Supplementary material for Bogen KT and Heilman JM, (2015). Reassessment of MTBE cancer potency considering modes of action for MTBE and its metabolites, Crit Rev Toxicol, 45, 1–56.

Supplementary/Online Materials:

Appendices A–E

Supplementary Appendix A: Genotoxicity Data Tables (Tables A-1 – A-5)

Tables summarizing genotoxicity data that are discussed in Section 3 of the text appear in this appendix. Specifically, Tables A-1 – A-5 summarize genotoxicity data obtained for MTBE, Formaldehyde, TBA, Methanol, and Formic Acid, respectively.

Supplementary Table A-1. Genotoxicity studies of MTBE^a

Mutation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Cinelli et al. 1992	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA 1538	–	–
Hüls 1991§	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA 1538	–	–
Kado et al. 1998	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA104, TA1535	–	–
Life Science Research 1989b§	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA 1538	ND	–
Litton Bionetics Inc. 1978; ARCO 1980	Reverse mutation	<i>Saccharomyces cerevisiae</i> D4	–*	–
Litton Bionetics Inc. 1978; ARCO 1980	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA 1538	–	–
McGregor et al. 2005	Reverse mutation	<i>S. typhimurium</i> TA102	–	–
RBM 1996 §	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	–	–
Williams-Hill et al. 1999	Reverse mutation	<i>S. typhimurium</i> TA102	[+]	+/[–]
Zhou et al. 1998, 2000	Reverse mutation	<i>S. typhimurium</i> TA98, TA100	–	–
ARCO 1980	Gene mutation	Mouse lymphoma cell line L5178Y TK+/-	+	–
Cinelli et al. 1992	Gene mutation	HPRT Chinese hamster V79 cells	–	–**
Life Science Research 1989a	Gene mutation in HPRT-locus	Chinese hamster V79 cells	–	–**
Mackerer et al. 1996	Gene mutation	Mouse lymphoma cell line L5178Y TK+/- (±S9) (+ formaldehyde (FA) dehydrogenase + NAD). Presence of FA dehydrogenase decreased genotoxic effect of MTBE indicating that the FA metabolite of MTBE is the species responsible for MTBE genotoxicity, at least under these conditions.	+/[–]	ND
Ward et al. 1995	Gene mutation in HPRT-locus	Mouse spleen lymphocytes	ND	–**

DNA Adducts, Fragmentation, or Oxidation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Casanova and Heck 1997	DPX and RFA formation with isotopically labeled MTBE	Female CD-1 mouse, male B6C3F ₁ mouse, and F344 rat hepatocytes with and without pretreatment to induce P450	ND	–
Chen et al. 2008	Comet assay for DNA strand breaks	Isolated human lymphocytes from a single donor	ND	+
Lee et al. 1998	Comet assay for DNA strand breaks	Rat lymphocytes	ND	+**
Lichtenberg-Fraté et al. 2003	DNA damage	<i>Saccharomyces cerevisiae</i> , strain modified to express green fluorescent protein upon DNA damage	ND	–**
Sgambato et al. 2009	Comet assay for DNA strand breaks; Oxidative DNA damage	Mouse embryo fibroblast C3H/10T _{1/2} /C1/8 cells	ND	+
Tang et al. 1997	DNA strand breakage	Human HL-60 leukemia cells treated <i>in vitro</i>	ND	+ ^b **
Du et al. 2005	AMS analysis of DNA radiolabel	Mice (Kunming, male); liver, lung, and kidney tissues 6 h after 1 ¹⁴ C-MTBE dose by gavage	ND	+**
Yuan et al. 2007	AMS analysis of DNA radiolabel	Mice (Kunming, male); liver, lung, and kidney tissues 6 h after 1 ¹⁴ C-MTBE dose by gavage	ND	+**
Unscheduled DNA Synthesis				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Cinelli et al. 1992	Unscheduled DNA synthesis	Primary rat hepatocytes	–	–
Life Science Research 1989c	Unscheduled DNA synthesis	Primary rat hepatocytes	ND	–
Vergnes et al. 1994; Mckee et al. 1997	Unscheduled DNA synthesis	CD-1 mouse hepatocytes	ND	–**
Zhou et al. 1998, 2000	Unscheduled DNA synthesis	Primary rat hepatocytes	ND	+**

Cytogenetic Damage or Cell Transformation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Litton Bionetics Inc. 1979	Chromosomal aberrations	Rat, Sprague Dawley, male, bone marrow	ND	—**
Litton Bionetics Inc. 1980	Chromosomal aberrations	Chinese hamster ovary cells	—	—
Litton Bionetics Inc. 1980	Sister chromatid exchange	Chinese hamster ovary cells, 95% MTBE	—	—**
Litton Bionetics Inc. 1980	Sister chromatid exchange	Chinese hamster ovary cells, 99% MTBE	—	—**
Kado et al. 1998	Micronucleus test	Swiss Webster Mice erythrocytes	ND	—**
Vergnes et al. 1993; McKee et al. 1997	Micronucleus test	CD-1 Mice erythrocytes	ND	—**
Zhou et al. 1998, 2000	Micronucleus test	Mouse, NIH/3T3, 98.8% MTBE	ND	—**
Semau 1989	Sex-linked recessive lethal test	<i>Drosophila melanogaster</i>	ND	—**
Vergnes et al. 1989; McKee et al. 1997	Bone marrow cytogenetic test	Rat, Fisher 344	ND	—**
Iavicoli et al. 2002	Cell transformation	Rat-1 fibroblasts	ND	+***

^a Studies reported in the EU ECB 2002 Risk Assessment (ECB 2002), updated with new studies identified via literature search through 2012. Primary publications were not obtained for all studies, only studies for which additional information was required to appropriately represent the study herein. Positive results in brackets indicate only negative results occurred in a repeat study. Comet assay = single cell gel electrophoresis (SCGE) assay; AMS = accelerator mass spectrometry without gel-electrophoresis confirmation of purported DNA adducts; LED = lowest effective dose, HID = highest ineffective dose, ND = no applicable data (assay not done).

^b Cruzan et al. 2007; Table 1. A positive result indicates a “significant response.” A negative result indicates “no significant response.”

^c Published in McKee et al. 1997.

§ = OECD or EU guideline study.

* = There was no positive control available in the activation assay for this strain.

= mL/plate (density = 0.74 at 20°C).

** = exogenous metabolic system not specified so none was assumed, or metabolic system was not appropriate for the specific test system, or AMS analysis without corresponding verification of purported adduct(s).

*** Occurred with substantial cell death.

Supplementary Table A-2. Genotoxicity studies of formaldehyde*

Mutation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Ashby et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA100	(+)	–
Ashby et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA97, 98, 1535, 1537, 1538	–	–
Connor et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA100	+	(+)
Connor et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA98	(+)	–
Connor et al. 1985	Reverse mutation	<i>S. typhimurium</i> UTH8414, UTH8413	–	–
Connor et al. 1983 ^a	Reverse mutation	<i>S. typhimurium</i> TA98, TA100	+	+
Connor et al. 1983 ^a	Reverse mutation	<i>S. typhimurium</i> UTH8414, UTH8413	–	–
Couch et al. 1982	Reverse mutation	<i>S. typhimurium</i> TA100	ND	(+)
De Flora et al. 1984	Reverse mutation	<i>S. typhimurium</i> TA102	+	+
De Flora, 1981	Reverse mutation	<i>S. typhimurium</i> TA98, 100, 1535, 1537, 1538	–	–
Dillon et al. 1998	Reverse mutation	<i>S. typhimurium</i> TA100, 104 ^d	+	+
Donovan et al. 1983	Reverse mutation	<i>S. typhimurium</i> TA97,98,100	+	+
Fiddler et al. 1984	Reverse mutation	<i>S. typhimurium</i> TA100	ND	+
Frei et al. 1984	Reverse mutation	<i>S. typhimurium</i> TA100	+	ND
Graves et al. 1994	Reverse mutation	<i>E. coli</i> K12 (AB1157) (WT)	ND	+
Graves et al. 1994	Reverse mutation	<i>E. coli</i> K12(AB1886)/ (uvrA) K12(AB2480)/recA/uvrA)	ND	–
Haworth et al. 1983	Reverse mutation	<i>S. typhimurium</i> TA100	+	–
Haworth et al. 1983	Reverse mutation	<i>S. typhimurium</i> TA1535, 1537	–	–
Haworth et al. 1983	Reverse mutation	<i>S. typhimurium</i> TA98	(+)	–
Jung et al. 1992	Reverse mutation	<i>S. typhimurium</i> TA102	+	ND
Le Curieux et al. 1993	Reverse mutation	<i>S. typhimurium</i> TA102	ND	+
Marnett et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA97, 98, 100	ND	–

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Marnett et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA102, 104	ND	+
Muller et al. 1993	Reverse mutation	<i>S. typhimurium</i> TA 102	+	ND
O'Donovan and Mee 1993	Reverse mutation	<i>S. typhimurium</i> TA 98, 100, 102	ND	+
O'Donovan and Mee 1993	Reverse mutation	<i>S. typhimurium</i> TA1535, 1537, 1538	ND	–
O'Donovan and Mee 1993	Reverse mutation	<i>E. coli</i> WP2 (pKM101)	ND	+
O'Donovan and Mee 1993	Reverse mutation	<i>E. coli</i> WP2uvrA (pKM101)	ND	ND
Oerstavik and Hongslo 1985	Reverse mutation	<i>S. typhimurium</i> TA98, 100	+	+
Ohta et al. 1999	Reverse mutation	<i>E. coli</i> (Lac+reversion) WP3101P, WP3106P	+	ND
Rydén et al. 2000	Reverse mutation	<i>S. typhimurium</i> TA102, 2638 ^d	ND	+
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA1535 ^c	–	–
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA1537 ^c	+	+
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA98, 100 ^c	+	–
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA97 ^c	ND	+
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA1535, 1537 ^d	–	–
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA98 ^d	+	+
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA100 ^d	–	+
Sarrif et al. 1997	Reverse mutation	<i>S. typhimurium</i> TA100 ^e	+	+
Schmid et al. 1986	Reverse mutation	<i>S. typhimurium</i> TA100	+	+
Takahashi et al. 1985	Reverse mutation	<i>S. typhimurium</i> TA98, 100, 102	ND	(+)
Takahashi et al. 1985	Reverse mutation	<i>E. coli</i> WP2, WP2 uvrA	ND	+
Takahashi et al. 1985	Reverse mutation	<i>E. coli</i> H/R30R, HS30RuvrA	ND	+
Takahashi et al. 1985	Reverse mutation	<i>E. coli</i> NG30recA, 016polA	ND	–
Watanabe et al. 1996	Reverse mutation	<i>S. typhimurium</i> TA102, 2638	ND	+
Watanabe et al. 1996	Reverse mutation	<i>E. coli</i> WP2 (pKM101), WP2uvrA (pKM101)	ND	+

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Wilcox et al. 1990	Reverse mutation	<i>S. typhimurium</i> TA102	ND	–
Wilcox et al. 1990	Reverse mutation	<i>E. coli</i> WP2 uvrA(pKM101)	ND	+
Wilcox et al. 1990	Reverse mutation	<i>E. coli</i> WP2 (pKM101)	ND	–
Zielenska and Guttenplan, 1988	Reverse mutation	<i>S. typhimurium</i> TA104	+	ND
Bosworth et al. 1987	<i>Reverse mutation</i>	<i>E. coli</i> D494uvrB	+	ND
Couch et al. 1982	Reverse mutation	<i>S. typhimurium</i> TM677	ND	(+)
Donovan et al. 1983	Reverse mutation	<i>S. typhimurium</i> TM677	+	+
Temcharoen and Thilly 1983	Reverse mutation	<i>S. typhimurium</i> TM677	+	+
Epstein et al. 1972	Dominant lethal mutation	Mouse, dominant lethal <i>in vivo</i>	ND	–
Epstein and Shafner 1968	Dominant lethal mutation	Mouse, dominant lethal <i>in vivo</i>	ND	–
Fontignie-Houbrechts 1981	Dominant lethal mutation	Mouse, dominant lethal <i>in vivo</i>	ND	(+)
Kitaeva et al. 1990	Dominant lethal mutation	Rat, dominant lethal <i>in vivo</i>	ND	(+)
Odeigah 1997	Dominant lethal mutation	Rat/albino spermatocytes, live implants <i>in vivo</i>	ND	+
Craft et al. 1987	Deletion mutation	Lymphoblast/TK	ND	+
Crosby et al. 1988	Deletion mutation	Lymphoblast/HPRT	ND	+ ^f
Crosby et al. 1988	Deletion mutation	<i>E. coli</i> GP120, GP120A 7-2, 33694	ND	+ ^f
Goldmacher and Thilly 1983	Deletion mutation	Lymphoblasts (TK6)	ND	+
Graftstrom et al. 1993	Deletion mutation	Hamster/chinese V79/Hprt	ND	+
Graftstrom 1990	Deletion mutation	Bronchial fibroblasts/epithelial cells (HPRT locus)	ND	+
Graftstrom et al. 1985	Deletion mutation	Human bronchial fibroblasts/epithelial cells (HPRT locus)	ND	+
Graftstrom et al. 1983	Deletion mutation	Human bronchial cell	ND	+
Graves et al. 1996	Deletion mutation	Hamster ovary/CHO-cell Hprt	+	–

