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Supplemental Material

Effects of Common Pesticides on Prostaglandin D2 (PGD2) Inhibition in SC5 Mouse Sertoli Cells, Evidence of Binding at the COX2 Active Site, and Implications for Endocrine Disruption

Subramaniam Kugathas, Karine Audouze, Sibylle Ermler, Frances Orton, Erika Rosivatz, Martin Scholze, and Andreas Kortenkamp

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Figure S3: Lack of cytotoxicity of test agents in the concentration ranges associated with PGD2 suppression. Shown are MTT responses (absorbance at 570 nm) in SC5 cells normalised to solvent-treated controls (absorbance readings in controls were set to 100%). The range associated with PGD2 suppression is depicted by the grey boxes. A clear downward trend in responses was not seen over the concentration ranges investigated.

Figure S4: Time course of PGD2 suppression in SC5 cells by OPP, ibuprofen and aspirin. SC5 cells were exposed to ibuprofen (IBU), aspirin (ASP) and o-phenylphenol (OPP) at their respective IC₅₀ concentrations (128 nM, 3426 nM and 175 nM of ibuprofen, aspirin and OPP, respectively) for the indicated time periods, and PGD2 concentrations measured. For each time point, PGD2 levels (average of 3 experiments performed in duplicate) are expressed as percentage of solvent controls. The last time point is 24 hours.

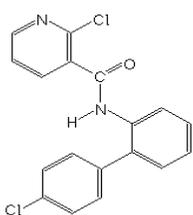
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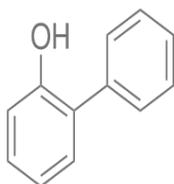
References

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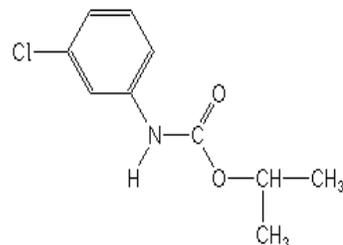
Pesticides inhibiting PGD2 synthesis in SC5 cells



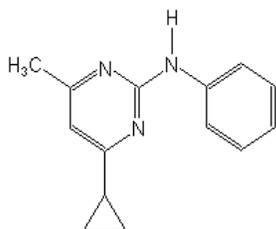
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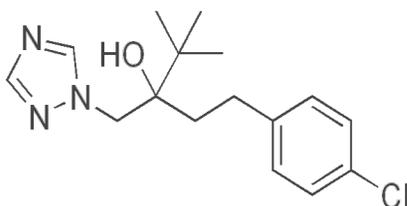
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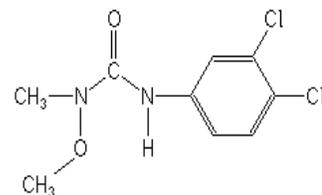
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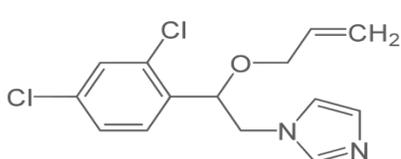
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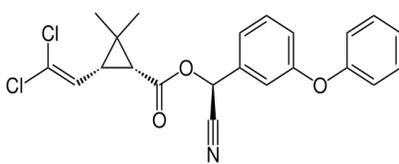
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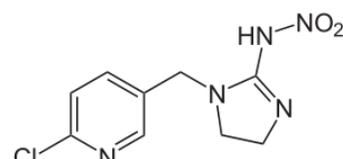
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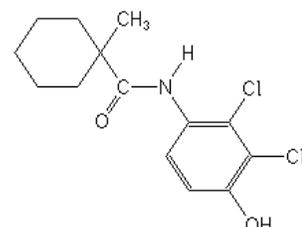
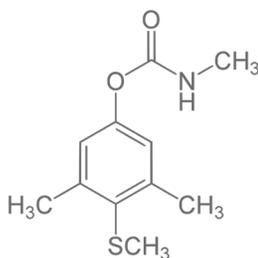
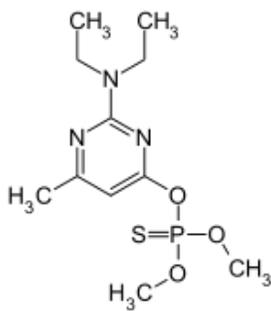
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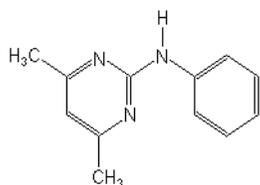
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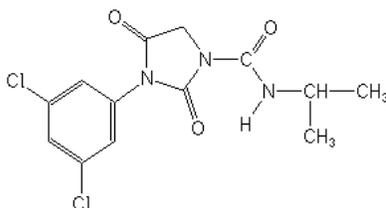
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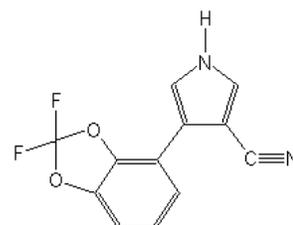
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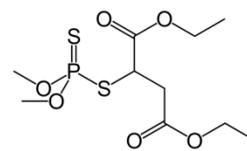
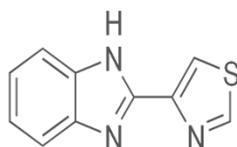
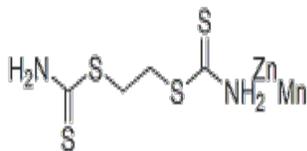


