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First experimental evidence of peritoneal and pleural mesotheliomas induced by fluoro-edenite fibres present in Etnean volcanic material from Biancavilla (Sicily, Italy)^(a)

Prima dimostrazione sperimentale di mesoteliomi peritoneali e pleurici indotti da fibre fluoro-edenitiche presenti nel materiale vulcanico etneo di Biancavilla (Sicilia)^(a)

Morando Soffritti, Franco Minardi, Luciano Bua, Davide Degli Esposti, Fiorella Belpoggi
Cancer Research Centre, European Ramazzini Foundation of Oncology and Environmental Sciences, Bologna, Italy

Summary

A total of 17 cases of pleural mesothelioma were ascertained in the town of Biancavilla (Sicily, Italy), on the south-west slopes of the volcano Etna, from 1980 to 1997. Occupational exposure to asbestos was considered possible in only 2 of them, though it could not be excluded in 5 other cases: for the remaining 10 cases the hypothesis of environmental exposure was deemed reasonable. On the basis of this evaluation, a mineralogic study was started in the area of Biancavilla, where incohesive volcanic material has largely been used in the building industry and road paving since the 1960s-1970s. This study led to the identification of the new fluoro-edenite amphibole with prismatic, acicular and fibrous asbestiform morphologies. With a view to acquiring more detailed information on the possible causal relationship between exposure to this mineral and the onset of mesotheliomas, the mineral was tested, by a single intraperitoneal or intrapleural injection, on groups of 80 Sprague-Dawley rats (40 males and 40 females) (fibrous fluoro-edenite) and on a group of 30 rats (15 males and 15 females) (powdered prismatic fluoro-edenite), 8 weeks old at the start of the study, at a dose of 25 mg in 1 cc of water; 1 cc of water was administered by intraperitoneal injection to 80 rats (controls). One hundred and nine weeks after the treatment, a high incidence of peritoneal and, to a lesser extent, pleural mesotheliomas was observed in the animals treated with fibrous fluoro-edenite; this incidence is comparable to that obtained with various kinds of asbestos. Up to now no cases of mesothelioma have been observed in the group treated with prismatic fluoro-edenite, or in the control group. Eur. J. Oncol., 9 (3), 169-175, 2004

Key words: asbestiform amphiboles, fluoro-edenite, lava stone, carcinogenicity, mesothelioma, rat

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Address/Indirizzo: Dr. Morando Soffritti, Centro di Ricerca sul Cancro, Fondazione Europea di Oncologia e Scienze Ambientali "B. Ramazzini", Castello di Bentivoglio, 40010 Bentivoglio (BO), Italia
Tel. 0039/051/6640460 - Fax 0039/051/6640223 - E-mail: crcfr@tin.it

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Riassunto

Nella città di Biancavilla (Sicilia), situata sul versante sud-occidentale dell'Etna, dal 1980 al 1997 sono stati accertati complessivamente 17 casi di mesotelioma pleurico. Un'esposizione professionale ad amianto è stata considerata possibile in 2 soli casi e non la si è potuta escludere in altri 5: per i restanti 10 casi si è ritenuta ragionevole l'ipotesi di un'esposizione ambientale. Sulla base di questa valutazione fu iniziato uno studio mineralogico nell'area di Biancavilla, località in cui materiale vulcanico incoerente è stato ampiamente utilizzato in edilizia e nella pavimentazione di strade a partire dagli anni 1960-1970. Questo studio ha portato all'identificazione del nuovo anfibolo con morfologia asbestiforme, prismatico, aciculare e fibroso: la fluoro-edenite. Al fine di acquisire più dettagliate informazioni sulla possibile relazione causale tra esposizione a questo materiale ed insorgenza di mesoteliomi, il minerale è stato studiato mediante una singola iniezione intraperitoneale o intrapleurica su gruppi di 80 ratti Sprague-Dawley (40 maschi e 40 femmine) (fluoro-edenite fibrosa) e su un gruppo di 30 ratti (15 maschi e 15 femmine) (fluoro-edenite prismatico polverizzata), di 8 settimane di età all'inizio dell'esperimento, alla dose di 25 mg in 1 cc di acqua; 1 cc di acqua è stato somministrato per via intraperitoneale a 80 ratti (controlli). Dopo 109 settimane dal trattamento è stata osservata un'alta incidenza di mesoteliomi peritoneali e in misura minore pleurici negli animali trattati con fluoro-edenite fibrosa; questa incidenza è simile a quella osservata con diversi tipi di amianto. Nessun caso di mesotelioma è stato finora osservato nel gruppo trattato con fluoro-edenite prismatico e nel gruppo di controllo. Eur. J. Oncol., 9 (3), 169-175, 2004

