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EXPERIMENTAL CARCINOGENICITY AND MUTAGENICITY OF NON-ASBESTOS NATURAL FIBRES – PRELIMINARY REPORT

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Abstract. The aim of this investigation was to quantify the carcinogenic and mutagenic activity of antigorite occurring in the form of admixtures in different mineral raw materials (serpentynite, magnesite, dolomite and nickel ore). The carcinogenicity of dusts was evaluated after intraperitoneal injections of 5 mg (mice) or 20 mg (rats) of dust suspended in saline.

A pathomorphological eaxmination was performed in all the dead animals. For two raw materials – serpentinite and nickel ore – their mutagenic potency was investigated (SCE test was used in this study). Results obtained in the experiments on animals (rats and mice) showed that the biological aggressiveness of the mineral raw materials tested was associated with the content of antigorite fibres. Particularly the frequency of mesothelioma (5–85%) was related to the number of antigorite fibres longer than 5 μm. Both of the investigated raw materials (serpentinite and nickel ore) were mutagenic in the SCE test.

INTRODUCTION

In Poland the problem of exposure to fibrous dusts other than asbestos occurring in the form of admixtures in deposits of the main mineral has been brought into light when occupational asbestosis was diagnosed in a worker employed in the nickel ore mining and processing plant located in Lower Silesia. Medical examinations of the remaining workers in that plant revealed numerous cases of asbestosis. The mean coefficient of incidence from asbestosis calculated for the past 20-year period amounted to about 20 cases per one thousand employees per year (4).

The situation found in the nickel ore mining and processing plant was a direct stimulus for undertaking investigations aimed at:

– mineralogical analysis of fibrous minerals admixtures taken from ten mines of mineral raw material in the Lower Silesia region;

– evaluation of carcinogenic and mutagenic properties of some fibrous dusts occurring in mines of Lower Silesia, in an experiment on animals.

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MATERIALS AND METHODS

Dusts

Samples of minerals from ten different mines of different minerals were ground to dust in a Fritsch planetery mill. Their crystalline phase content was than estimated by the x-ray diffraction method (5). The morphology of dust particles was determined using TEM. The number of respirable fibres in a dust dose was determined using the phase-contrast light microscopy technique.

Carcinogenic effect evaluations

Male and female white Wistar rats aged 6–8 weeks were used in our tests of the carcinogenic effect of the dusts whereas for pure antigorite dust tests both Balb C mice and rats were used.

The rats were intraperitoneally administered a single 20 mg dose of the dust suspended in 1.2 cm^3 of 0. 9% NaCl while the dose for the mice was 5 mg. The animals were left for survival. All organs from all deceased animals were taken for morphologic examination (3).

Mutagenicity effect evaluation

The ability of the dusts to induce mutagenic damage *in vivo* was evaluated by assessing the induction of sister chromatid exchanges (SCEs) in bone marrow cells of mice. In the SCE assay performed according to the method of Allen et al. (1) male Balb C mice, 8–10 weeks old were used.

The test dusts at doses of 5 mg/animal were administered i.p. to mice which had been subcutaneously implanted with a tablet of bromodeoxyuridine. Twenty three hours after the treatment with the test agents (and two hours after i.p. injection of Colcemid to inhibit mitotic division of cells) the mice were killed and standard bone marrow preparations were performed. Coded slides were prepared, differentially stained (2), and 50 second-generation metaphase cells per animal were analyzed for SCE.

RESULTS

Table 1 presents the results of the estimations of crystalline phases and respirable fibres content in 20 mg of the dust administered to the animals. From the data comprised in the Tables it can be seen that antigorite was found in 5 samples tested. Minerals of the amphibole group were found in two samples.

On the other hand neither serpentinite nor amphibole group minerals were found in the samples from the granite mine and from the coal mine. Nevertheless, these dusts caused the development of peritoneum mesothelioma. The respirable fibres content in 1 mg of the dust samples investigated differed even by as much as two orders of magnitude. The greatest fibre content was found in the sample of dust taken from the nickel ore mine which contained antigorite only (Fig. 1).

Table 1. The content of the crystalline phases and respirable fibres in dusts used for the experiments

Mine	Crystalline phases found	Content of respirable fibres in dust dose (20 mg) × 10^6
1	2	3
Nickel ore, Szklary (I)	antigorite, quartz	210.0
Serpentinite, Naslawice (II)	antigorite, quartz	18.0
Serpentinite, Glinica (III)	chlorite, amphibole, trydmite	116.0
Magnesite, Wiry (IV)	magnesite, talc, antigorite, serpentine	114.0
Magnesite, Grochow (V)	magnesite, talc, antigorite, chlorite, ripidolite	6.0
Gabbro, Braszowice (VI)	chlorite, ripidolite	24.0
Granite, Strzegom (VII)	biotite, quartz, orthoclase, microcline	0.4
Syenite, Pilawa (VIII)	muscovite, crocidolite, quartz, orthoclase	0.1
Dolomite Romanow (IX)	dolomite, antigorite	6.0
Coal, Nowa Ruda (X)	illite, kaolinite, quartz	2.8

Table 2 presents the results of the carcinogenic properties of the dusts. From the experiments on animals it has become evident that all dust samples taken from 10 different Lower Silesia mines of various mineral materials caused the development of neoplasm-peritoneum mesothelioma (Figs 2 and 3).

