

# FLUORO-EDENITE

## 1. Exposure Data

### 1.1 Chemical and physical properties

#### 1.1.1 Nomenclature

*Mineral name:* Fluoro-edenite [in allusion to its composition with dominant fluorine and its relationship with edenite] ([Mindat, 2014](#))

*Agreed name:* Fluoro-edenite fibrous amphibole, with compositional variability appearing to be similar to that of calcic amphiboles such as tremolite, winchite, and richterite

*Synonyms:* Fluor edenite; fluoredenite; IMA1994-059; IMA2000-049 ([Jambor et al., 1999](#); [Mindat, 2014](#))

*Chemical formula:*  $\text{NaCa}_2\text{Mg}_5\text{Si}_7\text{AlO}_{22}(\text{F},\text{OH})_2$  ([INS-Europa, 2014](#))

*Empirical formula:*

$\text{Na}_{0.9}\text{K}_{0.2}\text{Ca}_{1.6}\text{Mg}_{4.7}\text{Fe}^{2+}_{0.2}\text{Fe}^{3+}_{0.1}\text{Si}_{7.4}\text{Al}_{0.6}\text{O}_{22}\text{F}_2$  ([INS-Europa, 2014](#))

*Relative molecular mass:* 837.63 ([INS-Europa, 2014](#))

#### 1.1.2 General description

Fluoro-edenite occurs in the volcanic products of the Monte Calvario locality of Biancavilla on the flanks of Mount Etna, Sicily, Italy, and is found in the cavities of benmoreitic lava metasomatized by hot fluids rich in fluorine. The “Monte

Calvario” quarry has been mined extensively for a sandy volcanic material that is used in the local building industry. This geological area is made up of domes and dikes associated with “auto-clastic breccia,” a fine-grained material in which fluoro-edenite has been found that was initially classified as intermediate phases between tremolite and actinolite ([Comba et al., 2003](#); [Mazziotti-Tagliani et al., 2009](#)). Fluoro-edenite is found as both prismatic and acicular millimetre-scale crystals and asbestiform (fibrous) fibres that are present as loose materials in rock cavities. The National Institute for Occupational Safety and Health (NIOSH) of the USA provided definitions for the morphology of elongated mineral particles: acicular – a mineral comprised of fine needle-like crystals; prismatic – a crystal with one dimension markedly longer than the other two; asbestiform – a mineral that is fibrous and composed of separable fibres ([NIOSH, 2011](#)).

Fluoro-edenite is generally associated with potassium feldspars and plagioclase, quartz, clino- and orthopyroxenes, fluoro-apatite, ilmenite, and abundant haematite ([Gianfagna & Oberti, 2001](#)). Due to its high content of fluorine and sodium, in comparison with that of tremolite and actinolite fibres, a new end-member of the amphibole calcic group of the edenite → fluoro-edenite series has been approved by the Commission on New Minerals and Mineral Names of the International Mineralogical Association (IMA, code 2000-049) ([Gianfagna & Oberti, 2001](#); [Comba et al., 2003](#)). The

mineralization process of fluoro-edenite has been suggested to have occurred elsewhere in other volcanic areas (Comba et al., 2003).

Fluoro-edenite is found in materials extracted from the Il Calvario quarry that are used in the local building industry (walls, plaster, mortar, and concrete) and in soil used to pave roads, plazas, and other areas (Paoletti et al., 2000; Burrigato et al., 2005). The quarry has been mined since at least the 1950s, with a peak in production around the 1960s and 1970s (Gianfagna et al., 2003; Bruno et al., 2006), and fibrous amphiboles have also been found outside the quarry around Biancavilla (Bruni et al., 2014).

Biancavilla is the first area in which the occurrence of amphiboles fibres in a volcanic environment was reported (Burrigato et al., 2005). However, a “fluor-edenite” compound was also found in the cavities of the Ishigamiyama lava dome of the Kimpo volcano, Kumamoto, south-western Japan, as acicular crystals several millimetres in length and associated with tridymite and magnetite (Tomita et al., 1994; Makino et al., 1996; Jambor et al., 1999).

### 1.1.3 Chemical and physical properties

*Density (specific gravity):*  $D_{\text{cal}} = 3.09 \text{ g/cm}^3$

*Hardness (Mohs' scale):* 5–6 (between apatite and orthoclase)

*Cleavage:* perfect on {110}

*Fracture:* conchoidal

*Lustre:* vitreous to resinous

*Diaphaneity (transparency):* transparent

*Colour:* yellow to intense yellow (prismatic); yellowish and grey-whitish (fibrous)

*Streak:* grey-white, yellowish white, parallel to the *c*-axis

*Tenacity:* brittle

*Refractive index:* 1.60–1.63

From Mindat (2014)

#### (a) Chemical properties

##### (i) Chemical composition

The chemical composition of fluoro-edenite crystals from Biancavilla is variable, as shown by scanning electron microscopy (SEM)-X-ray microanalysis in several different studies (see Table 1.1). The chemical composition of “fluor-edenite” acicular crystals from Kumamoto which differs to a certain extent from that of Biancavilla is (wt%): SiO<sub>2</sub>, 48.92; TiO<sub>2</sub>, 1.32; Al<sub>2</sub>O<sub>3</sub>, 5.43; FeO, 7.17; MnO, 0.18; MgO, 19.14; CaO, 11.01; Na<sub>2</sub>O, 2.77; K<sub>2</sub>O, 1.03; F, 3.19; and Cl, 0.12 (Makino et al., 1996; Jambor et al., 1999). The chemical formula of the Kumamoto fluor-edenite is  $\text{K}_{0.190} \text{Na}_{0.776} \text{Ca}_{1.704} \text{Mg}_{4.121} \text{Fe}^{2+}_{0.866} \text{Ti}_{0.143} \text{Mn}_{0.022} \text{Al}_{0.924} \text{Si}_{7.066} \text{O}_{22} \text{F}_{1.455} \text{OH}_{0.545}$  (Tomita et al., 1994) and the crystal-chemical formula of the fluoro-edenite from Biancavilla is  $^{\text{A}}(\text{Na}_{0.56} \text{K}_{0.15}) ^{\text{B}}(\text{Na}_{0.30} \text{Ca}_{1.62} \text{Mg}_{0.03} \text{Mn}_{0.05}) ^{\text{C}}(\text{Mg}_{4.68} \text{Fe}^{2+}_{0.19} \text{Fe}^{3+}_{0.10} \text{Ti}^{4+}_{0.03}) ^{\text{T}}(\text{Si}_{7.42} \text{Al}_{0.58}) \text{O}_{22} \text{O}^3(\text{F}_{1.98} \text{Cl}_{0.02})_2$  (Gianfagna & Oberti, 2001). Although fibrous and prismatic fluoro-edenite have similar chemical compositions, some compositional differences exist between prismatic fluoro-edenite and fibres with regard to their contents of magnesium and calcium (higher in the prismatic variety) and silica and iron (higher in fibres), and the variability in composition is greater in fibrous species (Gianfagna et al., 2007). Despite these differences, optical, chemical, and X-ray analyses of the fibres confirm their similarity to the yellow prismatic fluoro-edenite. According to the Leake amphibole classification (Leake et al., 1997), the composition of the fibres ranged from fluoro-edenite (60%) to a lower proportion of winchite (24%), tremolite (12%), and richterite (4%) (Fig. 1.1). The variable chemical composition of fluoro-edenite and the presence of different components complicate the classification of the fibres and the definition of their mineral species (Andreozzi et al., 2009), similarly to the amphiboles observed in Montana (USA) and Libby amphiboles (winchite, ~84%;

**Table 1.1 Chemical composition of samples of fluoro-edenite from Biancavilla, Sicily, Italy (wt%)**

Component	Sample 1	Sample 2	Sample 3 F <sup>a</sup>	Sample 3 P <sup>b</sup>	Sample 4 <sup>c</sup>	Sample 5 F <sup>a,d</sup>	Sample 5 P <sup>b,e</sup>
SiO <sub>2</sub>	52.92	53.08	54–56	52.31	52.66–54.12	53.85	52.83
TiO <sub>2</sub>	0.29	NR	ND	0.28	ND–0.06, 0.02–0.03	0.59	0.55
Al <sub>2</sub> O <sub>3</sub>	3.53	3.65	3.8–4.2	3.87	1.95–2.91	3.55	3.81
FeO <sub>t</sub>	2.60	2.67 <sup>f</sup>	2.6–3.2	2.65	3.59–5.98 <sup>g</sup>	4.25 <sup>h</sup>	2.25 <sup>i</sup>
MnO	0.46	NR	ND	0.40	0.44–0.56	0.53	0.46
MgO	22.65	22.62	19–23	22.78	20.50–22.63	20.44	23.60
CaO	10.83	10.71	7–8	10.86	8.48–10.20	10.06	10.73
Na <sub>2</sub> O	3.20	3.33	4.2–5	3.10	2.29–3.10	2.96	3.04
K <sub>2</sub> O	0.84	1.12	1.2–1.4	0.89	0.52–0.54	0.88	0.82
F	4.35	4.54	4.2–4.7	4.47	4.40	4.46	4.11
Cl	0.07	NR	ND	0.08	0.06	0.08	0.05

<sup>a</sup> F, amphibole fibres

<sup>b</sup> P, prismatic fluoro-edenite

<sup>c</sup> Range of average chemical composition of four samples of fibrous amphiboles corresponding to 52 (1<sup>st</sup> sample), 40 (2<sup>nd</sup> sample), 42 (3<sup>rd</sup> sample), and 58 (4<sup>th</sup> sample) analyses

<sup>d</sup> Average chemical composition derived from 25 analyses of the fibrous amphibole

<sup>e</sup> Average chemical composition from three analyses of the prismatic fluoro-edenite crystal

<sup>f</sup> FeO, 1.71%; Fe<sub>2</sub>O<sub>3</sub>, 0.95%

<sup>g</sup> FeO, 0.29–2.50%; Fe<sub>2</sub>O<sub>3</sub>, 3.26–5.25

<sup>h</sup> FeO, 1.39%; Fe<sub>2</sub>O<sub>3</sub>, 3.17%

<sup>i</sup> FeO, 0.19%; Fe<sub>2</sub>O<sub>3</sub>, 2.29%

Al<sub>2</sub>O<sub>3</sub>, aluminium oxide; CaO, calcium oxide; Cl, chlorine; F, fluorine; FeO, ferrous oxide; FeO<sub>t</sub>, iron oxides; Fe<sub>2</sub>O<sub>3</sub>, ferric oxide; K<sub>2</sub>O, potassium oxide; MgO, magnesium oxide; MnO, manganese oxide; Na<sub>2</sub>O, sodium oxide; ND, not detected; NR, not reported; SiO<sub>2</sub>, silicon dioxide; TiO<sub>2</sub>, titanium dioxide; wt, weight

Sample 1, From [Gianfagna & Oberti \(2001\)](#); Sample 2, From [INS-Europa \(2014\)](#); Sample 3, From [Gianfagna et al. \(2003\)](#); Sample 4, From [Pacella \(2009\)](#), [Mazziotti-Tagliani et al. \(2009\)](#); Sample 5, From [Gianfagna et al. \(2007\)](#)

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richterite, ~11%; and tremolite, ~6%) ([Meeker et al., 2003](#)).

