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COMMENTARY

The fibres with fluoro-edenitic composition in Biancavilla (Sicily, Italy): health impact and clues for environmental remediation

Pietro Comba^(a), Salvatore Scondotto^(b) and Loredana Musmeci^(a)

(a) Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy

(b) Dipartimento Attività Sanitarie e Osservatorio Epidemiologico della Regione Sicilia, Palermo, Italy

Abstract

Subsequently to the observation of a localized excess of mortality from malignant pleural neoplasms in the town of Biancavilla (Sicily), previously unknown amphibolic fibres with fluoro-edenitic composition were detected as naturally occurring soil contaminants. Less than two years after the initial report, ISS provided a set of public health recommendations that were complied by regional and local institutions. The recognition of Biancavilla as a National Priority Contaminated Site in 2002 opened the way to clean-up interventions. An up-dating of epidemiological studies, exposure assessment investigations and *in vivo* and *in vitro* mechanistic studies on fluoro-edenite fibres is provided in this issue. Scientific evidence can provide a sound foundation to public health action and environmental remediation. Finally, it is now necessary to properly tune the response of the health system to the community needs in terms of diagnostic procedures and medical treatment.

Key words

- fluoro-edenite
- mesothelioma
- asbestiform fibre exposure
- environmental clean-up

Environmental health requires a multidisciplinary approach apt to integrate exposure science, epidemiological investigations and mechanistic studies in order to throw light on the role of the environment in the causation of disease in humans, and to subsequently promote appropriate and effective remedial action. This approach requires, among else, cooperation between central and local institutions with responsibilities in the domains of both environmental protection and health prevention.

An example of the aforementioned approach is illustrated in the mini-monograph on the health impact of exposure to amphibolic fibres with fluoro-edenitic composition published in the present issue of *Annali dell'Istituto Superiore di Sanità*. In essence, subsequently to the observation of a localized excess of mortality from malignant pleural neoplasms, a proxy of pleural mesothelioma incidence, in the town of Biancavilla (Sicily), located at the slopes of Etna Volcano, in the absence of detectable occupational exposure to asbestos, three lines of research produced novel scientific knowledge that can be summarized as follows.

Epidemiologists, as reported in this issue by Bruno *et al.* [1] and by Conti *et al.* [2], soon after the initial observation published in 1996 [3], firstly described the case series of pleural mesothelioma among Biancavilla residents, characterized by a comparatively high presence of women, low age at diagnosis and lack of evidence on occupational asbestos exposure. They then provided quantitative estimates of the excess risk of mesothelioma incidence, mortality and hospitalization, and more recently estimated an excess mortality and hospitalization from non-malignant respiratory diseases, namely from pneumoconiosis, thus corroborating the notion of the occurrence of environmental lung fibrosis.

Exposure scientists detected previously unknown amphibolic fibres with fluoro-edenitic composition as naturally occurring soil contaminants. These fibres contaminated among else an area characterized by the presence of a quarry where building materials extensively employed in Biancavilla used to be caved. Exposure to these fibres could thus be determined both by outdoor and indoor sources (see Bruni *et al.* [4] in this same issue).



In vivo carcinogenicity of Biancavilla fibres was demonstrated in a study performed by the Ramazzini Institute in the Bentivoglio experimental laboratories [5] by means of intraperitoneal administration in Sprague-Dawley rats followed by a dramatic increase in peritoneal mesothelioma. *In vitro* studies mainly performed at Istituto Superiore di Sanità (ISS) concurrently documented the cellular and molecular mechanisms underlying the fibres' carcinogenicity, namely multi-nucleation, pro-survival activity and pro-inflammatory cytokine release, as discussed by Ballan *et al.* [6] in this issue.

Interestingly enough, less than two years after the initial report, when the Biancavilla fibre was still believed to be an intermediate phase between actinolite and tremolite, ISS provided a set of public health recommendations that were complied by regional and local institutions, and included termination of quarrying activities, removal of construction industry byproducts and waste from the newly urbanized Biancavilla districts, asphalt paving of roads originally covered by inert material originating from the quarry area. These recommendations were followed by local institutions, and some studies concerning the fibres' air concentration showed a consistent decrease over time [4]. The abovementioned recommendations by ISS were thus formulated on the basis of scientific evidence and at same time founded on a precautionary approach [7], considering the large amount of uncertainty unavoidably affecting the available evidence.

A major breakthrough, in this frame, was represented by the previously quoted *in vivo* experiment [5], that demonstrated the carcinogenicity of fluoro-edenite fibres in rats. It is noteworthy to emphasize the proactive role of Ramazzini Institute that designed and realized the experiment, responding to a request by ISS, in the absence of any financial support from public institutions.

The recognition of Biancavilla as a National Priority Contaminated Site in 2002 opened the way to clean-up interventions whose effectiveness in reducing fibre exposure is well documented [4]. This act was a cornerstone, not only for environmental remediation, but also in terms of environmental justice. The Biancavilla municipality was recognized as a community deserving support by the national and regional governments, in terms of prevention, health care and further epidemiological monitoring.

Several detailed mortality and morbidity studies (the latter performed through the analysis of hospital discharge records) were produced by the Epidemiological Observatory of Sicily. The most recent release reports an analysis of mortality from 2004 through 2011 and analysis of hospitalization from 2007 through 2011. The only neoplastic disease in excess is pleural mesothelioma. Circulatory and respiratory diseases show excess risk in terms of both mortality and hospitalization [8].

Sicilian Region Health Authorities have recently designed an *ad hoc* plan of health intervention in the National Priority Contaminated Sites of Biancavilla [9]. The plan first of all recommends fostering epide-

miological surveillance with special emphasis on mesothelioma, pneumoconiosis, other asbestos-related radiological alterations and CO diffusion alterations. Health promotion will concurrently be pursued by anti-smoking campaigns and health education. Improvements of diagnostic and therapeutic interventions in the domain of chronic respiratory disease will also be ensured.

The health profile of Biancavilla community, as discussed in this issue [1, 2] and briefly summarized in the present commentary, shows similarities with the corresponding health profile of other populations characterized by environmental asbestos exposure due to residence in contaminated sites [10]. Tremolite-induced pleural plaques and mesothelioma were consistently reported in South Eastern Europe [10], including Italy [11, 12], New Caledonia [13], and the well-known case of Libby, Montana [14, 15].

The section concerning Biancavilla in the last report of SENTIERI study, the national epidemiological surveillance project on the population resident in National Priority Contaminated Sites, recommends to focus further epidemiological studies on the prevalence of lung fibrosis and pleural plaques, and to pursue a better characterization of exposure to fibres with fluoro-edenitic composition [16]. In the meanwhile, environmental clean-up must proceed, and its efficacy in terms of exposure mitigation has to be monitored as extensively discussed in this issue [4].

The improvement in exposure assessment should lead to three main goals: a better understanding of the location and characteristics of major sources of fibres in the Biancavilla territory, a better appreciation of activities and circumstances that may determine elevated, even if short-term, airborne fibres levels, and a better insight into indoor sources of fibres. This issue can benefit from innovative approaches, like the use of sentinel animals as biological indicators of fibre exposure, following the pilot study by De Nardo (17) on fluoro-edenitic fibres concentration in sheep lung samples. Progress in these domains may contribute to priority setting in environmental remediation by optimizing resource allocation in a health prevention perspective.

With respect to epidemiological surveillance, the top priority is completing the collection of residential and occupational histories of mesothelioma cases, since the proportion of missing data is still much too high. Subsequently to this achievement, it will be possible to perform etiologic studies aimed at testing hypotheses of causal interest, such as the contribution of outdoor vs indoor exposure to the overall mesothelioma risk, or the weight of exposure in specific time-windows, namely in childhood.

A further comment concerns the perspective of a permanent scientific cooperation between national and regional institutions in charge of environmental protection and health promotion. This goal is pursued by ISS as shown, among else, by the qualified presence of colleagues from Sicilia Region Environmental Protection Agency, Epidemiological Observatory and



Operational Centre for the National Mesothelioma Registry among the Authors of this mini-monograph.

As stated at the start of this Commentary, advances in causal reasoning require integration of epidemiology, exposure science and understanding of biological mechanisms. As a second step, scientific evidence as a whole can provide a sound foundation to public health action and environmental remediation. Finally, it is now necessary to properly tune the response of

the health system to the community needs in terms of diagnostic procedures and medical treatment. In order to be effective, preventive, diagnostic and therapeutic interventions will have to be characterized not only by sound scientific background, but also by well-designed communication strategies, in order to actively involve the whole community in a participatory process.

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Incidence of pleural mesothelioma in a community exposed to fibres with fluoro-edenitic composition in Biancavilla (Sicily, Italy)

Caterina Bruno^(a), Rosario Tumino^(b), Lucia Fazzo^(a), Giuseppe Cascone^(b), Achille Cernigliaro^(c), Marco De Santis^(a), Maria Concetta Giurdanella^(b), Carmela Nicita^(b), Patrizia Concetta Rollo^(b), Salvatore Scodotto^(c), Eugenia Spata^(b), Amerigo Zona^(a) and Pietro Comba^(a)

^(a) *Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy*

^(b) *Registro Tumori, Dipartimento di Prevenzione Medica, ASP Ragusa, Ragusa, Italy*

^(c) *Dipartimento Attività Sanitarie e Osservatorio Epidemiologico, Regione Sicilia, Palermo, Italy*

Abstract

Introduction. Amphibolic fibres with fluoro-edenitic composition characterize Biancavilla soil, including the major quarry from which building materials have been extensively extracted. These fibres induce mesothelioma in experimental animals and their *in vitro* biological action is similar to that of crocidolite.

Materials and methods. Malignant mesothelioma case series and incidence were examined to evaluate the disease burden on Biancavilla inhabitants.

Results. The incidence of pleural mesothelioma in Biancavilla is steadily higher than in the Sicilian Region, risk estimates are more elevated in women than in men, the most affected age class is constituted by subjects aged less than 50.

Discussion and conclusions. Environmental exposure to fibres with fluoro-edenitic composition appears to be causally related to the elevated mesothelioma occurrence in Biancavilla. In this frame, environmental clean-up is the main goal to be pursued in public health terms. A contribution of scientific research to public health decision making with respect to priority setting for environmental clean-up can derive from some further selected epidemiological investigations.

Key words

- fluoro-edenite
- pleural mesothelioma
- peritoneal mesothelioma
- prevention

INTRODUCTION

In the frame of the national programme of epidemiological surveillance of mortality from malignant pleural neoplasms (MPN), a proxy of pleural mesothelioma incidence, conducted by Istituto Superiore di Sanità (ISS), with reference to the time-window 1988-92, an excess risk based on four observed death versus 0.9 expected was reported in the town of Biancavilla, located in the Province of Catania (Sicily), at the slopes of the Etna volcano. Although based on a small number of cases, the excess was regarded as statistically significant since the lower limit of the confidence interval was greater than one, and thus Biancavilla was included in the list of Italian municipalities at risk of mesothelioma, where assessment of asbestos exposure was recommended [1]. The Major and the local health authorities initially challenged this inclusion, since they could easily certify that no relevant asbestos exposure occurred in that town of about 20 000 inhabitants where orange and lemon growing

were the prevailing economic activities. It was anyway agreed to check if further deaths from mesothelioma had been reported, if cases were hystologically confirmed and if they had been occupationally exposed to asbestos; in about one year time (1997 and beginning of 1998) it was ascertained that further cases had been reported also between 1993 and 1997, that most of them were hystologically confirmed and that no consistent occupational exposure to asbestos was detected. Concurrently, asbestos fibres were searched in the neighbouring stone quarry of Monte Calvario where material employed in the local building industry was caved. This investigation lead to the finding of an amphibolic fibre occurring in the relatively soft material located in-between lavic rocks; that soft stone material was extensively used to produce sand, cement and plaster. Based on these results, in May 1998 the Director of the ISS wrote to the Sicilian Regional Government, to the Major of Biancavilla and to the local health authorities recommending to terminate quarrying

activities in Monte Calvario, to remove building material by-products present near newly-constructed houses and to cover with asphalt all roads previously paved with Monte Calvario quarry waste material [2]. A detailed report on these activities was then published [3].

The Biancavilla fibre was initially regarded as an intermediate mineralogical phase between tremolite and actinolite [3]. In the meanwhile, though, a series of investigations performed by Gianfagna and coworkers lead to the identification of a new amphibole end-member, fluoro-edenite [4, 5] as extensively discussed by Bruni *et al.* in this same issue [6].

The environmental remediation process started in 2002, with the official recognition of Biancavilla as a National Priority Contaminated Site, as discussed by Bruni *et al.* [6] in this same issue. Being aware that this decision would provide in the long run a major contribution to risk-reduction in Biancavilla, epidemiologists from ISS together with colleagues from other institutions designed and realized some further studies aimed at pursuing a better insight in the health impact of environmental exposure to fibres with fluoro-edenitic composition. The main steps of this second phase of investigations can be summarized as follows.

Biggeri *et al.* [7] performed a geographic mortality study in all 36 municipalities located in the volcanic area of Mount Etna, in order to investigate the association between chronic obstructive pulmonary disease (a nosological entity in which cases of unrecognized lung fibrosis, if present, might have been allocated) and pleural mesothelioma. The association was observed, especially among women, after adjustment for urban-rural gradient and lung cancer mortality, thus suggesting an etiological role of fluoro-edenite exposure in the occurrence of non-malignant respiratory disease. Putzu *et al.* [8] performed a pilot study on the determination of fibres in the sputum of chronic bronchitis patients resident in Biancavilla as an indicator of exposure to fluoro-edenite; preliminary findings showed that six out of twelve subjects had at least one of three samples positive for fluoro-edenite. Bruno *et al.* [9] through a collaborative work with Sicilian Region Mesothelioma Registry, provided the first estimate of mesothelioma incidence in Biancavilla (5.4 x 100 000). This figure is about 10 times higher than the Sicilian incidence rate. For detailed review and critical discussion of this set of studies, the reader is referred to Bruno *et al.* [10].