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Mackerer et al. 1996	Deletion mutation	Mouse lymphoma L5178Y cells (Tk ^{+/-} locus)	ND	+
Merk and Speit 1998	Deletion mutation	Hamster/Chinese V79 cells (Hprt locus)	ND	—
Merk and Speit 1999	Deletion mutation	Hamster/Chinese V79 cells (Hprt locus)	ND	—
Shaham et al. 2003	Deletion mutation	Peripheral blood lymphocytes <i>in vivo</i>	ND	+
Speit and Merk 2002	Deletion mutation	Mouse Lymphoma L5178Y cells	ND	+
Blackburn et al. 1991	Point mutation	Mouse lymphoma cell/TK +/-	+	+
Crosby et al. 1988	Point mutation	<i>E. coli</i> GP120, GP120A 7-2, 33694	ND	+
Graves et al. 1996	Point mutation	Hamster/Chinese ovary Hprt	ND	+
Liber et al. 1989	Point mutation	Human lymphoma TK6	ND	+
Wangeheim and Bolcsfoldi, 1988	Point mutation	Mouse lymphoma cell /TK +/-	ND	+
Crosby et al. 1988	Insertion mutation	<i>E. coli</i> GP120, GP120A 7-2, 33694	ND	+
Graves et al. 1996	Insertion mutation	Hamster/Chinese ovary Hprt	ND	+
Liu et al. 2009	Heritable mutation	Mouse heritable mutation <i>in vivo</i>	ND	+
Boreiko and Ragan 1983	Cell transformation	Mouse embryo fibroblast C3H/10T _{1/2} cells	ND	[+]
Frazelle et al. 1983	Cell transformation	Mouse embryo fibroblast C3H/10T _{1/2} cells	ND	[+]
Plesner and Hansen 1983	Cell transformation	Hamster kidney cell/BHK-21cl.13	+	+
Ragan and Boreiko 1981	Cell transformation	Mouse embryo fibroblast C3H10T _{1/2} cells	ND	+ ^m
Bermudez et al. 1994	P53 mutation and/or p53 protein expression	Rats/F344 Nasal squamous cell carcinomas	ND	+
Meng et al. 2010	p53 mutation, K-Ras mutation	Male F344/NCrl rats exposed via inhalation	ND	—
Recio et al. 1992	P53 mutation and/or p53 protein expression	Rats/F344 Nasal squamous cell carcinomas <i>in vivo</i>	ND	+ ⁿ

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Shaham et al. 2003	P53 mutation and/or p53 protein expression	Human peripheral blood lymphocytes <i>in vivo</i>	ND	+
Wolf et al. 1995	P53 mutation and/or p53 protein expression	Rats/F344 Nasal squamous cell carcinomas <i>in vivo</i>	ND	+ ^o
Grogan et al. 2012	Insertions	<i>Saccharomyces cerevisiae</i> , wildtype and strains lacking repair genes. Assay was negative for wildtype strains.	ND	—
Jakab et al. 2010	HPRT mutant variant frequency	Human peripheral blood lymphocytes of pathology workers exposed mainly to formaldehyde and possibly other solvents	ND	—
Hayes et al. 1997	DNA repair enzyme activity	Human peripheral lymphocytes <i>in vivo</i>	ND	—
DNA-Protein Crosslinks (DPX)				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Andersson et al. 2003	DPX formation <i>in vitro</i>	Human peripheral blood lymphocytes	ND	+++
Bermudez and Delehanty 1986	DPX formation <i>in vitro</i>	Rat/F344 nasal epithelium	ND	+++
Blasiak et al. 2000	DPX formation <i>in vitro</i>	Human gastric mucosa cells	ND	+++
Brutlag et al. 1969	DPX formation <i>in vitro</i>	<i>In vitro</i> nucleohistone	ND	+++
Casanova et al. 1997	DPX formation <i>in vitro</i>	Mouse hepatocytes	ND	+++
Casanova and Heck 1997	DPX formation <i>in vitro</i>	Mouse hepatocytes	ND	+++
Casanova and Heck 1997	DPX formation <i>in vitro</i>	Rat/F344 hepatocytes	ND	+++
Casanova and Heck 1997	DPX formation <i>in vitro</i>	B6C3F ₁ hepatocytes	ND	+++
Casanova-Schmitz and Heck 1983 ^h	DPX formation <i>in vitro</i>	Rat/F344 nasal mucosa	ND	+++
Cosma et al. 1988	DPX formation <i>in vitro</i>	Rat/F344 primary tracheal epithelial cells	ND	+++
Cosma and Marchok 1988	DPX formation <i>in vitro</i>	Rat/unspecified C18 tracheal epithelial cell line	ND	+++

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Costa et al. 1997	DPX formation <i>in vitro</i>	Human EBV-Burkitt's lymphoma cells	ND	+ ^g **
Craft et al. 1987	DPX formation <i>in vitro</i>	Human lymphoblastoid/TK6	ND	+**
Doenecke 1978	DPX formation <i>in vitro</i>	<i>In vitro</i> nucleohistone	ND	+**
Emri et al. 2004	DPX formation <i>in vitro</i>	Human primary skin fibroblasts and keratinocytes	ND	+**
Fornace et al. 1982	DPX formation <i>in vitro</i>	Human Lung/bronchial epithelial cells	ND	+**
Garcia et al. 2009	DPX formation <i>in vitro</i>	Hamster/Chinese ovary cells	ND	+**
Graftstrom et al. 1986, 1990	DPX formation <i>in vitro</i>	Human Lung/bronchial epithelial cells	ND	+**
Graftstrom et al. 1983	DPX formation <i>in vitro</i>	Human bronchial epithelial cells	ND	+**
Graftstrom et al. 1984	DPX formation <i>in vitro</i>	Human Lung/bronchial epithelial cells, fibroblasts, skin fibroblasts	ND	+**
Li et al. 2004	DPX formation <i>in vitro</i>	Human buccal epithelial cells	ND	+**
Lin et al. 2005	DPX formation <i>in vitro</i>	Rat/Wistar aortic endothelial cells	ND	+**
Liu et al. 2006	DPX formation <i>in vitro</i>	Human peripheral blood lymphocytes	ND	+**
Liu et al. 2006	DPX formation <i>in vitro</i>	Human HeLa cells	ND	+**
Magana-Schwenche and Ekert 1978	DPX formation <i>in vitro</i>	Yeast <i>S. cerevisiae</i>	ND	+**
Marinari et al. 1984	DPX formation <i>in vitro</i>	Hamster/Chinese Ovary cells	ND	+**
Merk and Speit 1998, 1999	DPX formation <i>in vitro</i>	Hamster/Chinese V79 lung epithelial cells	ND	+**

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Neuss et al. 2010a	DPX formation <i>in vitro</i>	Human nasal epithelial cells. Positive with single treatment, negative for DPX upon repeated treatments.	ND	+/-
Neuss et al. 2010b	DPX formation <i>in vitro</i>	Coculture of nasal epithelial cells and lymphocytes – demonstrated that DPX form in directly treated lymphocytes but cannot be passed from epithelial cell to lymphocyte as hypothesized in some literature.	ND	+
O'Connor and Fox 1987	DPX formation <i>in vitro</i>	Rat/unspecified Yoshida lymphosarcoma cells	ND	+++
Ohba et al. 1979	DPX formation <i>in vitro</i>	<i>In vitro</i> nucleohistone	ND	+++
Olin et al. 1996	DPX formation <i>in vitro</i>	Hamster/Chinese Ovary cells	ND	+++
Olin et al. 1996	DPX formation <i>in vitro</i>	Human bronchial/skin fibroblasts	ND	+++
Quievryn and Zhitkovich 2000	DPX formation <i>in vitro</i>	Human peripheral blood lymphocytes	ND	+++
Ross et al. 1981	DPX formation <i>in vitro</i>	Mouse leukemia L1210 cells	ND	+++
Ross and Shipley 1980	DPX formation <i>in vitro</i>	Mouse leukemia L1210 cells	ND	+++
Saito et al. 2005	DPX formation <i>in vitro</i>	Human jurkat E6-1 cells	ND	+++
Saladino et al. 1985	DPX formation <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Schmid and Speit 2007	DPX formation <i>in vitro</i>	Human whole blood cultures	ND	+++
Shaham et al. 1996	DPX formation <i>in vitro</i>	Human white blood cells	ND	+++
Snyder and Van Houten 1986	DPX formation <i>in vitro</i>	Human foreskin fibroblasts	ND	+++
Speit et al. 2010	DPX formation <i>in vitro</i>	Human lung carcinoma (A549) cells	ND	+++
Speit et al. 2008	DPX formation <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Speit et al. 2007	DPX formation <i>in vitro</i>	Hamster/Chinese V79 lung epithelial cells	ND	+++

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Speit et al. 2000	DPX formation <i>in vitro</i>	Human fibroblasts	ND	***
Speit et al. 2010	DPX formation <i>in vitro</i>	Human A549 lung cells	ND	+
Swenberg et al. 1983	DPX formation <i>in vitro</i>	Hamster/Chinese V79 lung epithelial cells	ND	***
Wilkins and McLeod, 1976	DPX formation <i>in vitro</i>	Bacteria <i>E. coli</i>	ND	***
Zhitkovich and Costa, 1992	DPX formation <i>in vitro</i>	Hamster/Chinese Ovary cells	ND	***
Casanova et al. 1991	DPX formation <i>in vitro</i>	Rhesus monkeys Nasal, larynx, trachea and carina	ND	***
Casanova et al. 1989, 1994	DPX formation <i>in vitro</i>	Rat nasal mucosa	ND	***
Casanova-Schmitz et al. 1984	DPX formation <i>in vitro</i>	Rat nasal mucosa	ND	***
Casanova-Schmitz and Heck 1983 ⁿ	DPX formation <i>in vitro</i>	Rat nasal mucosa	ND	***
Cosma et al. 1988	DPX formation <i>in vitro</i>	Rat tracheal implants	ND	***
Heck et al. 1986	DPX formation <i>in vitro</i>	Rat nasal mucosa	ND	***
Heck and Casanova 1987	DPX formation <i>in vitro</i>	Rat nasal mucosa	ND	***
Lam et al. 1985	DPX formation <i>in vitro</i>	Rat nasal mucosa	ND	***
Neuss et al. 2008	DPX formation <i>in vitro</i>	Rat bronchoalveolar lavage cells	ND	—**
Shaham et al. 1996, 2003	DPX formation <i>in vitro</i>	Human peripheral blood lymphocytes	ND	***
Shaham et al. 1996	DPX formation <i>in vitro</i>	Human white blood cells	ND	***
Speit et al. 2009	DPX formation <i>in vitro</i>	Rat peripheral blood cells	ND	—**

DNA Adducts				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Beland et al. 1984	DNA adduct formation <i>in vitro</i>	Hamster CHO cells	ND	+++
Beland et al. 1984	DNA adduct formation <i>in vitro</i>	Calf thymus DNA <i>in vitro</i>	ND	+++
Casanova et al. 1989	DNA adduct formation <i>in vitro</i>	Rats nasal epithelial cells	ND	+++
Cheng et al. 2008	DNA adduct formation <i>in vitro</i>	Cell-free system deoxyribonucleosides	ND	+++
Cheng et al. 2003	DNA adduct formation <i>in vitro</i>	Cell-free system Guanosine	ND	+++
Cheng et al. 2003	DNA adduct formation <i>in vitro</i>	Cell-free system <i>in vitro</i>	ND	+++
Fennell 1994	DNA adduct formation <i>in vitro</i>	Cell-free system <i>in vitro</i>	ND	+++
Fennell 1994	DNA adduct formation <i>in vitro</i>	Rats nuclei	ND	+++
Heck and Casanova 1987	DNA adduct formation <i>in vitro</i>	Rats nuclei	ND	+++
Kennedy et al. 1996	DNA adduct formation <i>in vitro</i>	Cell-free system Guanosine	ND	+++
Lu et al. 2011	DNA adduct formation <i>in vitro</i>	HeLa S3 cells	ND	+
McGhee and von Hippel 1975a	DNA adduct formation <i>in vitro</i>	Cell-free system <i>in vitro</i>	ND	+++
McGhee and von Hippel 1975b	DNA adduct formation <i>in vitro</i>	Cell-free system <i>in vitro</i>	ND	+++
McGhee and von Hippel 1977a	DNA adduct formation <i>in vitro</i>	Cell-free system <i>in vitro</i>	ND	+++
McGhee and von Hippel 1977b	DNA adduct formation <i>in vitro</i>	Cell-free system <i>in vitro</i>	ND	+++
Von Hippel and Wong 1971	DNA adduct formation <i>in vitro</i>	Calf thymus DNA <i>in vitro</i>	ND	+++
Zhong and Que Hee 2004a	DNA adduct formation <i>in vitro</i>	Placental DNA <i>in vitro</i>	ND	+++
Zhong and Que Hee 2004b	DNA adduct formation <i>in vitro</i>	Human nasal epithelial cells	ND	+++
Alderson 1985	DNA adduct formation <i>in vitro</i>	<i>Drosophila</i> larvae	ND	+++

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Lu et al. 2010	DNA adduct formation <i>in vivo</i>	Rat/F344 nasal epithelium	ND	+++
Lu et al. 2010	DNA adduct formation <i>in vivo</i>	Rat/F344 white blood cells, bone marrow cells, lung, liver, spleen, thymus	ND	—**
Moeller et al. 2011	DNA adduct formation <i>in vivo</i>	Nasal mucosa and bone marrow of cynomolgous macaques exposed by inhalation. Exogenous adducts detected in nasal mucosal cells but not bone marrow.	ND	+/-
Wang et al. 2009	DNA adduct formation <i>in vivo</i>	Human white blood cells	ND	+++
Wang et al. 2007	DNA adduct formation <i>in vivo</i>	Rats indirect evidence	ND	+++

