Supplementary Information

Sonochemically Synthesized CuO NPs: Individual and Synergistic Toxicity Assessment with Sodium Arsenite in Adult Zebrafish

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Supplementary Methodology

1.0 Bliss independence model

The synergistic and antagonistic interaction between $NaAsO_2$ and CuO-NPs were quantified using Bliss independence model. It assumes that two toxicants acts independently through distinct mechanisms [1]. For each biochemical endpoints, the data were normalized to fractional responses (0-1 scale). The expected combined effects under independent action was calculated as:

$$\hat{y}_{AB} = y_A + y_B - y_A y_B$$

where y_A and y_B denote the fractional effects induced by $NaAsO_2$ and CuO-NPs alone respectively, and $\hat{y}_{AB}$ is the predicted effect under independent action [2]

Mathematical test for synergy and antagonism

The observed combined effect (y_{AB}) was obtained experimentally from the co-exposure group ($NaAsO_2$ + CuO-NPs). The interaction term is calculated as:

$$\Delta_{Bliss} = y_{AB} - \hat{y}_{AB}$$

or equivalently referred to as the Bliss Synergy Index (BSI):

$$BSI = y_{AB} - (y_A + y_B - y_A y_B)$$

According to the established criteria, BSI > 0 indicates synergistic interaction, BSI = 0 indicates additive (independent) interaction, and BSI < 0 indicates antagonistic interaction [2, 3] . This deviation-based formulation is applicable to single-concentration mixture designs.

Supplementary Tables

Table S1 Average crystallite size calculation using Debye Scherrer equation									
2θ	FWHM (Deg)	(θ)	Cos θ	FWHM (Rad)	βCos θ	Kλ	kλ/βCos θ (A)	D (nm)	D (nm)
32.5	0.852	16.25	0.960	0.015	0.014	1.386	97.069	9.706	Average particle size is 10.56 nm
35.4	35.511	17.70	0.952	0.620	0.590	1.386	2.349	0.235	
35.5	0.505	17.75	0.952	0.009	0.008	1.386	165.333	16.533	
38.7	38.756	19.35	0.944	0.677	0.638	1.386	2.171	0.217	
38.9	0.486	19.45	0.942	0.009	0.008	1.386	173.352	17.335	
46.1	0.638	23.05	0.920	0.011	0.010	1.386	135.243	13.524	
48.8	0.526	24.40	0.910	0.009	0.008	1.386	165.791	16.579	
51.3	2.193	25.65	0.901	0.038	0.035	1.386	40.197	4.020	
53.5	1.098	26.75	0.892	0.019	0.017	1.386	81.076	8.107	
58.3	0.719	29.15	0.873	0.013	0.011	1.386	126.556	12.656	
61.5	0.612	30.75	0.859	0.011	0.009	1.386	151.111	15.111	
65.2	0.724	32.60	0.842	0.013	0.011	1.386	130.237	13.024	
66.3	1.056	33.15	0.837	0.018	0.015	1.386	89.834	8.983	
68.1	0.812	34.05	0.828	0.014	0.012	1.386	118.036	11.803	

Supplementary References

1. C. I. Bliss, *Annals of applied biology*, 1939, **26**, 585-615.
2. Q. Liu, X. Yin, L. R. Languino and D. C. Altieri, *Statistics in biopharmaceutical research*, 2018, **10**, 112-122.
3. W. R. Greco, *Pharmacol Rev*, 1995, **47**, 331-385.