL in
30 phosphate-buffered saline (PBS)] was added to terminate cell growth, followed by incubation
31 at 37 ºC for 4 h. The solution was then removed, and 150 µl dimethylsulfoxide was added to
32 each well. After shaking with a micromixer for 10 min, the absorbance was measured at 490
33 nm using a Bio-Rad Model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA,
34 USA). The viability of JEG-3 cells after exposure to 5×10^{-6} mol/L rac-BF or the two
35 enantiomers was also determined every 24 h for 4 days using the same method. Each
36 treatment was performed at least 4 times. The results were expressed as the relative viability,
37 which was the OD490 value of each exposure group relative to the vehicle control.

38

39 **Real-time quantitative PCR**

40 Total RNA was extracted with TRIzol reagent (Gibco BRL, Grand Island, NY, USA),
41 and the RNA quality was evaluated by the ratio of the absorbance at 260 nm and 280 nm as
42 well as agarose gel electrophoresis. Reverse transcription was carried out using a qPCR RT
43 Kit (Toyobo, Shanghai, China). Specific oligonucleotide primers of progesterone receptor
44 (PR), human leukocyte antigen G (HLA-G), GnRH type-I (GnRHI), GnRH receptor type-I

45 (GnRHRI), cytochrome P450c17 (CYP17), cytochrome P450c19 (CYP19), 3

46 beta-hydroxysteroid dehydrogenase (3βHSD), 17 beta-hydroxysteroid dehydrogenase

47 (17βHSD) and the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (GAPDH)

48 were designed by beacon designer 2.1 software (**for sequences, see Table S1**) and

49 synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai,

50 China). The real-time quantitative PCR reactions were performed on a 7300 real-time PCR

51 system (Applied Biosystems, Foster City, CA, USA) using Realtime PCR Master Mix kit

52 (Toyobo, Shanghai, China) with the follow protocol: denaturation (95 ºC for 10 s),

53 amplification and quantification for 40 cycles (95 ºC for 5 s, and then 60 ºC for 30 s). Data

54 were collected at the end of each extension step. Each treatment was performed at least in

55 triplicate and the relative expression level was analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak and

56 Schmittgen 2001).

57

58 **References**

59 Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time

60 quantitative PCR and the 2(T)(-Delta Delta C) method. Methods 25(4): 402-408.

61 **Table S1** Primer sequences for real-time quantitative PCR.

Gene	Sequence of primers
3β-HSD	Forward 5′-TTCCAGAGGCTCTTCTTCGTG-3′
	Reverse 5′-TGCCAGTCTTCATCTACACCA-3′
17β-HSD	Forward 5′-TGTGTCTCCCACGCAATCTC-3′
	Reverse 5′-CTCCCTCTGACCAGCAACC-3′
CYP17	Forward 5′-TCACCGATGCTGGAGTCAAC-3′
	Reverse 5′-AGCCGCACACCAACTATCAG-3′
CYP19	Forward 5′-TGGTGGAATCGGGTCTTTATGG-3′
	Reverse 5′-AGG TGCTATTGGTCATCTGCTC-3′
GAPDH	Forward 5′-AAATCAAGTGGGGCGATGCTG-3′
	Reverse 5′-GCAGAGATGATGACCCTTTTG-3′
GnRHI	Forward 5′-GCCTTA GAATGA AGCCAATTCAA-3′
	Reverse 5′-TCCACGCACGAAGTCAGTAGA-3′
GnRHRI	Forward 5′-ACCGCTCCCTGGCTATCAC-3′
	Reverse 5′-ACTGTCCGACTTTGCTGTTGCT-3′
HLA-G	Forward 5′-GGGCAGGGAAGACTGCTT-3′
	Reverse 5′-AGCTGTGGTGGTGCCTTC-3′
PR	Forward 5′-CCCCAATGAGACCAATGAAATC-3′
	Reverse 5′-CAGCCCATCTTTGATGAGCTT-3′

62

63