Pyrimethanil

Iprodione

Fludioxonil

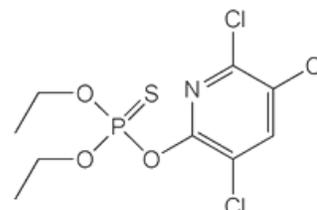
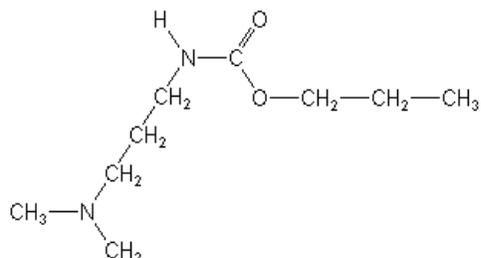
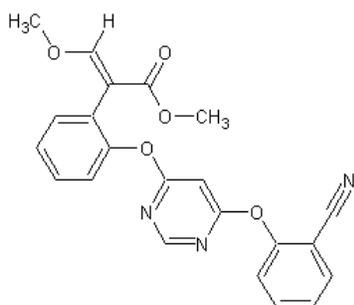
Pesticides tested but not inhibiting PGD2 synthesis in SC5 cells



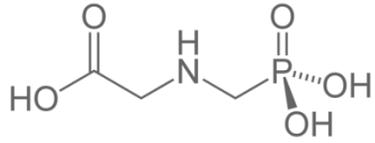
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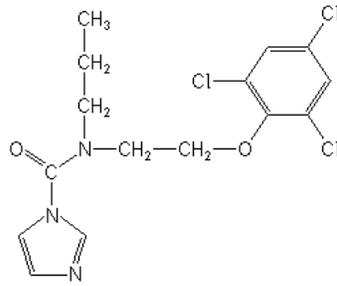
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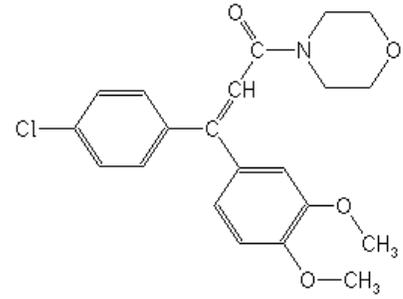
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Glyphosate

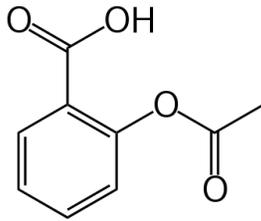


Prochloraz

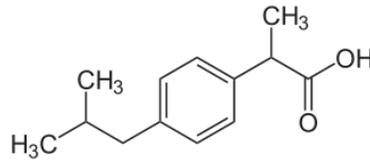


Dimethomorph

Common NSAIDs



Aspirin



Ibuprofen

Figure S2: Suppression of PGD2 synthesis by pesticides in the mouse Sertoli cell assay

