RECENT RESULTS OF CARCINOGENICITY BIOASSAYS OF FIBRES AND OTHER PARTICULATE MATERIALS

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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.

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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)	+	+	+	

[a]Experiments still in progress

[b]Implantation.

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	-	-

[a]Sprague-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.

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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	-	-

[a]Sprague-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	-	-

[a]Sprague-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.

[b]Supplied by IRDA.

Table 6. Gross fi non-asbestos fib

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Code	Type
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Chr 7	Chrysotil (Canada,
Chr 8	Chrysotil (Canada,
9	Fiberfrax
10	Kevlar fil
11	Water (c

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[b]Supplied by IRDA

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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	-	-

[a]Sprague-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.

[b]Supplied 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	-	-

[a]Swiss 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.

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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.

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Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats

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Key words: carcinogenicity; metal compounds; dust; mineral fibres; vitreous fibres; plastic fibres; heavy metals; glass microfibres; asbestos; attapulgite; erionite; wollastonite; cadmium; nickel; ferric oxides; corundum; titanium dioxide; chrysotile; crocidolite; quartz; benzo(a)pyrene; polyvinylchloride; polyvinylpyridine-N-oxide; wood dust; inhalation experiments; intratracheal instillation; intraperitoneal injection

Summary

About 50 dusts were examined on their carcinogenicity in rats mainly after intraperitoneal injection and some after intratracheal instillation. In the i.p. test, very low doses between 0.05 and 0.5 mg asbestos led to tumour incidences of about 20 to 80%. Polyvinylpyridine-N-oxide prolonged the tumour latency after injection of actinolite. 60 mg attapulgite from three sources with short fibre lengths were not shown to be carcinogenic but an attapulgite sample with longer fibres had a moderate effect. Relatively thick rock and *ceramic fibres (median* $> 1\,\mu m$) induced tumours, but slag and wollastonite fibres did not, probably because of their better solubility. Intratracheal instillations of glass microfibres (20 × 0.5 mg) led to lung tumours in 5 of 34 rats (0 in control). The carcinogenic potency of an inorganic fibre depends on its size and persistency, and possibly also on other properties, especially on the surface.

Nickel powder, nickel oxide, nickel subsulfide and cadmium sulfide were all found to be carcinogenic in the two tests. Cadmium chloride and cadmium oxide could only be administered in very low doses because of their high acute toxicity. A high amount of magnetite (15 × 1.5 mg i.tr.) led to an *unexpected lung tumour incidence* of 69%.

The i.p. test in rats proved to be very sensitive for detecting the carcinogenic potency of non-acute toxic natural and man-made mineral dusts as well as metal compounds. This means that, if a high dose of one of these dusts does not induce tumours in this test, no suspicion of carcinogenic potency can be substantiated.

Introduction

When in 1972/73 the first experimental results on the tumour-inducing effect not only of asbestos but also of other fibrous dusts were published, the results strongly supported the old hypothesis that the elongated shape of asbestos particles may be the cause of their carcinogenicity (Nordmann 1938; Linzbach and Wedler 1941; Stanton and Wrench 1972; Pott and Friedrichs 1972; Wagner et al. 1973). Moreover the question was raised of a possible carcinogenic effect of non-asbestos fibres in humans, especially of man-made mineral fibres, because their production has risen steadily since the fifties. As a consequence, epidemiologic studies on the man-made mineral fibre industry were carried out over the past ten years. The results were reviewed recently by Saracci (1986) and Doll (1986). They support the hypothesis that man-made mineral fibres – as present in the workplace atmo-

Dedicated to Dr. A. Bonnaire on the occasion of his 60th birthday

sphere of early slag wool rock wool production — may have played a role in the causation of lung cancer.

Up to now, in inhalation experiments with very fine man-made mineral fibres (glass microfibres 100 and 104), no statistically significant tumour rates have been detected (Le Bouffant 1986; Goldstein 1984; McConnell et al. 1984; Muhle et al. 1986; Smith et al. 1986; Wagner et al. 1984a). The strong carcinogen, crocidolite, has similarly produced only a low tumour rate in inhalation experiments. Therefore, this test model cannot be considered as sensitive for carcinogenic fibres. The positive results with chrysotile were observed after exposure to *higher fibre concentrations* (Davis et al. 1978; Davis et al. 1986; McConnell et al. 1984; Wagner et al. 1984a). Moreover it was demonstrated, that after a period of two years the number of chrysotile fibres deposited in the rat lung can increase more than tenfold by splitting (Bellmann et al. 1986). However, this multiplication cannot happen with crocidolite and vitreous fibres. Most carcinogenicity studies with mineral fibres in laboratory animals were carried out by means of intrapleural or intraperitoneal administration. By these routes of application, a high number of fibres can become active in the serosal tissue which is obviously very susceptible to fibre-related carcinogenic stimulation. This high sensitivity exists also in humans since the relation of lung carcinomas to mesotheliomas in asbestos workers amounts on average to about 3 to 1, although after inhalation contact of fibres with the thoracic or abdominal serosa should be much more difficult than with the bronchial epithelium.

The experimental results should lead to a generally valid definition of carcinogenic fibres. This aim is not yet reached. On the one hand there is a general consensus that length, diameter and bio-persistence represent the most important criteria for the carcinogenic potency of mineral fibres (Stanton et al. 1981; Wagner 1986; Davis 1986; Pott 1987). On the other hand, the relationship between the degree of these three characteristics and the expected tumour incidence is not sufficiently known. There is a continuous transition from the non-carcinogenicity of fibres which are too short, too thick, or too soluble to the maximum possible carcinogenic potency of fibres which have the "ideal" size and are persistent enough. However, it is an open question at which point the threshold is located between fibres which are too short, too thick or too short-lived in the tissue and fibres which are sufficiently long, thin and persistent regarding their carcinogenic potency for humans. Furthermore, for regulations, we need not only the distinction between carcinogenic and non-carcinogenic fibres but also criteria for a grading of carcinogenic potency.

For many years there has been the theory that the surface properties of fibres are the decisive agents for the tumour induction (Bignon and Jaurand 1983; Bonneau et al. 1986a, b; Cralley 1971; Cralley and Lainhart 1973; Dunnigan 1984; Fisher et al. 1985; Langer and Nolan 1985; Mossman 1983). This theory is mainly based on the results of *in vitro* testing where altered dust surfaces were seen to influence certain cytotoxic effects. But there are significant differences between a cytotoxic reaction *in vitro* and tumour induction *in vivo*. Often, good parallels were found between results from carcinogenicity tests and some *in vitro* tests (Davis et al. 1985). Nevertheless, up to now *in vitro* tests have not been able to provide reliable statements on fibre carcinogenicity (Wagner et al. 1985).

Recent results after combined injection of asbestos and the antisilicotic effective substance polyvinylpyridine-N-oxide indicate that the surface properties of asbestos fibres might not only be important for its cytotoxicity *in vitro* but also for its carcinogenicity (Pott et al. 1985).

Since the carcinogenic potency of fibres is composed of, at least, their length, diameter and durability, and as the spectrum of fibre dimensions in a single sample is wide ranging, it is very difficult to explain a particular tumour rate using only one specific parameter. With these difficulties always in mind, we are going to describe a number of results which may add some pieces to the puzzle. The experiments were carried out over the last 15 years and only some of them have already been published, although not with all the details now described.

A further question concerns the relation of the carcinogenic effect of fibres to chemically carcinogenic substances in the applied test systems. For comparison, a number of carcinogenic, non-carcinogenic and questionable carcinogenic substances were tested on their carcinogenicity after intraperitoneal injection and intratracheal instillation. In this context, additional carcinogenicity studies with some nickel and cadmium compounds are desirable because up to now there is no consensus on their carcinogenic potential. Of course, numerous reports on nickel carcinogenesis have been published in various reviews (IARC 1976; Raithel and Schaller 1981; Sunderman 1981; Sunderman 1984). Nickel powder and nickel oxide have been known to be chemical carcinogens for a long time. But recently, following a review of the epidemiologic and experimental results, it was proposed to classify these two substances as non-carcinogenic for humans and animals (Longstaff et al. 1984). Cadmium is also suspected to be carcinogenic in the relevant compounds cadmium oxide and cadmium sulfide, since inhalation of relatively low concentrations of cadmium chloride induced lung tumours in rats (Takenaka et al. 1983).

To widen the basis of comparison, further dusts were included in the study e.g. corundum, titanium dioxide, ferric oxides, polyvinylchloride, and wood dust.

Materials and Methods

1. Laboratory animals and animal keeping

In most of the experiments female Wistar rats (Wistar-WU Kißlegg) from the breeding farm S. Ivanovas (Kißlegg, Allgäu, F.R.G.) were used and only in experiments no. 10 and 12 Sprague-Dawley SIV 50 rats from the same breeding farm. Before each experiment the animals were randomly allocated to plastic cages on wood granule bedding ("T-grob", Buntenbach, Solingen, F.R.G.), and numbered mostly 8–10 to a cage at the start. As male rats grow much bigger than female rats and need more space, in only one experiment (no. 7) male rats were also used.

The animals were maintained under conventional conditions and these could be improved in the late 70ies. A standard pelleted laboratory diet (RMH-TM, Fa. Hope-Farms Woerden, NL) and water were given *ad libitum*.

