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Final Risk Evaluation for Trichloroethylene

Systematic Review Supplemental File:

Data Extraction and Evaluation Tables for Genotoxicity Studies

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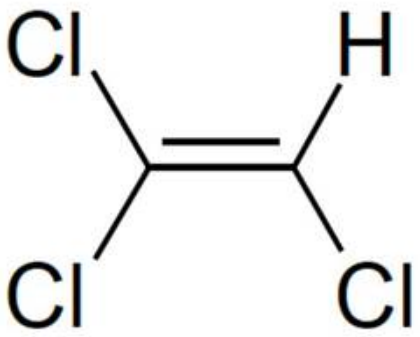

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The tables below present all relevant genotoxicity studies for TCE and various important metabolites. Relevant metabolites were selected based on the species most closely associated with a potential mutagenic mode of action for cancer target sites (i.e., conjugative metabolites for kidney, CH for liver, see Section 3.2.4.2.2 of the Risk Evaluation). Studies that score unacceptable or were not evaluated are indicated in red text.

Table 1. TCE genotoxicity in bacterial, yeast, and fungal systems

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
BACTERIAL SYSTEMS						
S. typhimurium (TA100)	0.1–10 µL (epoxide-free)	–	–	Plate incorporation assay	Henschler et al. (1977)	Unacceptable
S. typhimurium (TA1535, TA100)	1–2.5% (epoxide-free)	+ (TA100) – (TA1535)	– (TA100) – (TA1535)		Simmon et al. (1977)	Medium
S. typhimurium (TA98, TA100)	0.5–10%	–	–	The study was conducted in sealed dessicator vials	Waskell (1978)	Unacceptable
S. typhimurium (TA100, TA1535)	1–3% (epoxide-free)	+ (TA100) ± (TA1535)	–		Baden et al. (1979)	High
S. typhimurium (TA100)	5–20% (v/v)	–	–	Negative under normal conditions, but twofold increase in mutations in a preincubation assay	Bartsch et al. (1979)	Low
	0.33–1.33% (epoxide-free)	+	–		Crebelli et al. (1982)	Not evaluated – foreign language
S. typhimurium (TA1535, TA100)	1–5% (higher and lower purity)	– (higher purity) + (lower purity)	–	Extensive cytotoxicity	Shimada et al. (1985)	Medium
S. typhimurium (TA98, TA100, TA1535, TA1537, TA97)	10–1,000 µL/plate	–	–	Preincubation protocol	Mortelmans et al. (1986)	High
S. typhimurium (TA98, TA100, TA1535)	≤10,000 µg/plate (unstabilized)	–	Not determined	Vapor assay	McGregor et al. (1989)	High
	≤10,000 µg/plate (oxirane-stabilized)	+	+	Vapor assay	McGregor et al. (1989)	High

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
S. typhimurium	≤10,000 µg/plate (epoxybutane stabilized)	Not determined	+	Preincubation assay	McGregor et al. (1989)	High
	≤10,000 µg/plate (epichlorohydrin stabilized)	Not determined	+	Vapor assay	McGregor et al. (1989)	High
S. typhimurium (YG7108)	1.000–3.000 µg/plate	Not determined	+	Microcolony assay/revertants	Emmert et al. (2006)	High
Escherichia coli (K12)	0.9 mM (analytical grade)	+	–	Revertants at arg56 but not nad113 or other loci	Greim et al. (1975)	Unacceptable
YEAST AND FUNGAL SYSTEMS						
Gene Mutation and Recombination						
Saccharomyces cerevisiae D7 and D4	15 and 22 mM; 1 and 4 hrs	Not determined	+ at 1 hr, D7 strain; – at 4 hrs, both D7 and D4	Gene conversion; CYP content fivefold greater in D7 strain; high cytotoxicity at 22 mM	Callen et al. (1980)	High
S. cerevisiae D7	11.1, 16.6, and 22.2 mM	–	–	Both stationary and log phase/production of phototropic colonies	Koch et al. (1988)	High
Schizosaccharomyces pombe	0.2–200 mM (“pure” and technical-grade)	–	–	Forward mutation, different experiments with different doses and time	Rossi et al. (1983)	High
S. cerevisiae D7 and D4		+	–	Point mutations and gene conversions in D7, gene conversion in D4 strain	Bronzetti et al. (1980)	Not evaluated – foreign language
A. nidulans		No data	+	Forward mutation	Crebelli et al. (1985)	High
S. cerevisiae D7 and D4	15 and 22 mM; 1 and 4 hrs	Not determined	+		Callen et al. (1980)	High
A. nidulans		Not determined	+	Gene cross over	Crebelli et al. (1985)	High
Mitotic aneuploidy						
S. cerevisiae D61.M	5.5, 11.1, and 16.6 mM	+	+	Loss of dominant color homolog	Koch et al. (1988)	High

