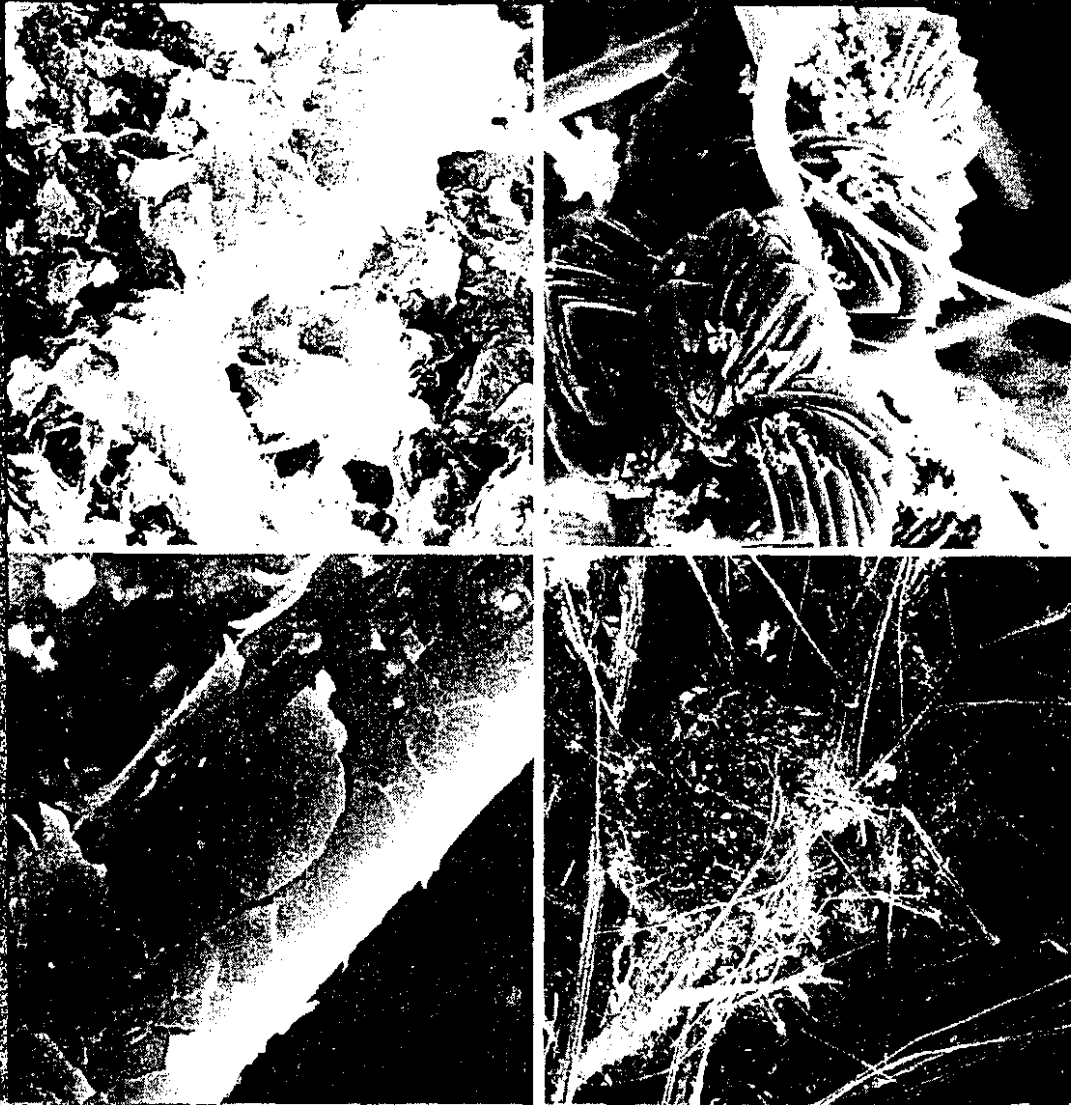


INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (WHO)
COMMISSION OF THE EUROPEAN COMMUNITIES



NON-OCCUPATIONAL EXPOSURE TO MINERAL FIBRES



EDITORS: J. BIGNON, J. PETO & R. SARACCI

IARC SCIENTIFIC PUBLICATIONS

N° 90
LYON, 1989

RECENT RESULTS OF CARCINOGENICITY BIOASSAYS OF FIBRES AND OTHER PARTICULATE MATERIALS

C. Maltoni & F. Minardi

Institute of Oncology, Bologna, Italy

Summary. Different types of natural, commercial and modified asbestos fibres were tested in a highly standardized manner by peritoneal injection into rats and mice in order to assess their carcinogenicity.

