Polish Journal of Occupational Medicine and Environmental Health Vol. 4, No. 1, 021-031, 1991

ORIGINAL PAPERS

THE FIBROGENIC ACTIVITY AND NEUROTOXICITY OF HEAT-TREATED CHRYSOTILE

HELENA WOŹNIAK, EDWARD WIĘCEK and GRAŻYNA BIELICHOWSKA-CYBULA

Department of Aerosols, Institute of Occupational Medicine, Lodz, Poland

Key words: Asbestos, Chrysotile, Thermal degradation, Fibrogenic activity, Neurotoxicity

Abstract. Thermal degradation of heated chrysotile results in dehydration and changes in its crystalline structure. The impact of heat treatment at 150–1200°C on the biological activity of chrysotile was tested in rats. Heating the chrysotile produced an increase in its biological aggressiveness measured in terms of animal survival rate and fibrogenic activity after intratracheal administration of the dust. The highest death rate (100% of the animals) was noted after administration of chrysotile heated at 600°C. Moreover, increased fibrogenic activity of chrysotile heated at 150°C up to 800°C was found. The biological effect of chrysotile heated at 1200°C did not differ from the effect exerted by unheated chrysotile. After intraperitoneal administration of the dust, the most violent reaction could be observed when chrysotile dust was heated at 600°C, which resulted in symptoms of nervous system impairment (of the hind legs, no reaction to nociceptive stimuli, drop of internal body temperature) and death of the test animals. In male rats, the period between dust administration and the manifestation of symptoms and death was found to be longer than in females.

INTRODUCTION

Because of its physical and chemical properties, asbestos is used for manufacturing various kinds of products used under conditions of high temperatures and pressure, and also in contact with acids, alkalies, solvents, etc. (Table 1). When heated, chrysotile undergoes thermal degradation resulting in dehydration (2) and changes in its crystalline structure (Table 2), which in turn may cause changes in the biological activity of chrysotile. This study was aimed at examining the biological effect of chrysotile exposed to temperatures most frequently occurring during the utilisation of asbestos product.

Address reprint requests to H. Woźniak, Department of Aerosols, Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

TABLE 1. Usage of some asbestos products.

Type of asbestos	Name of product	Usage	Temperature to which the product is exposed °C
Chrysotile	asbestos wool	heat insulation for water and steam boilers, pipes and heat exchangers	up to 500°
Chrysotile	asbestos yarn	semi-product for the production of cords, tapes, fabric, packings and unwoven asbestos cloth	from 260° up to 450°
Crocidolite or Chrysotile	asbestos cardboard	packings in the environment of gas, acids and lyes, acid-proof board, thermal insulation, fireproof cardboards	from 150° up to 600°
Chrysotile	cardboards	fireproof cardboards	about 1000°
Chrysotile	asbestos frictional products	for the production of brakes, clutches, brake bands	from 200° up to 400°

TABLE 2. Scheme of thermal changes of chrysotile due to dynamic heating (2).

Chrysotile with surplus of water and brucite	$Mg_6Si_4O_{10}(OH)_8 \cdot xH_2O \cdot yMg(OH)_2$
	$-\ xH_2O$; $100 - 250°C$ ↓
Brucite dehydration	$Mg_6SIO_4O_{10}(OH)_8 \cdot yMg(OH)_2$
	$-\ xH_2O$; $400°C$ ↓
Chrysotile dehydration	$Mg_6SiO_4O_{10}(OH)_8 + (yMgO)$ →
	$-\ 4H_2O$; $600 - 780°C$ ↓
Dehydrated chrysotile	Mg_6SiO_{14}
	$800°C$ ↓
Forsterite, amorphous silica	$3MgSiO_4 + SiO_2$ →
	$1000°C$ ↓
Forsterite, enstatite	$2MgSiO_4 + Mg_2SiO_6$

MATERIALS AND METHODS

Dust: The sample of chrysotile asbestos imported from the USSR and processed in Poland was divided into 6 portions. Five of them were exposed for 24 hours to one of the following temperatures: 150, 450, 600, 800, 1200°C, respectively; the remaining sample was left unheated (9). Next, all the samples of dust were subjected to x-ray difraction (8) and microscopic examination (6).

