APPENDIX

Cancer has been induced in rodents by the inhalation, instillation or implantation of solid materials, including mineral fibres, crystalline silica and other poorly-soluble particulates. In this section, hypotheses that seek to explain the mechanisms of cancer induction by these substances are reviewed with the objective of identifying whether they provide any insight to mechanisms that might be involved in the induction of cancer by other types of solid, implanted materials.

Asbestos fibres

Naturally occurring asbestos fibres, talc containing asbestiform fibres and non-asbestiform fibres (erionite) have been classified as carcinogenic to humans (IARC, 1977, 1987a,d). Inhalation of these fibrous minerals causes scarring and cancer of the lungs and pleura (reviewed in Kane, 1996a). The histopathology and pathogenesis of these fibrotic and carcinogenic processes in the lungs and mesothelium are somewhat different. Inhalation of asbestos and erionite fibres at high doses causes diffuse interstitial fibrosis or asbestosis that is usually more prominent in the lower lobes of the lungs. This fibrotic reaction develops slowly but progressively, beginning 10 years after the initial exposure. It is hypothesized that oxidants and proteases released from alveolar macrophages activated by phagocytosis of fibres damage the alveolar epithelial lining. In addition, fibres may become translocated across the damaged epithelium into the interstitium of the alveolar walls. This injury is repaired by a combination of epithelial regeneration by Type II alveolar cells plus proliferation of fibroblasts with collagen deposition in the interstitium. Up-regulation of growth factor expression has been observed at sites of asbestos fibre deposition in rat lungs: platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β are hypothesized to trigger fibroblast proliferation and collagen synthesis, respectively, while TGF-α is mitogenic for alveolar epithelial cells (reviewed in Brody *et al*., 1997b). Fibres also translocate to the pleural space following inhalation and accumulate near lymphatic openings on the parietal pleura and dome of the diaphragm (Boutin *et al*., 1996); these are the anatomical sites where fibrotic or calcified pleural plaques develop. Pleural plaques are considered as a marker of prior asbestos exposure; they can occur even in the absence of asbestosis. Diffuse fibrosis of the visceral pleura can also occur, usually following repeated episodes of pleural effusion that is also called benign asbestos pleurisy. It is hypothesized that asbestos-induced pleural effusions are caused by release of chemokines such as interleukin (IL)-8 from mesothelial cells (Boylan *et al*., 1992). Pleural fibrosis and pleural plaques are hypothesized to develop after injury to mesothelial cells and destruction of the basement membrane. This injury is

then repaired by mesothelial and submesothelial cell proliferation and deposition of collagen (Davila & Crouch, 1993).

Inhalation of asbestos fibres contributes to the development of lung cancer arising from the bronchial and alveolar epithelium. Asbestos and cigarette smoke most likely act as co-factors in the induction of lung cancer (Table 63). The anatomical location, histological type of tumour and molecular alterations in oncogenes and tumour-suppressor genes in smokers who have also been exposed to asbestos are similar to those of lung cancers that arise in smokers with no history of exposure to asbestos (Lee *et al*., 1998). No reports are available that describe the pathology and molecular lesions in asbestos-related lung cancers that are not associated with cigarette smoking. The point mutations in the K-*ras* oncogene (Husgafvel-Pursiainen *et al*., 1993) and *p53* tumour-suppressor gene (Wang *et al*., 1995), as well as deletions at the chromosome 3p14 locus are consistent with the known mutagenic spectrum of carcinogens in cigarette smoke (Nelson *et al*., 1998). The molecular mechanisms leading to the development of lung cancer and diffuse malignant mesothelioma after asbestos exposure are probably different. Human malignant mesotheliomas do not have point mutations in the *ras* oncogene or *p53* tumour-suppressor gene; 40% of cases have mutations at the *NF2* tumour-suppressor gene locus and 70–100% show co-deletions of the *p15* and *p16* tumour-suppressor genes (reviewed in Lechner *et al.*, 1997). The molecular alterations

