

Guidelines/Criteria	
Reference:	Zhao D, Chen Y, Zhou K, Cheng S, Ma T, Jiang C, Yan W, Zhu L, Gu X, Zhu X, Wu B, Zhang Y, Zhang X. 2011a. Reproductive toxicity in male mice exposed to Nanjing City tap water. <i>Ecotoxicology</i> 20(5):1057-1064.
In vivo Study Type Route of Administration Species & age of animals	Ip male Kunming mice, 7-9 weeks old
Study Duration	5 days
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	no yes dissimilar action assumed Reproduction toxicity
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Reproduction toxicity (testes and epididymides weights, testes enzymes, histopathology, sperm counts, sperm morphology) adverse
Individual Components Characterisation of individual compounds Name, exact chemical name, CAS no.	No Organic extracts in tap water: 2100 l of tap water were collected from the Jialing River and concentrated using solid phase extraction. The eluates were evaporated to dryness and the dry residue was dissolved in DMSO and used for animal testing. In parallel an amount was dissolved and subjected to GC/MS analysis. The amount of extract in 1 ml of DMSO was equal to the amount of organic compounds in 200 l of tap water. OE stock solutions were dissolved in DMSO (200 l/ml) and then diluted in DMSO to the required concentration of 2.5, 5, and 10 l/ml before use. According to the volume of intraperitoneal injection 0.005 l/kg bw/day in mice, the OE-treated mice were administered OE intraperitoneally at doses of 12.5, 25 and 50 l/kg bw/day (OE 12.5, 25, and 50 l represents OE from 12.5, 25, and 50 l of tap water, respectively). Main organic compounds in the OE of Tap water: Cichloroethane, 2,5-Cyclohexadiene 1,4-dione, 2,6 bis(1,1-dimethylethyl), 2,6-di(t-Butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-One, 4-Butyl-1,2-dimethoxybenzene, 2,5-Bis(2-methylpropyl)-thiophene, 2-(tert-Butyl)-4,6-dimethylphenol, Tetrahydropyrene, 13C-Methylbenzene, Dibenz[b,f]azepine-5-carbonylchloride, 1,1,1,3,3-Pentachloropropane, 3,5-Bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoic acid methylester, Butylated hydroxytoluene, 2-Propenoic acid, 2-methyl-2-sulfoethylester, Diisobutylphthalate, Di-(2-methyloxyethyl)phthalate, Dibutylphthalate, Ditrdecylphthalate.
Were dose responses established for individual components?	Three doses of organic extracts were used (12.5, 25, 50 l/kg bw/day). 0.005 l/kg bw/day was injected.
Were no effect levels established?	No
Were doses below the NO(A)ELs investigated?	Presumably not
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>)	DMSO, ip injections representing 12.5, 25, 50 l/kg bw/day not applicable no applicable 8 animals/diet group
Observations/Findings	Number of epididymal sperm in the high OE group was decreased, frequency of sperm abnormalities in all treated groups was increased, Serum testosterone and follicle-stimulating hormone levels in the treated groups were decreased, in mid and high OE doses histological changes in testes and were observed (seminiferous tubule deformation, seminiferous epithelial degeneration, Sertoli cell vacuolisation, Leydig cell abnormalities)
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	IP is a parenteral route of exposure with little relevance to human oral exposure. The tap waters of the river were heavily concentrated. The substance mixture administration is not representing environmentally or human-relevant exposure situation.