Animals: White Wistar rats of both sexes were used for biological testing; 150 male rats were used for testing fibrogenic properties; the dust samples were administered intratracheally at a single dose of 25 or 50 mg suspended in 0.6 cc of 0.9% NaCl solution. Each group consisted of 20 rats. After 3 months the animals were killed and the hydroxyproline content in the lungs was measured (11). Sixty rats of both sexes were used for testing neurotoxic properties; 100 mg chrysotile suspended in 1.2 cc of 0.9% NaCl solution was administered intraperitoneally, and the animals' behaviour was observed and analyzed according to the following criteria: external features, mode of locomotion, balance on an inclined plane, the grasping reflex of the front and hind limbs, reaction to nociceptive stimulus, mode of solid and liquid food intake, internal body temperature control, macroscopic lesions of internal organs (10). All the dead animals were dissected. The control group was given only the NaCl physiological solution.

RESULTS

Dust characteristics. Crystalline phases and respirable fibres content in unheated and heated chrysotile are presented in Table 3. It is to be noticed that

TABLE 3. Crystalline phases and respirable fibres content in chrysotile.

Temperature °C	Crystaline phases	Asbestos fibre content in 1 mg dust
20	chrysotile	10×10^6
150	chrysotile	10×10^6
450	chrysotile	10×10^6
600	chrysotile*	10×10^6
700	amorphous	
800	forsterite	12×10^6
900	forsterite	
1200	forsterite + enstatite	1.5×10^6

*20% of the sample preserves crystalline strusture
+ 80% converted into amorphous substance

the asbestos samples unheated and heated at 150, 450 and 600°C contain only $Mg_6Si_4O_{10}(OH)_8 \times H_2O$ chrysotile. Twenty-four hour heat-treatment of chry-

Fig. 1. Electron micrograph of the chrysotile unheated.

Fig. 2. Electron micrograph of the chrysotile heated at 600°C.

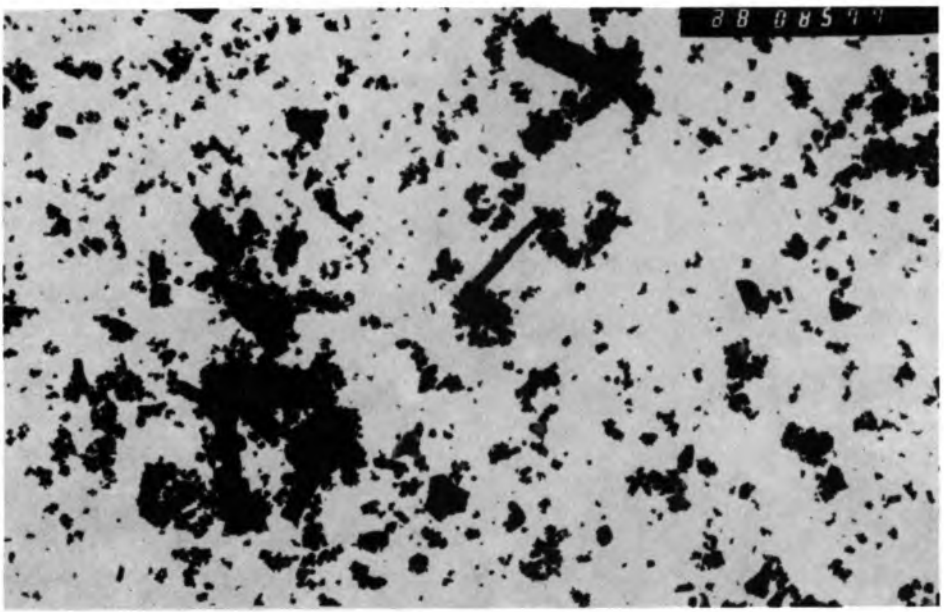


Fig. 3. Electron micrograph of the chrysotile heated at 1200°C.

sotile at 800 and 1200°C converts it into forsterite. However, different samples of chrysotile varied with respect to the number of fibres in 1 mg of dust; the lowest number of fibres was found in a chrysotile sample heated at 1200°C. The electron microscope pictures of the chrysotile unheated and then heated at 600°C and 1200°C,,respectively are presented in Figs 1, 2 and 3.