The purpose of the present paper is to update the previous incidence study and to review the complete case-serie of mesothelioma cases (1988-2011), with the aim of achieving a better insight in the characteristics of fluoro-edenite induced mesothelioma.

MATERIALS AND METHODS

Biancavilla malignant mesothelioma case series

In 1997 ISS, together with Catania Local Health Unit, collected Biancavilla malignant mesothelioma cases through municipality mortality Registrar, family doctors and hospital records. If available, histology slides were collected and reviewed by a single expert pathologist. Information about clinical history, occupation, relevant behaviour and residential history were

collected by Local Health Unit physicians through the subjects (if alive) or, in most cases, through next-of-kins.

Since 1998, mesothelioma incidence in Biancavilla and previous exposures of registered cases have been taken in charge by the Sicilian Operative Regional Centre (COR) of the National Mesothelioma Registry (ReNaM), a nation-wide system for malignant mesothelioma monitoring. Information have been collected through a structured questionnaire by trained interviewers.

ReNaM classifies mesotheliomas by level of diagnostic certainty:

1. "certain malignant mesothelioma" characterized by the following condition: microscopic examination on material (histological or cytological with centrifugation of the sediment) enclosed in paraffin, with characteristic morphological pattern

(1.1) with immunohistochemistry or

(1.2) immunohistochemistry not carried out or not defined;

2. "probable malignant mesothelioma"

(2.1) with histological or cytological examination with enclosure in paraffin carried out, but which did not give a result indicating mesothelioma in a clear and reliable way (doubtful case) or

(2.2) with cytological examination not enclosed in paraffin, with characteristic pattern and report expressed in terms clearly indicative of mesothelioma;

3. "possible malignant mesothelioma"

(3.1) characterized by indicative clinical and radiological data with diagnosis of discharge of mesothelioma or

(3.2) Death Certificate Only (DCO) with presence on the death certificate of the term "mesothelioma".

Asbestos exposure has been assessed and codified according to the ReNaM criteria which comprises three levels of certainty of occupational exposure (1. certain, 2. probable, 3. possible); three other modalities of exposure (4. household exposure, 5. environmental exposure, 6. non-professional exposure during leisure time activities). Other endpoints can be: 7. unlikely exposure, 8. unknown exposure (incomplete and insufficient information), 9. exposure definition in progress; 10. unclassified exposure (no information is available nor will presumably be available).

Biancavilla malignant mesothelioma incidence

Malignant mesothelioma incidence in Biancavilla municipality was compared to the regional incidence rates. Standardized Incidence Ratios (SIRs), with their 95% Confidence Intervals (95% CI) were computed, by applying the indirect standardization method by use of STATA software. For the computation of the Sicilian Region incidence rates, the population and cases resident in Biancavilla municipality were excluded. SIRs were computed for total malignant mesothelioma, pleural and peritoneal mesothelioma, in the two genders, separately. Data source for cases was the Sicilian Operative Regional Centre (COR) of the National Mesothelioma Registry. All cases diagnosed between 01/01/1998 through 31/12/2011, resident in Biancavilla at time of diagnosis, and classified as "certain", "probable" or "possible" were eligible for the study. At 05/03/2014, 24

pleural mesotheliomas and two peritoneal mesotheliomas were collected in the COR database.

RESULTS

Biancavilla malignant mesothelioma case series

Since 1988 to the end of 2011 45 cases of mesothelioma were detected among Biancavilla residents. Before 1998 a total of 17 cases of pleural mesothelioma had been collected by ISS researchers and Local Health Unit physicians (*Table 1*). For nine cases out of 17, histology slides were found and were available to be reviewed by an experienced pathologist: all diagnoses were confirmed. No certain occupational exposure to asbestos fibres was detected in any case, two subjects had been employed for a short time in industrial sectors in which asbestos might have been used. All cases but one were long-time residents in Biancavilla and eleven of them always resided in the town.

Since 1998 Sicilian COR has taken charge of the investigation on Biancavilla mesotheliomas, using standardized procedures, questionnaires and classifications [11].

Between 1998 and 2011, 28 mesothelioma cases were detected, even if two of them were not eligible to be included in the Biancavilla COR cases as they were not resident there at time of diagnosis (*Table 2*). In eight cases, only environmental exposure to Biancavilla fibres was ascertained, and an investigation to identify a concurrent occupational or non occupational exposure to asbestos fibres outside Biancavilla, if any, is ongoing for eleven cases. No one experienced a certain professional exposure to asbestos, one subject worked in an industrial sector in which asbestos had been used, and three subjects worked in industrial sectors in which asbestos presence could occur. Incomplete and insufficient information (unknown exposure), were collected for two more subjects, even if one of them, a farmer, experienced working in the quarry as a temporary job.

Finally, one case refused to be interviewed (information not available). All cases were long-time resident in Biancavilla and all but five always resided there.

Twenty-six mesothelioma cases were diagnosed in subjects resident in the municipality of Biancavilla at time of diagnosis and collected by COR, 13 in men and 13 in women. In two of them, one male and one female, peritoneum was affected, the other mesotheliomas were localized in the pleura. Mean age at diagnosis was about 60 in men and 70 in women (*Table 3*). The difference between mean age in men and women was noticeable, in particular for pleural mesothelioma. The subjects with peritoneal mesothelioma, one man and one woman, were respectively 63 and 65 years old.

Biancavilla mesothelioma incidence

Table 4 clearly shows that the Standardized Incidence Ratios (SIRs) for mesothelioma in Biancavilla were steadily high: the overall SIR was 5.76 (95% CI 3.76-8.44) respectively, 3.69 (95% CI 1.97-6.32) in men and 13.08 (95% CI 6.97-22.00) in women. An increased risk of pleural mesothelioma was also demonstrated (Overall SIR 5.65, 95% CI 3.62-8.41), both in men (SIR 3.63, 95% CI 1.87-6.34), and in women (SIR 12.75, 95% CI 6.59-22.00). The highest overall SIR was found for peritoneal cases (SIR 7.92, 95% CI 0.96-20.00), based on two observed cases.

With regard to pleural mesothelioma, when SIR estimates were stratified by age (*Table 5*), extremely high figures were shown in the younger age groups: the overall SIR in subjects less than 50 years old was 21.34 (95% CI 6.93-50.00) (four cases in men and one in a woman). These findings were strengthened by the results in the less than 40 years age group (overall SIR 62.88, 95% CI 13.00-180.00, 3 cases).

These findings are consistent with a strong excess of mesothelioma incidence among Biancavilla inhabitants

Table 1

Mesothelioma cases in Biancavilla municipality. *Ad hoc* case reports collection (before 1998)

	Year of diagnosis	Age at diagnosis	Resid years diagnosis	Gender	Level of diagnostic certainty	Known exposure to asbestos fibres	Site
1	1988	40	40	M	Indicative clinical and radiological data	0	Pleura
2	1988	55	19	M	Death Certificate Only (DCO)	Possible	Pleura
3	1991	62	62	F	Death Certificate Only (DCO)	0	Pleura
4	1992	45	45	M	Death Certificate Only (DCO)	0	Pleura
5	1992	42	42	M	Histological/cytological revised specimen	0	Pleura
6	1993	66	66	F	Death Certificate Only (DCO)	0	Pleura
7	1994	62	62	M	Histological/cytological revised specimen	0	Pleura
8	1994	44	32	M	Histological/cytological revised specimen	0	Pleura
9	1994	68	67	M	Histological/cytological revised specimen	Possible	Pleura
10	1995	63	57	M	Histological/cytological revised specimen	0	Pleura
11	1996	29	29	M	Indicative clinical and radiological data	0	Pleura
12*	1996	63	2	F	Death Certificate Only (DCO)	No information available	Pleura
13	1997	77	77	F	Histological/cytological revised specimen	0	Pleura
14	1997	68	68	M	Histological/cytological revised specimen	0	Pleura
15	1997	77	17	F	Histological/cytological revised specimen	0	Pleura
16	1997	86	86	F	Histological/cytological revised specimen	0	Pleura
17	1997	71	71	F	Death Certificate Only (DCO)	0	Pleura

* Resident in Biancavilla since two years before diagnosis.

in the absence of known occupational sources of asbestos exposure.

The cases sex ratio was 1:1, the Standardized Incidence Ratio was elevated in men and even more elevated in women. It is also important to note that a ten years lower mean age at diagnosis was experienced by the male population and to remark the extremely high SIR in the younger age groups.

DISCUSSION

The Italian National Mesothelioma Registry was created to establish a nation-wide system for malignant mesothelioma monitoring because of the massive utilization of asbestos which took place in Italy, followed by a severe health impact. ReNaM objectives are in particular centred on estimating mesothelioma incidence in Italy, and on contributing to the assessment of the effects of industrial use of asbestos and to the identification of sources of asbestos exposure; however environmental exposures to asbestos fibres have been investigated as well. In the present study, the same methodology has been used to identify or exclude occupational exposures to asbestos in Biancavilla resident cases of mesothelioma, while the definition of "environmental exposure" refers to fibres with fluoro-edenitic composition.

In vitro studies showed that fibres with fluoro-edenitic composition behave similarly to crocidolite, as discussed by Balan *et al.* in this issue [12], human lung carcinoma cells have been exposed to two different materials: prismatic fluoro-edenite and fibres with fluoro-edenitic composition. Only in the second case, they exhibit features typical of transformed cells. Most of the results for fibres with fluoro-edenitic composition were comparable to those obtained with crocidolite, used as a "positive control". Accordingly, *in vivo* studies demonstrated that the fluoro-edenitic composition fibre sample induces mesotheliomas [13].

Malignant mesothelioma (MM) incidence rates have been reported to be 1.2-9 times higher among men than among women because of occupational exposures [14]. Besides occupational settings, MM is also associated with domestic exposure in family members of asbestos workers [15]. The role of environmental exposure [16, 17] related to asbestos factories, mines, or naturally occurring asbestos (NOA) has been investigated in Greece, [18], Cyprus [19], Turkey [20-24], Corsica [25, 26], Italy [27], California, US [28], Libby, Montana, US [29] and China [30].

The sex ratio of about 1:1 both in our previous studies [3, 9] and in this issue corroborates the hypothesis

Table 2

Mesothelioma cases in Biancavilla Municipality from COR (Regional Operative Centre of National Mesothelioma Registry) (since 1998)

	Year of diagnosis	Age at diagnosis	Residence years	Gender	Level of diagnostic certainty (ReNaM)	Known exposure to asbestos fibres	Levels of certainty and modalities of exposure (ReNaM)	Site
18	1998	59	59	M	3.1	0	Not available	Pleura
19	1998	56	56	M	1.1	0*	Unknown exposure	Pleura
20	1998	39	39	M	1.2	1	Probable	Pleura
21	1999	48	48	M	1.1	1	Possible	Pleura
22	2000	87	87	M	2.2	?	Undergoing definition	Pleura
23	2002	72	72	F	3.1	0	Environmental	Pleura
24	2002	71	71	F	1.1	0	Environmental	Pleura
25	2002	70	70	F	1.1	0	Environmental	Pleura
26	2003	55	55	F	3.1	0	Undergoing definition	Pleura
27	2003	62	64	F	1.1	0	Environmental	Pleura
28	2004	66	66	F	2.2	?	Undergoing definition	Peritoneum
29	2004	77	77	F	1.1	0	Environmental	Pleura
30	2004	44	44	M	1.1	0	Environmental	Pleura
31	2005	63	48	M	1.2	?	Undergoing definition	Peritoneum
32	2005	73	73	F	1.1	?	Undergoing definition	Pleura
33	2006	84	84	M	3.2	0	Unknown exposure	Pleura
34	2006	81	81	F	1.1	0	Undergoing definition	Pleura
35	2007	74	74	F	1.1	0	Environmental	Pleura
36	2007	84	84	F	3.1	0	Undergoing definition	Pleura
37**	2009	57	53	M	=	=	=	Pleura
38	2009	27	27	F	2.1	?	Environmental	Pleura
39	2009	75	75	M	1.1	?	Undergoing definition	Pleura
40**	2009	68	28	F	=	=	=	Pleura
41	2009	61	48	M	1.2	?	Possible	Pleura
42	2009	64	64	M	1.2	?	Undergoing definition	Pleura
43	2009	90	90	F	3.1	?	Undergoing definition	Pleura
44	2011	33	31	M	1.1	?	Possible	Pleura
45	2011	57	57	M	1.1	?	Undergoing definition	Pleura

* Farmer, quarry worker as temporary job; ** Previously resident and exposed to Biancavilla fibers but not living there at time of diagnosis: data not available to the Regional Operative Centre (COR).

of an environmental exposition rather than an occupational source of asbestos exposure. In Cappadocia, Turkey, a very high incidence of pleural mesothelioma in three villages was found at the end of the 1970s. From 1970 to 1987, 108 cases of pleural mesothelioma were identified, and an annual incidence of more than 8,000 cases/million was calculated. The incidence was identical for men and women (the ratio M/W ranged between one and two), and the mean age was roughly 50, with a range of 26 to 75 years [31]. These cases of MM were caused by exposure to erionite fibers, which are not NOA, being fibrous zeolites [32]. Baris and Grandjean [33] confirmed this high incidence. Erionite fibers in pulmonary biopsy and sputum samples from mesothelioma patients were found [34, 35]. In Biancavilla six out of twelve subjects had samples positive for fluoro-edenitic fibres in the sputum [8].