Parole chiave: anfiboli asbestiformi, fluoro-edenite, roccia lava, cancerogenicità, mesotelioma, ratto

Introduction

In the framework of a national survey on mortality from pleural mesothelioma in Italy, a cluster of cases was detected in Biancavilla (Sicily, Italy), a town of about 23,000 inhabitants situated on the south-western slope of the volcano Etna (fig. 1), where 4 cases of pleural mesothelioma were observed in the years 1980-1993^{1,2}. In the period 1988-92¹, the expected cases in Biancavilla, according to data based on the resident population in Sicily, were 0.9 (SMR 417; 95% CI 142-954).

After this preliminary finding, an epidemiological study was started, which led to identification of a total of 17 cases (10 males and 7 females) of pleural mesothelioma among the inhabitants of the town in the years 1980-1997². This study indicated that for 2 of these cases asbestos exposure was possible and in 5 more cases such exposure could not be completely excluded. The remaining 10 cases could not be explained by known exposure to asbestos and it was postulated that the source might be in the general environment³.

A careful mineralogical investigation was duly started in the area of Biancavilla, where incohesive volcanic materials have been widely used for the building industry and for road paving, particularly in the 1960s and 1970s.

The studies carried out detected prismatic, acicular and fibrous amphiboles in the quarry material⁴. The prismatic crystals were later identified as fluoro-edenite $[\text{NaCa}_2\text{Mg}_5\text{Si}_7\text{AlO}_{22}(\text{OH},\text{F})_2]$, a new end-member of the calcium amphibole group, whose mineralogical and crystal-chemical data were reported on by Gianfagna and Oberti⁵. The fluoro-edenite of Biancavilla is a calcium amphibole with a high fluorine content (4% by weight), of transpar-

ent and intense yellow colour. Recently Gianfagna *et al.*⁶ reported mineralogical data (based on optical, chemical and X ray investigations) obtained from asbestiform amphiboles from Biancavilla and showed them to be closely related to the associated prismatic and acicular fluoro-edenite. These fibres, which are 0.5-1 μm in thickness and more than 10 μm in length, are intimately mixed with microcrystals of feldspar, pyroxene, and Fe-Ti oxides.

The Ramazzini Foundation (RF) cancer research programme, aimed at identifying carcinogenic agents of industrial and environmental origin, began a large, integrated project of long-term bioassays on fibrous materials at the end of 1970s.

Among the materials studied, there are many types of asbestos of different origin, various types of natural (sedimentary and hydrothermal) zeolites including erionite, several synthetic zeolites, and other organic, natural and man-made solid compounds, such as silica, alumina, talc, kaolin, man-made mineral fibres, propylene fibres, etc. The aim of the project is not only to verify the carcinogenicity of the compounds, but also to provide information regarding their relative carcinogenic potency. For this purpose all the compounds are tested under the same experimental conditions.

In order to acquire more detailed information on the relationship between exposure to fluoro-edenite compounds present in the area of Biancavilla and the onset of mesothelioma, an experimental study was started in our laboratory in 2002. The experimental plan and the preliminary results, 109 weeks after the start of the experiment, are presented in this report.

