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ORIGINAL PAPERS

# FIBROGENIC AND CARCINOGENIC EFFECTS OF ANTIGORITE

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**Key words: Mineral fibrous dusts, Antigorite, Fibrogenic and carcinogenic activity**

**Abstract.** The deposits of nickel ore and some deposits of paving stones in Poland contain admixtures of fibrous minerals.
In experiment on animals (white rats) the fibrogenic and cancerogenic effects of three fibrous mineral dust samples taken from a nickel ore deposit, emitted from chimneys of a nickel metallurgical plant and taken from paving stone deposits were tested. A relationship was found between the number of antigorite fibres (fibrous mineral from serpentine group) and the fibro- and cancerogenic effect of the dust.
The coefficient of correlation for the relationship between the number of fibres and the hydroxyproline content in lungs equaled $r = 0.96$; and $r = 0.83$ for the relationship between lung weight and the content of fibres more than 5 µm long. All dust samples after intraperitoneal administration induced the development of mesothelioma — dust from the nickel ore deposit in 81.6% of animals; from paving stone deposits in 19% of animals and the dust emitted from the chimney in 2.8% of animals.

## INTRODUCTION

Up to this moment it has not been explicitly determined which of its physico-chemical properties result in asbestos' biological aggressiveness. As some examinations show (3, 8, 9, 10) the content of fibres of a specific size determines the biological effects of exposure to asbestos. In Poland, admixtures of fibrous minerals are to be found in the deposits of some raw materials.

The discovery of asbestosis and tumors of the respiratory system (12) in workers employed in the mining and processing of raw materials with admixtures of fibrous minerals made us determine to start examinations aimed at the identification of the fibrous minerals found in nickel ore and paving stone deposits and the evaluation of their fibrogenic and cancerogenic activity by means of experiments on animals.

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**MATERIAL AND METHOD**

**Dusts.** All dust samples used for biological examinations were obtained by grinding the examined minerals in an agate ball mill KM-1 and sifting them through a sieve with 75 μm mesh. In samples thus prepared, the following items were determined: the content of cristalline phases by X-ray and spectrometry IR methods (15), the content of some metals (Fe, Ni, Cu) by X-ray fluorescence spectrophotometry (17) and the content of free crystalline silica by the colorimetric method according to Woźniak et al (16). The number of fibres more than 5 μm long in the dust mass unit and also the form and size of the dust were determined using a transmission electron microscope and a phase contrast optical microscope (11).

**Fibrogenic activity.** Experimental pneumoconiosis was induced in white male Wistar rats (body weight 180—200 g) by bloodless intratracheal administration of 50 mg of dust suspended in 0.6 $cm^3$ 0.9% NaCl solution: Group I was given the antigorite from a nickel ore, desposit $A_{Ni}$; Group II — the antigorite collected from a paving stone mine $A_{St}$; Group III — the mixed dust emitted from the chimney of a nickel metallurgic plant $Ch_{Ni}$; Group IV — 0.6 $cm^3$ 0.9% solution NaCl. Each group consisted of 15 to 29 rats. After 3, 6 and 9 * months, the animals were killed and the following factors were evaluated:

a) quantitative changes — wet lung weight and hydroxyproline content in lungs;

b) qualitative changes — development of and morphological character changes in lungs and mediastinum lymph glands (POCM) and ultrastructure of tissue material (TEM).

**Carcinogenic activity.** Carcinogenic properties were examined in white male Wistar rats. The animals were given intraperitoneal injections of 20 mg dust suspended in 0.6 $cm^3$ 0.9% NaCl solution.

The groups consisted of 30—45 animals, 6—8 weeks old. Group V was given the antigorite collected from a nickel ore mine. Group VI — the dust emitted from a nickel metallurgic plant; Group VII — the antigorite collected from a paving stone mine: Group VIII was given only the NaCl physiological solution and was considered to be the control group. The animals were left to survive.

Once a month, the animals were weighed and their health was checked every day. Dead animals or those in bad health underwent an autopsy. For microscopic examinations, all the internal organs and a segment of the abdominal wall were collected. The kind and submicroscopic charac-

* group II and IV.

teristic features of tumor were determined on the basis of ultrastructural examinations using an electron microscope JAM-100C by JOEL.

