cc: R. Buccafusco
K. Macon
E. Rogers
G. Rusch
T. Stewart

From: J. Charm 7/28/86

Sent

ENVIRONMENTAL RESEARCH 40, 84–91 (1986)

The Adsorption of Polyaromatic Hydrocarbons on Natural and Chemically Modified Asbestos Fibers

HUGUES MENARD,* LUC NOEL, JALEH KHORAMI, JEAN-LOUIS JOUVE, AND JACQUES DUNNIGAN†

Département de chimie, Faculté des sciences, Université de Sherbrooke, and †L'Institut de l'amiante, Division de la recherche, Sherbrooke, Province of Quebec J1K 2R1, Canada

Received June 1, 1984

Many reports indicate that the carcinogenic (genotoxic) potential of benzo[*a*]pyrene (B[a]P) may be enhanced several-fold by the promoter (epigenetic) effect of asbestos particles. This promoting effect could be related to the fact that when B[a]P is adsorbed onto the particles, there is a resulting enhanced transport and uptake of the carcinogen into microsomial membranes. These *in vitro* data bear relevance to the epidemiological studies which indicate an association between exposure to inhaled asbestos dusts and the high incidence of pulmonary cancers in smokers. Using HPLC, it has been observed that B[a]P has great affinity for natural asbestos fibers, and that chemical modification of natural chrysotile with $POCl_3$ results in the complete loss of this adsorption potential of chrysotile for benzo[a]pyrene.

INTRODUCTION

It is generally recognized that carcinogenesis is a multistep process, which occurs mainly in two sequential stages: initiation and promotion. The initiation stage corresponds to some induced alteration in the cell, associated with a damaged or modified DNA replication system. The promotion stage encompasses a number of conditions necessary for malignancy to be expressed in an "initiated" tissue. This scheme has formed the basis for the distinction of chemical agents into two categories: those which damage genetic material directly, the so-called genotoxic agents; and those that operate by indirect, nongenotoxic, or epigenetic mechanisms. A review on the subject has been published recently by Weisburger and Williams (1983). The authors explain that genotoxic agents undergo a series of competing reactions, ultimately reacting with DNA, which appears to be the critical event in carcinogenesis. Once cell duplication with the generated abnormal DNA has occurred, the effect is basically irreversible. In contrast, the action of agents operating by epigenetic mechanisms, which are as yet unclear and require much more research, usually necessitates their presence at high levels for a long time and, indeed, is reversible up to a certain point.

Substances operating on cell systems as epigenetic agents act by diverse mechanisms that are definitely different from those involving genotoxic pathways. A case in point is the situation where numerous well-known genotoxic agents have been used experimentally, both *in vivo* and *in vitro*, in combination with asbestos fibers. One of the best studied agents is benzo[*a*]pyrene (B[a]P). It has been reported by many authors that the mutagenic and carcinogenic potentials of this chemical are enhanced considerably when associated with particles. According

0013-9351/86 $3.00

to some authors, this promoting effect of particles could be related to the fact that when B[a]P is adsorbed onto the particles, there is a resulting enhanced transport and uptake of the carcinogen into microsomal membranes (Kandaswami and O'Brien, 1980; Lakowicz and Bevan, 1980).

The resulting effect of such particle-enhanced transport of carcinogens has been illustrated recently by Reiss *et al.* (1983), who studied the comutagenicity of chrysotile asbestos and B[a]P. The authors found that exposure of adult rat liver epithelial cells to chrysotile alone did not increase the mutant incidence, whereas B[a]P alone was mutagenic. Simultaneous exposure of the cells to chrysotile and B[a]P results in a greatly enhanced mutant recovery compared to either of these substances alone. The authors indicate that these results "extend their previous studies and strenghthen the proposal that asbestos is not a genotoxic carcinogen capable of altering DNA."

These results are essentially consistent with the previously expressed views of Mossman and Craighead (1981). These authors found that aryl hydrocarbon hydroxylase (AHH) activity was not changed in hamster tracheal epithelial organ culture after exposure to crocidolite (AHH system is required to metabolize a procarcinogen into a reactive, or "ultimate" carcinogen). However, it was observed that crocidolite fibers potentiated the effects of 3-methylcholanthrene (3 MC). Consistent with these results, the authors found that carcinomas developed after 12 to 52 weeks from tissues exposed to crocidolite with surface bound 3 MC, whereas neoplasms failed to evolve from organ cultures exposed to crocidolite in the absence of 3 MC (Mossman and Craighead, 1979). The authors suggest that "asbestos fibers might serve as a physical carrier of chemical carcinogens, providing a means of introducing polycyclic hydrocarbons into the cells, assuming these cells are adsorbed to the fibers before phagocytosis." Furthermore, they suggest that crocidolite, due to its chemical constitution, might also have a direct, potentiating effect on the AHH system. Thus, according to Eastman *et al.* (1983) asbestos "resembles a classical tumor promoter." In partial contradiction to the effect just reported by Mossman and Craighead on AHH inducibility, it may come as a surprise to learn that crocidolite and chrysotile have been found to *inhibit* AHH (Kandaswami and O'Brien, 1980); these authors interpret their finding as yet another mechanism, whereby asbestos fibers would retard the rapid metabolism of B[a]P, and thus prolong the retention of the carcinogen in the tissue, thereby increasing the risk of induction of carcinoma. Clearly, these opposite views in the data, and the ensuing interpretation in terms of mechanism of action, will have to be sorted out. However, both groups of workers are in total agreement as to the importance of the phenomenon of adsorption of B[a]P on asbestos fibers.

