

Guidelines/Criteria	
Reference:	Wade MG, Foster WG, Younglai EV, McMahon A, Leingartner K, Yagminas A, Blakey D, Fournier M, Desaulniers D, Hughes CL. 2002b. Effects of subchronic exposure to a complex mixture of persistent contaminants in male rats: systemic, immune, and reproductive effects. Toxicol Sci 67:131-143.
In vivo Study Type Route of Administration Species & age of animals	oral gavage Male Sprague Dawley rats (45 days of age)
Study Duration	70 days (daily gavage)
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	no yes dissimilar action assumed (ubiquitous persistent environmental substances based on quantifications in human reproductive tissues) systemic toxicity, immunotoxicity, reproduction toxicity
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	liver (liver microsomal enzyme activity, histology), genotoxicity (Micronucleus assay in bone marrow) kidney, Immunotoxicity (spleen, thymus gland, splenocyte function, NK cell activity mitogen-induced lymphocyte blastogenesis) and reproduction toxicity (pituitary gland, adrenal glands, major organs of reproductive tract, daily sperm production, sperm chromatin structure assay, flow cytometric analysis of testis cells) Body weight, Locomotor activity, concentrations of dopamine (DA), dihydroxyphenylacetic acid - metabolite of dopamine (DOPAC), homovanillic acid - metabolite of dopamine (HVA) in striatal sections, tyrosine hydroxylase immunoreactivity (TH densimetry) in brain sections, lung histopathology
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 Aldrin, p,p'-DDT, p,p'-DDE, Dieldrin, Endosulfan, Heptachlor, Hexachlorobenzene, Hexachlorocyclohexane, Mirex, Methoxychlor, 1,2,3-Trichlorobenzene, 1,2,4-Trichlorobenzene, 1,2,3,4-Tetrachlorobenzene, Pentachlorobenzene, TCDD, PCB (as Arochlor 1254), Cadmium chloride, Lead chloride No Almost Yes

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<p>Mixtures Investigated</p> <p>Number of dose levels</p>	<p>Three dose levels were administered: 1X, 10X, 100X, 1000X a mixture of the compounds at their MRL/RfD/TDI levels (established by US-EPA) with one exception: TCDD was dosed at the NOAEL level:</p> <p>Aldrin 30 ng/kg bw/day p,p'-DDT 30 ng/kg bw/day p,p'-DDE 570 ng/kg bw/day Dieldrin 50 ng/kg bw/day Endosulfan 50 ng/kg bw/day Heptachlor 0.5 µg/kg bw/day Hexachlorobenzene 0.3 µg/kg bw/day Hexachlorocyclohexane 0.3 µg/kg bw/day Mirex 0.8 µg/kg bw/day Methoxychlor 2 µg/kg bw/day 1,2,3-Trichlorobenzene 0.77 µg/kg bw/day 1,2,4-Trichlorobenzene 2.3 µg/kg bw/day 1,2,3,4-Tetrachlorobenzene 0.2 µg/kg bw/day Pentachlorobenzene 0.5 µg/kg bw/day TCDD 1 ng/kg bw/day (this value represents the NOAEL value of TCDD) PCB (as Arochlor 1254) 1 µg/kg bw/day Cadmium chloride 0.7 µg/kg bw/day Lead chloride 0.1 ng/kg bw/day</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>The lowest administered dose represents the reference doses of each component in the mixture (with the exception of TCDD, which is contained at the NOAEL level in the mixture). The next higher doses are 10x, 100x and 1000x multiples of the low dose mixture.</p> <p>not applicable</p> <p>10 (9 animals in control group)</p>
<p>Observations/Findings</p>	<p>General toxicity: No effects on body weights, no signs of stress or discomfort</p> <p>Liver: ≥ 100x: Urea nitrogen ↓, BROD/EROD ↑, rel. liver weight ↑ 1000 x: increased weights, LDH activity ↓, cholesterol ↑, total protein ↑, albumin ↑, serum uric acid ↓, serum phosphorus ↑ (was decreased at 10x); no evidence for hepatic cell damage, histopathological lesion scores ↑</p> <p>Micronuclei in bone marrow: no effects</p> <p>Kidney: 1000x: increased weights</p> <p>Immunotoxicity: 1000x: proliferation of splenic T cells ↓, NK cell lytic activity ↓ (at 1x an increase was observed)</p> <p>Reproduction toxicity: ≥ 100x: abs. and rel. total and caput epididymis weights (cauda epididymis weights not affected)</p> <p>1000x: rel number of testicular spermatids ↑, total epididymal sperm ↓, Sperm/g cauda ↓</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>Number of animals and investigated parameters sufficient. Observation well described and reproducible. Low doses were investigated. It is suggested by the study authors, that the MRLs, TDIs or RfDs set by the US-EPA, ATSDR and CEPA provide adequate protection for adult male animals for those systems examined.</p>