Biological examination

1. The first reaction of animals to unheated and heated chrysotile administered intratracheally.

a. Reaction to unheated dust. All animals tolerated the intratracheally administered standardized dose (950 mg) of dust.

b. Reaction to dust heated at different temperatures:

- at 150°C – 20% of rats died during the first day of experiment: during a month the mortality rate of the animals reached 50% of the initial number of rats in the group;
- at 450°C – 100% of rats died immediatelly after intratracheal administration of 50 mg of dust suspension, whereas the dust sample reduced to a half dose (25 mg) was well tolerated by the animals;
- at 600°C – 100% of animals died immediately at the moment of administering 50 mg of dust into trachea, 50% reduction of the dose did not reduce the mortality rate of the animals;
- at 800°C – the reaction of animals was approximately the same as that in the group exposed to dust heated at 450°C;
- at 1200°C – administration of 50 mg dose did not cause any deaths.

The fatal reaction of rats to the dust heated at 600°C administered intratracheally suggests that heating chrysotile at this temperature is conductive to the formation of soluble compounds with a strong toxic effect on the respiratory tract; it caused the death of animals at the moment of intratracheal administration of dust suspension. However, the result of the experiment carried out to verify the hypothesis on the formation of soluble toxic compounds was negative. The experiment consisted of preparing a standard suspension of the dust heated at 600°C (50 mg in 0.6 cc of 0.9% NaCl) and shaking it for 24 hours, then centrifuging it; the supernatant was removed to another glass tube and the residue was re-suspended. Rats were given either the supernatant or the re-suspended residue. Only the residue exhibited a lethal effect.

2. Fibrogenic activity. The results of studies on the fibrogenic activity of the examined dust marked with a hydroxyproline content in the lungs are presented in Fig. 4. It can be concluded, from the data presented in the Figure, that heating dust at the temperatures from 150°C to 800°C increases its fibrogenic activities, whereas, as can be concluded from this studies, the dust heated at 450°C has the highest fibrogenic activity; half of the standard dose (25 mg) of this dust induced a higher increase of hydroxyproline content in the lungs than the full dose (50 mg) of unheated dust, or dust heated at 1200°C. The fibrogenic activity of dust heated at 1200°C was approximate to the fibrogenic activity of unheated dust.