Table 63. Direct mechanisms of asbestos fibre carcinogenesis

Mechanism	Experimental end-points	References
Genotoxic	Oxidized bases	Chao *et al*. (1996); Fung *et al.* (1997a)
	DNA breaks	Reviewed in Jaurand (1996)
	Aneuploidy	Reviewed in Jaurand (1996); Jensen *et al*. (1996)
	Mutations	Park & Aust (1998)
Non-genotoxic		
Mitogenic	Target cell proliferation	BéruBé *et al*. (1996); Goldberg *et al*. (1997); Mishra *et al*. (1997)
	Binding to or activation of surface receptors	Boylan *et al*. (1995); Pache *et al*. (1998)
	Growth factor expression	Liu *et al*. (1996); Brody *et al*. (1997b); Kane *et al*. (1997)
	Intracellular signalling pathways	Zanella *et al*. (1996); Fung *et al*. (1997b); Mossman *et al*. (1997)
Cytotoxic	Apoptosis	Broaddus *et al*. (1996); Goldberg *et al*. (1997); Levresse *et al*. (1997)
	Necrosis	Reviewed in Kane (1996a)

characteristic of lung tumours and malignant mesotheliomas induced by asbestos may develop during later stages of tumour progression and may not reflect the direct genotoxic effect of fibres on the target cell population.

The mechanisms leading to the induction of lung cancers and malignant mesothelioma by exposure to asbestos fibres are unknown. Both physical and chemical parameters of fibres are related to their biological activity: fibre geometry and dimensions, biopersistence in the lungs, chemical composition and surface reactivity (reviewed in Everitt, 1994; Kane, 1996a, 1998). Asbestos fibres may act as direct or indirect carcinogens (see Tables 63 and 64). Direct genotoxic and mitogenic effects of asbestos, as well as of some man-made fibres, have been detected in in-vitro assays. These assays have been widely used because they are relatively inexpensive, simple and rapid. However, they have limitations and often produce conflicting results depending on the cell type, species and conditions of exposure. The most serious shortcoming is that direct exposure of target cells to high doses of fibres *in vitro* may not accurately reflect responses of the same target cells in the lung under conditions of chronic, low-dose exposure. Surface modification of fibres and mechanical dissolution or breakage that have been observed *in vivo* are not easily reproduced in in-vitro models (reviewed in Kane *et al*., 1996). However, some recent studies using animal models have confirmed the results of some of the in-vitro assays (Table 64). For example, hydroxyl (Schapira *et al*., 1994) and lipid radicals have been measured in rat lungs after intratracheal instillation of asbestos fibres (Ghio *et al*., 1998). Up-regulation of the nuclear factor (NF)-κB transcription factor has been detected in rat lung epithelial and mesothelial cells after short-term inhalation of asbestos (Janssen *et al*., 1997), as has increased expression of c-*fos* and c-*jun* proto-oncogenes in rat pleural mesothelial cells and c-*jun* in Syrian hamster tracheal epithelial cells (Heintz *et al*., 1993). Target cell proliferation and up-regulation of growth factor expression in proliferating cells have been confirmed in the lungs and mesothelium after in-vivo exposure (Table 64). Persistent proliferation of mesothelial cells observed after direct intraperitoneal injection of asbestos fibres in mice has been correlated temporally and spatially with persistence

Table 64. Indirect mechanisms of asbestos fibre carcinogenesis

Mechanism	References
Co-factor with cigarette smoke	Reviewed in Kane (1996a); Lee *et al*. (1998)
Co-factor with SV40 virus	Carbone *et al*. (1997); Testa *et al*. (1998)
Persistent inflammation with secondary genotoxicity	Donaldson (1996); Reviewed in Driscoll *et al*. (1997); Vallyathan & Shi (1997)
Persistent inflammation with release of cytokines and growth factors	Rosenthal *et al*. (1994); Brody *et al*. (1997b); reviewed in Driscoll *et al*. (1997); Kane *et al*. (1997); Simeonova *et al*. (1997)

of fibres and persistent inflammation (Macdonald & Kane, 1997). In chronic inhalation studies in rats, fibre persistence is also correlated with induction of lung tumours (Hesterberg *et al*., 1996). These recent chronic inhalation studies include man-made fibres such as ceramic fibres that were previously classified as possibly carcinogenic to humans (Group 2B) (IARC, 1988).

These recent in-vitro and in-vivo studies provide consistent evidence that persistent, nondegradable fibres can trigger proliferation of target cells in the lungs and pleura of rodents. Three mechanisms have been proposed for the mitogenic effects of fibres: direct activation of growth factors or their receptors followed by triggering of intracellular signalling pathways and induction of proto-oncogene expression; compensatory cell proliferation in response to apoptosis or necrosis; and indirect stimulation of cell proliferation by cytokines and growth factors released from inflammatory cells. It is likely that several mechanisms contribute to the proliferative effects of fibres, although it is uncertain to what extent each of these mechanisms may become activated under conditions of low-dose, chronic exposure in humans (reviewed in Kane *et al*., 1996).