In New Caledonia an excess incidence of MM was identified in a period of 10 years (1978-1987) [36]. In the same region, Baumann *et al.* [37], studying 109 MM cases recorded between 1984 and 2008, found in the Houailou area, where an important asbestos mining activity was located during the 1960s-1970s, a world age-standardized rate of 128.7 per 100 000 person-years [95% confidence interval (CI) 70.41-137.84]. They evaluated the presence of serpentinite on roads as a major environmental risk factor for mesothelioma.

In a case-control study conducted in the province of Sivas, Turkey, Bayram *et al.* [38] demonstrated a quantitative relationship between the risk of either malignant or non malignant pleural diseases and the proximity of the residence at birth to ophiolites, rocks known as sources of NOA.

The Biancavilla population showed excesses for pleural mesothelioma in mortality and in hospital discharges and non malignant respiratory diseases such as chronic respiratory diseases (in women) and only for hospital discharges from all respiratory diseases and in particular pneumoconiosis [39]. Diseases of circulatory system showed excess mortality among both genders.

As regards the occurrence in Biancavilla of MM cases in young people this finding is consistent with Baris *et al.* [31] findings concerning subjects exposed to erionite. In our study even the figures (four cases out 45 aged less than 40 years old, two of them in their late twenties) are surprisingly high: it needs to be considered for example that, in Australia, in 2008 out of 543 men diagnosed with mesothelioma there was only one man in his early thirties but none younger, and similarly out of 118 women diagnosed with mesothelioma there was only one woman aged in her early thirties but none younger [40].

Latency period for most of malignant mesothelioma of occupational origin is considered to be in a range between 20 to 40 years: in a review of 21 articles by Lanphear & Buncher [41] 96% had a latent period of at least 20 years and the estimated median latent period was at least 32 years after the initial exposure. Latency periods were examined in 312 cases of malignant pleural mesothelioma, diagnosed in the Trieste-Monfalcone area, Italy [42]; they ranged from 14 to 72 years (mean 48.7, median 51). Latency time analysis for 2544 cases

Table 3
Mesothelioma cases in Biancavilla Municipality: mean age at diagnosis

	Obs	Mean	Std. Dev
Pleural Mesotheliomas			
Men	12	58.93	16.70
Women	12	70.43	16.71
Overall	24	64.68	17.36
All Mesotheliomas			
Men	13	59.25	16.03
Women	13	70.04	16.06
Overall	26	64.65	16.65

Table 4
Mesothelioma cases in Biancavilla Municipality: standardized incidence ratios and confidence intervals

	Obs	Exp	SIR	95% CI
Pleural Mesotheliomas				
Men	12	3.31	3.63	1.87-6.34
Women	12	0.94	12.75	6.59-22.00
Overall	24	4.25	5.65	3.62-8.41
Peritoneal Mesotheliomas				
Overall	2	0.25	7.92	0.96-20.00
All Mesotheliomas				
Men	13	3.52	3.69	1.97-6.32
Women	13	0.99	13.08	6.97-22.00
Overall	26	4.51	5.76	3.76-8.44

Table 5
Pleural mesothelioma cases in Biancavilla Municipality: standardized incidence ratios by age groups

	Obs	Exp	SIR	95% CI
Age < 40				
Overall	3	0.05	62.88	13.00-180.00
Age < 50				
Overall	5	0.23	21.34	6.93-50.00
Age ≥ 50				
Men	8	3.14	2.55	1.10-5.02
Women	11	0.88	12.56	6.27-22.00
Overall	19	4.01	4.74	2.85-7.39

diagnosed during the period 1993-2001 and eligible for the analysis was performed by ReNaM [43]. Median latency was 44.6 years (95% CI 44.1-45.0). Latency increased constantly during the observed period with respect to the year of diagnosis: estimated mean latency period among pleural MM cases diagnosed in 1993 and in 2001 was 41.7 and 46.2 years, respectively. In the hypothesis of an asbestos-like behaviour of the Biancavilla fibres with fluoro-edenitic composition, exposure to fibres had to occur about 30 years before the mesothelioma outbreak.

The latency period of mesothelioma in fibres with fluoro-edenitic composition has not been studied yet; if it was similar to the latency of asbestos exposed mesothelioma cases, the occurrence of pleural MM in young adults would require exposure in childhood or in teenage years. Conti *et al.* [39] in this issue showed that the mortality for malignant pleural neoplasms under 50 concerns Biancavilla and two neighbouring communities.

The disparity of ten years in mean age at diagnosis in men and in women in pleural MM observed in Biancavilla cases and the occurrence of more male cases in younger ages have to be investigated taking into account the source population, as recommended by Consonni [44]. The possible role of fibre exposure associated to outdoor recreational activities should be assessed in this frame.

This study is the first report of an excess incidence of peritoneal mesothelioma in Biancavilla, even if based on two cases; this finding is noteworthy in view of the induction of peritoneal mesothelioma in rats after intraperitoneal administration of fibres with fluoro-edenitic composition that was reported by Sofritti *et al.* [13].

An analysis of different behaviours in Biancavilla adults and children in their everyday lives could be useful to figure out the way to reduce fibres exposures.

CONCLUSIONS

In light of the evidence that was illustrated and discussed in the present paper, some conclusions appear to be warranted.

The incidence of pleural mesothelioma in Biancavilla is steadily higher than in the Sicilian Region, risk estimates are more elevated in women than in men, the most affected age class is constituted by subjects aged less than 50.

Analysis of individual data shows that the contribution of asbestos exposure to this localized high occurrence of mesothelioma is substantially irrelevant.

Amphibolic fibres with fluoro-edenitic composition characterize Biancavilla soil, including the major quarry from which building materials have been extensively extracted. These fibres induce mesothelioma in experimental animals and their *in vitro* biological action is similar to that of crocidolite.

Environmental exposure to fibres with fluoro-edenitic composition appears to be the causally related to the elevated mesothelioma occurrence in Biancavilla.

In this frame, environmental clean-up is the main goal to be pursued in public health terms.

A contribution of scientific research to public health decision making with respect to priority setting for environmental clean-up can derive from some further selected investigations concerning, namely:

- the incidence of new cases and an in-depth collection of their occupational, residential and life style histories;
- mortality and hospitalization, also in order to evaluate their temporal trends;
- mean age of mesothelioma cases in relation to the population characteristics;
- health condition profile of residents in Biancavilla to elucidate the characteristics of non neoplastic effects on respiratory system (functionality and imaging) and to study the cardiovascular diseases [39];
- investigation on modality of exposures in the environmental context in the everyday lives of adults and children [6].

The authors also recommend to adopt guidelines and rules in building sector and soil materials handling to minimize fibre dispersion.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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Environmental concentrations of fibers with fluoro-edenitic composition and population exposure in Biancavilla (Sicily, Italy)

Biagio Maria Bruni^(a), Maria Eleonora Soggiu^(a), Giovanni Marsili^(a), Antonio Brancato^(b), Marco Inglessis^(a), Lorenzo Palumbo^(a), Augusta Piccardi^(a), Eleonora Beccaloni^(a), Fabrizio Falleni^(a), Simona Mazziotti Tagliani^(c) and Antonio Pacella^(c)

^(a)Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy

^(b)ARPA Sicilia Struttura Territoriale di Catania, Catania, Italy

^(c)Dipartimento di Scienze della Terra, Sapienza Università di Roma, Rome, Italy

Abstract

Introduction. The town of Biancavilla (Sicily) was included in the National Priorities List of Contaminated Sites due to environmental dispersion of amphibole fibers owing to the extraction of materials from a local quarry. The present report summarizes results from several, hitherto unpublished, environmental surveys carried out in the area, as well as from published analyses of the chemistry and composition of fibers.

Methods. Data included here comprises environmental fiber concentrations by scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) analysis in soil, indoor and outdoor air, personal monitoring, as well as a chemical characterization of the fibers. The full chemical structure and spectroscopic characterization of fibers were obtained through a multi-analytical approach: SEM-EDS, X-ray powder diffraction (XRPD), as well as Mössbauer (MS) and Fourier transform infrared (FT-IR) spectroscopies.

Results. Data analyzed provided a spatial and temporal picture of fiber concentrations in Biancavilla, and a qualitative assessment of population exposure. Results suggest that until 2000, the population had been exposed to high levels of amphibole fibers. Mitigation measures adopted since 2001, gradually reduced exposure levels to about 0.1-0.4 ff/l. Previous studies on fibrous amphiboles from Biancavilla reported considerable chemical variability. Differences in composition, especially concerning the presence of Si, Ca, Fe, and Na, were found both within and between samples. Compared to the previously investigated prismatic fluoro-edenite, these fibrous fluorine amphiboles consistently showed higher average values of Si and Fe content, whereas Ca was significantly lower, which we consider a distinctive characteristic of the fluorine fibrous variety.

Conclusions. The population of Biancavilla had been highly exposed to a suite of fibrous amphiboles for over 50 years. Dust mitigation measures have gradually reduced exposure, but continuous environmental follow-up is necessary in order to monitor exposure levels and prevent adverse health effects for future generations.

Key words

- fluoro-edenite
- fibrous amphiboles
- Biancavilla
- population exposure
- air fiber concentrations
- topsoil fiber concentrations

INTRODUCTION

A study on mortality from malignant pleural mesothelioma in Italy from 1988 to 1997 reported an unusual cluster among people living in Biancavilla [1, 2], a small town (about 20 000 inhabitants) located on the southwestern slopes of Mt. Etna in Sicily. Environmental and mineralogical surveys in Biancavilla showed no asbestos exposure either from occupational activities or from the use of manufactured products. Nevertheless, environ-

mental exposure from a quarry located in the area of Mt. Calvario, southeast of Biancavilla, was documented. The quarry had been widely exploited by the local construction industry for the extraction of sand and rubble. This altered the morphology of the region by pulling down the hill of Mt. Calvario, which from a geological point of view was composed of brecciated domes of highly viscous, benmoreitic lavas [3]. An abundance of altered incoherent and very friable materials was found in the quarry,



originating from both brecciated benmoreitic lavas and pyroclastic deposits. Mineralogical analysis of these materials revealed the presence of a fluorine amphibole with acicular and prismatic habit, identified as fluoro-edenite - a new end-member of the calcic amphibole group [4].

Amphibole minerals with fibrous habit, on the other hand, were found in proximity to the Mt. Calvario quarry [5, 6]. Comba *et al.* 2003 [7] therefore suggested that the unusual mesothelioma cluster in Biancavilla may have been caused by exposure to these fibrous amphiboles.



Figure 1
Sampling points of the 2004-2005 survey. The red line delimits the contaminated site, as per NPL-CS.

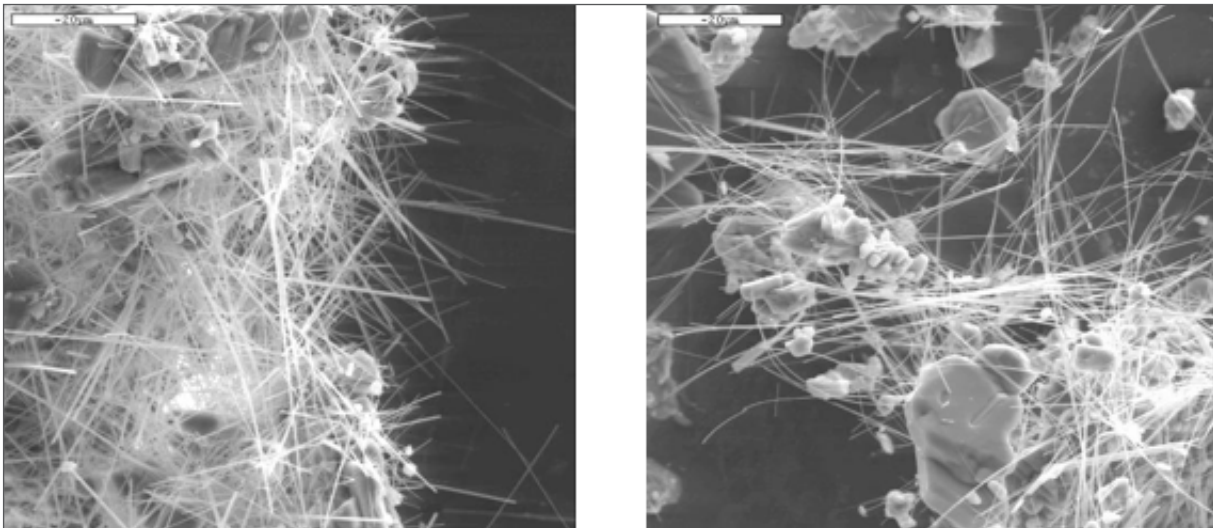


Figure 2
SEM photographs of two fibrous amphibole samples from Biancavilla: sample 3 (left), and sample 4 (right). The other associated minerals are mostly alkali-feldspars, clinopyroxenes, fluorapatite and Fe-Ti oxides. Reproduced with kind permission from [10].

Since 2000, the area of Biancavilla has been extensively studied in order to assess the environmental concentration of fibers in soil, as well as in outdoor and indoor air. In addition, personal monitoring was performed, and the fiber content of plaster/mortar in construction materials analyzed. High environmental concentrations of fibers were found, highlighting the need to adopt a series of dust mitigation measures in order to reduce population exposure. As of 2009, ongoing monitoring has documented a descending trend in environmental fiber concentrations in Biancavilla.