The Fe<sup>3+</sup>/Fe<sub>total</sub> ratios evaluated by Mössbauer spectroscopy reflect the different iron oxidation states, with a greater prevalence of Fe<sup>3+</sup> than Fe<sup>2+</sup> ([Mazziotti-Tagliani et al., 2009](#)). The reactivity of the fibrous samples, measured by the production of the [DMPO, HO]· radical adduct in electron paramagnetic resonance spectroscopy, was lower than that of UICC crocidolite (highly reactive) but comparable with that of fibrous tremolite ([Fantauzzi et al., 2012](#)).

### (ii) Crystal structure

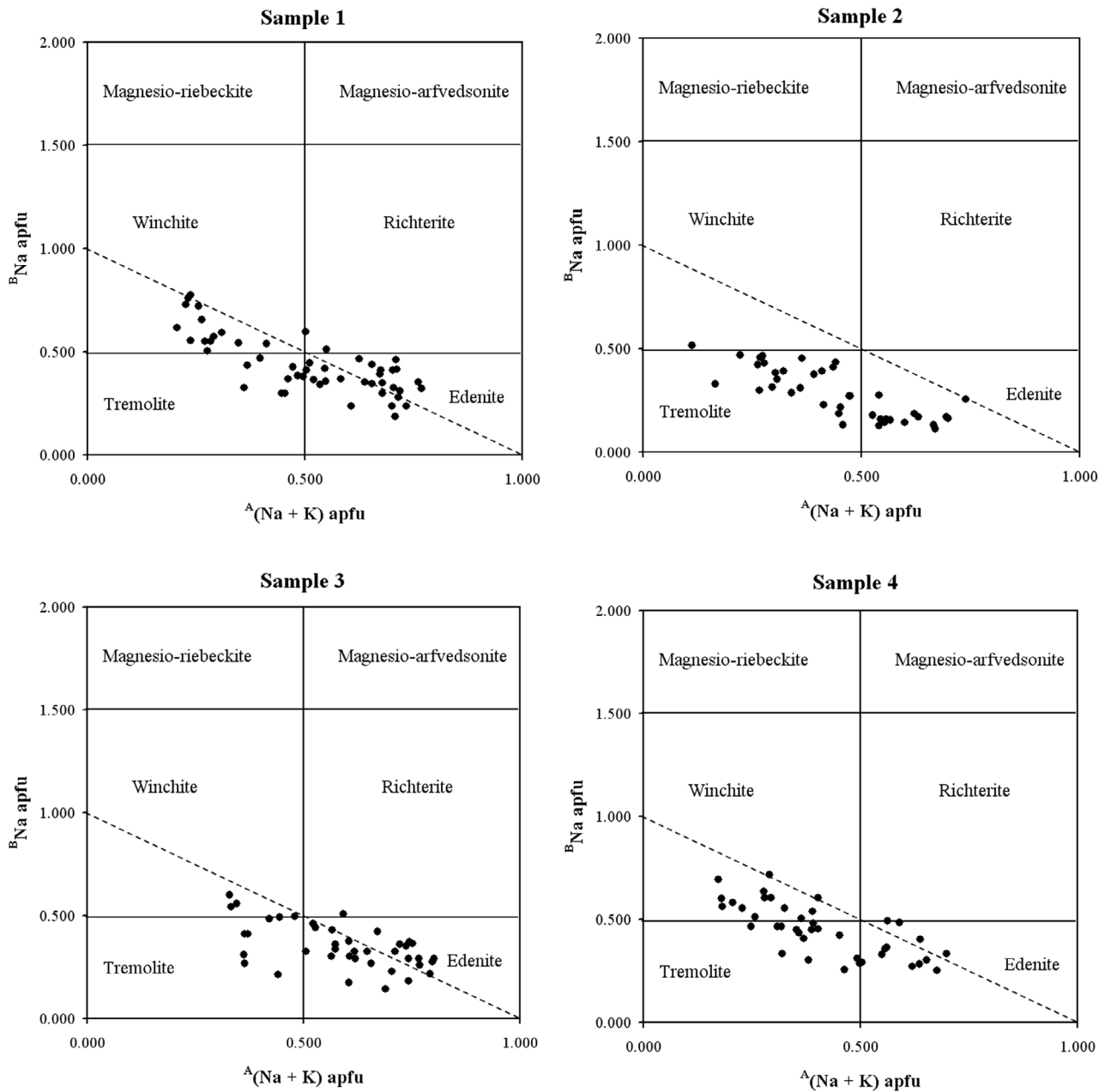
Relevant mineralogical data (unit-cell parameters) for prismatic and fibrous fluoro-edenite described in various studies are presented in [Table 1.2](#). Fluoro-edenite is monoclinic, with

space group C2/m and a space group number of 12. In plane-polarized light, fluoro-edenite is birefringent (first order), biaxial negative,  $\alpha = 1.6058(5)$ ,  $\beta = 1.6170(5)$ ,  $\gamma = 1.6245(5)$ ,  $2V_{\text{calc}} = 78.09^\circ$ , with no visible pleochroism ([Gianfagna & Oberti, 2001](#)), and refractive indices vary from 1.60 to 1.63. Fibrous and prismatic fluoro-edenite are quite similar according to optical, chemical, and Rietveld analyses ([Burrigato et al., 2005](#)).

### (b) Physical properties

Different morphologies that have been found for fluoro-edenite are shown in [Fig. 1.2](#). Biancavilla fluoro-edenite is transparent, deep yellow with prismatic to acicular properties. Fibrous and asbestiform fluoro-edenite amphibole fibres are also found in abundance as loose

**Fig. 1.1 Composition of four samples of fluoro-edenite fibrous amphibole from Biancavilla, plotted against  $A(\text{Na} + \text{K})/\text{BNa}$ , according to the classification of [Leake et al. \(1997\)](#)**



From [Mazziotti-Tagliani et al. \(2009\)](#) © Mazziotti-Tagliani et al. 2009. License: CC BY 4.0

**Table 1.2 Unit-cell parameters for fluoro-edenite**

Lattice parameters (Å)			Crystallographic axe $\beta$	Volume (Å <sup>3</sup> )	Morphology	References
a	b	c				
9.847(2)	18.017(3)	5.268(2)	104.84(2)°	903.45	Prismatic	<a href="#">Gianfagna &amp; Oberti (2001)</a>
9.8445(3)	18.0091(6)	5.2772(2)	104.813(2)°	904.5(6)	Prismatic	<a href="#">Gianfagna et al. (2003)</a>
9.815(1)	17.992(3)	5.2733(6)	104.547(9)°	901.4(3)	Fibrous	<a href="#">Gianfagna et al. (2003)</a>
9.8125(3)	18.0188(6)	5.2781(2)	104.620(2)°	903.00(5)	Fibrous	<a href="#">Gianfagna et al. (2007)</a>
9.8056(4)	18.0105(7)	5.2725(2)	104.406(3)°	901.86(6)	Fibrous	<a href="#">Andreozzi et al. (2009)</a>
9.8112(3)	18.0162(6)	5.2774(2)	104.624(2)°	902.61(5)	Fibrous	<a href="#">Andreozzi et al. (2009)</a>
9.8272(1)	17.9899(2)	5.2756(1)	104.596(1)°	902.57(2)	Fibrous	<a href="#">Andreozzi et al. (2009)</a>
9.7935(3)	17.9728(5)	5.2746(1)	104.403(2)°	899.23(5)	Fibrous	<a href="#">Andreozzi et al. (2009)</a>
9.861(4)	18.05(1)	5.282(1)	104.841(1)°	908.9(6)	Acicular	<a href="#">Tomita et al. (1994)</a> , <a href="#">Jambor et al. (1999)</a>

Å, 0.1 mm

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fibres with variable lengths of up to 100–150  $\mu\text{m}$  and a thickness or width < 1  $\mu\text{m}$  (200–600 nm) in the pores of grey-red altered benmoreitic lavas ([Gianfagna et al., 2003](#); [Mazziotti-Tagliani et al., 2009](#)).

Fluoro-edenite fibres are highly asymmetrical (thickness, < 1  $\mu\text{m}$ ; length, > 10  $\mu\text{m}$ ); the shorter fibres are rigid and hard, whereas longer fibres are tensile, elastic, and flexible. The dimensions of the fibres are micrometric to submicrometric and correspond to respirable fibres ([Mazziotti-Tagliani et al., 2009](#)).

## 1.2 Sampling and analytical methods

See [Table 1.3](#)

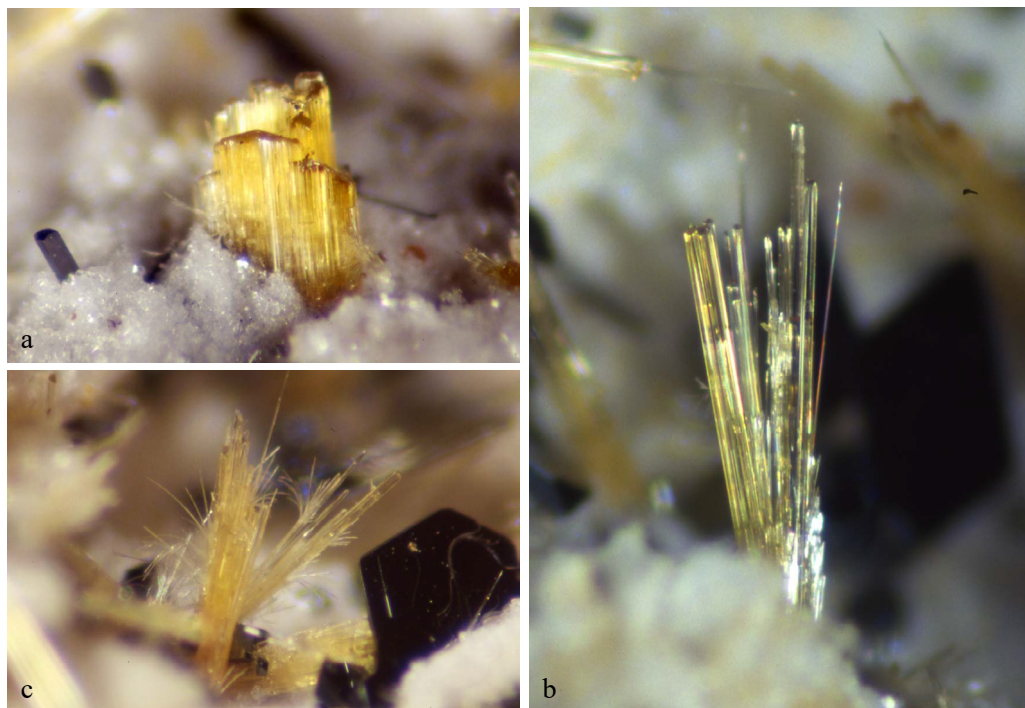
The sampling and analytical methods for fluoro-edenite are identical to those for conventional asbestos. Bulk samples are prepared by removing the debris and grinding the remainder in an agate mortar to produce fine particles, followed by further processing with a mesh or gravimetric sedimentation in water. The resulting suspension is filtered and then mounted for observation using an appropriate analytical device. Air samples are obtained using a vacuum

pump equipped with a membrane filter to obtain a representative air volume, and the filter is then processed according to the analytical method. Biological specimens, such as lung tissues, lymph nodes, and sputum, are digested with sodium hypochlorite or hydrogen peroxide or a combination thereof, and the mineral components are recovered on a filter for analysis. The tissue samples are ashed using a low-temperature plasma and then filtered before analysis.