The weight of the animals was determined before the start of the experiment and the average age of the groups was calculated according to tables of the breeding farm. The average age of the animals is given for each experiment in the tables of results.

2. Substances used

Some of the dusts or chemicals administered were bought from manufacturers or their agencies and used in the condition delivered. Many materials, especially the man-made mineral wools, were delivered from manufacturers or agencies or other persons and prepared for the animal experiments by cutting and milling. Sometimes the milled dust was fractionated by sedimentation. This work and the measurement of the fibre sizes (mentioned only as median values in tables 1 and 3) was performed in several laboratories, especially by Dr. Bellmann and Dr. Muhle (Fraunhofer Institut für Toxikologie und Aerosolforschung, Hannover, F.R.G.), Dr. Friedrichs (Medizinisches Institut für Umwelthygiene an der Universität Düsseldorf, F.R.G.), Dr. Rödelsperger (Institut für Arbeits- und Sozialmedizin der Universität Gießen, F.R.G.), and Dr. Spurny (Fraunhofer Institut für Umweltchemie und Ökotoxikologie, Schmallenberg-Grafschaft, F.R.G.). The UICC samples were described by Rendall (1970) and Timbrell (1970).

Actinolite, F.R.G. Origin: A diabas quarry near Schmallenberg, F.R.G. Preparation: Dr. Spurny (method see Spurny et al. 1979a). Measurement of fibre sizes: Dr. Bellmann, Dr. Muhle.

Actinolite, (Canada). Origin unknown; a small rock was obtained from the Mineralienkontor Bonn, F.R.G. Preparation: Dr. Friedrichs. The macroscopically fibrous structure was mainly destroyed by milling in an agate ball mill. A part of this sample had already been used in another experiment (Pott et al. 1976).

Anthophyllite, UICC. – Origin: Finland.

Attapulgite, Caceres. – Origin: Torrejon de Rubio Caceres, Spain. Preparation of a very fine fraction (reference No. 82 I) by H. PÉZERAT (Université P. et M. Curie, Paris, France). Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Attapulgite, Georgia. — Origin: Georgia, U.S.A. Delivered under the name "Pharmasorb colloidal" from Chemie-Mineralien KG, Bremen, F.R.G., and a product of Engelhard Minerals & Chemicals Corporation, Edison, N. J., U.S.A. Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Attapulgite, Lebrija. – Origin: Lebrija, Spain. Delivered with the label "Polvo fabrica" from Tolsa S.A., Madrid, Spain. According to the analysis of RÖDELSPERGER et al. (1987) the sample was identified as attapulgite with high probability. It was used in the condition obtained. Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Attapulgite, Mormoiron. – Origin: Mormoiron, France. The sample used was the drug "gastropulgite" which contains 83% attapulgite and is produced by Beaufour, Dreux, France. The fibre sizes measured came from a sample "Gastropulgite 50" which is produced under licence of Beaufour by Schwabe, Karlsruhe, F.R.G. Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Basalt wool. – Producer: Grünzweig – Hartmann und Glasfaser AG, Ludwigshafen, F.R.G. Preparation: Dr. SPURNY (method see SPURNY et al. 1979b). Measurement of fibre sizes: Dr. BELLMANN, Dr. MÜHLE.

Benzo(a)pyrene. – Delivered from Fluka AG, Neu-Ulm, F.R.G. Code-No. 12780. Purity 97%.

Brucite, $Mg(OH)_2$. – This mineral is the granular form of magnesium hydroxide (fibrous form: see nemalite). Preparation: Dr. FRIEDRICHS (FRIEDRICHS 1974).

Cadmium chloride ($CdCl_2 \cdot H_2O$). — Delivered from Merck, Darmstadt, F.R.G. Purity 99%.

Cadmium oxide. — Delivered from Merck, Darmstadt, F.R.G. Purity not indicated.

Cadmium sulfide. – Delivered from Aldrich-Chemie GmbH & Co. KG, Steinheim, F.R.G. Purity 99.999%.

Ceramic wool, Fiberfrax. — Producer: Carborundum, Düsseldorf, F.R.G. Delivered from Hoecker Werke GmbH & Co. KG, Weil, F.R.G. Preparation: Dr. SPURNY (method see SPURNY et al. 1979b). Measurement of fibre sizes: Dr. BELLMANN, Dr. MÜHLE.

Ceramic wool, M.N. – Producer: Manville Corporation, Denver Co., U.S.A. Delivered from Gossler, Hamburg, F.R.G. Preparation: Dr. SPURNY. Measurement of fibre sizes: Dr. BELLMANN Dr. MÜHLE.

Chrysotile, Calidria. Origin: California, U.S.A. The material was prepared for the production of asbestos paper. The sample used was obtained from Dr. ROBOCK, Asbest-Institut, Neuß, F.R.G. Measurement of fibre sizes: MÜHLE et al. 1986.

Chrysotile, UICC A. – Origin: Zimbabwe (formerly Rhodesia). Measurement of fibre sizes: FRIEDRICHS (1978).

Chrysotile, UICC A, HCl-treated. The standard sample was boiled in 1 N HCl for 8 h. The magnesium content probably was leached almost completely. The loss of weight amounted to 46%. The fibres were shortened and partially split up.

Chrysotile, UICC A, milled. The standard sample was dry milled in an agate ball mill for 4 h. Measurement of fibre sizes: FRIEDRICHS (1978). A part of this sample was used in larger doses in other experiments (PORT et al. 1972, 1976).

UICC B. – Origin: Canada. Measurement of particle sizes: TIMBRELL (1970). It has to be underlined that the results of measurement of the twisted chrysotile fibres are rather doubtful. Extensive treatment with ultrasound homogenizes the suspension and changes the fibre number by splitting of the bundles.

Chrysotile, UICC B, milled. The standard sample was dry milled in an agate ball mill for 3.5 h. Measurement of fibre sizes: Dr. BELLMANN Dr. MÜHLE.

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Corundum. The aluminium oxide dust was delivered from Maschinen- und Schleifmittelwerke, Offenbach, F.R.G.

Crocidolite, South Africa. - Origin: South Africa (like UICC crocidolite but fibre lengths greater). Preparation: Dr. RENDALL, Johannesburg, South Africa. Measurement of fibre sizes: MÜHLE et al. 1986.

Erionite, Oregon. - Origin: Oregon, U.S.A. The dust sample was supplied by Dr. WAGNER, Medical Research Council Pneumoconiosis Unit, Penarth, Wales, U. K., as representative of the material used by him in his experiments (WAGNER et al. 1985). Measurement of fibre sizes: Dr. RÖDELSPERGER.

Erionite, Turkey. Origin: Karain, Turkey. A small rock of the fibrous material was sent from Dr. BARIS, Ankara to Dr. SPURNY and prepared by Dr. SPURNY. Measurement of fibre sizes: Dr. BELLMANN Dr. MÜHLE.

γ-Ferric oxide hydrate. - The extremely short fibrous material was obtained from a producer of tapes. Estimation of the fibre sizes according to a microphotograph with a magnification of 1:50,000.

γ-Ferric oxide hydrate (1). - The fibrous material was obtained from a producer of tapes. Measurement of fibre sizes: Dr. MÜHLE.

γ-Ferric oxide hydrate (2). - The fibrous material was obtained from a producer of tapes. Estimation of the fibre sizes according to a microphotograph with a magnification of 1:50,000.

Glass fibres, 100 Pen. - Producer: Manville Corporation, Denver, Co., U.S.A. The dust sample was supplied by Mr. SKIDMORE, Medical Research Council Pneumoconiosis Unit, Penarth, Wales, U. K., as representative of the material used in his animal experiments (WAGNER et al. 1984a). Measurement of fibre sizes: Dr. SPURNY.

Glass fibres, 100, L & V. - Producer: Manville Corporation. Delivered from Lehmann & Voss, Hamburg, F.R.G. After cutting with scissors, the fibres were ground in a knife mill for 20 min. It is not known whether this sample has the same chemical composition as 100 Pen mentioned above. Measurement of fibre sizes: Dr. SPURNY.

Glass fibres, 104/1974. Producer: Manville Corporation. Delivered from Lehmann & Voss in 1974 (no further data). The chemical composition was analyzed with proton induced X-ray emission by E. BOMBELKA und Dr. F.-W. RICHTER, University of Marburg (published in BELLMANN et al. 1986); this analysis yielded about the same composition as reported by Manville Corporation for the glass fibre Tempstran E Glass Electrical Alkalifree. After cutting with scissors the wool was ground in distilled water in an agate mill for 1 h (charge 1) or 2 h (charge 2) and then dried at 80 °C. Measurement of fibre sizes: Dr. SPURNY. These samples were also used in other experiments (POTT et al. 1980, 1984a, 1984b).

Glass fibres, 104 1974, HCl- or NaOH-treated. — 500 mg of the fibres (see above) were incubated in 20 ml 1.4 N HCl or 1.4 N NaOH for 2 or 24 h in a magnetic stirrer at room temperature. After 10 min centrifuging at 1,200 × g they were filtered through Nucleopore filters (pore diameter 0.1 μm). The sediment was washed twice with 20 ml distilled water in each case, the residue filtered and the fibres dried at 80 °C for 16 h. The centrifuged particles and the particles on the filter were then weighed. The loss in weight 2 and 24 h after treatment with HCl amounted to 25 and 33 %, respectively, after treatment with NaOH to 1.7 and 6.8 %, and after treatment with distilled water to 1.7 % (POTT et al. 1984a). Measurement of fibre sizes: Dr. SPURNY.