Most reports dealing with this phenomenon are in agreement to underline the compelling relevance of this observation to epidemiological data, which clearly indicate an association between exposure to the asbestos dust and the high incidence of lung carcinoma in smokers. For instance, Selikoff *et al.* (1968) have established that the incidence of pulmonary cancers among asbestos workers who are cigarette smokers is 92 times that of the general population, whereas the increase in disease among nonsmoking asbestos workers is quite low. This particular situation, in which the experimental data correlate so remarkably well with

epidemiological data, has prompted us to measure the "carrier" properties of chrysotile asbestos for B[a]P and other polyaromatic compounds, and to study the effect on these properties of a chemical modification of asbestos after treatment with phosphorus oxychloride ($POCl_3$).

EXPERIMENTAL

$POCl_3$-treated (Lalancette and Dunnigan, 1981) and untreated samples of chrysotile asbestos fibers were prepared from two grades: a commercial grade used in asbestos–cement application, "Québec Standard Grade (QS 4T)" and a shorter commercial grade, "Québec Standard Grade (QS 7D)," used in felt applications.

These four different samples were studied by infrared spectroscopy, thermal analysis (TG/DTG), surface area (BET), and surface charge (zeta potential) measurements. Adsorption isotherms of polyaromatic compounds were obtained by high-pressure liquid chromatography.

The ir Fourier transform spectroscopy was performed using a Nicolet FT-ir-spectrometer, Model MX-1. For the thermal analyses, the following conditions were used: heating rate of 20°C/min; dynamic dry nitrogen atmosphere of 50 cm^3/min. The analyses were carried out using a Perkin–Elmer TGS-2 apparatus. Surface area data (BET) were obtained on a Quantachrome Model S-10 apparatus, and zeta potential measurements were carried out on a Zeta reader, Model ZR-11 (Komline–Sanderson).

The determination of isotherms was carried out using a HPLC system (Beckman, Model 100-A), coupled with a uv detector (Altex–Hitachi, Model 100-40), and a 20 μl flow cell volume. Isotherms were recorded (Varian, Model 9176) and coupled with an integrator (Hewlett–Packard, Model 3390A). The column was filled using an air-driven fluid pump (Haskel). The tubing was 3.5 cm long with an internal diameter of 4.6 mm. Other details of the procedure for obtaining adsorption isotherms have been published (Ménard *et al.*, 1984).

RESULTS AND DISCUSSION

Ir Spectroscopy

The ir spectra of phosphated (1.8 and 0.8% by weight respectively for 7D and 4T QS grades) fibers are given in Fig. 1. The comparison of ir spectra of phosphated and nontreated fibers indicate an increase in the intensities of OH^- vibrations at 3400 and 1650 cm^{-1}, and a broadening of the peaks at 1020 and 1080 cm^{-1}, attributed to the Si–O stretching modes of chrysotile. The increase in the intensities of bands at 3400 and 1650 cm^{-1} corresponding to free hydroxyl stretching vibrations is attributed to hydrated phosphate compound formation. The decrease in intensities of the peaks at 1020 and 1080 cm^{-1} is due to the superposition of the P–O–P stretching vibrations between 900 to 1200 cm^{-1}.

Thermogravimetry Analysis

Figure 2 shows the thermograms of phosphated and untreated samples. The thermograms indicate an increase of weight loss between 30 to 250°C for phosphated fibers, and a sharply reduced weight loss of brucite (specially for QS 4T fibers, rich in brucite) associated originally with the chrysotile fibers. Brucite decomposition appears in the zone between 250 and 400°C. The major dehydrox-

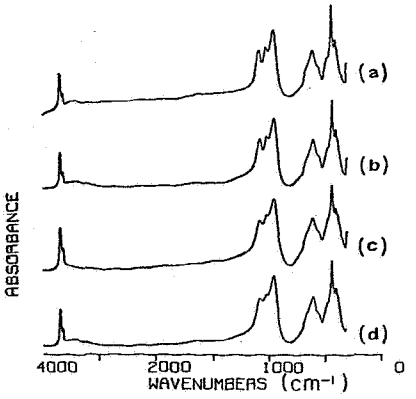

FIG. 1. Infrared spectra of different chrysotile fibers in KBr disk. (a) QS grade 7D, (b) $POCl_3$-treated QS grade 7D, (c) QS grade 4T, and (d) $POCl_3$-treated QS grade 4T.

ylation peak (DTG) between 500 and 780°C was modified for the treated samples. On the other hand, it has been proved that the magnesite and calcite in asbestos decompose in the same zone as chrysotile. The thermograms also indicate a slight change in the rate of dehydroxylation of chrysotile. These observations indicate that $POCl_3$ reacts initially with alkaline impurities ($Mg(OH)_2$, $MgCO_3$, and $CaCO_3$) associated with the chrysotile fibers, forming hydrated magnesium phosphate. The slower rate of dehydroxylation indicates the adsorption or adhesion of magnesium phosphate coming from the impurities reacting with $POCl_3$, and the formation of a phosphate layer involving one or more Mg–O–P bands on the surface of chrysotile.

These modifications of ir spectra and TG/DTG thermograms are more striking in the case of phosphated short fibers, with a higher specific surface area than long fibers (Lalancette and Dunnigan, 1981). Chemical analysis of phosphated short fibers shows a higher quantity of phosphate associated.