All processed samples on filters can be analysed using a phase-contrast microscope to count the fibres, according to [WHO \(1997\)](#) or NIOSH method 7402 ([NIOSH, 1994a](#)).

The sample-loaded filters can also be mounted on a stub and analysed using SEM-energy dispersive X-ray analyser (EDX) ([Bruni et al., 2006](#); [Putzu et al., 2006](#)) or mounted on a transmission electron microscopy (TEM) grid and analysed using TEM-EDX according to NIOSH method 7402 ([NIOSH, 1994a](#)). TEM allows the analysis of crystal structure and the identification of mineral fibres using electron diffraction and a comparison with reference minerals.

Powdered bulk samples can be analysed using X-ray diffraction based on NIOSH method 9000 ([NIOSH, 1994b](#)). To obtain the weight percentage

**Fig. 1.2 Morphology of fluoro-edenite crystals: (a) prismatic; (b) acicular; (c) fibrous**

From [Gianfagna et al. \(2003\)](#)

of fluoro-edenite or other amphiboles, standard fluoro-edenite or amphiboles should be prepared.

### 1.3 Production and use

Fluoro-edenite only occurs naturally. It is a contaminant of an ore that is extensively used in the building industry in Sicily, Italy (see Section 1.1.2).

### 1.4 Environmental occurrence

#### 1.4.1 Natural occurrence

Fluoro-edenite is a newly defined mineral species ([Gianfagna & Oberti, 2001](#); [Comba et al., 2003](#); [Gianfagna et al., 2003](#)) that is found near Biancavilla in eastern Sicily, Italy, located in a volcanic area near Mount Etna. Early environmental investigations of the area in and around

Biancavilla identified local quarries in a nearby area called Monte Calvario as the primary source of fluoro-edenite in the locality ([Paoletti et al., 2000](#)). Fluoro-edenite fibres have also been identified occurring naturally in and around the larger Biancavilla area ([Bruni et al., 2006](#)). According to [Comba et al. \(2003\)](#), the complex volcanic processes that produced the fluoro-edenite near Biancavilla may not be unique and may have occurred elsewhere.

#### 1.4.2 Air

Fluoro-edenite fibres have been found in air samples in Biancavilla ([Famoso et al., 2012](#); [Bruni et al., 2014](#)) and are considered to originate from the local quarry products ([Manna & Comba, 2001](#)) that have been used in building materials for local structures since at least the 1950s ([Bruni et al., 2006](#)). Unpaved roads made

**Table 1.3 Selected methods of analysis of fluoro-edenite in various matrices**

Sample matrix	Sample preparation	Assay method	Reference
<i>Bulk sample</i>			
Volcanic materials	Suspension filtered on polycarbonate filter 25 mm (0.4 µm pore), mounted on SEM-stub	SEM-EDX	<a href="#">Bruni et al. (2006)</a>
Plasters and mortar	Suspension filtered on polycarbonate filter 25 mm (0.4 µm pore), mounted on SEM-stub	SEM-EDX	<a href="#">Bruni et al. (2006)</a>
<i>Air sample</i>			
	Polycarbonate filter 25 mm (0.8 µm pore), mounted on SEM-stub	SEM-EDX	<a href="#">Bruni et al. (2006)</a> , <a href="#">Famoso et al. (2012)</a>
	Acetone–triacetin-treated filter	PCOM	<a href="#">Famoso et al. (2012)</a>
<i>Biological sample</i>			
Human lung	Digestion of lung tissue; filtration on polycarbonate filter 25 mm (0.45 µm pore), mounted on SEM-stub	SEM-EDX	<a href="#">Bruni et al. (2006)</a>
Sputum	Digestion of sputum; filtration through mixed cellulose ester filter (25 mm, 0.45 µm pore), mounted on SEM-stub or slide	PCOM SEM-EDX	<a href="#">Putzu et al. (2006)</a> <a href="#">Putzu et al. (2006)</a>
Sheep lung sample	Chopped with scissors and lancet; lipid dissolution by acetone; filtration through polycarbonate filter 25 mm (0.8 µm pore), washed in pure water	SEM-EDX	<a href="#">DeNardo et al. (2004)</a>
Sheep lung lymph nodes	SEM-stub	SEM-EDX	<a href="#">Rapisarda et al. (2005)</a>

EDX, energy dispersive X-ray analyser; PCOM, phase contrast optical microscopy; SEM, scanning electron microscopy  
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from local quarry products have also been a primary source of airborne fluoro-edenite fibres ([Manna & Comba, 2001](#)). [Bruni et al. \(2014\)](#) described three environmental sampling surveys that were conducted in Biancavilla, in 2000, 2004–05, and 2009–13 and limited the analyses to those with data obtained by SEM. Outdoor samples taken in 2000 (before mitigation efforts) showed amphibole concentrations (of unknown average age) ranging from 0.4 to 8.2 fibres/L, with a mean of 1.76 fibres/L. Peak concentrations were associated with the use of unpaved roads by heavy traffic with concentrations as high as 93–183 fibres/L. Indoor sampling during the same period identified concentrations ranging from < 0.4 fibres/L to 4.8 fibres/L, with a mean of 1.18 fibres/L. The outdoor sampling conducted in 2004–05 demonstrated amphibole concentrations ranging from 0.01 fibres/L to 4.19 fibres/L, with a mean of 0.35 fibres/L, and more recent

samplings yielded mean concentrations of 0.46 fibres/L in 2009 and 0.1 fibres/L in 2013.

Although the sampling period was not noted, and therefore these sample may not be independent of those mentioned by [Bruni et al. \(2014\)](#), [Famoso et al. \(2012\)](#) reported the results obtained from 860 air samples collected in a 3.3-km<sup>2</sup> area of Biancavilla and analysed by phase-contrast optical microscopy and SEM-EDX; in 21% of samples, concentrations greater than 0.4 fibres/L were detected while 6% of samples contained concentrations greater than 0.8 fibres/L.

#### 1.4.3 Water

Water flow is undoubtedly important to the fate and transport of fibres from the quarries near the Monte Calvario locality of Biancavilla, and from the anthropogenic use and dispersion of these materials over decades of construction activities and road building. However, few data

are available that describe the detection of fluoro-edenite fibres in and around Biancavilla. In one report, all of 10 samples from five water sources in Biancavilla, including two springs and three wells, contained fluoro-edenite fibres ([Famoso et al., 2012](#)).

#### 1.4.4 Soil

As mentioned above, environmental investigations have identified the quarries near Monte Calvario as a primary source of fluoro-edenite fibres ([Paoletti et al., 2000](#); [Bruni et al., 2006](#)). Analysis of 840 samples of top soil and 90 samples of roadside dust in Biancavilla showed that approximately 90% contained fluoro-edenite ([Famoso et al., 2012](#)).

### 1.5 Exposure of the general population

Exposure to fluoro-edenite mainly occurs via inhalation and ingestion. Inhalation is the primary route of exposure for the general population in Biancavilla, where outdoor air is contaminated with fluoro-edenite fibres ([Famoso et al., 2012](#)). Indoor air may also be contaminated from the use of contaminated local quarry products in plaster and mortar. [Bruni et al. \(2006\)](#) took friable plaster samples from 38 local buildings known to have been constructed using materials from the Monte Calvario quarries and found that 71% of these buildings were contaminated. Although the local quarries that are thought to be the primary source of fluoro-edenite were closed in 2001, historical exposures could also include those of family members of quarry and construction workers exposed to dust from working clothes.

Of 12 long-term residents of Biancavilla who were at least 45 years of age and had been hospitalized for exacerbation of their symptoms of chronic obstructive pulmonary disease, six had detectable fluoro-edenite fibres in their sputum

samples ([Putzu et al., 2006](#)). Of these, four were women who were all housewives, one was a man who was a farmer and the other was a man who was a mason. The fibre concentrations found in the sputum ranged from 0.04 to 10 fibres/g, with a length of 20–40 µm and a diameter of < 0.5 µm ([Putzu et al., 2006](#)).

Several mineral fibres identified as the tremolite/actinolite amphibole fibres found in the quarries and building materials in Biancavilla were also detected in the lung tissue of a woman aged 86 years who died from mesothelioma and was a housewife who had always lived in Biancavilla ([Paoletti et al., 2000](#)).

## 2. Cancer in Humans

### 2.1 Introduction

The available epidemiological evidence on the risk of cancer in humans associated with exposure to fibres composed of fluoro-edenite resulted from a sequence of community studies of the mortality from and incidence of pleural mesothelioma, and from two case series of individual patients with mesothelioma, all of which were conducted and reported in the same location – Biancavilla, a municipality of about 20 000 inhabitants situated in a rural setting on the slopes of the Mount Etna volcano in Sicily, Italy.

After the publication of several reports on pleural mesothelioma in Biancavilla, a few studies also investigated the occurrence of other asbestos-related diseases, namely lung cancer, in the same community, but no case-control or cohort studies have been conducted to date.

### 2.2 Mesothelioma

The first indication of an excess of mesothelioma in Biancavilla was given in a 1996 report of the national programme of epidemiological



surveillance of mesothelioma mortality in Italy for the period 1988–92 ([Di Paola et al., 1996](#)); the programme examined mortality from malignant pleural neoplasms in all 8000 Italian municipalities to detect cases with ascertained or suspected exposure to asbestos. The excess of mesothelioma in Biancavilla was based on four observed deaths versus 0.96 expected [standardized mortality ratio (SMR), 4.17; 95% confidence interval (CI), 1.13–10.67] using the Sicilian region as a reference population. Subsequent reports of this programme confirmed the original findings. [Mastrantonio et al. \(2002\)](#) reported an SMR of 5.80 (95% CI, 2.99–10.13) based on 12 observed deaths for the period 1988–97, and [Fazzo et al. \(2012\)](#) reported SMRs of 4.39 (90% CI, 1.91–8.67 [95% CI, 1.61–9.55]) based on six observed deaths in men and 6.12 (90% CI, 2.09–14.01 [95% CI, 1.67–15.66]) based on four observed cases in women, for the period 1995–2002. All of these studies used the Ninth Revision of the International Classification of Diseases that has a category of “malignant pleural neoplasms” (code 163). Subsequently, Italy began to use the 10th Revision, which includes a more specific code for pleural mesothelioma (code 45.1). Thus, for the time window of 2003–09, [Fazzo et al. \(2012\)](#) reported the mortality from pleural mesothelioma and found SMRs of 6.54 (90% CI, 2.58–13.75 [95% CI, 1.61–9.55]) in men and 22.93 (90% CI, 9.04–48.22 [95% CI, 2.72–15.70]) in women based on five cases in each sex.