Mineral fibres have been shown to catalyse the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cell-free models and in-vitro cell cultures (reviewed in Fubini, 1996). Ex-vivo studies have confirmed generation of RNS by rat alveolar macrophages after inhalation of asbestos fibres (Quinlan *et al*., 1998). Fibres may catalyse the generation of oxidants directly from molecular oxygen, or indirectly from ROS and RNS generated by inflammatory cells in the lungs or mesothelial lining. Some of the genotoxic, mitogenic and cytotoxic activities of asbestos fibres have been shown to be mediated by oxidants generated by target cells directly in the absence of inflammatory cells (Table 64). Surface reactivity and availability of iron to catalyse formation of hydroxyl radicals are important determinants of the biological activity of fibres as assessed in in-vitro systems (Fubini, 1996; Park & Aust, 1998). In addition to asbestos and erionite fibres, man-made fibres including ceramic and silicon carbide can generate free radicals in cell-free assays. No data are available for graphite fibres (Fubini, 1996). Ceramic and silicon carbide fibres are carcinogenic when injected intraperitoneally or intrapleurally in rats (Pott *et al*., 1987; Johnson & Hahn, 1996); no data are available for graphite or carbon fibres. The surface reactivity can be modified by coating asbestos or man-made fibres with IgG, surfactant or lung lining fluid. Adsorption of these macromolecules to the surface of fibres can either enhance (Chao *et al*., 1996) or diminish their ability to stimulate oxidant generation (Brown *et al*., 1998). It is uncertain whether sufficient oxidant generation occurs *in vivo* to induce genotoxicity in target cells. Inhalation of asbestos fibres by rats induces antioxidant defences (Holley *et al*., 1992; Janssen *et al*., 1992); in mesothelial cells *in vitro*, exposure to asbestos also induces expression and activity of a DNA repair enzyme and redox factor, apurinic/apyrimidinic (AP)-endonuclease or redox factor 1 (Fung *et al*., 1998). Changes in glutathione content of lung epithelial cells, alveolar macrophages and bronchoalveolar lavage fluid were variable after

inhalation of asbestos or anhydrous gypsum by rats (Clouter *et al*., 1997). *In vitro*, asbestos exposure caused efflux of glutathione from human lung epithelial cells (Golladay *et al*., 1997). These conflicting data raise questions about the roles of oxidant stress and induction of antioxidant defences in the pathogenesis of lung tumours and malignant mesotheliomas induced by exposure to asbestos in humans. Polymorphisms of glutathione-*S*-transferases (GSTs) are frequent in human populations and the null phenotype has been reported to be associated with an increased risk of lung cancer and malignant mesothelioma following exposure to asbestos (Anttila *et al*., 1995).

The *p53* tumour-suppressor gene is an important regulator of apoptosis and induction of cell-cycle arrest and repair in response to DNA damage (reviewed in Kane, 1996a). Short-term inhalation of asbestos fibres in rats induced expression of p53 protein at sites of fibre deposition in the lungs (Mishra *et al*., 1997). This is the first evidence that exposure to asbestos may induce DNA damage in target cells of the lung after in-vivo exposure. Although point mutations or deletions of the *p53* gene are rare in human malignant mesotheliomas (reviewed in Lechner *et al*., 1997) or in rat mesotheliomas induced by direct intraperitoneal injection of asbestos fibres (Unfried *et al*., 1997), the p53 protein is frequently over-expressed in human malignant mesotheliomas (Mayall *et al*., 1992; Ramael *et al*., 1992). In some cases, SV40 T-antigen has been detected by immunohistochemistry (Testa *et al*., 1998). This viral oncoprotein has been reported to bind to p53 protein, prolong its half-life, and inhibit p53 functional activity (reviewed in Carbone *et al*., 1997). Whether SV40 virus can act as a co-factor with asbestos in the induction of malignant mesothelioma in humans is highly controversial (Galateau-Salle *et al*., 1998). SV40 virus has also been found in spontaneous human osteosarcomas, as well as in brain and pituitary tumours.