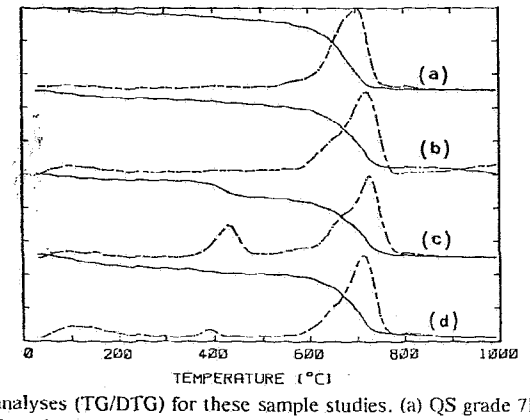

FIG. 2. Thermal analyses (TG/DTG) for these sample studies. (a) QS grade 7D, (b) $POCl_3$-treated QS grade 7D, (c) QS grade 4T, and (d) $POCl_3$-treated QS grade 4T.

Specific Surface Area

Measurements of specific surface area for the four samples give the following data: 10 and 13 $m^2 \cdot g^{-1}$ respectively for the untreated QS grades 7D and 4T, and 5 and 4 $m^2 \cdot g^{-1}$ respectively for the corresponding $POCl_3$-treated samples. From these results we can see a decrease in available surface for phosphated fibers. This lower specific surface area is attributed to a coating of magnesium phosphate salts on the surface, and can confirm the encapsulation of the fibers.

Zeta Potential

Measurements of zeta potentials for the four samples gave the following results: +20 and +18 mV respectively for the untreated QS grades 7D and 4T, and −39 and −36 mV respectively for the corresponding $POCl_3$-treated samples.

Adsorption Isotherms

The results obtained by HPLC with dry toluene solvent as mobile phase (Ménard *et al.*, 1984) and using Chuduk's (1981) calculation technique have enabled us to determine the adsorption isotherms of polyaromatic hydrocarbons, especially B[a]P. Table 1 shows the retention times for untreated and $POCl_3$-treated QS 7D fibers. Figure 3 shows the corresponding adsorption isotherms and demonstrates that phosphated fibers adsorb none or very little of the B[a]P, whereas natural fibers show a very high adsorption potential. In Table 1, B[a]P has a net retention time of 28.0 min for the natural fibers, with only 0.08 min for the phosphated fibers. Moreover, the required pressure to drive the solvent through the column filled with phosphated fibers is 10 times smaller than for the natural fiber.

Natural and phosphated 4T fibers were studied in the same fashion as the grade 7D fibers. Therefore Table 2 gives the net retention times for the polyaromatic hydrocarbons, especially B[a]P. The net retention time for B[a]P on phosphated 4T fibers shows no adsorption, while there is a net retention time of 25 min for QS 4T natural fibers. Figure 4 shows the two adsorption isotherms.

TABLE 1
RETENTION TIME OF POLYAROMATIC HYDROCARBONS ON NATURAL AND $POCl_3$-TREATED QS 7D CHRYSOTILE FIBERS

Natural QS grade 7D fibers		$POCl_3$-treated QS grade 7D fibers	
Agent injected (20 nmole)	T_R[a] (min)	Agent injected (20 nmole)	T_R (min)
Benzo[*a*]pyrene	≈28.0 ± .5	Benzo[*a*]pyrene	0.08 ± .01
Fluoranthrene	1.32 ± .03	Fluoranthrene	0.00 ± .01
Pyrene	1.07 ± .02	Pyrene	0.00 ± .01
Phenanthrene	0.40 ± .01	Phenanthrene	0.00 ± .01
Anthracene	0.38 ± .01	Anthracene	0.00 ± .01
Fluorene	0.18 ± .01	Fluorene	0.00 ± .01
Naphtalene	0.04 ± .01	Naphtalene	0.00 ± .01

[a] T_R = reduced retention time.

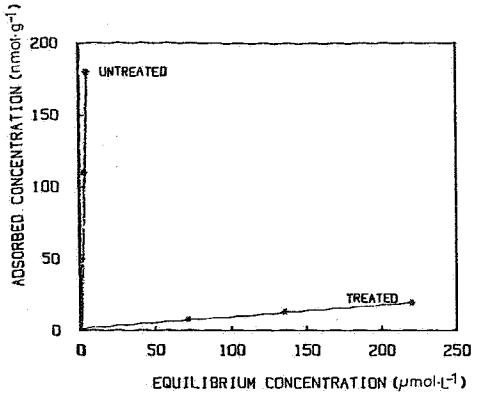

FIG. 3. Adsorption isotherms of benzo[*a*]pyrene obtained by HPLC technique on the QS 7D fibers.

DISCUSSION

Because of the apparent pathogenicity of asbestos fibers, there has been a general reaction of the public and certain health authorities regarding the use of products containing asbestos fibers. This has led to a certain amount of research to modify asbestos fibers in such a way as to reduce as much as possible the undesirable biological effects of asbestos fibers.

Various materials have been examined which interact with the surface of asbestos fibers and reduce its hemolytic activity. Such materials include EDTA, simple phosphates, disodium versenate, polyvinylpyridine *N*-oxide and aluminum (Macnab and Harington, 1967), and certain acidic polymers (Schnitzer and Pundsack, 1970). More recently, it has also been found that asbestos fibers with at least one metal molybdate (Pezzoli, 1979a) or metal tungstate (Pezzoli, 1979b) deposited thereon have reduced hemolytic activity in comparison with untreated asbestos fibers.

