

**The cytotoxic effects of a single and combined exposure to the mycotoxins, deoxynivalenol and zearalenone on a rat
Leydig cell line (LC-540)**

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Supplementary file

DON Dose-Response Curve

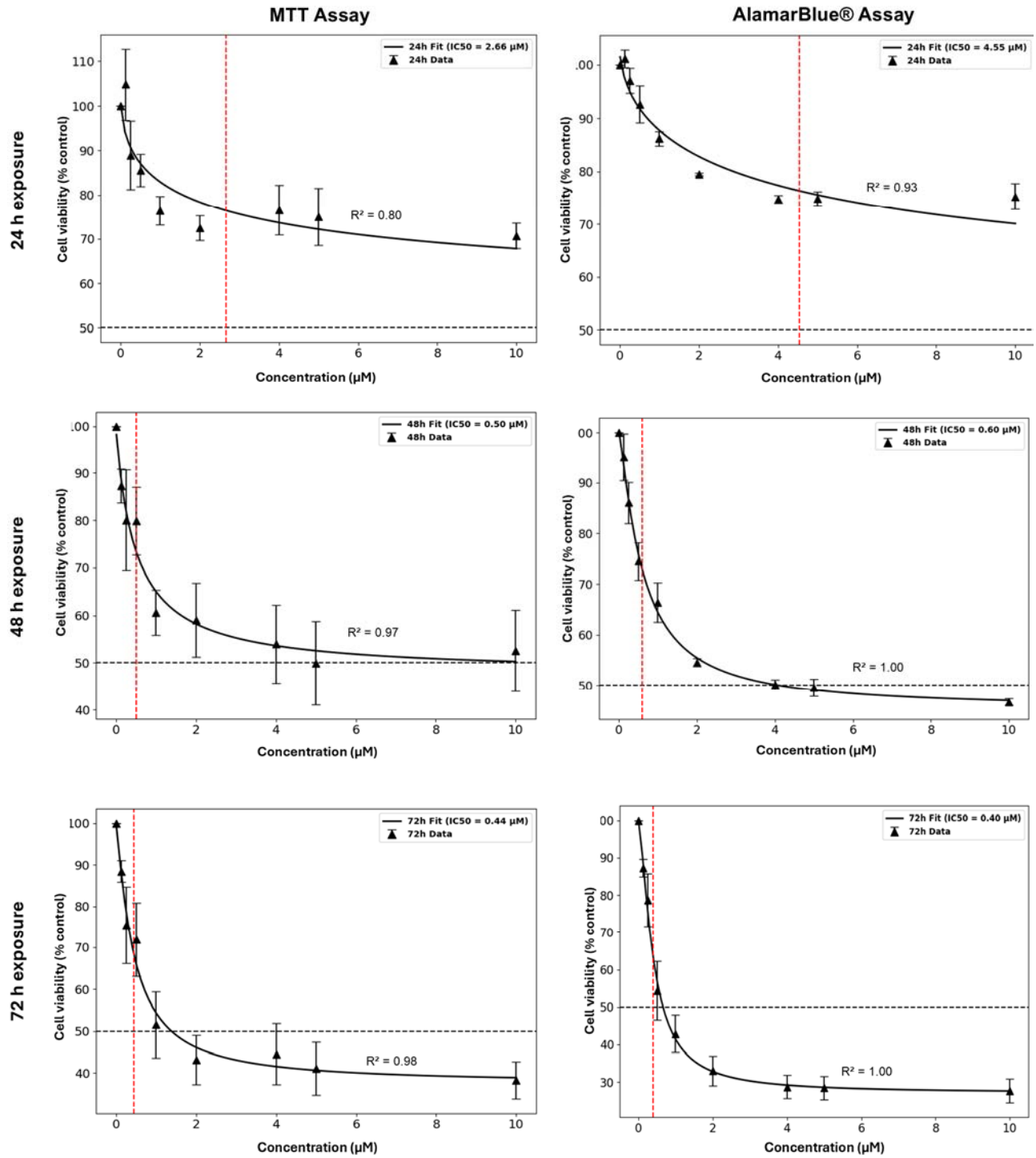


Fig. S1: : Dose-response curves of deoxynivalenol (DON) on LC-540 Leydig cell viability. Cells were treated with increasing concentrations (0.125 –10 µM) of DON for 24, 48, and 72 hours, and viability was assessed using MTT and AlamarBlue® assays.