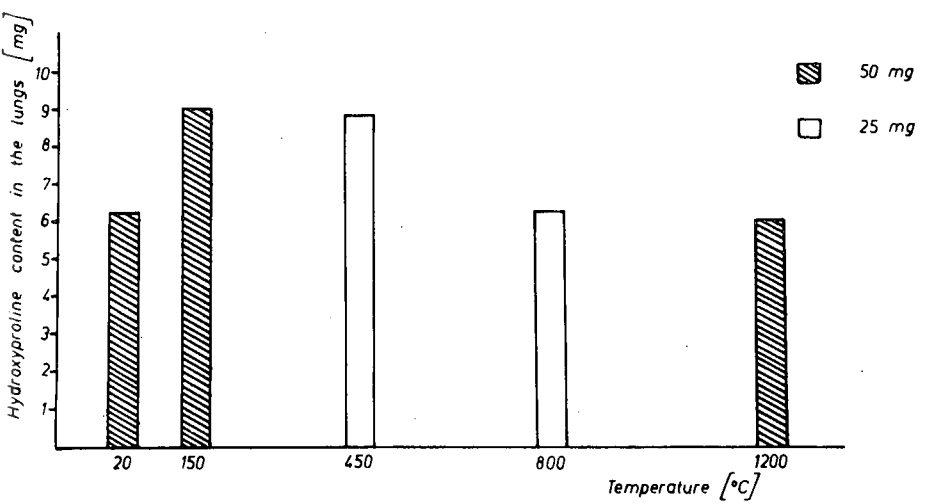


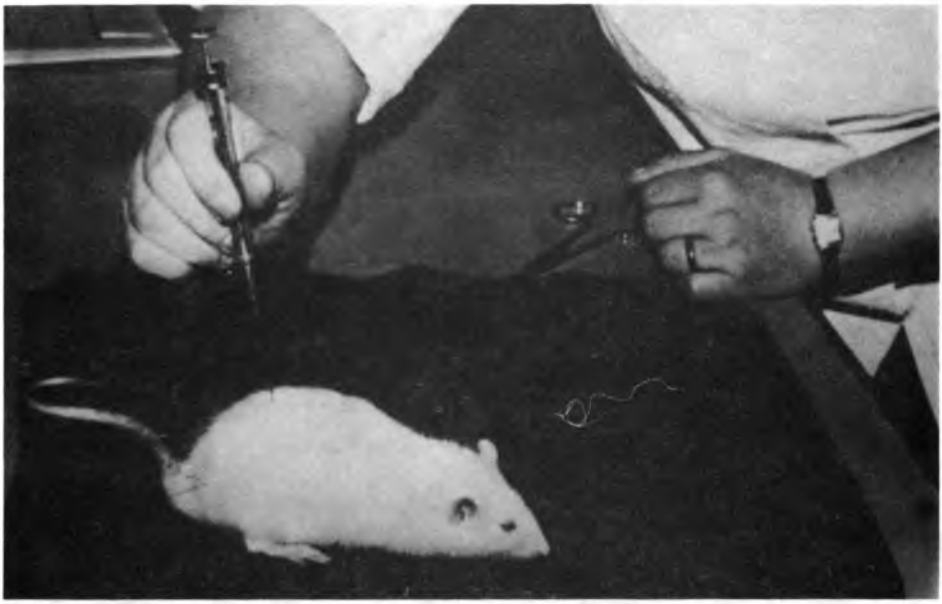
Fig. 4. Fibrogenic activity of the examined dust marked with hydroxyproline content in the lungs.

3. The neurotoxic effect of chrysotile dust. The results of examinations aimed at finding out the neurotoxic effect of chrysotile are shown in Table 4. No abnormal behaviour was to be observed in the rats from the control group (1.2 cc 0.9% NaCl). Within 3 hours of intraperitoneal introduction of unheated chrysotile, rats developed such symptoms as: apathy, erected hair, balance disturbances manifested by swerving and dragging of the

TABLE 4. Some reactions of rats after the intraperitoneal administration of heated chrysotile dust.

Temp. (°C)	Time between dust administration and disorders development (in minutes)							
	Apathy and hair erection	Dragging the hind limbs	Grasping reflex inhibition	Negative reaction to nociceptive stimuli	Difficulty in change of body position	Maximum body temperature decrease	Death	Withdrawal of all distemper
20	10–17	All symptoms were slightly manifested from 80 to 180 minutes			no			10 hr
150	15–25	125	88–90	120	no	196–235 (0.7°C)	*	4 days
450	15	170	178	168	190	180 (3°C)	no	5 days
600	immediately	immediately	25**–35	20**–23	23**–45	40**–420 (9.2°C) (4.5°C)	45**–3360	no
800	10–15	80	50	no	no	180 (1.6°C)	no	4 days
control (1.2 cm^3 saline)	no	no	no	no	no	no	no	10 min