Glass fibres, 104 475. - Producer: Manville Corporation. Type: Tempstran 475. Delivered from Lehmann & Voss. After cutting with scissors, the wool was ground in a knife mill for 30 min. Measurement of fibre sizes: MÜHLE et al. 1986.

Glass fibres, 104 475, HCl-treated. — The fibres mentioned before were treated for 24 h with 1.4 N HCl as described for glass fibres 104 1974. The loss in weight amounted to 0.5 %. The resistance of Tempstran 475 is much larger than that of the glass fibres 104 1974 mentioned above.

Glass fibres, 106. – Producer: Manville Corporation. Delivered from Schleicher und Schüll, Dassel, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS. The material was also used for other studies (POTT and FRIEDRICHS 1972; POTT et al. 1976).

Glass filaments, ES 3. – The (endless) textile fibre was delivered from Gewetex, Düsseldorf, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS. The variation in fibre diameters was in a limited range: 10"$_{o}$ 3.5 μm, 90"$_{o}$ $\leq$ 4.2 μm. Length: 10"$_{o}$ $<$ 6 μm, 90"$_{o}$ $<$ 50 μm (FRIEDRICHS 1978).

Glass filaments, ES 5 and 7. – These (endless) textile fibres were obtained from Klöckner & Schott, Dortmund, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS. In these samples the diameter variations were in a limited range: ES 5: 10"$_{o}$ $<$ 4.8 μm, 90"$_{o}$ $\leq$ 6.3 μm; Es 7: 10"$_{o}$ $<$ 6.8 μm, 90"$_{o}$ $\leq$ 8.1 μm. Length Es 5: 10"$_{o}$ $\leq$ 24 μm, 90"$_{o}$ $\leq$ 80 μm; Es 7: 10"$_{o}$ $\leq$ 23 μm, 90"$_{o}$ $\leq$ 102 μm (FRIEDRICHS 1978).

Glass (granular). – Pieces of glass milled to a fine dust. Preparation: Dr. FRIEDRICHS.

Kevlar (Trademark for aramide fibres). – Producer: E. I. du Pont de Nemours and Company, Newark, Delaware, U.S.A. It was not possible to make a suspension with separated fibres from the flaky material. The suspension of sample (1) for experiment 13 was prepared by ultrasonic treatment only. For sample (2) used in experiment 15 an attempt was made to get finer fibres and better suspension by drying, milling and ultrasonic treatment. This difficult preparation was carried out by Dr. SPURNY. However, the suspension injected was not homogeneous like that of mineral fibres.

Magnetite. Delivered under the name "Ferroso Ferric Oxide" from Research Organic Inorganic Chemical Corporation, Bellville, N.J., U.S.A. and obtained from Dr. OBERDÖRSTER, University of Rochester, N. Y. The particles are very small and cannot be measured by light microscopy.

Nemalite, $MgOH_2$. – This mineral is the fibrous form of magnesium hydroxide. It was obtained from the Mineralienkontor, Bonn, F.R.G. (Impurity with chrysotile possible.) Preparation and measurement of fibre sizes: Dr. FRIEDRICHS (FRIEDRICHS 1978).

Nickel oxide (NiO). — Delivered from Aldrich-Chemie GmbH & Co. KG, Steinheim, F.R.G. Purity 99.99"$_{o}$.

Nickel powder. — Delivered from Inco Metals Company, Missisauga, Ontario, Canada to Dr. MUHLE. Purity not indicated.

Nickel subsulfide (Ni_3S_2). — Delivered from Inco Metals Company, Missisauga, Ontario, Canada. Purity not indicated.

Polypropylene. The fibrous dust was obtained ready milled by Rhodia AG, Freiburg im Breisgau, F.R.G. Measurement of fibre sizes: Dr. BELLMANN Dr. MUHLE.

Polyvinylchloride. — Produced by Chemische Werke Hüls, Marl, F.R.G. Particle sizes 90"$_{o}$ $<$ 2.5 μm. Measurement: Dr. BELLMANN Dr. MUHLE.

2-Polyvinylpyridine-N-oxide (PVNO). – Produced by Bayer AG, Wuppertal, F.R.G. as a 2"$_{o}$ solution probably in distilled water (Charge V 3504) for silicosis research in the sixties.

Quartz DQ12. – Origin: Dörentrup, F.R.G. The "Ground Product No. 12" (in short: DQ12) with a size distribution of $<$ 60 μm was delivered from Dörentrup Sand- und Tonwerke GmbH of Dörentrup. A $<$ 5 μm size fraction was prepared from this material by centrifugal separation in air (RONOCK 1973). The fine dust was obtained from Steinkohlenbergbauverein, Essen, F.R.G. This quartz specimen has often been used by German institutes involved in silicosis research.

Rock wool, Sweden. – Produced in Sweden. Prepared for animal inhalation studies at the Medical Research Council Pneumoconiosis Unit, Penarth, Wales, U.K. (WAGNER et al. 1984a). A sample was obtained from Mr. SKIDMORE. One part was used in its delivered form, another was fractionated by Dr. SPURNY to obtain a sample with finer fibres (method SPURNY et al. 1979b). Measurement of fibre sizes: Dr. SPURNY.

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Results

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Slag wool, RH. — Produced by Rheinstahl, Gelsenkirchen, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS (FRIEDRICHS 1978).

Slag wool, ZI. - Produced by Zimmermann, Sprockhövel 2, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS (FRIEDRICHS 1978).

Titanium dioxide. - Delivered from Degussa, Frankfurt, F.R.G. signed P25. The crystal form is anatas.

Volcanic ash, St. Helen's. - Obtained from Dr. RAMBE, University of California, Davis, California, U.S.A.

Wollastonite. - Origin: India. A dust sample with relatively large particles signed D-1 was obtained from Osthoff-Petrasch KG, Hamburg, F.R.G. Preparation of a fine fraction by Dr. SPURNY. Measurement of fibre sizes: Dr. BELLMANN Dr. MÜHLE.

Wood dust, beech. - Signed "Buchenmehl Größe 00". Delivered from "brüder schulte", Olsberg, F.R.G. Commercially used for bread baking. Particle sizes about 10 -100 μm.

3. Method of administration

The dusts were dispersed in 0.9% NaCl solution with ultrasound, most of them for about 1–3 min. The intraperitoneal injections were given without anaesthesia, the intratracheal instillations under CO_2 anaesthesia. The animals received 0.3 ml per i.tr. instillation and mostly 2 ml per i.p. injection. Only 0.8 or 1 ml was injected intraperitoneally in the very young rats in experiments 7, 11, 13, and in those groups of experiment 15 which received only a very small amount of asbestos. If more than one i.p. injection was given, it was done weekly. The 10 to 20 i.tr. instillations were also applied weekly. The large amounts of relatively thick glass filaments in experiments 4 and 5 were inoculated in 4 ml saline by laparotomia in nembutal anaesthesia.

Polyvinylpyridine-N-oxide (PVNO) was applied in experiments 13 (table 1) and 15 (table 3). Three groups received actinolite i.p. suspended in 1 ml PVNO-solution (2% or 0.4%). Two of these groups were not further treated: one group was additionally i.p. injected four times with 1 ml 2% PVNO-solution 4, 8, 12, and 16 months later. The other groups in experiment 13, labelled "PVNO separately", received 1 ml PVNO solution intraperitoneally five times: one day before the i.p. injection of asbestos and after 4, 8, 12, and 16 months.

4. Method of examination

The animals died spontaneously or were killed when in a bad health condition. In some experiments, the surviving animals were sacrificed about 2.5 years after treatment. Post mortem examination was made of the abdominal cavity of rats injected intraperitoneally. Parts of tumours or organs with suspected tumour tissue were fixed in formalin (6%) for histological examination. In the intratracheal experiments the lungs and tracheas were wholly fixed in formalin for histological tumour evaluation. The number of rats examined (listed in tables 1–4) includes all autopsied rats and does not include those animals lost through cannibalism or during anaesthesia. The percentage of dead rats does not comprise rats lost during anaesthesia but it does include those lost through cannibalism.

Results

Table 1 lists in chronological order the results of 13 experiments using the intraperitoneal test. All rats with sarcoma, mesothelioma or carcinoma of the abdominal cavity were counted as tumour-bearing rats. The most frequent diagnoses were sarcomas as in previous experiments (PORT et al. 1976; SCHEUER et al. 1973); only a few carcinomas were found. These three tumour-types could not always be differentiated with certainty histologically; sometimes two tumour-types occurred together. Since 5–10% of the control group showed malignant tumours of the uterus, part of them with metastases, rats with malignant uterine tumours were not counted as tumour-bearing rats. Thus it is possible that a tumour was classified as a spontaneous uterine tumour even though it was induced by the substance administered and occurred accidentally alongside with a malignant neoplasm of the uterus.

