Supplementary Information (SI) for Environmental Science: Advances.

Lithium Nickel Manganese Cobalt Oxide Particles Cause Developmental Neurotoxicity in *Caenorhabditis elegans*

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Supplementary information

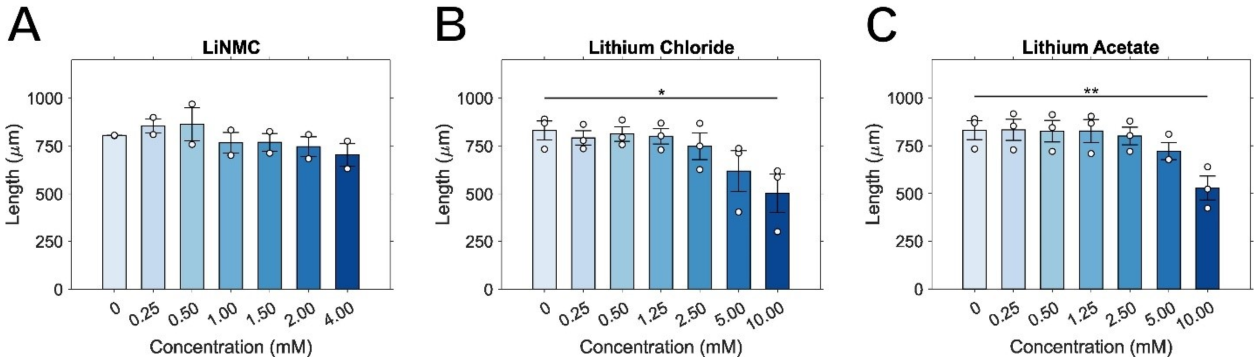
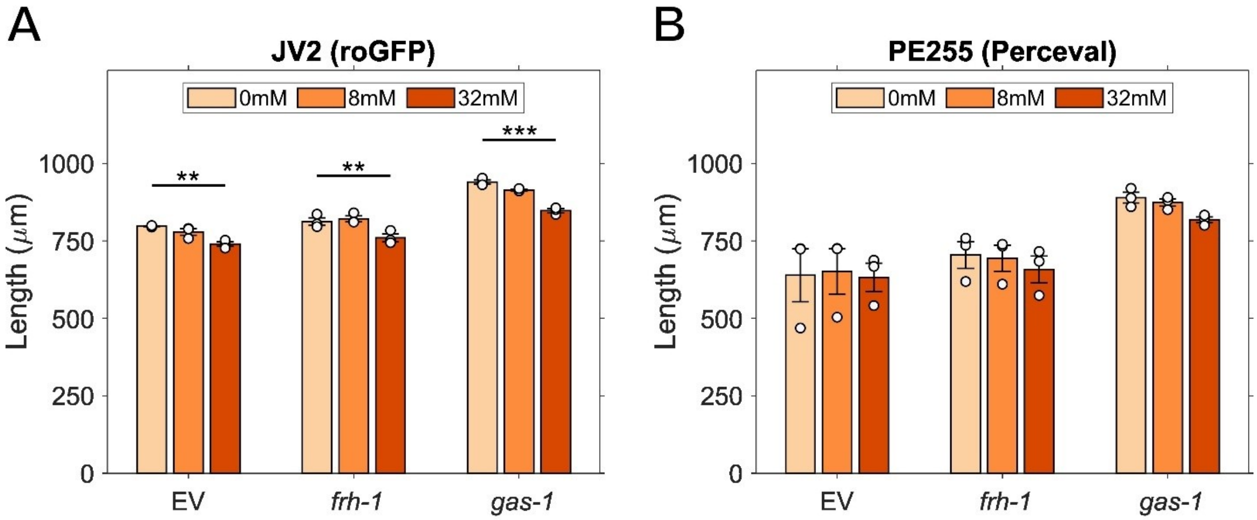