Materials and methods

Two samples of fluoro-edenite, fibrous and prismatic (powdered), were supplied by Dr. Gianfagna (Department of Earth Sciences, "La Sapienza" University, Rome).

Male and female Sprague-Dawley rats from the colony of the Cancer Research Centre (CRC) of the RF were used. These rats have been experimentally used in the Bentivoglio (BT) laboratory for the last 30 years. Data are available on more than 15,000 historical controls, kept under observation for their life span and submitted to systematic necropsy and standardised histopathological examination. Data are therefore available on the expected incidence of the different types of tumours and their fluctuations in control animals. Mesotheliomas are extremely rare (about 1%) among the untreated rats of this colony.

After weaning, at 4-5 weeks of age, the experimental animals were identified by ear punch, randomised in order to have no more than one male and one female of each litter in the same group, and housed 5 per cage. The animals were 8 weeks old at the start of the experiment.

The fibrous fluoro-edenite was administered to groups of 80 rats (40 males and 40 females) by intraperitoneal or intrapleural injection, *una tantum*, at the dose of 25 mg suspended in 1 cc of water. The powdered prismatic fluoro-edenite was administered to 30 rats (15 males and 15 females) by intraperitoneal injection, *una tantum*, at the dose of 25 mg suspended in 1 cc of water. A group of 80 rats (40 males and 40 females), injected with water alone, serves as controls. The plan of the experiment is presented in the Table 1.

The animals were kept under observation until spontaneous death. In order to detect all gross lesions, they are clinically con-



Fig. 1. Location of the town of Biancavilla, south-west of the volcano Etna.

Table 1 - Long-term carcinogenicity bioassay on fibrous^(a) and powdered prismatic fluoro-edenite, administered *una tantum* to 8-week-old male (M) and female (F) Sprague-Dawley rats (Exp. BT 2117). Plan of the experiment.

Group	Material	Dose (mg/cc H ₂ O)	Route of administration	Animals	
				Sex	No.
I	Fibrous fluoro-edenite	25	Intraperitoneal injection	M	40
				F	40
				M+F	80
II	Fibrous fluoro-edenite	25	Intraleural injection	M	40
				F	40
				M+F	80
III	Powdered prismatic fluoro-edenite	25	Intraperitoneal injection	M	15
				F	15
				M+F	30
IV	H ₂ O (control)	0	Intraperitoneal injection	M	40
				F	40
				M+F	80
Total					270

^(a) Material containing 25-30% fibrous fluoro-edenite, feldspars, haematite and pyroxenes (from Gianfagna *et al*⁶)

trolled every 2 weeks, and weighed every 4 weeks. All gross changes are registered. Animal status and behaviour are examined 3 times daily.

A systematic necropsy is performed on each animal. Histopathological examination is carried out on the tissue at the site of injection, and on the brain, thymus, lung, heart, liver, spleen, pancreas, kidneys, adrenal glands, stomach, uterus, gonads, subcutaneous, mediastinal and mesenteric lymph nodes, and any other organs with pathological changes. All organs and tissues are preserved in 70% ethyl alcohol. The specimens are trimmed following the standard procedures of the BT laboratory, and then processed as paraffin blocks; 3-5 µm sections of every specimen are obtained and routinely stained with haematoxylin-eosin.

Results

The preliminary results of the study are shown in Table 2.

After 109 weeks of observation following treatment, the 80 animals treated by intraperitoneal injection had all died. Among them, 66 cases of mesothelioma (37 in males and 29 in females) were observed, with an average latency time of 63.7 weeks (the first case was observed in a male rat 36 weeks after the injection).

In the same period, 70 of the 80 animals treated by intraleural injection died. Among them, 10 cases of pleural mesothelioma (4 in males and 6 in females) were observed with an average latency time of 72.1 weeks (the first case was observed in a male rat 51 weeks after the injection).

Table 2 - Long-term carcinogenicity bioassay on fibrous and powdered prismatic fluoro-edenite administered *una tantum* to 8-week-old male (M) and female (F) Sprague-Dawley rats (Exp. BT 2117). Preliminary results 109 weeks after treatment.