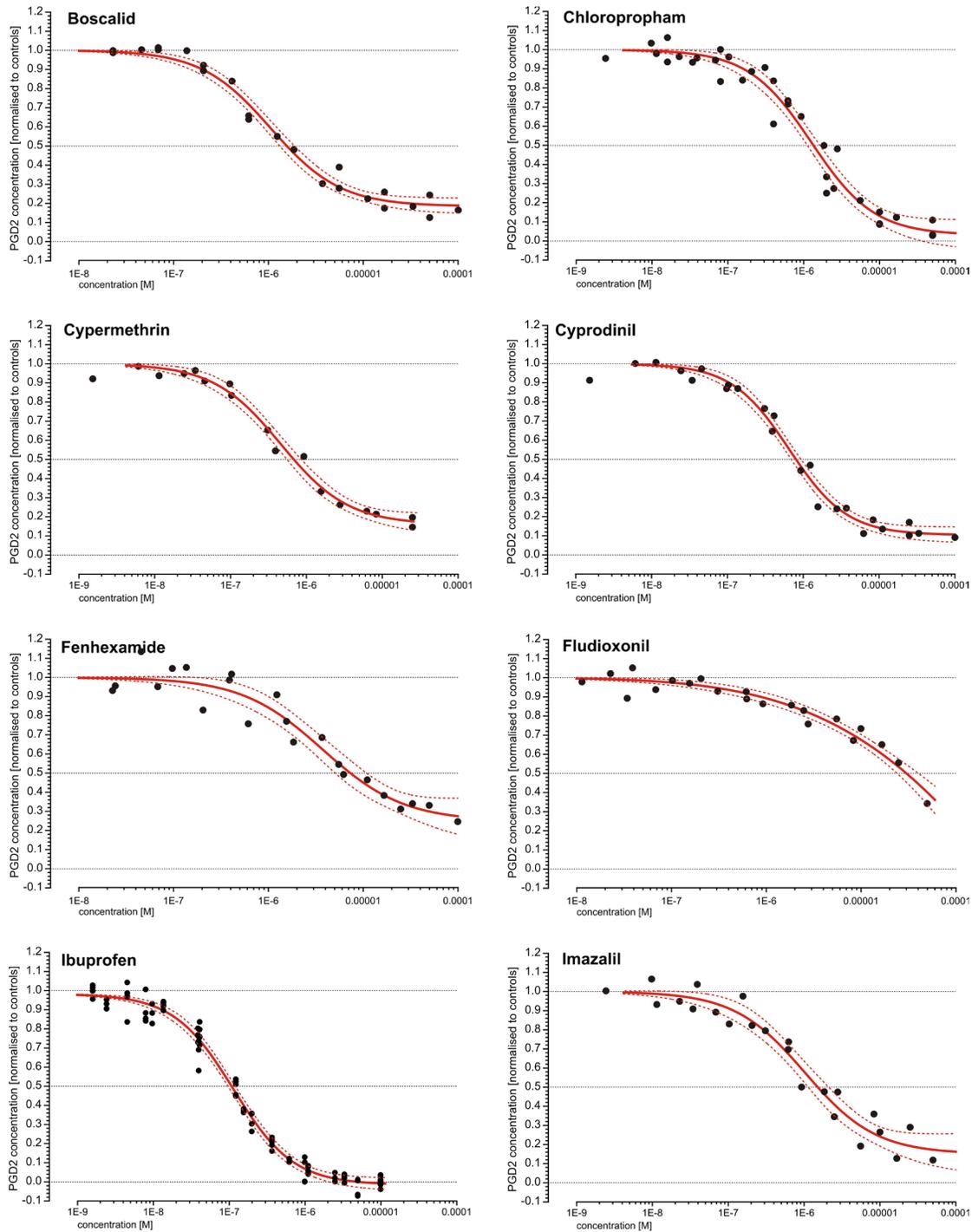
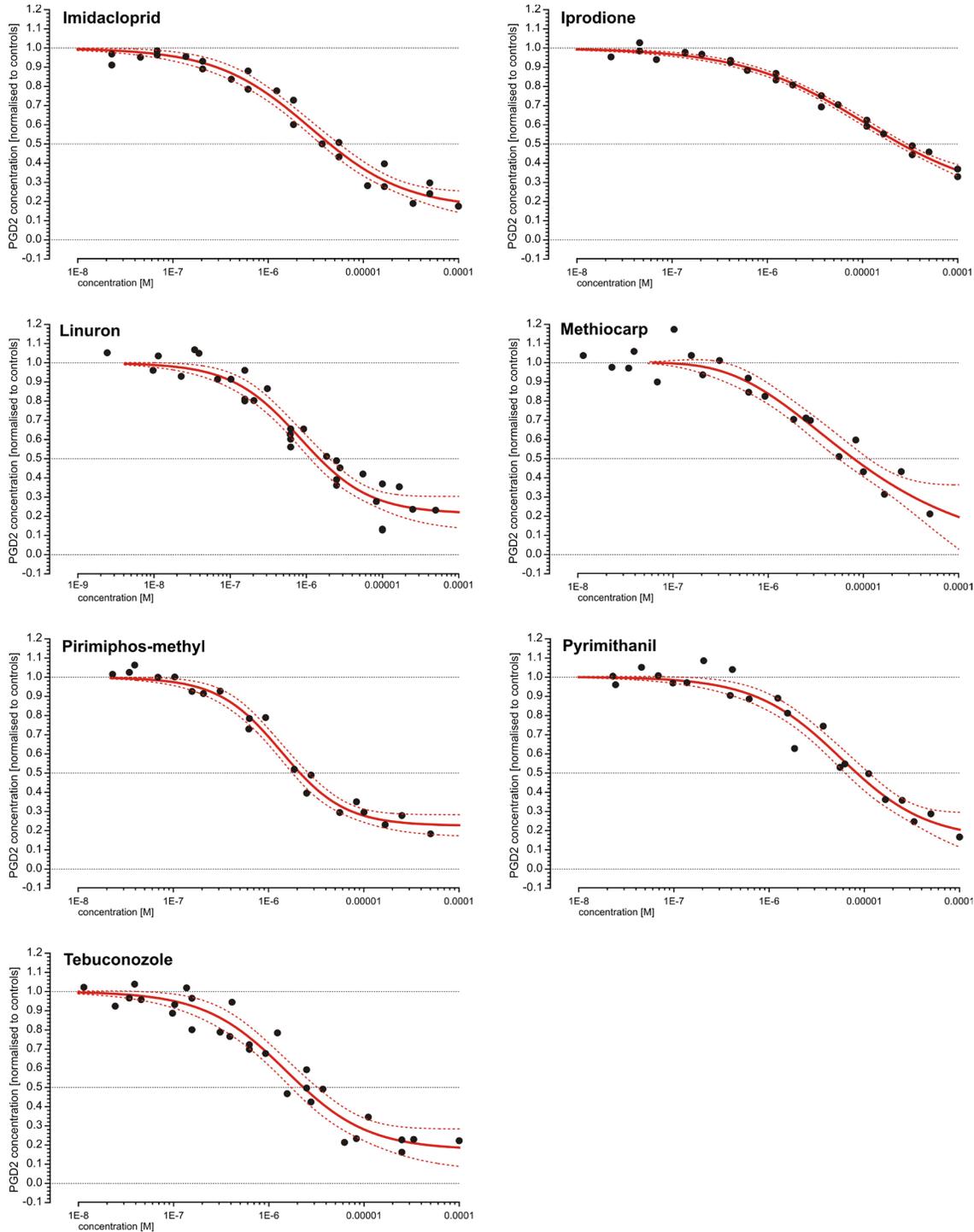