The present report summarizes the results of previous scientific publications on the chemical and structural characterization of fibrous amphiboles from Biancavilla, as well as results of environmental surveys on fiber concentrations in the town.

METHODS

Geological background

On the lower southwestern flank of Mt. Etna, Romano [3] identified three aligned domes of the early alkaline volcanic activity of Mt. Etna (Mt. Calvario, Santa Maria di Licodia and Ragalna). The Mt. Calvario lava dome, near Biancavilla, is well known for the occurrence of fluorine-rich mineral phases, specifically, fluoro-edenite, fluorophlogopite and fluorapatite [4, 8]. Fluorine-rich mineral phases are unusual for Mt. Etna, although similar phases have been found close to the mountain's summit, in lavas of the so-called Ellittico volcano, a caldera dating back to the Pleistocene [9].

Mineralogical and geochemical investigations have been carried out to ascertain the areal extent of these volcanic materials, and identify the genetic process underlying the crystallization of fluorine-rich minerals,

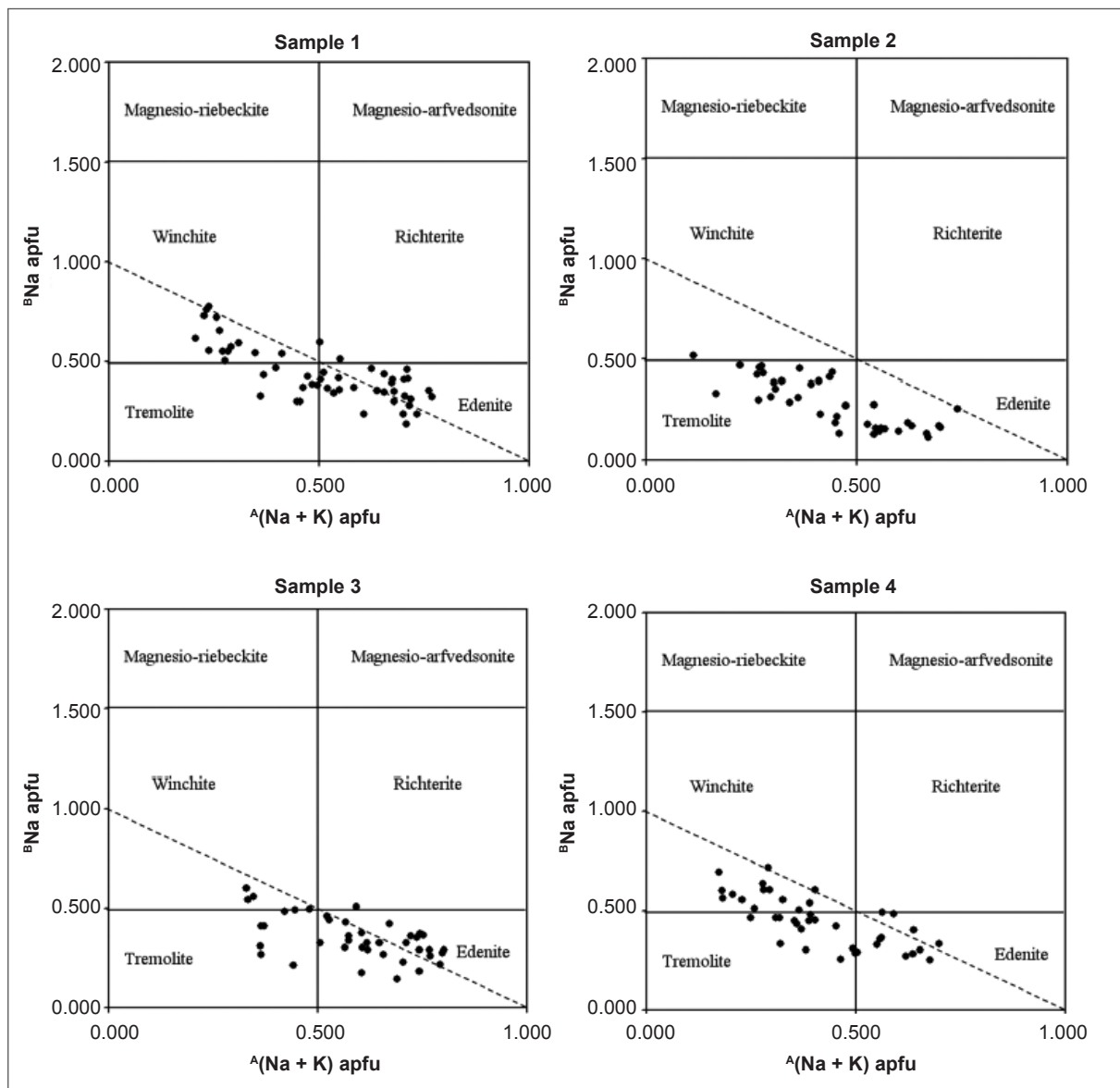


Figure 3
All results obtained from the four fibrous amphibole samples from Biancavilla, plotted against the ²³(Na+K)/²³Na diagram (Leake *et al.*, 1997). Reproduced with kind permission from [10].

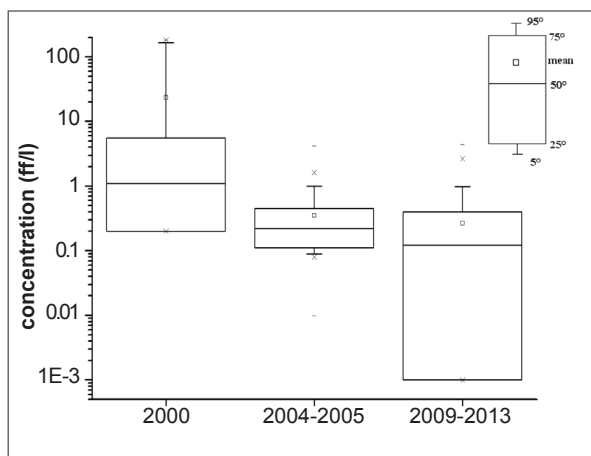


Figure 4
Distributions of air fibres concentrations acquired during the different surveys.

particularly those of fibrous morphology [9]. The dome and dyke complex consists of locally metasomatized benmoreitic and autoclastic breccias (*i.e.*, highly viscous lavas fragmented by the degassing of volatile components). The primary mineral assemblage of benmoreite lava consists of plagioclase, clinopyroxene, olivine, fluorapatite and iron oxides, whereas secondary mineralization comprises fluoro-edenite and fluorophlogopite, ferroan-enstatite, hematite, pseudobrookite and tridymite. Fluoro-edenite was found mainly in the metasomatized portion of the dome. The central portion of the structure is intensely fractured and is characterized by the presence of fluoro-edenite of variable morphology, from prismatic to acicular. The external portion of the dome consists of breccia with fibrous amphibole of micrometric dimensions. Importantly, the fluoro-edenite fibers, dispersed in a grey-reddish friable matrix, were not homogeneously distributed.

Geochemical data revealed enrichment in several major and trace elements (*e.g.*, Fe, Ti, P, and alkali), mainly fluorine and chlorine, which cannot be attributed solely to magma differentiation processes in the feeding system. The transfer of magmatic fluids from the deeper to the upper portions of the magma storage zones [9] has been suggested as a possible explanation for the presence of these elements. In the Mt. Calvario dome, selective enrichments in certain elements led to the crystallization of fluorine-rich mineral phases during the syn- and post-eruptive stages.

Fiber characterization

The location of sampling points is reported in *Figure 1*. As reported by Mazziotti-Tagliani *et al.* [10], the starting material for fiber characterization consisted of lava pieces in a fine and friable matrix, which contained an assemblage of amphibole fibers and other minerals of micrometric and sub-micrometric dimensions. To avoid or at least reduce any influence of other minerals on the experimental data, fiber characterization was performed on samples enriched in amphibole fibers. Briefly, a fiber content of > 90% in samples was obtained through a simple sedimentation in water, followed by a withdrawal of the supernatant solution after 30 to 35 hours.

Figure 2 displays scanning electron microscopy (SEM) photographs of two amphibole samples from Biancavilla. These fluorine-rich fibrous amphiboles are generally less than 1 μm (200 to 600 nm) thick, and up to 100-150 μm long. Samples 1, 2 and 3 were characterized by an acicular-fibrous morphology and shorter fibers (mean length, ca. 50 μm), while sample 4 showed a more filamentous-asbestiform morphology with length up to 150 μm [10].

The full chemical structural and spectroscopic characterization of the fibrous amphibole samples from Biancavilla was performed using a multi-analytical approach: SEM-EDS, X-ray powder diffraction (XRPD), as well as Mössbauer (MS), and Fourier transform infrared (FT-IR) spectroscopies. Quantitative chemical analyses (SEM-EDS) were performed by Mazziotti-Tagliani *et al.* [10], applying Paoletti and co-workers' standardization procedure [11]. In addition, cation site partitioning was performed by Adreozzi *et al.* [12] by optimizing average chemical, XRPD, MS, and FT-IR data.

Environmental characterization

In the past 15 years, *ad-hoc* surveys of environmental dispersion of amphibole fibers in Biancavilla have been carried out, with the aim of determining the levels of outdoor pollution, indoor contamination and personal exposure. Only data obtained by SEM-EDS were included in the present analysis. The first survey, in 2000, studied fiber levels in indoor and outdoor air, as well as the fiber content of plaster/mortar in Biancavilla buildings [13]. Later, in 2004, the University of Catania was charged with characterizing the area of Biancavilla [14], which was included, by law, in the National Priorities List of Contaminated Sites (NPL-CS) [15]. The survey in question, carried out between August of 2004 and July of 2005, aimed, among other things, at assessing the efficacy of the dust mitigation measures adopted after 2001, through the measurement of amphibole fiber concentrations in outdoor air and topsoil (0-50 cm). Airborne fiber concentrations were measured, and the fiber content of particulate depositions analyzed at 90 sampling points distributed throughout the area, of which 55 were located within the built-up area, 9 were in and around the quarry and 26 near sensitive targets such as schools and hospitals. Of the 90 particulate deposition samples, 22 were analyzed by SEM-EDS. Topsoil analysis was carried out at 840 sampling points. Two-hundred-and-fifty-seven of these samples were analyzed by SEM-EDS. *Figure 1* shows the geographical distribution of air and topsoil sampling points.

Data from an ongoing environmental monitoring system operated, since 2009, by the Regional Environmental Agency of Sicily (ARPA Sicilia, Dipartimento Provinciale di Catania), were also included. ARPA airborne fiber sampling is performed 1-3 times a week. Here, we analyze 465 samples collected between July 2009 and October 2013.

RESULTS

Fiber characterization

Upon FT-IR spectroscopy, none of the samples showed absorption bands in the O-H stretching region (3800-3600 cm^{-1}), indicating the complete substitution



Table 1

Chemical composition of the four samples of fibrous amphibole from Biancavilla. Reproduced with kind permission from [10]

N. Analyses	Sample 1		Sample 2		Sample 3		Sample 4	
	Average	Range	Average	Range	Average	Range	Average	Range
SiO ₂	54.12	52.39-56.70	52.66	50.61-55.19	53.49	51.47-5.56	53.86	51.68-55.92
TiO ₂	0.03	0.00-0.06	0.03	0.01-0.05	0.02	0.00-0.06	0.03	0.00-0.06
Al ₂ O ₃	1.95	1.38-3.28	2.91	1.96-3.81	2.51	1.56-3.27	2.31	1.52-3.32
MgO	21.34	19.28-23.11	20.50	19.07-21.36	22.63	21.21-24.19	21.94	20.56-23.39
CaO	8.48	7.03-9.88	10.20	9.01-11.32	9.19	7.62-10.84	8.59	7.22-10.58
MnO	0.56	0.00-1.06	0.45	0.18-0.79	0.49	0.23-0.78	0.44	0.00-1.00
FeO _{tot}	5.43	3.74-6.68	5.98	4.36-7.28	3.59	1.88-5.18	5.03	3.41-6.74
Na ₂ O	3.10	2.30-3.82	2.29	1.49-3.10	3.08	2.02-3.80	2.78	2.09-3.76
K ₂ O	0.53	0.18-1.30	0.52	0.00-0.79	0.55	0.11-1.02	0.54	0.16-1.04
^a F	4.40		4.40		4.40		4.40	
^a Cl	0.06		0.06		0.06		0.06	
^b Total	100.00		100.00		100.00		100.00	
^c Fe ₂ O ₃	3.26		4.45		3.67		5.25	
^c FeO	2.50		1.97		0.29		0.30	

^a fixed values for all samples; ^b normalized analyses; ^c obtained through Mössbauer investigation.