As a consequence of the first report on mortality from pleural mesothelioma ([Di Paola et al., 1996](#)), Biancavilla was included in the list of Italian municipalities at risk for mesothelioma, for which an assessment of exposure to asbestos was recommended. Pending this assessment, the occurrence of additional cases that died in 1993–97 was examined and their pathological diagnoses were reviewed.

In a report of a case series of mesothelioma, [Paoletti et al. \(2000\)](#) emphasized that the process of case ascertainment might not have been

exhaustive due to the absence (before 1998) of a validated system of mesothelioma surveillance in Sicily. The source of the cases included the mortality records of the municipality of Biancavilla, family doctors, and hospital records; histological specimens, where available, were reviewed by an expert pathologist; and information on the occupation and residence of the cases was collected by physicians in the local health unit through interviews with the cases (when alive) or, more frequently, with closest relatives. The case series included 10 men and seven women; histology slides were available for nine of the 17 cases, and all diagnoses were confirmed by the pathologist. One case was diagnosed at the age of 29 years, four cases were aged between 40 and 50 years, and the remaining 12 cases were aged over 50 years. All cases except one were long-term residents in Biancavilla. Information on occupation was available for all cases; none had confirmed occupational exposure to asbestos, but two had been employed in industries where exposure was considered to be probable. Asbestiform fibres were detected at a large stone quarry located close to the urbanized area of Biancavilla, from which materials used in the local building industry and road paving were extracted. Several mineral fibres were detected in the lung autopsy samples from a woman aged 86 years who died from pleural mesothelioma and was a resident in Biancavilla, and were identified as the fibrous amphiboles found in the quarries and building materials used in Biancavilla. The patient was a housewife who had been married to a farmer. No chrysotile, crocidolite, or amosite fibres were found in her lung tissue. The fibres were regarded as mineralogical phase intermediates between sodium- and fluorine-rich tremolites and actinolites ([Paoletti et al., 2000](#)). This material was subsequently identified as a new mineral species that was called fluoro-edenite ([Gianfagna & Oberti, 2001](#)). Further analyses of the mineral material demonstrated that amphibole fibres from Biancavilla had a dominant fluoro-edenite

component with significant tremolite and minor winchite components (Bruni et al., 2014).

Since 1998, the surveillance of mesothelioma in Sicily has been conducted by the Sicilian Operative Regional Centre of the Italian National Mesothelioma Registry; the procedures used by the centre for case ascertainment have been discussed extensively elsewhere (INAIL, 2010). A total of 28 cases were detected during the period 1998–2011 (Bruno et al., 2014). Two of these were not resident in Biancavilla at the time of diagnosis, and thus did not meet the criterion to be included in the case series of Biancavilla residents reported by the centre or in the subsequent computation of incidence rates; one had lived in Biancavilla from birth until the age of 53 years and the other from birth until the age of 28 years. Of the remaining 26 cases, two were peritoneal (one man and one woman) and 24 were pleural (12 men and 12 women). One case was diagnosed at the age of 27 years, one at the age of 33 years, one at the age of 39 years, two cases between the ages of 40 and 50 years, and the remaining cases after 50 years of age. Only environmental exposure to Biancavilla fibres was established for eight subjects. None of the cases had definite occupational exposure to asbestos, but one had worked in an industrial sector where asbestos had been used and three had been employed in industries where exposure could occur. Occupational information was insufficient for two cases, although there was an indication that one had worked temporarily in the Biancavilla quarry. No information about past exposures could be obtained for the remaining 12 cases. [Although observations in case series generally provide weak evidence of causality, the young ages of the cases, the equal numbers of men and women, and the lack of documented history of exposure to asbestos for most cases are consistent with an environmental cause.]

The incidence of mesothelioma in Biancavilla was computed and compared with that of the Sicilian region by estimating standardized

incidence ratios (SIRs) with their 95% CIs. Table 2.1 shows a large excess of both pleural (SIR, 5.65; 95% CI, 3.62–8.41) and peritoneal (SIR, 7.92; 95% CI, 0.96–20.0) mesothelioma, although the latter was based on only two cases and had a wide confidence interval (Bruno et al., 2014). SIRs for pleural mesothelioma were particularly elevated in women and were highest in the younger age groups: 21.34 (95% CI, 6.93–50.00; 5 cases) and 62.88 (95% CI, 13.00–180.00; 3 cases) for women under the ages of 50 years and 40 years, respectively.

[No data on individual exposures were available for the population of Biancavilla. However, the high incidence of mesothelioma in younger age groups and women is suggestive of environmental exposure. The consistently larger rate ratios for mesothelioma in women than those in men could most probably be explained by the much higher background rate of mesothelioma in men than in women; thus relative rates would be higher in women than in men even if the absolute excess rates were the same. This explanation is strongly supported by the observation of the same number of cases of mesothelioma in men and women in the reports of the Epidemiological Study of Residents in National Priority Contaminated Sites (SENTIERI). These findings are also unlikely to be explained by a greater susceptibility of women than that of men to the effects of exposure, which has not been observed in studies of populations exposed to asbestos.]

### 2.3 Cancer of the lung

The occurrence of cancer of the lung in the municipality of Biancavilla, where exposure to fibres composed of fluoro-edenite can occur, has been the object of geographical studies of mortality, cancer incidence, and hospitalization (Table 2.1).

Mortality from lung cancer in Biancavilla during the periods 1995–2002 and 2003–10 was

**Table 2.1 Occurrence of mesothelioma, and cancers of the lung, larynx, and ovary among Biancavilla (Italy) residents**

Reference, follow-up period	Total No. of subjects	Exposure assessment	Organ site	Exposure categories	No. of exposed cases	Relative risk	Covariates	Comments
Bruno et al. (2014) 1998–2011		Residence in Biancavilla at time of death/diagnosis	Pleural mesothelioma	Men and women	24	5.65 (95% CI, 3.62–8.41)		
				Men	12	3.63 (95% CI, 1.87–6.34)		
				Women	12	12.75 (95% CI, 6.59–22.00)		
				Age < 40 yr	3	62.88 (95% CI, 13.00–180.00)		
				Age < 50 yr	5	21.34 (95% CI, 6.93–50.00)		
				Age > 50 yr	19	4.74 (95% CI, 2.85–7.39)		
				Men, age > 50 yr	8	2.55 (95% CI, 1.10–5.02)		
				Women, age > 50 yr	11	12.56 (95% CI, 6.27–22.00)		
				Peritoneal mesothelioma	2	7.92 (95% CI, 0.96–20.00)		
				Conti et al. (2014) 2003–2010 national census)	23 703 (2011 national census)			
Men	5	3.79 (90% CI, 1.49–7.97)						
Women	6	11.28 (90% CI, 4.91–22.26)						
<i>Hospitalization (2005–10):</i>								
Men	7	2.61 (90% CI, 1.22–4.89)						
Women	7	7.80 (90% CI, 3.66–14.64)						
Lung								
<i>Mortality:</i>								
Men	35	0.86 (90% CI, 0.63–1.14) [95% CI, 0.62–1.20]						
Women	11	1.28 (90% CI, 0.72–2.12) [95% CI, 0.70–2.31]						
Larynx				<i>Hospitalization (2005–10):</i>				
				Men	53	1.10 (90% CI, 0.87–1.39) [95% CI, 0.84–1.44]		
				Women	13	1.16 (90% CI, 0.68–1.84) [95% CI, 0.67–2.00]		
				<i>Hospitalization (2003–10):</i>				
				Men	7	0.86 (90% CI, 0.40–1.62)		
				Mortality	4	0.77 (90% CI, 0.26–1.77)		
Ovary				<i>Hospitalization</i>				
				<i>Hospitalization</i>	11	0.80 (90% CI, 0.45–1.32)		

**Table 2.1 (continued)**

Reference, follow-up period	Total No. of subjects	Exposure assessment	Organ site	Exposure categories	No. of exposed cases	Relative risk	Covariates	Comments
<a href="#">Pirastu et al. (2014)</a> 2003–2005	23 703 (2011 national census)	Residence in Biancavilla at time of diagnosis	Lung	Men	21	0.72 (90% CI, 0.48–1.03) [95% CI, 0.47–1.10]	Age and socioeconomic status	Incidence
				Women	10	1.67 (90% CI, 0.91–2.84) [95% CI, 0.90–3.10]		
			Mesothelioma	Women	4	14.41 (90% CI, 4.90–32.94)		Mesothelioma incidence not reported for men
			Ovary	Women	3	0.65 (90% CI, 0.18–1.67)		

CI, confidence interval; yr, year  
Compiled by the Working Group

investigated as a part of the SENTIERI project ([Pirastu et al., 2011, 2014](#)). Sicilian regional mortality rates were used as the reference population and SMRs were adjusted for socioeconomic status. In 1995–2002, a deficit in mortality from lung cancer in men (SMR, 0.80; 90% CI, 0.60–1.04 [95% CI, 0.59–1.09]; 40 cases) and an excess in women (SMR, 1.33; 90% CI, 0.72–2.26 [95% CI, 0.72–2.47]; 10 cases) were observed. A more detailed report based on data from the 2003–10 SENTIERI project ([Conti et al., 2014](#)) showed moderate but imprecise increases in mortality from lung cancer (SMR, 1.28; 90% CI, 0.72–2.12 [95% CI, 0.70–2.31]) and hospitalization (standardized hospitalization ratio, 1.16; 90% CI, 0.68–1.84 [95% CI, 0.67–2.00]) in women and a deficit in mortality (SMR, 0.86; 90% CI, 0.63–1.14 [95% CI, 0.62–1.20]) and a small excess of hospitalization (standardized hospitalization ratio, 1.10; 90% CI, 0.87–1.39 [95% CI, 0.84–1.44]) in men. [The Working Group calculated that the pooled SMRs for lung cancer for the entire 1995–2010 period were 0.83 (95% CI, 0.66–1.03) in men and 1.30 (95% CI, 0.85–1.60) in women.]

A recent report by the Sicilian Region Department of Health ([Cernigliaro et al., 2013](#)) showed that mortality from lung cancer (2004–11) in Biancavilla was lower than that expected from local reference populations in men (SMR, 0.91; 95% CI, 0.69–1.18) but was higher than that expected compared with a local reference population in women (SMR, 1.77; 95% CI, 1.10–2.71).

The incidence of lung cancer (2003–05) was also examined in the SENTIERI project and showed a moderate excess in women (SIR, 1.67; 90% CI, 0.91–2.84 [95% CI, 0.90–3.10]) and a deficit in men (SIR, 0.72; 90% CI, 0.48–1.03 [95% CI, 0.47–1.10]) ([Pirastu et al., 2014](#)). [The only statistically significant finding for lung cancer was in women when local reference rates were used. An apparent deficit in lung cancer was observed among men when regional reference rates were used, but this was largely removed when local reference rates were used.

