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Guidelines/Criteria	
Reference:	van Birgelen APJM, Fase KM, van der Kolk J, Poiger H, Brouwer A, Seinen W, van den Berg M. 1996. Synergistic effect of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic porphyrin levels in the rat. Environ Health Perspect 104(5):10 pp.
In vivo **Study Type**	
Route of Administration	oral diet
Species & age of animals	female Sprague Dawley rats (7 weeks old)
Study Duration	13 weeks
Type of Mixture	
Binary	Yes (binary mixtures each containing TCDD were investigated)
>2 components	No
Similar acting or dissimilar	similar action assumed
What Mode of Action was investigated?	liver porphyrin accumulation and liver enzyme activation
Parameters/End points Measured	
Target organs/Critical effects	Liver (hepatic porphyrin levels, Cyp P450 measurements, Cyp 1A2 activity)
Pharmacological changes or adverse effects	pharmacological changes
Individual Components	
Characterisation of individual compounds	yes
Name, exact chemical name, CAS no.	Individual testing of PCB 153 (2,2', 4,4',5,5'-hexachlorobiphenyl), PCB 156 (2,3,3',4,4',5-hexachlorobiphenyl), PCB 126 (3,3',4,4',5-pentachlorobiphenyl), TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) and combinations of TCDD, PCB 126 and PCB 156 and PCB 153
Were dose responses established for individual components?	Yes
Were no effect levels established?	Yes:
Were doses below the NO(A)ELs investigated?	Yes (the TCDD doses were at or close to the NOAELs and the doses of the PCBs were below the NOAELs for PCB 153 only)

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Mixtures Investigated Number of dose levels	Doses of individual testing: TCDD: 0, 14, 26, 47, 320, 1024 ng/kg bw/day PCB 153: 0, 0.72, 2.07, 6.61 mg/kg bw/day PCB 126: 0, 0.47, 3.18, 10.1 µg/kg bw/day PCB 156: 0, 81, 365, 729 µg/kg bw/day Combination testing: TCDD/PCB 153: 33.9 ng/kg bw/0.68 mg/kg bw (doses close to the NOAEL of TCDD) 32.6 ng/kg bw/1.95 mg/kg bw (doses close to the NOAEL of TCDD) 32.0 ng/kg bw/6.40 mg/kg bw (doses close to the NOAEL of TCDD) 318 ng/kg bw/0.64 mg/kg bw 301 ng/kg bw/1.81 mg/kg bw 293 ng/kg bw/5.85 mg/kg bw TCDD/PCB 126 27 ng/kg bw/0.48 µg/kg bw (doses close to the NOAEL of TCDD and PCB 126) 26 ng/kg bw/3.25 µg/kg bw 23 ng/kg bw/10.4 µg/kg bw 315 ng/kg bw/0.44 µg/kg bw 306 ng/kg bw/3.06 µg/kg bw 126 ng/kg bw/9.68 µg/kg bw TCDD/PCB 156 317 ng/kg bw/76 µg/kg bw 305 ng/kg bw/366 µg/kg bw 290 ng/kg bw/696 µg/kg bw Mixture experiment for Cyp 1A2 induction did not test combinations below effect levels for TCDD.
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*)	Doses in mixtures were mostly close or at the NOAEL of the single compounds for this effect. not applicable 8 or 9
Observations/Findings	Results of single substance testing: LOAEL for Porphyrin accumulation: TCDD: 47 ng/kg bw/day PCB 153: 6.61 mg/kg bw/day (highest dose tested) PCB 126: 3.18 µg/kg bw/day PCB 156: 365 µg/kg bw/day TCDD/PCB 153 All combinations showed increased porphyrin levels TCDD/PCB 126 No statistically significantly increased Porphyrin levels were observed in the dose mixture (27 ng/kg bw TCDD + 0.48 µg/kg bw PCB 126). However at the next higher dose level 26 ng/kg bw TCDD + 3.25 µg/kg bw PCB 126 strongly increased levels are observed (pointing to a more than additive action).
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Sufficient number of animals/group; the doses used in the mixture experiments were always close or at the NOAEL for TCDD only, non-adverse endpoint.