DNA Breaks or Repair Inhibition

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Cosma et al. 1988	DNA single strand breaks <i>in vitro</i>	Rat/F344 trachea/tracheal epithelial cell/primary culture	ND	+++
Demkowicz-Dobrzanski and Castonguay 1992	DNA single strand breaks <i>in vitro</i>	Rat hepatocytes	ND	+++
Emri et al. 2004	DNA single strand breaks <i>in vitro</i>	Human skin keratinocytes/fibroblasts	ND	—**
Fornace et al. 1982	DNA single strand breaks <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Graftstrom 1990	DNA single strand breaks <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Graftstrom et al. 1984	DNA single strand breaks <i>in vitro</i>	Human bronchial cell/Skin fibroblast	ND	+++

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Graftstrom et al. 1984	DNA single strand breaks <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Liu et al. 2006	DNA single strand breaks <i>in vitro</i>	Human peripheral blood lymphocytes	ND	+++
Liu et al. 2006	DNA single strand breaks <i>in vitro</i>	Human HeLa cells	ND	+++
O'Connor and Fox 1987	DNA single strand breaks <i>in vitro</i>	Rat yoshida lymphosarcoma cells	ND	+++
Ross et al. 1981	DNA single strand breaks <i>in vitro</i>	Mouse leukemia L1210 cells	ND	—**
Ross and Shipley 1980	DNA single strand breaks <i>in vitro</i>	Mouse leukemia L1210 cells	ND	(+)**
Saladino et al. 1985	DNA single strand breaks <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Speit et al. 2007	DNA single strand breaks <i>in vitro</i>	Hamster/Chinese V79 epithelial cells	ND	—**
Snyder and Van Houten 1986	DNA single strand breaks <i>in vitro</i>	Human skin fibroblast	ND	+++
Vock et al. 1999	DNA single strand breaks <i>in vitro</i>	Human lung/bronchial epithelial cells	ND	+++
Wang et al. 2012	DNA strand breaks <i>in vitro</i>	EBf-H9 cells (human embryonic cells)	ND	+
Su et al. 2007	DNA strand breaks <i>in vivo</i>	Lung tissue from specific pathogen-free male SD rats exposed to 5 or 10 ppm formaldehyde 6 hr/day, 5 days/wk for 2 wk	NA	+
Costa et al. 2011	DNA strand breaks <i>in vivo</i>	Peripheral blood lymphocytes from occupationally exposed individuals	ND	+
Im et al. 2006	DNA single strand breaks <i>in vivo</i>	Rats/Sprague Dawley via inhalation, peripheral blood lymphocytes and liver	ND	+++
Jiang et al. 2010	DNA strand breaks <i>in vivo</i>	Peripheral blood lymphocytes from occupationally exposed individuals	ND	+
Neuss et al. 2010	DNA strand breaks <i>in vivo</i>	Male F344 rats exposed by inhalation	ND	—
Wang and Liu 2006	DNA single strand breaks <i>in vivo</i>	Mouse liver (maternal)	ND	+++
Wang and Liu 2006	DNA single strand breaks <i>in vivo</i>	Mouse liver (fetal)	ND	+++

Reference	Assay	Test System	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Zeller et al. 2011	DNA strand breaks, <i>in vivo</i>	Peripheral blood cells from non-smoking, human volunteers exposed under controlled conditions	ND	—**
Emri et al. 2004	DNA repair inhibition	Human Skin fibroblasts/keratinocytes	ND	+**
Graftstrom et al. 1984	DNA repair inhibition	Human Bronchial epithelial cells/skin fibroblasts	ND	+**
Speit et al. 2000	DNA repair inhibition	Human normal fibroblasts (MRC5CV), XPA cell line, and FA cell line	ND	+**

Unscheduled DNA Synthesis

Reference	Assay	Test System	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Bermudez and Allen 1984	Unscheduled DNA synthesis	Rat/F344 Nasal epithelial cells	ND	+**
Bermudez and Delehanty 1986	Unscheduled DNA synthesis	Rat/F344 Nasal epithelial cells	ND	+**
Doolittle et al. 1985	Unscheduled DNA synthesis	Human Bronchial epithelial cells	ND	+**
Hamaguchi and Tsutsui 2000	Unscheduled DNA synthesis	Hamster/Syrian embryo cells	ND	+**
Martin et al. 1978	Unscheduled DNA synthesis	Human HeLa cells	ND	+**
Williams et al. 1989	Unscheduled DNA synthesis	Rat/F344 hepatocytes	ND	+**

Cytogenetic Damage				
Reference	Assay	Test System	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Dallas et al. 1992	Chromosomal aberrations	Rats/Sprague Dawley bone marrow	ND	—**
Dallas et al. 1992	Chromosomal aberrations	Rats/Sprague Dawley pulmonary lavage cells	ND	+**
Dresp and Bauchinger 1988	Chromosomal aberrations	Hamster/Chinese ovary cells	ND	—
Dresp and Bauchinger 1988	Chromosomal aberrations	Human peripheral blood lymphocytes	ND	+
Fontignie-Houbrechts et al. 1981	Chromosomal aberrations	Mice/Q strain spermatocyte	ND	—**
Fontignie-Houbrechts et al. 1982	Chromosomal aberrations	Mice/Q strain spermatogonia	ND	—**
Galloway et al. 1985	Chromosomal aberrations	Hamster/Chinese ovary cells	(+)	(+)
Garcia et al. 2009	Chromosomal aberrations	Hamster/Chinese ovary cells	ND	+
Hagiwara et al. 2006	Chromosomal aberrations	Hamster/Syrian embryo cells	ND	+
Hikiba et al. 2005	Chromosomal aberrations	Hamster/Syrian embryo cells	ND	+
Ishidate Jr et al. 1981	Chromosomal aberrations	Hamster/Chinese lung fibroblasts	ND	+
Kitaeva et al. 1990	Chromosomal aberrations	Rats/Wistar bone marrow	ND	+**
Kligerman et al. 1984	Chromosomal aberrations	Rats/F344 lymphocytes	ND	—**
Kuehner et al. 2012	Chromosomal aberrations	Myeloid progenitor cells from healthy subjects cultivated in vitro with FA. In particular chromosomes 6, 7, and 8 were analyzed to directly test the findings of Zhang et al. 2010.	ND	—
Jakab et al. 2010	Chromosomal aberrations	Peripheral blood lymphocytes from occupationally exposed pathology workers	ND	+

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Levy et al. 1983	Chromosomal aberrations	Human fibroblasts	ND	+
Migliore et al. 1989	Chromosomal aberrations	Rats/Sprague Dawley gastric epithelial cells	ND	+**
Miretskaya and Shvartsman 1982	Chromosomal aberrations	Human peripheral blood lymphocytes	ND	+
Natarajan et al. 1983	Chromosomal aberrations	Hamster/Chinese ovary cells	ND	+
Natarajan et al. 1983	Chromosomal aberrations	Mice/CBA polychromatic erythrocytes	ND	—**
Natarajan et al. 1983	Chromosomal aberrations	Mice/CBA spleen Cells	ND	—**
Pongsavee 2011	Chromosomal aberrations	Human blood exposed <i>in vitro</i> , T-lymphocytes showed loss of chromosomes	ND	+
Santovito et al. 2011	Chromosomal aberrations	Peripheral blood lymphocytes from occupationally exposed pathology workers	ND	+
Schmid et al. 1986	Chromosomal aberrations	Human peripheral blood lymphocytes	+	+
Speit et al. 2009	Chromosomal aberrations	Rats/ F344 peripheral blood cells	ND	—**
Speit and Merk 2002	Chromosomal aberrations	Mouse lymphoma cells	+	+
Costa et al. 2011	Micronucleus test	Peripheral blood lymphocytes from occupationally exposed individuals	ND	+
Emri et al. 2004	Micronucleus test	Human fibroblasts	ND	+
Gocke et al. 1981	Micronucleus test	Mouse/NMRI bone marrow		—**
Jerbi et al. 2011	Micronucleus test	Peripheral blood isolated from European sea bass treated <i>in vivo</i>		+
Jiang et al. 2010	Micronucleus test	Peripheral blood lymphocytes from occupationally exposed individuals	ND	+
Ladeira et al. 2011	Micronucleus test	Peripheral lymphocytes and buccal cells from occupationally exposed individuals	ND	+

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Merk and Speit 1998	Micronucleus test	Hamster/Chinese V79 lung epithelial cells	ND	+
Migliore et al. 1989	Micronucleus test	Rats/Sprague Dawley gastric epithelial cells		+**
Natarajan et al. 1983	Micronucleus test	Mice/CBA femoral polychromatic erythrocyte and spleen cells		—**
Neuss et al. 2010	Micronucleus test	M F344 rats treated by inhalation	ND	—
Schmid and Speit 2007	Micronucleus test	Human whole blood cultures	ND	+
Speit et al. 2007	Micronucleus test	Hamster/Chinese V79 lung epithelial cells	ND	+
Speit et al. 2000	Micronucleus test	Human MRC5Cv (normal) and XP (Repair-deficient) and FA (repair-deficient) cell lines	ND	+ ⁱ
Speit et al. 2011a	Micronucleus test	Nasal epithelial cells from F344 rats treated with FA by inhalation. Positive control also did not result in detection of micronuclei formation	ND	—
Speit et al. 2011b	Micronucleus test	A549 lung cells and cultured human lymphocytes. Positive in binucleated cells, but not mononucleated cells indicating clastogenicity but not aneugenic activity.	ND	+/-
Speit et al. 2012	Micronucleus test	Peripheral lymphocytes from occupationally exposed workers. Although results from the system were positive authors' test of reliability of analysis shows that MNT is unreliable.	ND	+
Viegas et al. 2010	Micronucleus test	Peripheral blood lymphocytes and exfoliated buccal mucosa cells from occupationally exposed persons	ND	+
Wang et al. 2012	Micronucleus test	EBf-H9 cells (human embryonic cells) treated in <i>in vitro</i>	ND	+
Ward et al. 1983	Micronucleus test	Mice/B6C3F1 bone marrow		+**

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Zeller et al. 2011	Micronucleus test	Peripheral blood cells and exfoliated nasal mucosal cells from non-smoking, human volunteers exposed under controlled conditions	ND	—**
Basler et al. 1985	Sister chromatid exchange	Hamster/Chinese V79 lung epithelial cells	(+)	+
Galloway et al. 1985	Sister chromatid exchange	Hamster/Chinese ovary cells	(+)	(+)
Garcia et al. 2009	Sister chromatid exchange	Hamster/Chinese ovary cells	ND	+
Garry et al. 1981	Sister chromatid exchange	Human lymphocytes	ND	+ ^j
Jakab et al. 2010	Sister chromatid exchange	Human peripheral blood lymphocytes of pathology workers exposed mainly to formaldehyde with potential exposure to other solvents	ND	—
Kligerman et al. 1984	Sister chromatid exchange	Lymphocyte	ND	—**
Krieger and Garry 1983	Sister chromatid exchange	Human lymphocytes	ND	+
Merk and Speit 1998, 1999	Sister chromatid exchange	Hamster/Chinese V79 lung epithelial cells	ND	+
Miyachi and Tsutsui 2005	Sister chromatid exchange	Hamster/Syrian embryo cells	ND	+
Nataranja et al. 1983	Sister chromatid exchange	Hamster/Chinese ovary cells	ND	+
Neuss and Speit 2008	Sister chromatid exchange	Hamster/Chinese V79 lung epithelial cells	ND	+
Neuss and Speit 2008	Sister chromatid exchange	Human A549 lung epithelial cells	ND	+
Neuss and Speit 2008	Sister chromatid exchange	Human A549 +V79 (co-cultivated)	ND	+ ^k
Neuss and Speit 2008	Sister chromatid exchange	Human A549 +V79 (co-cultivated)	ND	— ^d
Obe and Beek 1979	Sister chromatid exchange	Hamster/Chinese ovary cells	ND	+
Obe and Beek 1979	Sister chromatid exchange	Human lymphocytes	ND	+

Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Schmid et al. 1986	Sister chromatid exchange	Human lymphocytes	ND	+
Schmid and Speit 2007	Sister chromatid exchange	Human whole blood cultures	ND	+
Speit et al. 2009	Sister chromatid exchange	Peripheral blood cells	ND	—**
Speit et al. 2007	Sister chromatid exchange	Hamster/Chinese V79 lung epithelial cells	ND	+
Wang et al. 2012	Sister chromatid exchange	EBf-H9 cells (human embryonic cells) treated <i>in vitro</i>	ND	+
Zeller et al. 2011	Sister chromatid exchange	Peripheral blood cells from non-smoking, human volunteers exposed under controlled conditions	ND	—**
Dresp and Bauchinger 1988	Premature chromosome condensation	Hamster/Chinese Ovary Cells	ND	+
Zhang et al. 2010	Leukemia-specific chromosomal changes monosomy 7 and trisomy 8	Myeloid progenitor cells isolated from human blood of exposed workers	ND	+++

* Adapted from the U.S. EPA (2010a) review, with additional publications captured in a 2010–2012 literature search. Primary publications were not obtained for all studies, only studies for which additional information was required to appropriately represent the study herein. + = positive test result, (+) = weak positive test result, – = negative test result, ND = no data in summary or test not done, NER = Nucleotide excision repair, NA = not applicable.

