Journal of Toxicology and Environmental Health, Part B, 14:179–245, 2011
Copyright © Taylor & Francis Group, LLC
ISSN: 1093-7404 print / 1521-6950 online
DOI: 10.1080/10937404.2011.556051

Taylor & Francis
Taylor & Francis Group

ROLE OF MUTAGENICITY IN ASBESTOS FIBER-INDUCED CARCINOGENICITY AND OTHER DISEASES

Sarah X. L. Huang[1], Marie-Claude Jaurand[2#], David W. Kamp[3#], John Whysner[1], Tom K. Hei[1,4#]

[1]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York, USA
[2]INSERM (Institut National de la Santé et de la Recherche Médicale), Paris, France
[3]Pulmonary & Critical Care Medicine, Northwestern University Feinberg School of Medicine, Jesse Brown VA Medical Center, Chicago, Illinois, USA
[4]Center for Radiological Research, Department of Radiation Oncology, College of Physicians and Surgeons, Columbia University, New York, New York, USA

The cellular and molecular mechanisms of how asbestos fibers induce cancers and other diseases are not well understood. Both serpentine and amphibole asbestos fibers have been shown to induce oxidative stress, inflammatory responses, cellular toxicity and tissue injuries, genetic changes, and epigenetic alterations in target cells in vitro and tissues in vivo. Most of these mechanisms are believe to be shared by both fiber-induced cancers and noncancerous diseases. This article summarizes the findings from existing literature with a focus on genetic changes, specifically, mutagenicity of asbestos fibers. Thus far, experimental evidence suggesting the involvement of mutagenesis in asbestos carcinogenicity is more convincing than asbestos-induced fibrotic diseases. The potential contributions of mutagenicity to asbestos-induced diseases, with an emphasis on carcinogenicity, are reviewed from five aspects: (1) whether there is a mutagenic mode of action (MOA) in fiber-induced carcinogenesis; (2) mutagenicity/carcinogenicity at low dose; (3) biological activities that contribute to mutagenicity and impact of target tissue/cell type; (4) health endpoints with or without mutagenicity as a key event; and finally, (5) determinant factors of toxicity in mutagenicity. At the end of this review, a consensus statement of what is known, what is believed to be factual but requires confirmation, and existing data gaps, as well as future research needs and directions, is provided.

In this review, “asbestos” is a commercial term often used as an identifier as related to regulatory definitions and refers to a group of naturally occurring mineral fibers, including amphiboles (crocidolite, amosite, tremolite, anthophyllite and actinolite) and chrysotile, the sole serpentine fibers (Case et al., 2011, this issue). Asbestos has been mined and used extensively in industry and households for decades globally. Occupational and environmental exposure of asbestos fibers induces several human diseases, including asbestosis, pleural plaques, lung cancer, mesothelioma, and possibly diseases in nonrespiratory organ systems (Currie

[#]This author has disclosed a potential conflict of interest as described by one or more of the following: He/She may have also received (and may also apply in future for) competitive-funding research grants from U.S. publicly financed, peer-reviewed grant approval process agencies concerning asbestos exposure and disease, including topics covered in all aspects of the workshop, including but not limited to research support from NIEHS.

This review article is part of the “Asbestos Workshop: A Science-Based Examination of the Mode of Action of Asbestos and Related Mineral Fibers” funded by NIEHS. This work was supported in part by grants from the National Institutes of Health R01-ES05786-12, Superfund P42 ES10349, NIEHS Center Grant ES09089 (TKH), and a Merit Review grant from the Department of Veterans Affairs (DWK). We thank David Bernstein, Thomas Hesterberg, Morton Lippmann, Roger McCellan, Günter Oberdörster, and Haining Yang for their critical reading of the draft article.

Address correspondence to Tom K. Hei, Center for Radiological Research, College of Physicians and Surgeons, Columbia University. 630 West 168th Street, New York, NY 10032, USA. E-mail: tkh1@columbia.edu

et al., 2009; IARC, 1987; Institute of Medicine (U.S.) Committee on Asbestos: Selected Health Effects, 2006; Straif et al., 2009). Asbestos has been classified as a Group I human carcinogen by the International Agency for Research on Cancer (IARC, 1987). The association between exposure to asbestos fibers and development of lung cancer and mesothelioma is well established in both humans and experimental animals (Kane, 1992; Mossman & Churg, 1998; Wagner & Berry, 1969; Yarborough, 2007). In addition, cigarette smoking enhances lung cancer incidence among asbestos workers in a synergistic fashion (Hammond et al., 1976; Saracci, 1987). As a result, the U.S. Environmental Protection Agency has restricted the industrial use of asbestos since the early 1970s and there has been legislation proposed in the United States over the years to ban the use of all types of asbestos (http://murray.senate.gov/public/index.cfm?p= BanAsbestos InAmerica). However, asbestos fibers continue to pose an important health concern due to the long latency period of asbestos-induced diseases. Moreover, asbestos is still widely used in many developing countries (Virta, 2003). Therefore, a better understanding of the pathogenic/carcinogenic mechanisms of asbestos is critical for the prevention and treatment of fiber-induced diseases including mesothelioma, an often fatal cancer with an average patient survival of less than 10 mo from the time of diagnosis.

The mechanisms by which asbestos fibers produce malignancy and fibrosis are not entirely clear at present. Various in vitro and in vivo studies suggested that fiber dimensions, surface properties, shape and crystallinity, chemical composition, physical durability, and exposure route, duration, and dose are important determinants of the biological activities of fibers (Mossman, 1990; Sanchez et al., 2009; Stanton et al., 1977). The variations in physical and chemical properties between different types of asbestos fibers are likely to account for differences in pathogenicity. For example, amphiboles might be more carcinogenic than serpentine (Browne, 2001; Carthew et al., 1992; Hodgson & Darnton, 2000; Rogers & Major, 2002). Nevertheless, all types of asbestos were found to be carcinogenic (Straif et al., 2009). There is evidence that the key events, including oxidative stress, chronic inflammation, and genetic and epigenetic alterations, as well as cellular toxicity and fibrosis, are induced by all types of asbestos fibers analyzed (Barrett, 1994; Hei et al., 2006; Kane, 1996; Mossman, 1993; Toyokuni, 2009). These events are not completely independent of, and may be interrelated with, each other. These events are involved in the complex mechanisms of asbestos-induced diseases; the contribution from each event might vary depending on the species and fiber and disease types.

The purpose of this review was to examine the role of mutagenicity—in a broader sense, genetic alterations—in asbestos fiber-induced diseases. This review focuses on the link between mutagenicity and fiber carcinogenicity mainly because it is more extensively studied and better understood, compared with the association of mutagenicity with noncancerous diseases. When examining fiber-induced noncancer-related health endpoints, an association with a broader term, "genotoxicity," rather than mutagenicity, was addressed. For the purpose of this review, studies of all six forms of asbestos are included. In addition, some of the studies on man-made fiber-induced or erionite (a naturally occurring fibrous mineral)-induced diseases, which share similar molecular pathways with and may aid to illustrate the mechanisms underlying asbestos-induced pathogenicity, are also reviewed in the current article. The majority of studies reviewed in this report were conducted using doses below the overload threshold. Five specific areas were addressed in this review, including (i) mutagenicity as a possible mode of action of asbestos and related mineral fibers; (ii) mutagenicity/carcinogenicity at low dose (presence of a possible threshold dose); (iii) biological activities that contribute to mutagenicity and impact of target tissue/cell type; (iv) health endpoints with or without mutagenicity as a key event; and finally, (v) determinant factors of toxicity in mutagenicity. After a review of the literature, a consensus statement is provided stipulating what is known for sure, what is believed to be factual but requires confirmation, and what areas are uncertain and require

additional research pertaining to mutagenicity as a possible mode of action for fiber pathogenicity.

IS THERE A MUTAGENIC MODE OF ACTION (MOA) IN FIBER CARCINOGENESIS?

Mutation refers to "permanent changes in the structure and/or amount of the genetic material of an organism that leads to heritable changes in its function, and includes gene mutations as well as structural and numerical chromosome alterations" (Eastmond et al., 2009). According to the current knowledge, cancer is a genetic disease that arises through a multistep process, and several mutations are required to convert a normal cell into a malignant one (Fearon & Vogelstein, 1990; Vogelstein & Kinzler, 1993). Cancer is the result of the accumulation of multiple mutations that result in ultimate autonomy, unlimited growth, and metastasis of target cells and tissues (Solomon et al., 1991). The mutations involved in carcinogenicity may be generated through two broad modes of actions (MOAs). The first MOA involves direct changes in the structure or content of the genome (mutagenic effects) and/or epigenome (composed of two modules: methylated cytosines located in the dinucleotide sequence CG of DNA, and the chromatin and its associated chromatin-modifying and remodeling activities). The second MOA is indirect alteration in cellular homeostasis, which promotes cell proliferation and growth, thus facilitating the accumulation of spontaneous mutations in the target cells (nonmutagenic mechanism) (Waters et al., 1999). Environmental carcinogens may increase the risk of neoplasms in humans or animals through mutagenic or nonmutagenic mechanisms or both. Thus far, data from existing literature collectively indicate that asbestos fiber-induced carcinogenicity involves both pathways (Barrett, 1992; Kane, 1996; Nymark et al., 2008; Walker et al., 1992). The current section reviews the experimental evidence of a mutagenic MOA in asbestos fiber-induced carcinogenicity. The existing evidence suggests that asbestos fibers are mutagenic carcinogens, which are defined as cancer-causing agents, chemical or physical, that induce mutations in an organism. The potential mechanisms of asbestos fiber mutagenesis are also summarized in this section.

It should be noted that most of the evidence linking a direct mutagenic effect of asbestos fibers is from in vitro studies. A noninvasive approach for obtaining biopsy tissues periodically from animal models or human subjects is still not available. The limited in vivo evidence of mutagenicity was identified from tumor tissues of humans or sacrificed animals. In this case, it was hard to establish the causal effect of the identified mutations on asbestos-induced cancers. As such, the current knowledge of fiber mutagenesis relies mostly on in vitro studies, where asbestos fiber doses were measured mostly in micrograms per square centimeter surface area of tissue culture dish and sometimes in micrograms per millilter. The advantages and disadvantages of in vitro versus in vivo assays for studying asbestos and others fibers are addressed in another review (Mossman et al., 2011, this issue). Although fiber length is usually not provided in these studies, it should be pointed out that most of them used standard reference samples with well-defined fiber dimensions (Bowes & Farrow, 1997; Timbrell et al., 1968; Timbrell & Rendall, 1972). The fiber count for a certain mass concentration of asbestos fibers was reported by (Health Effects Institute-Asbestos Research [HEI-AR], 1991). For example, the NIEHS chrysotile (geometric mean length 1.2 μm, Jeffery Mine, Quebec, Canada) has a total fiber count of approximately 10^5 fibers/cm^3 (fiber mass concentration = 10 mg/m^3) when measured by scanning electron microscopy (SEM) (Mast et al., 1995). Thus, 1 μg asbestos corresponds to 10^7 fibers measured by SEM, while transmission electron microscopy (TEM) measurement of the same mass concentration consistently showed a more than 10-fold higher number of fibers (Breysse et al., 1989). The discrepancy is often due to the detection limit/resolution of the former methodology. Nonetheless, measurement data could be used as reference for calculating the cellular exposure doses under in vitro conditions. Thus, doses reported as micrograms per square centimeter surface area could be converted into fibers per cell, given a known cell density.

Evidence of Asbestos-Induced Mutagenicity

Although there is considerable evidence that asbestos fibers induce chromosome aberrations (CA), micronucleus (MN) formation, and aneuploidy, indicative of mutational events, earlier experimental data failed to detect any asbestos-induced genetic mutations in short-term assays. These include gene mutation assays in bacteria, and in vitro mammalian mutation assays at the hypoxanthine guanine phosphoribosyltransferase *(HPRT),* thymidine kinase *(tk),* and *ouabain* (*oua*) loci using cells from humans, mouse lymphomas, and Chinese hamsters (Chamberlain & Tarmy, 1977; Reiss et al., 1982; Schins & Hei, 2006). Subsequent studies suggested that this might be a result of multilocus deletions induced predominantly by asbestos that are not compatible with the survival of the mutants (Hei et al., 1992; Okayasu et al., 1999b). In recent years, several mutagenic assays that are proficient in detecting either large deletions, homologous recombinations, or score mutants located on nonessential genes were used successfully to demonstrate the mutagenic potential of various fiber types (Both et al., 1995; Park and Aust 1998; Rihn et al., 2000). Asbestos fibers were found to induce large gene mutations, CA, and aneuploidy in mammalian cells in vitro (Jackson et al., 1993; Jaurand 1997; Waters et al., 1999). In March 2009, IARC reassessed the carcinogenicity of asbestos fibers based on cohort studies of women heavily exposed to asbestos in the workplace. It was concluded that asbestos induced mutagenicity, CA, aneuploidy, polyploidy, and epigenetic alterations in the disease process (Straif et al., 2009). In vitro and in vivo evidence, as well as human studies of asbestos fiber-mediated mutagenesis at both gene and chromosomal levels, are summarized in this section (Table 1).

In Vitro Evidence of Asbestos-Induced Gene Mutations

In the human–hamster hybrid A_L cell system, chrysotile and crocidolite asbestos induced both intragenic and multilocus mutations in a concentration-dependent manner within the tested concentration range (0.5–8 $\mu g/cm^2$) (Hei et al., 1992; Xu et al., 2002). Southern analysis confirmed that the majority of the induced mutants contained large deletions ranging from a few thousand to several million base pairs. In another system, crocidolite and erionite (50 or 200 $\mu g/ml$ for 72 h)-treated human lymphocytes were shown to have increased mutations arising from loss of heterozygosity (LOH) at the autosomal HLA-A locus and other distal loci (Both et al., 1994). Crocidolite exposure at the same doses (equivalent to 18.75 and 75 $\mu g/cm^2$) for 72 h produced similar LOH in a human mesothelioma cell line (Both et al., 1995). Park and Aust (1998) observed a twofold rise in the mutation frequency at the glutamate-pyruvate transaminase (*gpt*) locus of G12 (Chinese hamster transgenic $hgprt^-$, gpt^+ V79) cells after exposure to 6 $\mu g/cm^2$ NIEHS crocidolite for 24 h. Consistently, in primary mouse embryo fibroblasts (MEFs) isolated from *gpt* delta transgenic mice, International Union Against Cancer (UICC) chrysotile asbestos treatment at 1–4 $\mu g/cm^2$ for 24 h significantly increased the number of lambda mutants (Xu et al., 2007b). Collectively, these gene mutations may arise from DNA damages, such as DNA adducts and DNA strand breaks, induced by asbestos (Jaurand, 1997).

In Vitro Evidence of Asbestos-Induced Chromosomal Mutations

The chromosomal mutations induced by asbestos fibers could be structural (deletions, translocations, inversions, duplications, and sister chromatid exchanges [SCE]) or numerical (aneuploidy, polyploidy, or hyperdiploidy). These mutations are derived from different types of chromosomal damages induced by asbestos fibers, including chromosomal breakages and fragments (micronuclei), lagging chromosomes, exchange of chromosomal segments between two chromosomes, and missegregation of chromosomes.

Numerous studies from the 1970s to 1990s, with the use of conventional

TABLE 1A. Dose-Effect Relationship of Asbestos-Induced Mutagenic/Carcinogenic Responses—DNA Alterations

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Cleveland, 1984)	Cells Prokaryote	*Escherichia coli*	Richterite (analogue of tremolite) Ames test w/wo rat liver homogenates (S9)	Not done	Mutations w/wo S9, 0.01g/plate		(0.01g/plate)[3]	DNA bacteria
(Faux et al., 1994)	Cells Prokaryote	*S. typhimurium* TA 102	UICC Ch, Cr: 5–500 μg/plate mutagenicity	Dose-effect	Cr = 10 μg/plate Ch = no effect		(10 μg/plate)[3]	DNA Bacteria
(Howden and Faux, 1996a)	Cells Prokaryote	S. typhimurium TA 104	UICC Ch: 0.4 mg, UICC Cr: 0.4, 0.8 mg—DNA adducts	Not done (1 dose)		Ch, Cr : 0.4 mg	(0.4 mg)[1]	DNA Bacteria
(Bertino et al., 2007)	Cells	Human normal mesothelial	UICC : B Ch, Am : (a) cytotoxicity : 1–10 μg/cm^2, (b) DNA synthesis : 0.5–10 μg/cm^2, (c) DNA adducts: 8-OH-dG (with AM 10 μg/cm^2), apoptosis, signaling, transformation (focus assay)	Dose-effect (a) plateau	(a) 1 μg/cm^2 no transformation	(c) 8-OH-dG / 10 μg/cm^2	(10 μg/cm^2)[1] (10 μg/cm^2)[4]	DNA
(Blake et al., 2007)	Cells	Mouse macrophages, MM; RAW264.7 cells; primary alveolar macrophages lavaged from C57BL/6 mice (PAM)	Libby amphibole fibers (6-mix), Cr (RTI, NC) (a) internalization 5 μg/cm^2 (b) ROS production 6.25–62.5 μg/cm^2 (c) 8-oxo-dG and DSBs (comet)	Not done (a) Dose-effect (b) Not done (c)	(a) 5 μg/cm^2 S6 mix uptake in RAW264.7 (c) 8-oxo-dG and DSBs, in RAW264.7: 62.5 μg/cm^2 Cr; 62.5 μg/cm^2 S6 mix n.s.	(b) 6.25 μg/cm^2 S6 mix in RAW264.7	(62.5 μg/cm^2)[1]	DNA
(Cavallo et al., 2004)	Cells	MeT-5A	NIEHS Cr: 1, 2, 5, 10 μg/cm^2 (Comet ± Fpg)	Dose-effect (+ Fpg) = oxidative)-plateau		1 μg/cm^2	(1.0 μg/cm^2)[1]	DNA
(Chao et al., 1996)	Cells	A549 human epithelial	Cr: 1.5, 3, 6 μg/cm^2 (a) cytotoxicity, (b) NO production, (c) 8OH-dG in DNA	Not done (≈ plateau, cytox—linear $n = 3$)	(a) 1.5 μg/cm^2 (b) Concentration not provided	(c) 1.5 μg/cm^2	(1.5 μg/cm^2)[1]	DNA
(Denizeau et al., 1985b)	Cells	Hepatocytes	UICCB Ch: 10, 100 μg/ml—UDS	No effect— linear negative slope (toxicity)			(100 μg/ml)[4]	DNA

(*Continued*)

TABLE 1A. (*Continued*)

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Denizeau et al., 1985a).	Cells	Hepatocytes	UICCB Ch: 10 $\mu g/ml$—UDS	No effect (1 point)			(10 $\mu g/ml$)[4]	DNA
(Dong et al., 1994)	Cells	Rat pleural meso	UICC: A Ch, Cr (a) cytotoxicity: 2, 5, 10, 20, 40 $\mu g/cm^2$; (b) proliferation: 2, 4, 10, 10 $\mu g/cm^2$; (c) UDS: 2, 4, 10, 10 $\mu g/cm^2$	(a) ≈ plateau (b) Not done (c) Not done	(a) 2 $\mu g/cm^2$	(b) 2 $\mu g/cm^2$ (c) Ch = 2 $\mu g/cm^2$; Cr = 4 $\mu g/cm^2$	[2 $\mu g/cm^2$ (Ch)][1] [2 $\mu g/cm^2$ (Cr)][2]	DNA
(Dong et al., 1995)	Cells	Rat pleural meso	UICC: A Ch, Cr (a) cytotoxicity: 0, 2, 5, 20, 40 $\mu g/cm^2$— (b) poly(ADP-ribose) synthesis: 0, 2, 5, 20, 40 $\mu g/cm^2$	(b) Ch: ≈ polynomial order 3; Cr: linear up to 20 $\mu g/cm^2$, then plateau	(b) 2 $\mu g/cm^2$		(2 $\mu g/cm^2$)[3]	DNA
(Howden et al., 1996a)	Cells	RFL-6	UICC Ch: 5 $\mu g/cm^2$, UICC Cr: 2, 5 $\mu g/cm^2$ DNA adducts	Not done (1 or 2 doses)	Ch: low effect at 5 $\mu g/cm^2$	Cr: 5 $\mu g/cm^2$	(5 $\mu g/cm^2$)[3]	DNA
(Kamp et al., 1995)	Cells	WI-26 (SV40-transformed human embryonal lung)	UICC Ch, Am— (a) cytotoxicity: 250 $\mu g/cm^2$ (or 500 $\mu g/ml$)— (b) .OH production: 0.5–500 $\mu g/cm^2$— (c) DNA-SB (alkaline unwiding): 0.25, 2.5, 25 $\mu g/cm^2$	Not done Am One dose Ch	(a) Am: no effect	(b) Am: 50 $\mu g/cm^2$— (c) Am: 0.25 $\mu g/cm^2$ (72 h of treatment); Ch: 25 $\mu g/cm^2$	(0.25 $\mu g/cm^2$)[1]	DNA
(Kamp et al., 1995)	Cells	A549 (human bronchoalveolar carcinoma-derived) Rat alveolar epithelial	UICC Am: DNA-SB (alkaline unwiding); 25, 250 $\mu g/cm^2$	Not done (2 points)		25 $\mu g/cm^2$ in both cell types	(0.25 $\mu g/cm^2$)[1]	DNA
(Kim et al., 2001)	Cells	A549 human epithelial	UICC Cr: 10, 50, 100 $\mu g/ml$ (in 6 well plates)— (a) 8-OH-dG— (b) OGG1 activity	Not done	(b) 100 $\mu g/ml$ (one dose)	(a) 50 $\mu g/ml$ no effect at 10 $\mu g/ml$	(50 $\mu g/cm^2$)[3]	DNA

(Levresse et al., 2000)	Cells	Rat pleural meso	UICC A Ch: 0, 0.5, 2, 5, 10 $\mu g/cm^2$; Cr: 0, 2, 10 $\mu g/cm^2$ Comet assay (tail length, MTM)	Dose response ≈ linear or log		Ch: 0.5 $\mu g/cm^2$ Cr: 10 $\mu g/cm^2$	(0.5 $\mu g/cm^2$)[1]	DNA
(Libbus et al., 1989)	Cells	Rat embryo cells	UICC Cr: 2 $\mu g/cm^2$—(a) DNA strand breaks (nick translation, morphology); (b) cell proliferation (^{3}HdThd incorporation)	(a) Not done (1 dose) (b) log	(b) 0.5 $\mu g/cm^2$	(a) 2 $\mu g/cm^2$	(2 $\mu g/cm^2$)[1]	DNA
(Liu et al., 2000)	Cells	Rabbit pleural mesothelial	NIEHS Cr: 10 $\mu g/cm^2$—DNA breakage	Not done		10 $\mu g/cm^2$	(10 $\mu g/cm^2$)[3]	DNA
(Msiska et al., 2009)	Cells	Normal human small airway epithelial (SAE) human bronchial epithelial cancer (A549)	Cr NIEHS: 100 $\mu g/ml$—(a) cytotoxicity, (b) ROS production, (c) DNA DSBs: γHzAZfoci H2AX ^{ion}, (d) apoptosis: caspase 3/7 activation, (e) pARP induction *if medium equivalent to 5 ml for 25 cm² as in flasks ≤ ~20 μg/cm²*	Not done	(b) 0.5 $\mu g/cm^2$	(a) 100 $\mu g/ml$: SAE cells— (b) 100 $\mu g/ml$: A549 & SAE— (c) 100 $\mu g/ml$: A549 & SAE— (d) 100 $\mu g/ml$: SAE	(100 $\mu g/ml$)[1]	DNA
(Murata-Kamiya et al., 1997)	Cells	J774 sarcoma cell line	UICC Ch, Cr, Am: 8-OH-dG (HPLC)	Dose-effect— ≈ linear (Cr) not done (1 dose) other fibers	Ch: n.s. at 100 $\mu g/ml$ (1 dose)	Cr: ≈ 10 $\mu g/ml$ Am: 100 $\mu g/ml$ (1 dose)	(10 $\mu g/ml$)[1] (100 $\mu g/ml$)[3]	DNA
(Okayasu et al., 1999a)	Cells	CHO and xrs-5 (DSB-repair deficient)	UICC A Ch 0, 8, 16 $\mu g/cm^2$—DSB	Dose-effect— ≈ linear in both cell types; enhanced in xrs-5/CHO		8 $\mu g/cm^2$	(8 $\mu g/cm^2$)[1]	DNA
(Ollikainen et al., 1999)	Cells	Human TagSV40 mesothelial (MeT-5A)	UICC Cr (a) cytotoxicity: 1, 2 $\mu g/cm^2$, (b) DNA breakage (comet): 1, 2, 4 $\mu g/cm^2$, (c) ROS production: 1, 2 $\mu g/cm^2$	Not done (a)	(a) Not toxic (c) No effect	(b) 1 $\mu g/cm^2$	(1 $\mu g/cm^2$)[1]	DNA

(*Continued*)

TABLE 1A. (*Continued*)

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Ollikainen et al., 2000)	Cells	A549 and MeT-5A	DNA breakage (comet): A549 = 2, 10 $\mu g/cm^2$; MeT-5A = 2, 4 $\mu g/cm^2$	Not done (2 doses)	2 $\mu g/cm^2$		(2 $\mu g/cm^2$)[3]	DNA
(Pietruska and Kane 2007)	Cells	Murine mesothelial cell lines D9, D7—malignant mesothelioma cell lines, MeT-5A	UICC Cr—10 $\mu g/cm^2$—DSBs (γ-H2AX foci formation)—micronuclei—senescence	Not done		10 $\mu g/cm^2$; all tests	(10 $\mu g/cm^2$)[1]	DNA
(Puhakka et al., 2002)	Cells	A549 and MeT-5A	UICC Cr: 2, 4 $\mu g/cm^2$—Comet (tail extent moment)	Not done (1 or 2 doses)		2 $\mu g/cm^2$	(2 $\mu g/cm^2$)[1]	DNA
(Takeuchi and Morimoto, 1994)	Cells	HL60 (human promyelocytic leukemia cell line)	Cr: 15, 50, 150 $\mu g/ml$	Linear	15 $\mu g/ml$		(15 $\mu g/ml$)[3]	DNA
(Turver and Brown 1987)	Cells	C3H10T1/2	UICC Cr 25–200 $\mu g/cm^2$—DNA-SB	Dose-effect—≈ linear		25 $\mu g/cm^2$	(25 $\mu g/cm^2$)[1]	DNA
(Topinka et al., 2004)	Cells ex vivo	Alveolar macrophages and type II from Big Blue	Am I.tr.: (a) 1, 2 mg; (b) 4 × 2 mg—(a) DNA-SB (comet); (b) micronucleus	(a) Dose-effect—≈ log		(a) 2 mg in both cell types (b) 2 mg in macrophages; n.s. in type II	(2 mg)[1]	DNA
(Xu et al., 1999)	Cells	A_L cells	UICC Cr (a) nonprotein sulfhydryl (NPSH) depletion; 4 $\mu g/cm^2$ (b) 80H-dG staining; 2–12 $\mu g/cm^2$			(b) 4 $\mu g/cm^2$	(4 $\mu g/cm^2$)[3]	DNA
(Jiang et al., 2008).	Animal	Rats	UICC: A Ch, Cr, Amosite (Am): 10 mg 8OH-dG	Not done	10 mg: higher level with all fibers		(5 mg/ml)[3]	DNA in vivo
(Jung et al., 2000)	Animal	Rats Sprague-Dawley	UICC Am: Itr 2.5 mg—DNA breaks in bronchiolar epithelial cells	Not done (1 dose)		2.5 mg	(2.5 mg)[3]	DNA in vivo
(Unfried et al., 2002)	Animal	Transgenic F344 *lacI* rats (Big Blue)	UICC Cr: analysis of the greater omentum: (a) mutations: 2, 5 mg; (b) 8-OH-dG: 1, 2 mg	Not done (2 doses)	(a) 5 mg (b) 1 mg		(1 mg)[3]	DNA

Note. #, Lowest effective dose: 1, sLOAEL: Surrogate lowest-observed-adverse-effect level. 2, sNOAEL: Surrogate no-observed-adverse-effect level. 3, pLOAEL: Potential lowest-observed-adverse-effect level. 4, pNOAEL: Potential no-observed-adverse-effect level. *Type of assay. "DNA" = 8-OH-dG, breaks, repair. DSB: Double Strand break

TABLE 1B. Dose-Effect Relationship of Asbestos-Induced Mutagenic/Carcinogenic Responses—Chromosomal Changes

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Achard et al., 1987)	Cells	Cells rat pleural meso	Cr 10, 15, 20 μg/cm^2; SCEs	Dose-effect— ≈ linear (in highest dose)		10 μg/cm^2	(10 μg/cm^2)[1]	CA
(Ault et al., 1995)	Cells	newt *Taricha granulosa* lung epithelial	Cr 0.5 mg/ml; mitosis dynamics, chromosome breakage	Not done	0.5 mg/ml		(0.5 mg/ml)[3]	CA
(Babu et al., 1980)	Cells	CHO	Ch 10, 25, 50, 100, 200 μg/ml, chromosome aberrations	Not done (plateau)	10 μg/ml		(10 μg/ml)[3]	CA
(Both et al., 1994)	Cells	Human lymphocytes	UICC: A Ch 50, 400 μg/ml; Cr 400 μg/ml (a) mutations, (b) LOH *HLA-A* locus	Not done (1 or 2 doses)	(a) Cr: no effect	(a) Ch 50 μg/ml (b) Cr 400 μg/ml	(400 μg/ml)[1]	CA
(Both et al., 1995)	Cells	Human mesothelioma	UICC Cr: 50, 200 μg/ml (a) mutations, (b) LOH *HLA-A* locus	Not done (2 points)	(a) Effect not significant	(b) 50 μg/ml	[(b) 50 μg/ml][1]	CA
(Casey 1983)	Cells	CHO-K1	UICC Cr, Ch SFA: 0.001-0.01 μg/ml SCEs	Not done	No effect		(0.01 μg/ml)[2]	CA
(Dopp et al., 1995b)	Cells	SHE	UICC: A Ch, Cr—micronucleus: Ch = 1, 5, 10 μg/cm^2; Cr = 1, 5, 10 μg/cm^2	Dose-effect ≈ plateau or decrease at the highest dose		1.0 μg/cm^2	(1.0 μg/cm^2)[1]	CA
(Dopp et al., 1997)	Cells	Human amniotic fluid cells	UICC: A Ch, Cr, Amosite (Am): 0.5, 1, 5, 10 μg/cm^2 micronuclei w/wo kinetochore analysis, polyploidy, breakage	Micronuclei: dose effect, plateau breakage, hyperploidy: not done	Micronuclei: 0.5 μg/cm^2 breakage, hyperploidy: 1 μg/cm^2		(0.5 μg/cm^2)[1]	CA
(Dopp et al., 1998)	Cells	SHE	UICC: A Ch, Crmicronucleus: 0.5, 1, 5, 10 μg/cm^2	Dose-effect plateau	All fibers = 0.5 μg/cm^2		(0.5 μg/cm^2)[1]	CA
(Dopp et al., 1998)	Cells	Human amniotic fluid cells	UICC: A Ch, Cr micronucleus: 0.5, 1, 5, 10 μg/cm^2	Dose-effect plateau	All fibers = 1 μg/cm^2		(1 μg/cm^2)[1]	CA
(Dopp et al., 2005)	Cells	V79	UICC Ch 1 μg/cm^2— micronucleus (± kinetochore Ab)	Not done (1 dose)		1 μg/cm^2	(1 μg/cm^2)[1]	CA