1 $T(Si_{7.676}^{IV}Al_{0.324})_{\Sigma 8.000} C(Fe_{0.002}^{VI}Al_{0.002}^{VI}Fe_{0.348}^{3+}Fe_{0.297}^{2+}Mg_{4.283}Ti_{0.003}Mn_{0.067})_{\Sigma 5.000} B(Mg_{0.227}Ca_{1.289}Na_{0.484})_{\Sigma 2.000} A(Na_{0.368}K_{0.096})_{\Sigma 0.464} O_3(F_{1.974}Cl_{0.014})_{\Sigma 1.988}$

2 $T(Si_{7.495}^{IV}Al_{0.488})_{\Sigma 7.984} C(Fe_{0.477}^{3+}Fe_{0.235}^{2+}Mg_{4.229}Ti_{0.003}Mn_{0.067})_{\Sigma 5.000} B(Mg_{0.121}Ca_{1.555}Na_{0.324})_{\Sigma 2.000} A(Na_{0.307}K_{0.094})_{\Sigma 0.401} O_3(F_{1.981}Cl_{0.014})_{\Sigma 1.995}$

3 $T(Si_{7.544}^{IV}Al_{0.417})_{\Sigma 7.961} C(Fe_{0.389}^{3+}Fe_{0.034}^{2+}Mg_{4.516}Ti_{0.002}Mn_{0.059})_{\Sigma 5.000} B(Mg_{0.242}Ca_{1.389}Na_{0.369})_{\Sigma 2.000} A(Na_{0.473}K_{0.099})_{\Sigma 0.572} O_3(F_{1.963}Cl_{0.014})_{\Sigma 1.977}$

4 $T(Si_{7.587}^{IV}Al_{0.384})_{\Sigma 7.971} C(Fe_{0.557}^{3+}Fe_{0.035}^{2+}Mg_{4.356}Ti_{0.003}Mn_{0.053})_{\Sigma 5.000} B(Mg_{0.256}Ca_{1.297}Na_{0.447})_{\Sigma 2.000} A(Na_{0.312}K_{0.097})_{\Sigma 0.409} O_3(F_{1.960}Cl_{0.014})_{\Sigma 1.974}$

Table 2

Fe³⁺/Fe_{tot} ratios, and Fe²⁺ and Fe³⁺ contents for the four analyzed samples. Reproduced with kind permission from [10]

Samples	1	2	3	4
Fe ³⁺ /Fe _{tot}	0.540	0.670	0.920	0.939
Fe ³⁺	0.348	0.477	0.390	0.557
Fe ²⁺	0.296	0.235	0.034	0.036
Sum (Fe ³⁺ + Fe ²⁺)	0.644	0.712	0.424	0.593

of the hydroxyl OH⁻ with fluorine in the fibrous amphiboles analyzed, as previously observed in prismatic fluoro-edenite samples [4, 12]. The compositional range (min-max), average chemical composition and mean crystal-chemical formula for each sample are reported in Table 1. Having plotted the results of their analyses on a ^A(Na+K)/^BNa diagram [12], Mazziotti-Tagliani *et al.* [10] concluded that the four samples had reasonably similar, albeit slightly different compositional trends (Figure 3). Point analyses of the four samples fell close to the 1:1 edenite-winchite line. A significant tremolite component is present in all samples. Sample 3 was the closest in composition to prismatic fluoro-edenite (Tables 1 and 2). Notably, the FeO_{tot} content of fibrous amphiboles was always higher than that observed for prismatic fluoro-edenite (Table 1). For each sample, Andreozzi *et al.* [12] reported possible site occupancies obtained by combining chemical data with Rietveld refinement results. A high level of agreement was observed between refined site scattering (s.s.) values, and those calculated from possible site occupancies, being the largest differences (for C sites) smaller than 3% [12]. Samples 2 and 4 showed refined s.s. values at C sites higher than samples 1 and 3, due to the larger amount of heavier atoms (e.g., Fe). Finally, the s.s. val-

ues for the A site in fibrous samples (Table 3) were significantly lower than those in prismatic fluoro-edenite [4, 10], due to the lower ^ANa content in the former.

Environmental characterization

The analysis of data acquired in 2000, before mitigation measures were taken, found amphibole contamination levels ranging from 0.4 to 8.2 ff/l, with an average of 1.76 ff/l. The highest concentrations were detected on unpaved roads covered with inert material, especially during heavy traffic, when up to 93-183 ff/l were measured.

In the same period, a survey on the indoor environment found concentrations ranging from < 0.4 ff/l to 4.8 ff/l, with an average of 1.18 ff/l. Starting from 2001, a series of dust mitigation measures were taken, notably roads paving, to address what had emerged as the main risk factor for population exposure.

The 2004-2005 survey carried out by the University of Catania, demonstrated the efficacy of these clean up interventions, measuring between 0.01 and 4.19 ff/l (mean, 0.35 ff/l) in outdoor air.

Presently, ARPA monitoring shows a descending trend in the concentrations of airborne fibers from an average 0.46 ff/l in 2009, to an average of 0.1 ff/l in 2013, although few peaks have been observed in concomitance

Table 3

Site scattering (s.s.) values for the fibrous amphiboles: experimentally obtained from the structural refinement (left); calculated from the possible site occupancy (right). Reproduced with kind permission from [12]

Sample 1			
	s.s. from refinement	Possible site-occupancy	s.s. from site-occupancy
A	4.6(2)	$K_{0.10}Na_{0.37}$	6.0
A(m)	–	–	–
Sum A sites	4.6(2)		6.0
M(4)	37.6(4)	$Ca_{1.29}Na_{0.48}Mn^{2+}_{0.07}Fe^{2+}_{0.16}$	37.0
Sum B sites	37.6(4)		37.0
M(1)	25.0(3)	$Mg_{1.90}Fe^{2+}_{0.10}$	25.3
M(2)	28.0(3)	$Mg_{1.61}Fe^{2+}_{0.04}Fe^{3+}_{0.35}$	29.5
M(3)	11.8(2)	$Mg_{1.00}$	12.0
Sum C sites	64.8(5)		66.8

Sample 2			
	s.s. from refinement	Possible site-occupancy	s.s. from site-occupancy
A	2.9(8)	$K_{0.09}$	1.7
A(m)	3.3(8)	$Na_{0.31}$	3.4
Sum A sites	6.2(11)		5.1
M(4)	37.3(4)	$Ca_{1.56}Na_{0.32}Mn^{2+}_{0.05}Fe^{2+}_{0.07}$	37.8
Sum B sites	37.3(4)		37.8
M(1)	25.3(4)	$Mg_{1.92}Fe^{2+}_{0.08}$	25.1
M(2)	30.6(4)	$Mg_{1.44}Fe^{2+}_{0.08}Fe^{3+}_{0.48}$	31.8
M(3)	12.2(3)	$Mg_{0.99}Fe^{2+}_{0.01}$	12.1
Sum C sites	68.1(6)		69.0

Sample 3			
	s.s. from refinement	Possible site-occupancy	s.s. from site-occupancy
A	3.3(3)	$K_{0.10}$	1.9
A(m)	4.4(3)	$Na_{0.47}$	5.2
Sum A sites	7.7(4)		7.1
M(4)	34.1(1)	$Ca_{1.39}Na_{0.37}Mn^{2+}_{0.06}Mg_{0.18}$	35.5
Sum B sites	34.1(1)		35.5
M(1)	25.3(1)	$Mg_{1.87}Fe^{3+}_{0.12}$	25.6
M(2)	27.7(1)	$Mg_{1.70}Fe^{2+}_{0.03}Fe^{3+}_{0.27}$	28.2
M(3)	11.8(1)	$Mg_{1.00}$	12.0
Sum C sites	64.8(2)		65.8

Sample 4			
	s.s. from refinement	Possible site-occupancy	s.s. from site-occupancy
A	6.8(2)	$K_{0.10}Na_{0.31}$	5.3
A(m)	–	–	–
Sum A sites			5.3
M(4)	30.8(3)	$Ca_{1.30}Na_{0.45}Mn^{2+}_{0.05}Mg_{0.20}$	34.6
Sum B sites	30.8(3)		34.6
M(1)	24.1(2)	$Mg_{1.96}Fe^{3+}_{0.04}$	24.6
M(2)	30.3(2)	$Mg_{1.48}Fe^{2+}_{0.04}Fe^{3+}_{0.48}$	31.3
M(3)	12.7(2)	$Mg_{0.96}Fe^{3+}_{0.04}$	12.6
Sum C sites	67.1(5)		68.5

Note: The possible site-occupancy is the result of combining data from averaged chemical composition, Mössbauer spectroscopy and Rietveld refinement.



with specific kinds of public works (projects involving excavation) or under certain meteorological conditions.

Figure 4 shows the downward trend in fiber concentrations, as observed in the three surveys carried out in 2000, 2004-2005 and 2009-2013, a trend mainly attributable to the dust mitigation measures adopted since 2001. It should be noted however, that the three surveys had different aims and followed different protocols. ARPA, for example, performs measurements not only in everyday situations, but also with the aim of monitoring the dispersion of airborne fibers in high-risk situations (e.g., excavation work), resulting in greater variability in the data observed.

The fact that for decades, starting from about 1950, material from the Mt. Calvario quarry had been widely used for urban construction, prompted a study to examine plaster/mortar for amphibole fiber levels [16]. Amphibole fibers were found in all samples from pre-1960 buildings, in about 70% of samples from buildings constructed between 1960 and 1990, and in about 40% of samples from post-1990 buildings. Mt. Calvario materials were no longer used after 1999. In addition, in 2000, a small study assessed the personal exposure of people working outdoors as traffic policemen and garbage collectors. The survey revealed personal exposure levels ranging from 0.4 to 4.6 ff/l. The highest levels of exposure were associated with work in areas with unpaved roads.

More recently (2008-2012), further mitigation measures were adopted as the remedy of plasters of some public buildings as schools, the town hall and the cemetery surrounding wall and the envelopment of the ridge rock, surrounding the quarry, with Spritz Beton, i.e. a material able to avoid the atmospheric dispersion of particulate.

Spatial analysis, on data acquired in 2000, revealed peak concentrations (mean, ca. 4 ff/l) in the northeast and northwest of Biancavilla and high concentrations in the south (mean, ca. 2 ff/l). The lowest concentrations (ca. 1 ff/l) were measured around the quarry. The 2004-2005 survey showed mean amphibole concentrations of about 0.34 ff/l and 0.31 ff/l in the north and south of town, respectively. In the east, including the Mt. Calvario quarry, fiber concentrations averaged 0.38 ff/l. Comparable fiber levels were observed in the old town, with an average value of 0.34 ff/l.

No fibers were identified in 9% of topsoil and in 32% of particulate deposition samples. Fiber concentrations in 234 topsoil samples ranged from 8.9 ppm to 3173.4 ppm, and in particulate deposition samples, from 183.7 ppm to 1541.9 ppm. The highest values were observed in the north and east of Biancavilla, in agreement with the spatial pattern of fiber concentrations in outdoor air.

DISCUSSION AND CONCLUSIONS

In the case of Biancavilla, the environmental and health relevance of amphibole fiber pollution has been clearly documented by epidemiological studies. Published results [10, 12] on the crystal-chemical characterization of amphibole fibers from Biancavilla revealed considerable variation in fiber composition. Specifically, while a fluoro-edenite component was dominant,

significant tremolite and minor winchite components were also present. According to Andreozzi *et al.* [12], this complicates both the classification of these fibers and the definition of the mineral species to which they belong. From the 1950s and until the late 1990s, the population of Biancavilla had been exposed to high concentrations of amphibole fibers, both indoors and outdoors. Exposure was at its highest between 1950 and 1970, when Mt. Calvario quarry materials were widely used for construction.

Population exposure to amphibole fibers in Biancavilla may be attributed to the following three factors: the outdoor concentration of fibers in residential areas, the fiber content of building materials, and fiber concentrations in places frequented by the residents for work and other daily activities. The relative weight of each of these factors in determining exposure may have changed over time. Conceivably, in the quarry's early years, occupational exposure and exposure through daily activities were the most significant, while since the 1990s, the area of residence may have assumed a greater relative importance.

Considering data from surveys carried out before 2000, different population exposure levels by area of residence can be roughly presumed:

- in the old part of town: a low level of both indoor and outdoor exposure;
- in the northwest and northeast of Biancavilla: high levels of airborne fibers, both indoors and outdoors;
- in the southern part of the town: lower levels of exposure compared to the north, but higher than in the old part of town;
- in the rural eastern part of town: exposure levels lower than the north, and comparable to the south.

The mitigation measures implemented since 2001 have reduced the exposure of the population to concentrations below 1 ff/l. The long duration of exposure and the long latency of pleural mesothelioma, however, suggest that cases will continue to be observed in the Biancavilla population in the next few years.

The continuous monitoring of the area remains necessary, however, in order to control air fiber dispersion and to prevent high population exposure.

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Conflict of interest statement

The Authors declare no potential conflict of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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Health impact of the exposure to fibres with fluoro-edenitic composition on the residents in Biancavilla (Sicily, Italy): mortality and hospitalization from current data

Susanna Conti^(a), Giada Minelli^(a), Valerio Manno^(a), Ivano Iavarone^(b),
Pietro Comba^(b), Salvatore Scondotto^(c) and Achille Cernigliaro^(c)

^(a) Ufficio di Statistica, Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute, Istituto Superiore di Sanità, Rome, Italy

^(b) Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy

^(c) Dipartimento di Attività Sanitarie e Osservatorio Epidemiologico della Regione Sicilia, Palermo, Italy

Abstract

Introduction. The objective of this chapter is to study the health impact of the exposure to fibres with fluoro-edenitic composition on the residents in Biancavilla (Sicily, Italy), in terms of mortality and hospitalization. The diseases which international scientific literature indicates as associated with asbestos exposure were taken into consideration: mesothelioma of pleura, peritoneum, pericardium and tunica vaginalis testis, malignant neoplasm of larynx, malignant neoplasm of trachea, bronchus and lung, malignant neoplasm of ovary, pneumoconiosis; moreover, in order to describe the health profile of the study population, large groups of diseases were taken into consideration.

Material and methods. Current data (available in the Data Bases of the Unit of Statistics of ISS) regarding mortality and hospitalization were analyzed. Standardized Mortality Ratios, Standardized Hospitalization Ratios and Age-standardized Death Rates were calculated. The demographic background of the population residing in Biancavilla was also outlined.