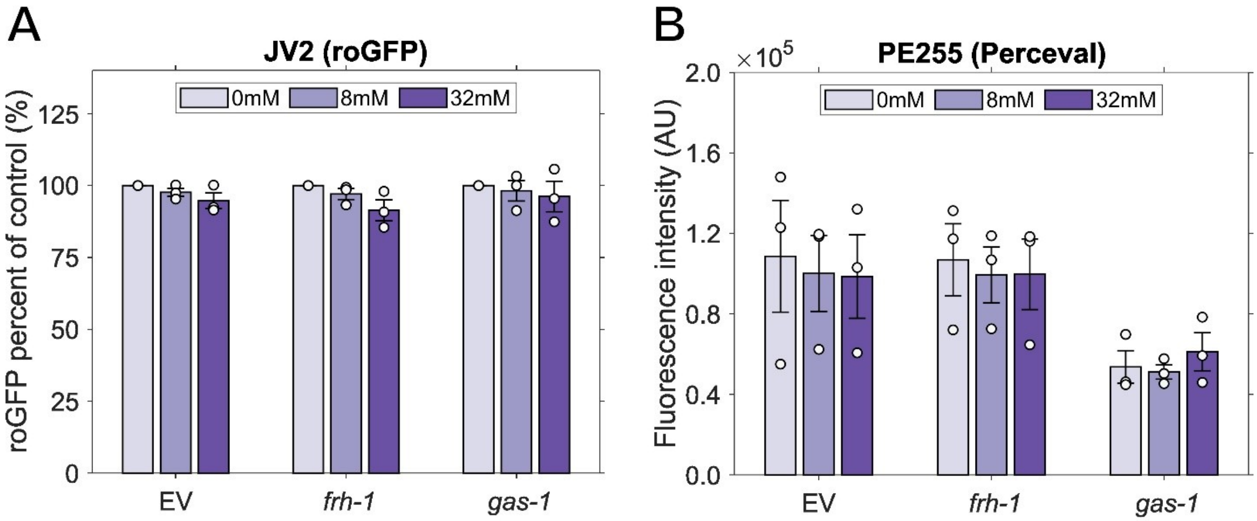
Figure S1: Length of worms after exposure to lithium compounds. Length of worms exposed during larval development to A) LiNMC, B) lithium chloride, or C) lithium acetate in liquid culture. One-way ANOVA with Dunnet's test, error bars are SEM, n = 3 biological replicates for lithium chloride and acetate, 2 biological replicates for LiNMC (150 - 300 worms per treatment), (*) $p < 0.05$, (**) $p < 0.01$.



C

	JV2 length	PE255 length
RNAi	< 0.001	< 0.001
LiNMC concentration	< 0.001	0.521
Interaction	0.090	0.975

Figure S2: Length of worms (not normalized) after exposure to LiNMC and fed RNAi. A) JV2 strain (roGFP reporter) and B) PE255 strain (Perceval reporter). C) Summary of the main effects of RNAi and LiNMC concentration in chemical uptake in worms. Two-way ANOVA with Tukey-HSD test, n = 3 biological replicates (100 worms per treatment), error bars are SEM, (**) $p < 0.01$, (***) $p < 0.001$. Note that *gas-1* worms were grown for 72 hours, vs 48 hours for N2 and *frh-1*, which is why they are larger (growth of *gas-1* worms is approximately 12 hours delayed at 48 hours post-hatch).



C

	roGFP	Perceval
RNAi	0.051	0.004
LiNMC concentration	0.676	0.905
Interaction	0.933	0.987

Figure S3: roGFP and ATP (not normalized) after exposure to LiNMC and fed RNAi. A) JV2 strain (roGFP reporter) and B) PE255 strain (Perceval reporter). C) Summary of the main effects of RNAi and LiNMC concentration in chemical uptake in worms. Two-way ANOVA with Tukey-HSD test, n = 3 biological replicates (100 worms per treatment).