(*Continued*)

TABLE 1B. *(Continued)*

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Durnev et al., 1993)	Cells	Blood lymphocytes, human	Ch Russia, 50 μg/ml—chromatid and chromosome breaks	Not done (1 dose)		50 μg/ml	(50 μg/ml)[1]	CA
(Emerit et al., 1991)	Cells	Blood lymphocytes	Clastogenicity of chrysotile-treated rat pleural mesothelial cellls, 0.2–2 μg/m^2	Dose-effect		0.2 μg/cm^2 (no effect = toxcity at 2 μg/cm^2)	(0.2 μg/cm^2)[1]	CA
(Hart et al., 1992)	Cells	CHO	UICC: A Ch, Cr (a) proliferation: Ch: 1, 2; Cr: 5 μg/cm^2— (b) colony forming efficiency (CFE): Cr: 5 μg/cm^2—(c) Nuclear abnormalities (NA): Ch: 1, 2, 5; Cr: 5 μg/cm^2	Not done (2 points)	UICC: A Ch, Cr (a) i proliferation: Ch: 1; Cr: 5 μg/cm^2 (b) i CFE: Cr: 5 μg/cm^2 (c) h NA: Ch: 1; Cr: 5 μg/cm^2		(1 μg/cm^2)[3]	CA
(Hesterberg et al., 1985)	Cells	SHE	Cr 1 μg/cm^2	Not done (1 dose)	1 μg/cm^2		(1 μg/cm^2)[3]	CA
(Huang et al., 1978)	Cells	CHL (Chinese hamster lung)	Cr: (a) survival: 10, 20, 40 μg/cm^2— (b) chromosomal aberrations: 5 μg/cm^2— (c) mutagenesis (6TG^R cells): 5 μg/cm^2 or 8 μg/cm^2 or 20 μg/cm^2	Dose-effect log (a) Not done (one dose (b)) Not done (one dose (c))	(a) 10 μg/cm^2 (at 50%)	(b) 5 μg/cm^2 (c) 8 μg/cm^2 (one among 3 experiments)	(5 μg/cm^2)[1]	CA
(Kelsey et al., 1986)	Cells	CHO fibroblasts	Cr: (a) ploidy, (b) clastogenicity, (c) SCEs (d) mutation HGPRT, and TK— 0, 5, 10 20, 50 μg/ml	Not done (1 or 2 doses)	(a) 5 μg/ml (b) No effect (c) No effect (d) No effect		(5 μg/cm^2)[1] (50 μg/ml)[2]	CA
(Korkina et al., 1992)	Cells	Human lymphocytes	Ch: 0.01, 0.05 mg/ml—abnormal metaphases	Not done (2 doses)		0.01 mg/ml	(0.01 mg/ml)[1]	CA
(Lavappa et al., 1975)	Cells	SHE	UICC Ch A: 0.1, 1, 10, 100 μg/ml	Dose-effect ≈ log		0.1 μg/ml	(0.1 μg/ml)[1]	CA

(Levresse, et al., 1998)	Cells	Rat pleural meso - normal - TagSV40	UICC: A Ch, Cr 10 $\mu g/cm^2$ cell cycle arrest,micronuclei, aneuploidy,	Not done (1 dose)	Positive effect		$(10\ \mu g/cm^2)^3$	CA
(Oshimura et al., 1984)	Cells	SHE	Ch, Cr (a) Mutation: 1, 2 $\mu g/cm^2$ (b) Transformation: 1, 2 $\mu g/cm^2$ (c) Cytogenetic (aneuploidy, polyploidy, binucleated cells, micronuclei): 0.5–2 $\mu g/cm^2$	(a) No mutation (b) No transformation (c) Dose effect		(c) 0.5 $\mu g/cm^2$: aneuploidy, polyploidy, binucleated; 1 $\mu g/cm^2$: micronuclei	$(0.5\ \mu g/cm^2)^1$	CA
(Palekar et al., 1987)	Cells	V79	UICC Ch, Cr: (2 ml in T25: 10, 40, 60, 100 $\mu g/ml$ = 0.8, 3.2, 4.8, 8 $\mu g/cm^2$)— (a) aneuploidy— (b) clastogenicity	(a) Dose-effect exon (b) Dose-effect (not simple)		(a) 0.8 $\mu g/cm^2$ (both types) (b) 3.2 $\mu g/cm^2$ (Cr)— 0.8 $\mu g/cm^2$ (Ch)	(0.8 $\mu g/cm^2$ 3.2 $\mu g/cm^2)^1$	CA
(Pelin et al., 1995b)	Cells	Cells MeT-5A meso human	UICC samples, micronuclei induction (a) Ch A 0.5–2 $\mu g/cm^2$ (b) Cr 0.5–2 $\mu g/cm^2$ UICC samples, binucelated cells (a) Ch A 0.5–2 $\mu g/cm^2$ (b) Cr 0.5–2 $\mu g/cm^2$	Micronuclei induction (a) Dose effect (b) Dose effect	Binucleated cells (a) 0.5 $\mu g/cm^2$; ≈ 10^6 F/cm² (b) 0.5 $\mu g/cm^2$; ≈ 10^6 F/cm²	Micronuclei induction (a) 0.5 $\mu g/cm^2$ (b) 0.5 $\mu g/cm^2$	$(0.5\ \mu g/cm^2)^1$	CA
(Pelin et al., 1995b)	Cells	Cells normal meso human	UICC samples, micronuclei induction (a) Ch A 0.5–2 $\mu g/cm^2$ (b) Cr 0.5–2 $\mu g/cm^2$ UICC samples, binucelated cells (a) Ch A 0.5–2 $\mu g/cm^2$ (b) Cr 0.5–2 $\mu g/cm^2$	Micronuclei induction (a) Dose effect (b) Dose effect	Binucleated cells (a) 0.5 $\mu g/cm^2$; ≈ 10^6 F/cm² (b) 0.5 $\mu g/cm^2$; ≈ 10^6 F/cm²	Micronuclei induction (a) 0.5 $\mu g/cm^2$ (b) 0.5 $\mu g/cm^2$	$(0.5\ \mu g/cm^2)^1$	CA

(*Continued*)

TABLE 1B. (*Continued*)

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Pelin et al., 1995b)	Cells	Cells rat liver epithelial (RLE)	UICC samples, micronuclei induction (a) Ch A 0.5–2 μg/cm^2 (b) Cr 0.5–2 μg/cm^2 UICC samples, binucelated cells (a) Ch A 0.5–2 μg/cm^2 (b) Cr 0.5–2 μg/cm^2	Micronuclei induction (a) Dose effect (b) Dose effect	Binucleated cells (a) 0.5 μg/cm^2; ≈ 10^6 F/cm^2 (b) 0.5 μg/cm^2; ≈ 10^6 F/cm^2	Micronuclei induction (a) 0.5 μg/cm^2 (b) 0.5 μg/cm^2	(0.5 μg/cm^2)[1]	CA
(Pelin et al., 1995a)	Cells	Human primary mesothelial cells from six patients (noncancerous)	UICC Am: 1, 2 μg/cm^2— chromosomal aberrations	Not done (2 doses)	1 or 2 μg/cm^2 in 2 patients		(1 μg/cm^2)[1]	CA
(Pietruska and Kane, 2007)	Cells	Murine mesothelial cell lines D9, D7—malignant mesothelioma cell lines, MeT-5A	UICC Cr—10 μg/cm^2— DSBs (γ-H2AX foci formation)— micronuclei— senescence	not done		10 μg/cm^2; all tests	(10 μg/cm^2)[1]	CA
(Poser et al., 2004)	Cells	Cells normal meso human	UICCA Ch, Cr 0.25–5 μg/cm^2(a) micronuclei formation	(a) dose-effect	(a) 0.25 μg/cm^2		(0.25 μg/cm^2)[3]	CA
(Price-Jones et al., 1980)	Cells	V79-4 (Chinese hamster cells)	UICC Cr: ≈ 0.25, 1;25, 3.75 μg/cm^2 (1, 5 or 15 μg/ml, 20ml/75cm^2)— (a) aneuploidy; (b) ECS	No effect			(3.75 μg/cm^2)[4]	CA
(Sincock and Seabright, 1975)	Cells	Chinese hamster cells	Ch SFA, UICC Cr: 10 μg/ml— chromosome aberrations	No effect (1 point)			(10 μg/ml) [4]	CA
(Sincock et al., 1982)	Cells	Primary human fibroblasts	Ch SFA, UICC Cr: 10 μg/ml (25-cm^2 flasks => likely 5 μg/cm^2)— chromosome aberrations		10 μg/ml (5 μg/cm^2 ?) only in hamster cells		(10 μg/ml) [3]	CA

(Valerio et al., 1983)	Cells	Human lymphocytes	Ch, CR:10 μg/ml— (a) aneuploidy: (b) chromatid and chromosome aberrations	Not done (1 dose)		(a) 10 μg/ml (b) 10 μg/ml	(10 μg/ml) [1]	CA
(Wang et al., 1987)	Cells	Cells rat pleural meso	UICC Ch A, Cr: 5 μg/ml = 1 μg/cm^2— Metaphase abnormalities	Not done (1 dose)	1 μg/cm^2		(1 μg/cm^2) [3]	CA
(Yegles et al., 1993)	Cells	Cells rat pleural meso	Aneuploidy (Aneu), Anaphase-Telophase Abnormalities (ATA). Ch UICCB:0.5-2 μg/cm^2; CR UICC: 1–6 μg/cm^2	Dose-effect		Aneu: Cr = 4 μg/cm^2— ATA: Cr = 7 μg.cm^2 (1.8 and 2 μg/cm^2 signif in 1/3 expt); Ch = 1.0 μg/cm^2	(4 μg/cm^2 7 μg/cm^2) [1]	CA
(Yegles et al., 1995)	Cells	Cells rat pleural meso	Anaphase-Telophase Abnormalities (ATA). UICC samples Ch: several doses	Ch ≈ linear	NOEL Stanton's fibers = 2.5×10^5/cm^2		(2.5×10^5/cm^2)[2]	CA
(Durnev et al., 1993)	Cells ex vivo	C57BL/6 mice, peritoneal fluid cells	Ch Russia IP: 50 mg/kg (≈ 1 mg/mice), then perit fluid and bone-marrow cells Chromatid and chromosome breaks	Not done (1 dose)		1 mg/mice	(1 mg/mice)[1]	CA
(Lavappa et al., 1975)	Cells ex vivo	bone marrow cells	UICC Ch A: 0.4, 4, 40, 400 mg/kgIP micronucleus	No effect	NOAEL = 400 mg/kg			CA in vivo
(Topinka et al., 2004)	Cells ex vivo	Alveolar macrophages and type II from Big Blue	Am I.tr.: (a) 1, 2 mg; (b) 4 × 2 mg— (a) DNA-SB (comet); (b) micronucleus	(a) Dose-effect— ≈ log		(a) 2 mg in both cell types (b) 2 mg in macrophages; n.s. in type II	(2 mg)[1 & 4]	CA

Note. #, Lowest effective dose: 1, sLOAEL: Surrogate lowest-observed-adverse-effect level. 2, sNOAEL: Surrogate no-observed-adverse-effect level. 3, pLOAEL: Potential lowest-observed-adverse-effect level. 4, pNOAEL: Potential no-observed-adverse-effect level. *Type of assay. "Chromo" = Chromosomal aberrations, SCEs, micronuclei.

TABLE 1C. Dose-Effect Relationship of Asbestos-Induced Mutagenic/Carcinogenic Responses—Mutagenesis

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Both et al., 1994)	Cells	Human lymphocytes	UICC: A Ch 50, 400 μg/ml; Cr 400 μg/ml (a) mutations *HLA-A* locus, (b) LOH *HLA-A* locus	Not done (1 or 2 doses)	(a) Cr: no effect	(a) Ch 50 μg/ml (b) Cr 400 μg/ml	Ch: (a) (50 μg/ml)[1] (b) (400 μg/ml)[2] Cr: 400 μg/ml)[2]	Mutagenesis
(Both et al., 1995)	Cells	Human mesothelioma	UICC Cr: 50, 200 μg/ml (a) mutations, (b) LOH *HLA-A* locus	Not done (2 points)	(a) Effect not significant	(b) 50 μg/ml	[(b) 50 μg/ml][1] [(a) 200 μg/ml][2]	Mutagenesis
(Hei et al., 1992)	Cells	A_L cells	UICC Ch— (a) survival: 1–40 μg/ml; (b) mutagenicity at HGPRT locus 20 μg/ml: (c) mutagenicity S1 locus	Dose-effect exp. (a) Not done (expression per survival) (b and c)	(a) 1 μg/ml = ≈ 0.2 μg/cm² (i 10%) (c) mutants at 2.5 μg/ml = ≈ 0.5 μg/cm²		(0.5 μg/cm²)[3]	Mutagenesis
(Hei et al., 1995)	Cells	A_L cells	UICC Ch— (a) survival: 1–40 μg/ml; (b) mutagenicity S1 locus 20 μg/ml— 40 μg/ml = ≈ 8 μg/cm²	Dose-effect exp. (a) Not done (1 dose) (b)	(a) 1μg/ml = ≈ 0.2 μg/cm² (i 10%) (b) 20 μg/ml = ≈ 4 μg/cm²		(4 μg/cm²)[3]	Mutagenesis
(Huang, 1979)	Cells	CHL CCL39 (Chinese hamster lung)	Ch, Cr, Am: 10 μg/cm² (a) Cell growth— (b) mutagenesis (6TG^R cells)	Not done (1 dose)		All types mutagenic 10 μg/cm²	(10 μg/cm²)[1]	Mutagenesis
(Huang et al., 1978)	Cells	CHL (Chinese hamster lung)	Cr: (a) survival: 10, 20, 40 μg/cm²— (b) chromosomal aberrations: 5 μg/cm²— (c) mutagenesis (6TG^R cells): 5 μg/cm² or 8 μg/cm² or 20 μg/cm²	Dose-effect log (a) Not done (one dose (b)) Not done (one dose (c))	(a) 10 μg/cm² (i 50%)	(b) 5 μg/cm² (c) 8 μg/cm² (one among 3 experiments)	(8 μg/cm²)[1]	Mutagenesis

(Kenne et al., 1986)	Cells	CHO	UICC Cr: 1.2, 7.6, 15.3, 23 23 μg/cm^2—mutagenicity (HGPRT)	Linear (3 lowest doses; highest doses toxic)	1.5 μg/cm^2		(1.5 μg/cm^2)[3]	Mutagenesis
(Okayasu et al., 1999b)	Cells	A_L cells	UICCA Ch, tremolite (Tr) (a) survival = Ch: 1–10 μg/cm^2, Tr: 5–40 μg/cm^2—(b) mutation HPRT = Ch: 1–10 μg/cm^2, Tr: 5–80 μg/cm^2— (c) mutation *S1*$^-$ = Ch: 1–2.5 μg/cm^2, Tr: 5–20 μg/cm^2	(a) Dose effect (b) No mutation (c) Mutation; Ch ≈ strong, Tr: dose effect	(a) Ch: 1 μg/cm^2; Tr: 15 μg/cm^2 (c) Ch: 1 μg/cm2; Tr: 5 μg/cm^2		[(c) 1 μg/cm^2 5 μg/cm^2][3] [(b) 10 μg/cm^2 80 μg/cm^2][4]	Mutagenesis
(Oshimura et al., 1984)	Cells	SHE	Ch, Cr (a) Mutation: 1, 2 μg/cm^2 (b) transformation: 1, 2 μg/cm^2 (c) cytogenetic (aneuploidy, polyploidy, binucleated cells, micronuclei): 0.5–2 μg/cm^2	(a) No mutation (b) No transformation (c) Dose-effect		(c) 0.5 μg/cm^2: aneuploidy, polyploidy, binucleated; 1 μg/cm^2: micronuclei	(2 μg/cm^2)[2]	Mutagenesis
(Park et al., 1998)	Cells	V79 (Chinese hamster cells) for *hgprt* G12 (transgenic Chinese hamster cells) for *gtp*	Cr : (a) survival G12: 3, 4.5, 6 μg/cm^2— (b) mutagenicity *hprt*— (c) mutagenicity *gpt* 3, 4.5, 6 μg/cm^2	(a) Dose-effect expo (c) Dose-effect expo	(b) No effect	(a) 3 μg/cm^2 (b) no effect (c) 4.5 μg/cm^2	(6 μg/cm^2)[1 & 2] (4.5 μg/cm^2)[1]	Mutagenesis
(Reiss et al., 1982)	Cells	Adult rat liver (ARL-18)	UICC B Ch 0.001, 0.1 μg/ml TG_r mutants	Not done	No increased mutation/ controls		(0.1 μg/ml)[4]	Mutagenesis
(Reiss et al., 1983)	Cells	ARL-6 and ARL-18	UICC Ch (0.75, 1 μg/ml), Cr (20, 30 μg/ml), Am (10, 20 μg/ml): mutation HGPRT (6-TG^R)	No effect			(1 μg/ml 30 μg/ml)[4]	Mutagenesis

(*Continued*)

TABLE 1C. (*Continued*)

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Xu et al., 2002)	Cells	A_L cells	UICC Cr (a) ROS production 2–9 μg/cm^2 (b) cytotoxicity (c) CD59 mutation 2, 4 μg/cm^2	(a) Peak (b) Not done (c) Not done	(a) 2 μg/cm^2—peak 6 μg/cm^2 (b) 2 μg/cm^2 (c) 2 μg/cm^2	2 μg/cm^2	(2 μg/cm2)[3]	Mutagenesis
(Xu et al., 2007b)	Cells	gpt delta transgenic mous primary fibroblasts	UICC Ch (a) cytotoxicity; 0.5–6 μg/cm^2 (b) *spi*$^-$ mutations; 0.5-4 μg/cm^2	(a) Exponential (b) ≈ Linear	(a) 0.5 μg/cm^2	(b) 1.0 μg/cm^2	(1.0 μg/cm2)[3] (0.5 μg/cm^2)[4]	Mutagenesis
(Xu et al., 2007a)	Cells	A_L cells karyoplasts, cytoplasts, whole cells	UICC Cr (a) ROS formation by cytoplasts 2–6 μg/cm^2 (b) NO induction whole cells 2–12 μg/cm^2 (c) CD59 mutation (karyo + cyto plasts)	(a) Dose effect (b) Peak (c) Not done	(a) 2 μg/cm^2 (b) 2 μg/cm^2—peak 6 μg/cm^2 (c) 4 μg/cm^2		(4 μg/cm^2)[1]	Mutagenesis
(Kociok et al., 1999)	Animal	Rats	Cr: 2 mg ip—mesothelioma, mutation analysis	Not done (1 dose)	2 mg [4 tumors with mutation (= deletions) in 27 tumors analyzed		(2 mg)[3]	Mutagenesis in vivo
(Rihn et al., 2000)	Animal	Transgenic F344 lacI rats (Big Blue)	Cr: nose-only exposure 5.75 mg/m^3—mutation	Not done (1 dose)		5.75 mg/m^3	(5.75 mg/m^3)[1]	Mutagenesis in vivo
(Topinka et al., 2004)	Animal	Transgenic F344 lacI rats (Big Blue)	Am I.Tr.: (a) 1, 2 mg; (b) 4 × 2 mg—mutagenesis	Not done (2 doses)		2 mg	(2 mg)[1]	Mutagenesis in vivo
(Unfried et al., 2002)	Animal	Transgenic F344 *lacI* rats (Big Blue)	UICC Cr: analysis of the greater omentum: (a) mutations: 2, 5 mg; (b) 8-OH-dG: 1, 2 mg	Not done (2 doses)	(a) 5 mg (b) 1 mg		(5 mg)[3]	Mutagenesis in vivo

Note. #, Lowest effective dose: 1, sLOAEL: Surrogate lowest-observed-adverse-effect level. 2, sNOAEL: Surrogate no-observed-adverse-effect level. 3, pLOAEL: Potential lowest-observed-adverse-effect level. 4, pNOAEL: Potential no-observed-adverse-effect level. *Type of assay. "Muta" = Mutagenesis.

TABLE 1D. Dose-Effect Relationship of Asbestos-Induced Mutagenic/Carcinogenic Responses—Tumorigenesis

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Altomare et al., 2005a)	Animal	Mice 129Sv/J Nf2 (+/−) Heterozygous and wild type	UICC Cr: ip total: 3.2 mg/mouse (repeated dose of 400 μg every 21 d for 8 rounds) mesos	Not done (1 dose)	Wild type: 3.2 mg = 59% Nf2 (+/−): 3.2 mg = 85%		(3.2 mg)^3	Tumor
(Altomare et al., 2009)	Animal	Mice Arf (+/−)	UICC Cr: ip total: 3.2 mg/mouse (repeated dose of 400 μg every 21 d for 8 rounds) mesos	Not done (1 dose)	Wild type: 81%, latent period 56 wk Arf (+/−): 96%, latent period 42		(3.2 mg)^3	Tumor
(Davis et al., 1988)	Animal	Rat wistar	Ch: long, short ip = 0.25, 2.5, 25—Peritoneal tumors	Dose-effect (plateau)	25 mg = 95% (long); 91.7% (short) 2.5 mg = 91.7% (long); 33.3% (short) 0.25 mg = 66.6% (long); 0% (short)		(0.25 mg function of size)^3	Tumor
(Davis et al., 1988)	Animal	Rat wistar	Ch long, short—Inh 10 mg/m^3—lung tumors + mesos	Not done (2 doses)	57.5% (long); 20% (short) 4.2% ctrl		(10 mg/m^3 function of size)^3	Tumor
(Davis et al., 1978)	Animal	Rat	Ch UICCA 2, 10 mg/m^3 Cr UICC 5, 10 mg/m^3 Am = 10 mg/m^3 tumor incidence	Dose-effect Ch Not done Cr	10 mg/m^3: 37.5% (Ch), 2.5% (Cr), 4.7% (Am) 2 mg/m^3: 21.4% (Ch), 6.9% (Cr) Ctrl = 0%	Ch 2 mg/m^3	(2 mg/m^3)^1	Tumor
(Davis et al., 1986)	Animal	Rat wistar	Am long, short—Inh 12 mg/m^3—lung tumors + mesos	Not done (1 dose)	35% (long); 2.4% (short)— Ctrl = 3.3%		(12 mg/m^3)^3	Tumor

(*Continued*)

TABLE 1D. *(Continued)*

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Davis et al., 1986)	Animal	Rat wistar	Am long, short—ip 25, 10 mg—Peritoneal tumors	Not done (2 doses)	25 mg = 95.8% (long); 4% (short) 10 mg = 88% (long); 0% (short)		(10 mg function of size)[3]	Tumor
(Davis et al., 1991)	Animal	RatAF/Han	Ch, Cr, Am eight doses: 0.01—15 mg	Dose response—log	Ch: 0.01 mg—total fibers: 9.0E+07—<0.25 μm: 3.4E+07—>5 μm, <0.25 μm: 6.2E+05		(10 μg)[3]	Tumor
(Davis et al., 1991)	Animal	RatAF/Han	Ch, Cr, Am eight doses: 0.01—15 mg	Dose response—log	Cr: 0.05 mg—total fibers: 1.1E+08—<0.25 μm: 9.0E+07− > 5 μm, <0.25μm: 5.3E+05		(50 μg)[3] (10 μg)[4]	Tumor
(Davis et al., 1991)	Animal	RatAF/Han	Ch, Cr, Am eight doses: 0.01—15 mg	Dose response—log	Am: 0.01 mg—total fibers: 8.3E+06—<0.25 μm: 5.3E+06—>5 μm, <0.25 μm: 2.9E+04		(10 μg)[3]	Tumor
(Fleury-Feith et al., 2003)	Animal	Mice FVB/N *Nf2*$^{KO3/+}$ heterozygous and wild type	Cr,length: 9.9 ± 7.8 μm and diameter: 0.3 ± 0 .2 μm,: total 5 mg in separated two doses (2nd injection was 2 mo apart from the 1st injection). Peritoneal tumors	Not done (1 dose)	Wild type: 5 mg = 17% Nf2 (+/−): 5 mg = 47%		(5 mg)[3]	Tumor
(Jaurand et al., 1987)	Animal	Rat Sprague-Dawley	UICC Am—20 mg—Pleural tumors	Not done (1 dose)	20/35		(20 mg)[1]	Tumor
(Lippmann, 1994)	Animal	Rodents—Tumorigenicity—data from inhalation studies by Davis et al. and Pott et al.	Several samples	Dose response—polynomial: $y = a + bf + cf^2$ with "c" << a and b	Correlation tumor incidence for fibers >5 μm length, >10 μm length		[≈ 1000 F/ml (L > 5 μm)][3]	Tumor

(Marsella et al., 1997)	Animal	Mice 129Sv *$p53^{-/-}$ $p53^{+/-}$ and $p53^{+/+}$*	UICC Cr: weekly ip of 200 μg for 35 wk mesos	Not done (1 dose)	Wild type: 32%, latent period 67 wk P53 (+/−): 76%, latent period 44 wk P53 (−/−): 12.5%, latent period 10 wk	(7 mg)[3]	Tumor
(Monchaux et al., 1981)	Animal	Rat Sprague-Dawley	UICC Cr—I Pleural 20 mg—Pleural tumors	Not done (1 dose)	21/32	(20 mg)[3]	Tumor
(Monchaux et al., 1981)	Animal	Rat Sprague-Dawley	ChA—I Pleural 20 mg—Pleural tumors	Not done (1 dose)	15/32	(20 mg)[3]	Tumor
(Vaslet et al., 2002)	Animal	*Mice* 129/Sv strain on a 75% C57B1/6 background, *$p53^{+/-}$ and $p53^{+/+}$*	UICC Cr: weekly ip of 200 μg for 35–66 wk until tumor develops	Not done (1 dose)	Wild type:32%, latent period 67 wk P53 (+/−): 76%, latent period 44 wk	(7–13.2 mg)[3]	Tumor
(Wagner et al., 1973)	Animal	Rat wistar	ChB—I Pleural 20 mg—Pleural tumors	Not done (1 dose)	10/32	(20 mg)[1]	Tumor
(Wagner et al., 1973)	Animal	Rat wistar	ChA—I Pleural 20 mg—Pleural tumors	Not done (1 dose)	7/31	(20 mg)[1]	Tumor
(Wagner et al., 1973)	Animal	Rat wistar	UICC Cr—I Pleural 20 mg—Pleural tumors	Not done (1 dose)	18/32	(20 mg)[1]	Tumor

(*Continued*)

TABLE 1D. *(Continued)*

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Wagner et al., 1974)	Animal	Rat wistar	UICC Ch A, Ch B—Inh 10 mg/m^3—lung tumors + mesos	Not done (1 dose)	11/21 (ChB), 11/17 (ChA)		$(10\ mg/m^3)^1$	Tumor
(Wagner et al., 1974)	Animal	Rat wistar	UICC Am—Inh 10 mg/m^3—lung tumors + mésos	Not done (1 dose)	13/21		$(10\ mg/m^3)^1$	Tumor
(Wagner et al., 1974)	Animal	Rat wistar	UICC Cr—Inh 10 mg/m^3—lung tumors + mesos	Not done (1 dose)	13/18		$(10\ mg/m^3)^1$	Tumor
(Hei et al., 1984)	Cells	C3H10T1/2	UICC Ch, Am	No effect (1 point)	5 μg/ml			Transformation
(Hesterberg and Barrett, 1984)	Cells	SHE	UICC Ch, Cr: 0.25–2 $\mu g/cm^2$ (a) survival— (b) transformation frequency	dose-effect expon. (a) dose-effect linear; slope ≈ 1; one hit compatible (b)	EC-50 (a) = 0.9 (Ch), 1.7 (Cr) $\mu g/cm^2$— (b): 0.25 $\mu g/cm^2$;		$(0.25\ \mu g/cm^2)^3$	Transformation
(Lu et al., 1988)	Cells	BALB/3T3	UICC Ch, Cr—Transformation assay Ch = 0.005-0.5 $\mu g/cm^2$ Cr = 0.1–10 $\mu g/cm^2$	Ch: dose effect Cr: no dose effect	Ch: 0.005 $\mu g/cm^2$ Cr ≈ 0.1 $\mu g/cm^2$		(0.005 $\mu g/cm^2$ 0.1 $\mu g/cm^2)^3$	Transformation
(Mikalsen et al., 1988)	Cells	SHE	Ch, Cr, Am, Anthophyllite: 0.5, 1.5, 3, 5 $\mu g/cm^2$—Transformation assay	Dose-effect ≈ plateau (depends on exptl conditions)	1% transformation in $\mu g/cm^2$: 0.15 (Ch), 2.6 (Cr), 3.0 (Am), 3.5 (Anth)	Cr = 1.5 $\mu g/cm^2$	$[1.5\ \mu g/cm^2]^3$ $[0.5\ \mu g/cm^2]^4$	Transformation
(Oshimura et al., 1984)	Cells	SHE	Ch, Cr (a) Mutation: 1, 2 $\mu g/cm^2$ (b) transformation: 1, 2 $\mu g/cm^2$ (c) cytogenetic (aneuploidy, polyploidy, binucleated cells, micronuclei): 0.5–2 $\mu g/cm^2$	(a) No mutation (b) Dose effect (c) Dose effect		(c) 0.5 $\mu g/cm^2$: aneuploidy, polyploidy, binucleated; 1 $\mu g/cm^2$: micronuclei	$(2\ \mu g/cm^2)^2$	Transformation

Note. #, Lowest effective dose: 1, sLOAEL: Surrogate lowest-observed-adverse-effect level. 2, sNOAEL: Surrogate no-observed-adverse-effect level. 3, pLOAEL: Potential lowest-observed-adverse-effect level. 4, pNOAEL: Potential no-observed-adverse-effect level. *Type of assay. "Tumor" = Tumor, "Transfo" = Transformation.