Conclusions. Our findings support the etiologic role of fibres with fluoro-edenitic composition in the occurrence of the above mentioned diseases, already observed in other studies.

Key words

- mortality
- hospitalization
- health profile
- asbestos
- fluoro-edenite

INTRODUCTION

The observation of an excess risk of pleural mesothelioma in the community of Biancavilla, subsequently attributed to the inhalation of fibres with fluoro-edenitic composition, as reported in this same issue by Bruno *et al.* and by Bruni *et al.*, was originally made in the frame of a national surveillance program of mortality from malignant pleural neoplasms in over 8000 Italian municipalities. The original observation concerned the time window 1988-1992 [1], and it was then confirmed by subsequent reports of the mesothelioma surveillance project, concerning, respectively, the years 1995-2002 [2] and 2003-2009 [3].

As extensively discussed by Bruno *et al.* in this same issue, incidence data concerning pleural mesothelioma have substantially confirmed the indications provided by mortality figures. Even if mortality is a proxy of

incidence, and can be affected by a higher degree of diagnostic misclassification, it has the advantage of being available for all Italian municipalities since 1980 (previously, mortality data could be investigated only at provincial level, that means a much lower specificity, since Italy has over 8000 municipalities versus 100 Provinces).

The objective of this paper is to study the health impact of the exposure to fibres with fluoro-edenitic composition on the residents in Biancavilla, in terms of mortality and hospitalization. The international scientific literature indicates some diseases as associated to asbestos exposure: among neoplastic diseases, mesothelioma of pleura, peritoneum, pericardium and tunica vaginalis testis, malignant neoplasm of larynx, malignant neoplasm of trachea, bronchus and lung, malignant neoplasm of ovary; among respiratory

diseases: pneumoconiosis (asbestosis) [4]. If the latter is not properly diagnosed, it is possible to detect excesses of chronic respiratory diseases and total respiratory disease among asbestos-exposed subjects.

Therefore, in this study, mortality and hospital discharge data due to the abovementioned diseases were analyzed. Moreover, in order to describe the health profile of the population at study, large groups of diseases were taken into consideration: infectious diseases, all neoplasms, diseases of the circulatory system, diseases of the digestive system and diseases of the genitourinary system.

The demographic background of the population residing in Biancavilla was also outlined.

MATERIAL AND METHODS

Demographic framework

Demographic data regarding the municipality of Biancavilla and Sicily Region were available from the Italian National Institute of Statistics (Istituto Nazionale di Statistica, ISTAT). The age and gender distribution of residents in Biancavilla was described showing the age-pyramid; in order to compare these distributions with the ones of the whole Sicilian population the Kolmogorov-Smirnoff test was used.

Mortality

The data source was the Italian Mortality Data Base, run by the Office of Statistics of ISS, based on official mortality and demographic data, released from ISTAT.

Mortality data, at international level, are codified according to the International Classification of Disease (ICD); it has been revised approximately every 10 years; the purpose of the revision is to stay abreast of medical advances in terms of disease nomenclature and etiology. In Italy, deaths have been codified according to the Ninth Revision (ICD-9) until 2002 [5]; since 2003, the Tenth Revision (ICD-10) has been adopted [6]. ICD-10 differs from ICD-9 in several respects, first of all, ICD-10 is far more detailed than ICD-9, with about 12 000 categories compared with about 5000 categories [7, 8]. Moreover, whilst the classification of neoplastic disease in ICD-9 was only topographic, ICD-10 allows also morphological distinction; therefore it is possible, since 2003 data, to study deaths from pleural and peritoneal mesothelioma (ICD-10 codes: C45.0 and C45.1 respectively). Until 2003, deaths due to mesothelioma were codified by a unique and non specific ICD-9 code: 163 "malignant neoplasm of pleura"; the code for this disease in ICD-10 is C38.4.

The following analysis of mortality data were performed:

a) estimate of standardized mortality ratio (SMR); reference population, Sicilian Region, study period 2003-2010

SMR_{*i*}, referred to residents in a selected geographic area *i*, is defined as the ratio between the number of

observed and expected deaths, $SMR_i = \frac{\sum_j e_j}{\sum_j T_j n_j} \times 100$

Where, for each age-group *j*: *e_j* are the observed events (deaths), *n_j* is the population and *T_j* is the

death rate of the reference population (regional population).

As well as the point estimation of SMR, also its confidence interval were calculated (CI 90%); if the observed deaths were less than 100, CI was estimated based on Poisson's distribution, otherwise, on the Byar method;

b) time trend 1980-2010 of age-standardized death rates (ASR) from malignant neoplasm of pleura: until 2002 it was codified by ICD-9 163, after 2002, two codes are to be taken into consideration: ICD-10 45.1 and ICD-10 38.4. The rates have been standardized using the direct method, having the 2001 Census population as reference population. The ASR were referred to 100 000 persons;

c) estimate of SMR from malignant neoplasms of pleura (MNP) in young adults (≤ 50 years) as a proxy for environmental exposure to fluoro-edenite fibres in childhood (reference population, Sicilian Region, study period 1980-2010: ICD-9, code 163 (1980-2002), and ICD-10, codes C45.0 and C38.4 (2003-2010).

In the abovementioned analyses, mortality data referred to the years 2004 and 2005 were not analyzed, given that they were not available from ISTAT.

Hospitalization

The source of data regarding hospitalizations in Italy is the Hospital Discharges Forms Data Base (HDF); in Italian, "schede di dimissione ospedaliera (SDO)". Information on hospitalizations contained in HDFs are collected by all Italian public and private hospitals and are then transmitted to the Ministry of Health. The data collected include information such as demographics (gender, date and place of birth, place of residence and so on), admission and discharge data, principal diagnosis and up to five secondary diagnoses. To code diagnoses, ICD 9-CM (International Classification of Diseases-Clinical Modification, Ninth Revision) is used [9].

In the HDF Data base that the Ministry of Health transmits to the Unit of Statistics of the Istituto Superiore di Sanità, each discharge form contains also an anonymous code, unique for each individual; therefore, it is possible to analyze the information regarding persons that are hospitalized, and not only the hospitalizations. Only the main diagnosis reported in the HDF was taken into consideration and if, during the study period, an individual had been hospitalized for the same diagnosis more than once, only the first hospitalization that occurred during the study period was considered [10, 11].

The following analysis was performed: estimate of standardized hospitalization ratio (SHR) reference population, Sicilian Region, study period 2005-2010; the diagnoses taken into consideration are the same analyzed for mortality study.

The definition of SHR is analogous to that of SMR; also for SHR CI 90% were calculated.

In geographical studies of environment and health, confounding from social and economic factors may occur. To control such confounding effect, standardization

**Table 1**

Number of observed deaths (Obs), standardized mortality ratio adjusted for deprivation (SMR DI), regional reference; 90% confidence interval (CI 90%); period: 2003-2010 (2004-2005 not available from ISTAT). Men and women

Causes of death	Men		Women	
	Obs	SMR DI (CI 90%)	Obs	SMR DI (CI 90%)
All causes (general mortality)	528	97 (90-104)	561	105 (98-113)
Infectious and parasitic diseases	4	59 (20-135)	4	66 (23-151)
All neoplasms	136	83 (72-96)	107	91 (77-107)
* Malignant neoplasm of larynx	< 3		< 3	
* Malignant neoplasm of trachea, bronchus and lung	35	86 (63-114)	11	128 (72-212)
* Pleural mesothelioma	5	379 (149-797)	6	1128 (491-2226)
* Peritoneal mesothelioma	< 3		< 3	
* Malignant neoplasm of ovary (F)			4	77 (26-177)
Malignant neoplasms, stated or presumed to be primary, of lymphoid, haematopoietic and related tissue	13	94 (56-150)	11	103 (58-171)
Diseases of circulatory system	251	123 (111-137)	297	121 (110-134)
Diseases of respiratory system	42	91 (69-118)	25	115 (80-161)
Acute respiratory diseases	< 3		< 3	
Chronic respiratory diseases	37	114 (85-150)	19	165 (108-242)
* Pneumoconiosis	< 3		< 3	
Diseases of digestive system	9	42 (22-73)	11	56 (32-93)
Diseases of genitourinary system	9	85 (44-148)	8	79 (39-143)

* Causes associated with exposure to asbestos.

Table 2

Number of observed cases (Obs), standardized hospitalization ratio adjusted for deprivation (SHR DI), regional reference; 90% confidence interval (CI 90%); period: 2005-2010. Men and women

Diagnosis on discharge	Men		Women	
	Obs	SHR DI (CI 90%)	Obs	SHR DI (CI 90%)
All causes (except complications of pregnancy, childbirth and puerperium)	5149	98 (96-100)	5521	98 (96-101)
Infectious and parasitic diseases	218	80 (71-89)	194	85 (75-95)
All neoplasms	384	89 (82-97)	347	97 (88-106)
* Malignant neoplasm of larynx	7	86 (40-162)	< 3	
* Malignant neoplasm of trachea, bronchus and lung	53	110 (87-139)	13	116 (68-184)
* Pleural mesothelioma	7	261 (122-489)	7	780 (366-1464)
* Malignant neoplasm of ovary (F)			11	80 (45-132)
Malignant neoplasms, stated or presumed to be primary, of lymphoid, haematopoietic and related tissue	37	86 (64-113)	27	75 (53-104)
Diseases of circulatory system	1136	98 (93-103)	971	96 (91-102)
Diseases of respiratory system	959	109 (103-115)	771	115 (109-122)
Acute respiratory diseases	281	80 (72-88)	274	96 (87-106)
Chronic respiratory diseases	196	145 (128-163)	187	242 (214-273)
Pneumoconiosis	7	396 (186-745)	5	1346 (530-2830)
* Diseases of digestive system	1010	89 (84-94)	862	85 (80-90)
Diseases of genitourinary system	298	79 (72-87)	284	89 (81-98)

* Causes associated with exposure to asbestos.

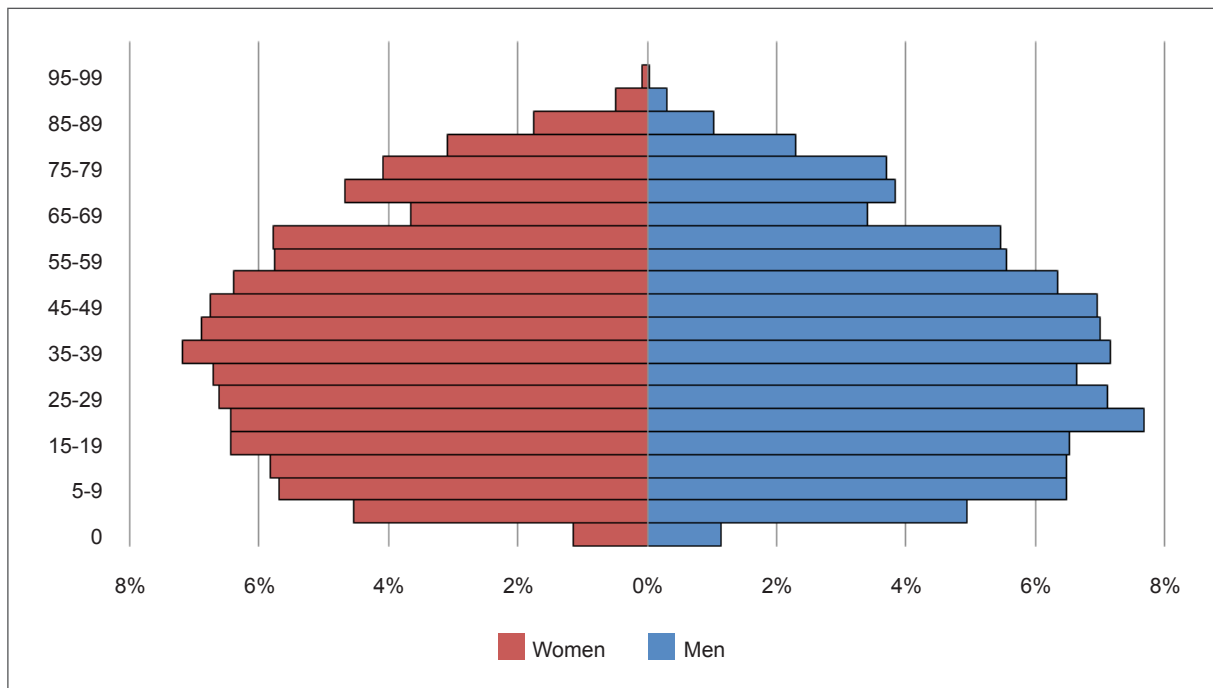


Figure 1
Age pyramid of the population of the municipality of Biancavilla (population at 2011 Census).