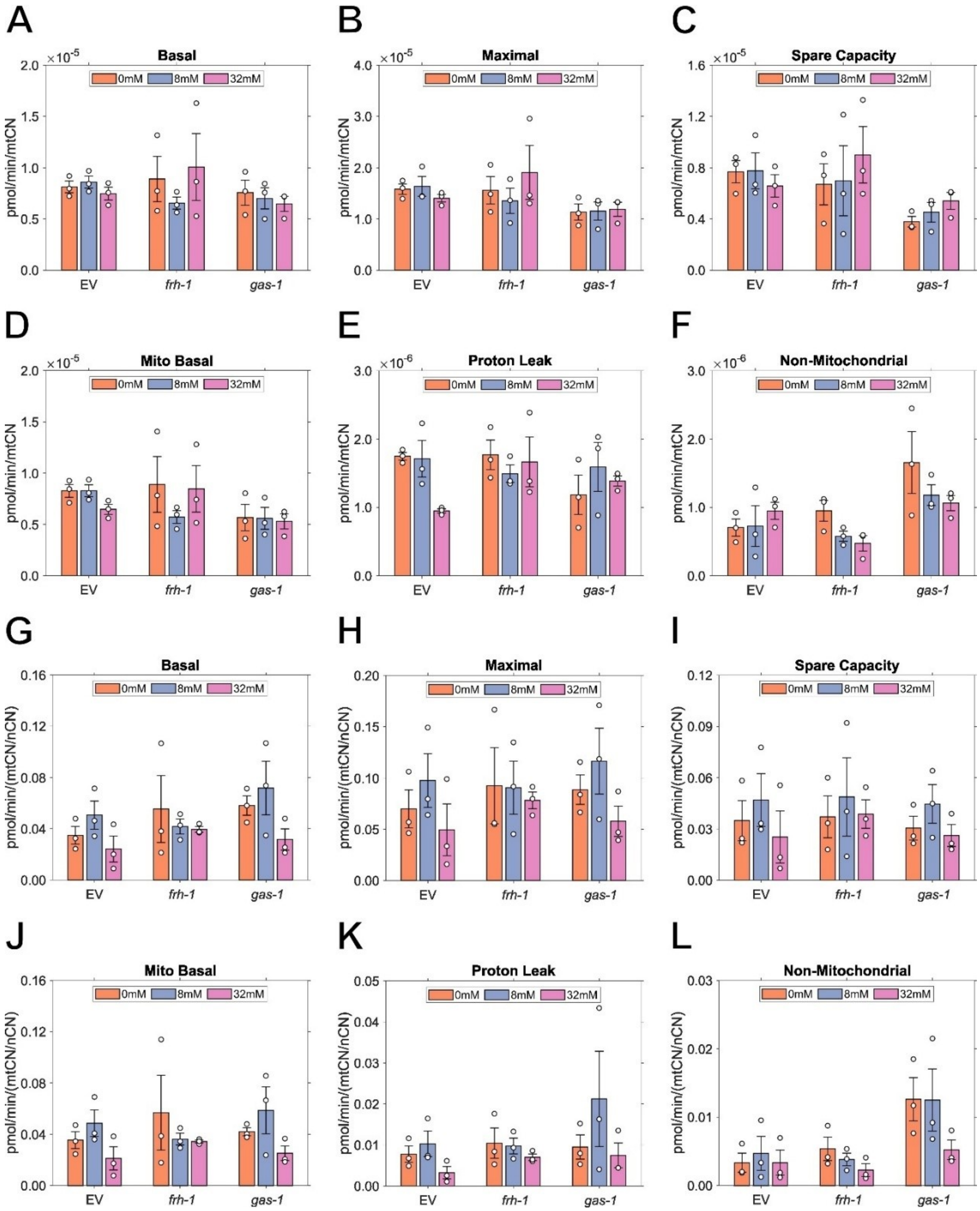


Figure S4: Additional OCR normalized to mtDNA copy number and the mtDNA to nDNA copy number ratio. Comparison of A) basal OCR, B) maximal OCR, C) spare OCR capacity, D) mitochondrial basal OCR, E) proton leak, and F) non-mitochondrial respiration between groups exposed to either 0, 8, or 32 mM LiNMC, and fed empty vector (EV), *frh-1* or *gas-1* RNAi bacteria and normalized to mtDNA copy number. Comparison of G) basal OCR, H) maximal OCR, I) spare OCR capacity, J) mitochondrial basal OCR, K) proton leak, and L) non-mitochondrial respiration between groups exposed to either 0, 8, or 32 mM LiNMC, and fed