TABLE 1E. Dose-Effect Relationship of Asbestos-Induced Mutagenic/Carcinogenic Responses—Cellular Responses

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Belitskaya-Levy et al., 2007)	Cells	Normal human bronchial epithelial	UICC Ch: 7, 12.7 μg:cm^{2}— gene expression	Not done (1 dose)	7 μg/cm^2		(7 μg/cm^2)^3	Cell response Gene expression
(Broaddus et al., 1996)	Cells	Cells meso rabbit	Cr, Am, Ch (a) decreased proliferation (b) enhanced apoptosis	Dose-effect		(a) Cr: 3 μg/cm^2— (b) Cr: 3 μg/cm^2, Am:15 μg/cm^2, Ch: 10 μg/cm^2		Cell response Apoptosis
(Cole et al., 1991)	Cells	*Taricha granulosa*—primary epith & mesoth lung cells	UICC Cr: 500 μg/ml	Not done (1 dose)	migration of <5 μm L fibers, not long fibers			Cell response Interaction with microtubules
(Dubes and Mack, 1988)	Cells	CLI (chimpanzee liver), KB human K, Eta monkey kidney, NIH 3T3	13 asbestos samples—1.42—142 μg/cm^2	Not done	not provided			Cell response Transfection of viral RNA
(Hart et al., 1994)	Cells	CHO	NIEHS Cr long, Cr med, Cr short—UICC Ch, Cr and MMVF [all types gathered according to size parameters]—≈ 1–200 μg/cm^2 or ≈12,000–150,000 fibers/cm^2 (a) Cytotoxicity (decreased proliferation) (b) Abnormal nucleus induction (ANI)	Dose-effect (a) and (b) dependence with length; slight dependence with diameter		(a) 0.68 to 3.0 μg/cm^2 or 0.8 to 45 × 10^5 fibers/cm^2		Cell response (viability)
(Jiang et al., 2008)	Cells	RAW264.7, HeLa, Met-5A, FRCC562 *(rat renal carcinoma ferric nitrilotriacetated-induced)*	UICC A ch, Cr, Am = 5 μg/cm^2 (a) phagocytosis— (b) ROS level in RAW264.7: 1–10 μg/cm^2	Not done	(a) Uptake: all fiber types in all cell types; fibers in the nucleus (b) Ch = n.s.	(b) Cr = 3 μg/cm^2; Am = 5 μg/cm^2		Cell response (genotoxic potential)

(*Continued*)

TABLE 1E. (*Continued*)

Reference	Study type	Assay system	Assay endpoints	Dose-response shape	Limit dose with response (significant?)	Limit significant (or dose)	Lowest effective dose#	Type of assay*
(Johnson et al., 1997)	Cells	A549 human epithelial	UICC Cr: 0.1, 0.5, 1, 2, 5 $\mu g/cm^2$—Cell cycle effects (a) h % G2 cells; h p21, h p53, h Gad43.	Dose response—log	0.5 $\mu g/cm^2$		$(0.5\ \mu g/cm^2)^3$	Cell response (genotoxic potential)
(Levresse et al., 1997)	Cells	Rat pleural meso	UICC A Ch: 0, 5, 10 $\mu g/cm^2$; Cr: 0, 10, 20 $\mu g/cm^2$	Dose-effect— ≈ linear		Ch: 5 $\mu g/cm^2$ Cr: 10 $\mu g/cm^2$	$(5\ \mu g/cm^2)^3$	Cell response (genotoxic potential)
(Lu et al., 1994)	Cells	V79 (Chinese hamster lung)	NIEHS Ch: intermediate, short treated with surfactant— (a) micronucleus— (b) SCEs	Not applicable				Cell response Chromo—fibers treated with surfactant
(Matsuoka et al., 2003)	Cells	A549 human epithelial	UICC ACh, Cr: 1–10 $\mu g/cm^2$ p53 phosphorylation	Dose-effect	1 $\mu g/cm^2$ (Western)			Cell response DNA—indirect
(Yang et al., 2006)	Cells	Cells normal meso human	(a) Cytotoxicity 0.2–5 $\mu g/cm^2$ (b) TNF-alpha and receptor production 6–24 $\mu g/cm^2$	(a) Dose effect (b) Dose effect (no stats) (c) Not done	(b) 6 $\mu g/cm^2$	(a) 0.5 $\mu g/cm^2$		Cell response Chromo (no dose provided)
(Rita and Reddy, 1986)	Cells ex vivo	Mice germinal cells	Ch—oral feeding					Cell response Chromo in germinal cells—oral feeding

Note. #, Lowest effective dose: 1, sLOAEL: Surrogate lowest-observed-adverse-effect level. 2, sNOAEL: Surrogate no-observed-adverse-effect level. 3, pLOAEL: Potential lowest-observed-adverse-effect level. 4, pNOAEL: Potential no-observed-adverse-effect level. *Type of assay. "Cell response" = cellular changes in response to asbestos treatment: i.e., phagocytosis, increased intracellular ROS level, changes in the expression/function of signaling molecules involved in cell cycle, apoptosis, DNA repair, or other pathways.

cytogenetic analyses, demonstrated that different types of asbestos fibers (chrysotile, crocidolite, amosite, or tremolite) induced various numerical chromosomal changes, or structural chromosomal aberrations in meta- and anaphases (lagging chromosomes, bridges, fragments, exchanges), in cultured mammalian cells, including human lymphocytes, Chinese hamster ovarian (CHO) cells, human fibroblast, human and rat pleural mesothelial cells, and Syrian hamster embryo (SHE) cells, after short-term treatments (6–96 h) (Athanasiou et al., 1992; Hesterberg & Barrett, 1985; Jaurand et al., 1986; Lavappa et al., 1975; Lechner et al., 1985; Livingston et al., 1980; Oshimura et al., 1984; Valerio et al., 1983; Yegles et al., 1993). The fiber concentrations used in these studies are arbitrary ranging from 0.5 to 4 $\mu g/cm^2$ or from 0.1 to 10 μg/ml. A couple of studies used a relatively higher dose of 100 μg/ml; however, a significant increase in overall incidence of aberrations was observed at a concentration as low as 0.1 μg/ml (Lavappa et al., 1975; Livingston et al., 1980). Some studies examined the effects of different types of asbestos and reported varied potency of these fibers. For example, 2 $\mu g/cm^2$ chrysotile was more potent in inducing cytogenetic effects and cell transformation in SHE cells than crocidolite (both types of asbestos were UICC fibers) and thin glass fibers at the same dosage, while thick glass fibers were much less potent and nonfibrous alpha-quartz had no effect (Oshimura et al., 1984). In another study, Canadian chrysotile was able to induce chromosomal abnormalities at a concentration of 2 $\mu g/cm^2$, while UICC crocidolite induced similar effects at a higher concentration (7 $\mu g/cm^2$) (Yegles et al., 1993). However, when fibers are counted by the number of long and thin fibers (length $> 8\ \mu$m, diameter $\leq 0.25\ \mu$m) per $\mu g/cm^2$, crocidolite was more potent than chrysotile (Yegles et al., 1993). Both studies indicated that physical characteristics of the fibers affected the ability to induce chromosomal mutations. A more complete list of these studies is reviewed elsewhere (Jaurand, 1997). In later studies, using a combination of the approaches including kinetochore staining, supravital ultraviolet (UV) microscopy, and fluorescence in situ hybridization (FISH) with tandem DNA probes, it was consistently shown that UICC standard amosite, chrysotile, and crocidolite asbestos fibers (0.5–10 $\mu g/cm^2$) significantly enhanced the induction of MN, chromosomal breakage and loss, and hyperdiploidy in human amniotic fluid cells (AFC) and hamster SHE cells in a concentration-dependent manner after 48 or 66 h of exposure (Dopp et al., 1995b, 1997; Dopp, & Schiffmann, 1998). Overall, these alterations are found to be concentration dependent in most of the studies, but also highly dependent on the cell and fiber type. The low dose effect is addressed later, in the section "Mutagenesis/Carcinogenesis at Low Dose."

Taken together, although some studies were not conducted in relevant lung or pleural target cells, the preceding in vitro evidence provides important molecular insights into asbestos mutagenicity and carcinogenicity. Many of the studies were conducted with concentrations higher than the occupational and environmental exposure in humans, which, however, is necessary for establishing dose- and time-response relationships between fiber exposure and the induction of mutagenic events. The overdose issues for both cell cultures and animal studies are addressed later, in the section "Effects of Different Fiber Dimensions and Types and Overdose Issue."

In Vivo Evidence of Asbestos-Induced Gene or Chromosomal Mutations

In contrast with the large amount of in vitro studies, limited in vivo data on asbestos-induced gene and chromosomal changes are available. A few studies examined CA, MN formation, or aneuploidy in bone-marrow or germline cells of rodents or monkeys, which are not akin to the relevant target cells of asbestos exposure, and results were controversial (Lavappa et al., 1975; Osgood & Sterling, 1991; Fatma et al., 1992). Kociok and colleagues (1999) observed DNA deletions in the peritoneal tumors developed in rats exposed to

a 2-mg dose of crocidolite injected intraperitoneally (ip) with a mutation frequency of 14.8% for all the tumors screened. The first nose-only inhalation study that demonstrated in vivo mutagenicity of asbestos was conducted in transgenic mice carrying the *lacI* reporter gene (Rihn et al., 2000). The animals were exposed to crocidolite asbestos (5.75 mg/m^3) for 5 consecutive days (6 h/d). In lung DNA, there was significantly increased mutation frequency in the *lacI* gene during 4 wk of exposure, but no increase in hydrophobic lung DNA adducts among asbestos-exposed animals. Similarly, intratracheally instillation of amosite (2 mg single dose, and 4 weekly doses of 2 mg) significantly elevated the mutation frequency in lung DNA in transgenic lambda-lacI (Big Blue) rats 16 wk after exposure (Topinka et al., 2004). Exposure of Big Blue mice to the same doses of rock wool (RW1) standard fibers (1700 fibers/μg, median length 23 μm, biological half life 3–4 mo) induced a significant dose-dependent increase in mutation frequency 16 wk after exposure (Topinka et al., 2006a). However, glass wool standard fibers MMVF10 (man-made vitreous fibers 10, 1 μg sample contains 8300 fibers longer than 5 μm, median length 26 μm, weighted half-life 37 d) at all doses did not produce any marked change in mutation frequency. These results suggest that durable fibers are more mutagenic in vivo than biodegradable fibers.

Evidence of Asbestos-Induced Gene or Chromosomal Mutations From Human Studies

The evaluation of mutagenicity in the relevant target tissues of human subjects exposed to asbestos or man-made fibers is technically difficult due to the lack of noninvasive approach in getting biopsy samples. More importantly, there is a high possibility of missing the mutagenic or precancerous cells, if taking random biopsies before tumor formation. Nevertheless, some supportive evidence is currently available. On one hand, several studies examined the mutagenic and cytogenetic alterations in peripheral blood lymphocytes of asbestos workers. The findings vary from each other and the type of asbestos exposure is not available in most of the studies, but in general the findings indicate an association between cytogentic alterations in lymphocytes and asbestos exposure. A significant correlation between SCE in group A chromosomes (large, metacentric human chromosomes, consist of chromosome pairs 1, 2, and 3) and asbestos exposure (a mean of 27.4 yr) was found in asbestos insulators (Rom et al., 1983). In Slovakia, 61 chronically exposed asbestos cement plant workers (exposure: 5–40 yr) had significantly higher numbers of CA and oxidized pyrimidines, compared with controls (Dusinska et al., 2004; Horska et al., 2006). The average rate of chromosomal aberrant cells in the peripheral blood lymphocytes in 31 Russian asbestos workers (both $<$1.5 yr and $>$10 yr of exposure groups) was not statistically different from that in the control (Bochkov et al., 1991). The frequency of MN in lymphocytes of 30 Turks (average 59.97, range 32–80 yr) environmentally exposed to chrysotile (exposure since born and terminated at 10 yr before the study was conducted) was not different from those in unexposed controls (Donmez-Altuntas et al., 2007). In mesothelioma patients, the MN frequency in blood lymphocytes was significantly increased, in comparison with lung cancer patients and patients with benign asbestos diseases (Bolognesi et al., 2002). In contrast, numerous chromosomal abnormalities and some gene deletions were identified in human mesothelioma and lung cancer tissues associated with mixed or single exposure to asbestos (Ivanov et al., 2009; Jaurand, 2006; Lindholm et al., 2006; Murthy & Testa, 1999; Musti et al., 2006; Neragi-Miandoab & Sugarbaker, 2009; Sekido, 2010). For example, these abnormalities include loss of heterozygosity (LOH), gene deletion, or change of methylation pattern at chromosome 9p21.3/cyclin-dependent kinase inhibitor 2A (CDKN2A, or p14^{ARF} and p16^{INK4a}) and CDKN2B genes, 22q/Neurofibromatosis type 2 (*NF2)* gene, and 3p/fragile histidine triad (FHIT) gene (Bianchi et al., 1995; Hirao et al., 2002; Lindholm

et al., 2007; Pylkkanen et al., 2002; Tug et al., 2006). For details, see the section "Health Endpoints With and Without Mutagenicity as a Key Event." The identification of these genetic alterations might lead to better therapeutic strategies and disease control for mesothelioma (Crispi et al., 2010).

Although peripheral blood lymphocytes are not the direct target cells, and a causal effect of the mutations identified in tumor tissues of animals and human subjects on asbestos-induced cancers cannot be easily established, there are some correlation between the observed in vitro and in vivo effects and human data. First, similar gene and chromosomal alteration patterns were identified in asbestos-induced murine and human mesothelioma models (Altomare et al., 2005a, 2005b; Andujar et al., 2007; Kane, 2006). The results indicate that the murine mesothelioma model can recapitulate the molecular features of human malignant mesothelioma and may be useful for human risk assessment. Therefore, gene alterations seen in tumors generated in fiber-exposed animals may be linked to the carcinogenesis of asbestos fibers. Second, a number of in vivo studies identified nonrandom mutagenic and/or cytogenetic changes in important genes (i.e., tumor suppressor genes) that are associated with asbestos-induced transformation (Barrett, 1994; Barrett et al., 1989). However, futures studies with well defined doses of various asbestos fibers (fiber numbers measured by TEM, or fiber surface area) are required in order for establishing a stepwise association between mutagenicity and carcinogenicity in relevant target tissues of animal models exposed to a variety type of asbestos fibers. A well-developed mutagenicity testing strategy with an appropriate selection of in vivo and in vitro tests will facilitate this process (Eastmond et al., 2009).

Potential Mechanisms of Asbestos-Induced Mutagenicity

Unlike chemical mutagens that generate genetic alterations by covalent binding to/modifying the DNA structures, asbestos-induced mutagenicity is mediated through direct or indirect pathways. Asbestos fibers may induce mutagenicity and genotoxicity directly through physical interaction with the mitotic machinery of dividing cells after being phagocytized by the target cells, or indirectly as a result of damaging of DNA and chromosome by asbestos-induced reactive oxygen and nitrogen species (ROS and RNS, respectively) (Jaurand, 1997; Kamp & Weitzman, 1999; Kane, 1996; Shukla et al., 2003a). ROS and RNS might be generated primarily (by asbestos fibers, i.e., surface iron [Fe] mediated Fenton reaction) or secondarily (through fiber-induced inflammation) (Gulumian, 2005; Hardy & Aust, 1995b; Aust et al., 2011, this issue). Thus, any physical or chemical properties of the fibers that might affect the ROS and RNS generating capability, or promote the physical interference with dividing cells by the fibers, such as surface iron content and fiber dimension, affect the mutagenicity of the fibers. One might expect that different types of fibers are likely to share same mutagenic mechanisms, except that the contribution of each of the mechanisms varies from one fiber type to another. In addition, there is evidence suggesting a synergistic effect of tobacco smoke and asbestos exposure on mutagenicity and lung cancer risks (Kamp et al., 1992; Nymark et al., 2008; Selikoff et al., 1968).

DNA Damage and Mutagenesis Resulting From Asbestos-Induced ROS and RNS

Asbestos-generated ROS and RNS may produce a variety of DNA and chromosomal damages, such as 8-hydroxydeoxyguanosine (8-OHdG), DNA single-strand breaks (SSB), and chromosome fragments (MN) (Jaurand, 1997; Kamp et al., 1992). The involvement of asbestos-generated ROS and RNS in asbestos mutagenicity is illustrated by the evidence that Fe chelator, free radical scavenger (dimethyl sulfoxide [DMSO]), or antioxidants (superoxide dismutase [SOD] and catalase [CAT]) attenuate the genotoxic effects of asbestos fibers. Additional supporting evidence came from the

findings that the mutation spectrum in asbestos fibers-treated cells was similar to that produced by hydrogen peroxide (H_2O_2). The contribution of ROS and RNS to asbestos fiber-induced DNA and chromosomal damage was addressed in cell-free systems, in vitro cultured cells, and in vivo animal models.

The observation of 8-OHdG formation, as a result of ROS/RNS generation by various asbestos fibers, in cell-free systems and cultured relevant target cells such as pulmonary epithelial cells and pleural mesothelial cells was reported and reviewed in many studies (Jaurand, 1997; Kamp et al., 1992; Shukla et al., 2003a; Upadhyay & Kamp, 2003). The protective effects of the Fe chelator deferoxamine (DEF), and antioxidants glutathione (GSH) and DMSO indicate that DNA damage and/or mutagenesis induced by asbestos fibers is mediated through reactive intermediate species (Faux et al., 1994b; Howden & Faux, 1996b; Xu et al., 1999). The inconsistent effect of Fe inhibitors reported in other studies is probably due to the effectiveness of different chelators and the valency state of the Fe (Lund & Aust, 1990; 1992; Kamp et al., 1992; Hardy & Aust, 1995b; Jaurand, 1997). Similarly, the induction of DNA strand breakages by asbestos was illustrated in plasmid DNA or calf thymus DNA (Jackson et al., 1987; Lund & Aust, 1992; Mahmood et al., 1994; Hardy & Aust, 1995a). Consistent results were found using cultured human and rodent target cells, and Fe chelators or CAT exerted protective effects (Burmeister, et al., 2004, Faux et al., 1994b; Kamp, et al., 1995; Turver & Brown, 1987; Xu, et al., 2007b). Iron associated with amosite asbestos bodies isolated from human lungs induced the formation of SSB in phi X174 RFI DNA (Lund et al., 1994). Similarly, crocidolite asbestos-induced mutagenesis at the CD59(S_1) locus of A_L cells or gpt locus of G12 cells was also shown to be attenuated by addition of antioxidants or radical scavengers (Hei et al., 1995; Park & Aust, 1998; Xu et al., 1999; 2002; 2007a, 2007b). The mutation spectra induced by crocidolite fibers in A_L cells or primary gpt delta transgenic MEF were similar to those produced by H_2O_2 (Xu et al., 2002, 2007b). In general, DNA lesions produced by hydroxyl radical (OH) significantly increased mutation frequency (McBride et al., 1991). In addition, the mutagenic effects of asbestos on lymphocytes were also suppressed by oxygen radical scavengers (Korkina et al., 1992). Furthermore, crocidolite and chrysotile asbestos-induced MN formation, an indicator of CA, in human mesothelial cells was significantly reduced after pretreatment of fibers with Fe chelators phytic acid and DEF, and/or simultaneous treatment of cells with asbestos fibers and radical scavengers dimethylthiourea or SOD (Poser et al., 2004). In another study, the addition of Fe chelators also reduced MN formation in chrysotile asbestos-exposed V-79 cells (Dopp et al., 2005). Finally, spindle deformation with disturbed meta- and anaphases was observed in amosite, chrysotile, or crocidolite asbestos-treated SHE cells, while the spindle fiber morphology appeared unchanged (Dopp & Schiffmann, 1998). The results from these studies show that asbestos fibers may induce both loss and breakage of chromosomes in the absence of direct interaction with spindle fibers, which, together with the data from antioxidant studies, provide evidence of the role of oxidative stress in producing CA in target cells.

The first in vivo evidence indicating the involvement of ROS and RNS in mediating DNA damage associated with fiber mutagenicity was demonstrated in mesothelial cells by Unfried et al. (2002). Using UICC crocidolite asbestos fibers (with a 59% fraction of carcinogenic fibers, aspect ratio $\geq$5), a significant increase in mutation frequency in peritoneal tissues of transgenic F344 big blue rats at 12 wk (5 mg fibers only) and 24 wk (2 mg and 5 mg fibers) after ip administration was noted. The mutational spectrum analysis showed a dominance of G $\rightarrow$ T transversions, which was different from those of spontaneous mutations. In a separate experiment in Wistar rats conducted by Unfried et al. (2002), a significant rise in 8-OHdG level after fiber treatment (1 mg or 2 mg) was observed at both 10 and 20 wk after exposure. These results indicate the involvement of hydroxyl radicals in crocidolite-induced mutagenesis in vivo.

Schurkes et al. (2004) observed similar levels of 8-OHdG induction 10 wk after ip administration of 14.7 and 29.4 mg of MMVF 11 (man-made vitreous fibers 11, diameter 0.08–4.20 μm; length 1.7–98.8 μm with a 25% fraction of carcinogenic fibers). Interestingly, the results showed that graded doses of MMVF11 (14.7, 29.4, 50, or 100 mg) induced a dose-dependent increase in 8-OHdG levels 20 wk after fiber administration. These data by Unfried and colleagues (2002) confirm the results from an earlier study, in which a single dose of UICC amosite (2.5 mg) induced a significant increase in DNA strand breaks 14 d after intratracheal instillation of the fibers (Jung et al., 2000). Jiang et al. (2008) demonstrated that at 4 wk after a single ip injection of each of the UICC chrysotile, crocidolite, and amosite asbestos fibers at 10 mg (5 mg/mL) into rats, these induced significantly higher levels of nuclear 8OHdG formation in mesothelium, spleen, liver, and kidneys. Furthermore, there were significant Fe deposits in the spleen, compared with control animals (Jiang et al., 2008). These findings provide evidence of DNA damage effects of asbestos fibers under in vivo conditions. However, ip or intratracheal instillation is not the normal physiological route of fiber exposure as it bypasses the mucociliary clearance processes that are associated with inhalation exposure and results in a higher exposure level (Mossman et al., 2010, this issue). Thus, inhalation studies are still required to validate the results observed in the current studies.

Asbestos Fibers Interfere Physically With Mitosis

Various asbestos fibers were reported to physically interfere with the mitotic machineries of cells. In order for fibers to interfere physically with mitosis of the target cells, two requirements need to be fulfilled: First, fibers need to be internalized through phagocytosis by the target cell population. Second, the target cell population must be proliferating (dividing) (Kane, 1996).

Direct physical interference is believed to be causal of numerical CA in asbestos fiber-exposed cells. The supportive evidence comes from the findings that (1) most types of cells phagocytize fibers, except lymphocytes, and (2) crocidolite asbestos was observed within mitotic cells, and in some cases directly interacting with the chromosomes (Reinis et al., 1979). Morphological studies, utilizing light, electron, or video-enhanced time-lapse microscopy, demonstrated that most of the internalized crocidolite asbestos fibers were surrounded by a phagolysosomal membrane, preventing direct interaction with chromosomes. However, long fibers were found to be trapped in the keratin microfilament cage protruding into the spindle region of mitotic cells (Ault et al., 1995; Jensen et al., 1994). Although a protruding fiber generally did not interfere with the segregation of chromosomes, it was able to impair chromosomal migration in some cases (Ault et al., 1995). These results indicate that physical interaction of asbestos fibers with the mitotic machinery induces missegregation of chromosomes during mitosis, resulting in aneuploidy. Furthermore, physical interference of asbestos with the chromosomes of dividing cells also produces structural CA. Anaphase aberrations including bridges and sticky and lagging chromosomes as a result of missegregation of chromosomes were detected in various asbestos fiber-treated mammalian cells (Hesterberg & Barrett, 1985; Palekar et al., 1987; Pelin et al., 1992; Yegles et al., 1993; 1995).

Effects of Fiber Characteristics, Fiber Types, and Overload Issue

As mentioned earlier, fiber dimensions, durability, and dose (the “three D’s”) as well as surface properties were shown to be important determinants of the biological activities of fibers (Mossman, 1990; Stanton et al., 1977). Different types of fibers possess varied physical and chemical prosperities; as a result, their pathogenicity, especially carcinogenicity, is different. A recent meta-analysis suggested that the variation in carcinogenic potency across published epidemiology studies, especially for mesothelioma, may be substantially reconciled after adjusting fiber size and mineral type (Berman, 2010).

First, fiber dimension is an important factor that affects the biological activity of asbestos fibers. Compared with the relatively large amount of literature demonstrating fiber dimension to be an important factor in asbestos carcinogenesis, there are only a few reported studies on fiber dimension and mutagenicity. However, given the known knowledge that long and thin fibers produce increased and prolonged release of ROS and RNS from phagocytes due to "frustrated phagocytosis" (repeated failing and trying to engulf fibers by phagocytes), long fibers are likely to be more genotoxic and mutagenic. In addition, there is evidence that long fibers are also more likely to interfere physically with mitosis in dividing cells. Jensen and colleagues (1996) demonstrated in monkey epithelial cells that long asbestos fibers (15 to 55 μm) impaired mitosis by interfering sterically with cytokinesis, disrupting cell division, while short fibers were found to move out of the cleavage zone. Using vertebrate epithelial cells, Cole et al. (1991) observed that internalized crocidolite fibers that were less than 5 μm in length underwent saltatory, jumplike transport, but not fibers over 5 μm in length. Transport of long crocidolite fibers was mediated by cytoplasmic microtubules. In addition, Hart and colleagues (1994) observed that long–thick glass fibers (MvL 475, 8 μm in diameter and >50 μm in length) did not induce abnormalities in the nucleus of CHO cells but provided substrate for cell attachment. In contrast, the long and thin glass fibers (MvL 901, average 1.4 μm in diameter and 22.2 μm in length) tended to pierce through several adjacent cells, while the shorter fibers (MvL 475, average 7 (diameter) × 18 (length) μm) were completely phagocytosed by the cells. Both groups of phagocytable fibers were associated with abnormal (micro- and poly-) nuclei (Hart et al., 1994). Current evidence suggests that respirable fibers with high aspect ratio (the ratio of the length to its diameter) are more potent in inducing chromosomal changes in exposed cells.

It is known that asbestos-associated catalytic Fe is one of the main sources of asbestos fiber-induced ROS production (Aust et al., 2011, this issue). Amphiboles have higher surface Fe content than serpentine. Compared to chrysotile, similar concentrations of amphiboles (e.g., crocidolite and amosite) produce more DNA damage in vitro that is directly proportional to the levels of redox-active Fe mobilized and the production of hydroxyl radical-like species (Kamp et al., 1992; Hardy & Aust, 1995a, 1995b; Shukla, et al., 2003a). Consistently, crocidolite and amosite, but not chrysotile, produced DNA double-strand breaks (DSB) and 8-OHdG formation in target mesothelial cells (Jiang et al., 2008). These results suggested that amphiboles are more mutagenic than serpentine and the difference in mutagenic potential might be accounted by the variance in surface Fe activity. The opposite result observed in some other studies may be due to differences in sample preparation. For example, the preparations of UICC and NIEHS standard asbestos fibers involved extensive grinding, which may alter the physical or chemical characteristics of these fibers. In addition, the characteristics of same type of fibers may vary depending on the sources/deposits (Berman, 2010). For example, low-Fe serpentine fibers from certain deposits may carry metal contaminants. Moreover, the valence state and mobility of Fe are important determinant factors of fiber mutagenic potential (Fubini et al., 2001; Gulumian et al., 1993; Hardy & Aust, 1995a, 1995b). Further investigations are necessary to better understand the physicochemical properties of different types of fibers.

The durability/biopersistence of asbestos fibers is known to contribute to the increased carcinogenicity of amphiboles observed in vivo (Berman & Crump, 2008a, 2008b). However, durability per se probably does not affect the in vitro short-term mutagenicity of different types of fibers. The clearance of both natural and synthetic durable fibers is markedly slower than that of less durable fibers, thus producing repeated damage and mutagenic events as well as prolonged inflammatory responses in exposed human subjects and animals. Consistently, when the clearance mechanism

is impaired, exposure to nondurable carbon black (CB, 52.8 mg/m^3) increased *hprt* mutations in the rat lungs (Driscoll et al., 1996). In contrast, subchronic inhalation of CB at 1.1 mg/m^3, a low dose that does not affect the clearance capability of the lung, did not elicit any detectable adverse lung effects. This may explain why some biodegradable glass fibers have short-term in vitro mutagenicity similar to that of carcinogenic fibers, but there is lack of evidence of these fibers producing carcinogenesis in vivo in humans and animals (Baan & Grosse, 2004; Hesterberg & Hart, 2001; Lipworth et al., 2009; Topinka et al., 2006a).

Lung overload is "a consequence of exposure that results in a retained lung burden of particles that is greater than the steady-state burden predicted from the deposition rates and clearance kinetics of particles inhaled during exposure" (ILSI Risk Science Institute Workshop Participants, 2000; Aust et al., 2011 this issue). Overload may impair macrophage activities and subsequently the clearance of fibers, producing retention of fibers in the body, promoting carcinogenesis, and changing the mechanisms of cellular toxicity (i.e., high dose facilitates necrosis while low dose of exposure induces apoptosis). Overload of low-toxicity particles such as carbon black (CB), titanium dioxide (TiO_2), and coal mine dust were noted to produce lung cancer in rodents, especially rats (Borm et al., 2004; Driscoll et al., 1996), though the relevance for human risk assessment is uncertain (ILSI Risk Science Institute Workshop Participants, 2000). Similarly, studies show that asbestos fibers and combustion particles (i.e., particulate matter, PM) coexposure slows the clearing of asbestos fibers from lungs (Oberdorster, 1995, 2002). There are many factors that affect particle loading, including cumulative effect of multiple exposure, species difference, individual susceptibility, and temporal factors.

It is of concern that fiber concentrations used in many published studies, especially in vitro studies, are much higher than the realistic environmentally relevant concentrations of occupational and environmental exposure in human subjects (Mossman et al., 2011, this issue; Case et al., 2011, this issue). Using the TEM measurements as reference, 1 μg asbestos would approximately correspond to 10^8 fibers (Breysse et al., 1989; Mast et al., 1995). When a concentration of 1 $\mu g/cm^2$ fibers is added to 10^6 cells grown on a 100-mm tissue culture dish (surface area 58.1 cm^2), theoretically, the cellular dose would be about 5800 fibers/cell if the fibers are 100% taken by the cells (in reality it is usually less). Based on this estimation, the concentration would be much higher than the human exposure level in reality, which is estimated to be less than 1 fiber/cell based on the threshold limit value of 0.1 f/cm^3 (ACGIH, 2001). However, Faux and colleagues (2003) demonstrated that overload of cells in culture usually does not occur when the concentration of asbestos fibers used is below 10 $\mu g/cm^2$ fibers. Typically, overload occurs in vivo at 1–3 mg or 200–300 cm^2 fiber surface area/rat lung (Borm et al., 2004). Therefore, in this review the majority of studies that were analyzed were conducted using concentrations below the overload threshold.