*One out of five rats died on 8th day
**Time reaction of female rats



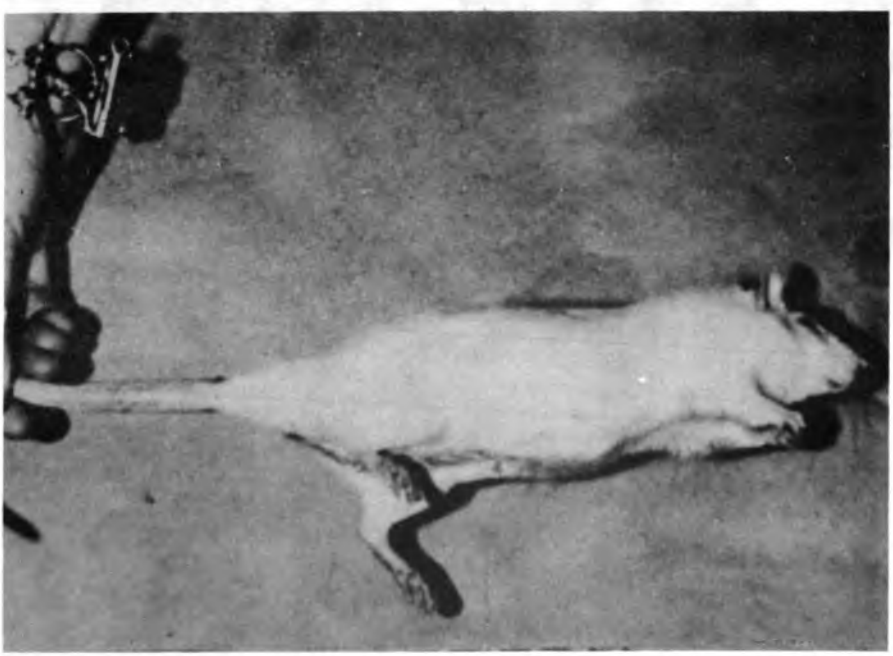


Fig. 5. Reaction of rats after intraperitoneal administration of heated chrysotile dust.

hind limbs, intermittent 20 minute phases of convulsions involving licking the treated sides accompanied by streightening out the hind limbs. In spite of these abnormalities the animals could keep their balance on an inclined plane. All symptoms were rather slightly manifested. Throughout the 10-hour experiment, reaction to nociceptive stimuli as well as the grasping reflex of the front and hind limbs were retained.

It can be concluded from the data presented in Table 2 that heating dust at temperatures from 150°C to 800°C increases its neurotoxic effect marked by the time between dust administration and the appearance of disturbance. The most violent reaction was to be observed in the case where chrysotile dust was heated at 600°C. Male rats developed such symptoms as apathy and erected hair immediately after intraperitoneal administration of chrysotile dust heated at 600°C. Within 1 hour, voluntary food intake ceased. Then cheek swelling occurred as well as 5–11 second convulsions recurring every 4 minutes. The animals developed also locomotor disturbances involving limb asthenia and dragging of the hind limbs. The animals slid off an inclined plane. It was found that the reaction to nociceptive stimuli within the cervical region of the spine was weaker compared to the respective reaction in the group exposed to unheated dust. Within another hour a negative reaction to nociceptive stimuli affected the whole spine, except for its cervical part. The animals' skin lost its elasticity and they assumed the so-called "seal position" (placid paralysis) (Fig. 5.)

The swallowing reflex was inhibited. The animals were given, by stomach tube, water with glucose (2.5 cc) to drink. During the sixth hour of the experiments a 4–8°C drop in body temperature was recorded. Next, the animals were placed on a heated plate. They exhibited difficulty in changing body position, lying on their sides or backs. Within the seventh hour, one case of cyanosis of natural body cavities and mucosa was recorded, and the body temperature of the rat dropped from 35°C (when the experiment started) to 25.8°C, followed by death within the eighth hour after chrysotile dust administration. Two days after the beginning of the experiment, four animals died. In females, a more violent reaction to the dust was observed as compared to male rats. The period between dust administration and development of the symptoms, as displayed in Table 2, amounted to 13 minutes after chrysotile administration. Impaired function of the hind limbs, manifested by grasping reflex disturbances, was found. Within another seven minutes a 3.5°C drop of internal body temperature occurred, as well as difficulty in changing body position in the animals placed on their sides or backs. 30 minutes after chrysotile administration, the reaction to pricking was inhibited within the whole spinal area. Within another 13 minutes, a further 1°C body temperature drop was observed and 2 minutes later, i.e. 45 minutes after chrysotile administration, the animals died. There were some insignificant differences in reaction time in particular animals. They ranged from 2 to 3 minutes. Dissection revealed that the macroscopic picture of the internal organs of the dead animals exposed to heated dust was similar in all rats. Dust clusters were observed in the intestines, mucosa, stomach, lipid tissue and the renal area. Moreover, blood vessels in the mesentery and large intestine were overfilled with blood and the lungs similarly congested. The experiment

indicates that heat-treatment of chrysotile results in its increased biological aggressiveness.