empty vector (EV), *frh-1* or *gas-1* RNAi bacteria and normalized to mitochondrial:nuclear DNA copy number. Two-way ANOVA with Tukey-HSD; error bars are SEM, n = 3 biological replicates (5 technical replicates per biological replicate, 20 - 30 L4 worms per technical replicate).

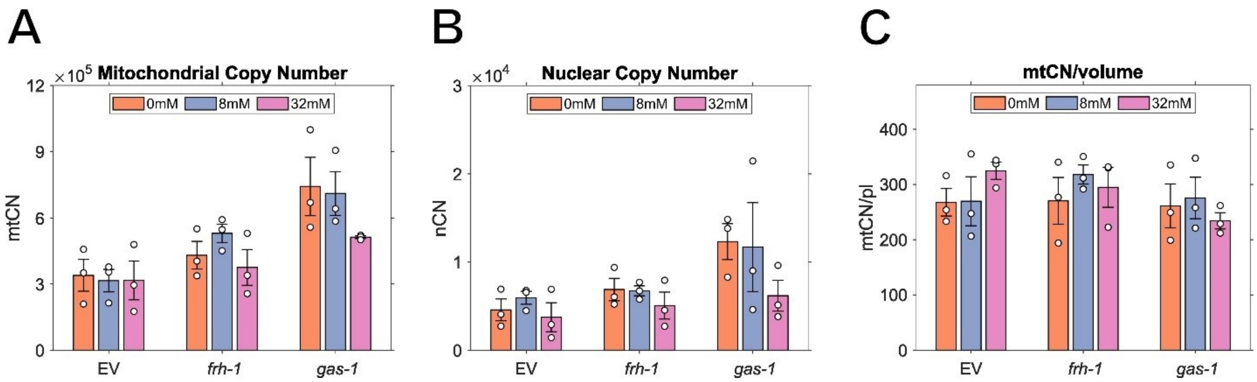


Figure S5: Additional DNA copy number visualization. A) Mitochondrial copy number, B) nuclear copy number, and C) mitochondrial copy number normalized to worm volume. Two-way ANOVA with Tukey-HSD; error bars are SEM, n = 3 biological replicates (5 technical replicates per biological replicate, 6 L4 worms per technical replicate).

