

Guidelines/Criteria	
Reference:	Simmons JE, Yang RSH, Svendsgaard DJ, Thompson MB, Seely JC, McDonald A. 1994. Toxicology studies of a chemical mixture of 25 groundwater contaminants: Hepatic and renal assessment, response to carbon tetrachloride challenge, and influence of treatment-induced water restriction. J Toxicol Environ Health 43(3):305-325.
In vivo Study Type Route of Administration Species & age of animals	drinking water, water control group, feed restricted group, water restricted group Male Fischer-344 rats, 65 days old
Study Duration	14 days
Type of Mixture Binary >2 components Similar acting or dissimilar What Mode of Action was investigated?	no yes dissimilar action assumed (chemicals mix to simulate groundwater supplies near hazardous dumps in combination with CCl ₄) repeated dose toxicity
Parameters/End points Measured Target organs/Critical effects Pharmacological changes or adverse effects	Kidney and liver, general toxicity, body weight determinations at day 1, 2, 5, 8, 10, 14 and 15 adverse
Individual Components Characterisation of individual compounds Name, exact chemical name, CAS no.	yes One mixture (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) was tested in combination with single applications of different concentrations of CCl ₄
Were dose responses established for individual components? Were no effect levels established? Were doses below the NO(A)ELs investigated?	Yes for the carbontetrachloride groups Yes Yes presumably
Mixtures Investigated Number of dose levels	Animals received for 13 days either ad libitum water, or restricted water or the groundwater mix (represents the groundwater mix summarized in NTP-35) and then at day 14 one gavage dose of carbontetrachloride (CCl ₄): 15 groups tested: Ad libitum water group: Controls, 0.0375, 0.050, 0.075, 0.150 mg/kg bw CCl ₄ Water restricted group: Controls, 0.0375, 0.050, 0.075, 0.150 mg/kg bw CCl ₄ Groundwater mix group: Target concentrations (ppm)/48 h value (ppm)/calculated dosage (µg/rat/d): Acetone: 53/43.5/626.4, Aroclor 1260:0.01/ND/-, Arsenic: 9.0/9.6/138.2, Benzene: 12.5/12.8/184.3, Cadmium: 51.0/50.0/720.0, Carbon tetrachloride: 0.4/0.35/5.04, Chloroform:7/6.9/99.4, Chlorobenzene:0.1/0.1/1.44, Chromium: 36/36.4/524.2, 1,1-Dichloroethane: 1.4/1.5/21.6, 1,1-Dichloroethylene: 0.5/0.46/6.62, 1,2-Dichloroethane: 40/42.4/610.6, 1,2-t-Dichloroethylene: 2.5/2.75/39.6, Di-2-ethylhexyl)-phthalate:0.015/ND/-, Ethylbenzene:0.3/0.34/4.90, Lead: 70/not determined/?, Mercury: 0.5/0.46/6.62, Methylene chloride: 37.5/34.1/491.0, Nickel: 6.8/6.7/96.5, Phenol: 29/28.4/409.0, Tetrachloroethylene: 3.4/3.0/43.2, Toluene: 7/7.1/102.2, 1,1,1-Trichloroethane:2/2.1/30.2, Trichloroethylen:6.5/7.3/105.1, Xylenes: 1.6/1.6/23.0 Controls: 0.0375, 0.050, 0.075, 0.150 mg/kg bw CCl ₄ Would have to be evaluated, NOAELs of individual compounds are not given.
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>)	not applicable 6 animals/group in 3 replicates --> 18 animals/groups

<p>Observations/Findings</p>	<p>Water and Food consumption of controls: Water and feed consumptions was decreased in the restricted water and in the 10% Mix control groups compared to ad libitum control group Liver toxicity: Ad libitum group: >= 0.0375 mg CCl4/kg bw: SDH ↑, ALT ↑ >= 0.050 mg CCl4/kg bw: AST ↑ >= 0.075 mg CCl4/kg bw: rel. Liver weight ↑ 0.150 mg CCl4/kg bw: ALKP ↑, 5'ND ↑, LDH ↑ Water restricted group: >= 0.0375 mg CCl4/kg bw: SDH ↑, ALT ↑, >= 0.050 mg CCl4/kg bw: rel. Liver weight ↑, 5'ND ↑, AST ↑ Groundwater mix group: >= 0.0375 mg CCl4/kg bw: SDH ↑, ALT ↑, AST ↑, ALKP ↑, 5'ND ↑ >= 0.050 mg CCl4/kg bw: rel. Liver weight ↑, 0.150 mg CCl4/kg bw: BILI ↑ When comparing the influence of groundwater mix to CCl4 induced liver toxicity it has been observed, that a small but statistically significant effect attributable to groundwater mix was detected for hepatocellular necrosis but not for hepatocellular degeneration. Kidney toxicity: No renal lesions occurred that could be attributed to any water treatment or water treatment + CCl4 combination. CCl4 had no significant effect on kidney weight, relative kidney weight, CREAT, or BUN/CREAT ratio in any water treatment group. In ad lib water rats, BUN was increased only at the highest dosage level, 0.150 mg CCl4/kg bw. CCl4 had no effect on BUN in groundwater mix or water restricted rats. No hepatic or renal lesions occurred that could be attributed to groundwater mix alone. Conclusion: The response to CCl4 in the restricted water rats was similar to that of groundwater mix rats, indicating that a substantial portion of the effect of groundwater mix on CCl4 hepatotoxicity is due to decreased water and feed intake.</p>
<p>Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)</p>	<p>Interesting study design. Gives evidence that some of the effects seen in different concentrations of groundwater mixture of 25 chemicals might be attributable to restricted feed and water intake caused by the test substance administration (via drinking water).</p>