TABLE 2
RETENTION TIME OF POLYAROMATIC HYDROCARBONS ON NATURAL AND $POCl_3$-TREATED QS 4T CHRYSOTILE FIBERS

Natural QS grade 4T fibers		$POCl_3$-treated QS grade 4T fibers	
Agent injected (20 nmole)	T_R[a] (min)	Agent injected (20 nmole)	T_R (min)
Benzo[*a*]pyrene, 1000 nmole	25.0 ± .5	Benzo[*a*]pyrene	≈0.00 ± .01
Fluoranthrene	2.53 ± .03	Fluoranthrene	0.00 ± .01
Pyrene	1.94 ± .02	Pyrene	0.00 ± .01
Phenanthrene	0.45 ± .01	Phenanthrene	0.00 ± .01
Anthracene	0.42 ± .01	Anthracene	0.00 ± .01
Fluorene	0.08 ± .01	Fluorene	000 ± .01
Naphtalene	≈0.02 ± .01	Naphtalene	0.00 ± .01

[a] T_R = reduced retention time.

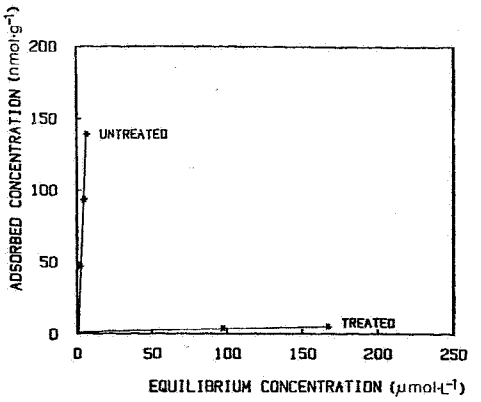

FIG. 4. Adsorption isotherms of benzo[*a*]pyrene obtained by HPLC technique on the QS 4T fibers.

The results reported here relate to a novel method for treating asbestos fibers: depositing phosphate groups on the asbestos fibers. Specifically, the data presented here indicate that the treatment results in modifications of infrared spectrum, and of thermal analysis and zeta potential data. More importantly, they also show a very important modification which relates to the alleged promoter, or cocarcinogenic, effect of asbestos fibers: the nearly complete loss of the treated fibers to adsorb and carry some known initiators of lung carcinogenesis present in tobacco smoke. The importance of this finding in a strategy to design safer fibers is of great significance and should be confirmed by appropriate cocarcinogenicity and comutagenicity biological assays.

REFERENCES

Chuduk, N. A., Eltekov, Yu. A., and Kiselev, A. V. (1981). Study of adsorption from solutions on silica by liquid chromatography method. *J. Colloid Interface Sci.* 84, 149–154.

Eastman, A., Mossman, B. T., and Bresnick, E. (1983). Influence of asbestos on the uptake of benzo[*a*]pyrene and DNA alkylation in hamster treacheal epithelial cells. *Cancer* 43, 1251–1255.

Kandaswami, C., and O'Brien, P. J. (1980) Effects of asbestos on membrane transport and metabolism of benzo[*a*]pyrene. *Biochem. Biophys. Res. Commun.* 97, 794–801.

Kimmerle, F.M., Khorami, J., and Choquette, D. (1982). Thermal analysis of phosphated chrytosile fibers. *In* "Proceedings, 7th Int. Conf. on Therm. Anal." Vol. 1, pp. 614–620.

Lakowicz, J. R., and Bevan, D. R. (1980). Benzo[*a*]pyrene uptake into rat liver microsomes: Effects of adsorption of benzo[*a*]pyrene to asbestos and non-fibrous mineral particulates. *Chem.-Biol. Interac.* 29, 129–138.

Lalancette, J. M., and Dunnigan, J (1981). "Novel Phosphated Asbestos Fibres." U.S. Patent No. 4,356,057, Oct. 26.

Macnab, G., and Harington, J. S. (1967). Hemolytic activity of asbestos and other mineral dusts. *Nature (London)* 214, 522–533.

Ménard, H., Noël, L., Kimmerle, F. M., Tousignant, L., and Lambert, M. (1984). Adsorption isotherms of polycyclic aromatic hydrocarbons on asbestos chrysotile by high-pressure liquid chromatography. *Anal. Chem.* 56, 1240–1242.

Mossman, B. T., and Craighead, J. E. (1979). Use of the hamster tracheal organ cultures for assessing the cocarinogenic effects of inorganic particulates on the respiratory epithelium. *Prog. Exp. Tumor Res.* 24, 37–47.

Mossman, B. T., and Craighead, J. E. (1981). Mechanisms of asbestos carcinogenesis. *Environ. Res.* 25, 269–280.

Pezzoli, P. A. (1979a). "Asbestos Treatment with Metal Molybdates." U.S. Patent No. 4,171,405, Oct. 16.

Pezzoli, P. A. (1979b). "Asbestos Treatment with Metal Tungstate." U.S. Patent No. 4,168,346, Sept. 18.

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.

Schnitzer, R. J., and Pundsack, F. L. (1970). Asbestos hemolysis. *Environ. Res.* 3, 1–13.

Selikoff, I. J., Hammond, E. C., and Chung J. (1968). Asbestos exposure, smoking and neoplasia. *J. Amer. Med. Assoc.* 204, 106–112.

Weisburger, J. H., and Williams, G. M. (1983). The distinct health risk: Analyses required for genotoxic carcinogens and promoting agents. *Environ. Health Perspec.* 50, 233–245.