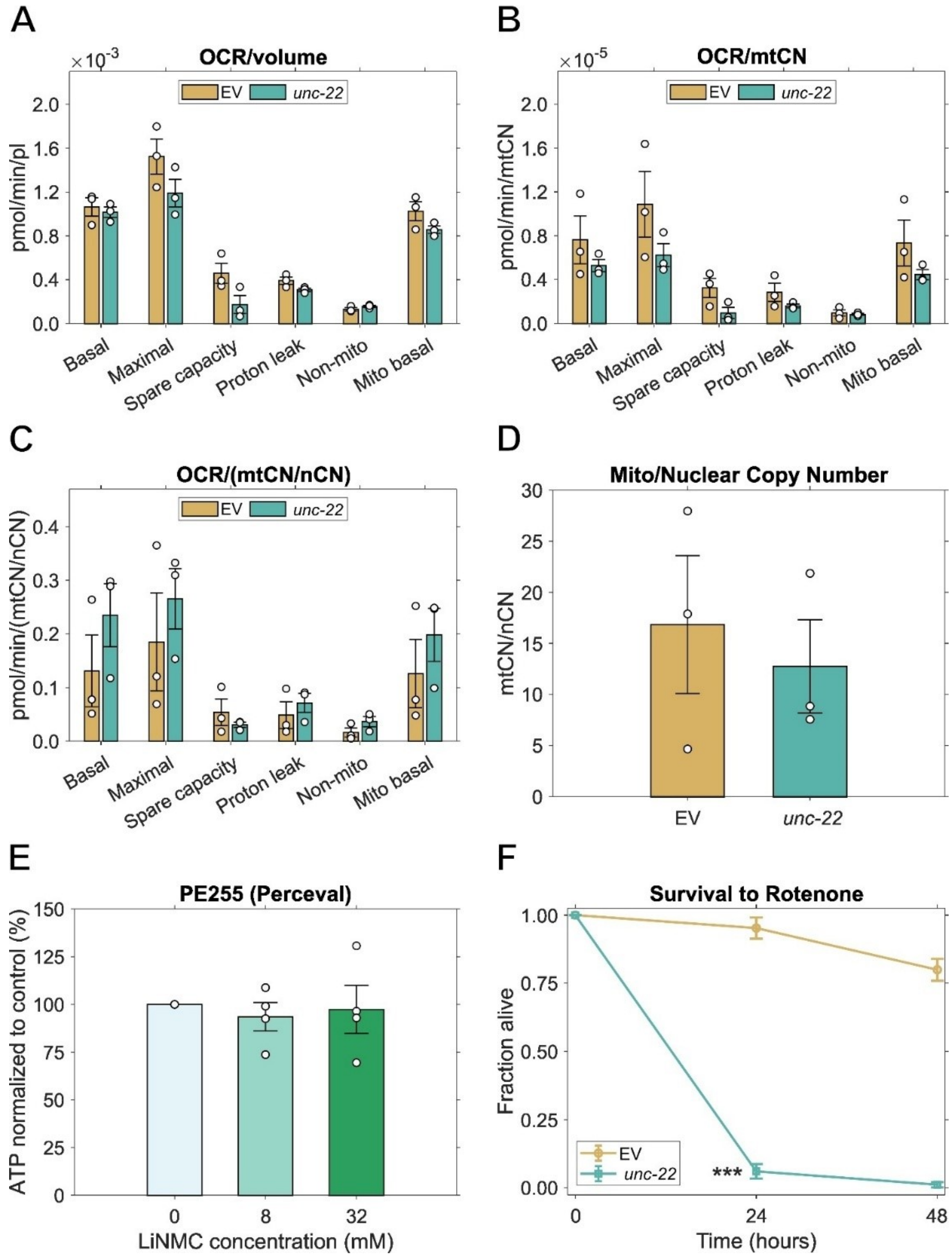


Figure S6: Characterization of the *unc-22* positive control used for RNAi experiments. A) OCR normalized to worm volume, B) OCR normalized to mitochondrial DNA copy number, C) OCR normalized to the mitochondrial:nuclear DNA copy number ratio, D) comparison of mitochondrial:nuclear DNA copy number, E) ATP normalized to control in PE255 strain (Perceval reporter) after exposure to LiNMC, and F) Survival to rotenone curves for worms fed empty vector or *unc-22* RNAi. Two-way ANOVA with Tukey-HSD for A, B, and C; One-way ANOVA with Dunnet's test for D and E; log-rank test for F; Error bars are SEM; n = 3 biological replicates (5 technical replicates per biological replicate, 20 - 30 L4 worms per technical replicate) for A, B, and C; n = 3 biological replicates (5 technical replicates per biological replicate, 6 L4 worms per technical replicate) for D; n = 4 biological replicates for E; and n = 3 biological replicates (100 worms per replicate); (***) $p < 0.001$.