Group	Material	Dose (mg/cc H ₂ O)	Route of administration	Animals			Mesotheliomas		
				Sex	No.	Deceased	No.	% ^(a)	Average latency (wks)
I	Fibrous fluoro-edenite	25	Intraperitoneal injection	M	40	40	37	92.5	61.6
				F	40	40	29	72.5	66.4
				M+F	80	80	66	82.5	63.7
II	Fibrous fluoro-edenite	25	Intraleural injection	M	40	37	4	10.8	71.0
				F	40	33	6	18.2	72.8
				M+F	80	70	10	14.3	72.1
III	Powdered prismatic fluoro-edenite	25	Intraperitoneal injection	M	15	13	0	-	-
				F	15	13	0	-	-
				M+F	30	26	0	-	-
IV	H ₂ O (control)	0	Intraperitoneal injection	M	40	33	0	-	-
				F	40	32	0	-	-
				M+F	80	65	0	-	-

^(a) Percentage referring to deceased animals

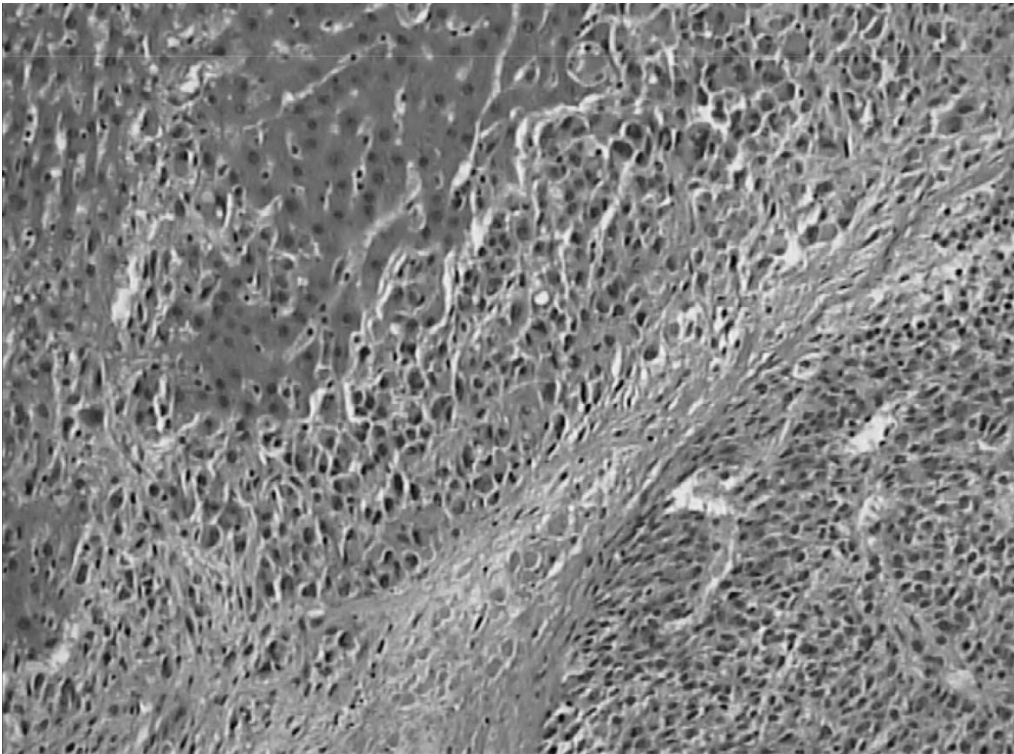


Fig. 2. Peritoneal mesothelioma with mixed pattern (epithelial solid and spindle cell). H.E. x200