Table 2. TCE genotoxicity in mammalian systems

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
Gene mutations (forward mutations)						
Schizosaccharomyces pombe	2 g/kg, 4 and 16 hrs	Not determined	–	Host-mediated: i.v. and i.p. injections of yeast cells	Rossi et al. (1983)	High
Gene mutations (mutations frequency)						
lac Z transgenic mice	0, 203, 1,153, or 3,141 ppm	No base changes or small deletions	No base changes or small deletions	Lung, liver, bone marrow, spleen, kidney, testicular germ cells used	Douglas et al. (1999)	Medium
Chromosomal aberrations[a]						
Chinese hamster ovary	745–14,900 µg/mL	Not determined	–	8–14 hrs	Galloway et al. (1987)	High
	499–14,900 µg/mL	–	Not determined	2 hrs exposure	Galloway et al. (1987)	High
C57BL/6J mice	5, 50, 500, or 5,000 ppm (6 hrs)	_	Not applicable	Splenocytes	Kligerman et al. (1994)	High
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm (6 hrs, single and 4-d exposure)	_	Not applicable	Peripheral blood lymphocytes	Kligerman et al. (1994)	High
Micronucleus						
Human hepatoma HepG2 cells	0.5–4 mM, 24 hrs	Not applicable	+		Hu et al. (2008)	High
Primary cultures of human and rat kidney cells	1.0, 2.0, or 4.0 mM	Not applicable	+	Dose-dependent significant increase	Robbiano et al. (2004)	High
Sprague-Dawley rats	3,591 mg/kg	+	–		Robbiano et al. (2004)	High
Male Sprague-Dawley rats; proximal tubule cells (in vivo)	4 mM/kg	+	Not applicable	Statistically significant increase in the average frequency of micronucleated kidney cells was observed.	Robbiano et al. (1998)	High
Chinese hamster ovary-K1 cells	0.8–1.4 ppm		+	Dose-dependent significant increase	Wang et al. (2001)	Medium
Male CD-1 mice	457 mg/kg	+	Not applicable	Bone marrow, correlated with TCOH in urine	Hrelia et al. (1994)	High
C56BL/6J mice	5, 50, 500, or 5,000 ppm (6 hrs)	–	Not applicable	Splenocytes	Kligerman et al. (1994)	High

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm (6 hrs)	+	Not applicable	Dose dependent; peripheral blood lymphocytes	Kligerman et al. (1994)	High
Male CD rats	50, 500, 2500, or 5000 ppm (6 hrs)	–	Not applicable	Bone marrow	Wilmer et al. (2014)	High
Sister Chromatid Exchanges						
Chinese hamster ovary	0.17%	–	Not determined	1 hr (vapor)	White et al. (1979)	Medium
	17.9–700 µg/mL	Not determined	+	25 hrs (liquid)	Galloway et al. (1987)	High
	49.7–14,900 µg/mL	+	Not determined	2 hrs	Galloway et al. (1987)	High
Human lymphocytes	178 µg/mL	Not determined	+		Gu et al. (1981a; 1981b)	Not evaluated – foreign language
Sprague-Dawley rats	5, 50, 500, or 5,000 ppm	–	Not applicable	Peripheral blood lymphocytes	Kligerman et al. (1994)	High
Peripheral blood lymphocytes from humans occupationally exposed	Occupational exposure	–	Not applicable		Nagaya et al. (1989a)	Unacceptable
C57BL/6J mice	5, 50, 500, or 5,000 ppm	–	Not applicable	Splenocytes	Kligerman et al. (1994)	High
Unscheduled DNA Synthesis						
Rat primary hepatocytes		Not determined	–		Shimada et al. (1985)	High
Human lymphocytes	2.5, 5, or 10 µL/mL	±	–	Increase was only in certain doses and maximum at 5 µL/mL concentration	Perocco and Prodi (1981)	Unacceptable
Phenobarbital-induced rat hepatocytes	2.8 mM	Not determined	+		Costa and Ivanetich (1984)	High
DNA strand breaks/protein crosslinks						
Primary rat kidney cells	0.5, 1.0, 2.0, or 4.0 mM	Not applicable	+	Dose-dependent significant increase	Robbiano et al. (2004)	High
Primary cultures of human kidney cells	1.0, 2.0, or 4.0 mM	Not determined	+	Dose-dependent significant increase	Robbiano et al. (2004)	High