Overload was shown to increase the mutagenic yield in CB-exposed animal lungs (Driscoll et al., 1996). It is of interest to know whether overload might change the mutagenic mechanisms of asbestos fibers. On one hand, asbestos fibers produced genotoxicity and cell toxicity in a concentration-dependent manner (Hart et al., 1994). Frequently, asbestos fiber-induced mutagenicity demonstrated a decrease at high concentrations due to cytotoxicity. In contrast, it is not known whether overload might change the spectrum of asbestos fiber-induced mutations, for example, in favor of one specific type of mutation (i.e., chromosomal changes) instead of another (i.e., gene mutations). Importantly, it should be noted that, despite the disadvantage of studies conducted with graded concentrations of fibers that are higher than environmentally relevant occupational and environmental exposure, these studies (1) are necessary for mechanistic investigation of fiber mutagenicity, (2) allow for a shortening of the experimental period, and (3) are important

for establishing a dose-response relationship between fiber-exposure and the induction of mutagenic events.

Enhanced Mutagenic Effects due to Coexposure to Tobacco Smoke

There is evidence that concurrent exposure to both asbestos fibers and cigarette smoke resulted in a DNA-damaging response that is synergistic in nature in both cell-free systems and lung epithelial cells in vitro (Kamp et al., 1992; 1998; Yuan et al., 2004; Testa et al., 2011, this issue). Furthermore, exposure of Sprague-Dawley rats to a combination of 2.5 mg UICC amosite (single dose) and cigarette smoke increased the number of DNA strand breaks in lung epithelial cells 2 and 14 d after exposure (Jung et al., 2000). Benzo[a]pyrene (BaP), a prototype carcinogen found in tobacco smoke, demonstrated significant synergistic mutagenic effects with amosite asbestos (single doses at 1, 2 mg, or multiple doses of 2 mg/dose/wk for 4 wk) in Big Blue rat lungs 4 wk after administration by the same route—intratracheal instillation (Loli et al., 2004). Similar effects were observed with coexposure of BaP and man-made fibers, including rock wool standard fibers RW1 (1700 fibers/μg, median length 23 μm, biological half life 3–4 mo) and glass wool MMVF10 standard fibers (1 μg sample contains 8300 fibers longer than 5 μm, median length 26 μm, weighted half-life 37 d) (Topinka et al., 2006b). The potential mechanisms include that (1) tobacco smoke carcinogens may be adsorbed on the surface of asbestos fibers, thereby increasing the uptake and retention time of these carcinogens, and (2) chemical carcinogens from cigarette smoke or other sources may augment the penetration of target cells by asbestos fibers via oxidative radicals mediated pathway (Churg et al., 1989; Fournier & Pezerat, 1986; Kamp et al., 1992; Nelson & Kelsey, 2002; Nymark et al., 2008). Nevertheless, further studies are needed to better understand the biologic basis for the well-established synergistic interaction between asbestos and tobacco smoke on the incidence of bronchogenic carcinoma.

Nonmutagenic Mechanisms of Asbestos Carcinogenesis

The influence of different types of asbestos fibers in inducing nonmutagenic alterations in relevant target cells and tissues was reviewed previously. Chronic inflammation is a recognized risk factor of human cancer (Federico, et al., 2007, Kundu & Surh, 2008, Weitzman & Gordon, 1990). Frustrated phagocytosis of long asbestos fibers by alveolar macrophages triggers inflammatory responses and the release of ROS and RNS from the cells (Branchaud et al., 1993; Kane, 1996). Reactive radical species further recruit more macrophage and other inflammatory cells to the lung. Thus, persistence of asbestos fibers in the lungs may trigger prolonged radical production and chronic inflammation at the sites of fiber deposition. In addition to inducing direct DNA damage and mutagenesis, these processes also lead to the sustained release of inflammatory mediators and growth factors, expression of transcription factors, and early response proto-oncogenes, which in turn regulate fibrosis and malignant transformation in asbestos-induced pathogenesis.

Asbestos-induced chronic inflammation and reactive radical species were found to activate multiple signaling cascades (Kamp & Weitzman, 1999; Kane, 1996; Manning et al., 2002; Mossman & Churg, 1998; Shukla et al., 2003a; Mossman et al., 2011, this issue). A rapid increase of mitogen-activated protein kinase (MAPK) signaling subsequently activated transcription factors such as activator protein-1 (AP-1) and nuclear transcription factor kappa-B (NFκB) in asbestos-exposed target cells. These transcription factors are known to be involved in the activation of a number of downstream genes that regulate apoptosis, inflammation, and proliferation changes, including cytokines, chemokines, adhesion molecules, growth factors, enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and proto-oncogenes such as c-fos, c-jun, and c-myc. These genes might be associated with asbestos-induced fibrogenesis and carcinogenesis. Asbestos-induced oxidative stress may also potentially regulate genes

involved in other signaling cascades, including DNA repair and cell cycle signaling, such as tumor suppressor gene p53 (Kamp & Weitzman, 1999, Kane, 1996). Recently, ROS-dependent inflammasome (a caspase-activating complex) activation was shown to be an important sensor of asbestos and trigger of pulmonary inflammation in target cells (Cassel et al., 2008; Dostert et al., 2008).

Importantly, asbestos-associated mutagenic and nonmutagenic pathways are interrelated with each other and may coordinate each other in promoting mutagenicity and carcinogenesis. For example, asbestos-triggered mutagenic events in critical genes associated with cell growth, DNA repair, or other functional processes may lead to modification of corresponding signaling pathways. Similarly, chronic inflammation may lead to the accumulation of mutagenic events by increasing proliferation and producing apoptosis-resistant cells. In addition, asbestos-induced biological activities, such as production of ROS and RNS, may induce both mutagenic effects and nonmutagenic signaling alterations in relevant target cells (Kamp & Weitzman, 1999; Kane, 1996; Shukla et al., 2003a).

MUTAGENESIS/CARCINOGENESIS AT LOW DOSE

The mutagenicity of multiple types of asbestos fibers was investigated in a wide range of in vitro cell systems and some in vivo experiments. Answering the question of the effects at low doses leads to the necessity for addressing a threshold issue. Threshold is defined as the lowest dose of a chemical (in this case asbestos fibers) at which a specified measurable effect (in this case mutagenicity/carcinogenicity) is observed and below which an effect is not observed. To address whether there is a threshold for asbestos-induced mutagenicity/carcinogenicity requires the knowledge of statistical dose-response relationships that can only be extracted from studies conducted with multiple doses of fibers. However, few of the current available animal and cell studies provide dose response relationships. That is, most experiments did not focus on the establishment of a threshold value but rather on mechanistic considerations and determination of the dependence of tissue or cell response on fiber characteristics, or the molecular mechanisms accounting for the observed effects. The existing experiments were generally carried out with a limited number of fiber doses. Therefore, determination of a threshold for asbestos fibers is limited by the existing database.

This section aims to summarize literature findings on mutagenicity and carcinogenicity focusing on relations between fiber doses (animal experiments) or fiber concentrations (cell systems) and DNA damage, chromosomal changes, or tumor yield, respectively. Studies are summarized in Tables 1A–1E. Types of assays were quoted as "DNA" (referring to 8-OHdG, DNA breaks, and DNA repair), mutagenesis, "chromo" (chromosomal aberrations, SCE and MN), tumorigenicity in animals (as "Tumor"), and transformation. In this process, several situations were encountered:

1. Animal data dealing with a small number of fiber doses. Statistical analyses were not always provided or possible.
2. In vitro data determining mutagenic effect in cell systems using a small number of fiber concentrations, with or without statistical analyses.
3. Experimental results providing dose-effect relationships. It needs to be noted that the presentation and format of data differ between different publications. Some provide dose-response curves while others provide table or histogram formats. As the shape of the dose-effect curve is of interest for threshold evaluation, in some cases where the curve was not provided, a dose-effect curve was constructed from estimated values reported in the paper.

Despite a rather low number of experiments carried out with a wide range of fiber doses, this review attempts to be as comprehensive

as possible to allow the comparison between different studies in terms of dose and effects. The idea was to determine an experimental dose level (or range of dose levels) in which the authors find/did not find an effect. Consensus reports that summarize and evaluate the relevant literature pertaining to the mutagenicity and carcinogenicity of asbestos fibers are available. The purpose of this review is not to detail each individual study analyzed by these consensus reports. Instead, this review primarily focuses on outlining the low-dose effects seen from these reports.

Ideally, it would have been interesting to determine LOAEL (lowest-observed-adverse-effect level) and NOAEL (no-observed-adverse-effect level). LOAEL is defined as the lowest level at which there are statistically or biologically significant increases in adverse effects in exposed population compared with controls. NOAEL is the highest exposure level at which there is no statistically or biologically significant increase in adverse effects, while comparing exposed population with controls. These values are generally estimated based on animal data with multiple doses. However, currently available asbestos mutagenesis studies were mainly carried out in cell systems with rather few fiber concentrations. Due to the aforementioned limitation of currently available data, some approximations have been employed when determining whether a threshold exists. First, a "surrogate" term is used when "canonical" data are not available. In this case, the LOAEL will be written as sLOAEL (surrogate LOAEL) and the NOAEL as sNOAEL (surrogate NOAEL). Second, when an insufficient number of doses was used or when statistical analyses were not given, lowest or highest doses were quoted as potential lowest-observed-adverse-effect level (pLOAEL) or potential (highest) no-observed-adverse-effect level (pNOAEL).

Animal Data on Tumor Incidence

The existing mutagenicity and carcinogenicity studies in animals used different routes of exposure including inhalation, intratracheal instillation, and intracavitary injection. Table 1D lists the experiments that were carried out in vivo to test the tumorigenicity in animals. Experiments reported by Stanton and colleagues (1981) examined the effects of both asbestos and nonasbestos fibers, after implanting the fibers in the pleural cavity of female Osborne-Mendel rats for more than 1 yr. For all types of fibers tested by these experiments, the probability of pleural tumors correlated best with the number of fibers with a size of less than 0.25 μm in diameter and greater than 8 μm in length, and correlated well with the number of fibers <1.5 μm in diameter and >4 μm in length. These data show that fiber dimensions play an important role in the incidence of pleural tumors. Nevertheless, data suggest that other factors are likely to play a role, since tumors are sometimes found in the absence of fibers of the indicated size. This observation is confirmed by post-analyses of the original data done by other groups (Bonneau et al., 1986; Wylie et al., 1987). Injection studies were also reviewed in a U.S. Environmental Protection Agency (EPA) report, and it was concluded that "reasonable dose-response curves have been generated using various sample masses of a single material in some of these studies" (Berman & Crump, 2003). In these studies, dose-response curves were demonstrated for UICC crocidolite and chrysotile "A" (Bolton et al., 1984). These results indicate that the relationship between tumor incidence and log(dose) may be linear and there is no effective threshold (Berman & Crump, 2003).

One study examined the relationship between the injected dose and the development of peritoneal mesothelioma in rats using the UICC standard reference samples of chrysotile, crocidolite, and amosite (Davis et al., 1991). Doses injected into the peritoneal cavity ranged from 0.005 to 25 mg (10 doses), thereby allowing the generation of a dose-response curve that was found to be logarithmic. Results demonstrated that "at the lower end of the dose range, chrysotile and amosite still produced a small proportion of tumors although crocidolite did not." In these

experiments, the NOAEL is estimated to be between 0.001 and 0.05 mg depending on the fiber type (Table 1D). The fiber mass was converted into number of fibers of different dimensions in order to evaluate (1) whether mass dose is the most appropriate dose parameter and (2) the importance of fiber dimensions. There were about 8×10^6–10^8 fibers in total, among them 3×10^4–6×10^5 fibers with a size of >5 μm in length and <0.25 μm in diameter, and 1.5–9 $\times 10^5$ fibers that are greater than 8 μm in length. Moreover, time to death from mesothelioma was analyzed using a standard hazard model. When dose was expressed as the number of long fibers injected (>8 μm in length), the hazard slopes for chrysotile, crocidolite, and amosite were relatively close to each other (Davis et al., 1991). This study estimated relative hazard between fibers but not absolute hazard. Nevertheless, data suggested that a sample containing far fewer than 1.5×10^5 "Stanton" fibers (>8 μm in length) rarely produces mesothelioma in rats. Other data from these experiments showed that the latent period for tumor induction increased as the dose decreased. These results suggest that a threshold exists, since at low doses the tumor did not develop within the animal lifetime. However, this finding does not imply that similar dose-relationship would be found in other species.

Berman et al. (1995) reviewed inhalation studies conducted with AF/HAN rats exposed to different types of asbestos fibers, and determined which exposure parameter(s) could be used to predict the observed lung tumor or mesothelioma incidence. A series of animal studies all employing a common inhalation protocol was conducted (Berman et al., 1995; Davis et al., 1978, 1980, 1985, 1986; Davis & Jones, 1988). Consistent with the limitations discussed in inoculation studies, no satisfying univariate measure of exposure was found, although the length parameter (>20 μm in length) was highly correlated with tumor incidence. In addition, the analysis defined an "optimum exposure index" attributing a weight to different classes of dimensions (Berman et al., 1995). No consistent dose response was noted between aerosol mass concentration of fibers or total number of fibers per cubic centimeter and lung tumor incidence, but a consistent dose-response relationship was identified between weighted airborne fiber concentration and lung tumor incidence. This relationship did not show a threshold.

Lippmann (1994) also reviewed published animal data on chronic inhalation studies in rats. Data were examined to determine dose-response relationships for lung cancer and mesothelioma according to fiber types and dimensions. Dose-response relationships for lung cancer, or both lung cancer and mesothelioma, did not show a threshold limit. A polynomial relationship of degree 2 was found for lung cancer, and the correlation coefficient was the best for fibers >20 μm in length.

Overall, limited animal data from chronic inhalation studies did not show a threshold limit for lung cancer and mesothelioma, according to the fiber types and dimensions tested (Lippmann, 1994). Intraperitoneal injection with a dose as low as 50 μg produced a significant increase in the incidence of mesotheliomas after exposure (Davis et al., 1991). It seems that the effective dose is different for mesothelioma and lung cancer induction, and for different types of fibers (i.e., chrysotile vs. crocidolite). Substantial efforts from future in vivo studies need to clarify this uncertainty.

Animal Data Mutagenicity and DNA Damage

Several in vivo mutagenic assays were conducted in animal studies as previously described (Kociok et al., 1999; Rihn et al., 2000; Topinka et al., 2004; Unfried et al., 2002) (Table 1C). In addition, several studies examined fiber-induced oxidative DNA damage or DNA strand breaks in vivo (Jung et al., 2000; Unfried et al., 2002; Schürkes et al., 2004; Jiang et al., 2008) (Table 1A and earlier discussion). Rihn and coworkers (2000) administered the fibers through inhalation at a daily dose of 5.75 mg/m^3, 6 h/d for 5 consecutive days, while in the rest of the studies, animals were

exposed to fibers through intratracheal instillation or ip inoculation, at either one or two of the single doses of 1, 2, 2.5, 5, or 10 mg, or a weekly dose of 2 mg for 4 wk, or both (Unfried et al., 2002; Schürkes et al., 2004; Jiang et al., 2008). It is noteworthy that time of exposure is also an important factor that affects the DNA damage or mutation yields. Although most of the studies detected significant effects at wk 10 to 24 after exposure but not earlier time points (i.e., 4 wk) (Kociok et al., 1999; Schürkes et al., 2004; Topinka et al., 2004; Unfried et al., 2002), in other studies asbestos fiber significantly increased DNA damage or mutation events after 4 wk or 14 d of exposure (Jiang et al., 2008; Jung et al., 2000; Rihn et al., 2000). In addition, the mutagenic effects of fibers did not always show a time-dependent increase. These results indicate that for DNA damage and mutagenic endpoints, the establishment of NOAEL is difficult because multiple time points need to be tested. Furthermore, only a limited number of doses was used in each of these studies. Therefore, only LOAEL, but not NOAEL, may be extracted from these data.

According to these few in vivo studies conducted using a limited dose range (1–10 mg single dose or repeated doses of 5.73 mg/m^3 crocidolite fibers), it seems that DNA damage and mutagenicity were observed with doses lower than those used in tumorigenetic assays. Both DNA damage and mutations were induced by single doses of 1 or 2 mg fibers following intratracheal instillation and ip inoculation, respectively (Kociok et al., 1999; Topinka et al., 2004; Unfried et al., 2002). Some studies detected no induction of mutation in animal lungs at the lowest dose tested (Kociok et al., 1999; Topinka et al., 2004). However, these negative results might due to the detection limit of the assay or the relatively short exposure periods. Overall, additional in vivo studies need to be conducted to test a sufficient number of low-dose groups.

Data Obtained With Cells in Culture

In vitro mutagenic studies provide important information that are relevant to carcinogenic potency of fibers by studying cancer-related cellular phenotypes and the step-wise processes involved in the mechanism of fiber carcinogenesis. Studies are summarized in Tables 1A–1E. Few studies were carried out using multiple fiber concentrations, rendering the establishment of dose-response relationship difficult, if not impossible. However, if dose responses are available, the curve is generally close to a linear or log shape when plotted on a linear scale. These data suggest that no threshold would be expected for asbestos-induced DNA damage and mutagenic effects in cultured cells, at least based on the current knowledge. In the following, highlights of the in vitro data cited in Tables 1A–1E, with an emphasis on doses and shape of dose-response curves, are provided.

Effects on DNA (as Assessed by Base Oxidation, Breakage) Studies reporting both LOAEL and NOAEL were not found. Based on the statistics from an in vitro study, a concentration of 0.25 μg/cm^2 enhanced DNA damage in comparison with untreated cells (Kamp et al., 1995). Meta-analysis on data from several publications identified effects on DNA with a dose of 2 μg/cm^2. When available, dose-response curves are mostly of a linear or approximate log shape. There are no sufficient in vivo data for comparison.

Mutagenesis (Bacteria, HGPRT on Eukaryotes, etc.)

Bacteria While early studies did not report mutagenicity in bacteria exposed to asbestos fibers (Chamberlain & Tarmy, 1977), more recent studies demonstrated an enhancement of revertants in the Ames test with crocidolite and chrysotile, or chrysotile only or with richterite (Cleveland 1984; Faux et al., 1994a; Howden & Faux, 1996a). Dose response was not investigated in any of the studies.

Eukaryotes In one study, both LOAEL (4.5 μg/cm^2) and NOAEL (3.0 μg/cm^2) were found for NIEHS crocidolite (Park & Aust, 1998). Other studies observed significant mutagenic effects with fiber concentrations higher than those produced DNA effects in exposed cells (Both et al., 1994; Hei et al., 1995; Huang et al., 1978; Huang, 1979; Okayasu et al.,

1999b; Reiss et al., 1983). A suggested shape of dose-response curves cannot be extracted from the currently available data.

Chromosomal Damage (Micronucleus, Aneuploidy) Significant enhancement of chromosome damage appears to be observed with concentrations of 0.5–1 $\mu g/cm^2$ for fibers examined (chrysotile, crocidolite or amosite, various sources). Concentration-response relationships are too few to suggest a shape. In addition, a trend for a threshold limit was not evident.

Transformation Although the statistical significance is not known, enhancement of cell transformation was found with low concentrations of asbestos fibers (1–5 $\mu g/cm^2$) (Hesterberg & Barrett, 1984; Lu et al., 1988; Mikalsen et al., 1988). These are similar to the concentration ranges at which DNA and chromosome damage and mutagenicity are observed.

The evidence from the in vitro studies just discussed suggests that there is no threshold concentration for asbestos-induced mutagenic effects. However, the determination of LOAEL and NOAEL as well as the shape of dose-response curves requires a sufficient number of low concentrations to be tested. This is generally not done in existing studies. This evidence, together with the fact that few in vivo mutagenic data are available, suggests that the mutagenic effect of asbestos fibers at low concentrations is still unknown and more studies are required to elucidate this uncertainty.

Summary It was demonstrated that the biological activity of fibers is dependent, at least partially, on fiber characteristics such as dimension. The parameters used to quantify fiber exposure usually are on a mass basis, rendering comparisons between different experiments difficult. Further studies need to consider both the mass and number/surface area of fibers while characterizing asbestos exposure. The mutagenic and carcinogenic effects of asbestos fibers at low doses are still not known. The concept of a threshold dose below which there is no mutagenic and carcinogenic responses upon fiber exposure is still controversial. A better knowledge of the dose-response relationship would provide a better understanding of the mechanisms underlying mutagenesis and cell transformation by asbestos. To carry out dose-response studies, it is necessary to use a wide range of doses of well characterized asbestos samples. Suggested dose range may be extracted from the numerous currently available data. Testing systems already established, such as in vitro chromosome mutations and mitosis impairment assay, in vivo mutagenesis, and large-scale genomic analyses, may be adopted to develop new mutagenic assays based on cellular responses to DNA/chromosome damage in fiber-exposed cells and tissues.

BIOLOGICAL ACTIVITIES THAT CONTRIBUTE TO MUTAGENICITY AND IMPACT OF TARGET CELL/TISSUE TYPE

Considerable in vitro and in vivo evidence show that asbestos fibers act as carcinogens on all the major pulmonary and pleural target cells (e.g., bronchial/alveolar epithelial and mesothelial cells) (Committee on Asbestos: Selected Health Effects, 2006). The implicated mechanisms underlying asbestos-induced carcinogenesis that are firmly established involve mutagenic and nonmutagenic (i.e., inflammation, mitogenesis, cell signaling alterations, and cytotoxic apoptosis/necrosis) pathways. Neither of these two mechanisms fully accounts for the complex biological abnormalities produced by asbestos fibers. Asbestos-induced pathophysiological responses are not completely independent of, and may be interrelated with, each other. Asbestos exerts pleiotropic cellular and tissue-type specific effects and nonmonotonic dose response at the cellular and molecular levels following exposure to physiologically relevant concentrations. Although the mechanisms underlying asbestos-induced mutagenicity are well established, this section further addresses this issue from the aspects relevant to asbestos-induced biological activities, tissue specificities, and the interrelations between nonmutagenic and mutagenic responses. The five plausible biological/pathological responses/activities

that may contribute to asbestos mutagenicity and carcinogenicity in relevant target cells, directly or indirectly, are: (1) ROS and RNS production, (2) DNA and chromosomal damage, (3) apoptosis, (4) p53 expression, and (5) inflammatory responses. The substantial in vitro and in vivo evidence are highlighted in this section.

ROS and RNS

ROS ROS including $O_2^{\cdot -}$, $^{\cdot}OH$, and H_2O_2, are important second messengers of asbestos toxicity that promote malignant transformation induced by multiple types of asbestos (Hardy & Aust, 1995b; Kamp et al., 1992; Shukla et al., 2003a; Aust et al., 2011, this issue). As recently reviewed by Liu et al. (2010), there are at least three mechanisms by which asbestos generates ROS that include the following: (1) reactions at the surface of mineral dusts, (2) activation of alveolar macrophages (AM) or polymorphonuclear neutrophils (PMN), inflammatory cells in the distal lung responsible for clearing the fibers, and (3) lung target cell mitochondrial dysfunction (e.g., lung epithelial cells). In cell-free systems, electron spin resonance (ESR) spectroscopy studies directly demonstrated that asbestos induces the production of ROS (Kamp et al., 1992; Weitzman & Graceffa, 1984). Following asbestos inhalation into the lung, asbestos bodies are formed as the fibers are coated with mucopolysaccharides and Fe on its surface, the latter of which is redox-active, can cycle between the oxidized and reduced forms, and triggers DNA damage (Hardy & Aust, 1995b; Kamp et al., 1992; Shukla et al., 2003a). Consistent with these observations suggesting that asbestos alters lung Fe homeostasis, increased levels of bronchoalveolar lavage fluid (BALF) Fe, transferrin, transferrin receptors, lactoferrin, and ferritin were detected in asbestos-exposed individuals (Ghio et al., 2008). Further, several lines of evidence suggest a crucial role for Fe-derived free radicals in mediating asbestos toxicity. First, several investigators showed that Fe chelators inhibit asbestos-induced $\cdot OH$ formation in cell-free systems as well as in relevant lung target cells (Kamp et al., 1992; Hardy & Aust, 1995b; Shukla et al., 2003a; Ghio et al., 2008). Second, asbestos promotes antioxidant enzyme (AOE) expression in lung epithelial and mesothelial cells, while exogenously administered AOE reduces asbestos-induced ROS production (Hardy & Aust, 1995b; Kamp et al., 1992; Shukla et al., 2003a). Finally, there are considerable in vivo data demonstrating that asbestos triggers ROS production—for example, a single intratracheal instillation of Fe-loaded chrysotile asbestos induces $\cdot OH$ in rat lungs as assessed by electron spin resonance (ESR) spectroscopy (Schapira et al., 1994). As reviewed in detail elsewhere, there is some in vivo evidence that Fe chelators and AOE may attenuate asbestos-induced mesothelial and epithelial cell toxicity. Collectively, these data demonstrate that asbestos augments ROS production in all the relevant lung target tissues and cells. Phagocytosis of fibers is often associated with extracellular oxyradical production from the target cells through activation of the membrane-associated enzyme complex NADPH (nicotinamide adenine dinucleotide phosphate) oxidases. Asbestos-induced ROS production is not simply the result of "frustrated phagocytosis" since, as noted earlier, asbestos induces cellular enzymes involved in ROS production and activates the mitochondria of target cells.

RNS Asbestos also induces the production of nitric oxide ($^{\cdot}NO$) and peroxynitrite ($ONOO^-$), which may be important in carcinogenesis (Thomas et al., 1994; Xu et al., 2007a). Inhibitors of nitric oxide synthase (NOS) prevent asbestos-induced formation of DNA adducts, such as 8-OHdG, in human A549 lung epithelial cells (Chao et al., 1996). A significant induction in iNOS protein expression and the formation of nitrotyrosine, a marker of $ONOO^-$ formation, in mesothelial cells was demonstrated after exposure to 4 $\mu g/cm^2$ NIEHS chrysotile and crocidolite fibers for 36 h, while nonfibrous carbonyl Fe sphere did not show any marked effect (Choe et al., 1998). NIEHS crocidolite asbestos, but not cristobalite silica, induced transactivation of iNOS in murine macrophage-like cells in vitro at all concentrations (2.5–10 $\mu g/cm^2$) 24 h after exposure (Quinlan et al., 1998).

There are also in vivo data implicating that asbestos induces RNS. For example, Quinlan et al. (1998) observed steady-state production of nitrites/nitrates by AM from rats 3 d after inhalation of 7–10 mg/m^3 NIEHS chrysotile and crocidolite fibers. In rats, ·NO level and NOS activity in AM of the lungs are increased following intratracheal instillation of UICC chrysotile asbestos (Iguchi et al., 1996). One week after inhalation of approximately 6.88 mg/m^3 fibers for 2 wk, NIEHS crocidolite fibers were found to translocate to the pleural space of the rat and augment generation of ROS and RNS that occurred in association with persistent pleural inflammation and cytokine production (Choe et al., 1997). Both NIEHS chrysotile and crocidolite fibers triggered NOS expression in macrophages by activating an Fe-sensitive transcription factor in rats exposed intermittently over 2 wk to 6.88–9.24 mg/m^3 fibers (Tanaka et al., 1998). Further, strong immunoreactivity for nitrotyrosine was detected in lungs of these rats at the alveolar duct bifurcations and within the bronchiolar epithelium (Tanaka et al., 1998). Thus, these findings firmly establish the presence of RNS following asbestos exposure (Zhu et al., 1998).

Overall, asbestos fibers may generate ROS and RNS production through several mechanisms: (1) asbestos-associated surface Fe-catalyzed ROS production, (2) release of ROS from phagocytes such as macrophages upon the activation of these cells by asbestos, (3) production of ROS from damaged/interrupted mitochondrial respiratory chain in target cells, and (4) induction of iNOS by asbestos and asbestos-induced oxidative stress (Kamp et al., 1992; Kamp & Weitzman, 1999; Kane, 1996; Panduri et al., 2004; Shukla et al., 2003b; Toyokuni, 2009). The in vitro and in vivo data addressing the causal role of ROS and RNS in asbestos-induced DNA/chromosomal damage and mutagenesis in target cells/tissues was previously summarized. The interrelations between ROS and RNS and other asbestos-induced biological activities (i.e., apoptosis, P53 expression and inflammation) are analyzed below, if available.

DNA and Chromosomal Damage

DNA and chromosomal damage is an early event in asbestos-exposed cells that may contribute to the malignant potential of fibers (Kamp et al., 1992; Hardy & Aust, 1995b; Shukla et al., 2003a). Over the last 20 yr, accumulating evidence have convincingly established that all types of asbestos induce DNA and chromosomal damage in all the relevant lung and pleural target cells as assessed using a variety of techniques. These effects occur in an asbestos concentration-dependent manner ranging from 0.05 to 250 $\mu g/cm^2$ (Jaurand, 1997; Upadhyay & Kamp, 2003). Compared to alveolar epithelial and mesothelial cells, bronchial epithelial cells have less DNA damage following asbestos exposure, which was attributed to their greater antioxidant capacity (Gadbois & Lehnert 1997; Nygren et al., 2004; Puhakka et al., 2002). Similarly, in cultured alveolar type 1 (WI-26 and primary isolated rat alveolar epithelial cells)- and type 2 (A549)-like epithelial cell lines, there is a suggestion that alveolar type 1 cells, which account for >90% of the lung surface area, are more susceptible to amosite asbestos (5–250 $\mu g/cm^2$) and cigarette smoke-induced DNA damage and subsequent poly-ADP-ribose polymerase (PARP) activation, an enzyme involved in DNA repair and apoptosis (Kamp et al., 1998, 2001).

As previously described, asbestos-induced DNA and chromosomal damage may be mediated through various mechanisms including asbestos-induced ROS and RNS, physical interference of asbestos fibers with mitosis, synergistic effect between asbestos and cigarette smoke, and altered cellular growth and death signaling pathways. Although fiber-induced DNA and chromosomal damages are frequently associated with cellular toxicity, there are occasions whereby non-critically damaged cells bypass DNA repair pathways, or apoptotic or other cellular death pathways, and successfully passing on the damaged genome to daughter cells. These lead to multiple mutagenic effects including point mutations, large deletion at gene and chromosomal levels, and loss of heterozygosity in various target cells

(Gulumian, 2005; Jaurand, 1997; Kamp et al., 1992; Kamp & Weitzman, 1999; Upadhyay & Kamp, 2003).

It should be noted that although the evidence from many studies indicates that ROS is one of the major causes of asbestos-induced DNA and chromosomal damage, there are no animal models or human studies showing that blocking any one of these effects is beneficial against mutagenesis. Strategies blocking site-specific ·OH and $ONOO^-$ production or targeting the various sources of ROS/RNS production may be important but need to be carefully considered. For example, the finding from large clinical trials (e.g., ATBC and CARET) that beta-carotene paradoxically increases the incidence of lung cancer in smoking asbestos workers, may be explained by beta-carotene acting as an antioxidant at low concentrations but as a prooxidant at high concentrations or in the setting of chronic oxidative stress (Palozza 2005).

