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Binding of environmental carcinogens to asbestos and mineral fibres

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ABSTRACT A rapid method has been developed for measuring the binding capacity of asbestos and other mineral fibres for environmental carcinogens. Benzo(α)pyrene (B(α)P), nitrosonornicotine (NNN), and N-acetyl-2-aminofluorene (NAAF) were assayed in the presence of Canadian grade 4T30 chrysotile, chrysotile A, amosite, crocidolite, glass microfibres, glasswool, attapulgite, and titanium dioxide. Chrysotile binds significantly more carcinogens than the other mineral fibres. This binding assay is reproducible with coefficients of variation of less than 8% and 6% respectively for inter and intra assay. The influence of pH was also studied, and there is good correlation between the carcinogen binding and the charge of the tested mineral fibres. The in vitro cytotoxicity on macrophage like cell line P388D$_1$ and the haemolytic activity of various mineral fibres were also measured: a good correlation was found between the binding capacity and the cytotoxicity of tested mineral fibres on P388D$_1$ cells. These results give some explanations for the reported synergism between exposure to asbestos and the smoking habits of workers.

The potentially harmful effects of all types of respirable mineral fibres are at present one of the most important fields of interest in industrial hygiene. Epidemiological data[1 2] suggest that most lung cancers in asbestos workers result from the synergistic effect between asbestos exposure and the smoking habit. In addition to being a physical carcinogen,[3] asbestos may also increase the effect of other potent carcinogens.[4 5] For the induction of lung cancer, the inhaled carcinogen must be retained by the lungs,[6] and this induction may be facilitated by absorption on to asbestos, thus retarding its pulmonary clearance and causing an enhancement of the total exposure level to the carcinogen.[7] Many investigators[8 9] have shown the absorptive capacity of asbestos for carcinogens and various organic compounds and ions.

Owing to its binding capacity for carcinogens present in the environment or in tobacco smoke, asbestos may be a cancer promoter. It is therefore necessary to identify which mineral fibres interact with carcinogens to induce neoplasia. The present data describe the binding capacity of mineral fibres for three carcinogens and the correlation with some in vitro biological assays.

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Materials and methods

All experiments were carried out with Rhodesian chrysotile A, crocidolite, and amosite obtained from International Union Against Cancer (UICC), Canadian grade 4T30 chrysotile and attapulgite (fibrous clay) obtained from the Research Institute for Asbestos Development (IRDA), and glass fibre (microfibre from GF/C microfilter), glasswool, and titanium dioxide (a synthetic amorphous sample) obtained from Fisher Scientific. Macrophage-like cell line P388D$_1$[10] was obtained from American Type Culture Collection (Rockville, Md, USA). Benzo(α)pyrene [^{3}H] (28 Ci/mmol), nitrosonornicotine, N'-[pyrrolidine-2-^{14}C] (18·4 mCi/mmol) and N-acetyl-2-aminofluorene [9-^{14}C] (46·16 mCi/mmol) were purchased from New England Nuclear (Montréal, Canada).

BINDING ASSAY

One millilitre of a suspension of each test material (2 mg/ml) was mixed on vortex with 400 μl of labelled carcinogen (1 μCi for B(α)P and 0·1 μCi for NNN and NAAF), washed five times, and counted. The buffer used in this experiment was Dulbecco's phosphate buffer saline[11] to which was added 10% ethanol and 0·25% (v/v) benzene for the B(α)P assay and 5% ethanol for NNN and NAAF assays.

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Table 1 *Specific surface areas of mineral fibres*

	Surface areas $m^2 g$
Glasswool	0·1963
Glass microfibres	2·864
Amosite	6·000
Titanium dioxide	9·088
Crocidolite	10·24
4T30 Canadian chrysotile	19·39
UICC chrysotile A	19·61
Attapulgite	123·0

This has been determined by solubility measurements.

The binding assay was performed in polypropylene centrifuge tubes obtained from Simport (Beloeil, Canada), and centrifugation was carried out with a Beckman Microcentrifuge B. Radioactivity was counted with Beta Rack counter (LKB, Wallac Oy, Finland).

The results are expressed in percentage of binding and each test was performed in triplicate.

EFFECT OF MINERAL FIBRES ON WATER ACIDITY

Speil and Leineweber have shown that the surface charge of mineral fibres is pH dependent.[12] It has also been shown that the binding of particular ligands on various surfaces is related to temperature, time, the ionisation status, and the available surface area.[13] To compare the surface charges of various mineral fibres, the pH change, caused by a constant amount of fibres, was measured for 15 minutes. Three hundred milligrams of fibres were added to 150 millilitres of deionised water at room temperature. Fibres were kept in suspension with a magnetic stirrer and the pH was measured at five minute intervals with a Corning Digital 110 pH meter using a polymer body combination electrode.