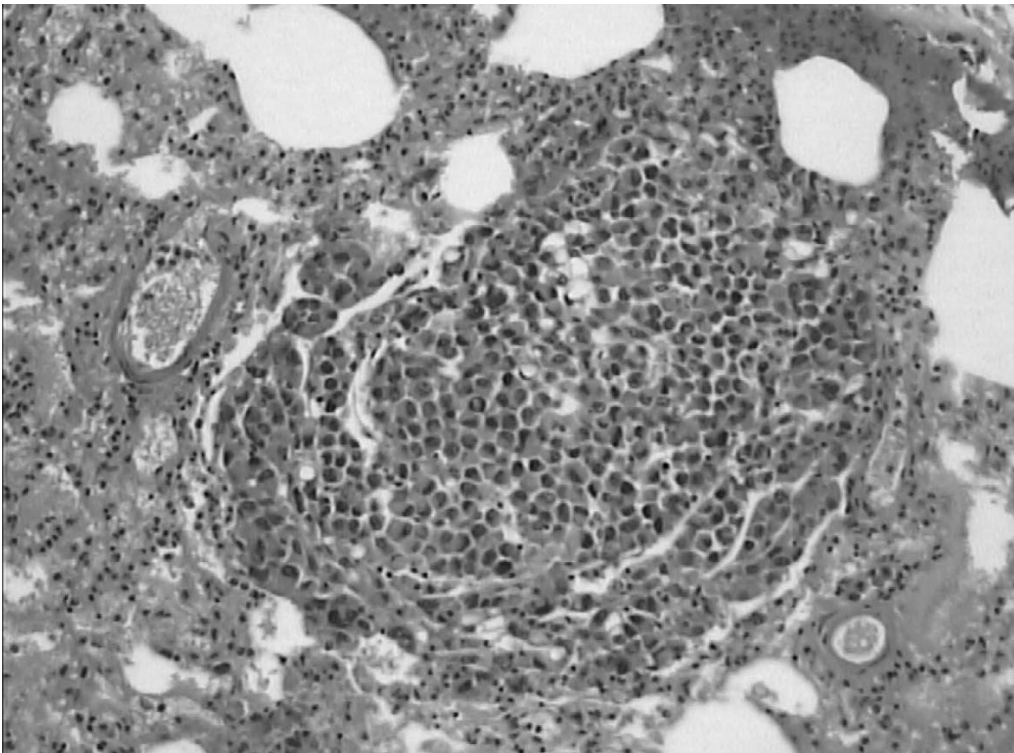


Fig. 3. Same case as fig. 2. Metastasis in the lung. H.E. x200

No cases of mesothelioma were observed among the 26 rats treated with powdered prismatic fluoro-edenite or among the 65 control animals that have so far died.

The peritoneal tumours usually involve the whole abdominal cavity, with whitish and yellowish tissue on the surface of all the organs. A serosal effusion is present in almost all cases.

The pleural tumours involve the visceral and/or parietal pleura. In 8 of the 10 mesothelioma cases the diaphragm is largely involved, with subsequent extension of the tumour into the peritoneal cavity.

Microscopically, the mesotheliomas are polymorphic, with a predominance of epithelial-solid and spindle cell patterns (figs. 2-