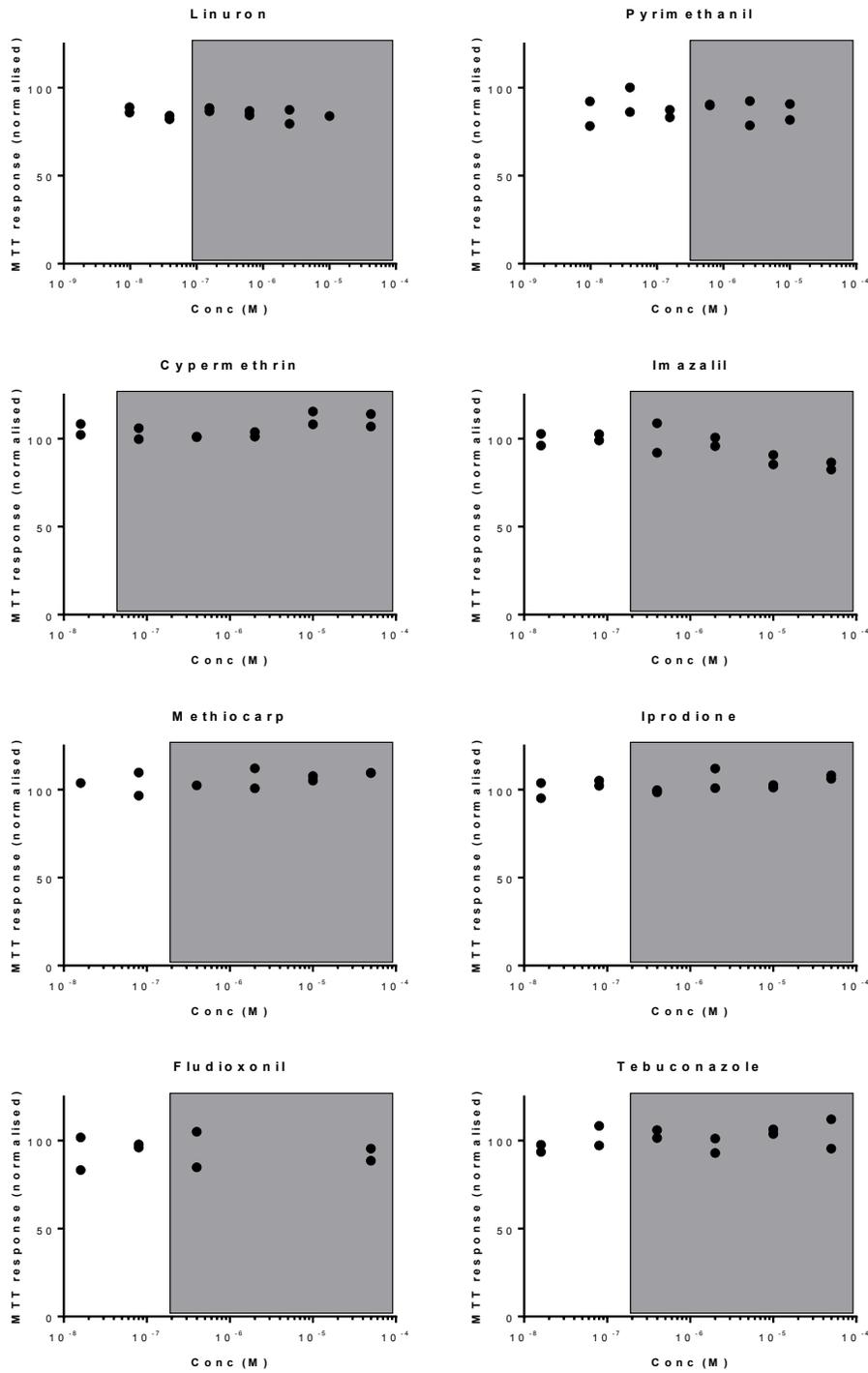


