Guidelines/Criteria	
Reference:	NTP. 1993a. NTP Technical Report on toxicity studies of a chemical mixture of 25 groundwater contaminants. NTP toxicity report series no. 35 - NIH Publication 93-3384. National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC, USA. Shelby MD, Tice RR, DeMarini DM, Yang RSH. 1990. Toxicity and mutagenicity of a mixture of 25 chemicals found in contaminated groundwater. IARC Sci Publ 104:314-332.
***In vivo* Study Type**	
Route of Administration	drinking water, water control group for the *in vivo* genotoxicity tests, and *in vitro* tests
Species & age of animals	B6C3F1 mice, and F344/rats 6 weeks
Study Duration	different study types: Ames assay, prophage-induction assay, cytogenetic studies of bone marrow and blood after *in vivo* exposure
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?	genotoxicity
Parameters/End points Measured	
Target organs/Critical effects	mutagenicity, chromosome aberration
Pharmacological changes or adverse effects	*In vivo* study: body weight at days 0, 1, 4, 7, 14, clinical observation, micronucleus (based on 1000 PCE and/or normochromatic erythrocytes/per animal and % PCE (based on 1000 peripheral blood erythrocytes of 200 bone marrow erythrocytes/animal), chromosome aberration
Individual Components	
Characterisation of individual compounds	yes
Name, exact chemical name, CAS no.	One mixture was investigated: Acetone, Aroclor 1260, Arsenic, Benzene, Cadmium, Carbon tetrachloride, Chloroform, Chlorobenzene, Chromium, 1,1-Dichloroethane, 1,1-Dichloroethylene, 1,2-Dichloroethane, 1,2-t-Dichloroethylene, Di/2-ethylhexyl)phthalate, Ethylbenzene, Lead, Mercury, 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 four dose levels were administered
Were no effect levels established?	Yes
Were doses below the NO(A)ELs investigated?	Yes presumably
Mixtures Investigated	
Number of dose levels	Target concentrations: Acetone: 5.3, 53, 106 ppm, Aroclor 1260: 0.001, 0.01, 0.02 ppm, Arsenic: 0.9, 9.0, 18 ppm, Benzene: 1.25, 12.5, 25 ppm, Cadmium: 5.1, 51, 102 ppm, Carbon tetrachloride: 0.04, 0.40, 0.8 ppm, Chloroform: 0.7, 7.0, 14 ppm, Chlorobenzene: 0.01, 0.10, 0.2 ppm, Chromium: 3.6, 36, 72 ppm 1,1-Dichloroethane: 0.14, 1.4, 2.8 ppm, 1,1-Dichloroethylene: 0.05, 0.5, 1 ppm, 1,2-Dichloroethane: 4.0, 40.0, 80 ppm, 1,2-t-Dichloroethylene: 0.25, 2.5, 5 ppm, Di-2-ethylhexyl)-phthalate: 0.0015, 0.015, 0.03 ppm, Ethylbenzene: 0.03, 0.3, 0.6 ppm, Lead: 7.0, 70.0, 140 ppm, Mercury: 0.05, 0.50, 1 ppm, Methylene chloride: 3.75, 37.5, 75 ppm, Nickel: 0.68, 6.80, 13.6 ppm, Phenol: 2.9, 29.0, 58 ppm, Tetrachloroethylene: 0.34, 3.40, 6.8 ppm, Toluene: 0.7, 7.0, 14 ppm, 1,1,1-Trichloroethane: 0.2, 2.0, 4 ppm, Trichloroethylen: 0.65, 6.50, 13 ppm, Xylenes: 0.16, 1.60, 3.2 ppm
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*)	10 animals/group

Observations/Findings	Mutagenicity in Salmonella: 2 experiments with and without S9, The high dose solution was used for this assay, concentrations from 5-1000 µg/plate were tested: cytotoxicity was observed at 1000 µg/plate without S9: no mutagenicity. Induction of prophage lambda: 2 experiments with and without S9, the high dose concentration was used, concentrations of 0.078 - 10% doses were used, cytotoxicity was observed at 1.25 (2.5 in the second experiment)% of the dose: no damage in *E. Coli* observed. Palatability study: 14 days exposure, three doses tested; decreased water consumptions are seen at all three doses; decreased body weight gains seen in rats high dose and in mice mid and high dose. Chromosome aberration *in vivo* (8 animals/sex/group) in rats and mice: no increased number of chromosomal aberrations. Micronucleus test in bone marrow in rats and mice (10 animals/sex/group): significantly increased frequency of MN-PCE in mice in the high dose, no increased incidences seen in rat bone marrow, decreased %PCE observed in female mice, not in male mice at all three doses; significant increase of %PCE observed in male and female rats at the high dose only. Micronucleus test in blood in rats and mice (10 animals/sex/group): no increased incidences of MN-PCE observed, significant increased %PCE observed in male and female mice at the two highest doses, significant increase %PCEin male rats observed in the high dose, not in females. SCE and AGT (average generation time) studies *in viv* o in bone marrow of mice, 3 doses tested, 8 animals/sex/group, significant increase of SCE/cell at all three doses in male mice, not in female mice; significant decreases in AGT in male and female mice at the two highest doses. Mitotic index in mice and rats, 3 doses tested, 10 animals/sex/group, increased mitotic indices in all three doses in male mice and at the top dose in female mice; increased mitotic index observed in male and female rats at the high dose only.
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.