^a Indicates the use of formalin in mutagenicity assay.

^b Indicates the use of hexamethylmelamine (HEMLA), a formaldehyde-releasing compound, in mutagenicity assay.

^c Indicates use of the standard plate method.

^d Indicates use of the preincubation plate method.

^e Indicates use of the suspension method.

^f Indicates loss of DNA.

^g Indicates that DNA-protein cross-links formed at cytotoxic concentrations

^h Used homogenates of nasal mucosa.

**Indicates that exogenous metabolic activation is not specified in summary, and it is therefore assumed that exogenous metabolic activation was not supplied.

ⁱ MN frequency increased in repair-deficient cell lines compared to normal cell lines.

^j Indicates SCE with significant loss of cell viability.

^k A549 cells exposed for 1 h with formaldehyde then co-cultivated with V79 cells.

^l A549 cells exposed for 1 h with formaldehyde, cells washed and then co-cultivated with V79 cells.

^m Positive only in the presence of 12-O-tetradecanoylphorbol 13-acetate (TPA).

ⁿ p53 mutations.

^o p53 mutated protein detected by immunohistochemistry.

[+] Indicates positive test result after TPA or *N*-methyl-*N*-nitro-*N*-nitrosoguanidine promoter treatment.

XPA = *Xeroderma pigmentosum* complementation group A (deficient in NER pathway).

FA = Fanconi's anemia cell line (has genetic defect leading to hypersensitivity to DNA-DNA cross links).

Supplementary Table A-3. Genotoxicity studies of TBA^a

Mutation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
EG&G Mason 1981a	Reverse mutation	<i>S. typhimurium</i> , TA98, TA100, TA1537 and TA1538	–	–
EG&G Mason 1981b	Reverse mutation	<i>S. typhimurium</i> , TA1535	+	+/–
EG&G Mason 1981c	Gene mutation	Mouse lymphoma L5178Y cells, tk locus <i>in vitro</i>	–	–
McGregor et al. 1988	Mutation	Rats F344/N metabolic activation, L5178Y tk ⁺ /tk [–] lymphoma cell line	ND	[+]
McGregor et al. 1988	Mutation	Rats F344/N metabolic activation, L5178Y tk ⁺ /tk [–] lymphoma cell line	–	–
McGregor et al. 1988	Gene mutation	Mouse lymphoma L5178Y cells, tk locus <i>in vitro</i>	–	–
McGregor et al. 2005	Reverse mutation	<i>S. typhimurium</i> , TA102	–	–
Williams-Hill et al. 1999	Reverse mutation	<i>S. typhimurium</i> , TA102	[+]/–	+
Zeiger et al. 1987	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	–	–
DNA Adducts, Fragmentation, or Oxidation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Sgambato et al. 2009	Comet assay for DNA strand breaks; DNA oxidative damage	Mouse embryo fibroblast C3H/10/T _{1/2} /C1/8 cells	ND	+
Tang et al. 1999	Comet assay for DNA strand breaks	Human HL-60 leukemia cells <i>in vitro</i>	ND	+
Yuan et al. 2007	AMS analysis of DNA radiolabel	Mice (Kunming, male); liver, lung, and kidney tissues 6 h after 1 ¹⁴ C-MTBE dose by gavage	ND	***

Cytogenetic Damage				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
NTP 1995	Chromosomal Aberration, <i>in vitro</i>	Hamster/Chinese ovary cells	–	–
EG&G Mason 1981d	Sister chromatid exchange	Hamster/Chinese ovary cells	–	+/-
NTP 1995	Sister chromatid exchange	Chinese hamster ovary 95% MTBE	–	[+]
NTP 1995	Sister chromatid exchange	Chinese hamster ovary 95% MTBE	–	–
NTP 1995	Micronucleus test	Peripheral blood of male and female mice following 13 wk treatment via drinking water	ND	–

^a Studies reported in NSF (2003) Oral Risk Assessment, updated with new studies via literature search through 2012, and from McGregor (2005, 2010). Primary publications were not obtained for all studies, only studies for which additional information was required to appropriately represent the study herein. Positive results in brackets indicate only negative results occurred in a repeat study. Comet assay = single cell gel electrophoresis (SCGE) assay; AMS = accelerator mass spectrometry without gel-electrophoresis confirmation of purported DNA adducts. Brackets indicate study results were all negative when repeated by different investigators (see text).

* A lower purity sample gave a significant response in the absence of metabolic activation.

** Exogenous metabolic system not specified so none was assumed, or metabolic system was not appropriate for the specific test system, or AMS analysis without corresponding verification of purported adduct(s).

Supplementary Table A-4. Genotoxicity studies of methanol^a

Mutation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Abbondandolo et al. 1980	Gene mutation	<i>Schizosaccharomyces pombe</i> , ade 6 locus	–	–
Chang et al. 1983	Mutagenic activity, (unspecified in WHO IPCS 1997 review)	Mouse, urine of animals dosed orally daily for 5 days	ND	–
Clark et al. 1983	Reverse mutation	<i>S. typhimurium</i> TA100, 98, 98 NR, 98 DNPR treated with gasoline particulate matter with or without added methanol	–	ND
De Flora et al. 1984	Reverse mutation	<i>S. typhimurium</i> TA1535, 100, 1538, 98, 1537	–	–
Heidelberger et al. 1983	Cell transformation	Rausher leukemia virus infected rat embryo cell (RLV/RE system)	ND	–
Kato 1989; NEDO 1987	Reverse mutation	unspecified	ND	–
McGregor et al. 1985	Mutation frequency	Mouse lymphoma cells, L5178Y	+	–
NEDO 1987; Kato 1989	Somatic mutation assay	CH-V79 cells.	ND	–
Pienta et al. 1977	Cell transformation	Syrian hamster embryo cells	ND	–
Simon et al. 1977	Reverse mutation	<i>S. Typhimurium</i> TA98, 100, 1535, 1537, 1538	–	–
DNA Adducts, DNA Oxidation, or DNA-Protein Crosslinks (DPX)				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Pereira et al. 1982	Covalent protein binding	Hemoglobin of rats orally administered up to 100 $\mu\text{mol/kg}$ methanol	ND	+

DNA Adducts & Oxidation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Lu et al. 2012	Hydroxymethyl DNA adduct formation with isotopically labeled methanol	Rats treated with 500 or 2000 mg/kg/day for 5 days. Adducts measured in several tissues, but nearly all due to endogenous formaldehyde rather than administered methanol.	ND	+ (see note under Test system)
Lutz et al. 1986	DNA-protein crosslinks	Male Sprague-Dawley rats treated with 1 g methanol by gavage, sacrificed 4 hours later.	ND	—
McCallum et al. 2011a	<i>In vivo</i> 8-oxo-dG formation from bone marrow and spleen	Male CD-1 mice, New Zealand rabbits, cynomolgus monkeys treated with 2.0 g/kg ip injection of methanol. Damage assessed 6h post-dose. Also assessed in the mice after 15 days of repeated dose administration.	ND	—
McCallum et al. 2011b	<i>In vivo</i> 8-oxo-dG formation from lung, liver or kidney.	Male CD-1 mice, New Zealand rabbits, cynomolgus monkeys treated with 2.0 g/kg ip injection of methanol. Damage assessed 6h post-dose. Also assessed in the mice after 15 days of repeated dose administration.	ND	—
Cytogenetic Damage				
Reference	Assay	Test system	Result	
			With exogenous metabolic activation	Without exogenous metabolic activation
Campbell et al. 1991	Micronucleus test	Mice treated with methanol by inhalation at up to 4000 ppm for 5 days, test in blood or lung cells	ND	—
Campbell et al. 1991	Sister chromatid exchange	Mice treated with methanol by inhalation at up to 4000 ppm for 5 days	ND	—
Campbell et al. 1991	Chromosomal aberrations in lung cells	Mice treated with methanol by inhalation at up to 4000 ppm for 5 days	ND	—

Reference	Assay	Test system	Result	
			With exogenous metabolic activation	Without exogenous metabolic activation
Campbell et al. 1991	Synaptonemal complex damage of spermatocytes	Mice treated with methanol by inhalation at up to 4000 ppm for 5 days	ND	–
Chang et al. 1983	Bone marrow cytogenetic analysis	Mice treated with 3 ip doses daily or 75-300 mg methanol/kg	ND	(+)
Crebelli et al. 1989	Chromosomal malsegregation	<i>Aspergillus nidulans</i> , diploid strain P1	ND	++
Fu et al. 1996	Micronuclei test	Pregnant CD-1 mice receiving oral gavage of methanol at 0 or 5000 mg/kg/day on GD 6-10	ND	–
Griffiths 1981	Aneuploidy	<i>Neurospora crassa</i> , dysfunction of linkage group I	ND	–
NEDO 1987; Katoh 1989	Chromosomal aberrations	Mice	ND	–
NEDO 1987; Katoh 1989	Sister chromatid exchange	Mice	ND	–
NEDO 1987; Katoh 1989	Micronuclei test	Mice	ND	–
Obe and Ristow 1977	Sister chromatid exchange	Hamster/Chinese ovary cells	ND	–
Pereira et al. 1982	Aneuploidy	Mice, treated with 1 g/kg methanol orally	ND	+
Pereira et al. 1982	Micronuclei test	Erythrocytes (polychromatic) of mice, treated with 1 g/kg methanol orally	ND	+
Pereira et al. 1982	Sister chromatid exchange	Mice, treated with 1 g/kg methanol orally	ND	+

^a Adapted from NTP (2002) review of methanol, supplemented by studies identified via literature search and in applicable review articles. GD = gestation day. Primary publications were not obtained for all studies, only studies for which additional information was required to appropriately represent the study herein. () = “some” evidence.

* Growth was reduced, potentially indicative of cell death, or occurred at cytotoxic dose.

Supplementary Table A-5. Genotoxicity studies of formic acid^a

Mutation				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Demerec et al. 1951	Reverse mutation	<i>Escherichia coli</i> , strains B/Sd-4/1, 3, 4, 5 and B/Sd-4/3, 4. Mutation rates in treated systems were 18.1 – 44.0 per 10 ⁸ , and 2.7 to 15.3 per 10 ⁸ in controls.	ND	+
NTP 1992	Reverse mutation	<i>S. typhimurium</i> , TA98, 100, 1535. Hamster S9 utilized.	–	–
NTP 1992	Reverse mutation	<i>S. typhimurium</i> , TA98, 100, 1535. Rat S9 utilized.	–	–
NTP 1992	Reverse mutation	<i>S. typhimurium</i> , TA97. Hamster S9 utilized.	Equivocal	Equivocal
NTP 1992	Reverse mutation	<i>S. typhimurium</i> , TA97. Rat S9 utilized.	–	–
Ragan and Boreiko 1981	Transformation	C3H/10T _{1/2} Cl8 mouse embryonic fibroblasts.	ND	–
Stumm-Tegethoff 1969	Sex-linked recessive lethal mutation	<i>Drosophila melanogaster</i> . Positive results were obtained with acid; pH-neutralization of treatment buffer or media yielded negative results.	ND	–
DNA Adducts				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Wang et al. 2004	DNA and hemoglobin adducts	Male Kunming mice administered formate by acute oral gavage. DNA adducts detected by AMS without adduct verification.	ND	+**

Cytogenetic Damage				
Reference	Assay	Test system	Results	
			With exogenous metabolic activation	Without exogenous metabolic activation
Basler et al. 1985	Sister chromatid exchange	Hamster V79 cells	—	—
Morita et al. 1990	Chromosomal aberrations	Chinese hamster ovary cells. It should be noted that neutralization of treatment buffer or media resulted in negative test indicating that acidity caused the test result.	—/+	—/+
Sipi et al. 1992	Sister chromatid exchange	Cultured human lymphocytes. Although pH (6.53) was considered to have been a potential factor in the positive results, authors concluded that other culturing condition factors may have been involved.	ND	+*

^a Adapted from NTP (1992) Toxicity Report Number 19 (Formic Acid), supplemented by studies identified via literature search through 2012. Primary publications were not obtained for all studies, only studies for which additional information was required to appropriately represent the study herein.

*Authors state that decreased cell proliferation was indicated at the effective treatment concentration and that the result should be interpreted with caution.

** AMS study performed without verifying putative adducts; authors recognized that detected atoms could have arisen indirectly via formate metabolism and subsequent nucleotide synthesis.

Supplementary Appendix B: Tumorigenicity Data Tables

Tables summarizing tumorigenicity data discussed in Section 4 of the text appear in this appendix. Specifically, Tables B-1 – B-4 summarize tumorigenicity data obtained for MTBE, formaldehyde, TBA, and methanol, respectively.