Significantly, an excess of lung cancer has not been consistently observed in other community studies of exposure to asbestos. The possible role of smoking as a confounder or effect modifier in these studies cannot be assessed directly because no data were available on smoking habits in Biancavilla. However, available reports have indicated that the proportion of current smokers in Catania Province, which includes Biancavilla, corresponded with the regional average in Sicily ([Regione Sicilia, 2014](#)).]

## 2.4 Other neoplasms

A non-significant deficit in hospitalization for laryngeal cancer based on seven observed cases in men was reported by [Conti et al. \(2014\)](#) ([Table 2.1](#)). Data on hospitalizations for women and mortality data for both sexes were not available because the observations were based on fewer than three cases and thus could not be published due to privacy regulations. No figures on mortality from laryngeal cancer were available in the previous edition of the SENTIERI project report for 1995–2002 ([Pirastu et al., 2011](#)). The incidence of laryngeal cancer was investigated in the most recent SENTIERI report for 2003–05 ([Pirastu et al., 2014](#)), but fewer than three cases per sex were observed, thus precluding publication of the data on this cancer.

A deficit in ovarian cancer based on four observed deaths (SMR, 0.77; 90% CI, 0.26–1.77) and 11 observed cases of hospitalization (standardized hospitalization ratio, 0.80; 90% CI, 0.45–1.32) was reported by [Conti et al. \(2014\)](#) for the period 2005–10 ([Table 2.1](#)). In the previous SENTIERI project report relative to 1995–2002, a deficit in mortality from ovarian cancer based on five cases was reported ([Pirastu et al., 2011](#)); a deficit in the incidence of ovarian cancer based on three observed cases (SIR, 0.65; 90% CI, 0.18–1.67) was reported in the last edition of the SENTIERI project that included data for the years 2003–05 ([Pirastu et al., 2014](#); [Table 2.1](#)).

[Potential confounding by tobacco smoking and alcohol consumption is a concern for laryngeal cancer, but data on these risk factors were not available for the study population. Although based on small numbers and statistically non-significant, the deficit in ovarian cancer appeared to be consistent.]

[The Working Group noted that 90% CIs rather than the more commonly adopted 95% CIs were used in the Biancavilla epidemiological studies that were included in the SENTIERI project. This choice was motivated by the focus on sites where specific, exposure-related conditions were expected to be in excess; the rationale was further elaborated by [Pirastu et al. \(2014\)](#). The Working Group computed 95% CIs for the associations that were reported to be statistically significant on the basis of 90% CIs, and all of them excluded unity.]

### 3. Cancer in Experimental Animals

See [Table 3.1](#) and [Table 3.2](#)

#### Rat

Only one study, described in two reports, was conducted to examine the potential carcinogenicity of fluoro-edenite in rats ([Soffritti et al., 2004](#); [Belpoggi et al., 2011](#)).

One group of 40 male and 40 female Sprague-Dawley rats (age, 8 weeks) received a single intraperitoneal injection of 25 mg of fibrous fluoro-edenite (the fibrous sample contained 25–30% of fluoro-edenite fibres [diameter, 0.5–1 µm; length > 10 µm] in addition to feldspars, haematite, and pyroxenes; in 1 mL of water) sampled from a quarry in Biancavilla, Sicily, Italy; a second group of 40 males and 40 females received a single intrapleural injection of 25 mg of fibrous fluoro-edenite (in 1 mL of water) through the chest wall into the pleural

cavity; a third group of 15 male and 15 female rats received a single intraperitoneal injection of 25 mg of powdered prismatic fluoro-edenite; and a fourth group of 40 males and 40 females received a single intraperitoneal injection of 1 mL of water only and served as vehicle controls. [No vehicle control group for intrapleural injection was provided.] The animals were observed until all those in group 1 that were treated with fibrous fluoro-edenite by intraperitoneal injection had died (109 weeks). At 109 weeks, 70 of the 80 rats treated by intrapleural injection (group 2) had died. The average time to the appearance of tumours in group 1 was 61.6 weeks for males and 66.4 weeks for females (see [Table 3.1](#)); a total of 37 out of 40 (92.5%) [ $P < 0.0001$ , Fisher exact test] males and 29 out of 40 (72.5%) [ $P < 0.0001$ , Fisher exact test] females developed mesothelioma. The average latency for the group that received an intrapleural injection of fibrous fluoro-edenite was 71.0 weeks in males and 72.8 weeks in females; 4 out of 37 (10.8%) males and 6 out of 33 (18.2%) females developed mesothelioma. None of the rats given water or powdered prismatic fluoro-edenite intraperitoneally had developed mesothelioma at 109 weeks ([Soffritti et al., 2004](#)).

The rats in group 2 (intrapleural injection of fluoro-edenite), group 3 (intraperitoneal injection of powdered prismatic fluoro-edenite), and group 4 (intraperitoneal injection of water) were further followed for a total of 122 weeks, at which time all of the rats had died or were killed (see [Table 3.2](#)). At 122 weeks, only 1 (female) out of 80 rats that received a single intraperitoneal injection of water alone had developed mesothelioma. None of the males or females exposed to powdered prismatic fluoro-edenite developed mesothelioma, but 6 out of 40 (15%) males and 7 out of 40 (17.5%) females in group 3 (intrapleural injection of fibrous fluoro-edenite) were found to have mesothelioma ([Belpoggi et al., 2011](#)). As reported in [Soffritti et al. \(2004\)](#), the incidence of mesothelioma was increased in males (37 out of 40) and females (29 out of 40) that

**Table 3.1 Induction of mesotheliomas by fluoro-edenite in Sprague-Dawley rats followed for up to 109 weeks**

Material	Route of administration (dose)	No. of rats (sex)	No. of deceased rats at 109 wk	Incidence of mesotheliomas (%)	Significance <sup>a</sup>	Mean latency (wk)
Fibrous fluoro-edenite	Intraperitoneal injection (25 mg/rat)	40 (M)	40	92.5 (37/40)	[ <i>P</i> < 0.0001]	61.6
Fibrous fluoro-edenite	Intraperitoneal injection (25 mg/rat)	40 (F)	40	72.5 (29/40)	[ <i>P</i> < 0.0001]	66.4
Fibrous fluoro-edenite	Intrapleural injection (25 mg/rat)	40 (M)	37	10.8 (4/37)	NA	71.0
Fibrous fluoro-edenite	Intrapleural injection (25 mg/rat)	40 (F)	33	18.2 (6/33)	NA	72.8
Powdered prismatic fluoro-edenite	Intraperitoneal injection (25 mg/rat)	15 (M)	13	0 (0/13)	[NS]	NA
Powdered prismatic fluoro-edenite	Intraperitoneal injection (25 mg/rat)	15 (F)	13	0 (0/13)	[NS]	NA
Water (control)	Intraperitoneal injection (1 mL)	40 (M)	33	0 (0/33)	–	NA
Water (control)	Intraperitoneal injection (1 mL)	40 (F)	32	0 (0/32)	–	NA

<sup>a</sup> Calculated by the Working Group, Fisher exact test  
 F, female; M, male; NA, not applicable; NS, not significant; wk, week  
 From [Soffritti et al. \(2004\)](#)

**Table 3.2 Induction of mesotheliomas by fluoro-edenite in Sprague-Dawley rats followed for up to 122 weeks**

Material	Route of administration (dose)	Total No. of rats (sex)	No. of rats deceased or killed at 122 weeks	Incidence of mesotheliomas (%)	Significance <sup>a</sup>	Mean latency (wk)
Fibrous fluoro-edenite	Intrapleural injection (25 mg/rat)	40 (M)	40	15 (6/40)	NA <sup>b</sup>	82.3
Fibrous fluoro-edenite	Intrapleural injection (25 mg/rat)	40 (F)	40	17.5 (7/40)	NA <sup>c</sup>	77.4
Powdered prismatic fluoro-edenite	Intraperitoneal injection (25 mg/rat)	15 (M)	15	0 (0/15)	[NS]	NA
Powdered prismatic fluoro-edenite	Intraperitoneal injection (25 mg/rat)	15 (F)	15	0 (0/15)	[NS]	NA
Water (control)	Intraperitoneal injection (1 mL)	40 (M)	40	0 (0/40)	–	NA
Water (control)	Intraperitoneal injection (1 mL)	40 (F)	40	2.5 (1/40)	–	122

<sup>a</sup> Calculated by the Working Group, Fisher exact test  
<sup>b</sup> Historical incidence in vehicle controls: 0/20 male Sprague-Dawley rats ([Maltoni & Minardi, 1989](#))  
<sup>c</sup> Historical incidence in vehicle controls: 0/20 female Sprague-Dawley rats ([Maltoni & Minardi, 1989](#))  
 F, female; M, male; NA, not applicable; NS, not significant; wk, week  
 From [Belpoggi et al. \(2011\)](#)

received an intraperitoneal injection of fibrous fluoro-edenite. The authors characterized the fluoro-edenite-induced mesotheliomas as epithelial, mixed, or sarcomatous. Rats given fibrous fluoro-edenite by intraperitoneal injection developed mesotheliomas of predominantly sarcomatous or mixed histology. In contrast, half of the mesotheliomas that resulted from pleural administration in males were morphologically epithelial. Many of the mesotheliomas had metastasized into the lung, lymph nodes, and throughout the abdomen. The incidence and latency were comparable with those of many types of fibre that have been demonstrated to cause mesothelioma (e.g. crocidolite and amosite) in Sprague-Dawley rats in that laboratory (Maltoni & Minardi, 1989). [The study described by Soffritti et al. (2004) and Belpoggi et al. (2011) was limited by the lack of statistical analysis and an appropriate control for the intrapleural injection experiment. However, the authors presented the mesothelioma response as a simple description to demonstrate the extent of an obvious effect. The Working Group also noted that 20 male and 20 female Sprague-Dawley rats that served as controls in a lifetime study conducted in the same laboratory (Maltoni & Minardi, 1989) did not develop mesotheliomas after a single intrapleural injection of water.]

## 4. Mechanistic and Other Relevant Data

### 4.1 Deposition, phagocytosis, retention, translocation, and clearance

#### 4.1.1 Humans

Paoletti et al. (2000) reported fluoro-edenite fibrous amphiboles (i.e. intermediate phases between tremolite and actinolite) in 71–72% of

the samples of sand and other building materials in Biancavilla, Sicily, Italy, that had the same type of amphibole phases observed in the local quarries. Similar fibrous amphiboles were also observed in the lung tissue from the autopsy of a woman aged 86 years who had been diagnosed with pleural mesothelioma, had lived in Biancavilla for her entire life, and did not have any reported occupational exposure to fibrous amphiboles. The fibre dimensions observed in the lung tissue were similar to those reported in sheep (Rapisarda et al., 2005), with diameters ranging from 0.4  $\mu\text{m}$  to 1  $\mu\text{m}$  and lengths ranging from 12  $\mu\text{m}$  to 40  $\mu\text{m}$  (Paoletti et al., 2000; Rapisarda et al., 2005).

#### 4.1.2 Experimental animals

Limited data are available on the mechanisms related to the deposition or disposition of inhaled fluoro-edenite in experimental animals. DeNardo et al. (2004) and Rapisarda et al. (2005) measured the burden of fluoro-edenite fibres in the lungs of sheep (Table 4.1) because of their anatomical and physiological similarity to human lungs (Bégin et al., 1981), and because the animals had potentially been exposed environmentally to fluoro-edenite amphibole fibres from a stone quarry near the town of Biancavilla.