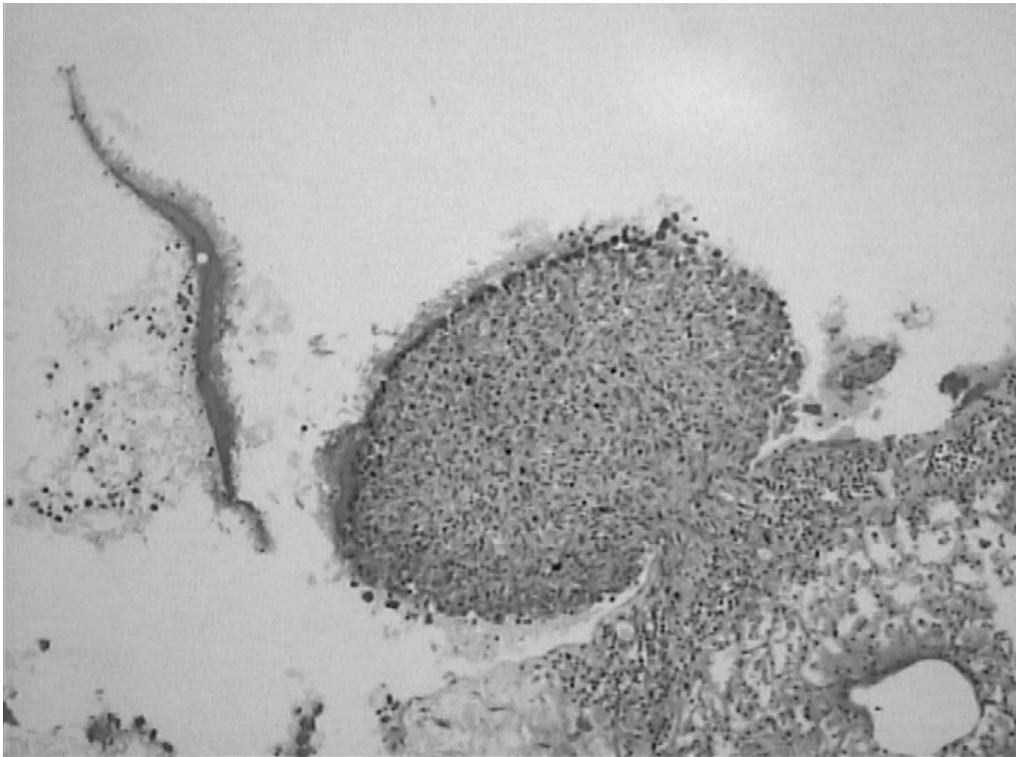


Fig. 4. Pleural mesothelioma with mixed pattern (epithelial solid and spindle cell). H.E. x100