Crystalline silica

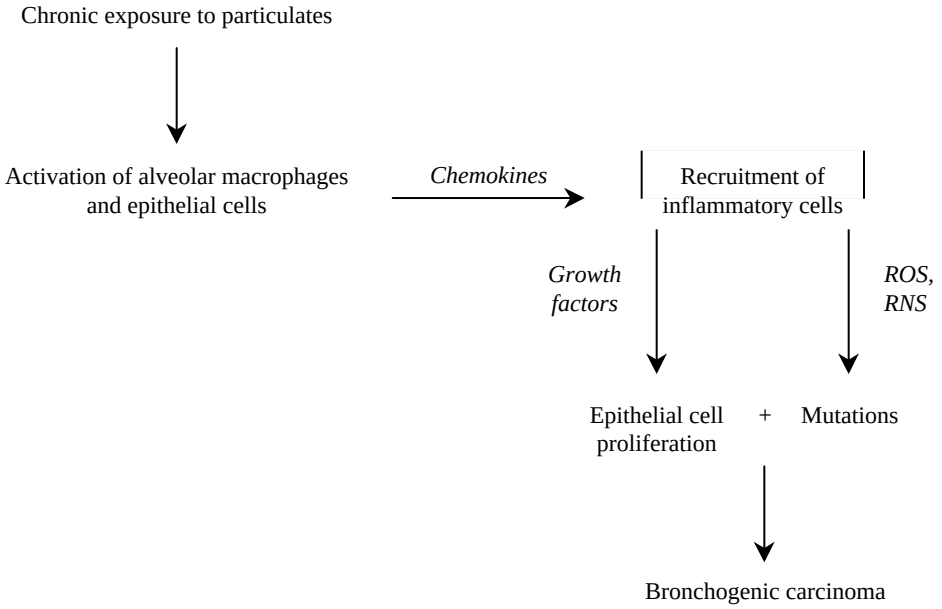
Workplace exposures to silicates and crystalline quartz are common (IARC, 1997a). Inhalation of silicate minerals (for example, pure talc, mica, kaolinite, wollastonite) that usually occur in sheets or platy forms causes a minimal fibrotic reaction in the lungs. Silicosis is characterized by irregular stellate lung scars composed of macrophages and multinucleated giant cells surrounding dust particles with mild chronic inflammation and deposition of collagen. In contrast, inhalation of crystalline silica can cause severe acute or chronic lung injury. Inhalation of freshly-fractured quartz is especially hazardous, resulting in extensive lung epithelial injury and accumulation of protein and lipid debris in the alveolar spaces. Acute silicosis or alveolar proteinosis is hypothesized to be mediated by free radicals generated at the surfaces of freshly-fractured quartz (Vallyathan *et al*., 1995). Chronic exposure to crystalline silica causes a characteristic fibrotic lesion, predominantly in the upper lobes of the lungs. Silicotic nodules are firm, round lesions with a central core of dense collagen surrounded by inflammatory cells and fibroblasts. This lesion is diagnostic of chronic or nodular silicosis. These nodules may enlarge and coalesce with formation of cavities; this is characteristic of progressive

massive fibrosis. In contrast to workers exposed to asbestos fibres or silicates, occupational exposure to crystalline silica greatly increases the risk of superimposed infection with *Mycobacterium tuberculosis* or fungi. The mechanisms responsible for formation of these nodular lesions are unknown; chronic release of cytokines such as tumour necrosis factor (TNF)-α is postulated to play a role (Piguet & Vesin, 1994). An imbalance between pro-inflammatory and anti-inflammatory cytokines has also been proposed to perpetuate the pulmonary fibrotic reaction and compromise lung defences against mycobacteria and fungi (reviewed in Kane, 1996b; Huaux *et al*., 1998).

Most silicates have been categorized by IARC as unclassifiable as to carcinogenicity to humans (Group 3). In contrast, inhaled crystalline silica (in the form of quartz or cristobalite from occupational sources) has been classified in Group 1 (carcinogenic to humans) (IARC, 1997a). The mechanisms responsible for induction of lung cancer under some conditions of occupational exposure to crystalline silica are unknown. It has been hypothesized that these are scar cancers associated with fibrosis, although the anatomical location and histopathological characteristics of nodular silicosis are quite distinct from diffuse interstitial pulmonary fibrosis or asbestosis. As with asbestos fibres, a role for ROS generated at the surfaces of crystalline silica has been proposed (reviewed in Vallyathan & Shi, 1997). Finally, an indirect mechanism for induction of lung cancer by crystalline silica has been proposed (Figure 2). This indirect mechanism is supported by chronic inhalation studies in rats that show a correlation between high lung burdens of particles, persistent inflammation, epithelial proliferation, fibrosis and late development of lung cancer. Oxidative stress has been proposed to increase expression of proinflammatory cytokines in the lungs of rats exposed to particulates. As with asbestos fibres, particulate-induced oxidant stress activates the NF-κB transcription factor that increases expression of cytokines such as TNF-α and chemokines, including macrophage inflammatory protein (MIP)-2 and IL-8. Persistent activation and recruitment of inflammatory cells into the lungs after chronic exposure to particulates trigger the chronic release of growth factors for epithelial cells and ROS and RNS that are genotoxic. The genotoxic effects of ROS released from neutrophils collected after in-vivo exposure to α-quartz has been documented (Driscoll *et al*., 1997). Chronic exposure of rats to crystalline silica elicits an influx of neutrophils into the lungs (30–50% of cells collected by bronchoalveolar lavage) compared with approximately 5% of neutrophils in bronchoalveolar lavage fluids obtained from human silicotics (IARC, 1997a). Therefore, it is uncertain whether this indirect inflammatory mechanism contributes to the increased incidence of lung cancer observed in some cohorts of workers exposed to crystalline silica.

