Heindel et al, 1995rats

Guidelines/Criteria	
Reference:	Heindel JJ, Chapin RE, George J, Gulati DK, Fail PA, Barnes LH, Yang RSH. 1995. Assessment of the reproductive toxicity of a complex mixture of 25 groundwater contaminants in mice and rats. Fundam Appl Toxicol 25:9-19.
In vivo **Study Type**	
Route of Administration	drinking water, water control group
Species & age of animals	Sprague-Dawley rats (10 week of age)
Study Duration	reproductive assessment by continuous breeding (7 days premating, 112 days breeding in breeding pairs, pairs are separated, selected F1 pups are reared in same sex groups for 88 days +/- 10 days of age, cohabitation for 7 days, housed singly until delivery of F2 generation
Type of Mixture	
Binary	no
>2 components	yes
Similar acting or dissimilar	dissimilar action assumed (chemicals mix to simulate groundwater supplies near hazardous dumps)
What Mode of Action was investigated?	reproduction toxicity (no specific mode of action)
Parameters/End points Measured	
Target organs/Critical effects	clinical signs, parental body weight, first and second generation fertility (number producing a litter/number of breeding pairs), litters per pair, live pups per litter, proportion of pups born alive, sex of live pups, pup body weights within 24 hr after birth, feed and water consumption, organ weight determination and histopathology of selected F1 organs, spermatology
Pharmacological changes or adverse effects	adverse effects
Individual Components	
Characterisation of individual compounds	yes
Name, exact chemical name, CAS no.	One mixture was investigated: Acetone, Aroclor 1260, Arsenic trioxide, Benzene, Cadmium acetate trihydrate, Carbon tetrachloride, Chloroform, Chlorobenzene, Chromium chloride hexahydrate, 1,1-Dichloroethane, 1,1-Dichloroethylene, 1,2-Dichloroethane, 1,2-t-Dichloroethylene, Di/2-ethylhexyl)phthalate, Ethylbenzene, Lead acetate trihydrate, Mercuric chloride, Methylene chloride, Nickel acetate tetrahydrate, Phenol, Tetrachloroethylene, Toluene, 1,1,1-Trichloroethane, Trichloroethylen, Xylenes
Were dose responses established for individual components?	No, only mixtures at three dose levels were administered
Were no effect levels established?	Yes
Were doses below the NO(A)ELs investigated?	Yes