Some animals were anesthetized through exsanguination from the ophtalmic vein, blood was used to determine alpha$_1$ — antitripsin (7) and Superoxide Dismutase SOD by a method based on the hampering of the luminal chemiluminescence process by SOD (6) in the ferricyanideluminol system.

## RESULTS

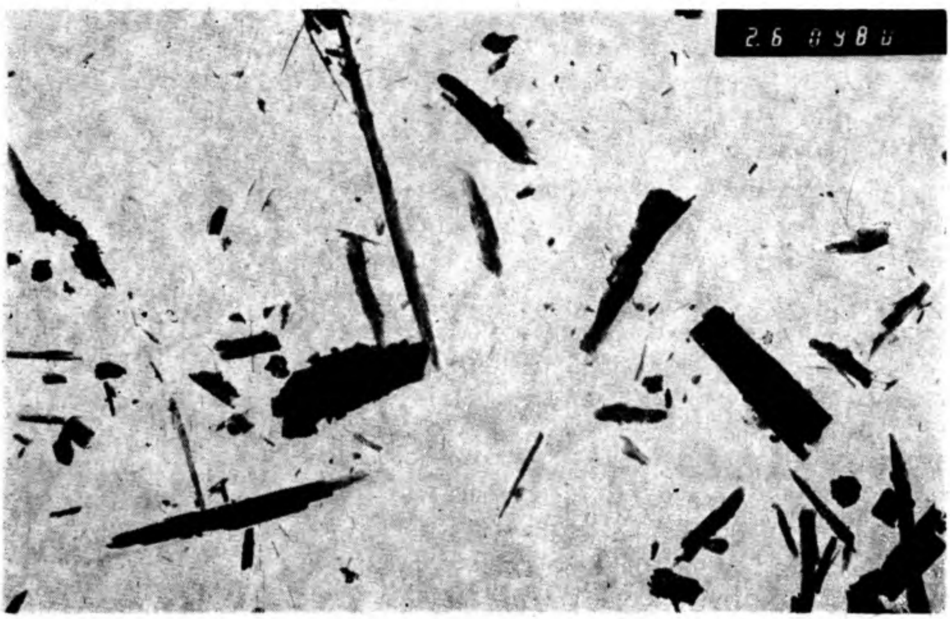
The physico-chemical properties of the examined dust samples is presented in Table 1. The pictures from the electron microscope of the examined dusts are presented in Fig. 1. Antigorite constituted the main crystalline phasis in the dusts from nickel ore and paving stone mines, while free crystalline silica in the form of alpha-quartz was a small admixture of about 2%. The dust emitted from the chimney was a mixture of antophyllite, quartz, antigorite, chrysotile and chamosite; free crystalline, silica in the from of alpha-quartz or crystobalite constituted about 7.8%. The dust emitted from the chimney contained about 3 times more iron and nickel than antigorite from the nickel ore deposit, and about 5 times more than the antigorite from the paving stone deposit. The smallest number of fibres more than 5 μm long were found in the dust emitted from the chimney and equaled $0.08 \times 10^6$fb.

TABLE 1. Physico-chemical Characteristics of Dusts

| Property | Dust | | |
|---|---|---|---|
| | $A_{Ni}$ | $Ch_{Ni}$ | $A_{St}$ |
| Crystalline phases | antigorite | antophyllite<br>quartz<br>antigorite<br>chrysotile<br>chamosite | antigorite<br>quartz |
| Metal content (%) | | | |
| Fe | 1.98 | 4.75 | 1.37 |
| Cu | 0.04 | — | — |
| Ni | 0.15 | 0.48 | 0.10 |
| Number of fibres (length $\geqslant 5$ μm) in 1 mg of dust | $10.5 \cdot 10^6$ | $0.08 \cdot 10^6$ | $0.9 \cdot 10^6$ |
| Crystalline silica $SiO_2$ content (%) | 2.3 | 7.8 | 2.0 |

In Table 2, the size distribution of the examined dust samples is presented. In the sample of dust emitted from the chimney, 64,8% of the

a)



b)

Fig. 1. Transmission electron micrograph of antigorite fibres in dust sample from nickel ore mine (a), paving stone mine (b). Total magnification 5200 X.