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
Sprague-Dawley rats	3,591 mg/kg	+	Not applicable	Single oral administration	Robbiano et al. (2004)	High
Sprague-Dawley rats (proximal tubule cell suspension)	500, 1,000, and 2,000 ppm	–	Not applicable	Comet assay	Clay (2008)	High
Cell transformation						
BALB/c 3T3 mouse cells	4, 20, 100, or 250 µg/mL	Not applicable	+	Weakly positive compared to other halogenated compounds tested in the same experiment	Tu et al. (1985)	High
Rat embryo cells		Not applicable	+		Price et al. (1978)	Unacceptable
Syrian hamster embryo cells	5, 10, or 25 µg/mL	Not applicable	–		Amacher and Zelljadt (1983)	Unacceptable

Table 3, CH genotoxicity in bacterial, yeast, fungal, and invertebrate systems

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference	Data Quality
		With activation	Without activation		
BACTERIAL SYSTEMS					
SOS chromotest, *Escherichia coli* PQ37	10,000	–	–	Giller et al. (1995)	Unacceptable
S. typhimurium TA100, TA1535, TA98, reverse mutation	10,000	–	–	Waskell (1978)	Unacceptable
S. typhimurium TA100, TA1537, TA1538, TA98, reverse mutation	1,000	+	+	Haworth et al. (1983)	High
S. typhimurium TA100, reverse mutation	5,000 µg/plate	–	–	Leuschner and Leuschner (1991)	Unacceptable
S. typhimurium TA100, reverse mutation	2,000 µg/plate	+	+	Ni et al. (1994)	Unacceptable
S. typhimurium TA100, reverse mutation, liquid medium	300	+	–	Giller et al. (1995)	Unacceptable
S. typhimurium TA100, TA104, reverse mutation	1,000 µg/plate	+	+	Beland (1999)	High
S. typhimurium TA104, reverse mutation	1,000 µg/plate	+	+	Ni et al. (1994)	Unacceptable
S. typhimurium TA1535, reverse mutation	1,850	–	–	Leuschner and Leuschner (1991)	Unacceptable
S. typhimurium TA1535, TA1537 reverse mutation	6,667	–	–	Haworth et al. (1983)	High
S. typhimurium TA1535, reverse mutation	10,000	–	–	Beland (1999)	High
S. typhimurium TA98, reverse mutation	7,500	–	–	Haworth et al. (1983)	High
S. typhimurium TA98, reverse mutation	10,000 µg/plate	–	+	Beland (1999)	High
YEAST AND FUNGAL SYSTEMS					
A.nidulans, diploid strain 35X17, mitotic cross-overs	1,650	Not tested	–	Crebelli et al. (1985)	High
A. nidulans, diploid strain 30, mitotic cross-overs	6,600	Not tested	–	Kafer (1986)	Medium
A. nidulans, diploid strain NH, mitotic cross-overs	1,000	Not tested	–	Kappas (1989)	High
A. nidulans, diploid strain P1, mitotic cross-overs	990	Not tested	–	Crebelli et al. (1991)	Medium
A. nidulans, diploid strain 35X17, nondisjunctions	825	Not tested	+	Crebelli et al. (1985)	High
A. nidulans, diploid strain 30, aneuploidy	825	Not tested	+	Kafer (1986)	Medium
A. nidulans, haploid conidia, aneuploidy, polyploidy	1,650	Not tested	+	Kafer (1986)	Medium
A. nidulans, diploid strain NH, nondisjunctions	450	Not tested	+	Kappas (1989)	High

