Casey et al group of 6 papers

Guidelines/Criteria	
Reference:	(Single study reported in all 6 papers listed here)
***In vivo* Study Type** Route of Administration Species & age of animals	 Single dose behavioural study Oral gavage Long-Evans rats
Study Duration	4.5 hours
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 No 5 component mixture Similar AchE inhibition
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 Locomotor activity (breaking photobeams) for 30 minutes, 4hrs after dosing Arguable - probably adverse
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?	 Acephate, diazinon, chlorpyrifos, dimethoate, malathion Yes 3mg/kg for acephate, 50 for diazinon, 10 for chlorpyrifos, 500 for malathion, 10 for dimethoate. Yes, except for acephate
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 (*in vitro*) No. of animals per dose group (*in vivo*)	 Six fixed ratio mixtures were tested plus a control, of which 2 were below the NOEL for all single components Components present at 0.13x, 0.0004x, 0.031x, 0.017x & 0.1x NOEL at the lowest mixture dose, and 0.73x, 0.0022x, 0.17x, 0.091x & 0.56x NOEL at the second lowest mixture dose. The fractions total 0.28x and 1.54x the NOEL. The proportions of each component were in proportion to estimated dietary exposure in the US. 8, 12 or 16 per group
Observations/Findings	No effect on locomotor activity when each component was present at or below its NOEL, but effects were seen for higher dose mixtures.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	 Seems like a very good study, however the data is generally presented as graphs of dose-response, rather than tabular form or as NOELs/LOELs. The NOELs were recalculated using the tables in Casey et al 2005 and a t-test with Dunnett's correction for comparison of multiple groups with a control.

Casey study paper 1: Casey M, Gennings C, Carter WH Jr, Moser VC, Simmons JE. 2004. Detecting interaction(s) and assessing the impact of component subsets in a chemical mixture using fixed-ratio mixture ray designs. J Agric Biol Environ Stat 9(3):339-361.	Casey study paper 2: Gennings C, Carter WH Jr, Casey M, Moser V, Carchman RA, Simmons JE. 2004a. Analysis of functional effects of a mixture of five pesticides using a ray design. Environ Toxicol Pharmacol 18:115-125.
Single dose behavioural study Oral gavage Long-Evans rats	Single dose behavioural study Oral gavage Long-Evans rats
4.5 hours	4.5 hours
No 5 component mixture Similar AchE inhibition	No 5 component mixture Similar AchE inhibition
Same study as to the left, but reporting a different endpoint, namely abnormal gait Arguable - probably adverse	Combining both endpoints in determining NOELs Arguable - probably adverse
Acephate, diazinon, chlorpyrifos, dimethoate, malathion Yes 10mg/kg for acephate, 75 for diazinon, 10 for chlorpyrifos, 500 for malathion, 10 for dimethoate. Yes	Acephate, diazinon, chlorpyrifos, dimethoate, malathion Yes 3mg/kg for acephate, 50 for diazinon, 10 for chlorpyrifos, 500 for malathion, 10 for dimethoate. Yes, except for acephate
Six fixed ratio mixtures were tested plus a control, of which 3 were below the NOEL for all single components Components present at 0.04x, 0.0003x, 0.031x, 0.017x & 0.1x NOEL at the lowest mixture dose, and 0.22x, 0.0015x, 0.17x, 0.091x & 0.56x NOEL at the second lowest mixture dose, and 0.4x, 0.003x, 0.31x, 0.17x & 1x NOEL at the third lowest mixture dose. The fractions total 0.19x, 1.03x and 1.9x the NOEL in each mixture, respectively. The proportions of each component were in proportion to estimated dietary exposure in the US. 8, 12 or 16 per group	Six fixed ratio mixtures were tested plus a control, of which 2 were below the NOEL for all single components Components present at 0.13x, 0.0004x, 0.031x, 0.017x & 0.1x NOEL at the lowest mixture dose, and 0.73x, 0.0022x, 0.17x, 0.091x & 0.56x NOEL at the second lowest mixture dose. The fractions total 0.28x and 1.54x the NOEL. The proportions of each component were in proportion to estimated dietary exposure in the US. 8, 12 or 16 per group
There was an effect on gait at all three mixture doses where each component was present at or below its NOEL. When malathion was removed from the mixture, but all other components remained at the same doses as before, there was no effect at the lowest mixture dose, but there remained an effect at the second and third lowest mixture doses. The sum of the NOEL fractions for the three mixture doses lacking malathion were 0.17x, 0.94x and 1.7x, respectively.	There was an effect on gait at both mixture doses where each component was present at or below its NOEL. When malathion was removed from the mixture, but all other components remained at the same doses as before, there was no effect at the lowest mixture dose, but there remained an effect at the second lowest mixture dose. The sum of the NOEL fractions for the two mixture doses lacking malathion were 0.26x and 1.45x, respectively.
Seems like a very good study. No stats were presented, but all 78 control animals had normal gait, so it is assumed that any animals with abnormal gait are indicative of a treatment effect, using data in Gennings et al 2004.	This is the appropriate analysis of this study for the purpose of this report. The result is interesting and potentially surprising based on an additivity assumption. When malathion is removed then the result is no longer surprising.

Casey study paper 3: Casey M, Gennings C, Carter WH Jr, Moser VC, Simmons JE. 2005. Ds-optimal designs for studying combinations of chemicals using multiple fixed-ratio ray experiments. Environmetrics 16:129-147.	Casey study paper 4: Moser VC, Casey M, Hamm A, Carter Jr WH, Simmons JE, Gennings C. 2005. Neurotoxicological and statistical analyses of a mixture of five organophosphorous pesticides using a ray design. Toxicol Sci 86(1)101-115.

Casey study paper 5: Stork LG, Gennings C, Carchman RA, Carter WH Jr, Pounds J, Mumtaz M. 2006. Testing for additivity at select mixture groups of interest based on statistical equivalence testing methods. Risk Anal 26(6):1601-1612.	Casey study paper 6: Moser VC, Simmons JE, Gennings C. 2006. Neurotoxicological interactions of a five-pesticide mixture in preweanling rats. Toxicol Sci 92(1)235-245.