Developmental neurotoxicity of fipronil and rotenone on a human neuronal in vitro test system

Online supplementary material

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Running title: developmental toxicity of fipronil in vitro

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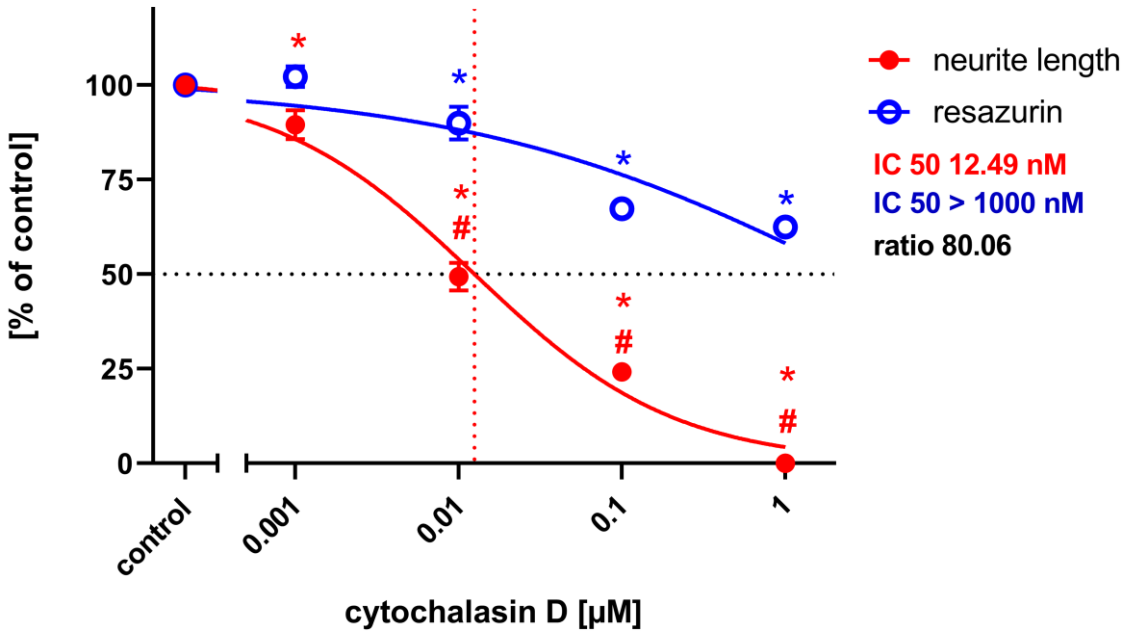
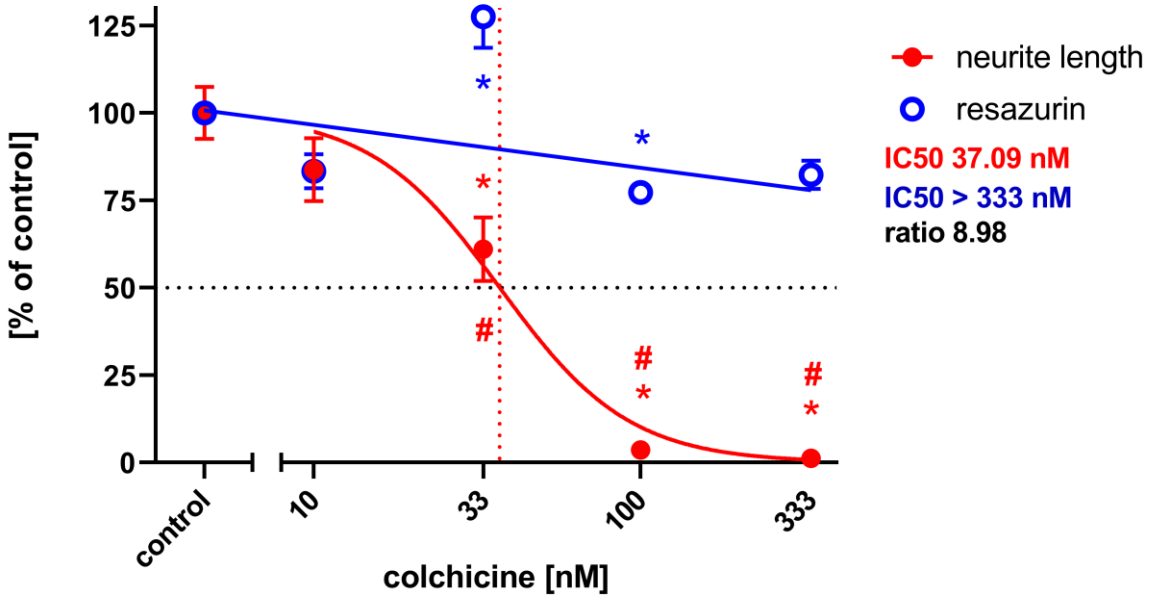
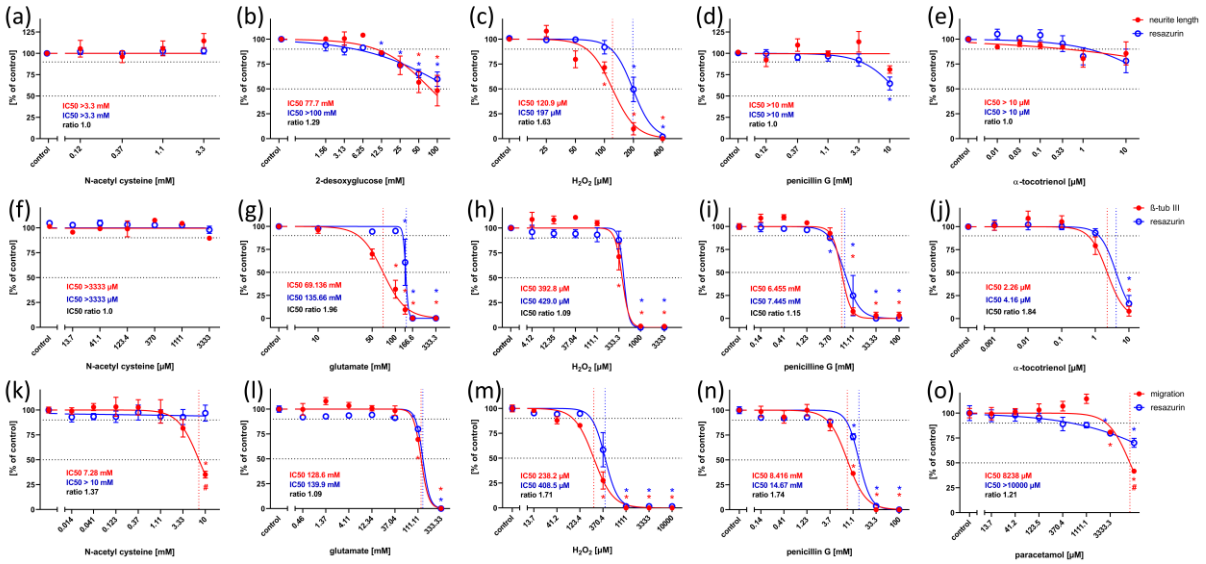
(a)