There are a few reports in the literature on the impact of temperature on abestos biological action. Szymczykiewicz (7), after administering chrysotile (heated at over 800°C) to guinea pigs, found a small decrease in fibrogenic activities as compared to unheated chrysotile.

Robock and Klostekötter (5) found that chrysotile after heating at 600 and 700°C was much more cytotoxic than unheated. On the basis of the examinations of absorption spectra (in infrared) they also found that, at these temperatures, a total destruction of the primary lattice took place. Koshi et al. (4) and Hayashi (1) after heating chrysotile at the temperature of about 650°C, found a considerable increase in its cytotoxicity and hemolytical properties. In the case of intraperitoneal administration of chrysotile heated at the temperature of 650°C to mice, the animals died within 48 hours after dust injection. Koshi et al., similarly as Robock and Klosterkötter, consider the changes of the biological properties of heated chrysotile to be due to changes of the crystalline structure of chrysotile in the process of its transformation into forsterite.

Juntunen et al. (3) found neurological changes of peripheral origin in workers exposed to asbestos as well as a certain CNS impairment. The incidence of neuropathy in the workers exposed was higher than in those from the reference group. The results of our studies provide evidence on the increased harmfulness of heated chrysotile as compared to that of unheated one. The products obtained at the temperature of 600°C were the most harmful, including the death of experimental animals in a short time after lung or peritoneal administration. The abnormal behaviour of the animals indicates that heated chrysotile produces irreversible nervous system impairment. The changes in the biological effect of chrysotile heated at different temperatures are undoubtedly due to the changes in its crystalline structure. It seems indispensable therefore to embark on a study aimed at explaining the higher aggressiveness of heat-treated asbestos as well as determining the range of temperatures at which the increased biological aggressiveness of chrysotile can be observed and, finally, determining the minimum dose of heated chrysotile that produces nervous system disorders.

ACKNOWLEDGEMENTS

The research was supported by the CPBR project 11.5 Cancer Control.

REFERENCES

1. Hayashi H. Cytotoxicity of heated chrysotile. Environ Health Perspect 9, 267–270, 1974.
2. Jolicoeur C, Duchesne D. Infrared and thermogravimetric studies of thermal degradation of chrysotile asbestos fibres evidence for matrix effects. Can J Chem 59, 1521–1526, 1981.
3. Juntunen J, Huuskonen M S, Matikainen F et al. Asbestosis, the nervous system and cancer. Ann US Acad of Med 13, 353–360, 1984.

4. Koshi K, Hayashi M, Sakabe H. Biological and mineralogical studies in serpentine minerals in heat treated state. Industrial Health 7, 66–85, 1969.
5. Robock K, Klosterkötter W. Untersuchungen über die Zytotoxizität von Azbest-Stäuben. Staub-Reinhalt Suft 33, 279–282, 1973.
6. Stroszejn-Mrowca G, Więcek E. Evaluation of exposure of workers to asbestos dust in asbestos processing plants Arch Immunol Therap Experiment 263–268, 1982.
7. Szymczykiewicz K. Some problems of the etiology and pathogenesis of asbestosis. Med Pracy 19, 152–173, 1968 (in Polish).
8. Więcek E. Identification of crystalline phases and the content of certain metals in fibrous minerals ("Polish Asbestos") accompanying nickel ore deposits. Med Pracy 30, 235–244, 1983 (in Polish).
9. Woźniak H, Więcek E. Biological effects of fibrous mineral dusts. I. Fibrogenic effects of chrysotile thermal degradation products. Med Pracy 36, 345–353, 1985 (in Polish).
10. Woźniak H, Więcek E, Owecka A. Biological effects of fibrous mineral dusts. Med Pracy 37, 139–150, 1986 (in Polish).
11. Woźniak H, Więcek E, Stetkiewicz J. Fibrogenic and carcinogenic effects of antigorite. Pol J Occup Med 1, 192–202, 1988.

Received for publication: November 15, 1990
Accepted for publication: March 29, 1991