Supplementary Table B-1. Cancer and tumor endpoints following MTBE exposure

Reference	Test system	Tumorigenic effects	Other related effects
Belpoggi et al. 1995, and 1998 (review of pathology)	Sprague-Dawley rats, M&F; received by gavage in oil 0, 250, or 1000 mg MTBE/kg BW/day on Mon., Tues, Thurs, and Fri. for 104 wk. Weak tolerance for the test substance at the high dose was used to justify the unusual dosing pattern.	Increased incidence of lymphoimmunoblastic dysplasia and lymphoma, and leukemia in treated F, however not sig. Authors stated that higher incidence of hyperplastic cells in mid dose group compared to high dose was indicative of progression from dysplasia to leukemia. M at 1000 mg/kg/day had sig. increased Leydig cell adenomas. Dose dependently increased Leydig cell hyperplasia also present but not sig.	Survival in M increased at 1000 compared to 250 mg/kg/day and control. At wk 88 survival displayed pattern of dose-related decrease in F, but by 120 wks, survival of all F groups was 20-25%.
Chun et al. 1992; Bird et al. 1997; CalEPA OEHHA, 1999	Fischer 344 rats, M&F; Inhalation exposure to 0, 400, 3000, or 8000 ppm MTBE, 6h/d, 5 d/wk, for 24 mo.	Nonsig. increased incidence of parathyroid adenomas M, 3000 (3/50) and 8000 ppm (1/50). Noted that it is unclear whether this finding would translate to humans. Increased renal tubular cell adenoma or carcinoma combined, M at 3000 (8/50, sig.) and 8000 ppm (3/50) vs. 1/50 at 0 ppm. Dose dependent sig. increase in Leydig cell tumors with 64% incidence at 0 ppm, 70% at 400, 82% at 3000 and 94% at 8000 ppm.	Decreased body weight at 8000 ppm. CPN in all dose groups, increasing in severity with increasing dose, but generally less severe in F than M. Decreased mean survival time and rate at 3000 and 8000 ppm in M, with survival less impacted in F. Increased relative liver and kidney weights in F at 3000 and 8000 ppm. Dose dependent increases in corticosterone, M.
Burleigh-Flayer et al. 1992; Bird et al. 1997; CalEPA OEHHA, 1999	CD-1 mice, M&F; Inhalation exposure to 0, 400, 3000, or 8000 ppm MTBE, 6 h/d, 5 d/wk, for 18 mo.	Sig. elevated 11/50 incidence of hepatocellular adenoma or carcinoma in F at 8000 ppm vs. 2/50 in controls (historical controls range 0-4%). Sig. elevated 8/42 hepatocellular carcinoma incidence in M at 8000 ppm vs. 2/44 in controls (adjusted for deaths prior to 1 st tumor occurrence). Dose-dependent sig. decrease in uterine endometrium hyperplasia in F. Decreased pituitary tumors in high dose M.	Mortality rates higher in M than F and higher than controls and other treated group in M at 8000 ppm. Sig. increased corticosterone, M. Also increased in F, but not sig. Decreased urine pH, M&F, at 8000 ppm at 59 and 79 weeks, respectively. Sig. increased liver weight in F at 3000 and 8000 ppm. Sig. increase kidney weight in M, all doses. Increased adrenal weight, M, 8000 ppm. Hepatocellular hypertrophy increased in M at 3000 and 8000 ppm and in F at 8000 ppm.

Reference	Test system	Tumorigenic effects	Other related effects
Dodd et al. 2011	Wistar rats, M&F; MTBE in drinking water at 0, 0.5, 3 mg/mL, or 7.5 mg/mL (M) or 15 mg/mL (F), equivalent to consumed MTBE doses of 0, 25, 140, or 330 mg/kg/day for M, and 0, 49, 232, or 1042 mg/kg/day for F, for 2 yr.	<p>Neoplastic lesions of the kidney were less than 1 per group, including controls and were therefore not evaluated for significance.</p> <p>Non-neoplastic changes of the kidney sig. increased incidence and severity of chronic progressive nephropathy (CPN) in high-dose M and F. Greater severity in M than F in the control and in the exposed rats.</p> <p>Brain astrocytomas observed but concluded to be within historical control range and not treatment-related.</p>	<p>No effects of treatment on survival or BW.</p> <p>Sig. kidney weight increases in M and F with evident dose-dependence.</p> <p>Commonly-encountered foot-pad infections were present in all exposure groups, requiring treatment with antibiotics and disinfectants.</p> <p>Significant increase in relative (but, except in the group exposed to the highest MTBE concentration, not absolute) weight gain observed in both kidneys of male rats, and in the absolute (but, except in the group exposed to the highest MTBE concentration, not relative) left (but, except in the group exposed to the highest MTBE concentration, not right) kidney of female Wistar rats exposed to MTBE in drinking water for two years at all non-control concentrations tested.</p> <p>Olfactory epithelial degeneration significantly elevated in M rats but not F.</p> <p>The authors note some issues of sampling bias, and from inspection of the data, there are some instances of large variability between responses of the control group used for condensate + MTBE group compared to the condensate only group. Two-sided instead of one-sided comparison tests were used.</p>
Benson et al. 2011	Fischer 344 rats, M&F; Inhalation of 0, 2, 10, or 20 g/m ³ gasoline condensate with (notated GMVC) or without (notated GVC) MTBE added at a concentration of ~23% for 6 h/d, 5 d/wk, for 104 wk.	<p>Renal tubule adenomas and carcinomas attributable to exposure to gasoline condensate with or without MTBE in M.</p> <p>Increased sig. trend for Leydig cell adenomas in MTBE group M, however controls in gasoline condensate-only group comparable.</p> <p>In F with MTBE, increased trend for fibroadenoma of the mammary gland, with sig. at the low and high dose.</p>	

Supplementary Table B-2. Cancer and tumor endpoints following formaldehyde exposure

Chronic Inhalation Studies			
Reference	Test system	Tumorigenic effects	Other effects or comments
Albert et al. 1982; Sellakumar et al. 1985	Sprague-Dawley rats; M, inhalation at 0 or 15 ppm, 6 h/d, 5 d/wk for lifetime.	25% incidence of squamous cell carcinoma formed in the nasomaxillary turbinates and nasal septum. Concluded evidence of carcinogenicity.	Squamous cell metaplasia and epithelial hyperplasia, polyps/papillomas.
Appelman et al. 1988	SPF Wistar rats; M, inhalation at 0, 0.1, 1, or 10 ppm for 6 h/d, 5d/wk for 13 or 52 wk. Half the noses of each study group were damaged via electrocoagulation, therefore only 5 animals treated with formaldehyde alone.	No tumors noted.	Focal changes to the respiratory and olfactory epithelium including rhinitis, hyperplasia and metaplasia at the high dose. Note that study was only 1 yr and full chronic rat study would be ~2 yr.
Dalbey 1982	Syrian golden hamsters; M, inhalation at 0 or 15 ppm for 5 h/d, 5 d/wk, for lifetime.	No tumors reported. Concluded no evidence of carcinogenicity.	Increased mortality and epithelial hyperplasia and metaplasia in treated animals (5% incidence of hyperplasia and metaplasia).
Dalbey 1982	Syrian golden hamsters; M, inhalation at 0 or 30 ppm for 5 h/d, 1 d/wk, for life +/- injections of DEN.	Increase in number of tumors per tumor-bearing animal in the tracheas of animals pre-treated with formaldehyde 48 h prior to DEN treatment but there was no increase in tumor bearing animals. Concluded evidence for formaldehyde as a promoter.	Increased mortality of formaldehyde + DEN compared to DEN only. Respiratory pathology not reported.
Holmstrom et al. 1989	Sprague-Dawley rats; F, inhalation at 0 or 12.4 ppm formaldehyde plus wood dust for 6 h/d, 5d/wk for 104 wk.	1/16 rats exposed to formaldehyde alone (no wood dust) developed nasal squamous cell carcinoma.	Squamous metaplasia and dysplasia.
Horton et al. 1963	C3H mice; sex not stated, inhalation at 0, 41, 82, or 163 ppm for 1h/d, 3 d/wk, up to 35 wk. Low and mid dose mice subsequently exposed at 122 or 244 ppm during wk 35-70.	No evidence of squamous cell carcinoma or carcinogenicity.	Histologic changes of the tracheobronchial epithelium including basal-cell hyperplasia, stratification squamous cell metaplasia and atypical metaplasia. Note that study was not full length 2-yr cancer bioassay.
Kamata et al. 1997	F344 rats; M, inhalation at 0, 0.3, 2, or 15 ppm, 6 h/d, 5 d/wk for 28 mo.	Nasal cavity squamous cell carcinoma in 13/32 animals in the high dose group, squamous cell papilloma in 3/32 and sarcoma in 1/32. Concluded evidence of carcinogenicity.	Squamous cell metaplasia and epithelial hyperplasia.

Reference	Test system	Tumorigenic effects	Other effects or comments
Monticello et al. 1996	F344 rats; M, inhalation at 0, 0.7, 2, 6, 10, or 15 ppm for 6 h/d, 5 d/wk, 24 mo.	Squamous cell carcinoma and polypoid adenoma of nasal cavity. Concluded evidence of carcinogenicity.	Olfactory degeneration, squamous metaplasia, epithelial hypertrophy and hyperplasia and mixed inflammatory cell infiltration.
*NTP 1992	B6C3F ₁ mice and F344 rats; M&F, inhalation of 0, 8, 16, 32, 64, or 128 ppm formic acid for 6 h/d, 5 d/wk, for 13 wk.	No unusual or gross lesions at necropsy in rats or mice. Squamous metaplasia and degeneration of the respiratory and olfactory epithelium observed in rats and mice and showed dose dependence.	Overall NTP concluded that results were consistent with irritant effects of formic acid. Body weight was significantly decreased at 64 and 128 ppm and there were 2 deaths, 1 M, 1 F at 128 ppm for mice. Systemic toxicity was not observed and lesions were generally confined to the areas of contact of the formic acid vapor.
Swenberg et al. 1980; Kerns et al. 1983; CIIT 1982; Battelle Columbus Laboratories 1981	B6C3F ₁ mice; M&F, inhalation at 0, 2, 5.6, or 14.3 ppm for 6 h/d, 5 d/wk for 24 mo with 6 mo recovery period and interim sacrifices at 6, 12, 18, 24 and 30 mo.	Nasal squamous cell carcinoma in M at 24 mos (2/17 animals). Concluded evidence of carcinogenicity.	Rhinitis with hyperplasia, dysplasia and squamous metaplasia of the nasal epithelium. Glandular adenitis and nasolacrimal duct hyperplasia and metaplasia.
Swenberg et al. 1980; Kerns et al. 1983; CIIT 1982; Battelle Columbus Laboratories 1981; Morgan et al. 1986	F344 rats; M&F, inhalation at 0, 2, 5.6, or 14.3 ppm, 6 h/d, 5 d/wk for 24 mo with 6 mo recovery period and interim sacrifices at 6, 12, 18, 24 and 30 mo.	Squamous cell carcinoma evident in nasal cavity at high dose with polypoid adenomas. Concluded evidence of carcinogenicity.	Nasal cavity lesions were primary effects with squamous metaplasia, epithelial dysplasia, hyperkeratosis, goblet cell hyperplasia, and rhinitis. Also noted atrophy, squamous metaplasia and sialadenitis of the salivary gland.
Tobe et al. 1985	F344 rats; M&F, inhalation at 0, 0.3, 2, or 14 ppm for 6 h/d, 5 d/wk, for 28 mo.	44% of rats at the highest dose had nasal squamous cell carcinoma.	Increased rhinitis, hyperplasia and squamous metaplasia of the nasal respiratory epithelium.
Reference	Test system	Tumorigenic effects	Other effects or comments
Woutersen et al. 1989	Wistar rats; M, inhalation at 0, 0.1, 2, or 10 ppm 6 h/d, 5 d/wk, for 28 mo or for 3 mo with 25 mo follow-up period. 2/3 rats from each group were subjected to electrocoagulation to damage the nasal cavity.	Nasal squamous cell carcinoma in 15/60 of rats with damaged noses at the high dose. Incidence of tumors low in other groups, regardless of nasal tissue damaged. Concluded results were evidence of carcinogenicity.	In rats with intact noses, squamous metaplasia was observed in the high dose group at 28 mo with degeneration of the olfactory epithelium.

Chronic Oral Studies

Reference	Test system	Tumorigenic effects	Other effects or comments
Soffritti et al. 1989	Sprague-Dawley rats; M&F, administered formaldehyde in the drinking water at up to 2500 ppm for 104 wk. Some animals were up to 25 wk of age at start of study.	Study noted increased incidence of leukemias and in stomach and total GI tumors in treated rats. Although not reported, increased total GI tumors had significant positive dose-related trends in M and F rats ($p = 0.0049$ and $p = 0.046$, respectively, by exact Cochran-Armitage trend tests—see text Section 4.2)	Effects other than GI neoplasms and leukemias not reported, except for survival. No marked effects on survival were noted between the control and formaldehyde treated rats. Leukemia data disputed (U.S. EPA 2012a).
Takahashi et al. 1986	Wistar (outbred) rats; M, an initiation/promotion protocol was used in which 100 mg/L of the carcinogen <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine (MNNG) was first administered in drinking water for 8 wk, followed by 32 wk of promotion via administering 0.5% formalin (containing 12–15% methanol) in drinking water.	Formaldehyde + MNNG: statistically increased the incidence of lesions in the forestomach and stomach in initiated animals. Increased adenocarcinoma in the pylorus observed as well as increased papilloma in the forestomach and increased adenomatous hyperplasia in the fundus. Formaldehyde alone: Significant increase in forestomach papillomas without lesions in the glandular stomach.	Formaldehyde exacerbated decreases in weight gain of the initiated group. Histopathologic investigations were limited to the GI tract.
Til et al. 1989	Wistar rats; paraformaldehyde administered in the drinking water with formaldehyde target doses of 0, 5, 25, or 125 mg/kg/day for M and 0, 1.8, 21, or 109 mg/kg/day for up to 104 wk with several interim sacrifices.	No evidence of carcinogenicity in either the GI tract or systemically.	At the high dose, papillary epithelial hyperplasia of the forestomach with hyperkeratosis on or near the limiting ridge in some cases and mucosal hyperplastic basal cells without atypical nuclei or other aberrant subcellular structures and chronic atrophic gastritis. Renal papillary necrosis also at the high dose but no evidence of chronic nephropathy with increasing dose. This was possibly related to decreased water intake.

