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Supplemental Material

Neurotoxicity of the Parkinson's Disease-Associated Pesticide Ziram Is Synuclein-Dependent in Zebrafish Embryos

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Figure S1: ZF $\gamma 1$ antibody is specific for ZF. Using denaturing conditions/SDS PAGE, a band for $\gamma 1$ was detected at 17 kDa for ZF adult brain (ZF) and purified ZF $\gamma 1$ (a). No ZF $\gamma 1$ signal was detected for mouse brain (MB). Using non-denaturing conditions/Native PAGE, a major band for ZF $\gamma 1$ was detected at 480 kDa and a minor band at 242 kDa (b). Peptide preincubated with the ZF $\gamma 1$ antibody is shown for SDS PAGE. No band for ZF $\gamma 1$ was detected after preincubation of $\gamma 1$ antibody with $\gamma 1$ peptide (c). ZF $\gamma 1$ MO reduced protein levels of ZF $\gamma 1$ as compared to scramble MO as determined by SDS PAGE (d).

Figure S2: Ziram is toxic in a concentration-dependent manner and causes notochord distortion. A significant change in toxicity was observed for embryos treated (24hpf) with 100nM and 1 μ M ziram by day 7 ($n = 50$, $p < .0001$) (a). ZF embryos treated with 50 nM ziram at 5 hpf, were found to have a shorter body axis, pericardial edema, and notochord distortion (b) as compared to vehicle treated ZF (c). $p < .0001$ Log-Rank test.

Figure S3: Ziram treatment results in reduction of TH-1 levels. Using Western blot analysis, the effect of ziram (50nM) on TH-1 levels was investigated. A 63% reduction in TH-1 was observed for embryos treated with 50nM ziram vs. controls (n=4; *p-value = .03 using two-tailed T test). Bars represent standard error of the mean.