Table 1. Results after intraperitoneal injection of various fibrous and granular dusts

Dust	Fibre dimens. [μm] length 50%-	diameter 50%-	Dose i.p. [μm]	Number of rats examined	Rats with tum. [%]*	Life-span [weeks] after first treatment of all rats 20%-	50%-	80%-	100%-	Rats with tum. first	average
Experiment 1 (Wistar, 12 weeks)**											
Chrysotile, UICC-A	9	0.15	6	34	77.1	58	71	79	101	19	71
Chrysotile, UICC-A	9	0.15	25	31	80.6	49	58	73	100	39	60
Chrysotile, HCl-treated			6	38	0.0	45	78	133	133		
Chrysotile, HCl-trated			25	40		0.4	0.6	0.7	1		
Saline				70	0.0	78	87	87	121		
						(life-span reduced by inf. in month 11)					
Experiment 2 (Wistar, 12 weeks)											
Glass filaments, ES 5	39	5.5	10	50	4.0	81	111	129	165	92	106
Glass filaments, ES 5	39	5.5	40 (2 × 20)	46	10.9	90	107	127	165	96	119
Glass filaments, ES 7	16	7.1	40 (2 × 20)	47	2.1	102	121	131	156	126	126
Glass	(granular)		40 (2 × 20)	45	4.4	102	119	136	165	121	129
Quartz, DQ 12	(granular)		40 (2 × 20)	41	22.0	88	101	115	133	45	91
Experiment 3 (Wistar, 15 weeks)											
Slag wool, RH	26	2.6	40 (2 × 20)	99	6.1	94	111	127	158	88	114
Slag wool, ZI	14	1.5	40 (2 × 20)	96	2.1	91	107	126	155	67	77
Nemalite, $Mg(OH)_2$	1.3	0.06	40 (2 × 20)	48	89.6	32	39	17	65	25	41
Brucite, $Mg(OH)_2$	(mainly gran.)		40 (2 × 20)	49	0.0	86	99	126	155		
Actinolite	(mainly gran.)		40 (2 × 20)	48	4.2	80	96	121	138	116	123
Saline			2 × 2 ml	48	0.0	85	101	129	150		
Experiment 4 (Wistar, 12 weeks)											
Glass filaments, ES 5	39	5.5	250 (lap.)	28	7.1	94	109	125	141	76	77
Experiment 5 (Wistar, 15 weeks)											
Glass filaments, ES 3	16.5	3.7	50 (lap.)	48	6.3	68	91	115	135	71	90
Glass filaments, ES 3	16.5	3.7	250 (lap.)	46	8.7	67	91	115	139	87	116
Glass	(granular)		50 (lap.)	48	8.3	70	88	115	139	62	91
Glass	(granular)		250 (lap.)	48	8.3	75	99	115	130	91	101
Saline			1 ml (lap.)	45	4.4	68	87	110	139	95	98
						(life-span reduced by inf. in month 15)					
Experiment 6 (Wistar, 12 weeks)											
Anthophyllite, UICC	2.6	0.61	2	37	10.8	71	84	105	139	102	112
Anthophyllite, UICC	2.6	0.61	10	39	43.6	71	84	105	133	67	94
Chrysotile, UICC-A milled	0.2	0.02	10	39	2.6	70	71	98	124	98	98
Glass fibres, 106	2.2	0.17	10	39	5.1	72	104	119	137	86	98
Nemalite	1.3	0.06	2	37	75.7	60	69	87	120	51	71
Nemalite	1.3	0.06	10	40	80.0	42	51	61	79	31	53
Quartz, DQ 12	(granular)		10	34	5.9	71	73	102	139	102	107
Corundum	(granular)		10	35	8.6	72	80	115	121	103	111

Experiment 5 (Wistar, 15 weeks)											
Glass filaments, ES 3	16.5	3.7	50 (lap.)	48	6.3	68	94	115	135	71	90
Glass filaments, ES 3	16.5	3.7	250 (lap.)	46	8.7	67	94	115	139	87	116
Glass	(granular)		50 (lap.)	48	8.3	70	88	115	139	62	91
Glass	(granular)		250 (lap.)	48	8.3	75	99	115	130	91	101
Saline			1 ml (lap.)	15	4.1	68	87	110	139	95	98
						(life-span reduced by inf. in month 15)					
Experiment 6 (Wistar, 12 weeks)											
Anthophyllite, UICC	2.6	0.61	2	37	10.8	71	84	105	139	102	112
Anthophyllite, UICC	2.6	0.61	10	39	43.6	71	84	105	133	67	94
Chrysotile, UICC/A milled	0.2	0.02	10	39	2.6	70	71	98	121	98	98
Glass fibres, 106	2.2	0.17	10	39	5.1	72	104	119	137	86	98
Nemalite	1.3	0.06	2	37	75.7	60	69	87	120	51	71
Nemalite	1.3	0.06	10	40	80.0	42	51	61	79	31	53
Quartz, DQ 12	(granular)		10	34	5.9	71	73	102	139	102	107
Corundum	(granular)		10	35	8.6	72	80	115	121	103	111
						(life-span reduced by inf. in month 16)					
Experiment 7 (Wistar, female (f) and male (m), 3 weeks)											
Glass fibres, 104/1974, Ch. 2	3.5	0.3	10	26 f	50.0	6	37	54	112	17	51
Glass fibres, 104/1974, Ch. 2	3.5	0.3	10	33 m	54.6	11	45	54	67	18	44
Experiment 8 (Wistar, 12 weeks)											
Chrysotile, UICC/B milled	0.56	0.06	50	41	2.4	48	62	78	119	99	99
Actinolite, F.R.G.	1.9	0.17	2.5	45	66.7	40	56	62	71	38	53
						(life-span reduced by inf. in month 10 - 14)					
Experiment 9 (Wistar, 9 weeks)											
Attapulgite, Mormoiron	0.7	0.07	60 (5 inj.)	114	3.5	92	116	138	161	17	92
Attapulgite, Lebuija	0.5	0.07	60 (5 inj.)	115	3.5	95	116	134	161	98	111
Attapulgite, Georgia	0.8	0.04	60 (5 inj.)	112	3.6	89	108	129	163	75	100
γ-ferric oxide hydrate (1)	0.5	0.07	135 (5 inj.)	111	18.9	96	121	138	160	62	111
Corundum	(granular)		90 (5 inj.)	115	3.5	93	112	135	161	95	119
Titanium dioxide	(granular)		90 (5 inj.)	113	5.3	96	120	137	161	92	119
Experiment 10 (Sprague-Dawley, 8 weeks)											
Glass fibres, 104/1974, Ch. 1	1.8	0.29	5	54	81.5	52	64	79	108	40	67
Glass fibres, HCl treated 2 h			5	54	59.3	75	88	105	133	68	93
Glass fibres, HCl-treated 24 h	5.3	0.5	5	54	7.4	78	99	108	142	102	111
Glass fibres, NaOH-treated 2 h			5	54	77.8	54	71	83	115	35	69
Glass fibres, NaOH-treated 24 h	5.4	0.5	5	53	86.8	61	72	81	106	12	72
Erionite, Turkey	2.9	0.38	1.25	53	71.7	64	83	97	130	41	82
Erionite, Turkey	2.9	0.38	5	53	81.1	45	52	68	115	32	54
Erionite, Turkey	2.9	0.38	20	53	69.8	35	41	51	72	30	44
Titanium dioxide	(granular)		5	52	3.8	77	99	109	142	86	97
Experiment 11 (Wistar, 4 weeks)											
Glass fibres, 104/1974, Ch. 1	1.8	0.29	5	45	44.4	7	34	53	65	31	19
Glass fibres, HCl-treated 24 h	5.3	0.5	5	45	4.4	104	113	131	146	112	126
Glass fibres, NaOH-treated 24 h	5.4	0.5	5	46	58.7	23	58	71	103	29	64
Erionite, Turkey	2.9	0.38	5	48	70.8	50	64	76	145	40	66
Actinolite, F.R.G.	1.9	0.17	0.5	59	91.5	57	66	80	122	45	69
Titanium dioxide	(granular)		5	47	0.0	81	102	120	145		

Table 1 (continued)