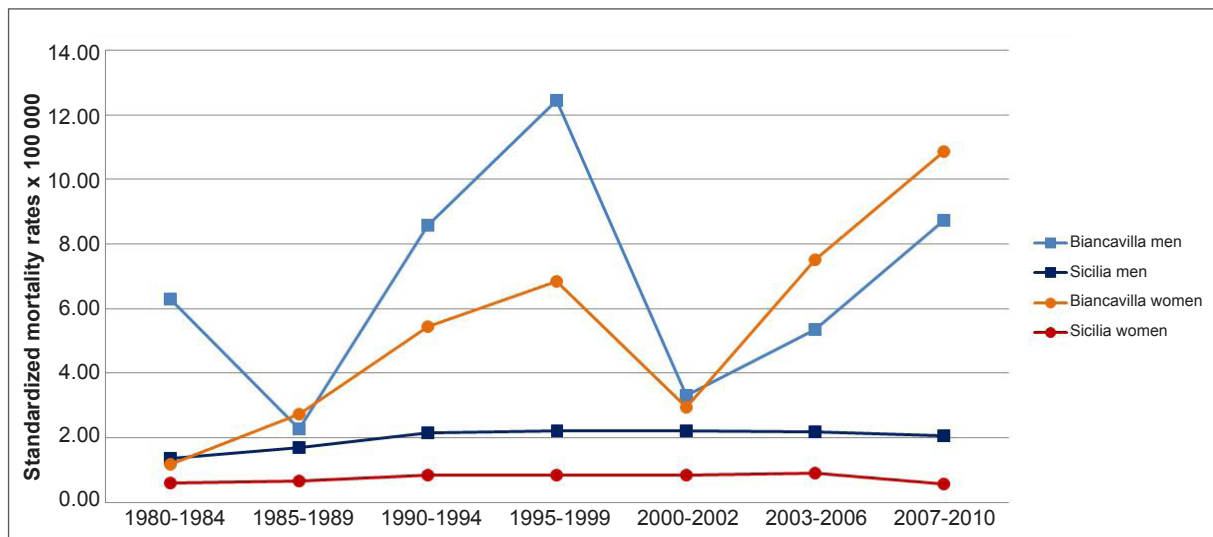


Figure 2
Trend of age-standardized mortality rates from malignant pleural cancer; period: 1980-2010 (2004-2005 not available from ISTAT). Biancavilla and Sicily, by gender.

techniques have been extensively used since the mid-1990s, taking into consideration the “deprivation”; deprivation can be defined as “a state of observable and demonstrable disadvantage relative to the local community or the wider society or nation to which an individual, family, or groups belong” [12]. Deprivation indices are area-based measures of material and social disadvantageous circumstances, that is, indicators of relative deprivation at population level. To account for possible confounding from socioeconomic factors in SENTIERI Project, an *ad hoc* deprivation index (DI) was built using the 2001 National Census variables representing the following socioeconomic domains: education, unemployment,

dwelling ownership, and overcrowding [13, 14]. The SENTIERI DI was used to adjust all the ratios (SMR and SHR) calculated in this paper.

RESULTS

Biancavilla has 23703 inhabitants at the National Census of 2011; the age structure of the population is shown in *Figure 1* (age pyramid); the population of Biancavilla is older than the Sicilian Reference Population ($p < 0.05$).

The number of observed cases, SMRs, SHRs and their confidence interval, for mortality and hospitalization, gender and specific causes are reported in *Tables 1 and 2*.



The analysis of mortality and hospitalization from all cancers does not show excesses; considering specific neoplastic diseases, only mortality and hospitalization from pleural mesothelioma show excesses (mortality: men SMR 379, women SMR 1128; hospital discharges: men SHR 261, women SHR 780). The analysis for respiratory diseases presents excesses of mortality and hospital discharges for chronic respiratory diseases (women: SMR 165, SHR 115) and only for hospital discharges from all respiratory diseases (men SHR 109, women SHR 115) and in particular pneumoconiosis (men: SHR 396, women: SHR 1346). When considering other large groups of diseases, used to describe the health profile of the population of Biancavilla (infectious and parasitic diseases, diseases of circulatory system diseases of digestive system, of genitourinary system), only diseases of the circulatory system show excess ratio of mortality among both genders (men: SMR 123, women: SMR:121).

In Figure 2 time trends of the age-standardized death rates (ASR) from malignant neoplasm of pleura in Biancavilla and in Sicily, by gender, are presented.

The fluctuating trend of ASRs among men and women in Biancavilla is depending on the low number of observed deaths occurred during the whole period (18 men and 16 women data not shown) but it is noteworthy that these values are, along the whole period, always higher than those observed in the Sicily Region.

As to mortality from malignant neoplasms of pleura (MNP) in young adults (≤ 50 years), in the period 1980-2010, 6 deaths *versus* 0.6 expected were observed (SMR = 1003, CI 90% 437-1980). Extending the analysis to municipalities located within a radius of 10 km from Biancavilla, the number of MNP deaths rose to 11 (SMR = 367, CI 90% 206-608).

DISCUSSION AND CONCLUSIONS

This study was conducted adopting the approach developed in the SENTIERI study [13]. The distinguishing feature of SENTIERI Project is the “*a priori* evaluation” of the epidemiological evidence of the causal association for each combination environmental exposure/cause of death selected for the analysis. This approach is interesting, since when performing epidemiologic studies, there is a risk for researchers to become data-driven. This can be the case when commenting results for causes showing an increase, possibly on the basis of statistical significance. In our study the pathologies taken into consideration were those indicated by the literature as associated with asbestos exposure.

When calculating standardized mortality/hospitalization rates, for the reference population the same data of the area units under study are needed: cases and populations stratified by gender and age categories. The reference population should be selected considering two different needs: a) it should be comparable to the studied populations for factors that can affect the health profile with the exception of the contamination at study; the differences in the

health profile between the compared populations should be ideally due only to the differences in environmental exposures, namely, to the contamination; b) it should be sufficiently numerous to obtain stable reference rates also for rare diseases. These two needs have opposite requirements, as the first one is usually negatively correlated with the dimension of the population, while the second one is positively correlated with the dimension of the population. The reference populations should be selected balancing these two needs. Usually one or two populations among the following are selected as reference population: national, and regional, local (*i.e.*, a population composed of populations residing in the neighborhood of the contaminated area). In the SENTIERI approach, and therefore in the present study, the regional population was used as reference.

The present study, as all epidemiological studies based on aggregate data, has limitations, but when considering diseases that according to few studies are considered as associated with the exposure to asbestos, it is noteworthy that both men and women residing in Biancavilla present excess mortality and hospitalization ratios from pleural mesothelioma; regarding the other diseases, excess in mortality from chronic respiratory diseases among women and hospitalization from diseases of the respiratory system, in particular chronic respiratory diseases. Our findings support the etiologic role of fibres with fluoro-edenitic composition in the occurrence of the above mentioned diseases, already observed in other studies [15-17]. Specifically, this report confirms the excess of hospitalization for pneumoconiosis firstly reported by Cernigliano *et al.* [16], that suggests the occurrence of environmental lung fibrosis. Furthermore, both mortality and hospitalizations from pleural mesothelioma present SMR and SHR higher among women than among men; this may reflect a higher level of fiber exposure for women, who are often engaged in activities such as sweeping of floors, balconies and sidewalks located in front of the houses [17].

Finally, the elevated excess mortality from malignant neoplasms of pleura among people aged 50 years or less, both in Biancavilla and among the municipalities surrounding Biancavilla, contributes to corroborate the hypothesis of the health impact of environmental exposure to fluoro-edenite fibres during childhood [18].

To conclude, the overall results of our study are supporting the association of exposure to fibres with fluoro-edenitic composition with mortality and morbidity from asbestos-related diseases.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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Mode of action of fibrous amphiboles: the case of Biancavilla (Sicily, Italy)

Giulia Ballan*, Antonella Del Brocco*, Stefano Loizzo, Alessia Fabbri, Zaira Maroccia, Carla Fiorentini and Sara Travaglione

Dipartimento del Farmaco, Istituto Superiore di Sanità, Rome, Italy

* These authors contributed equally

Abstract

Background. The inhalation of fibrous amphiboles can result in pulmonary fibrosis, lung cancer and mesothelioma. Although these fibres have the same disease-causing potential, their different morphologies and chemical composition can determine different biological activities. An unusual cluster of mesothelioma was evidenced in Biancavilla (Sicily) where no inhabitant had been significantly exposed to asbestos.

Objective. We herein discuss the mechanism of action of amphiboles, focusing on the fibres identified in the study area.

Results. Human lung carcinoma cells have been exposed to two different materials: prismatic fluoro-edenite and fibres with fluoro-edenitic composition. Only in the second case, they exhibit features typical of transformed cells, such as multinucleation, pro-survival activity and pro-inflammatory cytokine release. Accordingly, *in vivo* studies demonstrated that the fibrous sample only could induce a mesotheliomatogenic effect.

Conclusions. Fibres with fluoro-edenitic composition behave similarly to the asbestos crocidolite, whose connection with inflammation and lung cancer is well established.

Key words

- fibrous amphiboles
- asbestos
- fluoro-edenite
- Biancavilla
- mesothelioma

INTRODUCTION

Asbestos is a generic term used to identify six well-known silicate minerals belonging either to amphiboles (amosite, crocidolite, anthophyllite, actinolite, tremolite) or serpentine (crysotile), two families that have in common a fibrous morphology [1]. Some of these minerals were of significant industrial and economic importance and have been widely used [2], especially in building industry. Occupational exposure to asbestos fibres can cause pulmonary fibrosis (asbestosis), lung cancer and malignant mesothelioma (MM) but also non-pulmonary diseases, including peritoneal mesothelioma, ovarian and larynx carcinoma, as well established in both humans and experimental animals [1]. The International Agency for Research on Cancer Asbestos has classified asbestos as belonging to Group I human carcinogens (IARC, 1987).

Nevertheless, asbestos fibres continue to pose an important health concern due to the long latency period of asbestos-induced diseases. Even if commercial use of asbestos has decreased, old asbestos-containing buildings, the importation of asbestos-containing products, as well as non-commercial asbestos and other elongate mineral particles, represent an important environmental problem. Indeed, there is evidence that the inhalation of these fibres can provoke two types of interconnected pathogenetic processes: chronic inflammation

and carcinogenesis, both involving the lung, after deposition of asbestos particles. *In vitro* and *in vivo* studies demonstrate that, although all types of asbestos fibres have the same disease-causing potential, important determinants for the biological activities of these fibres are their dimensions, surface properties, shape and crystallinity, chemical composition, physical durability, exposure route, duration of the exposure, and dose. For example, amphiboles might be more carcinogenic than serpentine [1].

In this context, to explain the increased pathogenicity of the amphiboles crocidolite and amosite in the induction of human mesothelioma, the "amphibole hypothesis" was advanced: while chrysotile fibres appear to dissolve or fragment over time, amphibole asbestos persisting at sites of tumour development, serve as a chronic stimulus necessary for neoplastic growth [3].

MODE OF ACTION OF FIBROUS AMPHIBOLES: STATE OF THE ART

Although the understanding of the sequence of events, starting with mineral fibres and cells' interaction and leading to a disease development is of primary importance, yet the cellular and molecular mechanisms by which asbestos fibres induce cancer or other diseases remain to be clarified.

Most evidence highlight asbestos fibres' ability to: i)

interfere with the mitotic apparatus; ii) stimulate host cells' proliferation; iii) induce genetic and epigenetic alterations, as well as cellular toxicity and fibrosis; iv) produce oxidative stress that results in DNA damage and inflammatory cytokines and growth factors' release [4, 5]. In particular, reactive oxygen species (ROS) are known to be involved in mediating asbestos-induced mesothelial cells injury by causing DNA strand break, lipid peroxidation, and activation of signal transduction pathways [6]. However, *in vitro* cell culture studies have provided paradoxical evidence that exposure of mesothelial cells to asbestos causes cytotoxicity or apoptosis rather than malignant transformation [7]. These authors explain their results hypothesizing that one of the principal mechanisms to generate ROS is associated with the contents of asbestos fibre, especially iron [8]. In fact, it has been shown that the iron associated with asbestos participates, via ROS, in the cell toxicity and probably in MM pathogenesis, but the molecular mechanisms largely remain unknown. In this context, some authors have shown that, in asbestos-exposed cells, molecular oxygen mainly located in mitochondria is converted into O_2^- by reduction catalyzed by the iron. This O_2^- is a moderately reactive species capable of generating H_2O_2 , which in turn can produce highly reactive hydroxyl radicals ($HO\bullet$) via iron-dependent catalytic reactions, Fenton reaction and/or Haber-Weiss reaction [6, 8]. Indeed, the chemical properties of asbestos fibres, especially iron content, can contribute to the formation of ROS that is closely related to asbestos-induced pathogenesis [8]. There exist intrinsic defence systems that counteract ROS toxicity in mammalian cells, including the major iron storage protein ferritin. This protein, which is composed of a heavy chain and a light chain, has enzymatic properties, converting Fe^{2+} to Fe^{3+} , as iron is internalized and sequestered in the ferritin mineral core. This function is an inherent feature of ferritin heavy chain (FHC) subunit that exhibits ferroxidase activity, required for iron sequestration. It has been shown that FHC regulates the intracellular iron which catalyses the formation of toxic ROS. In fact, it was demonstrated that FHC works as an anti-apoptotic protein against toxic asbestos and oxidative stress in human mesothelial cells and MM cells [9]. Indeed, MeT-5A human mesothelial cells stably expressing FHC generated less amount of hydrogen peroxide (H_2O_2), one of the main ROS, after asbestos exposure and were more resistant to apoptosis induced by H_2O_2 compared with the cells transfected with the empty vector. These results suggest the contribution of FHC to apoptosis resistance of the MM cells and the potential role of FHC in the pathogenesis of asbestos-induced mesothelioma.

It is worth noting that the events involved in the complex mechanisms of asbestos-induced diseases are not completely independent one from each other and the contribution of each of them might vary depending on the species and on fibre and disease types.

