

Mechanisms of the combined effect of asbestos and smoking in the etiology of lung cancer

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VAINIO H, BOFFETTA P. Mechanisms of the combined effect of asbestos and smoking in the etiology of lung cancer. *Scand J Work Environ Health* 1994;20:235-42. The joint effects of exposure to two known lung carcinogens, tobacco smoking and asbestos, are reviewed. The variable pattern of interaction — ranging from supramultiplicative to less than additive — may reflect the fact that both asbestos and smoking are complex carcinogens which can affect more than one stage of lung carcinogenesis. The joint effect of two such agents will depend on the relative magnitude of the effects at each stage. The epidemiologic evidence from studies of insulation workers with high exposures suggests an interaction that approximates the multiplicative model, indicating that each of the two factors has an independent action on the multistage process of carcinogenesis. Very limited information is available on the interaction between these two agents in causing specific histological types of lung cancer. Both tobacco smoke and asbestos fibers can be genotoxic and cytotoxic and cause proliferative lesions in the lungs. Tobacco smoke is known to contain carcinogens that bind to critical genes in DNA (deoxyribonucleic acid) and cause mutations. Asbestos fibers may cause chronic inflammation of the lungs, which releases various cytokines and growth factors, and therefore may provide a possible selective growth advantage for mutated cells.

KEY TERMS — adenocarcinoma, cancer, interaction, lung, review, tobacco smoking.

Lung cancer is the leading cause of death from cancer throughout the world (1). The major histological types of lung cancer are small-cell, squamous-cell, and large-cell carcinomas and adenocarcinomas. Tobacco smoking causes all types of lung cancer, but the risk for squamous-cell and small-cell carcinomas of the lung is higher than that for adenocarcinoma (2). While tobacco smoking is the most important single cause of lung cancer, it is not a condition *sine qua non*. Increased risks for lung cancer have been demonstrated in nonsmoking populations with various occupational exposures, and increases in risk occur for both smokers and nonsmokers exposed occupationally to asbestos fibers (3). The risk for lung cancer associated with tobacco smoking is substantially increased in conjunction with exposure to asbestos, however, and therefore smoking and asbestos seem to have a more than additive action in causing lung cancer.

Lung cancer is not a single disease, and the various histological types have unique characteristics and may have specific etiologies. In this review, we examine the evidence for interaction between tobacco smoking and exposure to asbestos in causing lung cancer in general and various histopathological types

of lung cancer in particular and discuss the possible underlying mechanisms of that interaction.

Tobacco smoke as a lung carcinogen

Carcinogenesis is conventionally regarded as a series of events that begins with the initiation of genetic alterations by an agent or agents that interact with deoxyribonucleic acid (DNA) and lead to heritable alterations. These "initiated" cells require further stimuli to divide during an extended period of tumor "promotion" and "progression." The long latency period between the initiation and diagnosis of cancer may reflect the latter stages.

The epidemiologic data on tobacco smoking and lung cancer are more extensive than those for any other cause of neoplasms in humans. Smoking can affect both early and late stages of carcinogenesis. Smoking in early life has a substantial effect on the risk for cancer in old age and therefore evidently affects at least one early stage. Giving up smoking in later life has a substantial effect on the risk five or ten years later, and therefore it also seems to affect at least one late stage (4).

Tobacco smoke contains more than 3500 chemicals (2). Smoking thus entails exposure to a variety of carcinogens, which include polycyclic aromatic hydrocarbons, such as benzo[a]pyrene, nitrosamines, and aromatic amines. Tobacco smoke is carcinogenic in animals. It induces micronuclei, sister chromatid exchanges, and cell transformation *in vitro* and has tumor-promoting activity in various test systems (2).

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Many of the carcinogens in tobacco smoke are activated metabolically into DNA-binding intermediates, mainly by reactions mediated by cytochrome P450. DNA adducts derived from tobacco smoke have now been detected in lung and other tissues of cigarette smokers (5–9).

In particular, adducts of benzo[a]pyrene diol epoxide to DNA have recently been detected in the lungs of smokers (10). This binding reaction is apparently activated via cytochrome P4501A1, the inducibility of which has been associated with a higher risk for lung cancer among smokers. (For a review, see reference 11.)