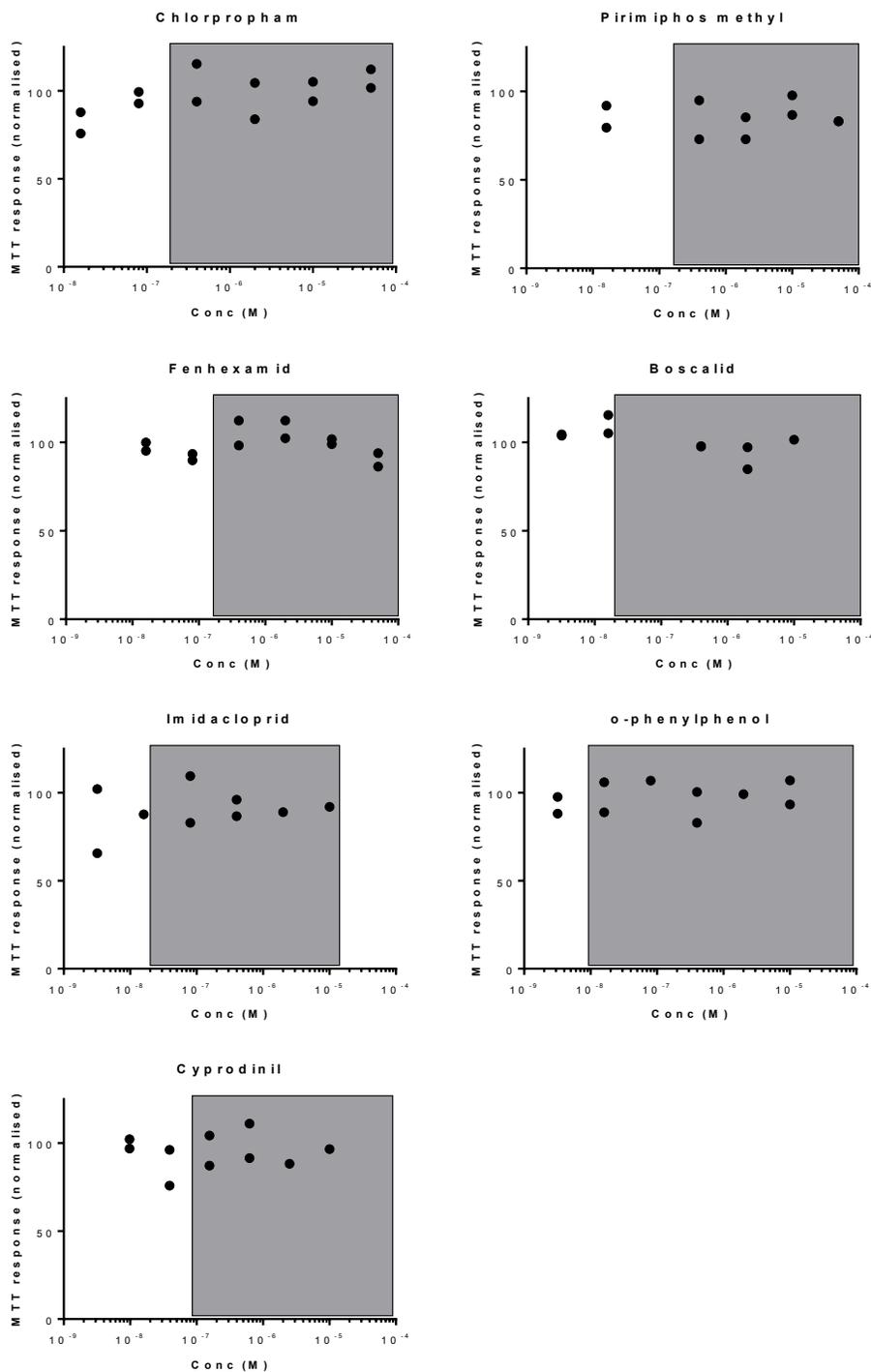
Figure S2 (contd):