Dust	Fibre dimens. [μm]		Dose i.p. [mg]	Number of rats examined	Rats with tum. [%][a]	Life-span [weeks] after first treatment of					
	length 50%	diameter 50%				all rats 20%	50%	80%	100%	rats with tum. first	average
Experiment 12 (Sprague-Dawley, 8 weeks)											
Glass fibres, 100/Pen	2.4	0.33	2	51	38.9	79	90	112	131	53	99
Glass fibres, 100/Pen	2.1	0.33	10	53	45.3	68	79	91	126	33	83
Glass fibres, 100/LAV	1.1	0.32	2	54	48.1	72	93	106	122	32	92
Rock wool, Sweden	23.0	1.9	75 (3 · 25)	63	71.4	61	77	96	134	39	79
Rock wool, Sweden, fine	1.1	0.64	10	45	13.3	86	97	115	131	86	109
Volcanic ash, St. Helen's	(granular)		10 (2 · 20)	54	5.6	93	116	131	131	79	87
NaCl-sol.			2 · 2 ml	54	5.6	91	109	131	131	91	100
Experiment 13 (Wistar, 5 weeks)											
Attapulgite, Caceres	1.3	0.07	10 (2 · 4 · 4)	30	40.0	91	109	132	142	74	116
Erionite, Oregon	1.8	0.21	0.5	31	48.4	81	108	129	142	63	98
Erionite, Oregon	1.4	0.21	2.0	31	90.3	60	79	93	118	51	80
Actinolite, F.R.G.	1.9	0.17	0.3	29	79.3	58	75	92	111	45	75
Actinolite, PVNO separately	1.9	0.17	0.3	32	65.6	61	80	106	142	39	77
Actinolite, in 1 ml 2% PVNO	1.9	0.17	0.3	29	48.3	97	117	135	142	95	121
PVNO separately											
Chrysotile, UICC/B	0.9	0.11	1.0	32	84.4	44	51	68	131	39	56
Chrysotile, PVNO separately	0.9	0.11	1.0	30	80.0	46	66	90	136	38	66
Chrysotile, Calidria	1.2	0.03	0.5	32	6.3	93	116	135	142	81	106
Crocidolite, South Africa	2.1	0.20	0.5	32	56.3	84	109	132	141	79	110
Crocidolite, South Africa	2.1	0.20	2.0	32	87.5	56	71	81	103	52	71
Glass fibres, 104/475	3.2	0.18	0.5	30	16.7	95	116	138	142	88	113
Glass fibres, 104/475	3.2	0.18	2.0	31	25.8	84	110	127	142	84	107
Glass fibres, 104-treated 24 h			2.0	32	50.0	93	107	129	141	56	105
Kevlar fibres (1)			10 (2 · 4 · 4)	31	12.9	109	121	139	142	128	135
Titanium dioxide	(granular)		10 (2 · 4 · 4)	32	0.0	103	130	142	142		
Saline			1 · 1 ml	32	6.3	97	120	142	142	113	117

[a] Animals with sarcoma, mesothelioma or carcinoma in the abdominal cavity excluding tumours of the uterus; percentage of rats examined

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* Animals with sarcoma, mesothelioma or carcinoma in the abdominal cavity excluding tumours of the uterus; percentage of rats examined

Of 204 rats injected intraperitoneally with saline alone, 5 (= 2.5%) had malignant tumours in the abdominal cavity other than uterine tumours (3 sarcomas, 1 mesothelioma, 1 carcinoma). In 394 rats injected with titanium dioxide or corundum, 15 neoplasms (= 3.8%) were found (8 sarcomas, 6 mesotheliomas, 1 carcinoma). Several other tumour-types that occurred occasionally in both the control and the test groups were also considered as spontaneous and were not included in the percentage of the tumour-bearing animals. Among those figured are adenomas, angiomas, fibromas, myomas, malignant lymphomas and others.

The typical tumours caused by fibres were accompanied by hemorrhagic ascites. In some cases, a hemorrhagic ascites was found but no tumour. Macroscopically, a few animals showed only a single tumour. Quite often there were several neoplasms that mostly originated from the upper abdominal serosa, especially from the omentum majus and omentum minus. In many cases, the primary tumour had spread diffusely within the abdominal cavity so that the visceral and parietal peritoneum was covered with numerous minute metastases often numbering more than a hundred.

The interval between injection and death of the first tumour-bearing animal and the average survival time of the tumour-bearing animals depended on the type of dust applied, and the dose and the age of the rats at the beginning of the experiment. The 7th experiment had the shortest latency period of 17 weeks. In this experiment, 3-week-old female and male rats were injected intraperitoneally with the relatively high dose of 10 mg glass fibres. A very long latency period was observed after the injection of 0.3 mg actinolite in PVNO-solution: the first tumour-bearing animal died after 95 weeks. Nevertheless, a tumour incidence of 48% was still reached.

Intraperitoneal injection of high doses of non-carcinogenic dusts can lead to inflammatory reactions with more or less distinct adhesions of abdominal organs. The degree of these alterations again depended on the type of dust, the dose and the age of the rats. For the experimental groups shown in table 3, a quantification is given which will be explained later in the text. Supplementary to table 1, only a few important findings concerning the degree of adhesion in some experimental groups should be mentioned. After correct intraperitoneal injection of NaCl-solution no irritant or inflammatory reaction occurs. But in non-anaesthetized rats, a puncture of the intestines with the injection needle cannot always be avoided and may subsequently lead to adhesive inflammation. Even after injection of small amounts, non-soluble dusts with a specific colour, such as titanium dioxide, corundum or ferric oxide, can be seen deposited on the peritoneum and especially on both sides of the omentum majus.

Only in certain cases does the degree af adhesion increase with the tumour rate. This happens with carcinogenic fibres in a medium range of doses. After injection of high doses some of the animals died from the adhesions before a tumour could develop. In the low-dose experiments (less than 1 mg per rat with a weight of more than 150 g) or after the injection of greater amounts of very short fibres, many animals showed such minor changes of the peritoneum that they could hardly be detected macroscopically or not at all. In spite of this, the tumour incidences after injection of 0.25 mg to 0.5 mg actinolite, chrysotile, crocidolite or erionite were between 50 and 80% (experiment 13 in table 1, experiment 15 in table 3). On the other hand, the injection of relatively thick glass filaments with diameters of 5 and 7 μm led to clearly visible adhesions of the abdominal organs, while a tumour inducing effect could not be proved (experiment 2).

Fibrosis caused by quartz (experiment 2) significantly differed in its macroscopic view from the superficially spreading fibrosis caused by natural and man-made mineral fibres as well as by metal compounds (experiment 14). After the injection of quartz, numerous nodules of one to several millimetres in diameter could be seen, especially in the omentum majus. The abdominal organs usually did not grow together.

As mentioned in the chapter "substances used", it was not possible to produce a homogeneous suspension of Kevlar fibres. Therefore, these fibres were more likely to occur in clumps in the peritoneal cavity than were the other dusts (experiments 13 and 15).

In those groups where the survival time was not shortened by the toxicity or tumour-inducing effect of the given substance, 20% of the animals died 80 to 100 weeks from the start of the experiment; 50% survived between 100 to 120 weeks and 20% up to about 140 weeks. As far as survival times are concerned it has to be borne in mind that the natural point of death was often not awaited for when the animals were in a bad condition. Most of the experiments were terminated after about two-and-a-half years. In some groups more than 20% of the rats were still alive after that period. Since the animals were not kept under SPF-conditions, the survival times are affected to a greater or lesser extent by infections. Furthermore, it has to be considered that the Sprague-Dawley rat which was used in experiments 10 and 12 has a lower life expectancy than the Wistar rat.

Table 2 shows the tumour rates and survival times after intraperitoneal injection of some nickel and cadmium compounds. The doses injected were chosen after examining the acute toxicity; they appeared to be the maximum tolerable amounts. Moderate to strong adhesions of the abdominal organs were observed in all groups.

Table 3 shows the preliminary results of a still uncompleted intraperitoneal experiment 28 months from outset. Although at this point many rats in several groups were still alive, the tumour rates already give interesting information on certain questions. For each group in this experiment not only the tumour incidence is given but also the degree of adhesions of the abdominal organs. To make a better survey and to quantify the adhesions, the great variety which was visible macroscopically had to be summarized and simplified. We classified the adhesions into the following grades:

Grade 0: No adhesions of abdominal organs.

Grade 1: Only punctual or very narrow adhesions up to about 10 mm^2. They are mostly found between the omentum majus and the liver or intestines. Sometimes the omentum is not extended normally, but slightly shrunken. This is probably due to a minimal local fibrosis.

Grade 2: Adhesions over small areas between 10 and 100 mm^2. They mostly affect the omentum and liver, the liver and diaphragm or the spleen and stomach, and less often the intestines. There are extensive adhesions between the omentum and stomach. The mesenterium may also be slightly deformed.

Grade 3: Adhesions of more than 1 cm^2. All abdominal organs are clearly distinguishable and, except for the omentum, only slightly deformed.

Grade 4: Adhesions over large areas with severe deformation of more than one abdominal organ.

Grade 5: The adhesions and deformation of the organs are too strong to allow their separation in the section.

Grades 4 and 5 did not occur in experiment 15 (table 2). They have been observed in rats after injection of 50 mg or more of asbestos or other corresponding fibres (Porr et al. 1976). Similarly strong adhesions also occurred after injection of 10 mg glass fibres into 3-week-old rats (experiment 7). The adhesions of the abdominal organs develop within the first weeks after intraperitoneal injection of the dusts. If in the later course of the experiment a tumour spreads in the abdomen, this growth also frequently leads to adhesions. Thus the original degree of adhesion cannot be judged correctly. The degree of adhesion after intraperitoneal injection of 1 mg of chrysotile has therefore been put into (1); in this group 86% of the rats showed tumours.

Table 4 shows the results after intratracheal instillation of crocidolite and glass fibres, benzo(a)pyrene and some metal compounds. As in the i.p. test with metal compounds, the doses applied were chosen after examining the acute toxicity (loss in weight, mortality). The difference in the acute toxicity of cadmium oxide and cadmium sulfide was not as high as after i.p. injection, but it can be estimated in the relation of 1 to 20.

