

Guidelines/Criteria	
Reference:	Lee GS, Liao X, Cantor RM, Collins MD. 2006. Interactive effects of cadmium and all-trans-retinoic acid on the induction of forelimb ectrodactyly in C57BL/6 mice. Birth Defects Res (Part A) 76:19–28.
In vivo Study Type Route of Administration Species & age of animals	IP Pregnant C57BL/6NCrIBR mice
Study Duration	dosing on GD 9.5 and foetuses collected on GD 18
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	binary no Postulated as similar (multiple mechanistic pathways that may perturb a common final pathway hypothesised.) forelimb ectrodactyly. Perturbance of bone homeostasis during limb development ?
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Postaxial forelimb ectrodactyly. (Other external malformations also reported but not the main aspect of the study). 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?	cadmium chloride (CdCl ₂ ; 10108-64-2) all-trans-retinoic acid (RA; 302-79-4) de-ionised water controls 8% ethanol/92% soybean oil controls CdCl ₂ : 0.5; 1; 2; 3; 5 mg/kg RA: 1; 3; 5; 7.5 mg/kg Mixtures: 0.5 CdCl ₂ + 1 RA; 0.5 CdCl + 3 RA; 1CdCl ₂ + 5 RA Yes (0.5 CdCl ₂ and 1 RA induced no effects) No.
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>)	3 different mixture ratios tested at 1 dose each. See cell C23 for details. Lowest mixture based on combination of lowest tested doses of CdCl ₂ and RA (No effect doses for each substance). 6 dams per dose. Litters were treated as the unit for statistical analysis
Observations/Findings	Reproductive effects: Mean foetal weights significantly lower than controls in all mixtures. RA had no effect on repro endpoints at all doses. CdCl ₂ induced lower foetal weights at 1, 2, 3, and 5 mg/kg. Forelimb defects: Lowest tested doses of individual substances caused no defects (referred to as 'subthreshold doses'). RA dose response not clear (although authors cite other publication demonstrating clear dose response between 12.5 and 75 mg/kg). All mixtures induced defects. In some cases, subthreshold combinations induced significant increases in mean litter % with forelimb defects. On the face of it, this appears to be potentiation in some cases and synergism in others.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Mixtures dosed as double volume of single doses, which were not controlled for. Group size adequate, doses established, but would be good to collate with Collins et al for full RA dose-response.