The graphs show data, best-fitting models (solid line) and 95% confidence belts (dashes line) for PGD2 synthesis inhibiting responses in SC5 (mouse Sertoli) cells after 24 hours exposure (3 replicates). All data were normalised to those of solvent controls.

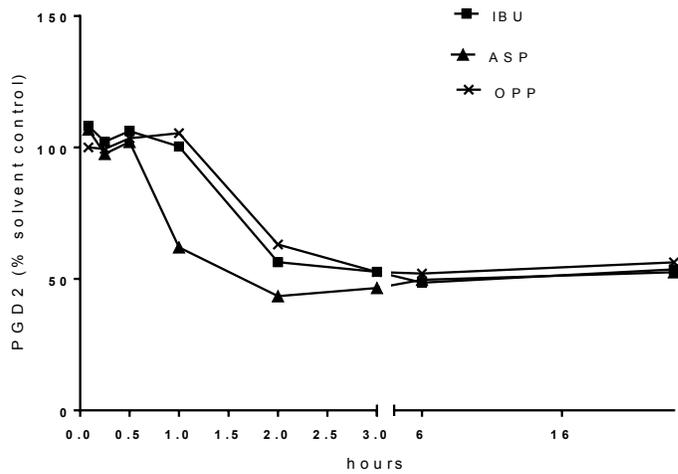
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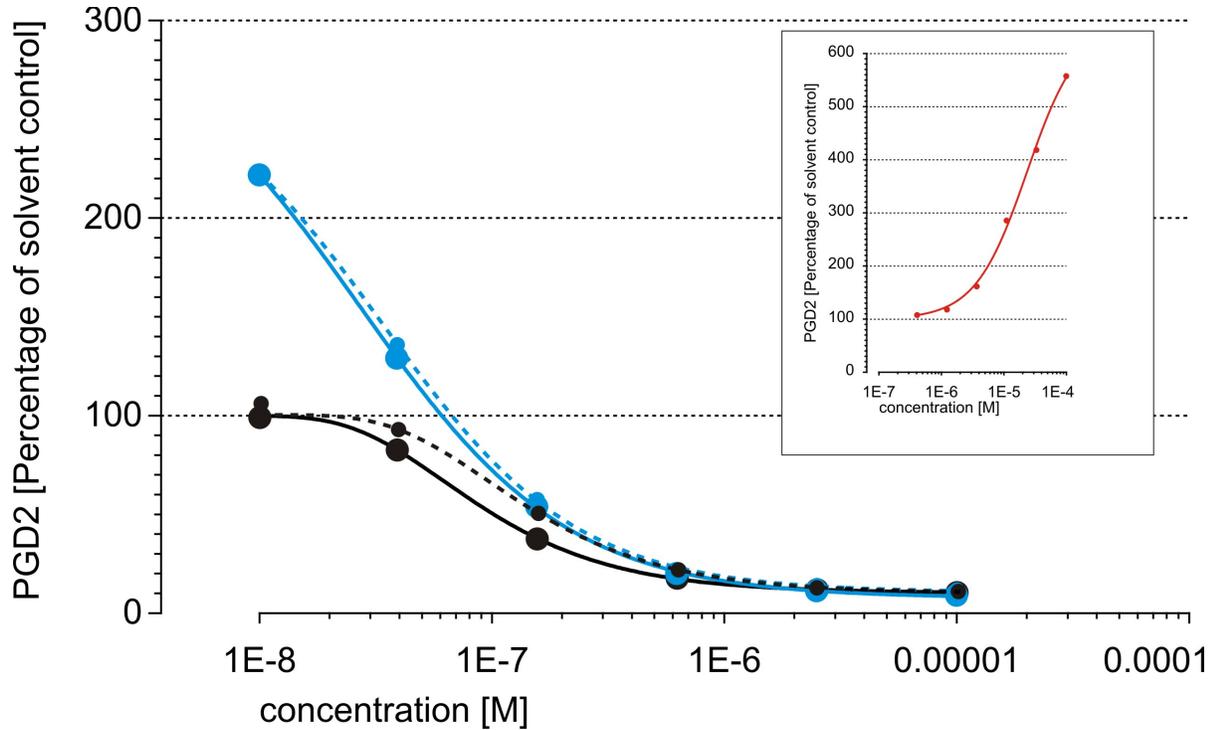
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PGD2 synthesis in mouse Sertoli cells after 24 h exposure to ibuprofen (black solid line) and o-phenylphenol (black dashed line) without AA. Supplementation of AA (10 μ M) during the last 2 hours of the 24 h exposure duration, with ibuprofen (blue solid line) or o-phenylphenol (blue dashed line). The inset figure (red line) shows the dose response curve of AA stimulation of PGD2 synthesis. The graphs show mean responses (dots, n=3) and their best-fitting regression models. Data were normalised against solvent controls.

