

Guidelines/Criteria	
Reference:	Vitalone A, Catalani A, Chiodi V, Cinque C, Fattori V, Goldoni M, Matteucci P, Poli D, Zuena AR, Costa LG. 2008. Neurobehavioral assessment of rats exposed to low doses of PCB 126 and methyl mercury during development. Environ Toxicol Pharmacol 25:103-113.
In vivo Study Type Route of Administration Species & age of animals	Perinatal study drinking water (MeHg) and Transgel consumption (PCB 126). Direct food consumption of pups was avoided by changing the cage configuration at PND 16 (addition of specific height enhancers) Female and male Wistar rats (acclimatized for 2 weeks and mated 2 F with 1 M); only the pregnant dams were dosed
Study Duration	Perinatal study, dosage from GD 7 to PND 21 (day of birth was denoted as PND 0)
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	yes (MeHg and PCB 126) no similar action assumed Neurobehaviour
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Effects on pregnancy (number of pregnancies, lengths of pregnancy, litter size, sex ratios) and body weight, Neurobehaviour: Pre-weaning observations: Ano-genital distance (PND1-21, Incisor eruption (PND7-21, Hair growth (PND7-21), eye opening (PND7-21, Pinnae detachment (PND1-4, Ear opening (PND7-21), negative geotaxis (PND7-15), righting reflex (PND7), cliff avoidance (PND 7-16), forelimb reflex (PND 7-10), pole grasping (PND7-10), homing behaviour (PND11), acoustic startle response (PND20) --> cohort 1 Post-weaning observations: open field (PND 30-110), sudden silence (PND 40), morris water maze (PND 75), novel object exploration (PND 80), rotarod (PND 90), elevated plus maze (PND 100), passive avoidance (PND 110) --> cohort 2 Adverse and non-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?	yes MeHg chloride (Sigma, Italy) and PCB126 (Larodan Fine Chemicals, Sweden) No Yes for selected endpoints Only for selected endpoints: Rationale for dose selection: MeHg dose of 0.5 mg/kg bw was chosen as a low dose as reported by others (Coccini et al., 2000, Roegge and Schanthez, 2006) The ten-fold higher dose of PCB 126 (1 µg/kg bw) caused body weight reductions in dams, prolongation of length of pregnancy, reduction of litter size, and a significant decrease in the number of alive pups

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<p>Mixtures Investigated</p> <p>Number of dose levels</p> <p>How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?)</p> <p>No. of technical replicates per exposure condition (<i>in vitro</i>)</p> <p>No. of animals per dose group (<i>in vivo</i>)</p>	<p>4 experimental groups: Control, MeHg 0.5 mg/kg day (target dose), PCB 126 100 ng/kg bw (administered via Transgel consumption), MeHg + PCB 126 (doses as above) Only one dose used.</p> <p>not applicable</p> <p>14-16 dams/group, culling at PND 1 --> 8 animals/litter (4 males and 4 females); these animals were divided into two cohorts: Cohort 1 was tested for developmental landmarks and used for pre-weaning reflex tests, cohort 2: evaluation in a series of behavioural tests starting on PND 30</p>
<p>Observations/Findings</p>	<p>The only effects, where the single component groups represented individual NOAELs were:</p> <ul style="list-style-type: none"> - pregnancy lengths - sex ratio at birth - physical development (at weaning) - attention, coordination and balance - object discrimination - spatial and conditioned learning <p>Also in the mixture group no effects were seen, when these parameters were evaluated.</p>
<p>Overall opinion</p> <p>(e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)</p>	<p>Good study design and well documented paper. The results do not support the hypothesis that co-exposure to MeHg and PCB 126 results in additive or synergistic effects.</p>