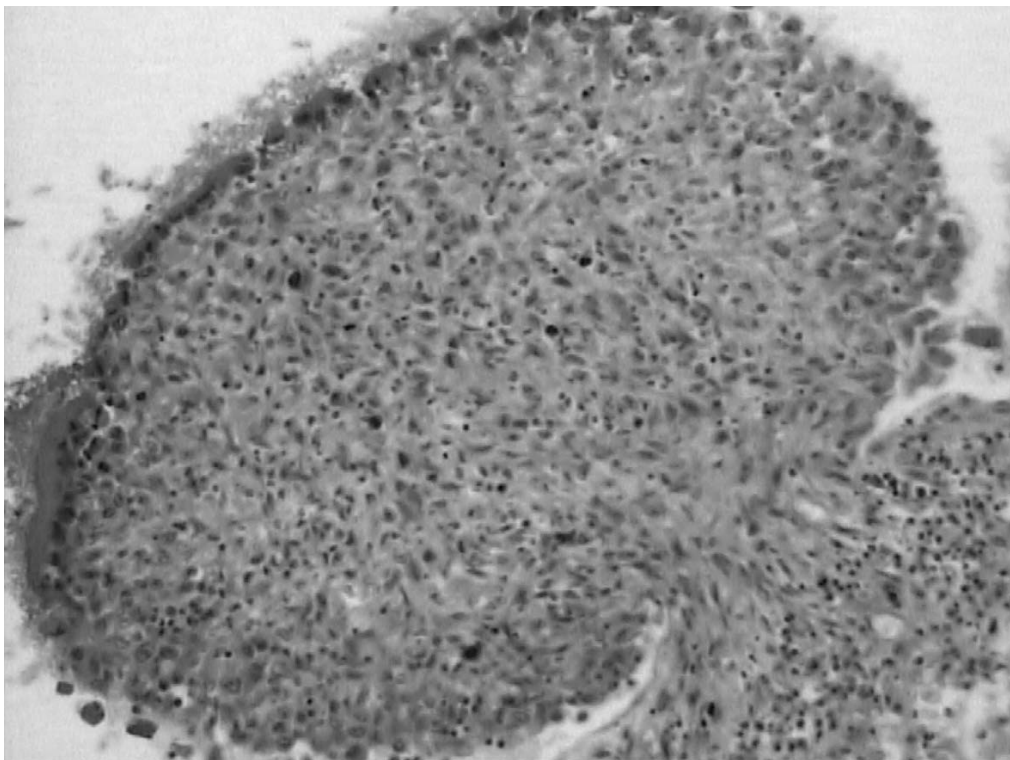


Fig. 5. Same case as fig. 4. H.E. x200

5). Metastases of the peritoneal tumours are localized in the mediastinal lymph nodes, mediastinum, lungs (fig. 3), pleura and pericardium. The presence of fibres has been detected in the neoplasms, as shown in fig. 6.

Conclusions

In our experimental conditions, fibrous fluoro-edenite has shown a strong mesotheliomatogenic effect on the peritoneum

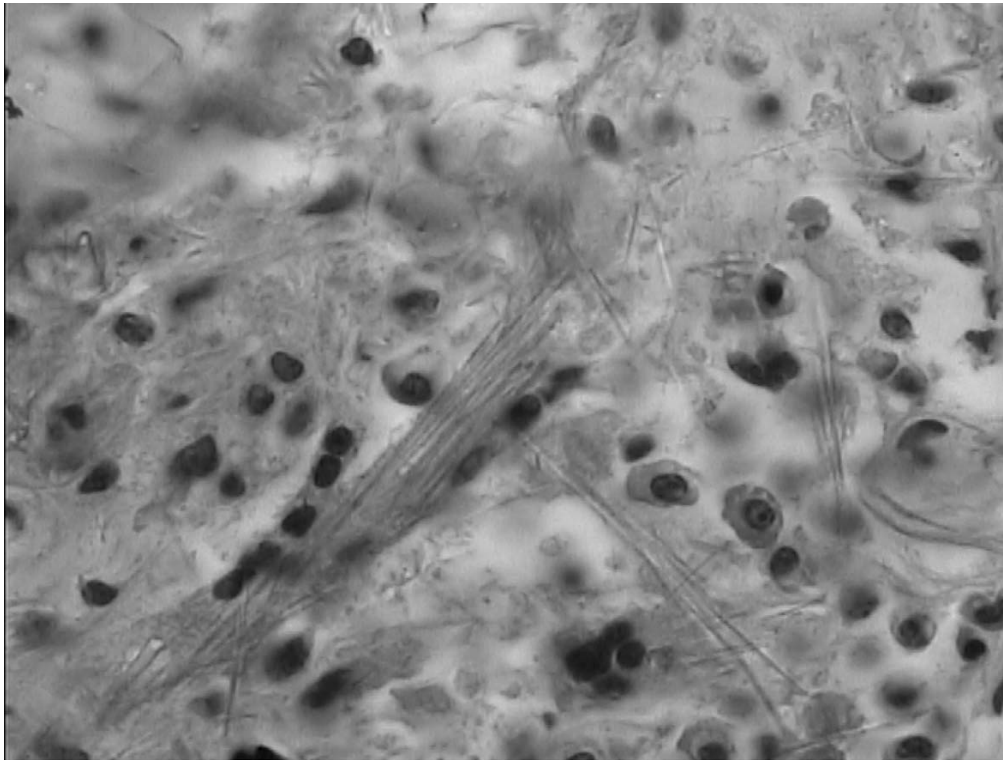


Fig. 6. Fluoro-edenite fibres in a pleural neoplastic nodule. H.E. x1000

Table 3 - Comparative incidence and latency time of *peritoneal* mesotheliomas after a single injection of different kinds of fibrous material and fibrous fluoro-edenite in male and female Sprague-Dawley rats