Heindel et al, 1995rats

Mixtures Investigated Number of dose levels	Each 3 dose groups containing the individual compounds at 1, 5, 10% of a stock solution: Acetone: 5.3, 26.5, 53 ppm, Aroclor 1260: 0.001, 0.005, 0.01 ppm, Arsenic trioxide: 0.9, 4.5, 9.0 ppm, Benzene: 1.25, 6.25, 12.5 ppm, Cadmium acetate trihydrate: 5.1, 25.5, 51.0 ppm, Carbon tetrachloride: 0.04, 0.20, 0.40 ppm, Chloroform: 0.7, 3.5, 7.0 ppm, Chlorobenzene: 0.01, 0.05, 0.10 ppm, Chromium chloride hexahydrate: 3.6, 18.0, 36.0 ppm 1,1-Dichloroethane: 0.14, 0.7, 1.4 ppm, 1,1-Dichloroethylene: 0.05, 0.25, 0.5 ppm, 1,2-Dichloroethane: 4.0, 20.0, 40.0 ppm, 1,2-t-Dichloroethylene: 0.25, 1.25, 2.5 ppm, Di-2-ethylhexyl)-phthalate: 0.0015, 0.0075, 0.015 ppm, Ethylbenzene: 0.03, 0.15, 0.3 ppm, Lead acetate trihydrate: 7.0, 35.0, 70.0 ppm, Mercuric chloride: 0.05, 0.25, 0.50 ppm, Methylene chloride: 3.75, 18.75, 37.5 ppm, Nickel acetate tetrahydrate: 0.68, 3.4, 6.80 ppm, Phenol: 2.9, 14.5, 29.0 ppm, Tetrachloroethylene: 0.34, 1.7, 3.40 ppm, Toluene: 0.7, 3.5, 7.0 ppm, 1,1,1-Trichloroethane: 0.2, 1.0, 2.0 ppm, Trichloroethylen: 0.65, 3.25, 6.50 ppm, Xylenes: 0.16, 0.8, 1.60 ppm Actual doses administered at the 10% concentrations are: Acetone: F0: 3646 µg/kg bw , F1: 3169 µg/kg bw, Aroclor 1260: F0: 0.7 µg/kg bw F1: 0.6 µg/kg bw, Arsenic trioxide: F0: 619 µg/kg bw F1: 538 µg/kg bw, Benzene: F0: 860 µg/kg bw F1: 747 µg/kg bw, Cadmium acetate trihydrate: F0: 3509 µg/kg bw F1: 3050 µg/kg bw, Carbon tetrachloride: F0: 27 µg/kg bw F1: 24 µg/kg bw, Chloroform: F0: 482 µg/kg bw F1: 419 µg/kg bw, Chlorobenzene: F0: 7 µg/kg bw F1: 6 µg/kg bw, Chromium chloride hexahydrate: F0: 2477 µg/kg bw F1: 2153 µg/kg bw, 1,1-Dichloroethane: F0: 96 µg/kg bw F1: 84 µg/kg bw, 1,1-Dichloroethylene: F0: 34 µg/kg bw F1: 30 µg/kg bw, 1,2-Dichloroethane: F0: 2752 µg/kg bw F1: 2392 µg/kg bw, 1,2-t-Dichloroethylene: F0: 172 µg/kg bw F1: 149 µg/kg bw, Di-2-ethylhexyl)-phthalate: F0: 1 µg/kg bw F1: 0.9 µg/kg bw, Ethylbenzene: F0: 21 µg/kg bw F1: 18 µg/kg bw, Lead acetate trihydrate: F0: 4816 µg/kg bw F1: 4186 µg/kg bw, Mercuric chloride: F0: 34 µg/kg bw F1: 30 µg/kg bw, Methylene chloride: F0: 2580 µg/kg bw F1: 2242 µg/kg bw, Nickel acetate tetrahydrate: F0: 468 µg/kg bw F1: 407 µg/kg bw, Phenol: F0: 1995 µg/kg bw F1: 1734 µg/kg bw, Tetrachloroethylene: F0: 234 µg/kg bw F1: 203 µg/kg bw, Toluene: F0: 482 µg/kg bw F1: 419 µg/kg bw, 1,1,1-Trichloroethane: F0: 138 µg/kg bw F1: 120 µg/kg bw, Trichloroethylen: F0: 447 µg/kg bw F1: 389 µg/kg bw, Xylenes: F0: 110 µg/kg bw F1: 96 µg/kg bw
How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?)	Would have to be evaluated, NOAELs of individual compounds are not given.
No. of technical replicates per exposure condition (*in vitro*)	not applicable
No. of animals per dose group (*in vivo*)	20 pairs/group (40 control pairs)
Observations/Findings	≥ 1%: decreased water consumption (presumably due to taste) ≥ 5%: F1 pup weight development during lactation decreased (males more affected than females) 10%: F1 generation: decreased body weight (m+f), increased relative kidney weights, decreased absolute liver (m+f), testis, epididymis, prostate weights (no changes in relative liver and prostate weights and increased testis and epididymis weights) -> no histopathological findings Reproduction F0: live pup weight decreased; no. Of males/total pups (0.50/0.50/0.50/0.45); no. Reproduction F1: Copulatory plugs/no. Cohabited decreased = mating index (14/20 compared to 18/20 in controls), live pup weight decreased, adjusted live pup weight decreased.
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Good study design, sufficient number of animals, relevant endpoints; NOAELs of individual compounds should be evaluated and compared to the actual doses administered.