Recent molecular studies of *p53* tumour-suppressor gene mutations and p53 protein expression in the lungs of patients with lung cancer and occupational exposure to crystalline silica and other dusts have been conducted. Mutations in the *p53* gene were detected at a frequency similar to those in smoking-related lung cancers (Husgafvel-Pursiainen *et al*., 1997). Expression of p53 protein can be detected by immunohistochemistry in preneoplastic epithelial lesions in the lungs of smokers. In patients with

Figure 2. Proposed mechanism for induction of lung cancer by nongenotoxic particulates[a]



[a] Modified from Oberdörster (1997)
ROS, reactive oxygen species
RNS, reactive nitrogen species

radiographic evidence of pneumoconiosis including silicosis, p53 protein expression was more widespread in bronchiolar dysplasias than in patients without pneumoconiosis (Katabami *et al.*, 1998). Most of the patients in both of these studies were current or former smokers. Similarly to the molecular studies in asbestos workers with lung cancer, no genetic lesion specifically associated with exposure to crystalline silica has been identified in human lung cancers.

Poorly-soluble particulates (PSPs) or low-toxicity dusts

Low-toxicity dusts such as carbon black, coal dust and titanium dioxide have been shown to induce lung tumours when administered at high doses in rats, but not in mice or hamsters. An indirect inflammatory mechanism has been proposed for induction of lung tumours by PSPs in rats (Figure 2). In contrast to crystalline silica, very high exposures to these particulates are required to induce lung tumours in rats. It is hypothesized that these high doses overwhelm lung clearance mechanisms and antioxidant defences, resulting in persistent inflammation and oxidant stress (Morrow *et al.*, 1996; Driscoll *et al.*, 1997; Warheit *et al.*, 1997). The reasons for the species differences in induction of lung tumours by PSPs are unknown. Several mechanisms have been proposed: dimi-

nished antioxidant defences, decreased adaptive responses, increased expression of pro-inflammatory cytokines and chemokines, decreased expression of anti-inflammatory cytokines, altered DNA repair mechanisms (reviewed in Oberdörster, 1997) and differences in generation of nitric oxide by alveolar macrophages (Jesch *et al*., 1997). The relevance of the rat inhalation model to assessment of the risk of PSPs in humans is controversial and was recently reviewed (ILSI Risk Science Institute Workshop Consensus Report, 2000). Coal dust and amorphous silica are not classifiable as to their carcinogenicity to humans (IARC, 1997a,d). Coal miners with prolonged exposure to bituminous coal develop stellate aggregates of dust macules in the centriacinar regions of the lungs with little inflammation or fibrosis. Under heavy exposures, these macules may coalesce into larger lesions called complicated coal workers' pneumoconiosis. Even these workers do not show an increased incidence of lung cancer (ILSI Risk Science Institute Workshop Consensus Report, 2000).

The tumours that develop in rats exposed to carbon black rarely show mutations in the K-*ras* oncogene or *p53* tumour-suppressor gene (Swafford *et al*., 1995). In contrast, human lung cancers show frequent mutations in K-*ras* and *p53* that are consistent with the genetic spectrum of chemical carcinogens present in cigarette smoke (reviewed in Perera, 1996).