There is some evidence that macrophages can replace the P450 system in activating benzo[a]pyrene (12). Benzo[a]pyrene-7,8-dihydrodiol can be oxidized by activated macrophages to derivatives that are mutagenic, bind covalently to DNA, and induce sister chromatid exchange. (For a review, see reference 13.) Benzo[a]pyrene has been reported to cause oxidative damage in DNA about 20 times more frequently than it forms adducts (14).

Action of asbestos in multistage carcinogenesis

Asbestos is not a single mineral. The term covers several fibrous inorganic minerals that share specific properties but differ in chemical composition, morphology, durability, and, therefore, biological effects. All of the main types of asbestos fibers have, however, been shown to be carcinogenic in humans (3).

Advances have been made recently towards understanding the molecular mechanisms of asbestos-induced carcinogenesis. The cytotoxic, genotoxic, and proliferative effects of asbestos seem to be mediated in part by active oxygen species — reactive metabolites of oxygen that are produced from phagocytic cells or catalyzed by iron on the fiber surface (15, 16). (For a review see references 17 and 18.) Asbestos fibers can induce neoplastic cell transformation (19) and chromosome changes in vitro (20); workers exposed occupationally to asbestos have an increased incidence of double-strand breaks in lymphocyte DNA (21).

Asbestos fibers can be phagocytized by macrophages, which then release a wide variety of cytokines and mediators of inflammation that modulate growth and differentiation of the target cells. Damage to DNA in the inflammatory milieu surrounding asbestos fibers may lead to genetic alterations, such as the activation of protooncogenes or the inactivation of tumor suppressor genes, and result in the initiation of carcinogenesis and enhanced growth. Interactions of asbestos fibers with the DNA of their target cells can occur through various mechanisms, including oxidant activity and direct interactions with chromosomes. As a result, chromosome alterations and point mutations may occur in cellular protoon-

cogenes and the expression of genes such as those coding for growth factors may be altered and ultimately lead to the development of tumors. (For a review, see references 17 and 18.)

Interaction between asbestos and tobacco smoking in causing lung cancer

In 1968, Selikoff and his co-workers (22) showed that the exposure of cigarette smokers to asbestos during insulation work was associated with a lung cancer risk far higher than that associated with each agent separately, a finding indicating an interaction between the two agents. Since the time of that seminal study, the asbestos-tobacco smoking-lung cancer paradigm has often been used as an example in methodological discussions of interactions.

In an epidemiologic study, interaction can be classified according to the statistical model that most closely describes the relationship between the observed relative risks among subjects exposed only to tobacco smoke (R_s), only to asbestos (R_a), and to both agents (R_{sa}), all relative risks being calculated in relation to subjects exposed to neither agent (23).

For any R_s and $R_a \leq 1$, the two simplest models or relationships of interaction are the additive model ($R_{sa} = R_s + R_a - 1$) and the multiplicative model ($R_{sa} = R_s \times R_a$) (24, 25). In this review, the absolute interaction magnitude, reconstructed from the figures available in the published reports, is classified with reference to these two models as follows: less than additive ($< A$) for $R_{sa} < -25\%$ of A ; near additive ($\sim A$) for R_{sa} within $\pm 25\%$ of A ; additive (A) for R_{sa} within $\pm 10\%$ of A ; intermediate (I) for $R_{sa} > 25\%$ of A but below -25% of M ; near multiplicative ($\sim M$) for R_{sa} within $\pm 25\%$ of M ; multiplicative (M) for R_{sa} within $\pm 10\%$ of M ; and more than multiplicative ($> M$) for $R_{sa} > 25\%$ of M .

The statistical variability of the measure of interaction was not taken into consideration, but the measures of interaction are very imprecise as the small numbers of lung cancers among nonsmokers exposed to the two risk factors strongly reduce the statistical power. Even though several studies show a similar pattern of interaction, the available data are not sufficient to reject alternative models formally.