Reference	Test system	Tumorigenic effects	Other effects or comments
Tobe et al. 1989	Slc Wistar rats; M&F, administered paraformaldehyde in the drinking water at formaldehyde target concentrations of 0, 10, 50 or 300 mg/kg/day for 24 mo.	Carcinogenicity not observed.	General condition of high dose animals was poor with mortality of 100% by 24 mos. At the high dose, hyperplasia of the squamous epithelium with or without hyperkeratosis in the forestomach (12/12 high dose animals) and erosions of ulcers with submucosal cell infiltrates in glandular stomach of 10/12 high dose animals. Evidence of regenerative changes in the glandular epithelium in 10/12 animals.

^a Adapted from U.S. EPA (2010a), adding Soffritti et al. 1989. New chronic studies were not identified in a literature search from 2010–2012.

* Study was performed with formic acid administered by inhalation, not formaldehyde. This 13-week study was the longest study located for formic acid. Chronic or carcinogenic studies were not identified.

Supplementary Table B-3. Cancer and tumor endpoints following TBA exposure

Reference	Test System	Tumorigenic Effects	Other Related Effects
Cirvello et al. 1995; NTP 1995	F344N rat; M&F, administered TBA in the drinking water for 2 yr at doses of 0, 1.25, 2.5, 5, mg/mL for M which delivered 0, 85, 195, or 420 mg/kg/d TBA, or 0, 2.5, 5, or 10 mg/mL for F which delivered 0, 175, 330, or 650 mg/kg/d to F.	<p>In M rats, a small number of renal tubule cell adenomas observed in treated animals that was not significant when standard sectioning methods were utilized, but became significant when step-sectioned kidneys were analyzed. Similar renal tubule tumors were not noted in F.</p> <p>Significant negative trends with dose for mammary gland adenoma, fibroadenoma, and carcinoma combined, and in the highest F dose group the decrease was significant compared to controls. In M a significant negative trend for pancreatic islet cell adenomas and carcinomas combined was observed.</p> <p>NTP concluded "some" evidence of carcinogenesis in M and "no" evidence in F.</p>	<p>Mean survival was similar between treated animals and controls however a trend towards a pattern of dose-dependent decrease in survival could be observed at the end of the study.</p> <p>Body weight effects of greater than 10% reduction were noted in M&F at the end of the study.</p> <p>Hyperactivity was noted in the high dose F.</p> <p>Alph2u-globulin accumulation was hypothesized to be a factor contributing to kidney tubule cell proliferation and tumors in exposed M.</p>
Cirvello et al. 1995; NTP 1995	B6C3F ₁ mouse; M&F administered TBA in the drinking water for 2 yr at doses of 0, 5, 10, or 20 mg/mL, which is approximately equivalent to 0, 535, 1035, or 2065 mg/kg/d in M and 0, 510, 1015, or 2105 mg/kg/d in F.	<p>Increased incidence of thyroid follicular cell adenoma in F mice in highest dose group (9/50) vs. controls (2/58) is not significant by 2-tail Fisher exact test ($p = 0.053$) but all F data show a significant positive trend with dose ($p = 0.010$, by 2-tail exact Cochran-Armitage trend test). In M incidence of thyroid follicular cell adenoma (A) and carcinoma (C) combined was marginally higher, in middle dose group only. Liver A (but not C or combined A or C) was significantly reduced in F mice, but combined A or C showed a significant negative trend with dose in M mice. Combined harderian gland A or C was significantly decreased in dosed M mice (6/30, 3/60, 10/60) compared to controls (1/60). Thyroid follicular cell hyperplasia was significantly increased in all treated M and in the two highest F dose groups.</p> <p>Concluded "some" evidence of carcinogenicity in F and "equivocal" evidence in M.</p>	<p>Slight body weight deficits observed in M and F on treatment.</p> <p>No significant clinical findings observed.</p> <p>Chronic inflammation of the bladder and epithelial hyperplasia were observed in high dose M and F; however, it was noted by comparison to the 13 wk NTP study that the hyperplasia was unlikely to result in neoplasia based on limited progression noted in the longer study compared to that observed in the 13 wk study.</p> <p>Incidence of hepatic steatosis was significantly increased in M mice in the high dose only.</p>

^a Adapted from NSF (2005); and McGregor (2010).

Supplementary Table B-4. Cancer and tumor endpoints following methanol exposure

Reference	Test System	Tumorigenic Effects	Other Related Effects
NEDO 1985a U.S. EPA 2009	F344 rat; M&F, inhalation at 0, 10, 100, or 1000 ppm methanol for 19.5 h/d, 7 d/wk, 104 wk. Study was GLP and followed OECD guidelines.	Although not reported by NEDO, there were significant dose-related trends for M lung nodes (2/52, 4/52, 5/52, 10/52; p-trend < 0.0036 ^b), and for M pulmonary adenoma/adenocarcinoma (1/52, 5/52, 2/52, 7/52; p-trend = 0.045 ^b). No significant difference between treated animals and controls in the incidences of leukemias and lymphomas.	No treatment-related signs of toxicity or effects on survival or feed consumption were reported. Transient bw effects were noted in F but not M. No changes in urinalysis, hematology, or clinical biochemistry were reported as treatment-related.
NEDO 1985b	B6C3F ₁ mouse; M&F, inhalation at 0, 10, 100, or 1000 ppm methanol for 19.1 h/d, 7 d/wk, 78 wk. During wk 43-54 a mechanical malfunction resulted in dosing of the 10 ppm group at 4 ppm.	No statistically significant increases in incidences of any gross lesions or organ weights; no significant differences from control for any pathological lesions including neoplasms. A suggestion of proliferative changes in the lung did not reach significance.	No treatment-related signs of toxicity and no effect on survival or bw. Slight decrease in food consumption at 7-12 mo in F. No significant effects on urinalysis, hematology, or clinical chemistry parameters.
Soffritti et al. 2002	Sprague-Dawley rat; M&F, methanol administered in drinking water. Estimated doses were 0, 55, 542, 1849 mg/kg/d for M, and 0, 67, 630, 2250 mg/kg/d for F. Water intakes measured once every 1-2 wk; animals housed 5/cage with shared water.	Lung pathologies in all treatment groups regardless of age of death including neoplasms, inflammation, and dysplasia; respiratory infections in all groups suspected (NTP 2011). Incidences of all cancers arising from blood cells were combined into one unconventional category, "total hemolymphoreticular tumors", including histiocytic sarcoma, mononuclear leukemia, myeloid leukemia, lymphoblastic lymphoma, and lympho-immunoblastic leukemia. 92% of these cancers identified occurred in the lungs; in F incidence was increased significantly, but not in M. Ear duct carcinomas and head/neck sarcomas/fibrosarcomas were reported as increased; however, the NTP (2011) PWG review does not support this finding.	Survival was generally increased in treated animals compared to controls; in some cases BW was greater for treated animals. Animals were not sacrificed in a controlled single sacrifice, but rather were allowed to die and were necropsied at time of natural death. Although autolysis would be expected considering once a day maximum morbidity/mortality observation of animals, no autolysis was reported. Externally recognized GLP compliance was not assured in this study.

^a Adapted from NTP (2002, 2011); Cruzan (2009); and TERA (2010)^b By exact Cochran-Armitage trend test.

Supplementary Appendix C: Historical Development of MOA Considerations for Chemical Carcinogens

Interim EPA procedures and guidelines adopted in 1976 enabled health risk assessments for suspected chemical carcinogens by implementing a linear no-threshold (LNT) approach to extrapolate increased cancer risks posed by exposures to environmental chemical carcinogens (U.S. EPA 1976; Anderson et al. 1983). This approach extrapolates risks from high doses down to lower doses beyond the region of observed risk and assumes that no dose is without some risk of cancer. The rationale for an LNT approach to risk extrapolation for chemical carcinogens was (and remains) that they may act by mechanisms involving accumulation of irreversible, somatically inherited (e.g., DNA) damage, similar to mutagenic and carcinogenic ionizing radiation. The LNT rationale was viewed as consistent with the somatic mutation theory of carcinogenesis, and specifically with the possibility that, like radiation, DNA-damaging chemicals may add to a “spontaneous” background of DNA damage that could, by this same theory, fully or substantially account for observed background rates of cancer (U.S. EPA 1976):

... Until the late 1950's, no agents, either chemical or physical, had been regulated in this country on the basis of their carcinogenic action with the sole exception of ionizing radiation, which had been known to cause cancer since the turn of the century. ... In the debate over the health effects of radioactive fallout from atomic weapons in the 1950's, the evidence for a no-threshold concept for cancer induction emerged, which supported the idea that there is no such thing as a completely safe dose. In other words any exposure, however small, will confer some risk of cancer on the exposed population.

Evidence has accumulated that indicates that the no-threshold concept can also be applicable to chemical carcinogens. ... We, thus [in the case of administering a risk-benefit-balancing environmental protection law, such as the Federal Insecticide, Fungicide, Rodenticide Act (FIRA)], have a comparable conceptual basis for the regulation of chemicals as for ionizing radiation where the philosophy has been to eliminate or reduce exposure to the greatest extent possible consistent with the acceptability of the costs involved.

Other federal agencies soon began to consider and establish varying approaches to chemical carcinogen risk assessment for the purpose of implementing recent and evolving statutes pertaining to environmental, occupational, food-related, pharmaceutical, and consumer-product aspects of public health and safety. In a landmark report known to students of risk assessment as

“the Red Book” (NRC 1983), the National Research Council (NRC) issued a report recommending that the process of risk assessment by federal agencies could be improved by the development of a coordinated set of general guidelines that clearly identify fundamental default assumptions to be adopted in order to allow regulatory decision making to proceed in a way that clearly separates procedures used for quantitative risk assessment from risk-acceptability decisions intrinsic to risk management. In particular, NRC (1983) urged that explicit, identified default assumptions be used to bridge the gaps in knowledge (e.g., pertaining to the true magnitude of low-level risk posed by exposure to environmental chemical carcinogens) that regulatory agencies must make in order to make scientifically plausible decisions about potential consequences that are inevitably fundamentally uncertain:

One may extrapolate to low doses by fitting a mathematical model to animal dose-response data and using the model to predict risks at lower doses corresponding to those experienced by humans. At present, the true shape of the dose-response curve at doses several orders of magnitude below the observation range cannot be determined experimentally. Even the largest study on record—the ED₀₁ study involving 24,000 animals—was designed only to measure the dose corresponding to a 1% increased risk in tumor incidence. However, regulatory agencies are often concerned about much lower risks (1 in 100,000 to 1 in 1,000). ... A difficulty with low-dose extrapolation is that a number of the extrapolation models fit the data from animal experiments reasonably well, and it is impossible to distinguish their validity on the basis of goodness of fit. [p. 24–25]

The primary problem with risk assessment is that the information on which decisions must be based is usually inadequate. Because the decisions cannot wait, the gaps in information must be bridged by inference and belief, and these cannot be evaluated in the same way as facts ... some limitations are inherent and unresolvable, and inference will always be required. Although we conclude that the mixing of science and policy in risk assessment cannot be eliminated, we believe that most of the intrusions of policy can be identified and that a major contribution to the integrity of the risk assessment process would be the development of a procedure to ensure that the judgments made in risk assessment, and the underlying rationale for such judgments, are made explicit. [p. 48–49]

Detailed methods EPA developed to perform quantitative cancer risk assessments in accordance with its 1976 interim guidelines, and with the emerging NRC (1983) recommendations, were first described by Anderson et al. (1983). This guidance emphasized that “the linear non-

threshold model has been used by the EPA to place plausible upper bounds on risk, not to establish actual risk” (Anderson et al. 1983). The somatic mutation theory of carcinogenesis again provided the scientific rationale for adopting an LNT approach to calculating conservative, plausible upper bounds on increased cancer risk, while acknowledging the fundamental scientific uncertainty inherent in this approach (Anderson et al. 1983):

There is no really solid scientific basis for any mathematical extrapolation model relating carcinogen exposure to cancer risks at the extremely low levels of concentration that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly using either animal experiments or epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time, the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response characteristic of mutagenesis is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence (from mutagenesis studies with both ionizing radiation and a wide variety of chemicals) that this type of dose-response model is the appropriate one to use. ...

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk assessments made with this model should be regarded as conservative, representing the most plausible upper limit for the risk (i.e., the true risk is not likely to be higher than the estimate, but it could be smaller).

The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the modified multistage model developed by Crump [1984]. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment. For this reason it may be called a “linearized” multistage model.

The LNT risk-extrapolation approach adopted (the “linearized multistage model”) assumed that an upper bound on any chemically induced increment over background risk is always strictly proportional to dose at sufficiently low doses (Anderson et al. 1983). The risk modeling approach adopted was thought to be justified by a mathematical demonstration by Crump et al. (1976) that “if carcinogenesis by an external agent acts additively with any already ongoing process, then under almost any model the response will be linear at low dose” and that “throughout the dose range where the extra risk is less than the spontaneous risk linear extrapolation [using the multistage model to extrapolate increased cancer risk] must be quite accurate.” However, Crump et al. (1976) also acknowledged that “if there were some type of threshold effect operating ... the argument would break down and [increased risk as a function of dose] would, in fact, not be linear at the lowest dose rates.”