Relevance of these mechanisms for evaluation of the carcinogenicity of surgical implants and prosthetic devices

Many of the materials that have been associated with solid-state carcinogenesis have been fabricated into surgical implants and prosthetic devices. In the experimental model of foreign-body carcinogenesis in rats and mice (Brand, 1982), non-metallic implants with smooth, continuous surfaces induce subcutaneous or intraperitoneal sarcomas. The mechanisms responsible for the induction of foreign-body tumours in this model are speculative and the biochemical and molecular events leading to the development of these tumours have not been identified. When similar materials are implanted subcutaneously or intraperitoneally in particulate form, they stimulate variable degrees of acute inflammation and fibrosis depending on the anatomical site and species. No tumours have been induced by these particulate non-metallic materials (Rigdon, 1975). Any analogy between foreign-body carcinogenesis in rats and mice and carcinogenicity of surgical implants and prosthetic devices in humans is limited by the following considerations. First, smooth films implanted subcutaneously or intraperitoneally in rodents induce sarcomas by an unknown mechanism. Isolated cases of sarcomas induced by surgical implants and prosthetic devices in humans have been reported; however, these have not provided any mechanistic information. Second, inflammatory and fibrotic reactions to foreign materials are common in experimental animals and humans. Despite the well-documented associations between chronic inflammatory conditions and carcinomas in humans (Table 65), it is uncertain whether this mechanism can be extrapolated to sarcomas induced by surgical implants and prosthetic devices. Human cancers associated with chronic inflammation are usually carcinomas (rather than sarcomas) that

Table 65. Some chronic inflammatory conditions that have been associated with cancer in humans[a]

Predisposing condition	Cancer
Chronic hepatitis[b]	Hepatocellular carcinoma
Chronic cystitis[c]	Bladder carcinoma
Ulcerative colitis	Colon carcinoma
Helicobacter pylori gastritis[d]	Gastric carcinoma and lymphoma
Chronic osteomyelitis	Cutaneous squamous-cell carcinoma
Chronic pancreatitis	Pancreatic cancer

[a] Reviewed in Brand (1982, 1987)
[b] Hagen *et al*. (1994), IARC (1994c)
[c] Kawai *et al*. (1993)
[d] IARC (1994d); Mannick *et al*. (1996)

develop at sites of repeated episodes of cell necrosis or apoptosis, followed by epithelial regeneration (for example, hepatocellular carcinoma associated with persistent viral infection, as discussed by Nakamoto *et al*., 1998). In these predisposing conditions, inflammation accompanied by local release of cytokines and oxidants is postulated to contribute to genotoxicity in proliferating epithelial cell populations (Kawai *et al*., 1993; Hagen *et al*., 1994; Mannick *et al*., 1996). In the examples of chronic inflammation associated with persistent viral infection (Nakamoto *et al*., 1998), bacterial infection (Mannick *et al*., 1996) or parasitic infestation (Brand, 1982), host immune defence mechanisms amplify tissue injury by inducing apoptosis of epithelial cells and exacerbating cytokine release. Nitric oxide has been proposed as an important mediator of chronic inflammation and DNA damage under these conditions; however, it may also be tumoricidal (Wink *et al*., 1998).

Asbestos fibres are established human carcinogens that cause lung cancer and malignant mesothelioma in humans and rodents (IARC, 1977, 1987a). Despite numerous experimental studies *in vitro* and *in vivo*, the mechanisms responsible for the development of these cancers are unknown. Fibrous minerals such as asbestos appear to be especially effective in inducing malignant mesotheliomas after inhalation or direct intrapleural or intraperitoneal injection. High doses of non-metallic particulates do not cause mesotheliomas even when injected intraperitoneally in rats (Pott *et al*., 1987). Asbestos fibres show genotoxic and mitogenic activities in several in-vitro systems; some of these activities have been confirmed in in-vivo models. However, a major limitation of all of these models is the use of high-dose exposures to produce tumours. It is uncertain whether the same mechanisms operate under conditions of chronic, low-dose inhalation exposure in humans. Lung carcinomas and malignant mesotheliomas are produced after inhalation of fibres; therefore, it is unwarranted to predict whether similar materials in fibrous form or incorporated into composites would be carcinogenic when implanted at other anatomical sites (Rigdon, 1975).

Correlations between persistence of fibres or particulates in the lungs, inflammation and fibrosis have been made in several model systems, especially in the rat. While these studies show consistent temporal associations, there has been no rigorous proof of a causal relationship between these parameters and the development of lung cancer (Kane *et al*., 1996). Rats appear to be extremely susceptible to induction of lung cancer by PSPs; extrapolation of this response to humans exposed to similar materials by inhalation must be done cautiously (ILSI Risk Science Institute Workshop Consensus Report, 2000). It would be premature to apply the proposed mechanism for the induction of rat lung tumours by PSPs to the evaluation of carcinogenicity of similar materials implanted at other anatomical sites in humans.