ZEA Dose-Response Curve

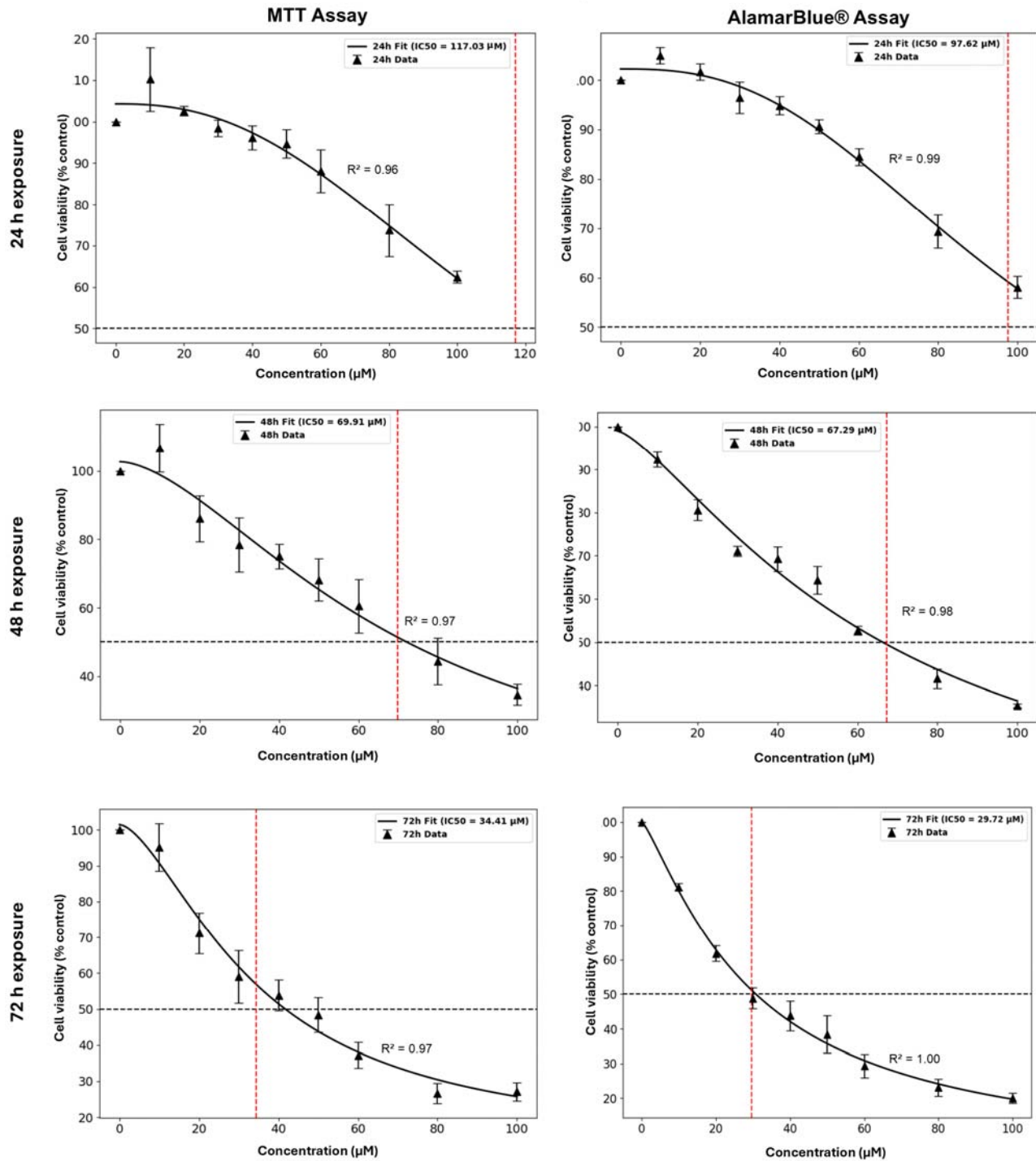


Fig. S2: Dose-response curves of zearalenone (ZEA) on LC-540 Leydig cell viability. Cells were exposed to increasing concentrations (10 –100 µM) of ZEA for 24, 48, and 72 hours. Cell viability was measured using MTT and AlamarBlue® assays.

Table S1: Bliss independence model analysis of zearalenone (ZEA) and deoxynivalenol (DON) interaction.

Concentration μM (DON)	Concentration μM (ZEA)	Exposure period h	Observed viability % (DON)	Observed viability % (ZEA)	Expected Viability % (DON+ZEA)	Observed Viability % (DON + ZEA)	Viability difference	Expected Effect Biss	Observed Effect	Interaction
0.125	10.0	24h	102.97	107.61	110.8	97.18	-13.61	-10.8	-10.8	Synergistic
		48h	91.2	99.54	90.78	81.52	-9.26	9.22	9.22	Synergistic
		72h	87.82	88.19	77.45	64.76	-12.69	22.55	22.55	Synergistic
0.5	20.0	24h	89.08	102.12	90.97	84.44	-6.53	9.03	9.03	Synergistic
		48h	77.21	83.34	64.34	66.36	2.02	35.66	35.66	Additive
		72h	63.18	66.52	42.02	42.36	0.33	57.98	57.98	Additive
2.0	30.0	24h	76.01	97.48	74.1	74.33	0.23	25.9	25.9	Additive
		48h	56.81	74.77	42.47	49.91	7.44	57.53	57.53	Antagonistic
		72h	38.08	53.91	20.53	30.31	9.78	79.47	79.47	Antagonistic
5.0	50.0	24h	74.97	92.64	69.45	70.63	1.18	30.55	30.55	Additive
		48h	49.71	66.3	32.96	43.63	10.67	67.04	67.04	Antagonistic
		72h	34.79	43.39	15.1	24.42	9.32	84.9	84.9	Antagonistic

Synergistic interaction: Observed viability < Expected viability (difference > 5%); Additive interaction: Observed viability \approx Expected viability (difference \pm 5%),

Antagonistic interaction: Observed viability > Expected viability (difference > 5%).