DeNardo et al. (2004) studied the lung burden of fibres in 27 sheep (age, 3 years or older) that lived and grazed near (1–3 km) the Monte Calvario stone quarries to the south east of Biancavilla. The sheep (all ewes) were selected on the basis of age and the longest air exposure in a flock of approximately 200 animals. Tissue samples from the lung tracheal lobes were analysed by SEM-EDX and the fibres were identified as fluoro-edenite by their chemical composition and the presence of fluorine, magnesium, and calcium. A positive identification of fluoro-edenite was based on an observed ratio of  $2 \leq \text{magnesium/calcium} \leq 3$ , and a probable identification based on a ratio of



**Table 4.1 Toxicokinetics of fluoro-edenite fibres in sheep**

Particle dimensions and surface area	Species (age and sex), number of animals	Route of exposure and dose/exposure concentration	Duration of study	Findings	Comments	Reference
NR Fluoro-edenite: confirmed, identified if ratio of $2 \leq \text{Mg}/\text{Ca} \leq 3$ ; probable, identified if $1.5 \leq \text{Mg}/\text{Ca} \leq 2$	Sheep ( $\geq 3$ yr, F), 27 exposed	Inhalation (environmental)	Sheep had lived and grazed near (1–3 km) the Monte Calvario stone quarries to the South-eastern of Biancavilla	Fluoro-edenite content was identified as 7.5% of the mineral species (2.7% confirmed and 4.8% probable)	Mineral species in lung tracheal lobe tissue samples (approximately 50 g) from each of 27 animals obtained from slaughterhouse	<a href="#">DeNardo et al. (2004)</a>
Fluoro-edenite: fibre length, 8–41 $\mu\text{m}$ ; fibre width: 0.4–1.39 $\mu\text{m}$	Sheep (NR), 60 exposed and 10 controls	Inhalation (environmental)	Exposed sheep randomly selected from six flocks that regularly grazed 3 km from the town of Biancavilla	Lymph nodes of all the exposed sheep contained fibres; no fibres found in lymph nodes of the controls Mean fibre number: $0.08 \pm 0.04 \times 10^6$ fibres/g of dry tissue		<a href="#">Rapisarda et al. (2005)</a>

F, female; NR, not reported; yr, year  
Compiled by the Working Group

$1.5 \leq \text{magnesium/calcium} \leq 2$ . Tissue analyses revealed the presence of 14 mineral species in the recovered particulate matter, and most of the minerals isolated from the lungs corresponded to those occurring geologically in the area; 5% of the total mineral species identified from the sheep were from exogenous materials, including man-made mineral fibres, and fluoro-edenite content was identified as 7.5% (2.7% confirmed and 4.8% probable).

[Rapisarda et al. \(2005\)](#) measured the concentration of fluoro-edenite in the lymph nodes because they considered that this site provided a better indication of previous exposure to asbestos than lung parenchymal tissues ([Dodson et al., 2000](#)). [Inhaled fibres may be cleared from the lungs by various processes, whereas the fibres that enter the lymph system would be retained and persist in the lymph nodes.] The lymph

nodes examined were from the tracheobronchial area and one from the middle mediastinum that drains the lung lobes. Sixty healthy sheep from six flocks that commonly grazed 3 km from the town of Biancavilla were randomly selected together with 10 unexposed (control) sheep. Using light microscopy, SEM, and EDX, the lymph nodes of all of the exposed sheep were found to contain fibres, while none were found in those of the control (unexposed) sheep. Some fibres were identified as fluoro-edenite by their crystallo-chemical characteristics, the dimensions of which were reported as 0.4–1.39  $\mu\text{m}$  in width and 8–41  $\mu\text{m}$  in length; the mean number of fibres was  $0.08 \pm 0.04 \times 10^6$  fibres/g of dry tissue.

## 4.2 Physico-chemical properties associated with toxicity

Because of the similarity between asbestos, fluoro-edenite, and some asbestiform minerals, the physico-chemical properties of mineral fibres associated with toxicity are briefly summarized below, but were reported in detail for asbestos in Volume 100C of the *IARC Monographs* ([IARC, 2012](#)).

### 4.2.1 Crystal structure and chemical composition

Fluoro-edenite exhibits considerable compositional variability, ranging from edenite [ $\text{NaCa}_2\text{Mg}_5\text{Si}_7\text{AlO}_{22}\text{F}_2$ ] to winchite [ $\text{NaCaMg}_4(\text{Al},\text{Fe}^{3+})\text{Si}_8\text{O}_{22}(\text{OH})_2$ ], with a variable content of tremolite [ $\text{Ca}_2(\text{Mg},\text{Fe}^{2+})_5\text{Si}_8\text{O}_{22}(\text{OH})_2$ ] ([Andreozzi et al., 2009](#)). The variability, which may influence pathogenic potency, has been ascribed to small differences in ambient conditions during the crystallization of the fluorine fibrous amphiboles ([Mazziotti-Tagliani et al., 2009](#)).

A set of three fibrous samples (Biancavilla) with variable compositions was tested to assess cytotoxicity using the MTT assay on human lung adenocarcinoma (A549) and human mesothelial (MeT-5A) cells ([Pacella, 2009](#)). Both cell lines were cultured in the presence of fibres (50 µg/mL) for 24 and 48 hours. On the basis of the average chemical formula, the three samples were identified as 25% tremolite and 30% winchite (a), 25% tremolite and 20% winchite (b), and 30% tremolite and 30% winchite (c). The iron content ranged from 3.6 to 6.0 wt% as ferrous oxide and the  $\text{Fe}^{3+}/\text{Fe}_{\text{total}}$  ratio was 92, 54, and 94% in the three samples, respectively. Despite the differences in composition, the three samples exhibited similar cytotoxic potency towards both cell lines.

Two specimens (length, 30 µm; average diameter, 5 µm) of fluoro-edenite fibres (Biancavilla) with a low ( $\text{Fe}^{3+}$ , 50%) and a high ( $\text{Fe}^{3+}$ , 70%)

total content of iron were tested using Met-5A and monocyte-macrophage J774 cells ([Cardile et al., 2007](#)). Reduction in cell viability (lactate dehydrogenase release and MTT assay) were investigated together with the induction of the heat shock protein 70, stimulation of reactive oxygen species (ROS) (dichloro-dihydro-fluorescein diacetate fluorescence assay) and nitrogen monoxide formation (analysis of nitrite release). Cells were exposed to 5, 50, or 100 µg of fibres/mL for 72 hours. An iron-free fibrous tremolite was used as a reference sample. High-iron fibres were more potent than low-iron fibres in stimulating ROS release; conversely, the low-iron sample had a remarkably stronger effect on nitrogen monoxide formation. Such differences were more pronounced in J774 macrophages than in Met-5A mesothelial cells. Low-iron fluoro-edenite was slightly more effective than high-iron fluoro-edenite in eliciting an increase in heat shock protein 70 expression in both cell lines and in reducing viability of Met-5A mesothelial cells. Both forms of fluoro-edenite were more potent than tremolite in eliciting all adverse cell responses. The authors inferred that the observed effects were mainly related to the differences in iron content.

Both low- and high-iron fluoro-edenite increased phospholipase C (PLC)β1 and PLCγ1 expression in A549 cells after exposure to 50 µg/mL for 48 hours; low-iron fluoro-edenite was more active than high-iron fluoro-edenite, crocidolite, and tremolite in the induction of PLCγ1 expression ([Loreto et al., 2009](#)). PLC expression did not depend on the total iron content of each fibre, but the increased level of PLCβ1 was associated with the highest content of ferrous ions. Both samples also induced the release of tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6. The induction of cytokines was positively correlated with the total iron content of the fibres in the following order: crocidolite > high-iron fluoro-edenite > low-iron fluoro-edenite > tremolite.

Edenite and fluoro-edenite fibres induced functional modifications (cell motility and distribution of polymerized actin) and the synthesis of vascular endothelial growth factor (VEGF) and  $\beta$ -catenin in A549 and MeT-5A cells. The level of cyclooxygenase (COX-2) and prostaglandin (PGE2) was examined in J774 cells ([Pugnaroni et al., 2007](#)). The fibre width ranged from about 1  $\mu\text{m}$  (mostly 0.5  $\mu\text{m}$ ) to 2–3  $\mu\text{m}$  (edenite) up to several millimetres (fluoro-edenite), and the length ranged from about 6–80  $\mu\text{m}$  (edenite) up to some hundred micrometres (fluoro-edenite). Differential alteration of cytoplasmic actin networks, cell motility, and the expression of VEGF and  $\beta$ -catenin was observed in treated A549 and MeT-5A cells, according to the different sensitivity of the two cell lines. Exposed J774 cells exhibited a significantly increased expression of COX-2 and PGE2 concentrations. [No information on the role of the two minerals (edenite/fluoro-edenite) was provided.]

#### 4.2.2 Form and size

Fluoro-edenite may have prismatic, acicular, or fibrous properties.

A pilot study ([Putzu et al., 2006](#)) on spontaneous sputum as an indicator of exposure to fluoro-edenite fibres was carried out on 12 subjects (age, > 45 years) who had been resident in Biancavilla for at least 30 years. In six subjects, fluoro-edenite fibres ranging between 20 and 40  $\mu\text{m}$  in length, and < 0.5  $\mu\text{m}$  in diameter were observed. Fibres of similar length (8–41  $\mu\text{m}$ ) and width (0.4–1.4  $\mu\text{m}$ ) were detected in sheep lymph nodes ([Rapisarda et al., 2005](#)). Fibres > 5  $\mu\text{m}$  in length were also detected in the lung and pleural tissues of residents of Biancavilla who died from pleural mesothelioma ([Paoletti & Bruni, 2009](#)); 95% and 98% of the fibres found in the lung and pleura were > 5  $\mu\text{m}$  in length, respectively.

[Travaglione et al. \(2006\)](#) showed that fibrous fluoro-edenite (average length, 15.5  $\mu\text{m}$ ; average

diameter, 0.45  $\mu\text{m}$ ) can induce multinucleation and spreading, which are common features related to cellular transformation, in A549 cells in contrast with prismatic (length, 20  $\mu\text{m}$ ; diameter, nearly 3  $\mu\text{m}$ ) fluoro-edenite ([Travaglione et al., 2003](#)).

Both prismatic fluoro-edenite and fibrous fluoro-edenite were cytotoxic. The cytotoxicity induced by prismatic fluoro-edenite was not accompanied by changes in morphology ([Travaglione et al., 2003](#)); in contrast, fibrous fluoro-edenite produced dramatic changes in cell morphology, similar to those caused by crocidolite ([Travaglione et al., 2006](#)).

Both prismatic and fibrous fluoro-edenite promoted the secretion of IL-6 [a multifunctional cytokine with immuno-regulatory and pro-inflammatory effects], within 48 hours of exposure; the fibrous form showed greater potency ([Travaglione et al., 2003, 2006](#)).