(b)



Online supplementary material Fig. 1 Neurite outgrowth assay, concentration-response curves of endpoint specific control compounds: (**a) Cytochalasin D** specifically inhibits neurite outgrowth. (**b) Colchicine** specifically inhibits neurite outgrowth. Each value is the average ± S.E.M. of three independent experiments normalized to the solvent control (0.25% DMSO). Hollow blue circles: general cytotoxicity (resazurin), filled red circles: total neurite length/neuron. Asterisks (*) indicate significant differences (at least $p<0.05$) from controls, (#) indicate significant differences between viability and neurite length at that concentration.



Online supplementary material Fig. 2 Concentration-response curves of five unspecifically toxic compounds for all three endpoints: **neurite outgrowth (a-e), neuronal differentiation (f-j), neural precursor cell migration (k-o).** Each value is the average ± S.E.M. of three independent experiments normalized to the solvent control (0.25% DMSO). Hollow blue circles: general cytotoxicity (resazurin), filled red circles: specific endpoint. Asterisks (*) indicate significant differences (at least $p<0.05$) from controls, (#) indicate significant differences between viability and neurite length at that concentration.

	neurite outgrowth		migration		neuronal differentiation	
	compound	ratio	compound	ratio	compound	ratio
	TOC	1,00	AAP*	1,21	PG*	1,15
	NAC	1,00	PG*	1,74	Glu*	1,96
	PenG	1,00	Glu*	1,09	NAC	1,00
	H_2O_2	1,63	NAC	1,37	TOC	1,84
	2DG	1,29	H_2O_2	1,71	H_2O_2	1,09
mean		1,18		1,42		1,41
s.d.		0,28		0,29		0,45
3x s.d.		0,84		0,88		1,36
mean + 3x s.d.		**2,02**		**2,30**		**2,77**
*: data taken from Stern et al. (2014) Arch. Toxicol. 88: 127–136						

Online supplementary material Table 1 Unspecific compounds: IC50 ratios between general cytotoxicity measured by resazurin reduction assay and specific endpoints. Each value is the ratio IC50 unspecific/IC50 specific derived from concentration-dependence curves (Online supplementary material Fig. 2) from three independent experiments on NT2 cultures as described in the materials and methods section. The mean + 3 standard deviations (s.d.) of five different unspecific compounds are used as threshold IC50 ratios. According to Krug et al. (2013), we consider a chemical compound DNT positive for any given endpoint, when the IC50 ratio exceeds this threshold. 2DG: 2-desoxy glucose, AAP: acetaminophen (paracetamol), Glu: sodium glutamate, H_2O_2: hydrogen peroxide, NAC: n-acetyl cysteine, PG: penicillin G, TOC: α-tocotrienol.

References:

Krug AK, Balmer NV, Matt F, Schonenberger F, Merhof D, Leist M (2013) Evaluation of a human neurite growth assay as specific screen for developmental neurotoxicants Arch Toxicol 87:2215-2231 doi:10.1007/s00204-013-1072-y

Stern M, Gierse A, Tan S, Bicker G (2014) Human Ntera2 cells as a predictive in vitro test system for developmental neurotoxicity Arch Toxicol 88:127-136 doi:10.1007/s00204-013-1098-1