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference	Data Quality
		With activation	Without activation		
A. nidulans, diploid strain P1, nondisjunctions	660	Not tested	+	Crebelli et al. (1991)	Medium
A. nidulans, haploid strain 35, hyperploidy	2,640	Not tested	+	Crebelli et al. (1991)	Medium
S. cerevisiae, meiotic recombination	3,300	Not tested	Inconclusive	Sora and Agostini Carbone (1987)	Unacceptable
S. cerevisiae, disomy in meiosis	2,500	Not tested	+	Sora and Agostini Carbone (1987)	Unacceptable
S. cerevisiae, disomy in meiosis	3,300	Not tested	+	Sora and Agostini Carbone (1987)	Unacceptable
S. cerevisiae, D61.M, mitotic chr. malsegregation	1,000	Not tested	+	Albertini (1990)	High
S. cerevisiae, D6, chromosome loss	1,000 - 5,000 (range tested)	Not tested	+	Parry et al. (1990)	Unacceptable
INVERTEBRATE SYSTEMS					
Drosophila melanogaster, somatic mutation wing spot test	825	NA	+	Zordan et al. (1994)	High
Drosophila melanogaster, induction of sex-linked lethal mutation	37.2 feed	NA	Inconclusive	Yoon (1985)	Medium
Drosophila melanogaster, induction of sex-linked lethal mutation	67.5 inj	NA	–	Yoon (1985)	Medium

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; inj = injection; NA = not applicable (*in vivo*).
+ = positive; – = negative
Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

Table 4. CH genotoxicity in mammalian systems

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference	Data Quality
		With activation	Without activation		
IN VITRO SYSTEMS					
Micronucleus formation					
Micronucleus formation (kinetochore-positive), Chinese hamster C1 cells, in vitro	165	NT	+	Degrassi and Tanzarella (1988)	Unacceptable
Micronucleus formation (kinetochore-negative), Chinese hamster C1 cells, in vitro	250	NT	–	Degrassi and Tanzarella (1988)	Unacceptable
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Parry et al. (1990)	Low
Micronucleus formation (kinetochore-positive), Chinese hamster LUC2 cells, in vitro	400	NT	+	Lynch and Parry (1993)	High
Micronucleus formation, Chinese hamster V79 cells, in vitro	316	NT	+	Seelbach et al. (1993)	High
Micronucleus formation, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	1,300	NT	–	Harrington-Brock et al. (1998)	Medium
Micronucleus formation, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	500	NT	+	Nesslany and Marzin (1999)	High
Micronucleus formation, human lymphocytes, in vitro	100	–	+	Van Hummelen and Kirsch-Volders (1992)	High
Micronucleus formation, human lymphoblastoid AHH-1 cell line, in vitro	100	NT	+	Parry et al. (1996)	Unacceptable
Micronucleus formation, human lymphoblastoid maximum contaminant level-5 cell line, in vitro	500	NT	–	Parry et al. (1996)	Unacceptable
Micronucleus formation (kinetochore-positive), human diploid LEO fibroblasts, in vitro	120	NT	+	Bonatti et al. (1992)	High
Micronucleus formation, human lymphocytes, in vitro	25	NT	+	Varshney et al. (2013)	High
Chromosomal aberrations					
Chromosomal aberrations, Chinese Hamster CHED cells, in vitro	20	NT	+	Furnus et al. (1990)	High
Chromosomal aberrations, Chinese Hamster ovary cells, in vitro	1,000	+	+	Beland (1999)	High
Chromosomal aberrations, mouse lymphoma L5178Y/TK $^{\pm}$ cells line, in vitro	1,250	NT	(+)	Harrington-Brock et al. (1998)	Medium
Aneuploidy and copy number aberrations					
Aneuploidy, Chinese hamster CHED cells, in vitro	10	NT	+	Furnus et al. (1990)	High
Aneuploidy, primary Chinese hamster embryonic cells, in vitro	250	NT	+	Natarajan et al. (1993)	High
Aneuploidy, Chinese hamster LUC2p4 cells, in vitro	250	NT	+	Warr et al. (1993)	High