Apoptosis Apoptosis (programmed cell death) is an important mechanism by which cells with DNA damage are eliminated without eliciting an inflammatory response (Shukla et al., 2003a; Upadhyay & Kamp, 2003; Kroemer et al., 2007; Kim et al., 2008; Franco et al., 2009). Apoptotic cells are characterized by nuclear chromatid condensation, endonuclease activation resulting in DNA fragmentation, translocation of phosphotidylserine to the outer plasma membrane, and generation of double-stranded DNA breaks. The two major pathways regulating apoptosis include the (1) mitochondrial (intrinsic) pathway and (2) death receptor (extrinsic) pathway. The mitochondrial death pathway mediates apoptosis induced by DNA damage. The Bcl-2 family of anti-apoptotic (e.g., Bcl-2, Bcl-xl, etc.) and pro-apoptotic proteins (e.g., Bax/Bak and BH3 only-like proteins) localize to the mitochondria and endoplasmic reticulum where they regulate intrinsic cell death. Diverse apoptotic stimuli, including asbestos fibers, trigger cytochrome *c* release from the mitochondria that binds apoptosis activating factor 1 (APAF-1) and caspase-9 to activate caspase-3 (Franco et al., 2009; Kim et al., 2008; Kroemer et al., 2007; Shukla et al., 2003a; Upadhyay & Kamp, 2003). Cytochrome *c* release results from a collapse in the mitochondrial membrane potential ($\Delta\psi_m$) generated by a H^+ gradient produced by the electron transport chain (ETC).

It was established that asbestos triggers apoptosis in all relevant lung target cells (Franco et al., 2009; Shukla et al., 2003a; Upadhyay & Kamp, 2003). Apoptosis occurs in an asbestos concentration-dependent manner (1.25–100 $\mu g/cm^2$) as detected by a variety of assays including the DNA-specific labeling and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining nuclear morphology, electron microscopy, DNA laddering, annexin V binding, caspase-3 activation, and a DNA fragmentation enzyme-linked immunosorbent assay (ELISA) (Aljandali et al., 2001; BeruBe et al., 1996; Broaddus et al., 1996; Buder-Hoffmann et al., 2009; Burmeister et al., 2004; Davis et al., 1997; Dopp et al., 1995a; Goldberg et al., 1997; Hamilton et al., 1996; Jimenez et al., 1997; Kido et al., 2008; Levresse et al., 2000; Marchi et al., 2000; Narasimhan et al., 1998; Panduri et al., 2003, 2004, 2006, 2009; Shukla et al., 2003b, 2003c; Stern et al., 2006; Yuan et al., 2004). In general, a relatively low dose of asbestos exposure (<1 $\mu g/cm^2$) results in proliferative signaling in lung epithelial and mesothelial cells, while higher asbestos doses result in apoptosis and proliferation blockade (Shukla et al., 2003a; Yuan et al., 2004). It is noteworthy that asbestos-induced necrosis (not addressed in this document), another form of cell death, usually occurs at high cytotoxic doses that put the cells under extreme physicochemical stress (Festjens et al., 2006). Compared to primary cultured mesothelial cells, malignant mesothelioma cell lines are resistant to apoptosis (Goldberg et al., 1997). Several studies implicated a role for Fe-derived ROS based on the protective effect of Fe chelators and free radical scavengers on asbestos-induced apoptosis (Aljandali et al., 2001; BeruBe et al., 1996; Broaddus et al., 1996; Hamilton et al., 1996; Jimenez et al., 1997). Furthermore, in vitro studies established that asbestos activated

mitochondria-regulated apoptosis in cultured alveolar epithelial cells (AEC) and mesothelial cells based upon the following findings: (1) Preventing activation and mitochondrial translocation of protein kinase C δ (PKCδ) is protective, (2) lung epithelial cells that are incapable of producing mitochondrial ROS (ρ^0 A549 cells) are resistant to asbestos-induced intrinsic apoptosis, and (3) overexpression of mitochondria-targeted 8-oxoG-DNA glycosylase (a DNA repair protein involved in base excision repair) attenuates asbestos-induced intrinsic apoptosis (Aljandali et al., 2001; Buder-Hoffmann et al., 2009; Kido et al., 2008; Panduri et al., 2003, 2004, 2006, 2009; Shukla et al., 2003b, 2003c; Stern et al., 2006). These in vitro studies are corroborated by in vivo evidence showing that asbestos (amosite, crocidolite, and chrysotile) induces apoptosis in cells at the bronchoalveolar duct junctions and AEC as assessed by TUNEL staining and TEM as well as in mesothelial cells as assessed by morphologic change in cytokeratin (BeruBe et al., 1996; Both et al., 1995; Jung et al., 2000; Marchi et al., 2000; Panduri et al., 2006; Stern et al., 2006). These findings suggest various types of asbestos induce apoptosis in all the relevant lung and pleural target cells.

Because the link between asbestos-induced apoptosis and mutagenesis is not completely known, cell and tissue type specific effects following exposure to different asbestos fiber types are lacking. Excess apoptosis probably has been involved in promoting excessive cell death, pulmonary injury and fibrosis in asbestos-exposed lungs. Studies showed that asbestos fibers (1–25 $\mu g/cm^2$) induced concentration-dependent apoptosis (up to over 30%) in lung epithelial cells and rat, rabbit, or human pleural mesothelial cells. This phenomenon was not observed after exposure of cells to a number of nonelongated particles at relatively high concentrations (10 or 25 $\mu g/cm^2$) (BeruBe et al., 1996; Broaddus et al., 1996; Kamp et al., 2002). However, failure of apoptosis of cells with unrepaired DNA and chromosomal damage after chronic exposure to asbestos may lead to permanent genetic alterations and trigger the development of a clone of cancerous cells, thus contributing to asbestos-induced mutagenicity and carcinogenicity (Thompson, 1995). Consistently, malignant mesothelioma cells are found to be apoptosis resistant (Fennell & Rudd, 2004; Narasimhan et al., 1998). Studies are underway exploring the underlying molecular mechanisms. Aung and colleagues (2007) showed that asbestos induces ferritin heavy chain (FHC) expression in human mesothelial cells and malignant mesothelioma (MM) cell lines. Notably, FHC acted as an anti-apoptotic protein against oxidative stress since silencing FHC using small interfering RNA promotes apoptosis in MM cells (Aung et al., 2007). In addition, taurolidine preferentially augmented apoptosis in mesothelioma cells but not in nonneoplastic human mesothelial cells by mechanisms involving an inhibition of oxidative stress and AKT signaling (Aceto et al., 2009). Altered apoptosis in immune cells may also be important. For example, a T-cell line rendered resistant to 10 $\mu g/ml$ chrysotile B-induced apoptosis following 8 mo of exposure was characterized by increased Bcl-2 expression, excess IL-10 expression, and STAT3 activation, all of which could be blocked to trigger apoptosis (Miura et al., 2006). One group showed that asbestos-induced intrinsic apoptosis in lung epithelial cells was blocked by overexpression of mitochondrial aconitase, a key enzyme in the Kreb's cycle implicated in preserving mitochondrial DNA (mtDNA) (Panduri et al., 2009). Furthermore, overexpression of mitochondria-targeted hOgg1 (define), which primarily functions to preserve mitochondrial aconitase, prevented asbestos- and H_2O_2-induced AEC mitochondrial dysfunction and intrinsic apoptosis (Panduri et al., 2009). Numerous groups are targeting a wide range of molecular pathways to overcome the resistance of malignant mesothelioma to apoptosis (Villanova et al., 2008). Additional studies are warranted to better understand the molecular mechanisms underlying asbestos-induced apoptosis, how apoptosis resistant cells are formed during mutagenesis, and whether there are unique molecular apoptotic signatures in the various lung target cells. Further, it will be important to determine the translational significance of these findings

in animal models and in humans exposed to asbestos.

p53 Altered p53 expression is also implicated in the pathophysiology of asbestos-associated malignancies. p53 is a transcriptional factor and a critical DNA damage response molecule that affects numerous genes involved in cell cycle arrest and apoptosis (when DNA damage is extensive) in part by the intrinsic death pathway (Janicke et al., 2008; Vousden & Prives, 2009). A normal functioning p53 response after exposure to DNA damaging agents prevents mutations from accumulating. Not surprisingly, the most common mutations in human tumors involve the p53 gene family members (Janicke et al., 2008; Vousden & Prives, 2009). Mutations in the p53 gene in lung cancer were associated with asbestos exposure (Liu et al., 1998; Nymark et al., 2008; Wang et al., 1995), while opposite results were also reported (Husgafvel-Pursiainen et al., 1999). Although the association between p53 mutation and asbestos exposure in lung cancer patients has been controversial, it is established that asbestos induces p53 expression in lung epithelial and mesothelial cells that results in cell cycle arrest and intrinsic apoptosis (Burmeister et al., 2004; Johnson & Jaramillo, 1997; Kopnin et al., 2004; Levresse et al., 1997; Matsuoka et al., 2003; Paakko et al., 1998; Panduri et al., 2006). $p21^{CIP1/WAF1/SD11}$ (p21) is a family of proteins (p21, p27 and p57) downstream of p53 expressed during a p53 genotoxic stress response following exposure to DNA damaging agents including asbestos, resulting in cell cycle arrest to allow time for DNA repair (Johnson et al., 1997; Levresse et al., 1997; Tuder et al., 2008). Activation of p53-dependent transcription is crucial for triggering asbestos-induced AEC mitochondria-regulated apoptosis (Panduri et al., 2006; Topinka et al., 2004). The levels of p53 accumulation in the respiratory epithelium of lung cancer patients with asbestosis and p53 point mutations are widely evident (Husgafvel-Pursiainen et al., 1997; Nuorva et al., 1994; Panduri et al., 2006; Topinka et al., 2004). Crocidolite asbestos (0.2–20 $\mu g/cm^2$) promotes p53 gene mutations predominantly in exons 9–11 in BALB/c-3T3 cells in a non-concentration-dependent manner (Lin et al., 2000). Furthermore, there are in vivo studies showing that a single dose of 5-h aerosol chrysotile asbestos exposure induces p53 expression in vivo in lung epithelial cells at the bronchiolar–alveolar duct junctions where fibers are initially deposited (Mishra et al., 1997). Finally, gene expression microarray studies confirmed a prominent role for p53 expression in mesothelial and lung epithelial cells (Hevel et al., 2008; Nymark et al., 2007). Collectively, these data indicate that asbestos fibers modify the p53 signaling pathway in exposed lung epithelium and mesothelium.

Despite the prominent role of p53 in mediating asbestos-induced mitochondrial dysfunction and intrinsic apoptosis in AEC, studies targeting p53 in order to modulate asbestos-induced toxicity show conflicting findings. For example, lung cancers and mesotheliomas that occur following asbestos exposure develop more rapidly in mice with compromised p53 function (Vaslet et al., 2002; Morris et al., 2004; Yee et al., 2008). Asbestos-induced p53 expression in the lung epithelium is blocked by phytic acid, an Fe chelator that inhibits asbestos-induced pulmonary fibrosis in vivo (Mishra et al., 1997; Panduri et al., 2006; Xu et al., 1999). However, asbestos-induced pulmonary fibrosis is increased in mice expressing a dominant-negative mutant form of p53, compared with control mice expressing wild-type p53 (Nelson et al., 2001). The explanation for these seemingly disparate findings requires further study but may in part be due to the diverse life and death functions of p53, and the dynamic expression of p53 over time. For example, mitochondrial-localized p53 enhances the accuracy of mtDNA synthesis, suggesting that p53 serves as a guardian of the mitochondrial genome (Bakhanashvili et al., 2008). In the absence of functional p53 proteins, it is likely that the resultant increase in genomic instability due to impaired control of DNA damage and cell cycle arrest may promote tumor yield. Thus, additional studies are necessary to better define the precise role of p53 in all the relevant lung and pleural target

cells following asbestos exposure. It is noteworthy that the change in expression of other genes that might be implicated in the pathogenesis of asbestos-induced carcinogenicity is previously reviewed.

Inflammation

Biopersistent asbestos fibers trigger chronic inflammatory responses both in animal models and in the lungs of patients with asbestos-related pulmonary diseases (Rom et al., 1991; Mossman & Churg, 1998; Kamp & Weitzman, 1999; Nymark et al., 2008). Asbestos-induced lung inflammation can promote secondary mutagenicity by releasing ROS/RNS from activated AM, lung epithelial cells, and mesothelial cells (Kamp & Weitzman, 1999; Mossman & Churg, 1998; Nymark et al., 2008; Rom et al., 1991). Kamp et al. (1992) demonstrated all forms of asbestos activate animal and human PMN and AM to release ROS. Activated lung target cells attempting to clear the fibers from the lung, especially AM and lung epithelial cells, produce cytokines, chemokines, proteases, and growth factors that promote cellular proliferation and tissue repair but, if persistently activated, lead to fibrosis and mutagenesis (Rom et al., 1991; Mossman & Churg, 1998; Kamp & Weitzman, 1999; Nymark et al., 2008). A murine laser capture microdissection study documented that 10 mg/m^3 chrysotile asbestos induced gene expression (tumor necrosis factor [TNF]-α, transforming growth factor [TGF]-β, and others) at the bronchiolar–alveolar duct junctions; this location is precisely where fiber deposition is the greatest in animals exposed for 2 or 3 d (Yin et al., 2007). TNF-α is prominently implicated in the direct association between inflammation and malignancy because it is crucial for mediating tumor promotion and cell transformation in various model systems (Balkwill, 2006; Suganuma et al., 1999). In human mesothelial cells, TNF-α prevented asbestos-induced cell death via a nuclear factor (NF)-κB-dependent mechanism despite the presence of cytogenetic abnormalities (Yang et al., 2006). It was established that asbestos-induced expression of TNF-α and other pro-inflammatory cytokines is mediated through MAPK pathways. For example, evidence shows that in human monocytes, increased TNF-α gene expression in patients with asbestosis is due to constitutively activated p38 and decreased phosphorylated extracellular signal-regulated kinase (ERK) when compared to normal subjects (Tephly & Carter, 2008). Asbestos-induced MAPK pathway members are important second messengers crucial for mediating cell surface signals to the nucleus (Davis et al., 1997; Jimenez et al., 1997). These studies underscore the importance of inflammation and, in particular, the role of MAPK, TNF-α, and NF-κB-dependent signaling pathways in the pathogenesis of asbestos-induced carcinogenesis.

Another recently described mechanism by which carcinogenic mineral fibers such as asbestos, but not inert particles, activate pulmonary inflammation is via ROS-mediated Nalp3 inflammasome sensing (Cassel et al., 2008; Dostert et al., 2008). Inflammasome, a complex of proteins that possess distinct roles in the innate defense system, is contained in macrophages and neutrophils and considered as cellular sensors of danger signals (Drenth & van der Meer, 2006; Tschopp et al., 2003). Nalp3 stands for Nacht domain-, leucine-rich repeat-, and pyrin domain (PYD)-containing protein 3. It is a member of the NLR (NOD-like receptor) family of more than 20 proteins that contain a caspase activation and recruitment domain (CARD) and activates caspase-1 in response to diverse stimuli (Petrilli et al., 2007). The Nalp3 inflammasome is formed when Nalp3 activation recruits the inflammasome adaptor apoptosis-associated speck-like protein containing a CARD (ASC) and then caspase-1 by CARD–CARD interactions. After exposure to 40 $\mu g/cm^2$ or 0.0.5–0.2 mg/ml chrysotile asbestos for 6 h, lipopolysaccharide (LPS)-primed macrophages that derived from murine bone marrow of wild-type C57BL/6 mice showed a significant activation of Nalp3 inflammasome and the subsequent secretion of the proinflammatory cytokine interleukin-1β (IL-1β). Similarly, LPS-primed macrophages derived from Nalp3,

ASC, or caspase-1 deficient mice showed a marked defect in their ability to secrete IL-1β in response to asbestos exposure (Cassel et al., 2008; Dostert et al., 2008). Similarly, in Nalp3, ASC, or caspase-1 deficient mice, NIEHS chrysotile asbestos (approximately 7 mg/m^3 air for 6 h/d for a total of 8 d, animals sacrificed on d 9) or silica (repeated dose of 20 mg/ml on d 0 and 14, animals sacrificed on d 30)-induced lung inflammatory cell recruitment and cytokine production (interleukin [IL]-1β and CXCL1 [chemokine (C-X-C motif) ligand 1]) are reduced compared to wild-type mice (Cassel et al., 2008; Dostert et al., 2008). The activation of inflammasome observed in these studies was triggered by ROS, which was generated by NADPH oxidase upon fiber phagocytosis or mitochondria oxidative phosphorylation, or both. These findings demonstrate a key role for Nalp3 inflammasome in recognizing fibers and other airborne pathogens and triggering chronic inflammation.

The direct link between asbestos-induced specific inflammatory stimuli and carcinogenesis is not established. Current data suggest that inflammation might mediate this process by promoting cell proliferation and resistance to apoptosis. In murine lung epithelium, disruption of MAPK-1 (MEK1) using a dominant-negative transgene targeted to the bronchiolar epithelium with the CC10 promoter inhibited asbestos-induced proliferation and procollagen gene expression (Manning et al., 2008). In addition, IL-1β and TNF-α, the major cytokines released by macrophages after inhalation of asbestos, were found to regulate human mesothelial cell proliferation by promote the transformation of these cells (Wang et al., 2004). Furthermore, inactivation of the AKT and MAPK pathways appears crucial for triggering asbestos-induced AEC apoptosis (Baldys et al., 2007). The AKT/mammalian target of rapamycin (mTOR) signaling pathways augment tumor formation in part by promoting the formation of cells that are resistant to apoptosis (Chiang & Abraham, 2007; Guertin & Sabatini, 2007). Wilson et al. (2008) showed that mTOR increased survival of MM cells and that rapamycin, an mTOR inhibitor, or silencing of p–S6K, a major downstream target of mTOR, augmented cell death of these otherwise apoptosis-resistant MM cells. Thus, targeted disruption of MKP-3 or activated p38 in macrophages may limit TNF-α gene expression or regulate AKT/MAPK/mTOR signaling in the lung epithelium or MM cells and yields novel approaches that merit additional studies.

Although asbestos triggers inflammatory responses in target tissues and cells, there are no animal models or human studies showing that blocking any one aspect of inflammation is beneficial against mutagenesis or malignant transformation. The link between inflammation and malignancies arising in humans exposed to asbestos merits further study, especially the role of the Nalp3 inflammasome. Future confirmatory studies are required to complete this link as well as to better characterize which inflammatory signal are crucial for asbestos-induced mutagenesis in humans.

Summary

Overall, five biological responses that might facilitate each other in promoting asbestos-induced mutagenicity in a highly interrelated fashion are described. Data from existing studies suggest that asbestos fibers induce ROS/RNS production, DNA and chromosomal damage response, p53 activation, apoptosis, and inflammation in all the relevant lung and pleural target cells. In addition, asbestos-induced responses show cell type specificity. Following asbestos inhalation, AM and lung epithelial cells rapidly internalize the relatively short fibers, while mesothelial cells also take up the fibers upon their migration to the pleura. In comparison with alveolar epithelial and mesothelial cells, bronchial epithelial cells are relatively resistant to asbestos-induced DNA damage, in part because of their greater antioxidant capacity (Gadbois & Lehnert, 1997; Nygren et al., 2004; Puhakka et al., 2002). Data suggest that alveolar type 1 epithelial cells are more susceptible than type 2 cells to asbestos-induced DNA damage and subsequent PARP activation (Kamp et al., 1998, 2001). Confirmatory studies are needed to

assess these findings in primary isolated human AEC for a better understanding of the mechanism involved.

The findings from existing studies confirm that asbestos-induced mutagenic and nonmutagenic pathways are overlapping and interrelated with each other. However, it is not clear how the complex overlapping signaling pathways regulating ROS/RNS production, DNA and chromosomal damage response, p53 activation, apoptosis, and inflammation are coordinated in promoting a mutagenic response following asbestos exposure. In addition, clear-cut evidence that blocking any one of the five known carcinogenic pathways would be beneficial against asbestos–induced mutagenesis has not been provided by any animal studies. In this regard, future studies using transgenic animal models and pharmacologic and genetic strategies targeting each of these five key components will help to better understand the crucial role of each in promoting mutagenicity in relevant target cells. Furthermore, with rare exceptions, there are inadequate human data validating the major in vitro and in vivo animal studies implicating ROS/RNS, DNA damage, apoptosis, and p53 activation in mediating asbestos-induced mutagenesis in each of the lung target cells. Therefore, human studies in asbestos-exposed individuals with or without a manifestation of asbestos-related diseases are critically important for validating the in vitro and in vivo findings.

HEALTH ENDPOINTS WITH AND WITHOUT MUTAGENICITY AS A KEY EVENT

The asbestos-induced biological/pathological effects may eventually manifest as different benign or malignant asbestos diseases, including asbestosis, pleural plaques, malignant lung cancer, and mesothelioma. It is believed that asbestos-induced benign diseases and cancers share some common underlying mechanisms, such as prolonged induction of radical species and chronic inflammation (Kamp & Weitzman, 1999; Mossman & Churg, 1998; Shukla et al., 2003a). However, asbestos-induced malignancies may occur through distinct mechanisms, as mutagenicity is at the center of neoplastic transformation induced by environmental carcinogens. The kinetics of the pathologies is clearly different in both humans and experimental animals. Although some epidemiological studies suggest that lung fibrosis is usually considered a precursor of lung cancer in humans, the results from six animals show that the presence of fibrosis is not correlated with the presence of lung tumors on the single-animal level (Borm et al., 2004). Evidence indicated that asbestos workers developed cancers without having asbestosis, and the asbestos burden in the lungs of asbestos-associated mesothelioma or lung cancer patients had huge variations (Dodson et al., 1997; Roggli et al., 2004). Asbestosis, on the contrary, is more directly associated with asbestos fiber burden in the lung and may develop without mutagenicity as a key event.

Pleural Plaques and Diffuse Pleural Thickening

Scars on the parietal pleura that become calcified are visualized on x-rays of the lung and are called pleural plaques (Kane et al., 2011, this issue). Scars (1) typically occur 20 to 30 yr after exposure, (2) are frequently bilateral and symmetric, and (3) occur primarily on the posterolateral chest wall between the fifth and eight ribs, over the mediastinal pleura, and on the dome of the diaphragm. The underlying mechanism is pleural inflammation that may result either from direct injury from fibers within the pleural cavity, or indirectly from cytokines or other factors released from the lung (Chapman et al., 2003). These pleural plaques are considered to be a biomarker of exposure to asbestos, but based on epidemiology studies do not appear to increase the risk of either mesothelioma or lung cancer (Kane, 1996). Therefore, this form of lung damage is not believed to play a role in carcinogenesis. Diffuse pleural thickening (DPT) refers to extensive fibrosis of the visceral rather than the parietal pleura (Miles et al., 2008). In this

form of asbestos injury, subpleural fibroblasts and mesothelial cells produce scar tissue and collagen deposition. DPT and pleural plaques frequently coexist, and although they produce different symptomatology, functional impairment, and prognosis, their underlying mechanisms may be similar. Both are believed to involve the cytokine TGF-β, which is expressed by mesothelial cells (Miles et al., 2008). The roles of other cytokines and growth factors and the role of ROS in pleural fibrosis were reviewed by Mutsaers et al. (2004). In addition, the activation Factor VII leading to fibrin deposition in the coagulation pathway contributes to fibrosis. There does not appear to be a role for mutagenesis in the development of pleural fibrosis. The lack of increased risk for lung cancer or mesothelioma in these diseases of pleural fibrosis argues strongly for a lack of mutagenesis in their development. In addition, our understanding of the role of pathways of gene expression in the pathogenesis of these diseases supports this conclusion.

In summary, there is reasonable certainty that mutagenesis does not play a role in the development of pleural plaques and diffuse pleural thickening produced by asbestos. Rather, these conditions appear to be produced by changes in gene regulation induced by the generation of ROS including inflammation.

Mesothelioma

One characteristic of mesothelioma is the long latency for the production of this cancer following asbestos exposure. In animal studies, such a characteristic has usually been associated with a role of tumor promotion by asbestos-induced alterations of redox-signaling pathways. However, there may also be a lag created by the required translocation time for asbestos fibers to reach the parietal pleura. Furthermore, multiple genetic alterations were initially found in mesotheliomas. These strongly suggest the operation of mechanisms that might primarily involve a direct mutagenic effect of asbestos fibers and a prevention of the repair of DNA damage through stimulation of cell proliferation.

Recent publications pointed to initial events involving mutagenicity. The studies that found ROS and 8-oxodG generation, chromosome damage, and mitosis impairment in rat and human mesothelial cells, upon the phagocytosis of asbestos fibers, were previously described. It is noteworthy that several in vivo studies observed increased levels of 8-OHdG, DNA strand breaks, or mutagenic events in animals exposed to asbestos fibers for a short term (Kociok et al., 1999; Rihn et al., 2000; Topinka et al., 2004; Unfried et al., 2002).

Specific chromosomal defects have now been discovered to be associated with asbestos-induced mesothelioma in humans that argue for specific mutations being initial events in this disease (Musti et al., 2006). The loss of one copy of chromosome 22 was reported to be a consistent aneuploidy found in mesothelioma, and cytogenetic alterations involving Neurofibromatosis type 2 (*NF2*), whose genetic position is 22q12, is found in up to 100% of mesothelioma cases but not in lung cancers (Toyooka et al., 2008). There is evidence that alterations in chromosomes 1, 14, 21, and 22 might be an early indicator of disease process (Hansteen et al., 1993). Other common cytogenetic abnormalities in mesothelioma involve the tumor suppressor genes for cyclin-dependent kinase (CDK) inhibitor proteins (Musti et al., 2006). Involvement of these genes in human mesotheliomas was further affirmed using specific gene knockout mice (Fleury-Feith et al., 2003; Altomare et al., 2005a, 2005b; Lecomte et al., 2005; Jongsma et al., 2008). It is believed that the continuing elucidation of genetic defects involved in mesothelioma may lead to better therapeutic strategies for this disease (Crispi et al., 2010). Inactivations of genes by epigenetic effects were described in melanoma involving methylation of tumor suppressor genes. The methylation profiles are different in mesothelioma and lung cancer; however, in some cases, these effects may be produced by infection of the SV40 virus (Toyooka et al., 2008). Other paracrine growth factors, including epidermal growth factor (EGF) and hepatocyte growth factor

(HGF), as well as increased expression of receptor proteins for EGF, HGF, and other growth factors, may be involved in regulating enhanced cell proliferation in asbestos-induced mesothelioma. Growing evidence suggests a role of asbestos-induced redox-dependent signal pathways in malignant mesotheliomas and lung cancer. A better understanding of the dysregulation of these signaling pathways may benefit the prevention and therapy of asbestos-related mesothelioma (Heintz et al., 2010).

In summary, specific genetic and chromosomal abnormalities are found in human and animal mesotheliomas. Asbestos-induced DNA and chromosomal damages, as well as the corresponding mutagenic events, were produced experimentally in human or rodent mesothelial cells. However, the direct causal link between asbestos-induced mutations and mesothelioma has not been established in animals or humans. In addition, alterations in gene expression and epigenetic changes may contribute to the pathogenesis of mesothelioma but additional information is needed to confirm the results.

Asbestosis

Fibrosis is the hallmark of asbestosis. Several lines of evidence suggest that asbestos and asbestos-induced ROS produce a number of sequential changes that do not involve mutagenic events, including inflammation, cell proliferation, and fibrosis, thereby producing asbestosis. Mossman (1990) found that crocidolite instillation produced signs of inflammation in rats, such as macrophages and neutrophils, which might be prevented by catalase subcutaneous administration. This finding suggested that H_2O_2 was involved in generating ROS through the Fenton reaction. Schapira et al. (1994) demonstrated that ·OH radicals were produced by instillation of Fe loaded chrysotile, which might be prevented by salicylate. The generation of ROS leads to alterations in the expression of various genes involved in redox-sensitive signaling pathways. For example, ROS could stimulate growth factors IGF1 (insulin-like growth factor 1) and PDG (platelet-derived growth factor) and increased proliferation of epithelial and interstitial cells, leading to a tissue mass rise and macrophage accumulation after instillation of chrysotile fibers (Brody & Overby, 1989; Donaldson, 1996). Depletion of GSH resulting from oxidative stress leads to activation of AP-1 and therefore cell proliferation as shown by in vitro studies (Donaldson, 1996; Janssen et al., 1995). The production of TNFα by macrophages that have phagocytosed asbestos fibers also leads to the depletion of GSH (Donaldson, 1996). The promotion of cell proliferation observed in these studies might be an intermediate process related to the generation of fibrosis. The other signaling pathways that might be potentially involved in asbestos-induced fibrosis, including altered p53 and p21 expressions, MAPK pathways, cytokines (i.e., TNF-α, TGF-β, IL-1β, and CXCL1), and inflammasome activation, were previously described here and by Mossman et al. (2011, of this issue).

Importantly, apoptosis has been related to the generation of fibrosis by asbestos (Albrecht et al., 2004; Kamp, 2009). The same mechanism that has been implicated in neoplasia, i.e., generation of ROS, may also produce genotoxicity that produces cell death through apoptosis or even necrosis. Excessive cell deaths are associated with pulmonary tissue injury and fibroblast proliferation, which leads to fibrosis (Albrecht et al., 2004; Kamp, 2009). Thus, there might be a role for DNA damage but not mutagenesis in the production of asbestosis.

In conclusion, there may be not be a direct role for mutagenicity in the pathways that produce asbestosis; however, genotoxicity may produce cell death and apoptosis, which may contribute to inflammation, cell proliferation and fibrosis. There is reasonable certainty that asbestos-induced genotoxicity and the subsequent alterations in cellular signaling produce asbestosis, especially those involved in inflammatory processes.

Lung Cancer

There are two primary types of lung cancer: small-cell carcinoma and non-small-cell

carcinoma. The latter includes three histological types: large-cell carcinoma, squamous-cell carcinoma, and adenocarcinoma. All these four histological types of lung cancer occur in asbestos-exposed patients (Churg, 1985). In recent years, there are an increasing number studies addressing the cytogenetic changes in asbestos-related lung cancer (Nymark et al., 2008). An early CA occurs in lung cancer is located in 2p and 3p (Nymark et al., 2006; Wikman et al., 2007). This specific CA occurs more frequently in asbestos-exposed patients. Other asbestos specific aberrations were detected in chromosomes 1, 5, 8, 9, and 19p. Microsatellite analysis found statistically significant differences in 19p13 allelic imbalance between asbestos-exposed and unexposed lung cancer cases (Wikman et al., 2007). Allelic imbalance was found for 9q31.3-q34.3 in 100% of asbestos-exposed versus 64% of unexposed cases (Nymark et al., 2009). Kettunen et al. (2009) demonstrated an association in lung cancer patients between asbestos exposure and DNA copy number loss and allelic imbalance at 2p16, which was one of those that differed most between asbestos-exposed and nonexposed patients. It is noteworthy that most of the asbestos-exposed subjects were also cigarette smokers. So far the available research has not located a specific genetic change that is clearly linked to asbestos exposure, but rather increases in changes that occur without asbestos exposure and usually in a background of cigarette smoking. Nevertheless, there is evidence that specific gene alterations, such as the inactivation of $p16^{INK4A}$ in asbestos-associated non-small-cell lung cancer, occur mainly through deletion. In contrast, inactivation by methylation is more often associated with non-asbestos-associated lung cancer cases (Andujar et al., 2010). In addition, mutations in TP53 in lung cancer patients have been positively associated with asbestos exposure (Nymark et al., 2008).