ENVIRONMENTAL RESEARCH 40, 84–91 (1986)

The Adsorption of Polyaromatic Hydrocarbons on Natural and Chemically Modified Asbestos Fibers

HUGUES MENARD,* LUC NOEL, JALEH KHORAMI, JEAN-LOUIS JOUVE, AND JACQUES DUNNIGAN†

Département de chimie, Faculté des sciences, Université de Sherbrooke, and †L'Institut de l'amiante, Division de la recherche, Sherbrooke, Province of Quebec J1K 2R1, Canada

Received June 1, 1984

Many reports indicate that the carcinogenic (genotoxic) potential of benzo[*a*]pyrene (B[a]P) may be enhanced several-fold by the promoter (epigenetic) effect of asbestos particles. This promoting effect could be related to the fact that when B[a]P is adsorbed onto the particles, there is a resulting enhanced transport and uptake of the carcinogen into microsomial membranes. These *in vitro* data bear relevance to the epidemiological studies which indicate an association between exposure to inhaled asbestos dusts and the high incidence of pulmonary cancers in smokers. Using HPLC, it has been observed that B[a]P has great affinity for natural asbestos fibers, and that chemical modification of natural chrysotile with $POCl_3$ results in the complete loss of this adsorption potential of chrysotile for benzo[a]pyrene.

INTRODUCTION

It is generally recognized that carcinogenesis is a multistep process, which occurs mainly in two sequential stages: initiation and promotion. The initiation stage corresponds to some induced alteration in the cell, associated with a damaged or modified DNA replication system. The promotion stage encompasses a number of conditions necessary for malignancy to be expressed in an "initiated" tissue. This scheme has formed the basis for the distinction of chemical agents into two categories: those which damage genetic material directly, the so-called genotoxic agents; and those that operate by indirect, nongenotoxic, or epigenetic mechanisms. A review on the subject has been published recently by Weisburger and Williams (1983). The authors explain that genotoxic agents undergo a series of competing reactions, ultimately reacting with DNA, which appears to be the critical event in carcinogenesis. Once cell duplication with the generated abnormal DNA has occurred, the effect is basically irreversible. In contrast, the action of agents operating by epigenetic mechanisms, which are as yet unclear and require much more research, usually necessitates their presence at high levels for a long time and, indeed, is reversible up to a certain point.

Substances operating on cell systems as epigenetic agents act by diverse mechanisms that are definitely different from those involving genotoxic pathways. A case in point is the situation where numerous well-known genotoxic agents have been used experimentally, both *in vivo* and *in vitro*, in combination with asbestos fibers. One of the best studied agents is benzo[*a*]pyrene (B[a]P). It has been reported by many authors that the mutagenic and carcinogenic potentials of this chemical are enhanced considerably when associated with particles. According

0013-9351/86 $3.00

to some authors, this promoting effect of particles could be related to the fact that when B[a]P is adsorbed onto the particles, there is a resulting enhanced transport and uptake of the carcinogen into microsomal membranes (Kandaswami and O'Brien, 1980; Lakowicz and Bevan, 1980).

The resulting effect of such particle-enhanced transport of carcinogens has been illustrated recently by Reiss *et al.* (1983), who studied the comutagenicity of chrysotile asbestos and B[a]P. The authors found that exposure of adult rat liver epithelial cells to chrysotile alone did not increase the mutant incidence, whereas B[a]P alone was mutagenic. Simultaneous exposure of the cells to chrysotile and B[a]P results in a greatly enhanced mutant recovery compared to either of these substances alone. The authors indicate that these results "extend their previous studies and strenghthen the proposal that asbestos is not a genotoxic carcinogen capable of altering DNA."

These results are essentially consistent with the previously expressed views of Mossman and Craighead (1981). These authors found that aryl hydrocarbon hydroxylase (AHH) activity was not changed in hamster tracheal epithelial organ culture after exposure to crocidolite (AHH system is required to metabolize a procarcinogen into a reactive, or "ultimate" carcinogen). However, it was observed that crocidolite fibers potentiated the effects of 3-methylcholanthrene (3 MC). Consistent with these results, the authors found that carcinomas developed after 12 to 52 weeks from tissues exposed to crocidolite with surface bound 3 MC, whereas neoplasms failed to evolve from organ cultures exposed to crocidolite in the absence of 3 MC (Mossman and Craighead, 1979). The authors suggest that "asbestos fibers might serve as a physical carrier of chemical carcinogens, providing a means of introducing polycyclic hydrocarbons into the cells, assuming these cells are adsorbed to the fibers before phagocytosis." Furthermore, they suggest that crocidolite, due to its chemical constitution, might also have a direct, potentiating effect on the AHH system. Thus, according to Eastman *et al.* (1983) asbestos "resembles a classical tumor promoter." In partial contradiction to the effect just reported by Mossman and Craighead on AHH inducibility, it may come as a surprise to learn that crocidolite and chrysotile have been found to *inhibit* AHH (Kandaswami and O'Brien, 1980); these authors interpret their finding as yet another mechanism, whereby asbestos fibers would retard the rapid metabolism of B[a]P, and thus prolong the retention of the carcinogen in the tissue, thereby increasing the risk of induction of carcinoma. Clearly, these opposite views in the data, and the ensuing interpretation in terms of mechanism of action, will have to be sorted out. However, both groups of workers are in total agreement as to the importance of the phenomenon of adsorption of B[a]P on asbestos fibers.