TABLE 2. Size Distribution of Fibres

| Fibre diameter μm | Fibre length μm < 1.06 % | 1.07—2.07 % | 2.08—5.03 % | 5.04—10.5 % | 10.6—15.08 % | > 15.08 % |
|---|---|---|---|---|---|---|
| < 0.18 | | | | | | |
| $A_{Ni}$ | 3.70 | 20.70 | 6.10 | 0.43 | — | — |
| $Ch_{Ni}$ | 24.30 | 21.60 | — | — | — | — |
| $A_{St}$ | 33.80 | 13.70 | 5.90 | — | — | — |
| 0.19—0.35 | | | | | | |
| $A_{Ni}$ | 0.47 | 14.50 | 15.40 | 4.20 | 0.47 | — |
| $Ch_{Ni}$ | — | 10.80 | 2.70 | — | — | — |
| $A_{St}$ | 0.98 | 8.30 | 9.80 | 0.49 | 0.49 | — |
| 0.36—0.53 | | | | | | |
| $A_{Ni}$ | — | 0.47 | 14.0 | 6.5 | 1.87 | — |
| $Ch_{Ni}$ | — | 5.40 | 5.40 | 5.40 | — | — |
| $A_{St}$ | — | 1.96 | 5.90 | 1.96 | 0.49 | — |
| > 0.53 | | | | | | |
| $A_{Ni}$ | — | 7.00 | 8.40 | 2.30 | 1.93 | — |
| $Ch_{Ni}$ | — | 2.70 | 13.50 | 5.40 | — | — |
| $A_{St}$ | — | 0.49 | 8.90 | 5.90 | 0.98 | — |

fibres did not exceed the length of 2.07 μm, while 45.9% of fibres were less than 0.18 μm in diameter. In the sample collected from the paving stone mine — 53.4% did not exceed a length of 5 μm and a diameter of 0.18 μm. The sample collected from the nickel ore mine contained the longest fibres — 4.3% of fibres were more than 10 μm long while fibres of 0.19—0.35 μm in diameter occurred most frequently.

The comparison of the most characteristic indices of the fibrogenic activity indices for UICC chrysotile and crocidolite are presented in Table 3.

It can be concluded from the data presented in Table 3 that the values of the examined fibrogenic activity indices obtained in the group of antigorite from the nickel ore mine did not differ from the values obtained under the effect of crocidolite and chrysotile. However, the antigorite sample collected from the paving stone deposits was weaker than the antigorite sample from the nickel ore deposit, despite the similar content of free crystalline silica in the dust of both antigorite samples. What differentiated the two antigorite samples was the content of fibres more than 5 μg long in 1 mg of dust (Table 1).

The values of coefficients of correlation between the indices of fibrogenic activity wet lung weight, and hydroxyproline content in lungs and the number of antigorite fibres in dust were high and equaled $r = 0.96$ for the relationship between the number of fibres and hydroxyproline content and r = 0.83 for the relationship between the lung weight and the content of fibres more than 5 μm long (Table 3).

It can be concluded, on the basis of the presented data, that there is a relationship between the period necessary to induce a definite degree of lung fibrosis and the number of fibres in the dust. The same hydroxyproline content in lungs, as in the case of antigorite samples containing about a $40 \times 10^6$ fibres dose, could be obtained in 3 times shorter the time by a 10-fold increase of the number of fibres in the sample dust.

The results of examinations on the carcinogenic effect are presented in Table 4. In Table 4, the number of tumors found in particular periods of the experiment are given. In the control group, tumors were found in 10 animals (24.3%), while 2 rats developed 2 kinds of tumors, one rat had leydigiomia testis, the other one nesidioma, and both developed lymphoma malignum pulmonary.