Fibrous, but not prismatic, fluoro-edenite induced mesotheliomas in Sprague-Dawley rats after intraperitoneal administration ([Soffritti et al., 2004](#)). The fibrous sample contained 25–30% of fluoro-edenite fibres [diameter, 0.5–1  $\mu\text{m}$ ; length > 10  $\mu\text{m}$ ] in addition to feldspars, haematite, and pyroxenes. [No information on the features of the prismatic sample was provided.]

The cytotoxicity of two samples of fluoro-edenite with a diameter of < 1  $\mu\text{m}$  (200–600 nm) and acicular-fibrous morphology, with an average length of ~50  $\mu\text{m}$ , or filamentous-asbestiform morphology, with a length of up to 150  $\mu\text{m}$ , was compared in A549 and in MeT-5A cells. Both cell lines were cultured in presence of 50  $\mu\text{g}/\text{mL}$  of fibres for 24 and 48 hours, and cell viability was evaluated using the MTT assay. All samples reduced the time-dependent loss of cell viability, but no significant differences in potency were noted ([Pacella, 2009](#)).

### 4.2.3 Surface reactivity

[Fantauzzi et al. \(2012\)](#) compared samples of fibres from Biancavilla and Libby, MT, USA. Comparison of the oxidation state (X-ray photoelectron spectroscopy) of surface iron with that of bulk iron revealed that the sample with the lowest bulk iron oxidation state was the most affected by surface oxidation. Biancavilla samples were highly heterogeneous (tremolite and winchite; see Section 4.2.1 samples (a) and (c); [Pacella, 2009](#)). Both samples had comparable iron content but with different oxidation states at the surface. Sample (a) had both ferrous and ferric ions ( $\text{Fe}^{2+}/\text{Fe}_{\text{total}} = 0.13$ ) and sample (c) had only fully oxidized iron. On both surfaces, ferric ions were predominantly in the form of oxyhydroxide ( $\text{Fe}^{3+}_{\text{hydr}}$ ), formed by weathering of the silicate surface:  $\text{Fe}^{3+}_{\text{ox}}/\text{Fe}_{\text{total}} = 0.33$  and  $\text{Fe}^{3+}_{\text{hydr}}/\text{Fe}_{\text{total}} = 0.54$  for sample (a), and  $\text{Fe}^{3+}_{\text{ox}}/\text{Fe}_{\text{total}} = 0.13$  and  $\text{Fe}^{3+}_{\text{hydr}}/\text{Fe}_{\text{total}} = 0.87$  for sample (c).

#### (a) Generation of free radicals

Surface reactivity was investigated by the production of hydroxyl ( $\text{HO}\cdot$ ) radicals released in the presence of hydrogen peroxide using the spin trapping technique ([Fantauzzi et al., 2012](#)). The two specimens from Biancavilla showed no significant difference in the production of  $\text{HO}\cdot$  radicals, but produced less  $\text{HO}\cdot$  than the Libby amphibole; crocidolite asbestos was used as a positive control. [The Working Group noted that  $\text{HO}\cdot$  release was evaluated at equal mass, but no data on the surface area of fluoro-edenite were provided.]

A quarry rock dust (Brunauer–Emmett–Teller area =  $0.5 \text{ m}^2/\text{g}$ ) and a house plaster dust (Brunauer–Emmett–Teller area =  $4.4 \text{ m}^2/\text{g}$ ) from Biancavilla were tested for their ability to generate  $\text{HO}\cdot$  radicals using the deoxyribose assay ([Rapisarda et al., 2003](#)). Both samples contained fluoro-edenite fibres and other minerals, including feldspar, quartz, haematite, ilmenite, and fluoro-apatite. The content of fluoro-edenite

was not quantified but the authors noted that the quarry rock dust was richer in fluoro-edenite than the house plaster dust. The two samples, tested at an equal surface area (doses ranging from  $2.5 \text{ cm}^2/\text{mL}$  to  $60 \text{ cm}^2/\text{mL}$ ), generated  $\text{HO}\cdot$  and the quarry rock dust was more reactive than the house dust.

#### (b) Bioavailability and biodeposition of metals

No data were available to the Working Group.

### 4.2.4 Fibre durability

No data on fibre durability (leaching, phagocytosis, dissolution, or breakage) were available to the Working Group.

## 4.3 Genetic and related effects

DNA strand breaks (assessed using the alkaline comet assay) were elevated in primary human lung fibroblasts, immortalized human lung adenocarcinoma (A549) cells, and mouse monocyte-macrophage (J774) cells exposed to  $5\text{--}100 \text{ }\mu\text{g}/\text{mL}$  of fluoro-edenite (Biancavilla) for 72 hours ([Cardile et al., 2004b](#)).

## 4.4 Other mechanistic data relevant to carcinogenesis

### 4.4.1 Release of cytokines, chemokines, and growth factors

#### (a) Experimental animals

Apoptosis was investigated in the lungs of healthy sheep grazing near Biancavilla through the detection of TNF-related apoptosis-inducing ligand (TRAIL) and its death receptor, DR5, and through the expression and localization of collagenases-3 (matrix metalloproteinase, MMP-13) [MMP-13 is an extracellular matrix component that plays an important role in the remodelling of the lung in inflammatory diseases]. The results

showed that MMP-13 was overexpressed, mainly in fibroblasts and epithelial cells, while positivity for TRAIL and DR5 was detected on alveolar cell surfaces and in the vascular stroma. In the lungs of sheep exposed to fluoro-edenite, the expression of the TRAIL receptor was most pronounced in areas of inflammatory cell infiltration and active fibrosis, revealed by the expression of MMP-13. These changes may reflect the activation of apoptotic processes through exposure to fluoro-edenite ([Martinez et al., 2006](#)). [TRAIL selectively induces apoptosis in a variety of tumour and transformed cells, but not in most normal cells ([Wang & El-Deiry, 2003](#)).]

#### (b) *Experimental systems in vitro*

Using the mouse monocyte-macrophage J774 cell line, [Pugnaloni et al. \(2007\)](#) found time-dependent increased expression of COX-2 and release of PGE2 after exposure to fluoro-edenite fibres (Monte Calvario) and edenite fibres with a wide range of diameters (from 0.5  $\mu\text{m}$  to several micrometres) and lengths (6  $\mu\text{m}$  to several hundred micrometres). Exposure to fluoro-edenite also increased the expression of VEGF in human lung adenocarcinoma A549 cells and in the immortalized human mesothelial Met-5A cell line, as assessed by immunocytochemistry ([Pugnaloni et al., 2007](#)). Exposure of J774 macrophages or Met-5A cells to two types of fluoro-edenite fibre from Biancavilla (sample 19, low-iron (50%  $\text{Fe}^{3+}$ ) or sample 27, high-iron (about 70%  $\text{Fe}^{3+}$ ); length, 30  $\mu\text{m}$ ; diameter,  $\sim 5 \mu\text{m}$ ) also induced the generation of ROS as indicated by dichlorofluorescein fluorescence and extracellular release of nitrite ([Cardile et al., 2007](#)). In J774 macrophages, exposure to fluoro-edenite (Biancavilla) in combination with lipopolysaccharide significantly enhanced the release of nitrite and increased the expression of inducible nitric oxide synthase ([Cardile et al., 2004a](#)).

[Travaglione et al. \(2003, 2006\)](#) compared the response of the human lung adenocarcinoma (A549) cell line to prismatic or fibrous forms

of fluoro-edenite (Monte Calvario) compared with crocidolite asbestos fluoro-edenite fibres. Prismatic fibres (length, 20  $\mu\text{m}$ ; diameter,  $\sim 3 \mu\text{m}$ ) were taken up by the cells and induced the cellular release of the cytokine IL-6 after 24–72 hours [no reference particles were included in this study] ([Travaglione et al., 2003](#)). Fibrous fluoro-edenite (length, 15.5  $\mu\text{m}$ ; diameter, 0.45  $\mu\text{m}$ ), as well as standard crocidolite asbestos fibres (NIOSH, USA) as a positive reference, at equivalent toxic doses were taken up by the cells resulting in a time-dependent release of the cytokines IL-6 and IL-8 ([Travaglione et al., 2006](#)). [Loreto et al. \(2009\)](#) confirmed the release of cytokines (IL-1 $\beta$ , TNF- $\alpha$ , and IL-6) from A549 cells after exposure to two samples of fluoro-edenite (sample 19, low-iron, or sample 27, high-iron) from Biancavilla, tremolite, or crocidolite asbestos fibres (OSHA standard) at a dose of 50  $\mu\text{g}/\text{mL}$  for 48 hours.

#### 4.4.2 Apoptosis

##### (a) *Experimental animals*

Programmed cell death *in vivo* was investigated by analysing the immuno-expression of bax protein and bcl-2 oncoprotein (which are involved in the early stages of apoptosis), caspase 3, poly (ADP-ribose) polymerase, and DNA fragmentation in lung tissues from sheep grazing near Biancavilla ([Loreto et al., 2008](#)). The results showed epithelial and interstitial overexpression of bax, especially in cells that were directly in contact with the fibres, and negative bcl-2 immuno-expression. Terminal deoxynucleotidyl transferase dUTP nick end labelling-positive cells characteristic of apoptosis were detected in the alveoli and in areas of fibrosis. [These data suggested an increase in the concentration of bax homodimers that enable apoptosis and were in agreement with the findings of [Narasimhan et al. \(1998\)](#), who documented nearly absent bcl-2 expression and uniform bax expression in malignant pleural mesothelioma cell lines. [Plataki et al. \(2005\)](#) found an increased expression

of pro-apoptotic and a reduced expression of anti-apoptotic molecules in epithelial cells from diseased lungs that may be responsible for inadequate and delayed re-epithelialization, which in turn contributes to fibroblast proliferation. Some evidence has also suggested that apoptosis is directly involved in the loss of alveolar epithelial cells ([Yokohori et al., 2004](#)). Recent studies indicated that epithelial apoptosis could be a key profibrotic event in lung fibrogenesis ([Li et al., 2004](#)), and that tumour growth is a result of cell resistance to apoptotic death ([Niehans et al., 1997](#)).

[These results support the hypothesis that apoptosis is an important mechanism for removing cells with irreparable fluoro-edenite-induced genetic changes that may result in a predisposition to the development of neoplasia.]

#### (b) *Experimental systems in vitro*

The exposure of several lung target cells (primary human fibroblasts, human lung adenocarcinoma A549 cells, murine monocyte-macrophage J774 cells, or Met-5A mesothelial cells) to fibrous fluoro-edenite from Monte Calvario ([Travaglione et al., 2006](#)), or Biancavilla ([Cardile et al., 2004b](#)), decreased cell viability, increased the intracellular generation of ROS, as indicated by dichlorofluorescein fluorescence, decreased mitochondrial activity, as indicated by the MTT assay, and induced plasma membrane damage, as indicated by the extracellular release of lactate dehydrogenase.