Most reports dealing with this phenomenon are in agreement to underline the compelling relevance of this observation to epidemiological data, which clearly indicate an association between exposure to the asbestos dust and the high incidence of lung carcinoma in smokers. For instance, Selikoff *et al.* (1968) have established that the incidence of pulmonary cancers among asbestos workers who are cigarette smokers is 92 times that of the general population, whereas the increase in disease among nonsmoking asbestos workers is quite low. This particular situation, in which the experimental data correlate so remarkably well with

epidemiological data, has prompted us to measure the "carrier" properties of chrysotile asbestos for B[a]P and other polyaromatic compounds, and to study the effect on these properties of a chemical modification of asbestos after treatment with phosphorus oxychloride ($POCl_3$).

EXPERIMENTAL

$POCl_3$-treated (Lalancette and Dunnigan, 1981) and untreated samples of chrysotile asbestos fibers were prepared from two grades: a commercial grade used in asbestos–cement application, "Québec Standard Grade (QS 4T)" and a shorter commercial grade, "Québec Standard Grade (QS 7D)," used in felt applications.

These four different samples were studied by infrared spectroscopy, thermal analysis (TG/DTG), surface area (BET), and surface charge (zeta potential) measurements. Adsorption isotherms of polyaromatic compounds were obtained by high-pressure liquid chromatography.

The ir Fourier transform spectroscopy was performed using a Nicolet FT-ir-spectrometer, Model MX-1. For the thermal analyses, the following conditions were used: heating rate of 20°C/min; dynamic dry nitrogen atmosphere of 50 cm^3/min. The analyses were carried out using a Perkin–Elmer TGS-2 apparatus. Surface area data (BET) were obtained on a Quantachrome Model S-10 apparatus, and zeta potential measurements were carried out on a Zeta reader, Model ZR-11 (Komline–Sanderson).

The determination of isotherms was carried out using a HPLC system (Beckman, Model 100-A), coupled with a uv detector (Altex–Hitachi, Model 100-40), and a 20 μl flow cell volume. Isotherms were recorded (Varian, Model 9176) and coupled with an integrator (Hewlett–Packard, Model 3390A). The column was filled using an air-driven fluid pump (Haskel). The tubing was 3.5 cm long with an internal diameter of 4.6 mm. Other details of the procedure for obtaining adsorption isotherms have been published (Ménard *et al.*, 1984).

RESULTS AND DISCUSSION

Ir Spectroscopy

The ir spectra of phosphated (1.8 and 0.8% by weight respectively for 7D and 4T QS grades) fibers are given in Fig. 1. The comparison of ir spectra of phosphated and nontreated fibers indicate an increase in the intensities of OH^- vibrations at 3400 and 1650 cm^{-1}, and a broadening of the peaks at 1020 and 1080 cm^{-1}, attributed to the Si–O stretching modes of chrysotile. The increase in the intensities of bands at 3400 and 1650 cm^{-1} corresponding to free hydroxyl stretching vibrations is attributed to hydrated phosphate compound formation. The decrease in intensities of the peaks at 1020 and 1080 cm^{-1} is due to the superposition of the P–O–P stretching vibrations between 900 to 1200 cm^{-1}.

Thermogravimetry Analysis

Figure 2 shows the thermograms of phosphated and untreated samples. The thermograms indicate an increase of weight loss between 30 to 250°C for phosphated fibers, and a sharply reduced weight loss of brucite (specially for QS 4T fibers, rich in brucite) associated originally with the chrysotile fibers. Brucite decomposition appears in the zone between 250 and 400°C. The major dehydrox-

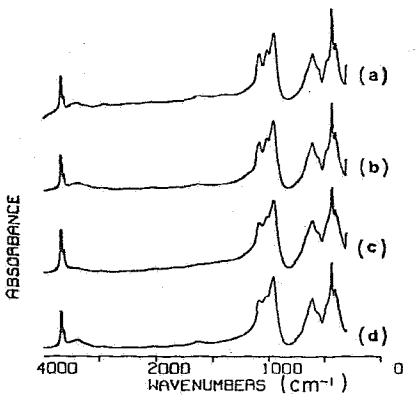

FIG. 1. Infrared spectra of different chrysotile fibers in KBr disk. (a) QS grade 7D, (b) $POCl_3$-treated QS grade 7D. (c) QS grade 4T, and (d) $POCl_3$-treated QS grade 4T.

ylation peak (DTG) between 500 and 780°C was modified for the treated samples. On the other hand, it has been proved that the magnesite and calcite in asbestos decompose in the same zone as chrysotile. The thermograms also indicate a slight change in the rate of dehydroxylation of chrysotile. These observations indicate that $POCl_3$ reacts initially with alkaline impurities ($Mg(OH)_2$, $MgCO_3$, and $CaCO_3$) associated with the chrysotile fibers, forming hydrated magnesium phosphate. The slower rate of dehydroxylation indicates the adsorption or adhesion of magnesium phosphate coming from the impurities reacting with $POCl_3$, and the formation of a phosphate layer involving one or more Mg–O–P bands on the surface of chrysotile.

These modifications of ir spectra and TG/DTG thermograms are more striking in the case of phosphated short fibers, with a higher specific surface area than long fibers (Lalancette and Dunnigan, 1981). Chemical analysis of phosphated short fibers shows a higher quantity of phosphate associated.