The largest epidemiologic studies of the interaction between tobacco smoking and exposure to asbestos are summarized in table 1, which also gives the absolute measure of interaction derived from the original reports. The overall evidence indicates an interaction in the multiplicative region, although the pattern across studies was not uniform. In particular, an additive interaction was observed for Canadian chrysotile miners and millers (26–28), while for Australian underground crocidolite miners a more than multiplicative interaction was seen (29). For workers exposed to amosite or a combination of

Table 1. Studies on the interaction between tobacco smoking and asbestos in the causation of lung cancer. (P = exposure information collected prospectively from study subjects, R = exposure information collected retrospectively from study subjects, O = exposure information collected from informants other than study subjects, for example, relatives, D = exposure information collected from documents, A = additive, M = multiplicative, I = intermediate)

Reference, design, location, years of observation, and exposure	Exposure assessment		Relative risk ^a		Interaction ^b
	Smoking	Asbestos	Smokers	Non-smokers	
Berry et al (30), cohort, United Kingdom, 1960–1970, mixed asbestos factory	D, O	D	2.3 ^c 7.4 ^d	5.0	[>M] [>M]
Selikoff & Hammond (35), cohort, United States, 1963–1974, chrysotile and amosite in insulation work	P	D	[6.0]	[0]	[>M]
Martischnig et al (39), case-referent, United Kingdom, 1973–1974, any asbestos exposure	R	R	[3.2]	1.1	>M
Blot et al (36), case-referent, United States, 1970–1976, shipyards	R, O	R, O	[1.6]	[1.3]	~M
Hammond et al (37), cohort, United States and Canada, 1967–1976, chrysotile and amosite in insulation work	P	D	[5.3]	[5.7]	M
Selikoff et al (31), cohort, United States, 1961–1977, amosite factory	P	D	4.7	25	I
Blot et al (38), case-referent, United States, 1972–1976, shipyards	O	O	[1.6]	[1.9]	~M
Pastorino et al (40), case-referent, Italy, 1976–1979, any asbestos exposure	R	R	[1.8]	[2.8]	I
Acheson et al (32), cohort, United Kingdom, 1947–1980, amosite factory	D	D	[1.6]	[2.0]	[~M]
Liddell et al (28), case-referent in cohort, Canada, 1950–1975, chrysotile mining and milling	O, P	D	[1.7]	[3.0]	~A
Baker (29), cohort, Australia, 1944–1981, crocidolite mining and milling	P	D	[5.0]	0.7	>M
Hilt et al (34), cohort, Norway, 1966–1973, maintenance workers exposed to asbestos	P	D	[4.3]	0	>M
Berry et al (33), cohort, United Kingdom, 1971–1980, mixed asbestos factory	P	D	2.0 ^c 4.3 ^d	6.3 12.5	[A] [I]
Kjuus et al (41), case-referent, Norway, 1979–1983, any asbestos exposure	R	R	[2.1]	[2.4]	~M
De Klerk et al (42), case-referent in cohort, Australia, 1979–1986, crocidolite miners	P	D	2.6	1.9	>M
Cheng & Kong (43), cohort, China, 1972–1987, mixed asbestos factories	D	D	1.6	1.6	M

^a Relative risk due to asbestos exposure; numbers in square brackets have been reconstructed from published data.

^b For definitions of the categories of interaction, see the text. The categories in square brackets have been based on the assumption of a relative risk due to smoking = 10.

^c Men.

^d Women.

amosite, chrysotile, and crocidolite in manufacturing, variations were seen in the size of the observed interaction (30–34). However, studies of highly exposed insulators (35–38) and case-referent studies of any type of asbestos exposure (39–41) showed a more uniformly multiplicative pattern.

A case-referent analysis nested in the cohort of Australian crocidolite miners (42) confirmed the pattern of a more than multiplicative interaction, while in a recently published study from China on workers exposed to chrysotile asbestos in various manufacturing industries, a multiplicative interaction was seen (43).

In conclusion, a variable pattern of interaction has been observed which may reflect both the fact that asbestos and smoking act at different stages of the

carcinogenic process and the fact that there are differences in the biological effects of different types of asbestos fibers (23). Overall, however, studies of workers exposed to high levels of asbestos, such as insulator workers, point to an interaction that approximates the multiplicative model.

Interaction between asbestos and tobacco smoking in causing different histological types of lung cancer

It is often claimed that adenocarcinoma is the histological type of lung cancer most frequently associated with exposure to asbestos. However, Churg (44) provided evidence that this claim may not be true.

He examined the studies in which the distribution of cases of lung cancer with an association to asbestos exposure and those without such an association was reported by cell type. Table 2 adds a few recent studies to the data reported by Churg and gives more details on the exposed and reference populations used in each study. When cases of squamous-cell carcinoma are taken as the reference group, a significant, positive association is seen between exposure to asbestos and small-cell carcinoma, but no association is seen with either adenocarcinoma or large-cell carcinoma. The data reported in table 2 do not allow any conclusion to be drawn with respect to specific types of asbestos fiber.