The first tumor in the control group (Group VIII) was found after 619 days of the experiment. All the examined dust samples caused the

TABLE 3. Comparison of the Values of Fibrogenic Effect Indices of Antigorite Dust with Chrysotile Dust and the Control Group

| Group | Survival time months | 95% confidence interval of geometric mean | | | |
|---|---|---|---|---|---|
| | | Wet lungs weight mg | | Hydroxyproline content in lungs mg | |
| | | LLCT | ULCI | LLCI | ULCI |
| $A_{Ni}$ | 3 | 2905.71 | 3436.33 | 7.91 | 9.68 |
| | 6 | 3157.20 | 3444.60 | 9.17 | 10.15 |
| $Ch_{Ni}$ | 3 | 1677.49 | 2392.62 | 2.80 | 3.63 |
| | 6 | 1707.16 | 2314.02 | 4.00 | 5.81 |
| $A_{St}$ | 3 | 1721.90 | 2093.96 | 4.62 | 5.73 |
| | 6 | 1787.25 | 2507.06 | 5.20 | 6.49 |
| | 9 | 2311.37 | 3584.43 | 7.31 | 10.04 |
| 0.9% NaCl | 3 | 1425.60 | 1749.80 | 3.35 | 3.93 |
| | 6 | 1473.30 | 2122.81 | 3.51 | 4.07 |
| | 9 | 1598.80 | 1854.00 | 3.89 | 4.41 |
| Chrysotile B | 3 | 1812.67 | 3182.86 | 6.21 | 10.11 |
| UJCC * | 6 | 2455.62 | 3814.92 | 7.72 | 12.31 |
| Crocidolite | 3 | 1052.91 | 2686.23 | 6.69 | 8.75 |
| UJCC * | 6 | 2480.47 | 3968.34 | 7.71 | 11.49 |

LLCI — Lower Limit of Confidence Interval
ULCI — Upper Limit of Confidence Interval
* Woźniak and Więcek, 1985

TABLE 4. Number of Tumors Found in Particular Periods of the Experiment and Number of Days which Passed before the First Tumor was Discovered

| Indices | Group | | | |
|---|---|---|---|---|
| | $^{V}A_{Ni}$ | $^{VI}Ch_{Ni}$ | $^{VII}A_{St}$ | $^{VIII}NaCl$ |
| Number of examined rats in the group | 38 | 68 | 42 | 41 |
| Number of tumors: | | | | |
| total | 32 | 10 | 15 | 12 |
| benign | 0 | 6 | 5 | 6 |
| mesothelioma | 31 | 2 | 8 | 0 |
| other malignant | 1 | 2 | 2 | 6 |
| Time to first tumor days | 238 | 588 | 298 | 619 |
| Number of tumors found in particular period of the experiments (days) | | | | |
| 201—300 | 7 | 0 | 1 | — |
| 301—400 | 14 | 0 | 0 | — |
| 401—500 | 7 | 0 | 0 | — |
| 501—600 | 4 | 2 | 4 | — |
| 601—700 | x | 0 | 6 | 2 |
| 701—800 | — | 4 | 5 | 4 |
| | — | 4 | x | 6 |
| Percentage of rats with tumors | 84.2 | 13.9 | 35.7 | 24.3 |
| Percentage of rats with mesothelioma | 81.6 | 2.8 | 19.0 | 0 |
| Number of fibres above 5 μm length in the dust × dose $10^6$ | 210.0 | 1.6 | 18.0 | 0 |

x all rats died

development of mesothelioma (Fig. 2 and 3) and in Group V mesothelioma developed in 81.6% of the animals, and there was one case of lymphoma malignum pulmonum (2.6%) found in this group, 22% of tumors developed during the period from 238 to 298 days, the last rat from this group died after 553 days of the experiment.

In Group VI, two cases of mesothelioma were found on the 742 day of the experiment and the first tumor developed after 588 days of experiment with carcinoma pulmonum. In Group VII, 16 tumors were found altogether including 8 mesothelioma cases; the first case of mesothelioma came after 513 days of the experiment.

Comparing the results of examinations in Group V with the results obtained in Group VII, it can be concluded that there is a relationship between the number of fibres more than 5 μm long and the carcinogenic effect of antigorite.