The incidence of peritoneum mesothelioma in animals exposed to dusts containing antigorite admixtures was close to (or higher than) those exposed to the UICC standard crocidolite (Table 2). Due to the high carcinogenic effect of the

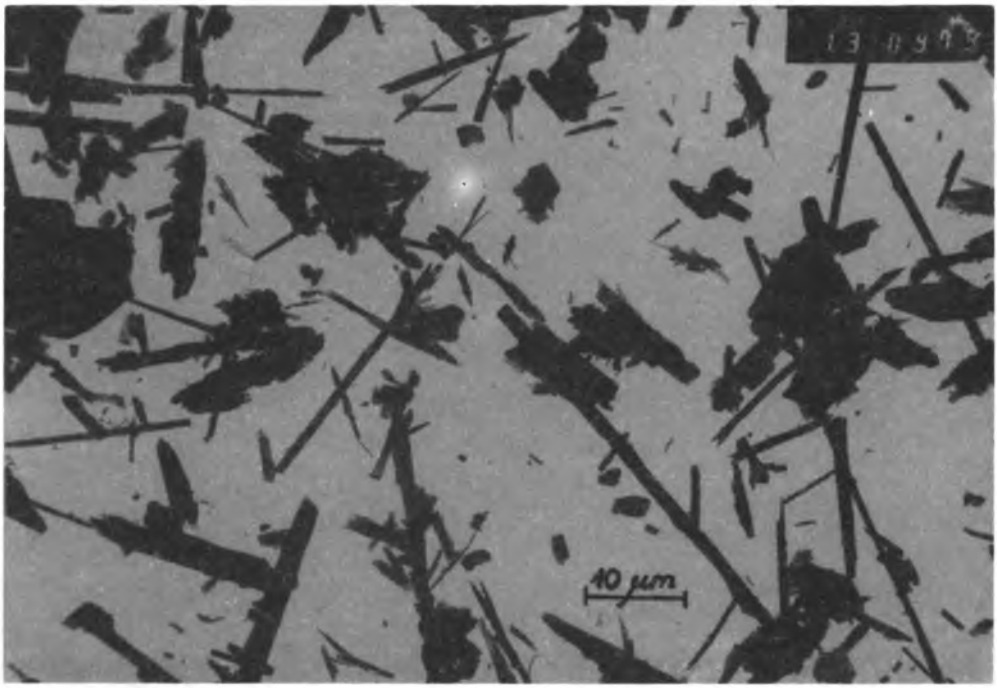

Fig. 1. Transmission electron micrograph of antigorite fibres in dust sample from nickel ore mine.

Table 2. The percentage of rats with tumors and survival time to the first tumor

Group	Number of rats	Percentage of animals with tumors			Time to first tumors (days)		
		T.	MT.	M.	B.	MT.	M.
1. Dust from mineral mines:							
– nickel ore (I)	45	80	80	77.8	–	238	238
– serpentinite (II)	76	30.3	18.4	13.1	404	298	513
– serpentinite (III)	60	44.1	30.5	8.5	443	354	354
– magnesite (IV)	41	48.8	36.6	29.3	582	462	462
– magnesite (V)	57	31.6	22.8	3.5	472	415	415
– gabbro (VI)	27	44.4	14.8	3.7	611	558	889
– granite (VII)	25	28.0	16.0	4.0	776	655	760
– syenite (VIII)	31	35.5	12.9	3.2	713	99	791
– dolomite (IX)	39	28.2	15.4	5.1	497	440	440
– coal (X)	28	46.4	10.7	3.6	146	325	892
2. Control group							
– NaCl	78	33.3	14.1	0.0	150	252	–
– chrysotile UICC	30	23.2	6.7	3.3	452	404	404
– crocidolite UICC	31	74.2	74.2	70.9	585	294	294

T – total number of tumors
MT – number of malignant tumors
M – mesotheliomas
B – benign tumors