Differences in mesotheliomatogenic effect were found between the various materials tested. Of particular interest is the finding that treatment of the fibres with phosphorus oxychloride and heating to 300°C markedly reduces the carcinogenicity of chrysotile fibres.

Introduction

A systematic and integrated study involving long-term experimental bioassays on particulate materials was started in January 1981 at the Bologna Institute of Oncology and is still in progress. The study covers a variety of fibrous and non-fibrous, natural and man-made materials, present in the occupational and/or general environment. Some of the materials studied are of major industrial importance.

The study is aimed at:

- identifying new potentially carcinogenic materials;
- assessing, in quantitative terms, the level of carcinogenic risk of a given material, and comparing the risks represented by different materials (assessment of the relative carcinogenic risk);
- helping to predict the target organs;
- defining the role in carcinogenesis of the physical and chemical characteristics of the test compounds.
- determining the role of different biological and experimental factors affecting the neoplastic response and, consequently, shedding some light on the pathogenesis of the possible oncogenic effects;
- helping to reconstruct the natural history of the tumours which may be induced by the test compounds.

Information on test materials and animals and the experimental procedures is given in Table 1. In view of the aims of the study, the experimental conditions are strictly standardized.

Table 1. Test materials, animals, method of treatment and experimental procedure

Test materials

Different types of natural, modified natural and man-made materials including: crocidolite (UICC), chrysotile (Canada, UICC), chrysotile (Rhodesia, UICC), chrysotile (California), amosite (UICC), anthophyllite (UICC), commercial chrysotiles (3 samples), modified chrysotiles (4 types), asbestos-cement, crystalline silica, amorphous silica, alumina, wollastonite, talc (2 samples), kaolin, bentonite, natural zeolites (13 minerals), man-made zeolites (20 types), rock wool (4 samples), carbon fibres, synthetic fibres (2 types).

Test animals

Groups of 40-100 (20-50 males and 20-50 females), 6-8-week-old Sprague-Dawley rats. For erionite and modified chrysotiles, also groups of 40 (20 males and 20 females) 8-week-old Swiss mice. A total of 10 760 rats and 900 mice have been tested.

Method of treatment

Intraperitoneal (i. pe.) (for all materials apart from carbon fibres), intrapleural (i. pl.) and subcutaneous (s. c.) (for most of the materials) injection, and ingestion (gavage) and intratracheal instillation (in the case of detergent 4A zeolite).

Experimental procedure

The animals are kept under observation (body weight and clinical controls) until they die naturally or until 2 years from the beginning of the experiments. All animals undergo complete autopsy and systematic histopathological examination.

A summary of the experimental work so far completed or in progress is given in Table 2.

In this paper the most recent results are presented of the bioassays of several types of natural, commercial and modified asbestos, administered as a single intraperitoneal (i. pe.) injection, to rats and, in a few instances, to mice.

The modified asbestos consisted of Canadian chrysotile of different fibre lengths and from different sources, treated with phosphorus oxychloride and heated at 300°C. This type of treatment was used in order to bring about physicochemical changes which might reduce the carcinogenicity of the natural material.

In rats, the peritoneum proved to be more responsive than the pleura to the mesotheliomatogenic effect of asbestos; this is the opposite of what is observed with erionite (Table 3).

The complete final results of the basic experiments on the effects of the asbestos materials studied in rats at a single dose of 25 mg, are available. Data on the effects of some forms of modified asbestos, as single administered doses of 10, 5 or 1 mg, to rats and mice, and of 25 mg to mice, are preliminary and based solely on gross examination, since the experiments were started only 76 weeks ago.

Table 2. Summary of experimental work so far completed or in progress

Materials	Injection			Ingestion and intratracheal instillation
	i.pe.	i.pl.	s.c.	
Crocidolite (UICC)	+	+	+	
Chrysotile (Canada, UICC)	+	+	+	
Chrysotile (Rhodesia, UICC)	+			
Chrysotile (California)	+			
Amosite (UICC)	+			
Anthophyllite (UICC)	+			
Commercial chrysotiles (3 types) ^a	+	+		
Modified chrysotiles (4 types) ^a	+	+		
Asbestos-cement	+	+	+	
Crystalline silica	+		+	
Amorphous silica	+		+	
Alumina	+	+	+	
Wollastonite	+	+	+	
Talc (2 samples)	+	+	+	
Kaolin	+		+	
Bentonite	+	+		
Natural zeolites (13 minerals)	+	+	+	
Man-made A zeolites (2 types)	+	+	+	+
Man-made X zeolite	+	+	+	
Man-made Z zeolite (17 types)	+	+	+	
Rock wool (4 samples)	+	+		
Carbon fibres ^b			+	
Synthetic fibres (2 types)	+	+	+	
Water (controls)	+	+	+	