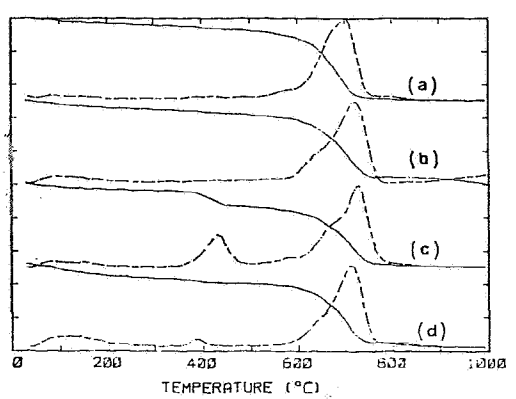

FIG. 2. Thermal analyses (TG/DTG) for these sample studies. (a) QS grade 7D, (b) $POCl_3$-treated QS grade 7D, (c) QS grade 4T, and (d) $POCl_3$-treated QS grade 4T.

Specific Surface Area

Measurements of specific surface area for the four samples give the following data: 10 and 13 $m^2 \cdot g^{-1}$ respectively for the untreated QS grades 7D and 4T, and 5 and 4 $m^2 \cdot g^{-1}$ respectively for the corresponding $POCl_3$-treated samples. From these results we can see a decrease in available surface for phosphated fibers. This lower specific surface area is attributed to a coating of magnesium phosphate salts on the surface, and can confirm the encapsulation of the fibers.

Zeta Potential

Measurements of zeta potentials for the four samples gave the following results: +20 and +18 mV respectively for the untreated QS grades 7D and 4T, and −39 and −36 mV respectively for the corresponding $POCl_3$-treated samples.

Adsorption Isotherms

The results obtained by HPLC with dry toluene solvent as mobile phase (Ménard *et al.*, 1984) and using Chuduk's (1981) calculation technique have enabled us to determine the adsorption isotherms of polyaromatic hydrocarbons, especially B[a]P. Table 1 shows the retention times for untreated and $POCl_3$-treated QS 7D fibers. Figure 3 shows the corresponding adsorption isotherms and demonstrates that phosphated fibers adsorb none or very little of the B[a]P, whereas natural fibers show a very high adsorption potential. In Table 1, B[a]P has a net retention time of 28.0 min for the natural fibers, with only 0.08 min for the phosphated fibers. Moreover, the required pressure to drive the solvent through the column filled with phosphated fibers is 10 times smaller than for the natural fiber.

Natural and phosphated 4T fibers were studied in the same fashion as the grade 7D fibers. Therefore Table 2 gives the net retention times for the polyaromatic hydrocarbons, especially B[a]P. The net retention time for B[a]P on phosphated 4T fibers shows no adsorption, while there is a net retention time of 25 min for QS 4T natural fibers. Figure 4 shows the two adsorption isotherms.

TABLE 1
RETENTION TIME OF POLYAROMATIC HYDROCARBONS ON NATURAL AND $POCl_3$-TREATED QS 7D CHRYSOTILE FIBERS

Natural QS grade 7D fibers		$POCl_3$-treated QS grade 7D fibers	
Agent injected (20 nmole)	T_R[a] (min)	Agent injected (20 nmole)	T_R (min)
Benzo[*a*]pyrene	=28.0 ± .5	Benzo[*a*]pyrene	0.08 ± .01
Fluoranthrene	1.32 ± .03	Fluoranthrene	0.00 ± .01
Pyrene	1.07 ± .02	Pyrene	0.00 ± .01
Phenanthrene	0.40 ± .01	Phenanthrene	0.00 ± .01
Anthracene	0.38 ± .01	Anthracene	0.00 ± .01
Fluorene	0.18 ± .01	Fluorene	0.00 ± .01
Naphtalene	0.04 ± .01	Naphtalene	0.00 ± .01

[a] T_R = reduced retention time.

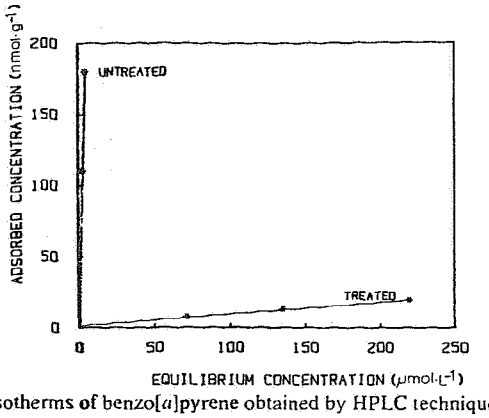

FIG. 3. Adsorption isotherms of benzo[*a*]pyrene obtained by HPLC technique on the QS 7D fibers.

DISCUSSION

Because of the apparent pathogenicity of asbestos fibers, there has been a general reaction of the public and certain health authorities regarding the use of products containing asbestos fibers. This has led to a certain amount of research to modify asbestos fibers in such a way as to reduce as much as possible the undesirable biological effects of asbestos fibers.

Various materials have been examined which interact with the surface of asbestos fibers and reduce its hemolytic activity. Such materials include EDTA, simple phosphates, disodium versenate, polyvinylpyridine *N*-oxide and aluminum (Macnab and Harington, 1967), and certain acidic polymers (Schnitzer and Pundsack, 1970). More recently, it has also been found that asbestos fibers with at least one metal molybdate (Pezzoli, 1979a) or metal tungstate (Pezzoli, 1979b) deposited thereon have reduced hemolytic activity in comparison with untreated asbestos fibers.