When compared on a mass basis, crocidolite asbestos fibres (OSHA standard) were more toxic at doses between 5 and 100 µg/mL after 24–72 hours ([Cardile et al., 2007](#)). Fluoro-edenite fibres (sample 19, low-iron, or sample 27, high-iron; Biancavilla) were more potent than fibrous tremolite (Val di Susa, Piemonte, Italy) in inducing the generation of ROS and mitochondrial and plasma membrane damage; fluoro-edenite sample 19 was more potent than fluoro-edenite sample 27 in inducing plasma

membrane damage in mesothelial Met-5A cells. All of the fibrous minerals induced the expression of heat shock protein 70 as a marker of a generalized stress-response pathway ([Cardile et al., 2007](#)). Exposure of A549 cells to fibrous fluoro-edenite (Monte Calvario) or standard crocidolite asbestos fibres (NIOSH, USA) induced time-dependent decreases in viability assessed by trypan blue exclusion, but no induction of apoptosis or altered expression of the pro-apoptotic protein, Bax, or the anti-apoptotic proteins, Bcl-2 or Bcl-X<sub>L</sub>, were detected ([Travaglione et al., 2006](#)).

#### 4.4.3 *Activation of intracellular signalling pathways*

##### *Human cells in vitro*

[Loreto et al. \(2009\)](#) demonstrated that exposure to fluoro-edenite samples 19 and 27 (low- and high-iron, respectively) from Biancavilla, tremolite, or crocidolite asbestos fibres induced PLCγ1 and -β1 protein expression in lung adenocarcinoma A549 cells; [this enzyme activates the signalling pathway that leads to the mobilization of intracellular calcium and activation of protein kinase C].

#### 4.4.4 *Cell proliferation*

##### *Human cells in vitro*

Exposure of human lung adenocarcinoma A549 cells to the prismatic ([Travaglione et al., 2003](#)) or fibrous ([Travaglione et al., 2006](#)) forms of fluoro-edenite (Monte Calvario) reduced cell proliferation for up to 72 hours with no disruption in cell-cycle progression, as assessed by flow cytometry. Neither fibrous fluoro-edenite nor standard crocidolite asbestos fibres (NIOSH, USA) altered the expression of p53, p21, or cyclin D1, as assessed by Western blot ([Travaglione et al., 2006](#)).

#### 4.4.5 Formation of granulomas and fibrosis

##### *Experimental animals*

Histopathological examination of the lung tissues collected from healthy sheep grazing near a town in the eastern region of Sicily where environmental exposure to fluoro-edenite was reported to occur revealed fibrosis, including the loss of alveolar architecture with honeycombing that are characteristic of the final stage of fibrosis ([Martinez et al., 2006](#)).

#### 4.4.6 Activation of oncogenes and inactivation of tumour-suppressor genes

##### (a) *Experimental animals*

The lungs of 10 ewes grazing 3 km from Biancavilla and 10 ewes grazing approximately 30 km from the local stone quarry were examined for apoptotic cell death in situ and the expression of Bax. Microscopic analyses detected the presence of fluoro-edenite fibres in the lungs of the exposed sheep. Focal Bax overexpression was observed in the alveolar epithelium and interstitium, particularly in alveolar epithelial cells in close contact with the fibres, and in the mucosal epithelium lining the terminal bronchioles. Increased expression of tumour-suppressor genes and Bax are consistent with increased apoptosis, as confirmed by the increased expression of caspase 3 and poly (ADP-ribose) polymerase immunoreactivity ([Loreto et al., 2008](#)).

The lungs of 10 sheep grazing 3 km from Biancavilla and 10 ewes grazing approximately 30 km from the local stone quarry were examined immunohistochemically for the expression of unphosphorylated (Rb) and phosphorylated (pRb) proteins. Microscopic analyses detected the presence of fibres in the lungs of exposed animals and pRb was overexpressed in the lungs of exposed sheep, especially in the cytoplasm of fibroblasts and epithelial cells, in comparison with those of control animals. pRb

overexpression was also detected in the alveolar epithelium and in the interstitium, especially in proximity to the fluoro-edenite fibres in the tissues, whereas Rb immunostaining was faint or absent ([Musumeci et al., 2010](#)). [These data suggested that the altered balance between pRb and Rb expression could favour the upregulation of the *RB* tumour-suppressor gene.]

##### (b) *Human cells in vitro*

The expression of several oncogenes and tumour-suppressor genes (cyclin D1, p21<sup>WAF1/CIP1</sup>, and p53) and pro- and anti-apoptotic genes was studied immunohistochemically in human lung carcinoma A549 cells exposed to fluoro-edenite or to NIOSH crocidolite fibres ([Travaglione et al., 2006](#)). No change in the levels of expression of p53, p21<sup>WAF1/CIP1</sup>, or cyclin D1 was found. The number of viable epithelial cells was decreased, but the loss of cell viability was not associated with apoptosis, as demonstrated by flow cytometry and the absence of changes in the expression of Bax, Bcl-2, and Bcl-X<sub>L</sub>. [These results demonstrated no alterations in the expression of oncogenes and tumour-suppressor genes under these experimental conditions.]

Human immortalized mesothelial MeT-5A and human lung adenocarcinoma A549 cells were incubated with fluoro-edenite at concentrations of 10, 50, and 100 µg/mL (2.12, 10.6, and 21.2 µg/cm<sup>2</sup>) and the expression of several proteins involved in cell-cycle regulation (Rb [unphosphorylated Rb], pRb [phosphorylated Rb], p27, and cyclin D1) was studied by Western blot analysis. In A549 cells, the two higher concentrations induced greater pRb expression in comparison with control cultures. In Met-5A cells, increased pRb expression was induced at the highest concentration only. The expression of cyclin D1, the product of the oncogene *CCND1*, was significantly increased in both A549 and Met-5A cells exposed to 50 and 100 µg/mL. Fluoro-edenite reduced the expression of p27<sup>Kip1</sup>, a negative regulator of cell-cycle progression

at G1 ([Musumeci et al., 2011](#)). [These results suggested that Rb phosphorylation could be upregulated to counteract the stimulation of cell-cycle progression.]

#### 4.4.7 Other mechanisms lacking evidence

No data on inflammasome activation, persistent inflammation, resistance to apoptosis, or depletion of antioxidants were available to the Working Group.

### 4.5 Susceptible populations

No data were available to the Working Group.

### 4.6 Mechanistic considerations

The environmental exposure of sheep to fluoro-edenite leads to the deposition and retention of these fibres in the lungs. Fluoro-edenite samples from Biancavilla can generate hydroxyl radicals directly in acellular assays. In-vitro exposure to fluoro-edenite induces DNA breaks and is associated with cytokine release in human epithelial cells and fibroblasts, and murine monocyte-macrophages. Overall, there is a paucity of mechanistic data for the carcinogenicity of fluoro-edenite.

## 5. Summary of Data Reported

### 5.1 Exposure data

Fluoro-edenite fibrous amphiboles – a new end-member of the calcic amphibole group composed of most fluoro-edenite – have been identified in the volcanic products of Mount Etna near Biancavilla in Sicily, Italy, and represent the first occurrence of amphibole fibres in a volcanic environment; a “fluoro-edenite” compound was also found in the lava dome of the Kimpo volcano, Kumamoto, Japan. Fluoro-edenite is found as

prismatic or acicular crystals or as asbestiform (fibrous) fibres.

The composition of samples of the amphibole fibres from Biancavilla included fluoro-edenite (60%), winchite (24%), tremolite (12%), and richterite (4%). The variable chemical composition of fluoro-edenite and the presence of different components complicate the classification of these fibres and the definition of their mineral species.

Fluoro-edenite and the associated fibrous amphiboles from Biancavilla occur naturally, and early environmental investigations identified the source as local quarry products that had been used in building materials for local structures since at least the 1950s. Unpaved roads made from the local quarry products have also been recognized as a primary source for airborne fluoro-edenite fibres.

Comparison of outdoor air samples taken before mitigation efforts were begun in 2000 with those taken in 2013 showed that mean amphibole concentrations have diminished from 1.76 fibres/L to 0.1 fibres/L. Indoor air may also be contaminated from the use of local quarry products in plaster and mortar. Few data are available to determine the occurrence of fluoro-edenite fibres in water supplies in and around Biancavilla other than one study, in which all 10 samples taken from several local wells and springs were reported to contain fluoro-edenite fibres. Analysis of samples of top soil and roadside dust in Biancavilla showed that approximately 90% of samples contained fluoro-edenite fibres.

### 5.2 Human carcinogenicity data

An excess of mortality from and incidence of mesothelioma has been documented in several surveillance studies in the Biancavilla municipality of Sicily, Italy, where exposure to fluoro-edenite from naturally occurring sources has been found. Although the exposure assessments were essentially ecological in nature, these studies provided strong evidence for a causal



association for several reasons. First, the magnitude of the rate ratios for mesothelioma was large and statistically stable, and thus chance is unlikely to explain these findings. Second, most of these cases did not have any history of occupational or environmental exposure to asbestos. Approximately 70–90% of cases of mesothelioma in the general population are believed to be attributable to exposure to asbestos; therefore, the fact that the majority of the cases in Biancavilla had no known exposure to asbestos strongly suggests another local exposure. The excess observed was similar in both sexes and was most prominent in young adults, which strongly indicates an environmental rather than an occupational cause. Finally, the cases were identified through a national surveillance programme using standardized procedures and their diagnoses were based on extensive pathological review; thus biases of ascertainment and diagnosis are unlikely to explain the findings.

The findings for cancer of the lung were much weaker than those for mesothelioma. A modest excess of mortality from cancer of the lung was observed in women and a modest deficit was observed in men, both with wide confidence intervals. The lack of any strong evidence of an effect on cancer of the lung may reflect that, in contrast to mesothelioma, the background rate of lung cancer is high and its causes are multiple. Furthermore, several studies of environmental exposure to asbestos in other countries have also failed to demonstrate an excess of lung cancer. The Working Group considered that the evidence for carcinogenicity in the lung was inadequate because of the small number of studies, the weak indication of an association, and the lack of controls for potential confounding.

### 5.3 Animal carcinogenicity data

The carcinogenicity of fluoro-edenite fibrous amphibole has been investigated in only one study in experimental animals. Intraperitoneal

injection of fibrous fluoro-edenite caused a significant increase in the incidence of mesothelioma in male and female rats. In the same study, intrapleural injection of fluoro-edenite resulted in a high incidence of mesotheliomas in male and female rats relative to historical controls from that laboratory.

### 5.4 Mechanistic and other relevant data

Fluoro-edenite is biopersistent in the lungs of sheep and is detected in the sputum of exposed humans. In-vitro exposure to fluoro-edenite induces DNA breaks and is associated with the release of cytokines in human epithelial cells and fibroblasts, and murine monocyte-macrophages. The available studies are consistent with the mechanisms proposed for fibre carcinogenicity (see [IARC, 2012](#)). Overall, the mechanistic data for the carcinogenicity of fluoro-edenite are moderate.

## 6. Evaluation

### 6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of fluoro-edenite fibrous amphibole. Fluoro-edenite fibrous amphibole causes mesothelioma.

### 6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of fluoro-edenite fibrous amphibole.

### 6.3 Overall evaluation

Fluoro-edenite fibrous amphibole is *carcinogenic to humans* (Group 1).

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