An 11-fold larger number of fibres in the Group V dust, when compared with the number of fibres in the dust administered in Group

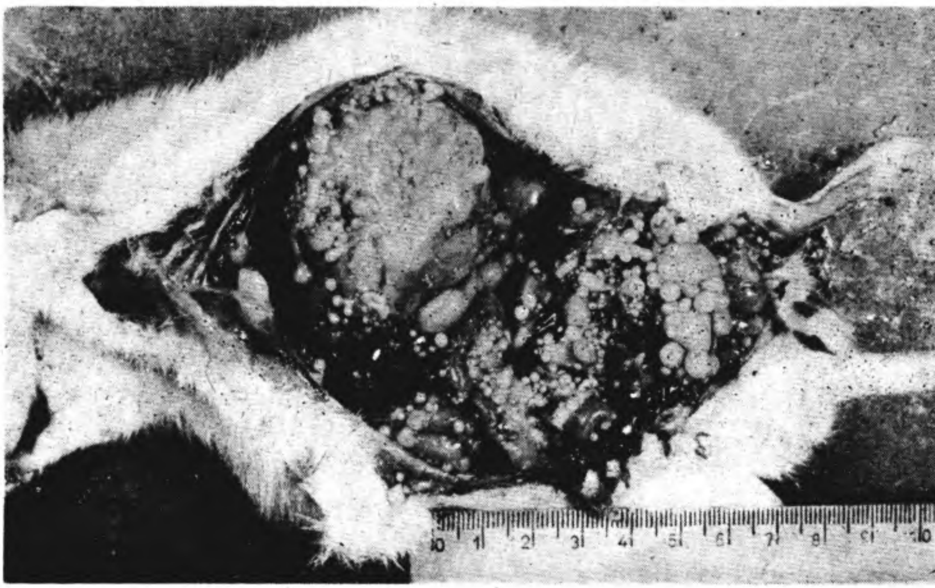


Fig. 2. Rat sacrificed on the 398th day after intraperitoneal administration of $A_{Ni}$ dust. On the surface of viseral and parietal peritoneum a great number of neoplasmic nodules is seen. Macrophotography.

VII, caused a biological response in the form of a 4-fold increase of mesothelioma cases in the animals. Simultaneously with the increase of the number of fibres, a two-fold decrease of the time which passed before the first mesothelioma case was found, took place. Nevertheless, the content of the metal admixtures did not have a decisive impact on the carcinogenic properties. Our examinations also show that antigorite may

TABLE 5. SOD Alpha-1 Antitripsin Activity

| | Group | Number of rats | Geometric mean | 95% confidence interval of geometric mean |
|---|---|---|---|---|
| SOD activity | Control NaCl without tumor | 42 | 66.4 | 47.3— 93.3 |
| | Control NaCl — animals with spontaneous tumors | 7 | 169.4 | 133.5—214.8 |
| | Antigorite — all rats with mesothelioma | 8 | 331.3 | 304.9—359.5 |
| Alpha-1 antitripsin activity | Control NaCl * | 18 | 2.08 | 2.02—2.13 |
| | $A_{Ni}$ * | 10 | 1.75 | 1.63—1.87 |
| | $Ch_{Ni}$ | 12 | 1.94 | 1.93—1.96 |

* 3 months after intratracheal administration 0.6 $cm^3$ NaCl or 50 mg of the dust