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Table 2. Results after intraperitoneal injection of cadmium and nickel compounds

Dust	Dose i.p. [mg]¹	Number of rats examined	Rats with tum. [%]²*	Life-span [weeks] after first treatment of all rats 20%	50%	80%	100%	rats with tum. first	average
Experiment 11 (Wistar, 12 weeks)									
Cadmium oxide	0.25 (2 × .125)	47	6.4	85	111	123	123	78	107
Cadmium sulfide	50	81	66.7	55	72	88	123	36	70
Nickel powder	75 (10 × 7.5)	48	95.8	27	30	33	88	25	31
Nickel oxide	1,000 (2 × 500)	47	97.9	25	28	36	73	22	31
Nickel subsulfide	25	42	64.3	18	28	42	123	18	33

¹ Dose of metal content in the compound
² Animals with sarcoma, mesothelioma or carcinoma in the abdominal cavity excluding tumours of the uterus; percentage of rats examined

Table 3. Preliminary results 28 months after intraperitoneal injection of various fibrous and granular dusts

Dust	Fibre dimens. [µm] length 50%-ile	diameter 50%-ile	Dose i.p. [mg]	Rats exam. + survivors	Rats with tum. [%] [1]	Degree of adhesions	Life-span [weeks] after first treatment of all rats 20%-ile	50%-ile	80%-ile	100%-ile	rats with tum. first	average
Experiment 15 [**] (Wistar, 8 weeks)												
Actinolite, F.R.G.	1.9	0.17	0.01	29 + 6 = 35	8.6	0	79	103	124		103	
Actinolite, F.R.G.	1.9	1.17	0.05	29 + 7 = 36	30.6	0	75	101	122		64	
Actinolite, F.R.G.	1.9	0.17	0.25	30 + 6 = 36	55.6	0–1	69	90	112		58	
Actinolite, in 1 ml 0.4% PVNO	1.9	0.17	0.25	32 + 3 = 35	25.7	0–1	77	96	113		69	
Actinolite, in 1 ml 2% PVNO	1.9	0.17	0.25	25 + 11 = 36	25.0	0–1	95	111			78	
Chrysotile, UICC-B	0.9	0.11	0.05	23 + 13 = 36	19.4	0–1	84	105			64	
Chrysotile, UICC B	0.9	0.11	0.25	30 + 4 = 34	61.8	0–1	80	90	115		58	
Chrysotile, UICC B	0.9	0.11	1.00	35 + 1 = 36	86.1	(1)	52	63	72		35	
Glass fibres, 104/475	3.2	0.18	5 (5–11)	52 + 1 = 53	66.0	3	53	67	85		37	
Basalt wool, G – H	20.0	1.8	75 (5–15)	53 + 0 = 53	60.4	3	64	79	92	111	54	83
Ceramic wool, Fiberfrax	8.3	0.91	15 (5–9)	47 + 0 = 47	68.1	3	38	51	63	95	30	51
Ceramic wool, MAN	6.9	1.1	75 (5–15)	53 + 1 = 54	22.2	2–3	71	91	106		60	
Wollastonite	5.2	1.1	100 (5–20)	34 + 20 = 54	0.0	1–2	88	107				
γ-ferric oxide hydrate (2)	0.5	0.03	250 (5–50)	39 + 10 = 49	12.2	2	67	98	120		88	
α-ferric oxide hydrate	0.1	0.01	250 (5–50)	34 + 17 = 51	2.0	2	83	112			64	
Kevlar fibres (2)	3.9	0.17	20 (5–40)	34 + 18 = 52	5.8	1–2	78	106			35	
Polypropylene fibres	7.4	1.1	50 (5–10)	35 + 16 = 51	2.0	2	86	108			117	
Polyvinylchloride	(granular)		500 (5–100)	37 + 14 = 51	9.8	1–2	83	107			62	
Titanium dioxide	(granular)		100 (5–20)	36 + 17 = 53	9.4	0	82	109			38	
Wood dust, beech	(granular)		250 (3 inj.)	23 + 30 = 53	0.0	1–2	104	124				
NaCl-sol.			5 × 2 ml	73 + 29 = 102	2.0	0	85	111			93	

[1] Rats with tumour(s) in the abdominal cavity excluding tumours of the uterus in % of the total of rats examined and survivors. Partly macroscopical diagnoses only.

[**] This experiment was partly supported by the Bundesanstalt für Arbeitsschutz, Dortmund, F.R.G.

Table 4. Results after intratracheal instillation of fibrous dusts and metal compounds into female Wistar rats.

Substance	Dose i.tr.	Number of rats ex-	Rats with lung tumours: adeno-matous	adeno-carc.	squamous cc.	mixed	total [%]	other tum. [*]	Life-span [weeks] after first instillation of: all rats [**]	rats with tum.

Titanium dioxide	(granular)	100 (5 20)	36	17	53	9.4	0	82	109		38	
Wood dust, beech	(granular)	250 (3 inj.)	23	30	53	0.0	1	2	101	124		
NaCl-sol.		5 2 ml	73	29	102	2.0	0	85	111		93	

' Rats with tumour(s) in the abdominal cavity excluding tumours of the uterus in % of the total of rats examined and survivors. Partly macroscopical diagnoses only.

' This experiment was partly supported by the Bundesanstalt für Arbeitsschutz, Dortmund, F.R.G.

Table 1. Results after intratracheal instillation of fibrous dusts and metal compounds into female Wistar rats.

Substance	Dose i.tr.	Number of rats examined	Rats with lung tumours						Life-span [weeks] after first instillation of					
			adenoma	adeno-carc.	squamous c.carc.	mixed	total [%]	other tum.**	all rats***				rats with tum.	
									20%	50%	80%	100%	first	average
Crocidolite, S. Africa	10 mg (20 × 0.5)	35	0	9	2	4	42.9	1	100	126	126	126	89	121
Glass fibres, 101/175	10 mg (20 × 0.5)	34	1	2	2	0	14.7	1	81	107	126	126	96	113
Benzo[a]pyrene	20 mg (20 × 1)	36	1	0	5	1	19.4	1	101	120	126	126	50	110
Cadmium chloride	20 µg* (20 1)	38	0	0	0	0	0.0	1	90	119	124	124	..	..
Cadmium chloride	60 µg* (20 × 3)	40	1	0	2	0	7.5	3	90	123	124	124	90	106
Cadmium chloride	135 µg* (15 × 9)	36	0	2	0	0	5.6	0	101	124	124	124	121	123
Cadmium oxide	20 µg* (20 1)	37	1	1	0	0	5.4	3	96	112	121	124	108	116
Cadmium oxide	60 µg* (20 3)	40	0	1	1	0	5.0	1	81	111	121	124	71	72
Cadmium oxide	135 µg* (15 9)	39	0	0	0	0	0.0	2	99	122	124	124	..	..
Cadmium sulfide	630 µg* (10 × 63)	39	1	1	0	0	5.1	3	91	123	124	124	124	124
Cadmium sulfide	2,500 µg* (10 × 250)	36	1	7	0	0	22.2	0	82	124	124	124	106	121
Cadmium sulfide	10,000 µg* (10 × 10^3)	36	0	4	3	0	19.4	0	32	92	124	124	97	115
Magnetite	225 mg (15 × 15)	34	0	1	14	7	69.4	0	86	118	126	126	74	115
Nickel oxide	50 mg* (10 × 5)	37	0	1	4	2	27.0	1	80	119	124	124	80	116
Nickel oxide	150 mg* (10 × 15)	38	0	0	12	0	31.6	3	67	101	124	124	102	115
Nickel powder	6 mg (20 × 0.3)	39	1	1	8	0	25.6	3	84	108	124	124	98	118
Nickel powder	9 mg (10 × 0.9)	32	0	3	4	1	25.0	1	89	117	124	124	117	122
Nickel subsulfide	0.94 mg* (15 × .063)	47	0	2	5	0	14.9	0	99	126	132	132	120	129
Nickel subsulfide	1.88 mg* (15 .125)	45	0	3	6	4	28.9	0	80	126	132	132	88	126
Nickel subsulfide	3.75 mg* (15 × .25)	40	0	7	4	1	30.0	0	99	120	132	132	99	125
Saline	20 0.3 ml	40	0	0	0	0	0.0	0	92	115	124	124	—	—

' Dose of the metal content in the compound

'' Other tumours in the lung: Fibrosarcoma, lymphosarcoma or lung metastases from tumours of other sites; these tumours are not included in the percentage of lung tumours

''' Age at start: 11 weeks

Discussion

The dusts which were examined for their carcinogenicity can be divided into 6 groups: (1) asbestos, (2) other natural mineral fibres, (3) man-made mineral fibres, (4) plastic fibres, (5) metal compounds and (6) other granular dusts. The results will be discussed in the given order. However, because of the large number of dusts applied, the wide explanations of their biological activities and the numerous connections to other studies can only be discussed briefly.

1. Asbestos

The experiments with asbestos have produced findings on the following points: dose response-relationships in very low dose ranges, significance of length and durability of fibres for their carcinogenic potency and effects of the prolonged latency period for tumour induction by PVNO.

