Guidelines/Criteria

Reference:	Hooth MJ, McDorman KS, Hester SD, George MH, Brooks LR, Swank AE, Wolf DC. 2002. The carcinogenic response of Tsc2 mutant Long-Evans (Eker) rats to a mixture of drinking water disinfection by-products was less than additive. Toxicol Sci 69:322-331.
***In vivo* Study Type**	
Route of Administration	oral via drinking water
Species & age of animals	Male and female Eker (Tsc2 Mutant Long-Evans) rats (10 weeks old)
Study Duration	4 and 10 months
Type of Mixture	
Binary	No
>2 components	4 components
Similar acting or dissimilar	dissimilar
What Mode of Action was investigated?	Potassium bromide: mutagenic, renal toxicant, renal carcinogen (MOA: oxidative DNA damage) Mutagen X: mutagenic, renal toxicant, no renal carcinogen (MOA: direct-acting mutagen) Chloroform: not mutagenic, renal toxicant, renal carcinogen (MOA: cytotoxicity/regenerative cell proliferation Bromodichloromethane: weakly mutagenic, renal toxicant, renal carcinogen (corn oil gavage) and no renal carcinogen (drinking water), (MOA: DNA damage, cytotoxicity/regenerative cell proliferation
Parameters/End points Measured	
Target organs/Critical effects	Renal carcinogenicity; total macroscopy; taken tissues were: adrenal glands, gross lesions, kidneys, large intestine, liver, spleen, testicles including surrounding membranes, thyroid gland, urinary bladder, and uterus; liver weights were recorded and all gross lesions were counted and measured (organs taken are based on the results of the individual chemicals toxicological studies)
Pharmacological changes or adverse effects	Adverse
Individual Components	
Characterisation of individual compounds	yes
Name, exact chemical name, CAS no.	Potassium bromate ($KBrO_3$) CAS 7758-01-2 Mutagen X CAS 77439-76-0 Chloroform ($CHCl_3$) CAS 67-66-3 Bromodichloromethane (BDCM) CAS 75-27-4
Were dose responses established for individual components?	Only two dose levels
Were no effect levels established?	Yes when compared to control. The mutant rat strain spontaneously developed renal lesions up to carcinoma in the duration of the study (4 and 10 months).
Were doses below the NO(A)ELs investigated?	Yes, see above
Mixtures Investigated	
Number of dose levels	

		$KBrO_3$		MX		$CHCl_3$		BDCM		Mixture	
Dose	Control	0.02 g/l	0.40 g/l	0.005 g/l	0.07 g/l	0.40 g/l	1.80 g/l	0.07 g/l	0.70 g/l	Low dose	High dose
Treatment time											
4 Months											
Male	10	8	8	8	8	8	8	8	8	10	10
Female	10	8	8	8	8	N/A	N/A	8	8	10	10
10 Months											
Male	10	8	8	8	8	8	8	8	8	14	14
Female	10	8	8	8	8	N/A	8	8	8	14	14

Note. Values are number of Eker rats in each group. N/A, groups not included in study design.

Chemical Consumption

Treatment (g/l)	Male (mg/kg/day)		Female (mg/kg/day)	
	Individual	Mixture	Individual	Mixture
$KBrO_3$ (0.02)	1.3 ± 0.05	1.2 ± 0.08	2.0 ± 0.1	1.8 ± 0.08
$KBrO_3$ (0.4)	23.1 ± 3.0	19.6 ± 1.6	36.1 ± 3.7	29.2 ± 2.7
MX (0.005)	0.28 ± 0.01	0.31 ± 0.019	0.45 ± 0.044	0.44 ± 0.032
MX (0.07)	4.2 ± 0.4	4.3 ± 0.7	7.2 ± 0.7	6.0 ± 0.5
$CHCl_3$ (0.4)	26.8 ± 1.2	26.0 ± 1.7	N/A	38.1 ± 2.1
$CHCl_3$ 1.8	102.1 ± 2.6	96.6 ± 8.1	158.0 ± 18.0	148.8 ± 16.8
BDCM 0.07	3.5 ± 0.1	3.8 ± 0.3	6.5 ± 0.4	5.6 ± 0.3
BDCM 0.7	35.0 ± 1.4	32.3 ± 2.4	55.6 ± 2.9	48.0 ± 4.9

Note. Chemical consumption was based on the measured chemical concentrations of the drinking water solutions, calculated time-weighted water consumption, and animal body weight. Values are mean ± SD for group.

How does the mixture make-up compare to individual components? (e.g. low dose) equivalents used?) No. of technical replicates per exposure condition (*in vitro*) No. of animals per dose group (*in vivo*)	Each one low dose and one high dose of the individual chemicals were tested and then a low dose and a high dose mixture group not applicable 8 - 10 per sex and group
Observations/Findings	No differences in animal survival; decreased mean group body weights was observed in males receiving the high dose mixture solution. Non-neoplastic lesions (after 10 month of treatment): The results of the low dose and high dose mixtures were similar to the results seen with the individual animals. Neoplastic lesions (after 4 month of treatment); Males: No increased incidences of renal adenomas and carcinomas observed with the individual chemicals and in the mixture groups. Females: Significant increased in adenomas and total tumours in the high dose mixture group compared to controls and to low dose mixture group. Not discussed by the authors. However, this finding did not carry through to the 10 month time point, where the MX high dose had a considerably higher adenoma incidence than the mixture high dose. At 4 months, the MX high dose adenoma incidence was somewhat increased, but not statistically significantly. It would be interesting to know if the difference between the mixtures and MX high dose groups at 4 months was statistically significant. Probably, the increase in adenoma incidence in the high dose mixture is rather a chance finding. And it is not relevant for the purpose of the ECETOC report, as only the high dose groups are concerned. Neoplastic lesions (after 10 months of treatment); Males: Increased incidences of renal adenomas and total tumours in the high dose MX and high dose mixture groups compared to controls and to low dose groups. Significant increase in the mean number of carcinomas in the low dose KBrO3 group compared to controls, but no effect observed in low dose mixture group or in the high dose KBrO3 group. Due to lack in dose-response, the low dose single component finding is probably coincidental. Females: Significant increase in adenomas and total tumours in the high dose MX group compared to control and low dose MX group. Number of adenomas in high dose mixture group was significantly increased compared to control animals. Treatment with the mixture produced on average no more tumours than with the individual compound with the greatest effect (note the 4 month female data discussed above). Proliferative lesions after 10 months of treatment: Increased in the number of splenic hemangiomas seen with KBrO3 in male rats only. Splenic lesion burden in the mixture groups was comparable to controls. Increased uterus leiomyomas was seen in low and high dose KBrO3 and MX groups and in the mixture groups. The lesion burden was lower in the both mixture groups compared to the individual KBrO3 and MX groups. Overall conclusion: Default assumption of additivity may overestimate the carcinogenic effect of chemical mixtures in water
Overall opinion (e.g. sufficient numbers of groups investigated, group sizes adequate, observations reproducible, low dose levels used investigated)	Small dose groups, only two doses, limited relevance of the test system due to increased spontaneous tumour rate. Overall conclusion only valid for kidney toxicity. Advantage is the inclusion of one dose representing the NOAEL for neoplastic lesion incidence. At that dose, neither the mixture (containing the same amounts of the individual components which were also tested separately) nor the individual components showed any significantly increased lesion incidence (with the one exception lacking dose-response discussed above). Due to the lack of established dose-response curves, the experimental design would not have been useful to evaluate any effects in the mixtures exceeding individual component effects. Luckily this did not occur, and no judgement on additive versus more than additive needs to be made.