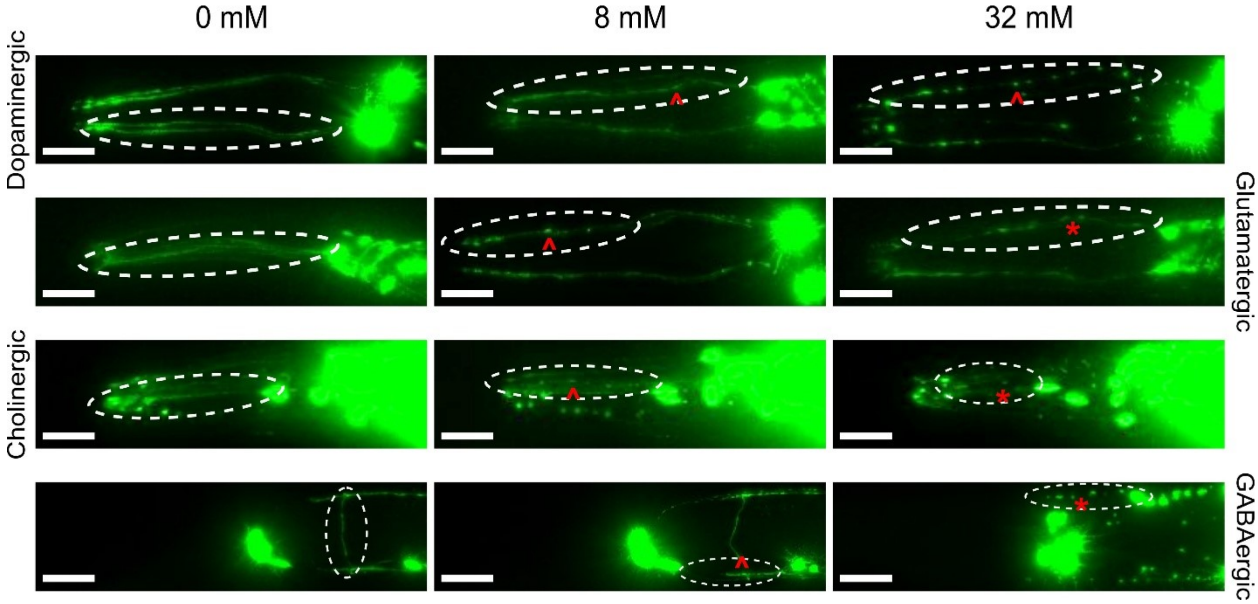


Figure S7: Morphological damage observed in neurons after exposure. Representative images of cephalic neurites under control conditions or after parental exposure to 8 mM or 32 mM LiNMC for dopaminergic, glutamatergic, cholinergic, and GABAergic neurons. White dashed circles delineate the regions scored, with examples of presence of blebs (red arrows) and breaks (red asterisks) for LiNMC exposures. Scale bars 20 μm.

Table S1: Elemental concentrations (μM) in worms exposed to LiNMC and fed RNAi containing bacteria.

	Empty vector			*frh-1* RNAi			*gas-1* RNAi		
	0 mM	**8 mM**	**32 mM**	**0 mM**	**8 mM**	**32 mM**	**0 mM**	**8 mM**	**32 mM**
Na	14703.957	16020.733	19752.947	15299.706	18607.660	20176.830	13096.046	12977.839	16047.997
Mg	4337.347	4980.859	6484.415	4525.568	4396.488	4890.320	3014.032	3322.734	3130.138
Al	145.972	201.150	508.801	94.397	96.863	221.517	48.349	119.093	68.969
P	43461.925	48780.077	50697.465	43895.198	44667.015	46349.289	32036.361	33806.393	34044.398
K	30479.166	35384.848	35808.877	31418.148	32599.155	33579.449	20232.774	20555.479	21252.190
Ca	5409.594	6998.811	77164.112	5884.066	5755.606	6604.098	5774.864	14004.121	6115.173
Fe	83.383	84.513	217.081	83.833	63.354	70.967	62.194	60.972	55.781
Cu	2.065	5.449	6.534	4.507	1.856	2.305	1.709	2.045	2.115
Zn	82.685	96.895	92.236	90.075	84.394	84.658	61.321	62.374	58.844
Se	<0.586	<0.612	<.0.776	<0.454	<0.441	<0.590	<0.271	<0.290	<0.360
Mo	<0.851	<0.890	<1.127	<0.660	<0.641	<0.858	<0.383	<0.419	<0.491