Even 0.05 mg actinolite or chrysotile UICC B had a clear carcinogenic effect after i.p. injection (experiment 15). This is the maximum amount of airborne chrysotile which is permitted in 1 m^3 at the workplace in the F.R.G. (TRK = Technische Richtkonzentration = technical guiding concentration). Certainly, the dose response-relationship found with the i.p. test cannot be extrapolated to the lung of humans; however, the results confirm in principle the hypothesis that there is no threshold for the carcinogenicity of asbestos down to an extremely low dose range. After i.p. injection of 0.05 mg actinolite or chrysotile, no adhesions were observed macroscopically. A histologically detectable fibrosis should therefore be extremely small. It is not common to call such a small, macroscopically undetectable fibrosis a scar. If such minor tissue alterations cause a tumour to develop with a relatively high probability, many other dusts should also have led to tumour development while they in fact only induced macroscopically detectable adhesions and fibrosis but no tumours. These observations contradict KUSCHNER's hypothesis (1986). According to this a scar is always the necessary morphologic base for the tumour development caused by asbestos. Should a fundamental difference exist between a minor fibrosis induced by asbestos and the fibrosis induced by non-carcinogenic dusts and how could this be substantiated? Already DAVIS (1984) stated that "the correct diagnosis of early asbestosis remains one of the greatest problems for pathologists".

BOLTON et al. (1984) also found tumours after i.p. injection of very small amounts of chrysotile and crocidolite. But mostly much higher doses were applied in serosal tests, e.g. 20 mg (WAGNER et al. 1973; WAGNER et al. 1984a) or 40 mg (STANTON et al. 1977, 1981) or up to 100 mg (POTT et al. 1972, 1976; SCHEUER et al. 1973). The strong fibrosis which is caused by large amounts of asbestos has encouraged KUSCHNER (1986) to revive the scar hypothesis developed about 50 years ago (LINZBACH and WEDLER 1941). At that time, it appeared a plausible explanation for cases of lung carcinoma that were accompanied by severe asbestos induced fibrosis. Large amounts of fibres shorten the tumour latency up to a certain degree. On the other hand by causing severe fibrosis they also shorten the animal or human life-span and thereby reduce the tumour incidence (see table 1, experiment 7).

It is remarkable that no tumours were observed after i.p. injection of 50 mg ground chrysotile UICC B with very short fibres (50 % $\leq 0.56\,\mu m$) (experiment 8). However, in this experiment, the average survival time of the rats was greatly reduced by an infection; nevertheless, in another group tumours were found in 67 % of the animals after injection of actinolite. In earlier studies, 100 mg of milled chrysotile UICC A had induced tumours in 40 or 32 % of the rats (POTT et al. 1972, 1976). At that time it had already been discussed whether the few remaining strongly carcinogenic long fibres or the preponderance of slightly carcinogenic short fibres are responsible for the effect. We still have no clear answer to this. 10 mg of the same sample did not lead to tumours in experiment 6.

In experiment 13, a Californian chrysotile (Calidria) was used, which had not previously been assessed in animal tests. The intraperitoneal injection of 0.5 mg did not show a clear carcinogenic effect. Perhaps the Californian chrysotile is less durable than the two UICC

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chrysotile samples. MÜHLE et al. (1986) also did not find tumours after inhalation of Calidria chrysotile.

We must turn to the cell biologists for an answer to the question how long fibres must persist in the bronchial wall or in the serosal tissue to alter the cells sufficiently for transformation into tumour cells without the further presence of fibres. Former chrysotile fibres which have lost their magnesium content through acid treatment can be regarded as a prototype for fibres with very low durability *in vivo*. Within one week, i.p. injection of 25 mg of these fibres led to the death of most animals, possibly because of an observed kidney damage; all animals given 6 mg survived and did not develop tumours (experiment 1). The half-life of acid-treated chrysotile fibres in rat lungs was only 2 days after intratracheal instillation (BELLMANN et al. 1986). Such magnesium-free fibres only consist of amorphous silic acid which easily disintegrates in the body; this has already been recognized by BEGER (1933) in his remarkable study, although he disposed of only simple instruments. MONCHAUX et al. (1981) found a reduced carcinogenic effect of chrysotile dependent of the intensity of the acid treatment. The authors did not interpret this effect as a consequence of a decreased durability but concluded that, in connection with fibre surface properties, magnesium plays an important role in tumour induction.

The inhibitory effect of PVNO on experimental silicosis was first described by SCHLIPKÖTER and BROCKHAUS (1960). Tests were performed to establish whether PVNO also inhibits the development of asbestosis and tumours induced by asbestos. The tumour induction time in two experiments was significantly prolonged when actinolite was injected together with a 2% PVNO-solution (experiments 13 and 15). A separate injection of PVNO did not lead to inhibition. It has to be clarified whether PVNO neutralizes a carcinogenic agent on the surface of actinolite fibres by adsorption, or whether some side effect, present only under experimental conditions, prolongs the tumour development. Maybe the polymeric substance leads to the formation of fibre aggregates in the abdominal cavity, so that during the first year of the experiment the fibres are not distributed normally in the tissue. The tumourigenic potency of chrysotile was not affected by PVNO, possibly because the fibres split up in the body, as demonstrated by BELLMANN et al. (1986).

2. Natural mineral fibres other than asbestos

The carcinogenic effect of erionite was first described in inhabitants of a region in Turkey, where this fibrous zeolite occurs naturally and was formerly used for house building (BARIS et al. 1978). These fibres not only induced tumours after intraperitoneal injection in mice and intrapleural injection in rats (SUZUKI 1982, WAGNER et al. 1985). After inhalation, they also caused mesotheliomas in 27 out of 28 rats, while inhalation exposure to crocidolite with similar fibre size distribution led to a tumour (squamous cell carcinoma) in only 1 of 28 rats (WAGNER et al. 1985). In experiments 10 and 11, Turkish erionite was injected and in experiment 13 an erionite sample from Oregon, which Dr. WAGNER had also used. The results correspond to what can be expected from the dose and fibre size. So far, there is no explanation for the unique mesothelioma-inducing effect produced in the inhalation experiment of WAGNER et al. (1985).

We could confirm the carcinogenic effect of nemalite (experiments 3 and 6) using several doses of the same material described earlier (POTT et al. 1976). Granular magnesium hydroxide, mineralogically signed brucite, did not induce any tumours (experiment 3).

Attapulgite (palygorscite) from four sources was examined. Three samples (experiment 9) had no carcinogenic effect, the fourth with longer fibres (experiment 13) had moderate effect in relation to the applied dose. HARVEY et al. (1984) found *in vitro* strong cytotoxicity and hemolytic activity in a sample from Georgia, U.S.A. Therefore, different mechanisms of cytotoxic and carcinogenic effects can be assumed.

The only wollastonite sample examined had a median diameter of 1.1 μm and this was clearly bigger than the other natural fibres. Despite the high dose of 100 mg, no tumours appeared during the first two years of the experiment. There were no severe adhesions either. Comparing the result with rock and ceramic fibres of a similar size which are discussed later

on. the effect of the tested wollastonite fibres is much smaller or even zero. Probably wollastonite has a low durability *in vivo*: taken 50 mg of the fibres tested in the animal experiment, 10 % dissolved after 24 h in 0.9 % buffered saline (*p*H 7.4) at 37 °C. The lack of carcinogenicity of 100 mg fibrous gypsum after i.p. injection has also been explained by its high solubility (Pott and Friedrichs 1972).

3. Man-made mineral fibres

Since 1972, glass "microfibres" with median diameters of $\leq 0.3\,\mu$m have been well known to be carcinogenic after intrapleural and intraperitoneal administration. They are produced in various chemical compositions. These differences could be important for thei carcinogenic potency as well as their sizes. The treatment of sample 104 475 with acid did not reduce the tumour rate obtained by 2 mg. On the contrary, it even seems to have increased it (experiment 13). This increase might be accidental. But in comparison with other results, it seems more likely that the tumour rate for untreated fibres is too low. The reaction to HCl-treated E-glass fibres type 104 1974 was quite different. The fibres lost 33 % of their weight by the 24 h HCl-treatment. This loss mainly involved aluminium oxide and calcium oxide, each of which makes up 15 –20 % of E-glass (Bellmann et al. 1986). The carcinogenicity of these fibres was strongly reduced and, in comparison with the control group, not statistically significantly increased (experiments 10 and 11). Also, the half-life in the lung was clearly shortened (Bellmann et al. 1986). It was surprising that after 2 h of HCl-treatment the weight loss already amounted to 25 %, while the decrease in carcinogenicity was much less significant.

After treatment of glass fibres 104 1974 with NaOH, there was only an initial prolongation of the tumour latency. A general inhibition could not be detected.

The administration of glass filaments with diameters of about 3, 5 and 7 μm did not lead to increased tumour rates (experiments 2, 4, 5). Because of their thickness, the actual number of fibres was small despite the great mass, and fibre-induced tumours were therefore unlikely to occur. An increased tumour rate could only have been expected if the glass filaments ES3 (experiment 5) were at least as effective as ceramic or basalt fibres (table 3).

As stated earlier (Pott et al. 1980), Sprague-Dawley rats may be more sensitive to the carcinogenic effect of glass fibres in the i.p. test than Wistar rats, but the difference does not appear to be significant. We prefer Wistar rats for the i.p. test, because they are thinner than Sprague-Dawley rats; therefore it is easier to diagnose ascites or abdominal tumours in the animals when they are still alive.