The interim EPA approach and NRC (1983) recommendations were incorporated into the U.S. EPA (1986) Guidelines for Carcinogen Risk Assessment that, again citing Crump et al. (1976), similarly adopted a default linear approach to extrapolating increased risk of chemically induced cancer at low environmental doses from experimental tumor-response data. The 1986 guidelines again emphasized the upper-bound nature of such estimates. In particular, these guidelines (at p. 13) pointed out that the true level of increased cancer risk at very low environmental exposure levels may be zero:

It should be emphasized that the linearized multistage procedure [of linear-no-threshold extrapolation of increased cancer risk] leads to a plausible upper limit to the risk that is consistent with some proposed mechanisms of carcinogenesis. Such an estimate, however, does not necessarily give a realistic prediction of the risk. The true value of the risk is unknown, and may be as low as zero. ... An established procedure does not yet exist for making “most likely” or “best” estimates of risk within the range of uncertainty defined by the upper and lower limit estimates.

In the Clean Air Act Amendments of 1990, Congress directed the EPA Administrator to engage the National Academy of Sciences in a review of EPA methods used to estimate toxicological risk. The resulting report, *Science and Judgment in Risk Assessment* (NRC 1994, also known as “the Blue Book”), updated the previous NRC (1983) report by focusing on how methods for quantitative characterization of uncertainty and inter-individual variability could be used to improve risk assessment for toxic environmental chemicals, particularly for environmental

chemical carcinogens. This report also noted the critical importance of decision-making context to appropriate considerations and characterizations of risk uncertainty. Specifically, the NRC report recommended that, for purposes of protective standard-setting, analyses of uncertainty in risk estimates that are based on conservative default assumptions (e.g., concerning how to model increased low-level risk) are appropriately performed, conditional on the set of default assumptions used to generate those estimates. In contrast, for decisions that require trade-offs (i.e., weighing or balancing of conflicting interests), the NRC report recommended that all aspects of uncertainty be analyzed in a way that ensures that such decisions do not increase the expected magnitude of risk:

To estimate cancer potencies, EPA currently uses the linearized multistage model ... essentially an empirical curvefitting procedure to describe the relationship between bioassay dose and response and to extrapolate the relationship to exposures below the experimental range. A statistical upper bound on the slope of the low-dose linear portion of the curve is considered to represent an upper bound on a chemical's carcinogenic potency. The multistage model is based on a theory of carcinogenic mechanism proposed in the early 1950s by Armitage and Doll [1954]. In essence, normal cells in a target organ are envisioned as undergoing a sequence of irreversible genetic transformations culminating in malignancy. ... No other potential effects of exposure or alternative mechanisms of carcinogenesis, such as induced cell proliferation or receptor-mediated alterations in gene expression, are included in the [1954, *multistage version of the*] Armitage-Doll model. One important consequence of this assumption about how exposure influences transformations is the linearity of risk at low doses, i.e., risk increases and decreases in direct proportion to the delivered dose. ... Thus, the normal processes of cell division, differentiation, and death are not taken into account by the model. [p. 123; *italicized clarifications added*]

Another cancer dose-response model that has been developed to estimate cancer potencies for risk assessment, but that is not used routinely for regulatory purposes, is the [*stochastic*] two-stage model ... developed by [Armitage and Doll 1957; and refined, e.g., by] ... Moolgavkar and Venzon 1979; Moolgavkar and Knudson 1981; Moolgavkar [1983,] 1988; Moolgavkar et al. 1988; [and Bogen 1989]. Unlike the Armitage-Doll [1954, *multistage*] model, it can explicitly account for many processes considered important in carcinogenesis, including cell division, mutation, differentiation, and death and the clonal expansion of populations of cells. [p. 123; *italicized clarifications added*]

As long as exposure to a chemical has no substantial effect on cell processes other than genetic change, one would not expect the exclusion of these processes from the multistage model to compromise the resulting cancer risk estimates. The model would likely be appropriate for "direct-acting" carcinogens—ones, such as radiation, that act by directly attacking cellular DNA and thereby causing genetic transformation. In recent years, however, it has become apparent that many substances alter the pharmacodynamics of cells and can be carcinogenic by mechanisms that do not involve direct covalent interaction with DNA at all, but involve indirectly caused alterations in gene expression. One consequence of such a change could be altered cellular dynamics in the target organ. Because genetic transformations can occur spontaneously, many target organs contain a background of continuing steps in the multistep carcinogenic process. Exposure to a chemical could augment those background carcinogenic processes by simply increasing the pool of cells that are susceptible to further transformation. Such augmentation might occur as a regenerative response to cellular injury among surviving cells or to the cell-killing that occurs after exposure to highly toxic substances. The augmentation of background carcinogenic processes could also occur as an indirect response to alterations in hormonal balances induced by exposure or as a response to a directly mitogenic substance, i.e., one that stimulates normal cell division. By increasing the rate of cell division, such substances can increase the overall probability of generating a mutation, even though they have no direct effect on the transformation probability per cell division. [p. 124–125]

The committee strongly supports the inclusion of uncertainty analysis in risk assessments ... [and] believes that a more explicit treatment of uncertainty is critical to the credibility of risk assessments and to their utility in risk management. [p. 184]

EPA should make uncertainties explicit and present them as accurately and fully as is feasible and needed for risk management decision-making. To the greatest extent feasible, EPA should present quantitative, as opposed to qualitative, representations of uncertainty. ... In this way, EPA can give its default models the primacy they are due under its guidelines, while presenting useful, but distinct alternative estimates of risk and uncertainty. ... In ... **risk characterizations (which will serve as one important input to standard-setting and residual-risk decisions under the Act)**, EPA risk assessors **should consider only the uncertainty conditional on the choice of the preferred models for dose-response relationships**, exposure, uptake, etc. [p. 185, emphasis added]

For any **decisions involving risk-trading or priority-setting (e.g., for resource allocation or “offsets”)**, EPA **should take into account information on uncertainty** in quantities being ranked so as **to ensure that such trades do not increase expected risk** and such priorities are directed at minimizing expected risk. [p. 186, emphasis added]

The biologically based stochastic two-stage model referred to in these NRC (1994) recommendations is a model that was inspired by and is fully consistent with the somatic mutation theory of carcinogenesis. This newer model differs from the older multistage model only by its more biologically realistic additional consideration of rates of cell replication and cell death, or terminal differentiation, that affect the size of modeled cell populations (see model-specific references cited above).

Importantly, the NRC (1994) recommendation contained in the last paragraph quoted above, regarding uncertainty analysis supporting trade-off or resource-allocation decisions, generally cannot be implemented without considering all sources of uncertainty that bear on the expected magnitude of predicted low-level risk, rather than considering only worst-case or plausible upper-bound estimates of the risks being compared. In the context of chemical carcinogen risk assessment at low levels of exposure, this recommendation requires explicit consideration of the full range of scientifically plausible carcinogenic modes of action, rather than (as done in the U.S. EPA carcinogen risk assessment guidelines) restricting consideration only to the most conservative plausible MOA as a default regulatory policy that is designed to address gaps in knowledge by consistently erring on the side of safety. More specifically, this NRC (1994) recommendation implies that decision makers generally must consider fundamental (“model”) uncertainty associated with predictions made by one or more low-dose risk-extrapolation models, whenever the resulting estimated magnitudes of risk must be weighed, ranked, balanced, or prioritized. Such balancing is typically required whenever the goal of a risk-management decision is to identify a policy that best addresses constraints imposed by limited resources and/or competing interests. The NRC recommendation concerning how to address uncertainty when competing risks must be balanced thus necessarily pertains directly to emergency-related and other environmental-health triage decisions (Bogen 2005), and to chemical risk assessments introduced in civil litigation to provide a factual basis required to judge and weigh the merits of alleged harms and legal claims for equitable remedy (Bogen et al. 2009).

When the 1986 EPA cancer risk assessment guidelines were revised nearly two decades later by U.S. EPA (2005), the new guidelines retained a default linear approach to extrapolate low-dose risk, again as a scientifically plausible default assumption based on the possibility that at least some chemical carcinogens, like radiation, could induce cancer by damaging DNA, in accordance with the somatic mutation theory of carcinogenesis. In accordance with the NRC (1994) recommendations discussed above, the U.S. EPA (2005) guidelines for carcinogen risk assessment placed a greater emphasis on uncertainty characterization. The new guidelines replaced the default “linearized” multistage cancer risk extrapolation model with a new “benchmark dose” (BMD) procedure that no longer involves the application of any biologically inspired mathematical model for risk extrapolation. Instead, the new BMD approach to default cancer risk extrapolation is a purely statistical procedure in which a “model-free” straight line is simply drawn from the lower end of an observed set of tumor-response data, in a way that uses the greatest slope that is statistically consistent with the observed data. As a practical matter, the new BMD procedure typically yields upper-bound estimates of increased cancer risk very similar to those produced by the “linearized” multistage model (Bogen 2011). However, the new U.S. EPA (2005) regulatory guidelines include some initial, explicit, albeit rather vague, criteria for determining when the default linear-BMD risk-extrapolation procedure can be replaced, or supplemented, by an alternative nonlinear approach. Specifically, the new guidelines allow an alternative nonlinear approach to be used when there are “sufficient” MOA-related supporting data (U.S. EPA 2005):

A linear extrapolation approach is used when the mode of action information is supportive of linearity or mode of action is not understood. The linear approach is used when a view of the mode of action indicates a linear response, for example, when a conclusion is made that an agent directly causes alterations in DNA, a kind of interaction that not only theoretically requires one reaction but also is likely to be additive to ongoing, spontaneous gene mutation. Other kinds of activity may have linear implications, for example, linear rate-limiting steps would also support a linear procedure. The linear approach is to draw a straight line between a point of departure [POD] from observed data ... and the origin (zero incremental dose, zero incremental response). This approach is generally considered to be public-health protective. ... The linear default is thought to generally provide an upper-bound calculation of potential risk at low doses ... [p. A-8 – A-9]

When adequate data on mode of action provide sufficient evidence to support a nonlinear mode of action for the general population and/or any subpopulations of concern, a different approach — a reference dose/reference concentration that assumes that nonlinearity — is used. The POD is again generally a BMDL [or lower confidence limit on a benchmark dose (BMD) associated with a benchmark-level (e.g., 10%) increment above background risk] when incidence data are modeled. A sufficient basis to support this nonlinear procedure is likely to include data on responses that are key events integral to the carcinogenic process. This means that the POD may be based on these precursor response data, for example, hormone levels or mitogenic effects rather than tumor incidence data. [p. A-9]

... In the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data: animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity. ... Some modes of action are anticipated to be mutagenic and are assessed with a linear approach. This is the mode of action of radiation and several other agents that are known carcinogens. ... The linear approach is used when: (1) there is an absence of sufficient information on modes of action or (2) the mode of action information indicates that the dose-response curve at low dose is or is expected to be linear. [p. 1-10, 1-11, 1-15]

A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity. Depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative at low doses and focus on a nonlinear approach ... [p. 3-22]

If toxicity or mortality is excessive at the high dose, interpretation depends on whether or not tumors are found. ... Studies that show tumor effects only at excessive doses may be compromised and may or may not carry weight ... Results of such studies, however, are generally not considered suitable for dose-response extrapolation if it is determined that the mode(s) of action underlying the tumorigenic responses at high doses is not operative at lower doses. [p. 2-18]

Trend tests and pairwise comparison tests are the recommended tests for determining whether chance, rather than a treatment-related effect, is a plausible explanation for an apparent increase in tumor incidence. ... By convention, for

both tests a statistically significant comparison is one for which p is less than 0.05 that the increased incidence is due to chance. Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result. ... A two-tailed test or a one-tailed test can be used. In either case a rationale is provided. [p. 2-19, 2-20]

Importantly, and consistent with NRC (1994) recommendations discussed above, the new EPA (2005) guidelines recognize explicitly that the appropriate definition of “sufficient”—for the purpose of determining whether there are “sufficient” MOA-related supporting data to justify adopting a nonlinear approach to chemical carcinogen risk extrapolation—depends on the context in which risk-management decisions are being made:

The weight of evidence that is sufficient to support a decision about a mode of action may be less or more, depending on the purpose of the analysis ... Generally, “sufficient” support is a matter of scientific judgment in the context of the requirements of the decisionmaker or in the context of science policy guidance regarding a certain mode of action. [p. 2-42]

More recently, the U.S. EPA (2007) reiterated its reliance on the somatic mutation theory of carcinogenesis as the sole scientific basis for its default regulatory approach of applying a linear extrapolation method to estimate increased cancer risk for genotoxic environmental chemical carcinogens:

It is well established that mutations in somatic cells play a key, early role in cancer initiation and may also affect other stages of the carcinogenic process. Since all cancer cells acquire multiple mutations during carcinogenesis, mutation induction or acquisition can be key events at some stage in all cancers. [p. 7]

The 2007 guideline statement above was not accompanied by a comprehensive or even summary consideration of relevant evidence that had accumulated by that time concerning the scientific merits of the somatic mutation theory of cancer, compared to competing theories of chemical carcinogenesis that generally predict non-linear (for example, S-shaped) relationships between increased risk and corresponding dose at very low (non-cytotoxic) dose levels, as discussed in the main text.