THE CASE OF BIANCAVILLA'S AMPHIBOLE

An epidemiological survey (carried out from 1988 to 1992) on the mortality due to malignant pleural neo-

plasm in Italy, highlighted an unusual epidemiological cluster of mesothelioma in Biancavilla, a village located in the Etnean Volcanic Complex (Catania, Italy) of eastern Sicily, where no inhabitants had been significantly and professionally exposed to asbestos. The possible cause of such unusual distribution of the pathology was proposed to be the stone quarries located in "Monte Calvario", in the southeast of Biancavilla. All the population, in fact, was exposed to the material derived from stone quarries that had been largely used in the local building industry. Among the minerals present in the benmoreitic lavas extracted from quarries, a new amphibole, initially referred as to an anomalous Na- and F-rich tremolite-actinolite intermediate phase, was discovered. A subsequent crystal-chemistry investigation of the Monte Calvario amphiboles [10] led to the identification of this mineral as "fluoro-edenite", a new end-member of the edenite \bar{P} fluoro-edenite series approved on 30 January 2001 by the CNMMN (Commission on New Minerals and Mineral Names, IMA: cod. 2000-049) [11].

In vitro studies

In this context, we have performed studies aimed at analyzing the effects of the different forms of fluoro-edenite in A549 cells, a tumour cell line from human lung carcinoma with properties of alveolar epithelial cells (Table 1). This cell line is largely acknowledged as a suitable model to study the interaction of environmental particulates with lung epithelial cells, epithelia representing the first line of defence against air dispersed harmful substances *in vivo*.

In the first study [4], we have focused on the prismatic form of fluoro-edenite, identified and sampled in the quarry of Mount Calvario. We found that epithelial cells showed a remarkable tropism toward fluoro-edenite material. In fact, the presence of fluoro-edenite in the culture medium induced an active response by epithelial cells, which developed membrane ruffles and filopodia. These structures established a first contact with the fibres, then progressively surrounded and subsequently wrapped around the material, thus engulfing it into the cell cytoplasm through a phagocytic-like process (Figure 1b). Surprisingly, the organization of the actin cytoskeleton, which represents the main engine of phagocytosis and one of the key target for a huge number of toxicants, was not handled by the contact with fluoro-edenite and its architecture remained unchanged, appearing well organized in stress fibres both in control and in treated A549 cells (Figures 1d and 1e). However, the prismatic fluoro-edenite was able to interfere with epithelial cell physiology, by reducing the proliferation rate without perturbing the passage of cells through the different phases of the cell cycle. Moreover, in analogy with other asbestos fibres, fluoro-edenite treatment ensued IL-6 secretion, a multifunctional cytokine with immunoregulatory and pro-inflammatory effects. Hence, in this paper we did not evidence any particular effects of the prismatic fluoro-edenite that could be somehow related to cellular transformation. In accordance with these findings, it has been reported that intraperitoneal administration of prismatic fluoro-

**Table 1**

Effects of prismatic fluoro-edenite and fibres with fluoro-edenitic composition, in comparison with crocidolite, in A549 cells

Fibre type cellular effects on:	Prismatic fluoro-edenite	Fibres with fluoro-edenitic composition	Crocidolite
- <i>actin cytoskeleton</i>	No change in the actin network, which remains well organized in stress fibres	Dramatic changes in the actin network structure	Dramatic changes in the actin network structure
- <i>phagocytic-like activity</i>	Cells develop actin-rich protrusions from the plasma membrane (ruffles and filopodia) to wrap the fibres	Close interaction and strong tropism of cells towards the amphibole material. These events are accompanied by an arising of actin-rich membrane ruffles from the cell surface	Evident close interaction and strong tropism of cells towards the amphibole material thanks to a plethora of actin-rich membrane ruffles stemming from the cell surface
- <i>antiproliferative capacity</i>	Dramatic decrease in the number of viable epithelial cells without arrest or interference with the cell cycle	Dramatic decrease in the number of viable epithelial cells without arrest or interference with the cell cycle	Dramatic decrease in the number of viable epithelial cells without arrest or interference with the cell cycle
- <i>induction of cytokines expression</i>	Increase of IL-6 secretion in the supernatant	Increase of both IL-6 and IL-8 secretion in the supernatant	More consistent secretion of IL-6

edenite failed in inducing the development of mesothelioma in experimental rats [12].

In contrast, when fibres with fluoro-edenitic composition are concerned, the same authors [12] reported their strong mesotheliomatogenic effect on the peritoneum and, to a much lesser extent, on the pleura. In keeping, when the activity of such a fibrous form was investigated in the same cell line, the A549 cells [13], we found that its effects were very different from those induced by prismatic fluoro-edenite. In particular, the newly characterized fibrous material provoked dramatic changes in the cell morphology, promoting the spread out of cells and the multinucleation [13], which most probably was caused by a failure in the formation of the actin contractile ring at the last stage of cytokinesis (Figures 1c and 1f). A close link between actin derangement and multinucleation has been already reported as occurring in cells challenged with renowned actin cytoskeleton-perturbing agents, either promoting the assembly or the breakdown of the cytoskeleton. Importantly, multinucleation has been indicated as a marker of cell transformation.

The above changes in morphology, which were accompanied by a dramatic decrease in the number of viable cells, did not interfere with the passage of the multinucleated cells through the cell cycle [13]. Moreover, cells were not condemned to cell death since no signs of apoptosis or changes in the expression of pro- or anti-apoptotic markers were evidenced. An important aspect that has to be taken into account is the fact that fibres with fluoro-edenitic composition promoted the secretion of IL-6, a multifunctional cytokine with immunoregulatory and pro-inflammatory effects, and IL-8, a potent chemo-attractant for polymorphonuclear leukocytes [13]. Interestingly, these fibres also regulate the expression of phospho-retinoblastoma protein to trigger a network of signals strictly connected with cell proliferation and neoplastic cell transformation [14]. Finally, a study by Cardile and colleagues reveals the

involvement of nitric oxide (NO) in the cytotoxic and genotoxic effects caused by fibres with fluoro-edenitic composition in the mouse monocyte-macrophage cell line J774. These effects are in line with those reported for other asbestos and have been ascribed to the permanence of the fibres in cultures for a long period of time, suggesting that inflammatory disorders apparently increase the risk for lung cancer induced by fluoro-edenite [15].

All the results reported are suggestive of a putative pro-transforming activity, which is strengthened by the fact that in lung epithelial cells most of the results obtained for fibres with fluoro-edenitic composition were comparable to those obtained with crocidolite, used as a "positive control". Crocidolite is indeed one of the best-characterized amphibole asbestos, with well-depicted cytotoxic properties and accredited carcinogenic capacity [16] and whose connection with severe inflammation and cancer of the lung is renowned. The ability of lung epithelial cells to progress throughout the cell cycle despite the presence of several nuclei in the same cytoplasm, could account for the carcinogenic properties of fibres with fluoro-edenitic composition and of crocidolite. In fact, the uninterrupted proliferation of multinucleated cells inevitably leads to aneuploidy, this nuclear content alteration being largely known to participate to cancer development.

In vivo studies

It is also important to underline that fibres with fluoro-edenitic composition have been found both in a patient died for mesothelioma [17] and in the pulmonary parenchyma of sheep living in Biancavilla area [18]. To strengthen these epidemiological data, an *in vivo* study has been carried out by Belpoggi and co-workers [19]. In this paper, Sprague-Dawley rats (males and females, at the same percentage) were treated "*una tantum*" with i) prismatic fluoro-edenite, ii) fibres with fluoro-edenitic composition or iii) the vehicle. A single

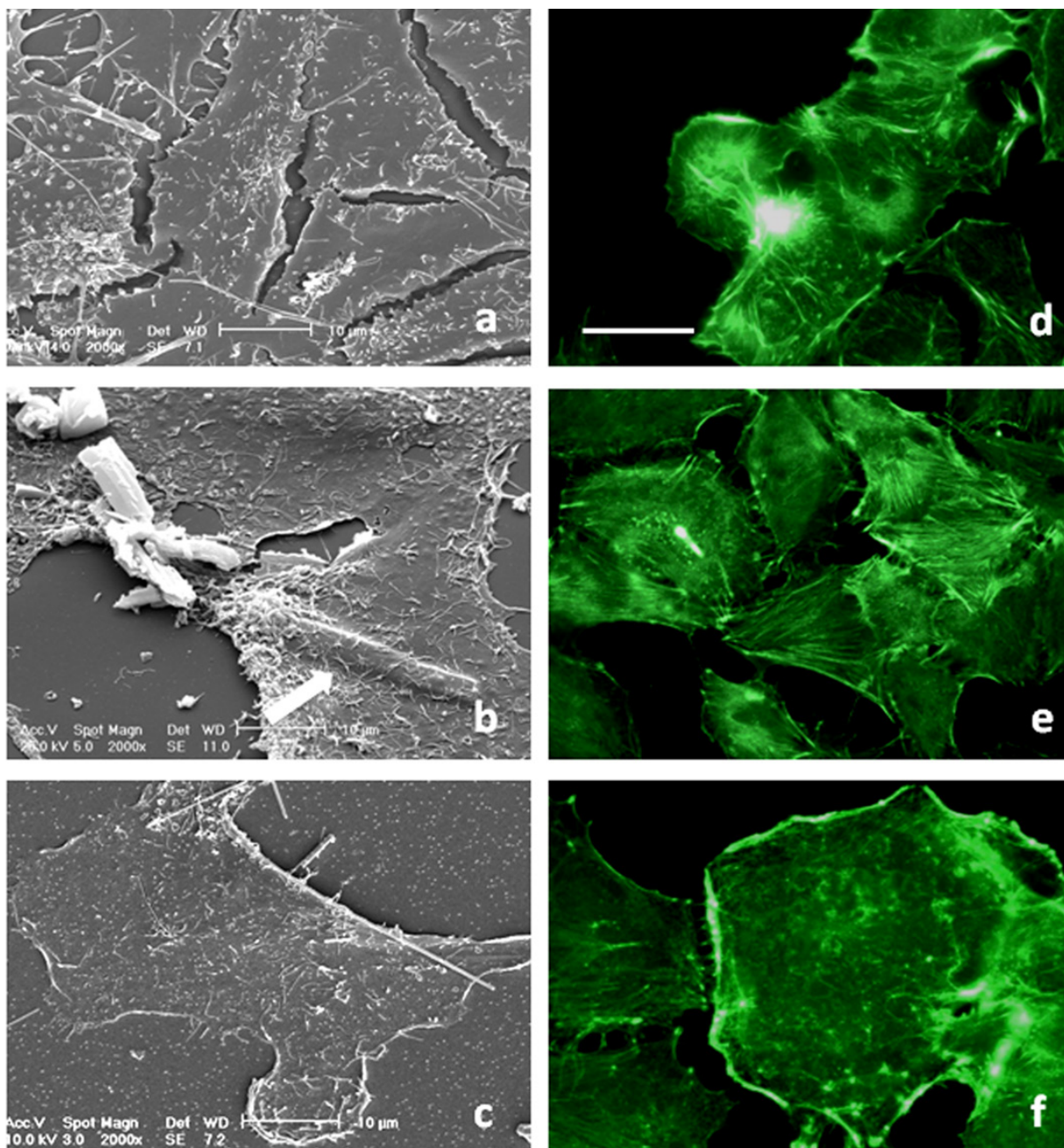


Figure 1
Morphological effects of prismatic fluoro-edenite and fibres with fluoro-edenitic composition on A549 lung epithelial cells.

(a-c) Scanning electron micrographs of (a) control cells, and (b, c) cells treated with (b) prismatic fluoro-edenite or (c) fibres with fluoro-edenitic composition.

(d-f) Fluorescence micrographs of cells stained with FITC-Phalloidin for F-actin detection. (d) Control cells; (e, f) cells treated with (e) prismatic fluoro-edenite and (f) fibres with fluoro-edenitic composition.

Although engulfed by an epithelial cell (arrow), the prismatic fluoro-edenite (b) does not induce morphological changes, in term of cell surface appearance (b) and F-actin distribution (e). By contrast, the exposure to fibres with fluoro-edenitic composition causes a striking modification of the cell morphology, mainly consisting in the enlargement of the cell body and the smoothing of the cell surface (c). These changes were accompanied by a derangement of the F-actin network (f).

dose was used (25 mg), administered by intrapleural or intraperitoneal injection. In line with previous preliminary results [15], these experiments have evidenced that fibres with fluoro-edenitic composition have a mesotheliomatogenic potential, independently from sex. Also,

they showed that the injection into peritoneum caused effects much stronger than those observable after injection into pleura. In contrast, treatment with the vehicle only or with the prismatic form of fluoro-edenite failed to induce a mesotheliomatogenic response, in accordance

to the criteria reported in Ref. [20]. Strikingly, the effects provoked by fibres with fluoro-edenitic composition are long-term and this proves the bio-persistence of fibres *in vivo*.

CONCLUSIONS

Nowadays, a plethora of evidence suggests a close link between cancer and chronic inflammation. In humans, inflammatory diseases in the lung can be associated with asbestos fibres exposure [21]. Cytokines and growth factors derived from alveolar macrophages are strongly implicated as mediators of asbestos-induced pathophysiological responses. Although best handled by specialized cells like macrophages, inflammation also characterizes the response of epithelial cells to external danger, which hence produce an array of mediators transmitting cellular signals. Moreover, the inflammatory signalling in epithelial cells results in their inappropriate survival and transformation. Hence, the tendency of fibres with fluoro-edenitic composition to act as a transforming agent is also supported by the ability of treated epithelial cells to produce pro-inflammatory cytokines. The inflammatory response is a double-edged sword: it explains acute disease severity but also the attempt of the host cells to obtain the clearance from the causative agent of inflammation. Possibly, surviving cells that continue to release IL-6 and IL-8, could trigger a chronic inflammatory process, a phenomenon known to be tightly related to many types of cancer.

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