HAEMOLYSIS

One millilitre of a 2% suspension of sheep erythrocytes in 0·02M Tris HCl buffer (with 0·12M NaCl) at pH 7·3 was added to 30 mg of each fibres sample in 3 ml of the same buffer. After a 50 minute period of incubation at 37°C, each test tube was centrifuged and the optical density (OD) of the supernatant was measured at 541 nm.

The percentage of haemolysis was calculated as follows:

$$\frac{\text{OD 541 (test)} - \text{OD 541 (0\% haemolysis)}^{*} \times 100}{\text{OD 541 (100\% haemolysis)}^{**}}$$

* 0% of haemolysis: RBC in Tris-HCl (without fibre).
** 100% of haemolysis: RBC in deionised water.

CYTOTOXICITY

Three hundred thousand macrophage-like cells P388D$_1$ were mixed with 1000 μg of the different fibres in test tubes containing 1 ml of RPMI 1640 medium without Fetal Bovine Serum. After four hours of incubation at 37°C, the acute cytotoxicity was measured by the Trypan blue dye exclusion method.[14] The results are expressed in relative toxicity and each test was performed in triplicate.

SURFACE AREA MEASUREMENTS OF MINERAL FIBRES (TABLE 1)

The specific surface of each mineral fibre tested was evaluated with BET apparatus[15] (Micrometrics 2100) using krypton for glass microfibres and glasswool and nitrogen for all other fibres.

Results

BINDING ASSAY

Figure 1 illustrates the binding capacity of different mineral fibres for benzo(α)pyrene, nitrosonornicotine, and N-acetyl-2-aminofluorene. Results are expressed in percentage of binding. Chrysotile binds significantly more carcinogens than other mineral fibres ($p < 0{\cdot}005$) except for attapulgite, a fibrous clay used as a substitute to asbestos which binds as much carcinogen as chrysotile.

EFFECT OF MINERAL FIBRES ON WATER ACIDITY

When mineral fibres were added to deionised water, a pH rise was recorded (fig 2); this change in pH was especially important for chrysotile and attapulgite.

HAEMOLYSIS AND CYTOTOXICITY (TABLE 2)

We found that titanium dioxide, glasswool, crocidolite, and amosite samples caused negligible haemolysis. This is in agreement with the results reported by Daniel and Le Bouffant.[16] Toxicity studies with the same fibres showed that chrysotile and attapulgite are the most toxic fibres. Titanium dioxide and crocidolite are also toxic. This is in contrast with the results obtained with the haemolysis assay or the ones reported by Wright *et al*.[17] The latter, however, measured the toxicity after 24 and 48 hours whereas our assay measures the acute effects on the cell membranes which influence Trypan blue uptake.

CORRELATION

We found a good correlation between the binding capacity and all other tests performed with mineral fibres (table 3). Furthermore, there is a strong correlation between the carcinogen binding capacity, the charge of the tested mineral fibres, the haemolytic activity, and the toxicity. These results are not in

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agreement with the results of *Wade et al*[18] and Daniel and Le Bouffant[16] where doubts have been raised on the value of haemolysis as a predictive assay system. We found a good correlation between the surface area of mineral fibres, the binding of NNN and NAAF, and the toxicity, whereas the correlation between the surface area and the other tests was not significant.

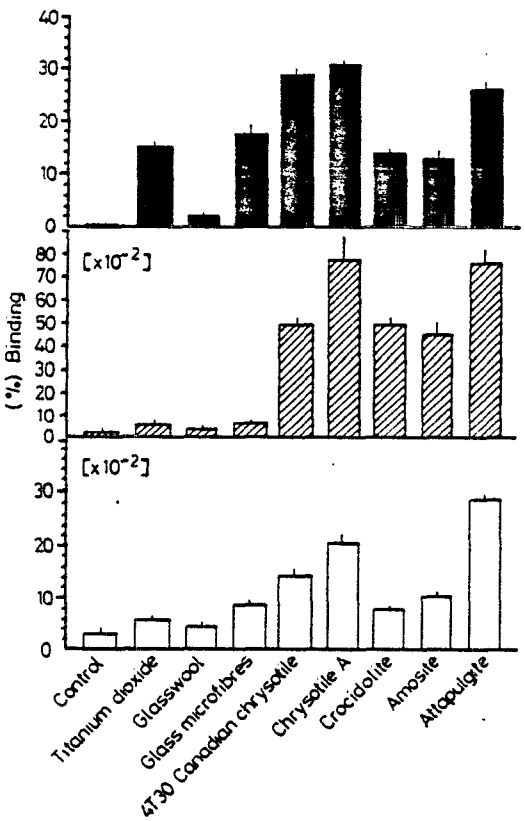

Fig 1 *Binding capacity of tested mineral fibres for benzo(α)pyrene (■), nitrosonornicotine (□) and N-acetyl-2-aminofluorene (□).*