Supplementary Appendix D: Cancer Potency and Benchmark Dose Calculations

D.1 Cancer Potency and Benchmark Dose Estimation

In the “linearized” multistage cancer risk model, cancer potency or “slope factor” (SF) is the initial slope of the multistage model or function $A(d)$ that relates values of predicted increased (or “extra”) cancer risk (on the Y-axis) to corresponding values of dose d (on the X-axis), at a dose value equal to zero. In other words, SF is the limiting predicted value of “increased risk per unit of dose” (i.e., slope) associated with $A(d)$ as d approaches a value of zero. In standard mathematical terms, SF is defined as the derivative of the function $A(d)$ with respect to dose d in the limit as d approaches zero. More specifically, SF represents an increased (“extra”) cancer risk $A(d) = [R(d) - R(0)]/[1 - R(0)]$ above tumor-specific background risk $R(0)$ per unit dose d (for very small risks $R(d) \ll 1$), assuming a mechanistically and statistically independent background risk $R(0)$ (U.S. EPA 1986). The traditional multistage cancer risk model is defined as $R(d) = 1 - \exp[-F(d)]$, in which $F(d)$ is a polynomial function of lifetime time-weighted average dose, d (in mg/kg/day), that sums over terms of the form $q_i d^i$ for $i = 0, 1, \dots, k$, where $SF = q_1$ (the linear coefficient in dose d , also denoted SF), k is the number of groups of dosed animals in addition to the control group, and the fitted coefficients are constrained such that $q_j \geq 0$ for $1 \leq j \leq k$ (Anderson et al. 1983; U.S. EPA 1986). It follows mathematically that $R(0) = 1 - \exp(-q_0)$, and therefore, that $A(d) = 1 - \exp[-F_A(d)]$ in which $F_A(d)$ is defined as the polynomial function $F(d)$ conditional on $q_0 = 0$. The one-tail upper 95% confidence limit on an estimated SF (or q_1) is denoted SF^* (or q_1^*). The greatest value of SF^* is then typically selected for risk extrapolation, among the set of SF^* values estimated for each significantly elevated tumor type in each species, sex, strain, and experiment for which tumor data of adequate quality are available (Anderson et al. 1983; U.S. EPA 1986). If this procedure yields a set of estimated SF^* values all fairly close in magnitude, sometimes the geometric mean SF^* value has been applied for risk extrapolation purposes. At a dose sufficiently low to ensure that increased risk $A(d)$ is very small (e.g., $A(d) \leq 0.001$), the linear-no-threshold dose-response relationship $A(d) = (SF d)$ provides a very accurate approximation of increased cancer risk $A(d)$ at dose d . An acceptable daily dose d^* is therefore approximated as $d^* = R_a/SF^*$, where R_a denotes a very low level of risk (typically 10^{-6} or 10^{-5}) that is specified by EPA to be acceptable for its risk-management purposes.

The Benchmark Dose (BMD) procedure is an alternative approach developed to model dose-response data, which EPA currently applies to extrapolate carcinogen risk (U.S. EPA 2005, 2010b, 2012b), as well as to characterize non-cancer risks using different procedures. The BMD procedure applied to chemical carcinogens typically involves fitting each observed set of dichotomous tumor-response data to a series of mathematical models, identifying a subset of fits that are adequate using statistical goodness-of-fit and (if needed) graphical criteria, and finally calculating for each good fit a BMD, which is defined as the dose for which the fitted model predicts a specified benchmark risk (BMR), where BMR is defined as a relatively low level of (typically a 10%) increased risk of response relative to background (U.S. EPA 2005, 2010b, 2012b). The one-tail lower 95% confidence limit on an estimated BMD is denoted BMDL. Then, among the set of BMDL values estimated for each significantly elevated tumor type in each species, sex, strain, and experiment for which tumor data of adequate quality are available, the minimum BMDL value is typically selected as a “point of departure” (POD) for the purpose of linear extrapolation of increased risk relative to background (U.S. EPA 2005, 2010b, 2012b). If this procedure yields a set of estimated BMDL values all fairly close in magnitude, sometimes the geometric mean BMDL value is selected as the POD BMDL that is assumed to be associated with an elevated risk equal to the specified BMR. An acceptable daily dose d^* is then defined by linear extrapolation as $d^* = R_a \text{ BMDL/BMR}$, with R_a defined as above (U.S. EPA 2005, 2010b, 2012b).

Note that in the special case that SF is estimated to be zero from data that exhibit a significant positive response, the fitted data typically exhibit upward curvature so great that the fitted multistage polynomial $F_A(d)$ has no linear coefficient in dose d , but rather involves a j^{th} -degree term $q_j d^j$ in which j is the smallest power of dose that appears in $F_A(d)$. In this special case, the approximation $A(d) = q_j d^j$ is very accurate. At a dose d^* sufficiently low to correspond to some small level of acceptable risk $R_a = A(d_a) \leq 0.001$, it follows that $d^* = (R_a/q_j)^{1/j}$ is also a very accurate approximation. The quantity $SF_a = R_a/(R_a/q_j)^{1/j}$ in this case represents an SF^* -equivalent value of potency that is well defined for all values of SF. If $SF > 0$, then $j = 1$ and $SF_a = SF = q_1$. In the special case that $SF = q_1 = 0$, SF_a is calculated *conditional* on $A(d) = R_a$. Thus $d^* = R_a/SF_a$ for all estimated values of SF, including the special case that SF is estimated to be zero.

As indicated in Sections 6 (introduction) and 6.2 of the main text, a modified version of the traditional multistage described above—called the Generic Hockey-Stick (GHS) dose-response model (Bogen 2011)—was used to calculate SF, SF*, BMD, and BMDL. The modification is that it allows up to $k+2$ terms $q_i d^i$ involving dose d with exponents $i \leq k+1$. This modification allows improved fits to dose-response data that exhibit abrupt upward curvature. Otherwise, resulting SF* and BMDL estimates obtained by the GHS model are mathematically equivalent to those obtained by fitting the traditional “linearized multistage” cancer-risk extrapolation model. GHS estimates of SF, BMD, SF*, and BMDL are typically similar to those obtained using U.S. EPA (2010b c) Benchmark Dose software. Bogen (2011) showed that GHS estimates of SF and BMD are at least as (if not more) accurate (i.e., unbiased), and that GHS estimates of SF* and BMDL exhibit at least as accurate statistical coverage, as those obtained using U.S. EPA (2010b c) Benchmark Dose software.

The GHS model (Bogen 2011) was used to obtain a complete cumulative probability distribution function (cdf) associated with each of the parameters SF and BMD, using bootstrap Monte Carlo methods conditional on assumed binomial sampling error associated with each observed set of tumor incidence data. These cdfs were used in Monte Carlo calculations that address (statistical) uncertainty in these parameters. Specifically, the expected value of potency ($\langle \text{SF} \rangle$) and (as applicable) of $\langle \text{BMD} \rangle$ was in each case calculated numerically as the mathematical expected value (i.e., arithmetic mean value) of the corresponding cdf, by applying the percentile method to evaluate the bootstrap Monte Carlo cdf generated by the GHS program. The value of SF* (or, as applicable, BMDL) was likewise calculated numerically as the 95th (or 5th) percentile of each corresponding cdf.

For all calculations reported in this assessment, the GHS code was implemented in a way that assumed no threshold (i.e., assumed a linear-no-threshold dose-response, as in the standard U.S. EPA multistage cancer model). In all calculations, optional “hormetic” or threshold-like features of the unconstrained GHS model described by Bogen (2011) were not implemented. This was accomplished by constraining all GHS-model coefficients (including the linear coefficient) in dose to be non-negative, analogous to the multistage cancer model that is applied to dichotomous tumor-response data to assess cancer risk by U.S. EPA (2010b c, 2012b) Benchmark Dose software.

D.2 Interspecies Extrapolation and Body-Weight Adjustment of Tumorigenic Potency

For a reference adult human with $BW = 70$ kg, the human-equivalent value of cancer potency estimated from a rodent potency value of SF^* , based on data involving rodents with adult body weight $BW = W$, is expected to be the product of SF^* and the dose-scaling factor $(70 \text{ kg}/W)^x$. EPA has typically applied a default assumption that $1-x = 3/4$, and therefore that doses are equivalent when normalized by $W^{3/4}$, implying an interspecies dose-scaling exponent of $x = 1/4$ (U.S. EPA 1992):

The aim of a dose-scaling methodology is to estimate administered daily doses to experimental rodents and humans that result in equal lifetime cancer risks. That is, the scaled doses are intended to be “toxicologically equivalent.” It is useful to recognize two components to this equivalence. The first, which might be termed “pharmacokinetic equivalence,” concerns adjustment of the administered dose to a rodent or human so that the corresponding tissues that constitute the targets of the agent's toxicity receive similar exposures to the toxin. The second, or “pharmacodynamic equivalence,” relates to the relative tissue doses that, when experienced daily for a lifetime, yield equal lifetime cancer risks. This latter aspect includes, but goes beyond the question of “sensitivity” to address species differences in the operation of the carcinogenic processes as they relate to tissue dose. ...

The proposed scaling of daily administered doses of putative carcinogens by $W^{3/4}$ is intended to be such an unbiased projection; i.e., it is to be thought of as a “best” estimate rather than one with some conservatism built in to assure that any error is on the side of being overly protective. It should not be interpreted as a “safety factor” or other intentional bias designed to “err on the side of safety.” Thus, it is to be expected that some individual compounds will have their human potencies overestimated by this procedure, while others will have them underestimated.

This having been said ... there is considerable uncertainty about the best scaling method to achieve this unbiased projection. In particular, the empirical data on comparative carcinogen potencies are also compatible with both body weight and surface area scaling, the methodologies that we propose to abandon in favor of $W^{3/4}$ scaling.

In its 2005 cancer risk guidelines, EPA described its default dose-scaling assumption as follows (U.S. EPA 2005, p. 3-6 – 3-7):

For oral exposures, administered doses should be scaled from animals to humans on the basis of equivalence of $\text{mg/kg}^{3/4}\text{-d}$ (milligrams of the agent normalized by the $3/4$ power of body weight per day) (U.S. EPA, 1992). The $3/4$ power is consistent with current science, including empirical data that allow comparison of potencies in humans and animals, and it is also supported by analysis of the allometric variation of key physiological parameters across mammalian species. ... When toxicokinetic modeling is used without toxicodynamic modeling, the dose-response assessment develops and supports an approach for addressing toxicodynamic equivalence, perhaps by retaining some of the cross-species scaling factor (e.g., using the square root of the cross-species scaling factor or using a factor of 3 to cover toxicodynamic differences between animals and humans, as is currently done in deriving inhalation reference concentrations).

These descriptions distinguish two components that each contribute to a dose-scaling factor: a component concerning more-readily-modeled pharmacokinetic differences between species, and a pharmacodynamic component that concerns hypothesized interspecies differences in the intrinsic sensitivity of target tissue to chemically induced tumorigenesis. The 2005 EPA cancer guidelines imply that, for example, if pharmacokinetic differences in biologically effective delivered dose are addressed directly by an empirically supported pharmacokinetic model, then residual interspecies pharmacodynamic differences can be addressed by a dose-scaling factor equal to the square root of $(70 \text{ kg} / W)^x$ —that is, equal to $(70 \text{ kg} / W)^{x/2}$ —which implies a dose-scaling exponent of $1/8$ using the default assumption that $x = 1/4$. A body-weight scaling assumption ($x = 0$) thus implies that human cancer potency is best estimated directly by rodent cancer potency when both are measured in units of $(\text{mg/kg/day})^{-1}$.

Although EPA has described its default assumption that $x = 1/4$ as an approach “to be thought of as a ‘best’ estimate rather than one with some conservatism built in to assure that any error is on the side of being overly protective,” EPA has nevertheless recognized that there is “considerable uncertainty about the best scaling method” that can be used to define an unbiased measure of toxicologically equivalent dose between species (U.S. EPA 1992). The EPA proposal that its default assumption is a “best” rather than “conservative” (i.e., upper-bound) estimate is not clearly consistent with its recognition that “the empirical data on comparative carcinogen potencies are also compatible with both body weight and surface area scaling” (U.S. EPA 1992). Body weight vs. surface area scaling correspond to assumptions that $x = 0$ and $x = 1/3$, respectively, so the EPA default assumption that $x = 1/4$ lies within the empirically supported

range $0 \leq x \leq 1/3$. However, the default assumption can represent a “best” estimate only if an alternative assumption within the empirically supported range does not provide a better fit to existing relevant data. The alternative assumption that $x = 0$ (corresponding to scaling by body weight) is the assumption that is most consistent with results from studies that directly compared quantitative estimates of human and rodent potency for up to 23 chemical carcinogens that are all positive in humans and rodents (Crouch and Wilson 1979; Allen et al. 1988a b; Crump et al. 1989; Goodman and Wilson 1991; Monroe and Mordenti 1995). There is considerable uncertainty in each of the estimated human and rodent potencies that were compared in these studies. Such uncertainty may appear to prevent firm conclusions about the most accurate approach to interspecies potency extrapolation, particularly regarding any interspecies differences there may be in pharmacodynamically equivalent dose. However, regulatory-policy interest typically focuses not on a broad range of potency estimates for each chemical carcinogen, but rather on a conservatively defined upper confidence limit (UCL) on each such estimated potency. A recent comparison of UCLs on estimated human vs. rodent potencies for 24 chemical carcinogens showed that these UCLs exhibit a pattern consistent with the hypothesis that $x = 0$, and more specifically that UCL estimates of human and rodent potency for chemical carcinogens are, on average, equal to within a factor of <3 with 95% confidence (Bogen 2014).

Supplementary Appendix E. References for Appendices A–D

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