A few recent studies also provide results on the risks for specific cell types of lung cancer associated with asbestos exposure. In a case-referent study in Japan, Minowa and his co-workers (50) found a significantly increased risk for Kreyberg I lung cancer

(small-cell and squamous-cell carcinoma) after exposure to asbestos, as estimated from job titles [odds ratio (OR) 3.40], and a lower, nonsignificant risk (OR 1.72 total number of exposed cases 38) for Kreyberg II lung cancer (adenocarcinoma). In a cohort study of Danish asbestos-cement workers, who were mainly exposed to chrysotile, the standardized incidence ratio (SIR) was higher for adenocarcinoma [SIR 3.31, 95% confidence interval (95% CI) 2.12–4.92, cases 24] than for squamous-cell carcinoma (SIR 1.67, 95% CI 1.18–2.31, cases 37); a trend was seen for adenocarcinoma, but not for squamous-cell carcinoma, according to the duration of exposure and latency (51). Finally, in a study on lung cancer cases in Finland, in which smoking was adjusted for, the odds ratio for adenocarcinoma associated with exposure to asbestos (measured as $\geq 3 \cdot 10^6$ fibers $\cdot$ g lung tissue⁻¹) was 3.4 (90% CI 0.7–15) when compared with squamous-cell carci-

Table 2. Number of cases of lung cancer by exposure to asbestos and cell type in selected studies.

Reference, location, time, exposed population	Unexposed population	Squamous-cell carcinoma		Small-cell carcinoma		Adeno-carcinoma		Large-cell carcinoma	
		Exposed cases	Un-exposed cases	Exposed cases	Un-exposed cases	Exposed cases	Un-exposed cases	Exposed cases	Un-exposed cases
Kannerstein & Churg (45), United States (period and source of cases not specified), any type of asbestos	Cases without history of asbestos exposure, matched by diagnostic procedure	11	12	11	14	11	9	6	8
Martschnig et al (39), United Kingdom, 1972–1973, cases from one hospital, self-reported exposure to any type of asbestos	Cases without self-reported exposure	26	77	22	27	1	8	—	—
Auerbach et al (46), United States, 1966–1979, three hospitals and a cohort of insulation workers, exposure to any type of asbestos derived from occupational history	Cases from same hospitals	96	335	48	102	29	122	20	89
Ives et al (47), United States, World War II, shipbuilding workers ^a	Cases from same hospitals	24	103	7	27	5	31	—	—
Baker et al (48), Australia, 1958–1978, crocidolite miners	Cases from same province, matched by age and year of diagnosis	21	76	9	46	9	64	6	32
Mollo et al (55), Italy, 1982–1986, exposure to any type of asbestos derived from occupational history	Definite or probable exposure versus possible or no exposure	22	205	1	21	15	97	4	33
Johansson et al (49), Sweden, 1953–1986, asbestos cement works (>95% chrysotile)	Cases from same hospital, matched by diagnostic procedure, age, year of duration and gender	11	42	5	28	9	13	4	13
Odds ratio for asbestos exposure ^b		1 (reference)		1.33		0.93		0.83	
95% confidence interval		—		1.00–1.76		0.69–1.25		0.56–1.23	

^a Data provided by W Blot (personal communication).

^b Mantel-Haenszel odds ratio.

noma, on the basis of nine and four exposed cases, respectively (52).

The differences in the results on the association between asbestos exposure and specific cell types of lung cancer may be due to inadequate control for confounding factors. The source of cases may be an important variable, as shown by Whitwell and his co-workers (53), who compared the proportions of different cell types in series of biopsy samples, surgical specimens, and necropsy samples. They found a lower proportion of adenocarcinomas in the biopsy series than in the necropsy series, and an opposite pattern for squamous-cell carcinoma. Other aspects that may play a role are the type of asbestos fiber and characteristics of the exposure pattern, such as duration and intensity.

Tobacco smoking increases the risk of all main types of lung cancer. However, the risks of squamous-cell and small-cell carcinomas of the lung are increased to a greater extent than that of adenocarcinomas (2).