TABLE 2
RETENTION TIME OF POLYAROMATIC HYDROCARBONS ON NATURAL AND $POCl_3$-TREATED QS 4T CHRYSOTILE FIBERS

Natural QS grade 4T fibers		$POCl_3$-treated QS grade 4T fibers	
Agent injected (20 nmole)	T_R[a] (min)	Agent injected (20 nmole)	T_R (min)
Benzo[*a*]pyrene, 1000 nmole	25.0 ± .5	Benzo[*a*]pyrene	≈0.00 ± .01
Fluoranthrene	2.53 ± .03	Fluoranthrene	0.00 ± .01
Pyrene	1.94 ± .02	Pyrene	0.00 ± .01
Phenanthrene	0.45 ± .01	Phenanthrene	0.00 ± .01
Anthracene	0.42 ± .01	Anthracene	0.00 ± .01
Fluorene	0.08 ± .01	Fluorene	000 ± .01
Naphtalene	≈0.02 ± .01	Naphtalene	0.00 ± .01

[a] T_R = reduced retention time.

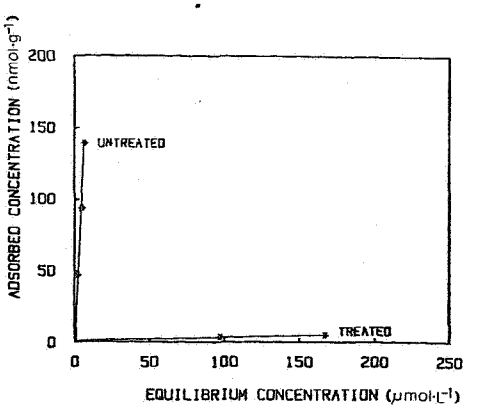

FIG. 4. Adsorption isotherms of benzo[*a*]pyrene obtained by HPLC technique on the QS 4T fibers.

The results reported here relate to a novel method for treating asbestos fibers: depositing phosphate groups on the asbestos fibers. Specifically, the data presented here indicate that the treatment results in modifications of infrared spectrum, and of thermal analysis and zeta potential data. More importantly, they also show a very important modification which relates to the alleged promoter, or cocarcinogenic, effect of asbestos fibers: the nearly complete loss of the treated fibers to adsorb and carry some known initiators of lung carcinogenesis present in tobacco smoke. The importance of this finding in a strategy to design safer fibers is of great significance and should be confirmed by appropriate cocarcinogenicity and comutagenicity biological assays.

REFERENCES

Chuduk, N. A., Eltekov, Yu. A., and Kiselev, A. V. (1981). Study of adsorption from solutions on silica by liquid chromatography method. *J. Colloid Interface Sci.* **84**, 149–154.

Eastman, A., Mossman, B. T., and Bresnick, E. (1983). Influence of asbestos on the uptake of benzo[*a*]pyrene and DNA alkylation in hamster treacheal epithelial cells. *Cancer* **43**, 1251–1255.

Kandaswami, C., and O'Brien, P. J. (1980) Effects of asbestos on membrane transport and metabolism of benzo[*a*]pyrene. *Biochem. Biophys. Res. Commun.* **97**, 794–801.

Kimmerle, F.M., Khorami, J., and Choquette, D. (1982). Thermal analysis of phosphated chrytosile fibers. *In* "Proceedings, 7th Int. Conf. on Therm. Anal." Vol. 1, pp. 614–620.

Lakowicz, J. R., and Bevan, D. R. (1980). Benzo[*a*]pyrene uptake into rat liver microsomes: Effects of adsorption of benzo[*a*]pyrene to asbestos and non-fibrous mineral particulates. *Chem.-Biol. Interac.* **29**, 129–138.

Lalancette, J. M., and Dunnigan, J (1981). "Novel Phosphated Asbestos Fibres." U.S. Patent No. 4,356,057, Oct. 26.

Macnab, G., and Harington, J. S. (1967). Hemolytic activity of asbestos and other mineral dusts. *Nature* (*London*) **214**, 522–533.

Ménard, H., Noël, L., Kimmerle, F. M., Tousignant, L., and Lambert, M. (1984). Adsorption isotherms of polycyclic aromatic hydrocarbons on asbestos chrysotile by high-pressure liquid chromatography. *Anal. Chem.* **56**, 1240–1242.

Mossman, B. T., and Craighead, J. E. (1979). Use of the hamster tracheal organ cultures for assessing the cocarinogenic effects of inorganic particulates on the respiratory epithelium. *Prog. Exp. Tumor Res.* **24**, 37–47.

Mossman, B. T., and Craighead, J. E. (1981). Mechanisms of asbestos carcinogenesis. *Environ. Res.* 25, 269–280.

Pezzoli, P. A. (1979a). "Asbestos Treatment with Metal Molybdates." U.S. Patent No. 4,171,405, Oct. 16.

Pezzoli, P. A. (1979b). "Asbestos Treatment with Metal Tungstate." U.S. Patent No. 4,168,346, Sept. 18.

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.

Schnitzer, R. J., and Pundsack, F. L. (1970). Asbestos hemolysis. *Environ. Res.* 3, 1–13.

Selikoff, I. J., Hammond, E. C., and Chung J. (1968). Asbestos exposure, smoking and neoplasia. *J. Amer. Med. Assoc.* 204, 106–112.

Weisburger, J. H., and Williams, G. M. (1983). The distinct health risk: Analyses required for genotoxic carcinogens and promoting agents. *Environ. Health Perspec.* 50, 233–245.