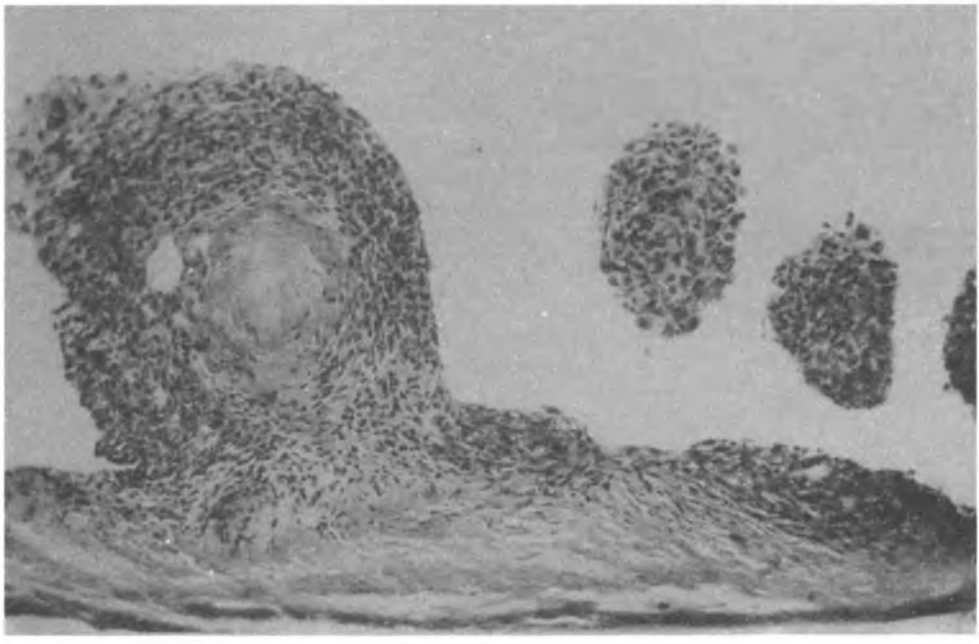

Fig. 2. Rat killed after 210 days since a single intraperitoneal injection of antigorite from nickel or deposit. Malignant epithelial and fusocellular mesothelioma of peritoneum. Hematoxylin and eosin staining. (Magn. 150x).

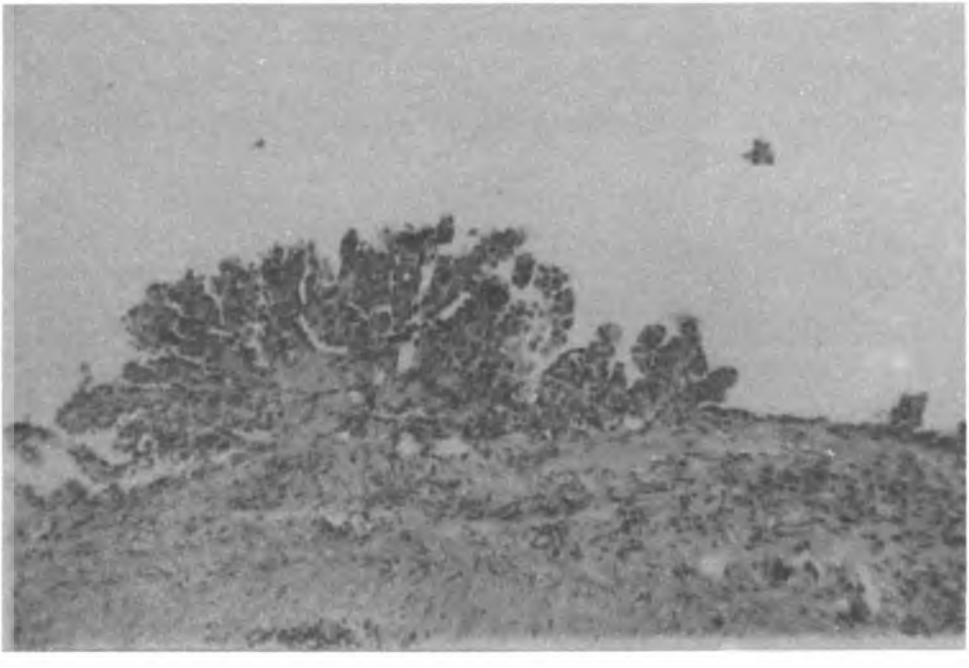

Fig. 3. Rat killed after 462 days since a single injection of magnesite from Wiry deposit. Malignant mesothelioma predominantely epithelial of peritoneum Hematoxylin and eosin staining. (Magn. 150x).

antigorite-containing dust, the latter was subject to additional examinations to confirm, by means of the SCE test, its mutagenic effect.

These additional examinations revealed that antigorite dust increased the frequency of the sister chromatid exchanges in the bone marrow of the mice as compared with the control (NaCl) group in a significant way. The frequency of the sister chromatid exchanges increased in proportion to dust dose and, the increasing number of the fibres contained in the dust (Table 3).

Table 3. Relationship between content of respirable fibres of antigorite in dust dose and Sister Chromatid Exchange (SCE) Frequency and number of mesothelioma in mice

Content of respirable fibres in the dust dose	Number of the mice with mesothelioma	SCE/cell X ± XSE
Control group – 0	0/29	4.66 ± 0.55
Antigorite 52.5×10^6	9/32	8.55 ± 1.38[x]
Control group – 0	0/29	3.95 ± 0.13
Antigorite 0.1×10^6	1/28	4.50 ± 0.33

X – arithmetic mean
SE – standard error
x – statistcally significant ($p < 0.01$)

CONCLUSIONS

The results of the tests allow the classification of the investigated samples with respect to their carcinogenic effect, into two groups:

1. antigorite-containing samples, with a carcinogenic effect similar to that of crocidolite, and

2. antigorite-free samples with a slight carcinogenic effect which is similar to that of chrystotile.

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