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference	Data Quality
		With activation	Without activation		
Aneuploidy, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	1,300	NT	–	Harrington-Brock et al. (1998)	Medium
Tetraploidy and endoreduplication, Chinese hamster LUC2p4cells, in vitro	500	NT	+	Warr et al. (1993)	High
Aneuploidy (double Y induction), human lymphocytes, in vitro	250	NT	+	Vagnarelli et al. (1990)	High
Aneuploidy (hyperdiploidy and hypodiploidy), human lymphocytes in vitro	50	NT	+	Sbrana et al. (1993)	High
Polyploidy, human lymphocytes, in vitro	137	NT	+	Sbrana et al. (1993)	High
Cell transformation assay					
Cell transformation, Syrian hamster embryo cells (24-hr treatment)	350	NT	+	Gibson et al. (1995)	High
Cell transformation, Syrian hamster dermal cell line (24-hr treatment)	50	NT	+	Parry et al. (1996)	Unacceptable
DNA single-strand breaks (SSBs)					
DNA SSBs, human lymphoblastoid cells, in vitro	1,650	NT	–	Chang et al. (1992)	High
DNA SSBs, rat primary hepatocytes in vitro	1,650	NT	–	Chang et al. (1992)	High
DNA crosslinking, gene mutation, SCEs, and aberrant mitosis					
DNA-protein cross-links, rat nuclei in vitro	41,250	NT	–	Keller and Heck (1988)	High
Gene mutation, mouse lymphoma L5178Y/TK $^{\pm}$, in vitro	1,000		(+)	Harrington-Brock et al. (1998)	High
Gene mutation, *tk and hprt* locus, human lymphoblastoid	1,000	NT	+	Beland (1999)	Medium
SCEs, Chinese hamster ovary cells, in vitro	100	+	+	Beland (1999)	High
SCEs, human lymphocytes, in vitro	54	NT	(+)	Gu et al. (1981a)	Not evaluated – foreign language
C-Mitosis, human lymphocytes, in vitro	75	NT	+	Sbrana et al. (1993)	High
Aberrant spindle defects, immortalized Chinese hamster cells, in vitro	500	NA	+	Parry et al. (1990)	Low
IN VIVO/EX VIVO SYSTEMS					
Micronucleus formation					
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	NA	–	Leuschner and Leuschner (1991)	High
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	NA	–	Russo and Levis (1992b)	Medium

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference	Data Quality
		With activation	Without activation		
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	NA	+	Russo and Levis (1992a)	Medium
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	NA	+	Russo et al. (1992)	Medium
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	NA	–	Leopardi et al. (1993)	Medium
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	NA	+	Allen et al., (1994)	High
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	NA	+	Marrazzini et al., (1994)	High
Micronucleus formation, B6C3F_1 mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	NA	+	Nutley et al. (1996)	High
Micronucleus formation, B6C3F_1 mouse spermatids after meiotic cell treatment	413, i.p.	NA	–	Nutley et al. (1996)	High
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	NA	–	Grawe et al. (1997)	Medium
Micronucleus formation, male B6C3F_1 mouse bone-marrow erythrocytes	500, i.p., × 3	NA	+	Beland (1999)	High
Micronucleus formation, infants, peripheral lymphocytes	50, oral	NA	+	Ikbal et al. (2004)	Medium
Chromosomal aberrations					
Chromosomal aberrations, male and female F1 mouse bone marrow cells	600, i.p.	NA	–	Xu and Alder (1990)	Medium
Chromosomal aberrations, male and female Sprague-Dawley rat bone-marrow cells	1,000, oral	NA	–	Leuschner and Leuschner (1991)	Medium
Chromosomal aberrations, BALB/c mouse spermatogonia treated	83, i.p.	NA	–	Russo and Levis, (1992a)	Medium
Chromosomal aberrations, F1 mouse secondary spermatocytes	82.7, i.p.	NA	+	Russo et al. (1984)	Medium
Chromosomal aberrations, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	NA	–	Marrazzini et al. (1994)	High
Chromosomal aberrations, ICR mouse oocytes	600, i.p.	NA	–	Mailhes et al. (1993)	High
Aneuploidy and copy number aberrations					
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	NA	–	Xu and Alder (1990)	Medium
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	NA	+	Miller and Adler (1992)	Medium
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	NA	–	Leopardi et al. (1993)	Medium

Test system/endpoint	Doses (LED or HID)[a]	Results		Reference	Data Quality
		With activation	Without activation		
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	NA	+	Marrazzini et al. (1994)	High
DNA single-strand breaks (SSBs)					
DNA SSBs, male Sprague-Dawley rat liver	300, oral	NA	+	Nelson and Bull (1988)	High
DNA SSBs, male F344 rat liver	1,650, oral	NA	–	Chang et al. (1992)	High
DNA SSBs, male B6C3F_1 mouse liver	100, oral	NA	+	Nelson and Bull (1988)	High
DNA SSBs, male B6C3F_1 mouse liver	825, oral	NA	–	Chang et al. (1992)	High

[a]LED = lowest effective dose; HID = highest ineffective dose; doses are in µg/mL for in vitro tests; inj = injection; NA = not applicable (*in vivo)*.
+ = positive; (+) = weakly positive; – = negative
Source: Table adapted from IARC monograph (2004b) and modified/updated for newer references.