^aExperiments still in progress^bImplantation.**Table 3. Final results of tests on crocidolite, chrysotile (Canada) and sedimentary erionite^a**

Material	Peritoneal mesotheliomas			Pleural mesotheliomas		
	Tumour-bearing animals		Average latency time (weeks)	Tumour-bearing animals		Average latency time (weeks)
	No.	%		No.	%	
Crocidolite	39	97.5	59.5	18	45.0	104.8
Chrysotile (Canada)	32	80.0	92.2	26	65.0	111.1
Sedimentary erionite	20	50.0	106.1	35	87.5	64.2
Water (controls)	0	-	-	0	-	-

^aSprague-Dawley rats (20 males and 20 females) were given a single intraperitoneal and intrapleural injection of the material (25 mg in 1 ml of water) and kept under observation for their full lifespan.

Materials and methods

The asbestos materials, obtained from Mount Sinai School of Medicine (New York, USA) (natural asbestos), Associazione Cemento-Amianto (Balangero, Italy) (asbestos-cement) and CERAM-SNA (Canada) (commercial and modified asbestos), were injected into the peritoneal cavity of 8-week-old Sprague-Dawley rats and Swiss mice. Groups of 40 animals (20 males and 20 females) were used for each species, material and dose level.

The animals were examined 3 times daily for general behaviour and were weighed and examined for gross changes every 2 weeks.

In experiment BT 2101 (Table 4), the animals were kept under observation for their full lifespan. In experiment BT 2106 (Table 5), the animals were kept for up to 104 weeks, at which time the survivors were sacrificed. Experiments BT 2111 (Table 6) and BT 2112 (Table 7) will also be terminated at 104 weeks.

Table 4. Final results of tests on various types of natural asbestos and asbestos-cement (experiment BT 2101)^a

Material	Peritoneal mesotheliomas		
	Tumour-bearing animals		Average latency time (weeks)
	No.	%	
Crocidolite (UICC)	39	97.5	59.5
Chrysotile (Canada, UICC)	32	80.0	92.2
Chrysotile (Rhodesia, UICC)	33	82.5	89.7
Chrysotile (California)	29	72.5	85.3
Amosite (UICC)	36	90.0	66.7
Anthophyllite (UICC)	35	82.5	73.3
Asbestos-cement	21	52.5	99.7
Water (controls)	0	-	-

^aSprague-Dawley rats (20 males and 20 females) were given a single intraperitoneal injection of the material (25 mg in 1 mg of water) and kept under observation for their full lifespan.

A complete autopsy is performed on all animals, whether dying naturally or sacrificed. A histopathological examination is carried out on the peritoneum (site of injection), brain, thymus, lungs, liver, spleen, kidneys, adrenals, stomach, uterus, gonads, mesenteric, mediastinal and subcutaneous lymph-nodes, and all pathological organs and tissues.

The strictly standardized experimental conditions and procedures allow quantitative comparisons between different groups in the same experiment and between different experiments.

Table 5. Final results of tests on various types of chrysotile and other non-asbestos fibres (experiment BT 2106)^a

Material		Treatment	Peritoneal mesotheliomas		
Code	Type		Tumour-bearing animals		Average latency time (weeks)
			No.	%	
Chr 1	Paperbestos 5 - 100 M + 200 M ^b	-	33	82.5	78.3
Chr 2	Paperbestos 5 - 100 M + 200 M ^b	POCl ₃ + 300°C	17	42.5	88.6
Chr 3	Paperbestos 5 - 100 M + 200 M from asbestos-latex paper ^b	-	17	42.5	87.5
Chr 4	Paperbestos 5 - 100 M + 200 M from asbestos-latex paper ^b	POCl ₃ + 300°C	15	37.5	94.7
Chr 5	Short chrysotile 7 (by water fractionation of Paperbestos 5) ^b	-	29	72.5	76.4
Chr 6	Short chrysotile 7 (by water fractionation of Paperbestos 5) ^b	POCl ₃ + 300°C	3	7.5	97.6
Chr 7	Chrysotile (Canada, UICC)	-	30	75.0	76.1
Chr 8	Chrysotile (Canada, UICC)	POCl ₃ + 300°C	20	50.0	90.0
9	Rock-wool fibres	-	3	7.5	79.8
10	Kevlar fibres	-	0	-	-
11	Water (controls)	-	0	-	-

^aSprague-Dawley rats (20 males and 20 females) were given a single intraperitoneal injection of one material (25 mg in 1 ml of water) and kept under observation for 104 weeks.

^bSupplied by IRDA.