For at least some cases of lung cancer, there appears to be a synergistic effect of cigarette smoking and asbestos (Erren et al., 1999). The fact that the mutagenic potential of cigarette smoking is promoted by the processes leading to asbestosis may be an explanation for synergy, which is observed experimentally in initiation–promotion studies of carcinogens. In such experiments, noncarcinogenic doses of tumor promoters greatly enhance the carcinogenic effects of mutagenic carcinogens.

In some cases, the presence of asbestosis is associated with increases the risk of lung cancer. However, it is uncertain whether the development of asbestosis is merely a dose-related biomarker for asbestos exposure or whether asbestosis produces a necessary or an enhanced environment for the production of cancer. There has been considerable debate regarding the requirement of asbestosis and cigarette smoke exposure for asbestos-related lung cancer development. In general, there have been studies supporting both possibilities, and so far there is no consensus whether or not asbestosis promotes lung cancer (Hessel et al., 2005; Kane, 1996). However, the development of both asbestosis and lung cancer might be produced by the same agents, i.e., ROS generated secondary to asbestos-induced inflammation. The alterations in the expression of genes involved in inflammation, cell proliferation, and apoptosis, such as p53 and p21, MAPK pathways, cytokines TNF-α, TGF-β, IL-1β, and CXCL1, may also be involved in the development of asbestos-associated lung cancer (Kamp, 2009).

In conclusion, there is some indication for the role of mutagenicity in asbestos-associated lung cancer development; further study is necessary to establish this mechanism. Increased incidences of allelic alterations found in asbestos-exposed cases are also present in many asbestos-unexposed patients. These changes may represent enhancement of mutagens from cigarette smoke or other exposures. There is a reasonable certainty of the involvement of nonmutagenic effects, that is, alterations in gene expression, in asbestos-induced lung cancer development.

DETERMINANT FACTORS OF TOXICITY IN MUTAGENICITY

Various in vitro and in vivo studies indicated that fiber dimensions, surface properties,

shape and crystallinity, chemical composition, and physical durability are important determinant factors of asbestos pathogenicity (Mossman, 1990; Sanchez et al., 2009; Stanton et al., 1977). The morphology and aspect ratios of inhaled asbestos fibers determine their deposition, subsequent translocation, and finally the primary cellular targets for mutagenic and genotoxic action. In addition, chemical composition and surface reactivity are known to modulate asbestos toxicity. Fibers that are rich in Fe content, e.g., crocidolite, are more reactive in ROS and RNS production and contribute to their mutagenic and genotoxic potential. Asbestos fibers or other aerosol particles that have an insoluble or poorly soluble core onto which adsorbed mutagens (or carcinogens) are carried from the environment into and throughout the human body can facilitate the each other's mutagenic response. Significant contributors to this mutagenic pathway include the polycyclic aromatic hydrocarbons (PAH) and tobacco carcinogens, which upon biotransformation convert into reactive intermediates that form bulky adducts to the DNA. This mode of action from tobacco carcinogen was postulated to account for the synergism in lung cancer incidence observed among asbestos workers who also smoked (Hammond et al., 1976). Furthermore, the ability of asbestos to remain physically intact in the lung is considered an essential criterion for fiber toxicity. As such, fibers that are durable, e.g., crocidolite and amosite, are significantly more biologically active than glass fibers and chrysotile (Hume & Rimstidt, 1992). Finally, it is noteworthy that fiber toxicity also depends on the exposure concentration and duration. These factors modify fiber mutagenicity and show bidirectional effects. On one hand, increases in the time or dose of fiber exposure enhance cellular toxicity and genotoxicity in target cells. However, at sufficiently high doses, the mutation yield in short-term assays was found to decrease due to cytotoxicity (Unfried et al., 2002; Xu et al., 1999, 2002). The chronic effects of cytotoxicity and cell/tissue injury are associated with inflammation, fibrosis, and cell proliferation, which eventually may contribute to asbestos carcinogenicity through nonmutagenic pathway (Mossman, 1993; Mossman & Churg, 1998). On the other hand, exposure at high concentration or chronic exposure may produce lung overload and impair the clearance of fibers by macrophages, enhancing the carcinogenic and mutagenic potential of asbestos fibers (Borm et al., 2004; Driscoll et al., 1996; ILSI Risk Science Institute Workshop Participants, 2000; Oberdorster 2002; Oberdorster et al., 2005). Another factor that might affect asbestos fiber toxicity and mutagenicity is individual susceptibility such as genetic background or nutrition status (Mossman et al., 2011, this issue; Case et al., 2011, this issue).

SUMMARY

What is Known

1. Asbestos is clearly mutagenic when assayed using a variety of in vitro and in vivo model systems and induces both gene and chromosomal mutations in target cells by mechanisms ranging from generation of oxidants to physical interaction with the mitotic apparatus of dividing cells.
2. Specific genetic and chromosomal abnormalities are found in human mesothelioma tissues associated with asbestos exposure. Similar mutation profiles have been identified in murine mesothelioma models.
3. Mutagenesis does not play a role in the development of pleural plaques and diffuse pleural thickening induced by asbestos. Rather, these conditions appear to be produced by changes in gene regulation produced by the generation of ROS and inflammation.
4. Asbestos-induced alterations in gene expression induce asbestosis, especially those involved in inflammatory processes.

What Is Postulated to Be True But Requires Confirmation

1. Asbestos fibers induce DNA damage and mutation in vivo in rat lung tissues after

ip or intratracheal instillation or, in limited cases, inhalation exposure. Additional long-term inhalation studies at doses that are relevant to human exposure experience are needed to confirm the incidence and spectrum of the mutagenic events in animals.
2. The mutagenic effects of asbestos may result primarily from fiber–cell interaction, or secondarily from fiber-induced ROS/RNS. The latter may involve a threshold depending on the exposure concentration and treatment period that induces chronic inflammation and overwhelms the antioxidant and DNA damage repair capacity of the target tissues. Further studies dissecting these pathways are required to reveal the precise molecular mechanisms of asbestos-induced mutagenesis.
3. Asbestos-induced biological responses, including ROS/RNS production, DNA and chromosomal damage response, p53 activation, apoptosis, and inflammation, that contribute to asbestos mutagenicity are observed in all the relevant lung and pleural target cells but show cell type specificity. In comparison with alveolar epithelial and mesothelial cells, bronchial epithelial cells are relatively resistant to asbestos-induced DNA damage in part because of their greater antioxidant capacity. Confirmatory studies are needed to provide a better understanding of the mechanism involved.
4. There is some evidence that alterations in gene expression and epigenetic changes may contribute to the pathogenesis of asbestos-associated mesothelioma but additional information is needed to confirm the results.
5. Genotoxicity may not be directly involved in the development of asbestosis; however, genotoxicity may produce cell death and apoptosis, which may contribute to inflammation, cell proliferation and fibrosis. The alterations in gene expression involved in these pathways may also be involved in lung cancer development, but more information is needed.

Areas of Uncertainties That Require Additional Research

1. Fiber dimension is one of the important determinant factors of asbestos carcinogenicity. Occupational exposure limit levels are based on determination of fibers greater than 5 μm in length. However, in most experiments, dose-effect relationships are generally considered on an asbestos mass basis, but not on number of fibers (total or size-adjusted) or fiber surface area. Although animal data suggest that "long" fibers are more carcinogenic than "short" fibers, it is not clear whether the mutagenic potency of "long" fibers is different from that of "short" fibers. In addition, many existing studies were conducted with fiber doses that impair the clearance of the fibers and produce "overload." It is not known whether "overload" will change the spectrum of asbestos fiber-induced mutations. Therefore, future in vivo and in vitro studies need to use well-characterized fibers (taking into consideration fiber mass, number, size distribution, and/or surface area) at realistic exposure levels. The results from these studies will be more reliable for assessing the potential health risks of exposure for humans.
2. The direct link between asbestos-induced mutagenesis and carcinogenesis is lacking. Further in vivo inhalation studies coupled with transformation endpoints using well-defined animal models and fibers at relevant exposure levels are required for studying the stepwise processes and identifying the critical tumor-initiating/—promoting mutations in exposed animals.
3. The mutagenic effect of asbestos fibers at low doses is still unknown. Some evidence from in vitro studies suggests that there is no threshold dose for asbestos-induced mutagenic effects. However, the determination of LOAEL and NOAEL as well as the shape of dose-response curves requires a sufficient number of low doses to be tested. These are generally not available in existing studies. More importantly, in vivo studies are few and conducted using a limited dose range (1–10 mg single dose or repeated doses of

5.73 mg/m^3 fibers) (Jiang et al., 2008; Jung et al., 2000; Kociok et al., 1999; Rihn et al., 2000; Topinka et al., 2004; Unfried et al., 2002). Some studies indicated no induction of mutation in the animal lungs at the lowest dose tested. However, these negative results might due to the detection limit of the assay, or the relative short exposure periods. Overall, future studies need to be conducted to test a sufficient number of low-dose groups in animals and cultured relevant target cells.

4. The concept of a threshold dose below which there is no carcinogenic response upon fiber exposure is still controversial. There is indication that the effective dose is different for mesothelioma induction and lung cancer induction, and for different types of fibers (i.e., chrysotile vs. crocidolite). Limited evidence from inhalation studies in rodents indicates that the dose-response relationship shows no threshold for asbestos carcinogenicity. Substantial efforts from future studies, both in vitro and in vivo, using well-characterized fibers (as described above in point 1) and several dose metrics, are needed to clarify this uncertainty.
5. Although ROS/RNS production and the downstream DNA damages are well established in asbestos-treated lung target cells and tissues, the relative contribution from each of the sources of ROS production is still not known. For example, the functional role of mitochondrial and mtDNA alterations in promoting asbestos induced mutagenesis is virtually unknown. More importantly, there are no animal models or human studies showing that blocking any one of these effects is beneficial against mutagenesis. Therefore, strategies targeting the various sources of asbestos-induced ROS production in lung epithelial and mesothelial cells are important.
6. Asbestos-associated mutagenic and nonmutagenic pathways are interrelated with each other. However, it is not known whether each of the pathways is sufficient in producing asbestos-associated cancers. In addition, it is not clear how the complex overlapping signaling pathways regulating ROS/RNS production, DNA damage response, p53 activation, apoptosis, and inflammation are coordinated in promoting a carcinogenic responses following asbestos exposure. In this regard, studies using murine transgenic models along with targeted over- and underexpression studies will continue to be important for furthering our understanding of the field.
7. Increased incidences of genetic allelic alterations found in asbestos-exposed lung cancer patients are also present in many non-asbestos-related cases, and these changes may represent enhancement of mutagens from cigarette smoke or other exposures. The causal relationship of specific allelic alterations in relation to fiber carcinogenesis in lung needs to be examined.

REFERENCES

Aceto, N., Bertino, P., Barbone, D., Tassi, G., Manzo, L., Porta, C., Mutti, L., and Gaudino, G. 2009. Taurolidine and oxidative stress: A rationale for local treatment of mesothelioma. *Eur. Respir. J*. 34:1399–1407.

ACGIH. 2001. *Asbestos: TLV chemical substances*, 7th ed. documentation. Cincinnati, OH: American Conference of Governmental Industrial Hygienists (ACGIH).

Achard, S., Perderiset, M., and Jaurand, M. C. 1987. Sister chromatid exchanges in rat pleural mesothelial cells treated with crocidolite, attapulgite, or benzo 3–4 pyrene. *Br. J. Ind. Med*. 44:281–283.

Albrecht, C., Borm, P. J. A., and Unfried, K. 2004. Signal transduction pathways relevant for neoplastic effects of fibrous and non-fibrous particles. *Mutat. Res./Fundam. Mol. Mech. Mutagen*. 553:23–35.

Aljandali, A., Pollack, H., Yeldandi, A., Li, Y., Weitzman, S. A., and Kamp, D. W. 2001. Asbestos causes apoptosis in alveolar epithelial cells: Role of iron-induced free radicals. *J. Lab. Clin. Med*. 137:330–339.

Altomare, D. A., Menges, C. W., Pei, J., Zhang, L., Skele-Stump, K. L., Carbone, M.,

Kane, A. B., and Testa, J. R. 2009. Activated TNF-alpha/NF-kappaB signaling via down-regulation of Fas-associated factor 1 in asbestos-induced mesotheliomas from Arf knockout mice. *Proc. Natl. Acad. Sci. USA* 106:3420–3425.

Altomare, D. A., Vaslet, C. A., Skele, K. L., De Rienzo, A., Devarajan, K., Jhanwar, S. C., McClatchey, A. I., Kane, A. B., and Testa, J. R. 2005a. A mouse model recapitulating molecular features of human mesothelioma. *Cancer Res*. 65:8090–8095.

Altomare, D. A., You, H., Xiao, G. H., Ramos-Nino, M. E., Skele, K. L., De Rienzo, A., Jhanwar, S. C., Mossman, B. T., Kane, A. B., and Testa, J. R. 2005b. Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. *Oncogene* 24:6080–6089.

Andujar, P., Lecomte, C., Renier, A., Fleury-Feith, J., Kheuang, L., Daubriac, J., Janin, A., and Jaurand, M. C. 2007. Clinico-pathological features and somatic gene alterations in refractory ceramic fibre-induced murine mesothelioma reveal mineral fibre-induced mesothelioma identities. *Carcinogenesis* 28:1599–1605.

Andujar, P., Wang, J., Descatha, A., Galateau-Salle, F., Abd-Alsamad, I., Billon-Galland, M. A., Blons, H., Clin, B., Danel, C., Housset, B., Laurent-Puig, P., Le Pimpec-Barthes, F., Letourneux, M., Monnet, I., Regnard, J. F., Renier, A., Zucman-Rossi, J., Pairon, J. C., and Jaurand, M. C. 2010. p16INK4A inactivation mechanisms in non-small-cell lung cancer patients occupationally exposed to asbestos. *Lung Cancer* 67:23–30.

Athanasiou, K., Constantopoulos, S. H., Rivedal, E., Fitzgerald, D. J., and Yamasaki, H. 1992. Metsovo-tremolite asbestos fibres: in vitro effects on mutation, chromosome aberration, cell transformation and intercellular communication. *Mutagenesis* 7:343–347.

Ault, J. G., Cole, R. W., Jensen, C. G., Jensen, L. C., Bachert, L. A., and Rieder, C. L. 1995. Behavior of crocidolite asbestos during mitosis in living vertebrate lung epithelial cells. *Cancer Res*. 55:792–798.

Aung, W., Hasegawa, S., Furukawa, T., and Saga, T. 2007. Potential role of ferritin heavy chain in oxidative stress and apoptosis in human mesothelial and mesothelioma cells: Implications for asbestos-induced oncogenesis. *Carcinogenesis* 28: 2047–2052.

Baan, R. A., and Grosse, Y. 2004. Man-made mineral (vitreous) fibres: Evaluations of cancer hazards by the IARC Monographs Programme. *Mutat. Res*. 553:43–58.

Babu, K. A., Lakkad, B. C., Nigam, S. K., Bhatt, D. K., Karnik, A. B., Thakore, K. N., Kashyap, S. K., and Chatterjee, S. K. 1980. In vitro cytological and cytogenetic effects of an Indian variety of chrysotile asbestos. *Environ. Res*. 21: 416–422.

Bakhanashvili, M., Grinberg, S., Bonda, E., Simon, A. J., Moshitch-Moshkovitz, S., and Rahav, G. 2008. p53 in mitochondria enhances the accuracy of DNA synthesis. *Cell Death Differ*. 15:1865–1874.

Baldys, A., Pande, P., Mosleh, T., Park, S. H., and Aust, A. E. 2007. Apoptosis induced by crocidolite asbestos in human lung epithelial cells involves inactivation of Akt and MAPK pathways. *Apoptosis* 12:433–447.

Balkwill, F. 2006. TNF-α in promotion and progression of cancer. *Cancer Metastasis Rev*. 25:409–416.

Barrett, J. C. 1992. Mechanisms of action of known human carcinogens. *IARC Sci. Publ*. 116:115–134.

Barrett, J. C. 1994. Cellular and molecular mechanisms of asbestos carcinogenicity: Implications for biopersistence. *Environ. Health Perspect*. 102(suppl. 5):19–23.

Barrett, J. C., Lamb, P. W., and Wiseman, R. W. 1989. Multiple mechanisms for the carcinogenic effects of asbestos and other mineral fibers. *Environ. Health Perspect*. 81:81–89.

Belitskaya-Levy, I., Hajjou, M., Su, W. C., Yie, T. A., Tchou-Wong, K. M., Tang, M. S., Goldberg, J. D., and Rom, W. N. 2007. Gene profiling of normal human bronchial epithelial cells in response to asbestos and

benzo(a)pyrene diol epoxide (BPDE). *J. Environ. Pathol. Toxicol. Oncol*. 26:281–294.

Berman, D., and Crump, K. 2003. *Final draft: Technical final draft: Technical support document for a protocol to assess asbestos-related risk. Support document for a protocol to assess asbestos-related risk*, ed. U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response. Washington, DC: EPA number 9345.

Berman, D. W. 2010. Comparing milled fiber, Quebec ore, and textile factory dust: Has another piece of the asbestos puzzle fallen into place? *Crit. Rev. Toxicol*. 40:151–188.

Berman, D. W., and Crump, K. S. 2008a. A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. *Crit. Rev. Toxicol*. 38(suppl. 1):49–73.

Berman, D. W., and Crump, K. S. 2008b. Update of potency factors for asbestos-related lung cancer and mesothelioma. *Crit. Rev. Toxicol*. 38(suppl. 1):1–47.

Berman, D. W., Crump, K. S., Chatfield, E. J., Davis, J. M., and Jones, A. D. 1995. The sizes, shapes, and mineralogy of asbestos structures that induce lung tumors or mesothelioma in AF/HAN rats following inhalation. *Risk Anal*. 15:181–195.

Bertino, P., Marconi, A., Palumbo, L., Bruni, B. M., Barbone, D., Germano, S., Dogan, A. U., Tassi, F., Porta, C., Mutti, L., and Gaudino, G. 2007. Erionite and asbestos differently cause transformation of human mesothelial cells. *Int. J. Cancer* 121:12–20.

BeruBe, K. A., Quinlan, T. R., Fung, H., Magae, J., Vacek, P., Taatjes, D. J., and Mossman, B. T. 1996. Apoptosis is observed in mesothelial cells after exposure to crocidolite asbestos. *Am. J. Respir. Cell Mol. Biol*. 15:141–147.

Bianchi, A. B., Mitsunaga, S. I., Cheng, J. Q., Klein, W. M., Jhanwar, S. C., Seizinger, B., Kley, N., Klein-Szanto, A. J., and Testa, J. R. 1995. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. *Proc. Natl. Acad. Sci. USA* 92:10854–10858.

Blake, D. J., Bolin, C. M., Cox, D. P., Cardozo-Pelaez, F., and Pfau, J. C. 2007. Internalization of Libby amphibole asbestos and induction of oxidative stress in murine macrophages. *Toxicol. Sci*. 99: 277–288.

Bochkov, N. P., Katosova, L. D., Platonova, V. I., Sapacheva, V. A., Titenko, N. T., and Tsurikova, G. V. 1991. Cytogenetic examination of workers engaged in manufacturing of asbestos technical products. *Gig. Tr. Prof. Zabol*. 12:10–11.

Bolognesi, C., Filiberti, R., Neri, M., Perrone, E., Landini, E., Canessa, P. A., Simonassi, C., Cerrano, P. G., Mutti, L., and Puntoni, R. 2002. High frequency of micronuclei in peripheral blood lymphocytes as index of susceptibility to pleural malignant mesothelioma. *Cancer Res*. 62:5418–5419.

Bolton, R. E., Davis, J. M. G., Miller, B., Donaldson, K., and Wright, A. 1984. The effect of dose of asbestos on mesothelioma production in the laboratory rat. *Proc VIth Int. Pneumonoconiosis Conference,* p. 2.

Bonneau, L., Malard, C., and Pezerat, H. 1986. Studies on surface properties of asbestos: II. Role of dimensional characteristics and surface properties of mineral fibers in the induction of pleural tumors. *Environ. Res*. 41:268–275.

Borm, P. J., Schins, R. P., and Albrecht, C. 2004. Inhaled particles and lung cancer, part B: paradigms and risk assessment. *Int. J. Cancer* 110:3–14.

Both, K., Henderson, D. W., and Turner, D. R. 1994. Asbestos and erionite fibres can induce mutations in human lymphocytes that result in loss of heterozygosity. *Int. J. Cancer* 59:538–542.

Both, K., Turner, D. R., and Henderson, D. W. 1995. Loss of heterozygosity in asbestos-induced mutations in a human mesothelioma cell line. *Environ. Mol. Mutagen*. 26:67–71.

Bowes, D. R., and Farrow, C. M. 1997. Major and trace element compositions of the UICC standard asbestos samples. *Am. J. Ind. Med*. 32:592–594.

Branchaud, R. M., Garant, L. J., and Kane, A. B. 1993. Pathogenesis of mesothelial reactions to asbestos fibers. Monocyte recruitment

and macrophage activation. *Pathobiology* 61:154–163.

Breysse, P. N., Cherrie, J. W., Addison, J., and Dodgson, J. 1989. Evaluation of airborne asbestos concentrations using TEM and SEM during residential water tank removal. *Ann. Occup. Hyg*. 33:243–256.

Broaddus, V. C., Yang, L., Scavo, L. M., Ernst, J. D., and Boylan, A. M. 1996. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. *J. Clin. Invest*. 98:2050–2059.

Brody, A. R., and Overby, L. H. 1989. Incorporation of tritiated thymidine by epithelial and interstitial cells in bronchiolar-alveolar regions of asbestos-exposed rats. *Am. J. Pathol*. 134:133–140.

Browne, K. 2001. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann. Occup. Hyg*. 45:327–329; author reply 336–338.

Buder-Hoffmann, S. A., Shukla, A., Barrett, T. F., MacPherson, M. B., Lounsbury, K. M., and Mossman, B. T. 2009. A protein kinase Cdelta-dependent protein kinase D pathway modulates ERK1/2 and JNK1/2 phosphorylation and Bim-associated apoptosis by asbestos. *Am. J. Pathol*. 174:449–459.

Burmeister, B., Schwerdtle, T., Poser, I., Hoffmann, E., Hartwig, A., Muller, W. U., Rettenmeier, A. W., Seemayer, N. H., and Dopp, E. 2004. Effects of asbestos on initiation of DNA damage, induction of DNA-strand breaks, P53-expression and apoptosis in primary, SV40-transformed and malignant human mesothelial cells. *Mutat. Res.*. 558:81–92.

Carthew, P., Hill, R. J., Edwards, R. E., and Lee, P. N. 1992. Intrapleural administration of fibres induces mesothelioma in rats in the same relative order of hazard as occurs in man after exposure. *Hum. Exp. Toxicol*. 11:530–534.

Casey, G. 1983. Sister-chromatid exchange and cell kinetics in CHO-K1 cells, human fibroblasts and lymphoblastoid cells exposed in vitro to asbestos and glass fibre. *Mutat. Res*. 116:369–377.

Cassel, S. L., Eisenbarth, S. C., Iyer, S. S., Sadler, J. J., Colegio, O. R., Tephly, L. A., Carter, A. B., Rothman, P. B., Flavell, R. A., and Sutterwala, F. S. 2008. The Nalp3 inflammasome is essential for the development of silicosis. *Proc. Natl. Acad. Sci. USA* 105:9035–9040.

Cavallo, D., Campopiano, A., Cardinali, G., Casciardi, S., De Simone, P., Kovacs, D., Perniconi, B., Spagnoli, G., Ursini, C. L., and Fanizza, C. 2004. Cytotoxic and oxidative effects induced by man-made vitreous fibers (MMVFs) in a human mesothelial cell line. *Toxicology* 201:219–229.

Chamberlain, M., and Tarmy, E. M. 1977. Asbestos and glass fibres in bacterial mutation tests. *Mutat. Res*. 43:159–164.

Chao, C. C., Park, S. H., and Aust, A. E. 1996. Participation of nitric oxide and iron in the oxidation of DNA in asbestos-treated human lung epithelial cells. *Arch. Biochem. Biophys*. 326:152–157.

Chapman, S. J., Cookson, W. O., Musk, A. W., and Lee, Y. C. 2003. Benign asbestos pleural diseases. *Curr. Opin. Pulm. Med*. 9:266–271.

Chiang, G. G., and Abraham, R. T. 2007. Targeting the mTOR signaling network in cancer. *Trends Mol Med* 13:433–442.

Choe, N., Tanaka, S., and Kagan, E. 1998. Asbestos fibers and interleukin-1 upregulate the formation of reactive nitrogen species in rat pleural mesothelial cells. *Am. J. Respir. Cell Mol. Biol*. 19:226–236.

Choe, N., Tanaka, S., Xia, W., Hemenway, D. R., Roggli, V. L., and Kagan, E. 1997. Pleural macrophage recruitment and activation in asbestos-induced pleural injury. *Environ. Health Perspect*..105(suppl. 5):1257–1260.

Churg, A. 1985. Lung cancer cell type and asbestos exposure. *J. Am. Med. Assoc*. 253:2984–2985.

Churg, A., Hobson, J., Berean, K., and Wright, J. 1989. Scavengers of active oxygen species prevent cigarette smoke-induced asbestos fiber penetration in rat tracheal explants. *Am. J. Pathol*. 135:599–603.

Cleveland, M. G. 1984. Mutagenesis of *Escherichia coli* (CSH50) by asbestos

(41954). *Proc. Soc. Exp. Biol. Med*. 177:343–346.

Cole, R. W., Ault, J. G., Hayden, J. H., and Rieder, C. L. 1991. Crocidolite asbestos fibers undergo size-dependent microtubule-mediated transport after endocytosis in vertebrate lung epithelial cells. *Cancer Res*. 51:4942–4947.

Committee on Asbestos: Selected Health Effects. 2006. Biological aspects of asbestos-related diseases (chapter 5) In *Asbestos: Selected cancers*, pp. 81–103 Washington, DC: National Academies Press.

Crispi, S., Cardillo, I., Spugnini, E. P., Citro, G., Menegozzo, S., and Baldi, A. 2010. Biological agents involved in malignant mesothelioma: relevance as biomarkers or therapeutic targets. *Curr. Cancer Drug Targets* 10:19–26.

Currie, G. P., S. J. Watt, and N. A. Maskell. 2009. An overview of how asbestos exposure affects the lung. *Br. Med. J*. 339: 506–510.

Davis, J. M., Addison, J., Bolton, R. E., Donaldson, K., Jones, A. D., and Miller, B. G. 1985. Inhalation studies on the effects of tremolite and brucite dust in rats. *Carcinogenesis* 6:667–674.

Davis, J. M., Addison, J., Bolton, R. E., Donaldson, K., Jones, A. D., and Smith, T. 1986. The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. *Br. J. Exp. Pathol*. 67: 415–430.

Davis, J. M., Beckett, S. T., Bolton, R. E., Collings, P., and Middleton, A. P. 1978. Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. *Br. J. Cancer* 37:673–688.

Davis, J. M., Beckett, S. T., Bolton, R. E., and Donaldson, K. 1980. A comparison of the pathological effects in rats of the UICC reference samples of amosite and chrysotile with those of amosite and chrysotile collected from the factory environment. *IARC Sci. Publ*.:285–292.

Davis, J. M., Bolton, R. E., Miller, B. G., and Niven, K. 1991. Mesothelioma dose response following intraperitoneal injection of mineral fibres. *Int. J. Exp Pathol* 72: 263–274.

Davis, J. M., and Jones, A. D. 1988. Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. *Br. J. Exp. Pathol*. 69:717–737.

Davis, W. P., Janssen, Y. M. W., Mossman, B. T., and Taatjes, D. J. 1997. Simultaneous triple fluorescence detection of mRNA localization, nuclear DNA, and apoptosis in cultured cells using confocal scanning laser microscopy. *Histochem. Cell Biol*. 108: 307–311.

Denizeau, F., Marion, M., Chevalier, G., and Cote, M. G. 1985a. Genotoxicity of dimethylnitrosamine in the presence of chrysotile asbestos UICC B and xonotlite. *Carcinogenesis* 6:1815–1817.

Denizeau, F., Marion, M., Chevalier, G., and Cote, M. G. 1985b. Inability of chrysotile asbestos fibers to modulate the 2-acetylaminofluorene-induced UDS in primary cultures of rat hepatocytes. *Mutat. Res*. 155:83–90.

Dodson, R. F., O'Sullivan, M., Corn, C. J., McLarty, J. W., and Hammar, S. P. 1997. Analysis of asbestos fiber burden in lung tissue from mesothelioma patients. *Ultrastruct. Pathol*. 21:321–336.

Donaldson, K. 1996. Short-term animal studies for detecting inflammation, fibrosis and pre-neoplastic changes induced by fibres. *IARC Sci. Publ*.140:97–106.

Dong, H. Y., Buard, A., Levy, F., Renier, A., Laval, F., and Jaurand, M. C. 1995. Synthesis of poly(ADP-ribose) in asbestos treated rat pleural mesothelial cells in culture. *Mutat. Res*. 331:197–204.

Dong, H. Y., Buard, A., Renier, A., Levy, F., Saint-Etienne, L., and Jaurand, M. C. 1994. Role of oxygen derivatives in the cytotoxicity and DNA damage produced by asbestos on rat pleural mesothelial cells in vitro. *Carcinogenesis* 15:1251–1255.

Donmez-Altuntas, H., Baran, M., Oymak, F. S., Hamurcu, Z., Imamoglu, N., Ozesmi, M., and Demirtas, H. 2007. Investigation of micronucleus frequencies in lymphocytes

of inhabitants environmentally exposed to chrysotile asbestos. *Int. J. Environ Health Res*. 17:45–51.

Dopp, E., Nebe, B., Hahnel, C., Papp, T., Alonso, B., Simko, M., and Schiffmann, D. 1995a. Mineral fibers induce apoptosis in Syrian hamster embryo fibroblasts. *Pathobiology* 63:213–221.

Dopp, E., Saedler, J., Stopper, H., Weiss, D. G., and Schiffmann, D. 1995b. Mitotic disturbances and micronucleus induction in Syrian hamster embryo fibroblast cells caused by asbestos fibers. *Environ. Health Perspect*. 103:268–271.

Dopp, E., and Schiffmann, D. 1998. Analysis of chromosomal alterations induced by asbestos and ceramic fibers. *Toxicol. Lett*. 96–97:155–162.

