Guidelines/Criteria	
Reference:	Institóris L, Kovács D, Kecskeméti-Kovács I, Lukács A, Szabó A, Lengyel Z, Papp A, Nagymajtényi L, Dési I. 2006. Immunotoxicological investigation of subacute combined exposure with low doses of PB, HG and CD in rats. Acta Biol Hung 57(4):433-439.
In vivo **Study Type** Route of Administration Species & age of animals	 oral gavage (5 mL/kg) Outbred male Wistar rats(4 weeks old)
Study Duration	4-week and 13-week dosing periods
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	 yes; three binary mixtures (Pb+Hg; Pb+Cd; Hg+Cd) tertiary mixture not stated not stated; simply investigating whether combined NOEL doses resulted in detectable effects.
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	 General toxicological; haematological; and immune system function effects. adverse effects
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?	 Heavy metals (inorganic): Lead acetate; Mercury chloride; Cadmium chloride As above. No CAS numbers provided in the publication Not in this publication. Individual chemicals were investigated in separate experiments (4-week treatments only) and NOELs established and reported in other publications. Reported in separate publications for 4-week treatments only. No. Tested mixtures of NOELs (established in 4 week studies) in both the 4 week and 12 week studies.
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*)	 One dose for each mixture Mixed at NOELs only. Pb-Acetate, 20 mg/kg HgCl2, 0.4 mg/kg CdCl2, 1.61 mg/kg Experiment 1 (tertiary mixture): 24 animals treated (8 used for toxicological/haematological studies; 8 used for PFC assay; 8 used for DTH reaction). The DTH treatment group was continued for another 8 weeks and then used for toxicological/haematological studies.) Experiment 2 (binary mixtures): Investigated using same experimental protocol as experiment 1.
Observations/Findings	At 4 weeks treatment: tertiary mixture: no effects on immune function. Significant increase in lung weight; decrease in popliteal lymph node (PLN). Binary mixtures: Pb+Hg decreased relative weight of PLN and adrenal weight; Pb+Cd decreased relative weight of PLN; Hg+Cd increased relative adrenal weight. At 12 weeks treatment: tertiary mixture: no changes in the toxicological and haematological parameters. binary mixtures: Pb+Hg significantly decreased the rel. thymus and adrenal weight, and increased rel. kidney weight; Pb+Cd resulted in increased rel. thymus weight, increased wbc count and decreases in both rbc count and haematocrit values.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Mixture based on NOELs established in separate (historic) studies. A rather confusing picture of effects, which as only tested at single doses cannot really be fully comprehended. Nevertheless, some effects on organ weights and haematological alterations were observed at NOEL combinations (Pb+Cd mixture), but it is not possible to determine the type of interaction.