Table 6. Gross findings after 76 weeks in tests on various types of chrysotile and other non-asbestos fibres (experiment BT 2111)^a

Material		Treatment	Dose (mg)	Peritoneal mesotheliomas		
Code	Type			Tumour-bearing animals		Average latency time (weeks)
				No.	%	
Chr 5	Short chrysotile 7 (by water fractionation of Paperbestos 5) ^b	-	10	11	27.5	57.0
			5	4	10.0	59.7
			1	0	-	-
Chr 6	Short chrysotile 7 (by water fractionation of Paperbestos 5) ^b	POCl ₃ + 300° C	10	0	-	-
			5	0	-	-
			1	0	-	-
Chr 7	Chrysotile (Canada, UICC)	-	10	14	35.0	61.6
			5	11	27.5	60.3
			1	0	-	-
Chr 8	Chrysotile (Canada, UICC)	POCl ₃ + 300° C	10	3	7.5	61.0
			5	1	2.5	67.0
			1	1	2.5	64.0
9	Fiberfrax	-	10	5	12.5	65.2
			5	0	-	-
			1	0	-	-
10	Kevlar fibres	-	10	0	-	-
			5	0	-	-
			1	0	-	-
11	Water (controls)	-	0	0	-	-

^aSprague-Dawley rats (20 males and 20 females) were given a single intraperitoneal injection of the material (in 1 ml of water) and kept under observation for 104 weeks.

^bSupplied by IRDA.

The bioassays of experiment BT 2106 (the first on modified asbestos) were performed in such a way that the nature of the materials tested was disclosed only when the biological data had already been obtained.

Table 7. Gross findings after 76 weeks in tests on various types of chrysotile (experiment BT 2112)^a

Material		Treatment	Dose (mg)	Peritoneal mesotheliomas		
Code	Type			Tumour-bearing animals		Average latency time (weeks)
				No.	%	
Chr 5	Short chrysotile 7 (by water fractionation of Paperbestos 5) ^b	-	25	6	15.0	65.7
			10	3	7.5	64.7
			5	1	2.5	71.0
			1	0	-	-
Chr 6	Short chrysotile 7 (by water fractionation of Paperbestos 5) ^b	POCl ₃ + 300° C	25	0	-	-
			10	0	-	-
			5	0	-	-
			1	0	-	-
Chr 7	Chrysotile (Canada, UICC)	-	25	6	15.0	58.5
			10	5	12.5	61.0
			5	0	-	-
			1	0	-	-
Chr 8	Chrysotile (Canada, UICC)	POCl ₃ + 300° C	25	3	7.5	70.0
			10	1	2.5	56.0
			5	0	-	-
			1	0	-	-
11	Water (controls)	-	0	0	-	-

^aSwiss mice (20 males and 20 females) were given a single intraperitoneal injection of the material (in 1 ml of water) and kept under observation for 104 weeks.

^b Supplied by IRDA.

Results

The results of the experiments are presented in Tables 4-7.

The bioassays of different natural asbestos fibres and of asbestos-cement, at a dose of 25 mg, show that the mesotheliomatogenic effect (evaluated on the basis of the incidence and latency time of tumours) of the materials tested decreases (Table 4) in the order:

- (1) crocidolite;
- (2) amosite;
- (3) anthophyllite;
- (4) chrysotiles;
- (5) asbestos-cement.

The chemical and heat treatment of asbestos fibres, tested at a dose of 25 mg, markedly reduces the carcinogenicity of 'short chrysotile 7' and to a lesser extent 'Paperbestos 5', while that of 'UICC Canada chrysotile' is reduced still less. The carcinogenic potency of fibres from 'asbestos-latex-paper' is lower than that for 'Paperbestos 5' and seems little affected by the chemical and heat treatment. These results may be due to the insulating effect of latex (Table 5).

The reduction in carcinogenic potency of 'short chrysotile 7' and also of 'UICC Canada chrysotile' seems also to be confirmed at the present stage of the study by the preliminary incomplete results of experiments BT 2111 and BT 2112, in which the materials are being tested at various doses on rats and mice (Tables 6 and 7).

Conclusions

The data reported here seem to indicate that experimental long-term bioassays may be used as a tool for quantifying the carcinogenic potency of particulate materials and, in the present case, specifically of different types of asbestos.

The findings presented may provide the basis for further analytical studies aimed at clarifying the possible mechanisms of asbestos carcinogenesis. They are also of technological interest; however, at present, it is difficult to predict their implications for the asbestos industry and, in general, for the manufacture and use of other fibres.

Acknowledgements

The study was supported in part by the EEC (Contracts Nos ENV/347/I-(S) and ENV/755/I) and in part by the Institut de Recherche et de Développement sur l'Amiante, Canada