Dopp, E., Schuler, M., Schiffmann, D., and Eastmond, D. A. 1997. Induction of micronuclei, hyperdiploidy and chromosomal breakage affecting the centric/pericentric regions of chromosomes 1 and 9 in human amniotic fluid cells after treatment with asbestos and ceramic fibers. *Mutat. Res*. 377:77–87.

Dopp, E., Yadav, S., Ansari, F. A., Bhattacharya, K., von Recklinghausen, U., Rauen, U., Rodelsperger, K., Shokouhi, B., Geh, S., and Rahman, Q. 2005. ROS-mediated genotoxicity of asbestos-cement in mammalian lung cells in vitro. *Part. Fibre Toxicol*. 2:1–9.

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. 2008. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320:674–677.

Drenth, J. P. H., and van der Meer, J. W. M. 2006. The inflammasome—A linebacker of innate defense. *N. Engl. J. Med*. 355: 730–732.

Driscoll, K. E., Carter, J. M., Howard, B. W., Hassenbein, D. G., Pepelko, W., Baggs, R. B., and Oberdorster, G. 1996. Pulmonary inflammatory, chemokine, and mutagenic responses in rats after subchronic inhalation of carbon black. *Toxicol Appl Pharmacol* 136:372–380.

Dubes, G. R., and Mack, L. R. 1988. Asbestos-mediated transfection of mammalian cell cultures. *In Vitro Cell Dev. Biol*. 24:175–182.

Durnev, A. D., Daugel-Dauge, N. O., Korkina, L. G., and Seredenin, S. B. 1993. Peculiarities of the clastogenic properties of chrysotile-asbestos fibers and zeolite particles. *Mutat. Res*. 319:303–308.

Dusinska, M., Collins, A., Kazimirova, A., Barancokova, M., Harrington, V., Volkovova, K., Staruchova, M., Horska, A., Wsolova, L., Kocan, A., Petrik, J., Machata, M.,Ratcliffe, B., and Kyrtopoulos, S. 2004. Genotoxic effects of asbestos in humans. *Mutat. Res*. 553:91–102.

Eastmond, D. A., Hartwig, A., Anderson, D., Anwar, W. A., Cimino, M. C., Dobrev, I., Douglas, G. R., Nohmi, T., Phillips, D. H., and Vickers, C. 2009. Mutagenicity testing for chemical risk assessment: Update of the WHO/IPCS Harmonized Scheme. *Mutagenesis* 24:341–349.

Emerit, I., Jaurand, M. C., Saint-Etienne, L., and Levy, A. 1991. Formation of a clastogenic factor by asbestos-treated rat pleural mesothelial cells. *Agents Actions* 34: 410–415.

Erren, T. C., Jacobsen, M., and Piekarski, C. 1999. Synergy between asbestos and smoking on lung cancer risks. *Epidemiology* 10:405–411.

Fatma, N., Khan, S. G., Aslam, M., and Rahman, Q. 1992. Induction of chromosomal aberrations in bone marrow cells of asbestotic rats. *Environ. Res*. 57:175–180.

Faux, S. P., Howden, P. J., and Levy, L. S. 1994a. Iron-dependent formation of 8-hydroxydeoxyguanosine in isolated DNA and mutagenicity in *Salmonella typhimurium* TA102 induced by crocidolite. *Carcinogenesis* 15:1749–1751.

Faux, S. P., Michelangeli, F., and Levy, L. S. 1994b. Calcium chelator Quin-2 prevents crocidolite-induced DNA strand breakage in human white blood cells. *Mutat. Res*. 311:209–215.

Faux, S. P., Tran, C.-L., Miller, B. G., Jones, A. D., Monteiller, C., and

Donaldson, K. 2003. *In vitro determinants of particulate toxicity: The dose-metric for poorly soluble dusts*. Research Report RR154. Sudbury, UK: Health and Safety Executive.

Fearon, E. R., and Vogelstein, B. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759–767.

Federico, A., Morgillo, F., Tuccillo, C., Ciardiello, F., and Loguercio, C. 2007. Chronic inflammation and oxidative stress in human carcinogenesis. *Int. J. Cancer* 121:2381–2386.

Fennell, D. A., and Rudd, R. M. 2004. Defective core-apoptosis signalling in diffuse malignant pleural mesothelioma: Opportunities for effective drug development. *Lancet Oncol*. 5:354–362.

Festjens, N., Vanden Berghe, T., and Vandenabeele, P. 2006. Necrosis, a well-orchestrated form of cell demise: Signalling cascades, important mediators and concomitant immune response. *Biochim. Biophys. Acta* 1757:1371–1387.

Fleury-Feith, J., Lecomte, C., Renier, A., Matrat, M., Kheuang, L., Abramowski, V., Levy, F., Janin, A., Giovannini, M., and Jaurand, M. C. 2003. Hemizygosity of Nf2 is associated with increased susceptibility to asbestos-induced peritoneal tumours. *Oncogene* 22:3799–3805.

Fournier, J., and Pezerat, H. 1986. Studies on surface properties of asbestos: III. Interactions between asbestos and polynuclear aromatic hydrocarbons. *Environ. Res*. 41:276–295.

Franco, R., Sanchez-Olea, R., Reyes-Reyes, E. M., and Panayiotidis, M. I. 2009. Environmental toxicity, oxidative stress and apoptosis: Menage a trois. *Mutat. Res*. 674:3–22.

Fubini, B., Fenoglio, I., Elias, Z., and Poirot, O. 2001. Variability of biological responses to silicas: effect of origin, crystallinity, and state of surface on generation of reactive oxygen species and morphological transformation of mammalian cells. *J. Environ. Pathol. Toxicol. Oncol*. 20(suppl. 1): 95–108.

Gadbois, D. M., and Lehnert, B. E. 1997. Cell cycle response to DNA damage differs in bronchial epithelial cells and lung fibroblasts. *Cancer Res*. 57:3174–3179.

Ghio, A. J., Stonehuerner, J., Richards, J., and Devlin, R. B. 2008. Iron homeostasis in the lung following asbestos exposure. *Antioxid. Redox Signal*. 10:371–377.

Goldberg, J. L., Zanella, C. L., Janssen, Y. M., Timblin, C. R., Jimenez, L. A., Vacek, P., Taatjes, D. J., and Mossman, B. T. 1997. Novel cell imaging techniques show induction of apoptosis and proliferation in mesothelial cells by asbestos. *Am. J. Respir. Cell Mol. Biol*. 17:265–271.

Guertin, D. A., and Sabatini, D. M. 2007. Defining the role of mTOR in cancer. *Cancer Cell* 12:9–22.

Gulumian, M. 2005. An update on the detoxification processes for silica particles and asbestos fibers: successess and limitations. *J. Toxicol. Environ. Health B* 8:453–483.

Gulumian, M., Bhoolia, D. J., Theodorou, P., Rollin, H. B., Pollak, H., and van Wyk. J. A. 1993. Parameters which determine the activity of the transition metal iron in crocidolite asbestos: ESR, Mossbauer spectroscopic and iron mobilization studies. *S Afr J Sci* 89:405–409.

Hamilton, R. F., Iyer, L. L., and Holian, A. 1996. Asbestos induces apoptosis in human alveolar macrophages. *Am. J. Physiol*. 271: L813–L819.

Hammond, E. C., Garfinkel, L., Seidman, H., and Lew, E. A. 1976. "Tar" and nicotine content of cigarette smoke in relation to death rates. *Environ. Res*. 12:263–274.

Hansteen, I. L., Hilt, B., Lien, J. T., Skaug, V., and Haugen, A. 1993. Karyotypic changes in the preclinical and subsequent stages of malignant mesothelioma: A case report. *Cancer Genet. Cytogenet*. 70:94–98.

Hardy, J. A., and Aust, A. E. 1995a. The effect of iron binding on the ability of crocidolite asbestos to catalyze DNA single-strand breaks. *Carcinogenesis* 16:319–325.

Hardy, J. A., and Aust, A. E. 1995b. Iron in asbestos chemistry and carcinogenicity. *Chem. Rev* 95:97–118.

Hart, G. A., Kathman, L. M., and Hesterberg, T. W. 1994. In vitro cytotoxicity of asbestos and man-made vitreous fibers: Roles of fiber length, diameter and composition. *Carcinogenesis* 15:971–977.

Hart, G. A., Newmen, M. M., Bunn, W. B., and Hesterberg, T. W. 1992. Cytotoxicity of refractory ceramic fibres to Chinese hamster ovary cells in culture. *Toxicol. In Vitro* 6:317–326.

Health Effects Institute–Asbestos Research. 1991. *Asbestos in public and commercial buildings: A literature review and synthesis of current knowledge. Report of the Literature Review Panel*. Cambridge, MA: Health Effects Institute–Asbestos Research.

Hei, T. K., Hall, E. J., and Osmak, R. S. 1984. Asbestos, radiation and oncogenic transformation. *Br. J. Cancer* 50:717–720.

Hei, T. K., He, Z. Y.,and Suzuki, K. 1995. Effects of antioxidants on fiber mutagenesis. *Carcinogenesis* 16:1573–1578.

Hei, T. K., Piao, C. Q., He, Z. Y.,, Vannais, D., and Waldren, C. A. 1992. Chrysotile fiber is a strong mutagen in mammalian cells. *Cancer Res*. 52:6305–6309.

Hei, T. K., Xu, A., Huang, S. X., and Zhao, Y. 2006. Mechanism of fiber carcinogenesis: From reactive radical species to silencing of the beta igH3 gene. *Inhal. Toxicol*. 18: 985–990.

Heintz, N. H., Janssen-Heininger, Y. M., and Mossman, B. T. 2010. Asbestos, lung cancers, and mesotheliomas: From molecular approaches to targeting tumor survival pathways. *Am. J. Respir. Cell Mol. Biol*. 42: 133–139.

Hessel, P. A., Gamble, J. F., and McDonald, J. C. 2005. Asbestos, asbestosis, and lung cancer: A critical assessment of the epidemiological evidence. *Thorax* 60:433–436.

Hesterberg, T. W., and Barrett, J. C. 1984. Dependence of asbestos- and mineral dust-induced transformation of mammalian cells in culture on fiber dimension. *Cancer Res*. 44:2170–2180.

Hesterberg, T. W., and Barrett, J. C. 1985. Induction by asbestos fibers of anaphase abnormalities: Mechanism for aneuploidy induction and possibly carcinogenesis. *Carcinogenesis* 6:473–475.

Hesterberg, T. W., and Hart, G. A. 2001. Synthetic vitreous fibers: A review of toxicology research and its impact on hazard classification. *Crit. Rev. Toxicol*. 31:1–53.

Hevel, J., L. Olson-Buelow, L., Ganesan, B., Stevens, J., Hardman, J., and Aust, A. E. 2008. Novel functional view of the crocidolite asbestos-treated A549 human lung epithelial transcriptome reveals an intricate network of pathways with opposing functions. *BMC Genomics* 9:376.

Hirao, T., Bueno, R., Chen, C.-J., Gordon, G. J., Heilig, E., and Kelsey, K. T. 2002. Alterations of the p16INK4 locus in human malignant mesothelial tumors. *Carcinogenesis* 23:1127–1130.

Hodgson, J. T., and Darnton, A. 2000. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann. Occup. Hyg*. 44:565–601.

Horska, A., Kazimirova, A., Barancokova, M., Wsolova, L., Tulinska, J., and Dusinska, M. 2006. Genetic predisposition and health effect of occupational exposure to asbestos. *Neuro Endocrinol Lett* 27(suppl. 2):100–103.

Howden, P. J., and S. P. Faux. 1996a. Fibre-induced lipid peroxidation leads to DNA adduct formation in *Salmonella typhimurium* TA104 and rat lung fibroblasts. *Carcinogenesis* 17:413–419.

Howden, P. J., and Faux, S. P. 1996b. Short communication: Glutathione modulates the formation of 8-hydroxydeoxyguannosine in isolated DNA and mutagenicity in *Salmonella typhimurium* TA100 induced by mineral fibres. *Carcinogenesis* 17:2275–2277.

Huang, S. L. 1979. Amosite, chrysotile and crocidolite asbestos are mutagenic in Chinese hamster lung cells. *Mutat. Res*. 68:265–274.

Huang, S. L., Saggioro, D., Michelmann, H., and Malling, H. V. 1978. Genetic effects of crocidolite asbestos in Chinese hamster lung cells. *Mutat. Res*. 57:225–232.

Hume, L. A., and Rimstidt, J. D. 1992. The biodurability of chrysotile asbestos. *Am. Mineral*. 77:1125–1128.

Husgafvel-Pursiainen, K., Kannio, A., Oksa, P., Suitiala, T., Koskinen, H., Partanen, R., Hemminki, K., Smith, S., Rosenstock-Leibu, R., and Brandt-Rauf, P. W. 1997. Mutations, tissue accumulations, and serum levels of p53 in patients with occupational cancers from asbestos and silica exposure. *Environ. Mol. Mutagen*. 30:224–230.

Husgafvel-Pursiainen, K., Karjalainen, A., Kannio, A., Anttila, S., Partanen, T., Ojajarvi, A., and Vainio, H. 1999. Lung cancer and past occupational exposure to asbestos. Role of p53 and k-ras mutations. *Am. J. Respir. Cell Mol. Biol*. 20:667–674.

IARC. 1987. .Overall evaluations of carcinogenicity: An updating of IARC monographs volumes 1 to 42. *IARC Monogr. Eval. Carcinogen. Risks Hum*. Lyon, France: International Agency for Research on Cancer, World Health Organization.

Iguchi, H., Kojo, S., and Ikeda, M. 1996. Nitric oxide (NO) synthase activity in the lung and NO synthesis in alveolar macrophages of rats increased on exposure to asbestos. *J. Appl. Toxicol*. 16:309–315.

ILSI Risk Science Institute Workshop Participants. 2000. The relevance of the rat lung response to particle overload for human risk assessment: a workshop consensus report. ILSI Risk Science Institute Workshop Participants. *Inhal. Toxicol*. 12:1–17.

Institute of Medicine (US) Committee on Asbestos: Selected Health Effects. 2006. *Asbestos: Selected cancers, The National Academies Collection: Reports funded by National Institutes of Health*. Washington, DC: National Academies Press.

Ivanov, S. V., Miller, J., Lucito, R., Tang, C., Ivanova, A. V., Pei, J., Carbone, M., Cruz, C., Beck, A., Webb, C., Nonaka, D., Testa, J. R., and Pass, H. I. 2009. Genomic events associated with progression of pleural malignant mesothelioma. *Int. J. Cancer* 124: 589–599.

Jackson, J. H., Schraufstatter, I. U., Hyslop, P. A., Vosbeck, K., Sauerheber, R., Weitzman, S. A., and Cochrane, C. G. 1987. Role of oxidants in DNA damage. Hydroxyl radical mediates the synergistic DNA damaging effects of asbestos and cigarette smoke. *J. Clin. Invest*. 80:1090–1095.

Jackson, M. A., Stack, H. F., and Waters, M. D. 1993. The genetic toxicology of putative nongenotoxic carcinogens. *Mutat. Res*.. 296:241–277.

Janicke, R. U., Sohn, D., and Schulze-Osthoff, K. 2008. The dark side of a tumor suppressor: Anti-apoptotic p53. *Cell Death Different*. 15:959–976.

Janssen, Y. M. W., Heintz, N. H., and Mossman, B. T. 1995. Induction of c-fos and c-jun proto-oncogene expression by asbestos is ameliorated by *N*-acetyl-L-cysteine in mesothelial cells. *Cancer Res*. 55:2085–2089.

Jaurand, M. C. 1997. Mechanisms of fiber-induced genotoxicity. *Environ. Health Perspect*. 105(suppl. 5):1073–1084.

Jaurand, M. C. 2006. 59 Asbestos, chromosomal deletions and tumor suppressor gene alterations in human malignant mesothelioma. *Lung Cancer* 54:S15–S15.

Jaurand, M. C., Fleury, J., Monchaux, G., Nebut, M., and Bignon, J. 1987. Pleural carcinogenic potency of mineral fibers (asbestos, attapulgite) and their cytotoxicity on cultured cells. *JNCI* 79:797–804.

Jaurand, M. C., Kheuang, L., Magne, L., and Bignon, J. 1986. Chromosomal changes induced by chrysotile fibres or benzo-3,4-pyrene in rat pleural mesothelial cells. *Mutat. Res*.. 169:141–148.

Jensen, C. G., Jensen, L. C., Rieder, C. L., Cole, R. W., and Ault, J. G. 1996. Long crocidolite asbestos fibers cause polyploidy by sterically blocking cytokinesis. *Carcinogenesis* 17:2013–2021.

Jensen, C. G., Jensen, L. C. W., Ault, J. G., Cole, R., and Rieder, C. C. 1994. Time-lapse video light microscopic and electron microscopic observations of vertebrate epithelial cells exposed to crocidolite asbestos. In *Cellular and molecular effects of mineral and synthetic dusts and fibres*, eds. J. Davis, M. G. Jaurand, et al., pp. 63–78 NATO ASI series. Series H:85, Cell biology. New York: Springer-Verlag.

Jiang, L., Nagai, H., Ohara, H., Hara, S., Tachibana, M., Hirano, S., Shinohara, Y., Kohyama, N., Akatsuka, S., and Toyokuni, S. 2008. Characteristics and modifying factors of asbestos-induced oxidative DNA damage. *Cancer Sci*. 99:2142–2151.

Jimenez, L. A., Zanella, C., Fung, H., Janssen, Y. M., Vacek, P., Charland, C., Goldberg, J., and Mossman, B. T. 1997. Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and H_2O_2. *Am. J. Physiol*. 273:L1029–L1035.

Johnson, N. F., and Jaramillo, R. J. 1997. p53, Cip1, and Gadd153 expression following treatment of A549 cells with natural and man-made vitreous fibers. *Environ. Health Perspect*. 105(suppl. 5):1143–1145.

Jongsma, J., van Montfort, E., Vooijs, M., Zevenhoven, J., Krimpenfort, P., van der Valk, M., van de Vijver, M., and Berns, A. 2008. A conditional mouse model for malignant mesothelioma. *Cancer Cell* 13: 261–271.

Jung, M., Davis, W. P., Taatjes, D. J., Churg, A., and Mossman, B. T. 2000. Asbestos and cigarette smoke cause increased DNA strand breaks and necrosis in bronchiolar epithelial cells in vivo. *Free Radical Biol. Med*. 28:1295–1299.

Kamp, D. W. 2009. Asbestos-induced lung diseases: an update. *Transl. Res*. 153:143–152.

Kamp, D. W., Graceffa, P., Pryor, W. A., and Weitzman, S. A. 1992. The role of free radicals in asbestos-induced diseases. *Free Radical Biol. Med*. 12:293–315.

Kamp, D. W., Greenberger, M. J., Sbalchierro, J. S., Preusen, S. E., and Weitzman, S. A. 1998. Cigarette smoke augments asbestos-induced alveolar epithelial cell injury: Role of free radicals. *Free Radical Biol. Med*. 25:728–739.

Kamp, D. W., Israbian, V. A., Preusen, S. E., Zhang, C. X., and Weitzman, S. A. 1995. Asbestos causes DNA strand breaks in cultured pulmonary epithelial cells: Role of iron-catalyzed free radicals. *Am. J. Physiol*. 268:L471–L480.

Kamp, D. W., Panduri, V. Weitzman, S. A., and Chandel, N. 2002. Asbestos-induced alveolar epithelial cell apoptosis: Role of mitochondrial dysfunction caused by iron-derived free radicals. *Mol. Cell. Biochem*. 234–235:153–160.

Kamp, D. W., Srinivasan, M., and Weitzman, S. A. 2001. Cigarette smoke and asbestos activate poly-ADP-ribose polymerase in alveolar epithelial cells. *J. Invest. Med*. 49:68–76.

Kamp, D. W., and Weitzman, S. A. 1999. The molecular basis of asbestos induced lung injury. *Thorax* 54:638–652.

Kane, A. B. 1992. Animal models of mesothelioma induced by mineral fibers: implications for human risk assessment. *Prog. Clin. Biol. Res*. 374:37–50.

Kane, A. B. 1996. Mechanisms of mineral fibre carcinogenesis. *IARC Sci. Publ*. 140:11–34.

Kane, A. B. 2006. Animal models of malignant mesothelioma. *Inhal. Toxicol*. 18: 1001–1004.

Kelsey, K. T., Yano, E., Liber, H. L., and Little, J. B. 1986. The in vitro genetic effects of fibrous erionite and crocidolite asbestos. *Br. J. Cancer* 54:107–114.

Kenne, K., Ljungquist, S., and Ringertz, N. R. 1986. Effects of asbestos fibers on cell division, cell survival, and formation of thioguanine-resistant mutants in Chinese hamster ovary cells. *Environ. Res*. 39: 448–464.

Kettunen, E., Aavikko, M., Nymark, P., Ruosaari, S., Wikman, H., Vanhala, E., Salmenkivi, K., Pirinen, R., Karjalainen, A., Kuosma, E., and Anttila, S. 2009. DNA copy number loss and allelic imbalance at 2p16 in lung cancer associated with asbestos exposure. *Br. J. Cancer* 100:1336–1342.

Kido, T., Morimoto, Y., Asonuma, E., Yatera, K., Ogami, A., Oyabu, T., Tanaka, I., and Kido, M. 2008. Chrysotile asbestos causes AEC apoptosis via the caspase activation in vitro and in vivo. *Inhal. Toxicol*. 20:339–347.

Kim, H. N., Morimoto, Y., Tsuda, T., Ootsuyama, Y., Hirohashi, M., Hirano, T., Tanaka, I., Lim, Y., Yun, I. G., and Kasai, H. 2001. Changes in DNA 8-hydroxyguanine levels, 8-hydroxyguanine repair activity, and hOGG1 and hMTH1 mRNA expression in

human lung alveolar epithelial cells induced by crocidolite asbestos. *Carcinogenesis* 22:265–269.

Kim, I., Xu, W. J., and Reed, J. C. 2008. Cell death and endoplasmic reticulum stress: Disease relevance and therapeutic opportunities. *Nat. Rev. Drug Discov*. 7:1013–1030.

Kociok, N., Unfried, K., Roller, M., and Dehnen, W. 1999. DNA fingerprint analysis reveals differences in mutational patterns in experimentally induced rat peritoneal tumors, depending on the type of environmental mutagen. *Cancer Genet. Cytogenet*. 111:71–76.

Kopnin, P. B., Kravchenko, I. V., Furalyov, V. A., Pylev, L. N., and Kopnin, B. P. 2004. Cell type-specific effects of asbestos on intracellular ROS levels, DNA oxidation and G1 cell cycle checkpoint. *Oncogene* 23: 8834–8840.

Korkina, L. G., Durnev, A. D., Suslova, T. B., Cheremisina, Z. P., Daugel-Dauge, N. O., and Afanas'ev, I. B. 1992. Oxygen radical-mediated mutagenic effect of asbestos on human lymphocytes: Suppression by oxygen radical scavengers. *Mutat. Res*. 265: 245–253.

Kroemer, G., Galluzzi, L., and Brenner, C. 2007. Mitochondrial membrane permeabilization in cell death. *Physiol. Rev* 87: 99–163.

Kundu, J. K., and Surh, Y. J. 2008. Inflammation: gearing the journey to cancer. *Mutat. Res*.. 659:15–30.

Lavappa, K. S., Fu, M. M., and Epstein, S. S. 1975. Cytogenetic studies on chrysotile asbestos. *Environ. Res*. 10:165–173.

Lechner, J. F., Tokiwa, T., LaVeck, M., Benedict, W. F., Banks-Schlegel, S., Yeager, H., Jr., Banerjee, A., and Harris, C. C. 1985. Asbestos-associated chromosomal changes in human mesothelial cells. *Proc. Natl. Acad. Sci. USA* 82:3884–3888.

Lecomte, C., Andujar, P., Renier, A., Kheuang, L., Abramowski, V., Mellottee, L., Fleury-Feith, J., Zucman-Rossi, J., Giovannini, M., and Jaurand, M. C. 2005. Similar tumor suppressor gene alteration profiles in asbestos-induced murine and human mesothelioma. *Cell Cycle* 4:1862–1869.

Levresse, V., Moritz, S., Renier, A., Kheuang, L., Galateau-Salle, F., Mege, J. P., Piedbois, P., Salmons, B., Guenzburg, W., and Jaurand, M.. C. 1998. Effect of simian virus large T antigen expression on cell cycle control and apoptosis in rat pleural mesothelial cells exposed to DNA damaging agents. *Oncogene* 16:1041–1053.

Levresse, V., Renier, A., Fleury-Feith, J., Levy, F., Moritz, S., Vivo, C., Pilatte, Y., and Jaurand, M. C. 1997. Analysis of cell cycle disruptions in cultures of rat pleural mesothelial cells exposed to asbestos fibers. *Am. J. Respir. Cell Mol. Biol*. 17:660–671.

Levresse, V., Renier, A., Levy, F., Broaddus, V. C., and Jaurand, M. 2000. DNA breakage in asbestos-treated normal and transformed (TSV40) rat pleural mesothelial cells. *Mutagenesis* 15:239–244.

Libbus, B. L., Illenye, S. A., and Craighead, J. E. 1989. Induction of DNA strand breaks in cultured rat embryo cells by crocidolite asbestos as assessed by nick translation. *Cancer Res*. 49:5713–5718.

Lin, F., Liu, Y. G., Liu, Y. Q., Keshava, N., and Li, S. Q. 2000. Crocidolite induces cell transformation and p53 gene mutation in BALB/c-3T3 cells. *Teratogen. Carcinogen. Mutagen*. 20:273–281.

Lindholm, P. M., Salmenkivi, K., Vauhkonen, H., Nicholson, A. G., Anttila, S., Kinnula, V. L., and Knuutila, S. 2007. Gene copy number analysis in malignant pleural mesothelioma using oligonucleotide array CGH. *Cytogenet. Genome Res*. 119:46–52.

Lindholm, P., Nymark, P., Wikman, H., Salmenkivi, K., Nicholson, A., Korpela, M.V., Kaski, S., Ruosaari, S., Hollmén, J., Vanhala, E., Karjalainen, A., Anttila, S., Kinnula, V., and Knuutila, S. 2006. 60 Asbestos-associatedmalignancies in the lung and pleura show distinct genetic aberrations. *Lung Cancer* 54:S15–S15.

Lippmann, M. 1994. Deposition and retention of inhaled fibres: Effects on incidence of lung cancer and mesothelioma. *Occup Environ Med* 51:793–798.

Lipworth, L., La Vecchia, C., Bosetti, C., and McLaughlin, J. K. 2009. Occupational exposure to rock wool and glass wool and risk of cancers of the lung and the head and neck: A systematic review and meta-analysis. *J. Occup. Environ. Med*. 51:1075–1087.

Liu, B. C., Fu, D. C., Miao, Q., Wang, H. H., and You, B. R. 1998. p53 gene mutations in asbestos associated cancers. *Biomed. Environ. Sci*. 11:226–232.

Liu, G., Beri, R., Mueller, A., and Kamp, D. W. 2010. Molecular mechanisms of asbestos-induced alveolar epithelial cell death. *Chem. Biol. Interact*. 188:309–318.

Liu, W., Ernst, J. D., and Broaddus, V. C. 2000. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage, and apoptosis in mesothelial cells. *Am. J. Respir. Cell Mol. Biol*. 23:371–378.

Livingston, G. K., Rom, W. N., and Morris, M. V. 1980. Asbestos-induced sister chromatid exchanges in cultured Chinese hamster ovarian fibroblast cells. *J. Environ. Pathol. Toxicol*. 4:373–382.

Loli, P., Topinka, J., Georgiadis, P., Dusinska, M., Hurbankova, M.., Kovacikova, Z., Volkovova, K., Wolff, T., Oesterle, D., and Kyrtopoulos, S. A. 2004. Benzo[a]pyrene-enhanced mutagenesis by asbestos in the lung of lambda-lacI transgenic rats. *Mutat. Res*. 553:79–90.

Lu, J., Keane, M. J., Ong, T., and Wallace, W. E. 1994. In vitro genotoxicity studies of chrysotile asbestos fibers dispersed in simulated pulmonary surfactant. *Mutat. Res*. 320:253–259.

Lu, Y. P., Lasne, C., Lowy, R., and Chouroulinkov, I. 1988. Use of the orthogonal design method to study the synergistic effects of asbestos fibres and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in the BALB/3T3 cell transformation system. *Mutagenesis* 3:355–362.

Lund, L. G., and Aust, A. E. 1990. Iron mobilization from asbestos by chelators and ascorbic acid. *Arch. Biochem. Biophys*. 278:61–64.

Lund, L. G., and Aust, A. E. 1992. Iron mobilization from crocidolite asbestos greatly enhances crocidolite-dependent formation of DNA single-strand breaks in phi X174 RFI DNA. *Carcinogenesis* 13:637–642.

Lund, L. G., Williams, M. G., Dodson, R. F., and Aust, A. E. 1994. Iron associated with asbestos bodies is responsible for the formation of single strand breaks in phi X174 RFI DNA. *Occup. Environ. Med*. 51: 200–204.

Mahmood, N., Khan, S. G., Athar, M., and Rahman, Q. 1994. Differential role of hydrogen peroxide and organic peroxides in augmenting asbestos-mediated DNA damage: Implications for asbestos induced carcinogenesis. *Biochem. Biophys. Res. Commun*. 200:687–694.

Manning, C. B., Sabo-Attwood, T., Robledo, R. F., MacPherson, M. B., Rincon, M., Vacek, P., Hemenway, D., Taatjes, D. J., Lee, P. J., and Mossman, B. T. 2008. Targeting the MEK1 cascade in lung epithelium inhibits proliferation and fibrogenesis by asbestos. *Am. J. Respir. Cell Mol. Biol*. 38:618–626.

Manning, C. B., Vallyathan, V., and Mossman, B. T. 2002. Diseases caused by asbestos: Mechanisms of injury and disease development. *Int. Immunopharmacol*. 2:191–200.

Marchi, E., Liu, W., and Broaddus, V. C. 2000. Mesothelial cell apoptosis is confirmed in vivo by morphological change in cytokeratin distribution. *Am. J. Physiol. Lung Cell. Mol. Physiol*. 278:L528–L535.

Marsella, J. M., Liu, B. L., Vaslet, C. A., and Kane, A. B. 1997. Susceptibility of p53-deficient mice to induction of mesothelioma by crocidolite asbestos fibers. *Environ. Health Perspect*. 105(suppl. 5):1069–1072.

Mast, R. W., McConnell, E. E., Anderson, R., Chevalier, J., Kotin, P., Bernstein, D. M., Thevenaz, P., Glass, L. R., Miiller, W. C., and Hesterberg, T. W. 1995. Studies on the chronic toxicity (inhalation) of four types of refractory ceramic fiber in male Fischer 344 rats. *Inhal. Toxicol*. 7:425–467.