Very little information is available on the interaction between tobacco smoking and exposure to asbestos in causing different histological types of lung cancer. In a case-referent study of white male patients admitted to a large hospital in New York State in the United States, occupational exposure to asbestos was assessed from the job titles reported by the patients (54). Figure 1 shows the number of cases and the relative risks according to cell type and exposure to smoking and asbestos. On the basis of the categories used in table 1, the interaction is ~M for squamous-cell carcinoma, ~A for small-cell carcinoma, and A for adenocarcinoma. A series of lung cancer patients seen at a hospital in Turin, Italy, was interviewed with respect to asbestos exposure and smoking habits (55), and asbestos bodies were counted in samples of normal lung tissue. A case-case analysis was then carried out in which cases of adenocarcinoma were compared with cases of squamous-cell carcinoma. The term for interaction between tobacco smoking (more than 20 cigarettes per day versus 0–20 cigarettes per day) and exposure to asbestos (definite or probable exposure versus possible or no exposure) was negative (OR 0.94, 95% CI 0.23–3.86), while that between smoking and asbestos body count (more than 10 000 bodies · g⁻¹ versus up to 10 000 bodies · g⁻¹) was positive (OR 1.82, 95% CI 0.08–41.7). Neither interaction term was statistically significant and therefore suggested that there was no difference according to histological type in the interaction between exposure to asbestos and tobacco smoking. Both terms were, however, highly imprecise.

The report of the study (52) on lung cancer cases from Helsinki, Finland, provided enough detail to allow an ad hoc analysis of the interaction between smoking (categorized as <50 pack-years versus more) and asbestos exposure (up to 3 · 10⁶ fibers · g lung tissue⁻¹ versus more) by cell type. The age-ad-

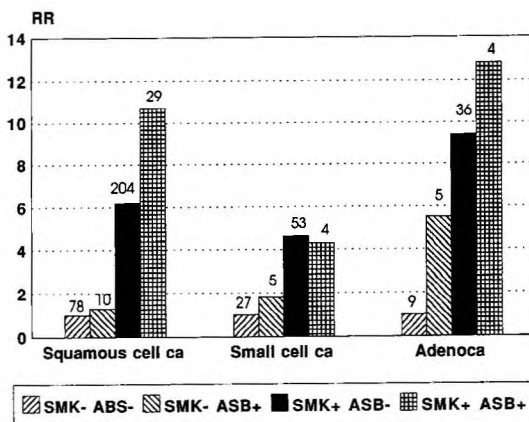


Figure 1. Numbers of cases and the relative risks according to lung cancer cell type and exposure to smoking and asbestos. The results derive from the case-referent study of Vena et al (49). The analysis was based on crude data. Smoking (SMK) exposure is categorized as 0–39 pack-years (SMK-) versus ≥40 pack-years (SMK+). Asbestos (ASB) exposure is categorized as never (ASB-) versus ever (ASB+) exposed. The reference category is SMK- ASB-. The numbers of referents were as follows: SMK- ASB- 718, SMK- ASB+ 72, SMK+ ASB- 304, SMK+ ASB+ 25. (ca = carcinoma, Adenoca = adenocarcinoma)

justed odds ratios for adenocarcinoma, using squamous-cell carcinoma as the reference, were 1.1 (95% CI 0.1–9.3) for the subjects exposed to asbestos but not to smoking, 0.4 (95% CI 0.1–2.8) for the subjects exposed to smoking but not to asbestos, and 3.2 (95% CI 0.6–20) for the subjects exposed to both, as compared with subjects exposed to neither agent, a finding suggesting a stronger interaction (ie, closer to >M than <A) between exposure to asbestos and smoking in the occurrence of adenocarcinoma than in the occurrence of squamous-cell carcinoma.

Biological basis of the combined effect

In an attempt to explain the multiplicative increase in the risk for lung cancer associated with combined exposure to asbestos and tobacco smoke, hamsters were given intratracheal instillations of asbestos fibers and benzo[a]pyrene. The substances induced tumors only when given in combination (56). The mechanisms of the joint effect are not known. It is possible that fibers enhance the penetration of tobacco smoke constituents (such as benzo[a]pyrene) into cells. Cigarette smoke condensate in combination with crocidolite asbestos induces the formation of hydroxyl radicals and DNA strand breaks in isolated DNA (57). Asbestos fibers can cause inflammatory reactions, oxygen radical bursts, and, after long-term exposure, fibrosis of the lungs. Tobacco smoke contains several carcinogenic chemicals that bind to DNA and activate protooncogenes and tumor suppressor genes. Activated oxygen species induce mu-