The intratracheal instillation of 20 0.5 mg glass microfibres 104 475 induced lung tumours in 5 of 34 rats (table 4). This 15 % tumour incidence is not very high but statistically significant, especially with regard to the zero-tumour-rate of non-dusted Wistar rats which also was found in other long term experiments (Heinrich et al. 1986a, b; Muhle et al. 1986). The result confirms earlier ones obtained after instillation of 8 · 1 mg glass fibres 104 1974 in Syrian golden hamsters (Pott et al. 1984b). In that experiment two samples with different lengths were administered (they were also used in experiments 7 and 10). Of 136 animals instilled with the longer fibres, 5 hamsters with lung carcinomas and 37 with mesotheliomas were found; the shorter fibres induced 6 lung carcinomas and 26 mesotheliomas in 138 hamsters. The high mesothelioma rate can be explained by the high amount of fibres which reached the pleura immediately after instillation. UICC crocidolite which contains relatively short fibres induced lung carcinomas in 9 and mesotheliomas in 8 of 142 hamsters. The tumour latency was rather long; nearly all tumour-bearing animals survived for a period of 18 months after the first instillation; about 50 % lived for longer than two years. The morphological aspects of the induced mesotheliomas were described by Mohr et al. (1984).

In a similar experiment of Fabian et al. (1985), hamsters received 26 1 mg of glass microfibres which resulted in stronger inflammation and a short survival time. The experiment has been finished already after 80 weeks without showing an increased tumour rate

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either with glass fibres or with crocidolite. The short life-span of the animals can explain the negative results.

While the very fine glass microfibres are only produced in small amounts, the common isolating wools from slag, glass, rock or aluminium silicate (ceramic) are used in great quantities. Their median diameter is several times as wide as that of microfibres. The thinner fibres from the wools were enriched for the animal experiments; the median diameter of the injected fibres was between 0.9 and 2.6 μm. Therefore one needs a much larger mass than with microfibres to achieve the same number of fibres. For example, a cylindric fibre with a diameter of 1.5 μm and a length of 14 μm (= median of the slag wool Z1 in experiment 31) has 300 times the mass of a fibre with 0.18 μm × 3.2 μm (= median of glass fibres 104 475 in experiment 13). Consequently, a zero effect of the slag wool sample in comparison with glass fibres 104 475 cannot be proven with the given dose.

The interpretation of the results on slag wool (Pott et al. 1984a) also has to be revised, since on re-examination of the fibre sizes a median diameter of 1.3 μm instead of the earlier measured 0.18 μm was found. Thus, the number of fibres quoted in the experiment was again too small to anticipate a carcinogenic effect in comparison with glass microfibres 100 or 104.

Nevertheless, slag wool seems to be less dangerous than rock or ceramic wools. If one compares the median sizes described in the experiments, the number of injected fibres per animal does not differ too much. Rock wool, basalt wool and the two ceramic wools (experiments 12 and 15) show a clear carcinogenic effect in contrast to the two slag wool samples (experiment 31). For a final interpretation we have to wait for the results of a planned verification and re-evaluation of the data on fibre sizes. In any case, it is remarkable that all four rock and ceramic wool samples induced tumours. Perhaps the relatively high percentage of very long fibres (> 20 μm) caused this unexpected effect. Davis et al. (1984) found one adenoma and three carcinomas in 48 Wistar rats after inhalation of ceramic fibres and tumours in the abdominal cavity in 3 of 32 rats after i.p. injection of 25 mg of the fibres.

Finally, the very short ferric oxide hydrate fibres used in the production of tapes should also be mentioned. The fibre tested in experiment 9 shows a statistically significant increased tumour rate compared to other groups. But in view of the high injected dose the effect has to be regarded as very small. Furthermore, it is not certain whether the effect was caused by the very low carcinogenicity of the very short fibres or by a chemical effect of the ferric compound. In an earlier i.p. test on 2 ferric oxides, no tumours were detected (Pott et al. 1976). Recent evaluation of ferric oxides gave no evidence for their carcinogenicity (Henschler 1984; Stokinger 1984), but the findings on magnetite (table 4), which will be discussed later, again raise this question.

4. Plastic fibres

A tumour inducing effect has not been found so far. But the interlacing of plastic fibres is much higher and their capacity for suspensions in water much smaller than that of mineral fibres. We must therefore make allowance for different conditions *in vivo*, and these favourable results should be judged with caution.

5. Metal compounds

The intraperitoneal experiment with nickel and cadmium compounds was designed to examine the carcinogenic effect of maximum tolerable doses. Cadmium oxide was found to be more than a 100 times as acutely toxic as cadmium sulfide. Nickel oxide was much less toxic than nickel powder and nickel subsulfide. With the exception of cadmium oxide, which could only be given in very low doses, high tumour rates were found with short latency periods (experiment 14, table 2). Therefore one can still expect increased tumour rates after injection of doses 20 to 50 times lower than the ones applied here. Running studies with lower doses will give more definite results on the relative carcinogenic potencies of these and other nickel compounds. They will make possible a comparison with the ranking order found by Sunderman (1984) after a single i.m. administration of 14 mg nickel

nickel compounds to Fischer rats. Cadmium sulfide also induced local sarcomas in rats after i.m. and s.c. injection (KAZANTZIS and HANBURY 1966).

All three nickel-containing dusts caused lung tumours after intratracheal instillation (table 4). In relation to the dose, nickel subsulfide had the strongest effect, nickel powder a slightly smaller one while nickel oxide was clearly the least effective.

Since cadmium sulfide has a considerably lower acute toxicity than cadmium chloride and cadmium oxide it could be dosed much higher and, depending on the dose level, led to lung tumours (table 4). The highest dose of 10 mg cadmium in cadmium sulfide caused early death of some animals. The tumour rate therefore appears to be too low. Also, after inhalation, cadmium sulfide seems to induce lung tumours in rats (OLDIGES and GLASER 1986) but not in hamsters and mice (HEINRICH et al. 1986c).

Most surprising was the high lung tumour rate of 69% after 15 intratracheal instillations of 15 mg each of very fine granular magnetite. This raises the question of whether an unspecific reaction of the rat lung to the large surface of the particles led to the tumours. This possibility is discussed for the inhalation of high concentrations of TiO_2 (LEE et al. 1985). But in contrast to other ferric oxides, magnetite could also have a chemically carcinogenic effect.

6. Other granular dusts

As non-carcinogenic control dusts we took corundum (experiments 6, 9), titanium dioxide (experiments 9, 10, 11, 13, 15), glass powder (experiments 2, 5) and volcanic ash (experiment 12). The tumour rates were between 0 and about 10%. Besides the above-mentioned metal compounds quartz was the only non-fibrous mineral dust which had a low tumour inducing effect after i.p. injection of a higher dose (40 mg, experiment 2). HOLLAND et al. (1986) found lung tumours in rats after inhalation of quartz dust. GROTH et al. (1986) after intratracheal instillation, and WAGNER and BERRY (1969) after intrapleural administration.

Polyvinylchloride (PVC) was injected in a very high dose (5 × 100 mg), but the preliminary result (experiment 15) shows no clear carcinogenic effect. Since the density of PVC is low, the animals received a high dust volume. In addition to a chemical carcinogenic effect, foreign body-induced tumours could be expected from the high mass which was deposited in clumps (OPPENHEIMER et al. 1948; BRAND 1986). The low tumour rate found after i.p. injection of high masses of granular dusts indicates that the carcinogenicity of fibres cannot be explained by a simple foreign body effect.

Beech and oak dusts are known to induce carcinomas in the nose in humans. In experiment 15 we tried to assess whether the i.p. test is sensitive for the unknown carcinogenic agent. The preliminary results (table 3) do not reveal an existing sensitivity.

Conclusions

The length and durability of fibres are of great significance for their carcinogenic potency. It should be re-examined how far this also applies to the diameter because the effect of relatively thick rock and ceramic fibres was unexpectedly strong. Further possible explanations could be surface properties or an especially strong carcinogenicity of very long fibres (> 20 μm), which have been quite numerous in the samples used. A second measurement of several of the used fibre samples is necessary for a better evaluation of the relation between fibre dimensions and carcinogenic effect. The available data of the fibre size distributions are not comparable in all cases because measurements were carried out at different times using different methods and by different working groups.

The intraperitoneal test in rats is easy feasible and is proved to be very sensitive for detecting the carcinogenic effect of durable natural and man-made mineral fibres as well as metal compounds with low acute toxicity. This means that, if a high dose of one of these dusts does not induce tumours in this test, no suspicion of carcinogenic potency worth mentioning can be substantiated. Maybe the present examples are too few to be certain

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that this is true for every negative result, but this seems to be highly probable. An important step would be to establish a simple test method that excludes with a high probability false negative results for a certain group of substances. It is more difficult to deduce whether a carcinogenic potency resulting from the i.p. test in principle applies also to the lung after inhalation of the dusts, and whether the observed order of carcinogenic potency is also transferable to the lung. As discussed *in detail, there exist substantial differences with clear* advantages and disadvantages for each between the inhalation test and the i.p. test (Pott 1984). The *inhalation test simulates* realistic exposure conditions but is open to the great risk of yielding false negative results (e.g. some negative or weak positive inhalation studies with crocidolite). The i.p. test has a high sensitivity of carcinogenic fibres and metal compounds but it cannot simulate the selection of particles which occurs physiologically after inhalation by deposition in different parts of the airways and by the clearance mechanisms. While these influences can be estimated more or less precisely, it is more difficult to measure the difference in the carcinogenic response of the bronchial epithelium and the serosa. This point needs further research.

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