Matsuoka, M., Igisu, H., and Morimoto, Y. 2003. Phosphorylation of p53 protein in A549 human pulmonary epithelial cells

exposed to asbestos fibers. *Environ. Health Perspect*. 111:509–512.

McBride, T. J., Preston, B. D., and Loeb, L. A. 1991. Mutagenic spectrum resulting from DNA damage by oxygen radicals. *Biochemistry* 30:207–213.

Mikalsen, S. O., Rivedal, E., Sanner, T. 1988. Morphological transformation of Syrian hamster embryo cells induced by mineral fibres and the alleged enhancement of benzo[a]pyrene. *Carcinogenesis* 9:891–899.

Miles, S. E., Sandrini, A., Johnson, A. R., and Yates, D. H. 2008. Clinical consequences of asbestos-related diffuse pleural thickening: A review. *J. Occup. Med. Toxicol*. 3:1–10.

Mishra, A., Liu, J. Y., Brody, A. R., and Morris, G. F. 1997. Inhaled asbestos fibers induce p53 expression in the rat lung. *Am. J. Respir. Cell Mol. Biol*. 16:479–485.

Miura, Y., Nishimura, Y., Katsuyama, H., Maeda, M., Hayashi, H., Dong, M., Hyodoh, F., Tomita, M., Matsuo, Y., Uesaka, A., Kuribayashi, K., Nakano, T., Kishimoto, T., and Otsuki, T. 2006. Involvement of IL-10 and Bcl-2 in resistance against an asbestos-induced apoptosis of T cells. *Apoptosis* 11:1825–1835.

Monchaux, G., Bignon, J., Jaurand, M. C., Lafuma, J., Sebastien, P., Masse, R., Hirsch, A., and Goni, J. 1981. Mesotheliomas in rats following inoculation with acid-leached chrysotile asbestos and other mineral fibres. *Carcinogenesis* 2:229–236.

Morris, G. F., Notwick, A. R., David, O., Fermin, C., Brody, A. R., and Friedman, M. 2004. Development of lung tumors in mutant p53-expressing mice after inhalation exposure to asbestos. *Chest* 125:85S–86S.

Mossman, B. T. 1990. In vitro studies on the biologic effects of fibers: Correlation with in vivo bioassays. *Environ. Health Perspect*. 88:319–322.

Mossman, B. T. 1993. Mechanisms of asbestos carcinogenesis and toxicity: The amphibole hypothesis revisited. *Br. J. Ind. Med*. 50:673–676.

Mossman, B. T., and Churg, A. 1998. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am. J. Respir. Crit. Care Med*. 157:1666–1680.

Msiska, Z., acurari, M., Mishra, A., Leonard, S. S., Castranova, V., and Vallyathan, V. 2009. DNA double-strand breaks by asbestos, silica, and titanium dioxide: Possible biomarker of carcinogenic potential? *Am. J. Respir. Cell Mol. Biol*. 43:210–219.

Murata-Kamiya, N., Tsutsui, T., Fujino, A., Kasai, H., and Kaji, H. 1997. Determination of carcinogenic potential of mineral fibers by 8-hydroxydeoxyguanosine as a marker of oxidative DNA damage in mammalian cells. *Int. Arch. Occup. Environ. Health* 70:321–326.

Murthy, S. S., and Testa, J. R. 1999. Asbestos, chromosomal deletions, and tumor suppressor gene alterations in human malignant mesothelioma. *J. Cell Physiol*. 180: 150–157.

Musti, M., Kettunen, E., Dragonieri, S., Lindholm, P., Cavone, D., Serio, G., and Knuutila, S. 2006. Cytogenetic and molecular genetic changes in malignant mesothelioma. *Cancer Genet. Cytogenet*. 170:9–15.

Mutsaers, S. E., Prele, C. M., Brody, A. R., and Idell, S. 2004. Pathogenesis of pleural fibrosis. *Respirology* 9:428–440.

Narasimhan, S. R., Yang, L., Gerwin, B. I., and Broaddus, V. C. 1998. Resistance of pleural mesothelioma cell lines to apoptosis: Relation to expression of Bcl-2 and Bax. *Am. J. Physiol*. 275:L165–L171.

Nelson, A., Mendoza, T., Hoyle, G. W., Brody, A. R., Fermin, C., and Morris, G. F. 2001. Enhancement of fibrogenesis by the p53 tumor suppressor protein in asbestos-exposed rodents. *Chest* 120:33S–34S.

Nelson, H. H., and Kelsey, K. T. 2002. The molecular epidemiology of asbestos and tobacco in lung cancer. *Oncogene* 21:7284–7288.

Neragi-Miandoab, S., and Sugarbaker, D. J. 2009. Chromosomal deletion in patients with malignant pleural mesothelioma. *Interact. Cardiovasc. Thorac. Surg*. 9:42–44.

Nuorva, K., R. Makitaro, E. Huhti, D. Kamel, K. Vahakangas, R. Bloigu, Y. Soini, and P.

Paakko. 1994. p53 protein accumulation in lung carcinomas of patients exposed to asbestos and tobacco smoke. *Am. J. Respir. Crit. Care Med*. 150:528–533.

Nygren, J., Suhonen, S., Norppa, H., and Linnainmaa, K. 2004. DNA damage in bronchial epithelial and mesothelial cells with and without associated crocidolite asbestos fibers. *Environ. Mol. Mutagen*. 44:477–482.

Nymark, P., Kettunen, E., Aavikko, M., Ruosaari, S., Kuosma, E., Vanhala, E., Salmenkivi, K., Pirinen, R.,. Karjalainen, A., Knuutila, S.,. Wikman, H., andAnttila, S. 2009. Molecular alterations at 9q33.1 and polyploidy in asbestos-related lung cancer. *Clin. Cancer Res*. 15:468–475.

Nymark, P., Lindholm, P. M., Korpela, M. V., Lahti, L., Ruosaari, S., Kaski, S., Hollmen, J., Anttila, S., Kinnula, V. L., and Knuutila, S. 2007. Gene expression profiles in asbestos-exposed epithelial and mesothelial lung cell lines. *BMC Genomics* 8:1–17.

Nymark, P., Wikman, H., Hienonen-Kempas, T., and Anttila, S. 2008. Molecular and genetic changes in asbestos-related lung cancer. *Cancer Lett*. 265:1–15.

Nymark, P., Wikman, H., Ruosaari, S., Hollmén, J., Vanhala, E., Karjalainen, A., Anttila, S., and S. Knuutila. 2006. Identification of specific gene copy number changes in asbestos-related lung cancer. *Cancer Res*. 66:5737–5743.

Oberdorster, G. 1995. Lung particle overload: implications for occupational exposures to particles. *Regul. Toxicol. Pharmacol*. 21:123–135.

Oberdorster, G. 2002. Toxicokinetics and effects of fibrous and nonfibrous particles. *Inhal. Toxicol*. 14:29–56.

Oberdorster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., Olin, S., Monteiro-Riviere, N., Warheit, D., and Yang, H. 2005. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part. Fibre Toxicol*. 2:1–35.

Okayasu, R., Takahashi, S., Yamada, S., Hei, T. K., and Ullrich, R. L. 1999a. Asbestos and DNA double strand breaks. *Cancer Res*. 59:298–300.

Okayasu, R., Wu, L., and Hei, T. K. 1999b. Biological effects of naturally occurring and man-made fibres: In vitro cytotoxicity and mutagenesis in mammalian cells. *Br. J. Cancer* 79:1319–1324.

Ollikainen, T., Linnainmaa, K., and Kinnula, V. L. 1999. DNA single strand breaks induced by asbestos fibers in human pleural mesothelial cells in vitro. *Environ. Mol. Mutagen*. 33:153–160.

Ollikainen, T., Puhakka, A., Kahlos, K., Linnainmaa, K., and Kinnula, V. L. 2000. Modulation of cell and DNA damage by poly(ADP)ribose polymerase in lung cells exposed to H_2O_2 or asbestos fibres. *Mutat. Res*.. 470:77–84.

Osgood, C., and Sterling, D. 1991. Chrysotile and amosite asbestos induce germ-line aneuploidy in *Drosophila*. *Mutat. Res*.. 261:9–13.

Oshimura, M., Hesterberg, T. W., Tsutsui, T., and Barrett, J. C. 1984. Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture. *Cancer Res*. 44:5017–5022.

Paakko, P., Ramet, M., Vahakangas, K., Korpela, N., Soini, Y., Turunen, S., Jaworska, M., and Gillissen, A. 1998. Crocidolite asbestos causes an induction of p53 and apoptosis in cultured A-549 lung carcinoma cells. *Apoptosis* 3:203–212.

Palekar, L. D., Eyre, J. F., Most, B. M., and Coffin, D. L. 1987. Metaphase and anaphase analysis of V79 cells exposed to erionite, UICC chrysotile and UICC crocidolite. *Carcinogenesis* 8:553–560.

Palozza, P. 2005. Can beta-carotene regulate cell growth by a redox mechanism? An answer from cultured cells. *Biochim. Biophys. Acta* 1740:215–221.

Panduri, V., Weitzman, S. A., Chandel, N. S., and Kamp, D. W. 2004. Mitochondrial-derived free radicals mediate asbestos-induced alveolar epithelial cell apoptosis.

Am. J. Physiol. Lung Cell. Mol. Physiol. 286:L1220–L1227.
Panduri, V., Liu, G., Surapureddi, S., Kondapalli, J., Soberanes, S., de Souza-Pinto, N. C., Bohr, V. A. Budinger, G. R. S., Schumacker, P. T., Weitzman, S. A., and Kamp, D. W. 2009. Role of mitochondrial hOGG1 and aconitase in oxidant-induced lung epithelial cell apoptosis. *Free Radical Biol. Med*. 47:750–759.
Panduri, V., Surapureddi, S., Soberanes, S., Weitzman, S. A., Chandel, N., and Kamp, D. W. 2006. P53 mediates amosite asbestos-induced alveolar epithelial cell mitochondria-regulated apoptosis. *Am. J. Respir. Cell Mol. Biol*. 34:443–452.
Panduri, V., Weitzman, S. A., Chandel, N., and Kamp, D. W. 2003. The mitochondria-regulated death pathway mediates asbestos-induced alveolar epithelial cell apoptosis. *Am. J. Respir. Cell Mol. Biol*. 28:241–248.
Park, S. H., and Aust, A. E. 1998. Participation of iron and nitric oxide in the mutagenicity of asbestos in hgprt-, gpt+ Chinese hamster V79 cells. *Cancer Res*. 58:1144–1148.
Pelin, K., Hirvonen, A., Taavitsainen, M., and Linnainmaa, K. 1995a. Cytogenetic response to asbestos fibers in cultured human primary mesothelial cells from 10 different donors. *Mutat. Res*. 334:225–233.
Pelin, K., Husgafvel-Pursiainen, K., Vallas, M., Vanhala, E., and Linnainmaa, K. 1992. Cytotoxicity and anaphase aberrations induced by mineral fibres in cultured human mesothelial cells. *Toxicol In Vitro* 6:445–450.
Pelin, K., Kivipensas, P., and Linnainmaa, K. 1995b. Effects of asbestos and man-made vitreous fibers on cell division in cultured human mesothelial cells in comparison to rodent cells. *Environ. Mol. Mutagen*. 25:118–125.
Petrilli, V., Dostert, C., Muruve, D. A., and Tschopp, J. 2007. The inflammasome: A danger sensing complex triggering innate immunity. *Curr. Opin. Immunol*. 19:615–622.
Pietruska, J. R., and Kane, A. B. 2007. SV40 oncoproteins enhance asbestos-induced DNA double-strand breaks and abrogate senescence in murine mesothelial cells. *Cancer Res*. 67:3637–3645.
Poser, I., Rahman, Q., Lohani, M., Yadav, S., Becker, H. H., Weiss, D. G., Schiffmann, D., and Dopp, E. 2004. Modulation of genotoxic effects in asbestos-exposed primary human mesothelial cells by radical scavengers, metal chelators and a glutathione precursor. *Mutat. Res*. 559:19–27.
Price-Jones, M. J., Gubbings, G., and Chamberlain, M. 1980. The genetic effects of crocidolite asbestos: Comparison of chromosome abnormalities and sister chromatid changes. *Mutat. Res*. 79:331–336.
Puhakka, A., Ollikainen, T., Soini, Y., Kahlos, K., Saily, M., Koistinen, P., Paakko, P., Linnainmaa, K., and Kinnula, V. L. 2002. Modulation of DNA single-strand breaks by intracellular glutathione in human lung cells exposed to asbestos fibers. *Mutat. Res*.. 514:7–17.
Pylkkanen, L., Sainio, M., Ollikainen, T., Mattson, K., Nordling, S., Carpen, O., Linnainmaa, K., and Husgafvel-Pursiainen, K. 2002. Concurrent LOH at multiple loci in human malignant mesothelioma with preferential loss of NF2 gene region. *Oncol. Rep*. 9:955–959.
Quinlan, T. R., BeruBe, K. A., Hacker, M. P., Taatjes, D. J., Timblin, C. R., Goldberg, J., Kimberley, P., O'Shaughnessy, P., Hemenway, D., Torino, J., Jimenez, L. A., and Mossman, B. T. 1998. Mechanisms of asbestos-induced nitric oxide production by rat alveolar macrophages in inhalation and in vitro models. *Free Radical Biol. Med*. 24:778–788.
Reinis, Z., Gvozdjak, J., Widimsky, J., and Prerovsky, J. 1979. The fiftieth anniversary of the Czechoslovak Society of Cardiology. *Cor Vasa* 21:445–449.
Reiss, B., Solomon, S., Tong, C., Levenstein, M., Rosenberg, S. H., and Williams, G. M. 1982. Absence of mutagenic activity of three forms of asbestos in liver epithelial cells. *Environ. Res*. 27:389–397.
Reiss, B., Tong, C., Telang, S., and Williams, G. M. 1983. Enhancement of

benzo[a]pyrene mutagenicity by chrysotile asbestos in rat liver epithelial cells. *Environ. Res*. 31:100–104.

Rihn, B., Coulais, C., Kauffer, E., Bottin, M. C., Martin, P., Yvon, F., Vigneron, J. C., Binet, S., Monhoven, N., Steiblen, G., and Keith, G. 2000. Inhaled crocidolite mutagenicity in lung DNA. *Environ. Health Perspect*.. 108:341–346.

Rita, P., and Reddy, P. P. 1986. Effect of chrysotile asbestos fibers on germ cells of mice. *Environ. Res*. 41:139–143.

Rogers, A., and Major, G. 2002. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure: The Wittenoom data. *Ann. Occup. Hyg*. 46:127–128; author reply 128–129.

Roggli, V. L., Oury, T. D., and Sporn, T. A. 2004. *Pathology of asbestos-associated diseases*, 2nd ed. New York: Springer.

Rom, W. N., Livingston, G. K., Casey, K. R., Wood, S. D., Egger, M. J., Chiu, G. L., and Jerominski, L. 1983. Sister chromatid exchange frequency in asbestos workers. *JNCI* 70:45–48.

Rom, W. N., Travis, W. D., and Brody, A. R. 1991. Cellular and molecular basis of the asbestos-related diseases. *Am. Rev. Respir. Dis*. 143:408–422.

Sanchez, V. C., Pietruska, J. R., Miselis, N. R., Hurt, R. H., and Kane, A. B. 2009. Biopersistence and potential adverse health impacts of fibrous nanomaterials: What have we learned from asbestos? *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol*. 1:511–529.

Saracci, R. 1987. The interactions of tobacco smoking and other agents in cancer etiology. *Epidemiol Rev* 9:175–193.

Schapira, R. M., Ghio, A. J., Effros, R. M., Morrisey, J., Dawson, C. A., and Hacker, A. D. 1994. Hydroxyl radicals are formed in the rat lung after asbestos instillation in vivo. *Am. J. Respir. Cell Mol. Biol*. 10:573–579.

Schins, R. P. F., and Hei, T. K. 2006. Genotoxic effects of particles. In *Particle toxicology*, eds. K. Donaldson and P. Borm, pp. 285–298. Boca Raton, FL: CRC Press/Taylor & Francis,

Schürkes, C., Brock, W., Abel, J., and Unfried, K. 2004. Induction of 8-hydroxydeoxyguanosine by man made vitreous fibres and crocidolite asbestos administered intraperitoneally in rats. *Mutat. Res./Fundam. Mol. Mech. Mutagen*. 553:59–65.

Sekido, Y. 2010. Genomic abnormalities and signal transduction dysregulation in malignant mesothelioma cells. *Cancer Sci*. 101:1–6.

Selikoff, I. J., Hammond, E. C., and Churg, J. 1968. Asbestos exposure, smoking, and neoplasia. *J. Am. Med. Assoc*. 204:106–112.

Shukla, A., Gulumian, M., Hei, T. K., Kamp, D., Rahman, Q., and Mossman, B. T. 2003a. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. *Free Radical Biol. Med*. 34:1117–1129.

Shukla, A., Jung, M., Stern, M., Fukagawa, N. K., Taatjes, D. J., Sawyer, D., Van Houten, B., and Mossman, B. T. 2003b. Asbestos induces mitochondrial DNA damage and dysfunction linked to the development of apoptosis. *Am. J. Physiol. Lung Cell. Mol. Physiol*. 285:L1018–L1025.

Shukla, A., Stern, M., Lounsbury, K. M., Flanders, T., and Mossman, B. T. 2003c. Asbestos-induced apoptosis is protein kinase C{delta}-dependent. *Am. J. Respir. Cell Mol. Biol*. 29:198–205.

Sincock, A. M., Delhanty, J. D., and Casey, G. 1982. A comparison of the cytogenetic response to asbestos and glass fibre in Chinese hamster and human cell lines. Demonstration of growth inhibition in primary human fibroblasts. *Mutat. Res*.. 101:257–268.

Sincock, A., and Seabright, M. 1975. Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres. *Nature* 257:56–58.

Solomon, E., Borrow, J., and Goddard, A. D. 1991. Chromosome aberrations and cancer. *Science* 254:1153–1160.

Stanton, F., Layard, M., Tegeris, A., Miller, E., May, M., and Kent, E. 1977. Tumorigenicity of fibrous glass: Pleural response in the rat in relation to fiber dimension. *J. Natl. Cancer Inst*. 58:587–603.

Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M.„ Morgan, E., and Smith, A. 1981. Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. *JNCI* 67:965–975.

Stern, M., Taatjes, D. J., and Mossman, B. T. 2006. Multifluorescence labeling techniques and confocal laser scanning microscopy on lung tissue. In *Cell imaging techniques: Methods and protocols*., eds. by B. T. M.. and D. J. Taatjes and B.T. Mossman. Methods in Molecular Biology vol. 319. Tutowa, NJ: Humana Press. pp. 67–76.

Straif, K., Benbrahim-Tallaa, L., Baan, R., Grosse, Y., Secretan, B., El Ghissassi, F., Bouvard, V., Guha, N., Freeman, C., Galichet, L., and Cogliano, V. 2009. A review of human carcinogens—Part C: Metals, arsenic, dusts, and fibres. *Lancet Oncol*. 10:453–454.

Suganuma, M., Okabe, S., Marino, M. W., Sakai, A., Sueoka, E., and Fujiki, H. 1999. Essential role of tumor necrosis factor α (TNF-α) in tumor promotion as revealed by TNF-α-deficient mice. *Cancer Res*. 59:4516–4518.

Takeuchi, T., and Morimoto, K. 1994. Crocidolite asbestos increased 8-hydroxydeoxyguanosine levels in cellular DNA of a human promyelocytic leukemia cell line, HL60. *Carcinogenesis* 15:635–639.

Tanaka, S., Choe, N., Hemenway, D. R., Zhu, S., Matalon, S., and Kagan, E. 1998. Asbestos inhalation induces reactive nitrogen species and nitrotyrosine formation in the lungs and pleura of the rat. *J. Clin. Invest*. 102:445–454.

Tephly, L. A., and Carter, A. B. 2008. Asbestos-induced MKP-3 expression augments TNF-α gene expression in human monocytes. *Am. J. Respir. Cell Mol. Biol*. 39:113–123.

Thomas, G., Ando, T., Verma, K., and Kagan, E. 1994. Asbestos-induced nitric oxide production: synergistic effect with interferon-gamma. *Ann. NY Acad. Sci*. 725:207–212.

Thompson, C. B. 1995. Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456–1462.

Timbrell, V., Gibson, J. C., and Webster, I. 1968. UICC standard reference samples of asbestos. *Int. J. Cancer* 3:406–408.

Timbrell, V., and Rendall, R. E. G. 1972. Preparation of the UICC Standard reference samples of asbestos. *Powder Technol*. 5: 279–287.

Topinka, J., Loli, P., Dusinska, M., Hurbankova, M., Kovacikova, Z., Volkovova, K., Kazimirova, A., Barancokova, M., Tatrai, E., Wolff, T., Oesterle, D., Kyrtopoulos, S. A., and Georgiadis, P. 2006a. Mutagenesis by man-made mineral fibres in the lung of rats. *Mutat. Res*. 595:174–183.

Topinka, J., Loli, P., Georgiadis, P., Dusinska, M., Hurbankova, M., Kovacikova, Z., Volkovova, K., Kazimirova, A., Barancokova, M., Tatrai, E., Oesterle, D., Wolff, T., and Kyrtopoulos, S. A. 2004. Mutagenesis by asbestos in the lung of lambda-lacI transgenic rats. *Mutat. Res*. 553:67–78.

Topinka, J., Loli, P., Hurbakova, M., Kovacikova, Z., Volkovova, K., Wolff, T., Oesterle, D., S., Kyrtopoulos, A., and Georgiadis, P. 2006b. Benzo[a]pyrene-enhanced mutagenesis by man-made mineral fibres in the lung of lamda-lacI transgenic rats. *Mutat. Res*.. 595:167–173.

Toyokuni, S. 2009. Mechanisms of asbestos-induced carcinogenesis. *Nagoya J. Med. Sci*. 71:1–10.

Toyooka, S., Kishimoto, T., and Date, H. 2008. Advances in the molecular biology of malignant mesothelioma. *Acta Med Okayama* 62:1–7.

Tschopp, J., Martinon, F., and Burns, K. 2003. NALPs: A novel protein family involved in inflammation. *Nat. Rev. Mol. Cell Biol*. 4:95–104.

Tuder, R. M., Yun, J. H., and Graham, B. B. 2008. Cigarette smoke triggers code red: p21CIP1/WAF1/SDI1 switches on danger responses in the lung. *Am. J. Respir. Cell Mol. Biol*. 39:1–6.

Tug, E., Tug, T., Elyas, H., Coskunsel, M., and Emri, S. 2006. Tumor suppressor gene alterations in patients with malignant

mesothelioma due to environmental asbestos exposure in Turkey. *J. Carcinogen*. 5:1–6.

Turver, C. J., and Brown, R. C. 1987. The role of catalytic iron in asbestos induced lipid peroxidation and DNA-strand breakage in C3H10T1/2 cells. *Br. J. Cancer* 56: 133–136.

Unfried, K., Schurkes, C., and Abel, J. 2002. Distinct spectrum of mutations induced by crocidolite asbestos: Clue for 8-hydroxydeoxyguanosine-dependent mutagenesis in vivo. *Cancer Res*. 62:99–104.

Upadhyay, D., and Kamp, D. W. 2003. Asbestos-induced pulmonary toxicity: Role of DNA damage and apoptosis. *Exp. Biol. Med. (Maywood)* 228:650–659.

Valerio, F., de Ferrari, M., Ottaggio, L., Repetto, E., and Santi, L. 1983. Chromosomal aberrations induced by chrysotile and crocidolite in human lymphocytes in vitro. *Mutat. Res*. 122:397–402.

Vaslet, C. A., Messier, N. J., and Kane, A. B. 2002. Accelerated progression of asbestos-induced mesotheliomas in heterozygous p53+/− mice. *Toxicol. Sci*. 68:331–338.

Villanova, F., Procopio, A., and Rippo, M. R. 2008. Malignant mesothelioma resistance to apoptosis: Recent discoveries and their implication for effective therapeutic strategies. *Curr. Med. Chem*. 15:631–641.

Virta, R. L. 2003. *Worldwide asbestos supply and consumption trends from 1900 to 2000*. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey.

Vogelstein, B., and Kinzler, K. W. 1993. The multistep nature of cancer. *Trends Genet*. 9:138–141.

Vousden, K. H., and Prives, C. 2009. Blinded by the light: The growing complexity of p53. *Cell* 137:413–431.

Wagner, J. C., and Berry, G. 1969. Mesotheliomas in rats following inoculation with asbestos. *Br. J. Cancer* 23:567–581.

Wagner, J. C., Berry, G., Skidmore, J. W., and V. Timbrell, V. 1974. The effects of the inhalation of asbestos in rats. *Br. J. Cancer* 29:252–269.

Wagner, J. C., Berry, G., and Timbrell, V. 1973. Mesotheliomata in rats after inoculation with asbestos and other materials. *Br. J. Cancer* 28:173–185.

Walker, C., Everitt, J., and Barrett, J. C. 1992. Possible cellular and molecular mechanisms for asbestos carcinogenicity. *Am. J. Ind. Med*. 21:253–273.

Wang, N. S., Jaurand, M. C., Magne, L., Kheuang, L., Pinchon, M. C., and Bignon, J. 1987. The interactions between asbestos fibers and metaphase chromosomes of rat pleural mesothelial cells in culture. A scanning and transmission electron microscopic study. *Am. J. Pathol*. 126: 343–349.

Wang, X., Christiani, D. C., Wiencke, J. K., Fischbein, M., Xu, X., Cheng, T. J., Mark, E., Wain, J. C., and Kelsey, K. T. 1995. Mutations in the p53 gene in lung cancer are associated with cigarette smoking and asbestos exposure. *Cancer Epidemiol Biomarkers Prev* 4:543–548.

Wang, Y. H., Faux, S. P., Hallden, G., Kirn, D. H., Houghton, C. E., Lemoine, N. R., and Patrick, G. 2004. Interleukin-1 beta and tumour necrosis factor-alpha promote the transformation of human immortalised mesothelial cells by erionite. *Int. J. Oncol* 25:173–178.

Waters, M. D., Stack, H. F., and Jackson, M. A. 1999. Genetic toxicology data in the evaluation of potential human environmental carcinogens. *Mutat. Res./Fundam. Mol. Mech. Mutagen*. 437:21–49.

Weitzman, S. A., and Gordon, L. I. 1990. Inflammation and cancer: Role of phagocyte-generated oxidants in carcinogenesis. *Blood* 76:655–663.

Weitzman, S. A., and Graceffa, P. 1984. Asbestos catalyzes hydroxyl and superoxide radical generation from hydrogen peroxide. *Arch. Biochem. Biophys*. 228:373–376.

Wikman, H., Ruosaari, S., Nymark, P., Sarhadi, V. K., Saharinen, J., Vanhala, E., Karjalainen, A., Hollmen, J., Knuutila, S., and Anttila, S. 2007. Gene expression and copy number profiling suggests the importance of allelic imbalance in 19p in

asbestos-associated lung cancer. *Oncogene* 26, V. :4730–4737.

Wilson, S. M., Barbone, D., Yang, T.-M., Jablons, R. Bueno, R., Sugarbaker, D. J., Nishimura, S. L., Gordon, G. J., and Broaddus, V. C. 2008. mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. *Am. J. Respir. Cell Mol. Biol*. 39:576–583.

Wylie, A. G., Virta, R. L., and Segreti, J. M. 1987. Characterization of mineral population by index particle: Implication for the Stanton hypothesis. *Environ. Res*. 43:427–439.

Xu, A., Huang, X., Lien, Y. C., Bao, L., Yu, Z., and Hei, T. K. 2007a. Genotoxic mechanisms of asbestos fibers: role of extranuclear targets. *Chem Res Toxicol* 20:724–733.

Xu, A., Smilenov, L. B., He, P., Masumura, K., Nohmi, T., Yu, Z., and Hei, T. K. 2007b. New insight into intrachromosomal deletions induced by chrysotile in the gpt delta transgenic mutation assay. *Environ. Health Perspect*. 115:87–92.

Xu, A., Wu, L. J., Santella, R. M., and Hei, T. K. 1999. Role of oxyradicals in mutagenicity and DNA damage induced by crocidolite asbestos in mammalian cells. *Cancer Res*. 59:5922–5926.

Xu, A., Zhou, H., Yu, D. Z., and Hei, T. K. 2002. Mechanisms of the genotoxicity of crocidolite asbestos in mammalian cells: Implication from mutation patterns induced by reactive oxygen species. *Environ. Health Perspect*. 110:1003–1008.

Yang, H., Bocchetta, M., Kroczynska, B., Elmishad, A. G., Chen, Y., Liu, Z., Bubici, C., Mossman, B. T., Pass, H. I., Testa, J. R., Franzoso, G., and Carbone, M. 2006. TNF-alpha inhibits asbestos-induced cytotoxicity via a NF-kappaB-dependent pathway, a possible mechanism for asbestos-induced oncogenesis. *Proc. Natl. Acad. Sci. USA* 103:10397–10402.

Yarborough, C. M. 2007. The risk of mesothelioma from exposure to chrysotile asbestos. *Curr. Opin. Pulm. Med*. 13:334–338.

Yee, H., Yie, T. A., Goldberg, J., Wong, K. M., and Rom, W. N. 2008. Immunohistochemical study of fibrosis and adenocarcinoma in dominant-negative p53 transgenic mice exposed to chrysotile asbestos and benzo(a)pyrene. *J. Environ. Pathol. Toxicol. Oncol*. 27:267–276.

Yegles, M., Janson, X., Dong, H. Y., Renier, A., and Jaurand, M. C. 1995. Role of fibre characteristics on cytotoxicity and induction of anaphase/telophase aberrations in rat pleural mesothelial cells in vitro: Correlations with in vivo animal findings. *Carcinogenesis* 16:2751–2758.

Yegles, M., Saint-Etienne, L., Renier, A., Janson, X., and Jaurand, M. C. 1993. Induction of metaphase and anaphase/telophase abnormalities by asbestos fibers in rat pleural mesothelial cells in vitro. *Am. J. Respir. Cell Mol. Biol*. 9:186–191.

Yin, Q., Brody, A. R., and Sullivan, D. E. 2007. Laser capture microdissection reveals dose-response of gene expression in situ consequent to asbestos exposure. *Int. J. Exp Pathol*. 88:415–425.

Yuan, Z., Taatjes, D. J., Mossman, B. T., and Heintz, N. H. 2004. The duration of nuclear extracellular signal-regulated kinase 1 and 2 signaling during cell cycle reentry distinguishes proliferation from apoptosis in response to asbestos. *Cancer Res*. 64:6530–6536.

Zhu, S., Manuel, M., Tanaka, S., Choe, N., Kagan, E., and Matalon, S. 1998. Contribution of reactive oxygen and nitrogen species to particulate-induced lung injury. *Environ. Health Perspect*.. 106:1157–1163.