Table 2 *Haemolytic activity and toxicity of mineral fibres*

	*Relative haemolysis**	*Relative toxicity**
Titanium dioxide	1·00	1·00
Glasswool	1·00	1·00
Glass microfibres	3·56	1·00
Amosite	1·26	1·00
Crocidolite	1·05	1·50
Attapulgite	3·72	4·26
UICC chrysotile A	5·00	4·63
4T30 Canadian chrysotile	2·44	5·00

* All results are expressed in relative values of toxicity or haemolysis ranging from 1·00 to 5·00.

Discussion

An increasing number of reports support the hypothesis that asbestos may not be a true carcinogen but a promoter. Miller *et al*[19] and Shabad *et al*[20] could not induce tracheal carcinoma by asbestos instillation alone whereas polycyclic aromatic hydrocarbons could induce bronchoalveolar carcinoma by themselves. The addition of both polycyclic aromatic hydrocarbons and asbestos increased the incidence of carcinoma. Mossman *et al* also suggested the promoter like action of asbestos fibres.[21] The presence of inhaled asbestos in the respiratory tract could cause an accumulation of environmental carcinogens, thus increasing their local concentration. Moreover, different reports[22,23] have shown that the presence of asbestos in the cellular environment changes benzopyrene transport and its metabolism by the cell and decreases the rate of DNA repair resulting in a larger amount of DNA adducts.

The binding capacity of various mineral fibres for carcinogens thus becomes an important factor in the evaluation of the promotion potential of a particular mineral fibre. We have shown above that chrysotile and attapulgite, a proposed substitute of asbestos, bind a larger proportion of B(α)P, NNN, or NAAF than any other mineral fibre tested. This residual carcinogen binding could not be changed significantly after five washing procedures; these binding properties thus represent physicochemical

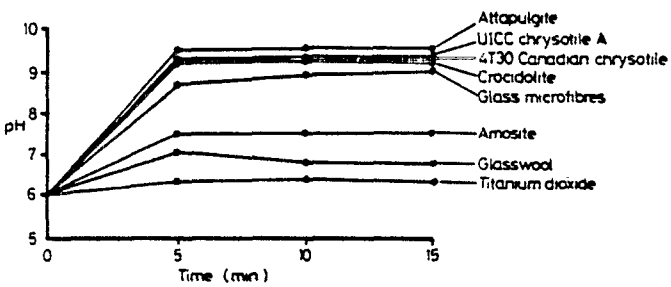

Fig 2 *Effect of mineral fibres on water acidity.*

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Table 3 *p Values for correlation coefficients between the different tests*

	Binding			*Haemolysis*	*Toxicity*	*pH*	*Specific surface area*
	B(α)P	*NNN*	*NAAF*				
Binding:							
B(α)P		<0·05	<0·05	<0·05	<0·01	<0·05	>0·20
NNN			<0·01	<0·20	<0·05	<0·05	<0·20
NAAF				<0·05	<0·05	<0·01	<0·01
Haemolysis					<0·10	<0·05	>0·20
Toxicity						<0·10	<0·20
pH							>0·20

B(α)pyrene = Benzo(α)pyrene.
NNN = Nitrosonornicotine.
NAAF = N-acetyl-2-aminofluorine.

differences in the structure of mineral fibres.

Fournier *et al* have shown that fibres with basic properties bind polycyclic aromatic hydrocarbons such as B(α)P more strongly[24]: this is the case for attapulgite and chrysotile. NNN and NAAF binding seems to be related to the adsorption properties of the fibres; we have found a positive correlation between the binding of NNN and NAAF and the specific surface of various fibres tested.

To evaluate the toxicity, the promotion effect, or the carcinogenicity of mineral fibres, we have also performed other tests already used to evaluate the in vitro effects of mineral fibres. Chrysotile and attapulgite were found to be more cytotoxic and haemolytic than other mineral fibres tested. We have also evaluated the charge present on the surface of mineral fibres by measuring the change in pH caused by a constant amount of fibres suspended in deionised water. There was a positive correlation between the apparent basic charge of the fibre and its cellular toxicity.

Various authors[25–27] and the present data suggest that the toxicity of asbestos is not only size related but that the various physicochemical properties of mineral fibres also play a major part in their toxicity. Recently, the cytotoxic properties of Canadian chrysotile were reduced by chemical treatment.[28]

These various in vitro tests to evaluate the toxicity of mineral fibres as a function of their physicochemical properties should not only produce some explanation for the biological effect of mineral fibres but they should permit a rapid evaluation of the potential toxicity of natural and modified chrysotile or its substitutes.

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