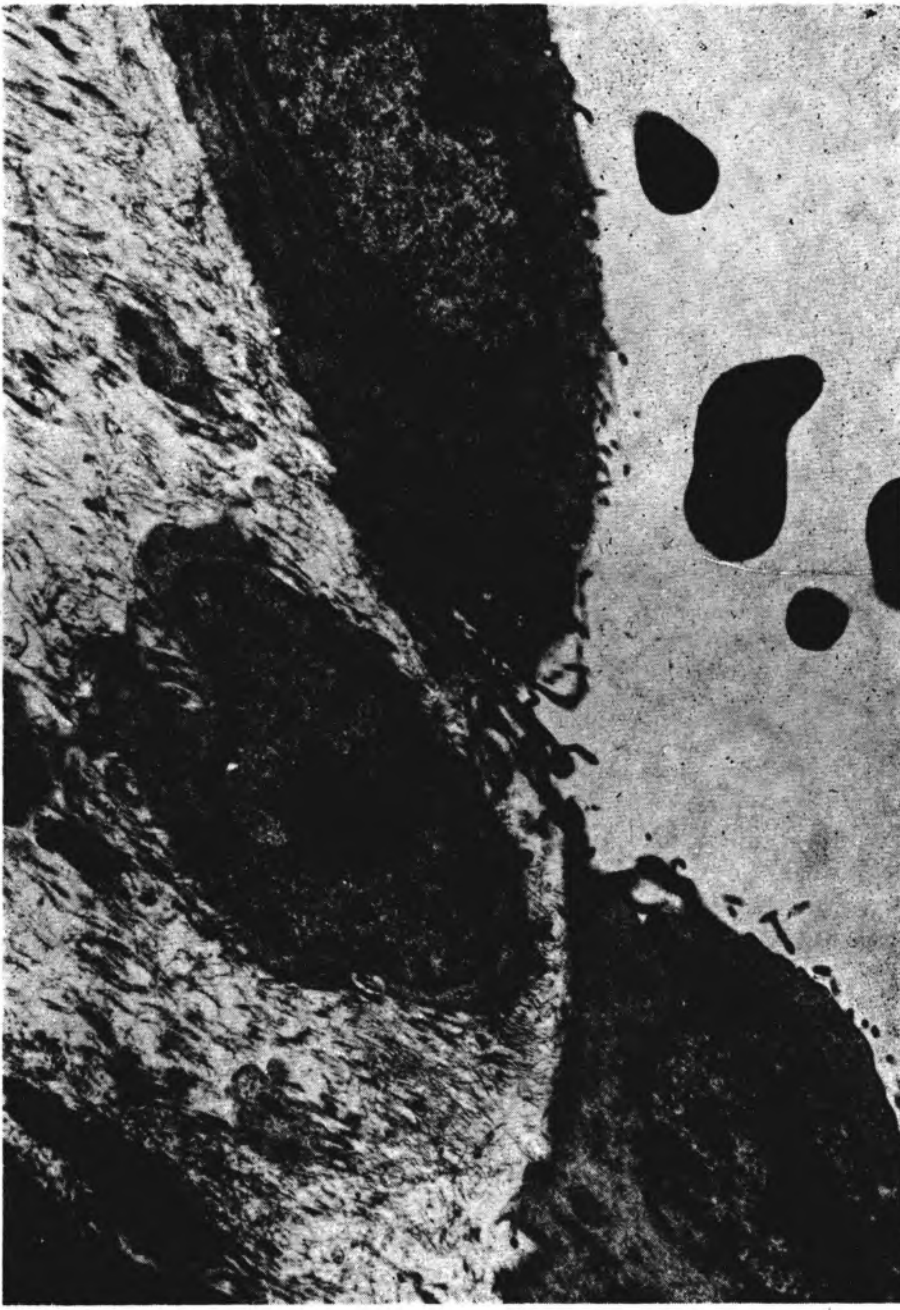


Fig. 3. Rat sacrificed on the 398th day after intraperitoneal administration of $A_{Ni}$. Ultrastructure of mesothelioma Fusiforme mesothelial neoplastic cell with a number of microvilli is seen. This cell produced the basal membrane. Magnification 6600 X.

have an effect on the activity of some enzymes such as alpha$_1$-antitrypsin and superoxide dismutase SOD (Table 5), which may result in the disturbance of some metabolic processes in the organism. It can be seen from the data presented in Table 6 that the activity of SOD increased in animals with a tumor, especially in animals with mesothelioma. Because of the small group of animals examinations are considered to be preliminary.

## DISCUSSION

The correlation between the size of fibrous dusts and their biological effect can be found in the results from, among others, the experiments of Stanton (10), Harrington (3), Oshimura et al (9), although, as the latest studies of Wagner (14) and Kolev (5) show, such a correlation cannot be determined for all kinds of fibres.

The results we have obtained differ from the data of other authors (4,2). In their examinations, they used non-fibrous antigorite and their results show a considerably weaker biological aggressiveness of antigorite as compared with chrysotile. Therefore, it seems that the difference between the results we obtained and those of the above quoted authors confirm the conclusion regarding the extremely important role of the fibrous structure of antigorite and thereby the number of fibres of a special length, in the biological aggressiveness of this mineral.

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