Material	Mesotheliomas		
	%	Average latency (weeks)	Latency of the first tumour (weeks)
Crocidolite	97.5	59.5	29
Amosite	90.0	66.7	44
Anthrophyllite	87.5	73.3	18 ^(a)
Chrysotile (Rhodesia)	82.5	89.7	48
Fibrous fluoro-edenite	82.5	63.7	36
Chrysotile (Canada)	80.0	92.2	65
Chrysotile (California)	72.5	85.3	39
Asbestos-cement	52.5	99.7	60
Erionite	50.0	106.1	67
Glass fibres	42.5	90.4	65
Ceramic fibres	32.5	84.9	58
Rock wool	10.0	78.7	69
Kevlar fibres	0	-	-

^(a) Microscopic finding in a rat deceased for another cause (phlogosis), not related to the injected agent

Table 4 - Comparative incidence and latency time of *pleural* mesotheliomas after a single injection of different kinds of fibrous material and fibrous fluoro-edenite in male and female Sprague-Dawley rats

Material	Mesotheliomas		
	%	Average latency (weeks)	Latency of the first tumour (weeks)
Erionite	87.5	64.2	32
Chrysotile (Canada)	65.0	111.1	80
Crocidolite	45.0	104.8	33
Asbestos-cement	35.0	113.1	82
Fibrous fluoro-edenite ^(a)	14.3 ^(b)	72.1	51

^(a)Ten animals out of 80 are still alive

^(b)Percentage referring to deceased animals

brous materials, such as natural and man-made zeolites, which have proved to have limited or no tumorigenic effect.

These results support the need for local administrators and public health officials at Biancavilla to take urgent preventive measures on behalf of the inhabitants.

References

1. Di Paola M, Mastrantonio M, Carboni M, *et al.* La mortalità per tumore maligno della pleura in Italia negli anni 1988-1992. Roma: Istituto Superiore di Sanità, Rapporti ISTISAN 96/40, 1996.
2. Paoletti L, Battisti D, Bruno C, *et al.* Unusually high incidence of malignant pleural mesothelioma in a town of eastern Sicily: an epidemiological and environmental study. Arch Environ Health 2000; 55: 392-8.
3. Pasetto R, Bruni B, Bruno C, *et al.* Problematiche sanitarie della fibra anfibolica di Biancavilla. Aspetti epidemiologici, clinici e sperimentali. Notiziario Istituto Superiore di Sanità 2004; 17(1): 8-12.

and to a much lesser extent on the pleura, which in humans seems to be the main target organ⁷.

This effect, indicated by tumour incidence as well as latency time, is comparable with that of various types of fibrous materials, tested in our laboratory under the same experimental conditions⁸ (Tables 3, 4).

The lack of any mesotheliomatogenic effect by powdered prismatic fluoro-edenite is not surprising in the light of the results of the experiments performed in our laboratory on a series of non-fi-

4. Gianfagna A, Paoletti L, Ventura P. Segnalazione di fibre di amianto anfibolico nei prodotti lavici metasomatizzati di Monte Calvario, Biancavilla (Sicilia orientale). *Plinius*, suppl. *Eur J Mineral* 1997; 18: 117-9.
5. Gianfagna A, Oberti R. Fluoro-edenite from Biancavilla (Catania, Sicily, Italy): crystal chemistry of a new amphibole end-member. *Am Mineral* 2001; 86: 1489-93.
6. Gianfagna A, Ballirano P, Bellatreccia F, *et al.* Characterization of amphibole fibres linked to mesothelioma in the area of Biancavilla, Eastern Sicily, Italy. *Mineralogical Magazine* 2003; 67(6): 1221-9.
7. Comba P, Gianfagna A, Paoletti L. Pleural mesothelioma cases in Biancavilla are related a new fluoro-edenite fibrous amphibole. *Arch Environ Health* 2003; 58: 229-32.
8. Maltoni C, Minardi F. Recent results of carcinogenicity bioassays of fibres and other particulate materials. In Bignon J, Peto J, Saracci R. *Non-occupational exposure to mineral fibres*. Lyon: IARC Scientific Publications No. 90, 1989, 46-53.