Table 5. GSH conjugation metabolite genotoxicity in all systems

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
Gene mutations (Ames test)						
S. typhimurium, TA100, 2638, 98	0.1–0.5 nmol	ND	+	DCVC was mutagenic in all three strains of *S. typhimurium* without the addition of mammalian subcellular fractions.	Dekant et al., (1986c)	High
S. typhimurium, TA2638	50–300 nmol	+	+	Increase in number of revertants in DCVC alone at low doses; further increase in revertants was observed in the presence of microsomal fractions. Toxicity as indicated by decreased revertants per plate were seen at higher doses.	Vamvakas et al. (1988b)	High
Mutation analysis						
In vitro—rat kidney epithelial cells, LOH in *Tsc* gene	10 µM	NA	–	Only 1/9 transformed cells showed LOH.	Mally et al. (2006)	Unacceptable
In vitro—rat kidney epithelial cells, *VHL* gene (exons 1–3)	10 µM	NA	–	No mutations in *VHL* gene. Note: *VHL* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis.	Mally et al. (2006)	Unacceptable
Unscheduled DNA Synthesis (UDS)						
Porcine kidney tubular epithelial cell line (LLC-PK1)	2.5 µM–5, 10, 15, 24 hrs; 2.5–100 µM	NA	+	Dose-dependent in UDS up to 24 hrs tested at 2.5 µM. Also, there was a dose-dependent increase at lower concentrations. Higher concentrations were cytotoxic as determined by LDH release from the cells.	Vamvakas et al. (1989)	High
Syrian hamster embryo fibroblasts		NA	+	Increase in UDS in treatment groups.	Vamvakas et al. (1988a)	Medium
DNA strand breaks						
Male rabbit renal tissue (perfused kidneys and proximal tubules)	0–100 mg/kg or 10 µM to 10 mM	ND	+	Dose-dependent increase in strand breaks in both i.v. and i.p. injections (i.v. injections were done only for 10 and 20 mg/kg) were observed. Perfusion of rabbit kidney (45-min exposure) and proximal tubules (30-min exposure) experiment resulted in a dose-dependent difference in the amount of SSBs.	Jaffe et.al. (1985)	Medium

Test system/endpoint	Doses tested	Results		Comments	References	Data Quality
		With activation	Without activation			
Primary kidney cells from both male rats and human	1–4 mM; 20 hrs exposure	NA	+	Statistically significant increase in all doses (1, 2, or 4 mM) both in rats and human cells.	Robbiano et al. (2004)	High
Male Sprague-Dawley rats—comet assay (proximal tubule cell suspensions analyzed)	1 or 10 mg/kg; single oral dose incubated for 2 hrs (both doses) or 16 hrs (10 mg)	+	NA	Two replications performed. In exp 1, both 1 or 10 mg to DCVC resulted in significant increase in tail length (only increase at 1 mg for other metrics) with no dose-response at 2 hr but ND at 16 hrs. In exp 2, significant increase for multiple metrics at 10 mg at both 2 hr and 16 hr.	Clay (2008)	High
Micronucleus						
Syrian hamster embryo fibroblasts		NA	–	No micronucleus formation.	Vamvakas et al. (1988a)	Unacceptable
Primary kidney cells from both male rats and human	1–4 mM; 20 hrs exposure	NA	+	Statistically significant increase in all doses (1, 2, and 4 mM) both in rat and human cells.	Robbiano et al. (2004)	High
Cell transformation						
Kidney tubular epithelial cell line (LLC-PK1)	1or 5 µM; 7 wks	NA	+	Induced morphological cell transformation at both concentrations tested. Furthermore, cells maintained both biochemical and morphological alterations remained stable for 30 passages.	Vamvakas et al. (1996)	High
Rat kidney epithelial cells (in vitro)	10 µM; 24 hrs exposure, 7 wks post incubation	NA	+	Cell transformation was higher than control; however, cell survival percentage ranged from 39 to 64%, indicating cytotoxicity.	Mally et al. (2006)	Medium
Gene expression						
Kidney tubular epithelial cell line (LLC-PK1)	1 or 5 µM clones, 30, 60, or 90 min	NA	+	Increased c-Fos expression in 1 and 5 µM exposed clones at three different times tested.	Vamvakas et al. (1996)	High
Kidney tubular epithelial cell line (LLC-PK1)		NA	+	Expression of c-Fos and c-Myc increased in a time-dependent manner.	Vamvakas et al. (1993)	Unacceptable

LDH = lactate dehydrogenase; ND = not determined; NA = not applicable