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Compound	IC ₅₀ (M)	
	PGD2 inhibition	AR-antagonism
Ethylparaben	7.59E-06	1.11E-04
<i>n</i> -Propylparaben	2.85E-06	7.01E-05
<i>n</i> -Butylparaben	2.43E-06	4.11E-05
Benzophenone 3	1.97E-07	1.79E-05
Bisphenol A	2.72E-06	4.07E-06
Flutamide	1.87E-06	1.56E-06
<i>p,p'</i> -DDE	6.11E-06	2.70E-06
Cyprodinil	8.03E-07	2.81E-05
Imazalil	1.51E-06	8.30E-06
Pirimiphos-methyl	2.14E-06	1.71E-05
Pyrimethanil	8.27E-06	9.86E-05
Fludioxonil	3.02E-05	2.62E-06
Fenhexamid	7.37E-06	7.08E-06
Chlorpropham	1.34E-06	1.92E-05
o-Phenylphenol	1.75E-07	9.57E-06
Methiocarb	7.85E-06	1.48E-05
Tebuconazole	2.32E-06	8.06E-06
Linuron	1.49E-06	6.63E-06

References

Ermler S, Scholze M, Kortenkamp A. 2011. The suitability of concentration addition for predicting the effects of multi-component mixtures of up to 17 anti-androgens with varied structural features in an in vitro AR antagonism assay. *Toxicol Appl Pharmacol* 257(2):189-197.

Kristensen DM, Skalkam ML, Audouze K, Lesné L, Desdoits-Lethimonier C, Frederiksen H et al. 2011b. Many putative endocrine disruptors inhibit prostaglandin synthesis. *Environ Health Perspect* 119(4):534-541.

Orton F, Rosivatz E, Scholze M, Kortenkamp A. 2011. Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens. *Environ Health Perspect* 119:794-800.