tations in mammalian cells (58). Recently, there has been a surge of interest in the role of nitrogen oxide and its derivatives in the pathobiology of chronic infection and its relationship to the carcinogenic process (59). Macrophages produce inflammatory cytokines and oxygen radicals when exposed to asbestos fibers, and it was reported recently that macrophages also produce nitric oxide in response to asbestos (60). Tobacco smoke contains up to 600 µg of nitric oxide and other nitrogen oxides per cigarette (2, p 95). Excess nitric oxide is mutagenic and induces DNA damage by deaminating nucleotide bases, such as 5-methylcytosine, and inducing strand breaks.

Damage to DNA is critical to the process of initiation in the multistage model of carcinogenesis, and the joint effect of exposure to tobacco smoke and asbestos depends on the relative magnitude of the effects on mutation rates and on the rate of clonal expansion of mutated cells (61). The DNA modification must be sufficiently tenacious to escape efficient repair processes but not so excessive that cell death results. Common sites for point mutations, in both the *K-ras* gene and the *p53* suppressor gene, are guanine:cytosine base pairs in deoxycytidine-3',5'-deoxyguanosine dinucleotide sequences (62, 63), so that guanine:cytosine base pairs in both tumor suppressor genes and protooncogenes may represent vulnerable targets for mutation. As has already been described, exposure to asbestos is associated with an excess occurrence of adenocarcinomas in some studies, and adenocarcinomas have a higher frequency of *K-ras* mutations than squamous-cell carcinomas do (52). In a Finnish study of lung adenocarcinoma patients, mutations in the *K-ras* gene were present in 46% (17 of 37), and guanine-to-thymine transversions were the predominant type of mutation (41%, 7 of 17) (Ridantpää et al, unpublished manuscript). Smoking is associated with guanine-to-thymine transversions in codon 12 of the *K-ras* gene, which can be caused, for example, by benzo[a]pyrene. Asbestos fibers can, under suitable circumstances, hydroxylate 2-deoxyguanosine to 8-hydroxydeoxyguanosine, mediated by hydroxyl radicals (64); the presence of 8-hydroxyguanine in DNA would also lead to guanine-to-thymine transversions. Guanine-to-adenine and cytosine-to-thymine transitions are also fairly common in lung tumors, especially in the *p53* gene (65). The deamination of 5-methylcytosine at cytosine-guanosine dinucleotides by nitric oxide has been suggested to cause cytosine-to-thymine transitions in vivo (66, 67). Exposure to asbestos may increase the frequency of transition and transversion mutations indirectly via oxygen radical or nitric oxide pathways (65) and therefore increase the likelihood of *K-ras* mutations in adenocarcinomas (52, 68). Another mechanism could be enhancement of clonal expansion, improved recruitment of mutated cells, and a selective growth advantage to mutated cells.

Concluding remarks

The joint effect of two exposures, both of which affect more than one stage of carcinogenesis, depends on the relative magnitude of the effects on early and late stages. Tobacco smoke and asbestos fibers may have interdependent effects on the multistage process of lung carcinogenesis. Tobacco smoke can act at early stages, inducing genetic alterations, DNA adducts, and mutations in genes critical to oncogenesis, and the epidemiologic evidence suggests that tobacco smoke may also act at later stages of carcinogenesis. Asbestos fibers can be cytotoxic and genotoxic and cause proliferative lesions in the lungs, mediated in part by oxygen radicals and nitrogen oxides. Chronic inflammation of the lungs can release various cytokines and growth factors which may provide a selective growth advantage to mutated cells.

The epidemiologic evidence for the interaction between asbestos and tobacco smoking is clearest in studies of workers exposed to high levels of asbestos (ie, asbestos insulation workers), and that evidence points to an interaction that approximates the multiplicative model. In other situations, a variable pattern of interaction has been observed. Tobacco smoking increases the risk for adenocarcinoma of the lung to a less extent than other major histological types. Asbestos exposure is associated with an excess occurrence of adenocarcinomas in some, but not all, studies. Unfortunately, the existing data for assessing the interaction for a particle histological subtype of lung cancer are weak, and further epidemiologic studies are warranted.

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