

Guidelines/Criteria	
	Reference: Peng J, Peng L, Stevenson FF, Doctrow SR, Andersen JK. 2007. Iron and paraquat as synergistic environmental risk factors in sporadic Parkinson's Disease accelerate age-related neurodegeneration. J Neurosci 27(26):6914–6922.
In vitro Study Type Route of Administration Species & age of animals	Rat dopaminergic cell line N27
Study Duration	24 hours
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	Binary No Arguably similar Redox cycling, oxidative stress and subsequent cell death
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects <i>In vitro</i>	Cell viability as % of control based on MTT assay
Individual Components Characterisation of individual compounds Name, exact chemical name, CAS no. Were dose responses established for individual components? Were no effect levels established? Were doses below the NO(A)ELs investigated?	Paraquat dichloride and iron dichloride Controls without paraquat or Fe are not shown properly. Estimating from the figures and relying on statements in the text, paraquat was tested at statistical NOECs, but those doses were already indicating a trend compared to control levels. No dose-response established for Fe, only one dose tested. Statistically 200uM paraquat. According to text 80uM FeCl ₂ . Data not shown. Around NOEC for paraquat dichloride. Claimed for FeCl ₂ , but no data to support that, might be based on previous experiments with unknown conditions.
Mixtures Investigated Number of dose levels How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used? No. of technical replicates per exposure condition (<i>in vitro</i>) No. of animals per dose group (<i>in vivo</i>)	4 levels of paraquat dichloride, 1 of iron dichloride one level of iron dichloride throughout, with 0.5, 1, 1.5 & 2x NOEC (?) for paraquat dichloride 5
Observations/Findings	Allegedly significant reduction in cell viability for all mixtures tested compared to control (controls not shown), but no effect with individual substances
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Mixtures were only examined as side aspect, emphasis on MoA. Might be a reasonable study, but the publication does not present the control data of the <i>in vitro</i> part properly. Also, dose-response data for FeCl ₂ itself not available / presented. Might thus be any type of interaction, claim of synergy not supported by data. Other <i>in vitro</i> endpoints only examined with clear effect doses of paraquat. The <i>in vivo</i> part of the study is non-relevant, as: exposures to carbonyl iron (one dose) occurred only on PNDs 10-17, while paraquat (one dose group) was only administered repeatedly during later life of the mice. The Fe dose was an effect dose at 24 months age and the paraquat dose was an effect dose throughout.