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## Environmental exposure to asbestos and other inorganic fibres by animal lung investigation

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1 **Environmental exposure to asbestos and other inorganic fibres**

2 **using animal lung model**

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## 22 **Abstract**

23

24 Professional exposure to asbestos fibres is widely recognized as very  
25 dangerous to human health and for this reason many countries have banned their  
26 commercial uses. People, nevertheless, continue to be exposed to low dose of  
27 asbestos from natural and anthropogenic sources still in loco, for which the  
28 potential hazard is unknown.

29 The aim of this research is to assess environmental exposure in an area with  
30 outcropping serpentinite rocks, which bear asbestos mineralizations, using  
31 sentinel animals which are a non-experimental animal model. We studied the  
32 burden of inorganic fibres in cattle lungs which come from two valleys in Italy's  
33 Western Alps bearing serpentinitic outcrops: Susa Valley with a heavy  
34 anthropization and Lanzo Valleys, with a minor human impact. The identification  
35 and quantification of inorganic fibres was performed by scanning electron  
36 microscope (SEM) and energy dispersive spectrometer (EDS). In comparison to  
37 humans, studies of animals have some advantages, such as no occupational  
38 exposure or history of smoking and, in the case of cattle, a sedentary life restricted  
39 to one region.

40 Results spotlight that over than 35% of inorganic fibres found both in Susa and  
41 Lanzo valleys, belong to asbestos mineralogical species (asbestos  
42 tremolite/actinolite, chrysotile s.s., asbestos grunerite, crocidolite). We also  
43 observed a higher concentration of artificial fibrous products in Susa samples  
44 showing a correlation with the level of anthropization.

45 These results confirm sentinel animals are an excellent model to assess  
46 breathable environmental background because it is possible to eliminate some  
47 variables, such as unknown occupational exposure.

48

49 **Key words:** asbestos, inorganic fibres, sentinel animals, environmental  
50 exposure, SEM-EDS

51

## 52 **Introduction**

53 According to International law six fibrous silicates, with length  $> 5 \mu\text{m}$ ,  
54 diameter  $< 3 \mu\text{m}$ , aspect ratio  $> 3:1$ , belong to the asbestos group: asbestos  
55 actinolite, asbestos tremolite, asbestos anthophyllite, asbestos grunerite,  
56 crocidolite and chrysotile (the first five are amphiboles, chrysotile is a serpentine).

57 -Directive CEE 18/2003. In the last century asbestos has been widely used in  
58 many countries: asbestos grunerite, chrysotile and crocidolite have been exploited  
59 for several industrial products (i.e. building materials, heat and noise insulators),  
60 asbestos anthophyllite was also used, but not in significant amounts (its  
61 mineralization are quite rare), asbestos tremolite and asbestos actinolite have not



62 been commercially used but are found as contaminants in mining areas and in  
63 some products, such as talc powder. Over this time people have experienced  
64 intense exposure to asbestos fibres and a correlation between exposure and some  
65 pathologies like asbestosis and mesothelioma, has been found for occupational  
66 exposure to high levels of fibre burden. As a result, commercial use of asbestos  
67 has been banned by many countries, but nevertheless, fibres are continuously  
68 airborne from both anthropogenic sources (e.g. asbestos- cement roof) and natural  
69 one (e.g. outcropping serpentinite rocks, bearing asbestos). Therefore low levels  
70 of asbestos are present everywhere and can be inhaled and deposited in the  
71 deepest parts of lungs.

72 In addition, other non-asbestos minerals can crystallize with fibrous habit and  
73 epidemiological evidence of mesothelioma clusters have been associated also to  
74 low level exposure to non-asbestos fibres (Skinner et al., 1988; Hillerdal, 1999).  
75 To date it has been impossible to define a threshold below which there is no risk  
76 for asbestos and fibrous mineral exposure (WHO, 1986).

77 The Western Alps of Italy in the region of Piedmont are very rich in serpentine  
78 rocks (Fig. 1), many of them outcropping, and some of which have been exploited  
79 in the past for the quarrying of asbestos (i.e. the mine at Balangero, closed in the  
80 1990). In recent decades many researchers have aimed to identify other fibrous  
81 species in these outcropping rocks, and altogether, 11 have been described. In  
82 decreasing order of frequency, these are: chrysotile (with polygonal serpentine),  
83 antigorite, asbestos tremolite, asbestos actinolite, diopside, carlosturanite,  
84 forsterite, balangeroite, sepiolite, brugnatellite and brucite. These fibrous species  
85 are intergrown at sub-micrometric scale to form fibrous bundles (Belluso and  
86 Ferraris, 1991).

87 The Piedmont Region, because of extensive industrialization, also has many

88 anthropogenic sources of asbestos such as roofs in public and private buildings,  
89 and shell flues.

90 In many Italian areas as in Piedmont Region, natural and anthropogenic  
91 contexts occur which interact. In fact many buildings are constructed on  
92 serpentinitic rocks and, at the same time, manufactured materials containing  
93 asbestos are placed in loco. The asbestos species commonly found in natural  
94 sources (i.e. rocks) is asbestos tremolite and asbestos actinolite, whereas from  
95 anthropogenic materials we commonly find crocidolite and asbestos grunerite;  
96 chrysotile fibres can have either origin. Inorganic fibres are continuously airborne  
97 from both natural and anthropogenic sources due to weather and/or human  
98 activities. Thus in areas as such as Piedmont, everyone is exposed to low doses of  
99 airborne asbestos, even if not professionally exposed.

100 The study of mineralogical burden in lung tissues is a useful tool to understand  
101 the natural background of the airborne breathable fibres, as well to monitor its  
102 fluctuations. For this kind of investigation a non-experimental animal model,  
103 defined as “sentinel animals” (De Nardo et al., 2004), is preferred to a human one.  
104 Animals are also exposed to airborne and potentially toxic pollutants, including  
105 asbestos, but they have some advantages like non-occupational exposure and/or  
106 habit to smoke and, in the case of cattle, they are sedentary and live their lives in a  
107 small region.

108 The aim of the present study is to evaluate the environmental background of  
109 breathable inorganic fibres and distinguish between fibres from natural and  
110 anthropogenic sources in two different areas of Western Alps in the Piedmont  
111 region: the Susa Valley and the Lanzo Valleys.

112

## 113 **Materials and Methods**

114 In collaboration with the Veterinary Services of Susa and Lanzo valleys, 39  
115 cattle's lung samples have been collected: 20 from Susa Valley and 19 from the  
116 Lanzo Valleys - both in Piedmont Region of North Western Italy - Fig. 2. In order  
117 to analyse the same part of the lung, the specimens were always taken from the  
118 right lower lobe. All animals were females, except two males from Susa cluster;  
119 the average age was 6 (range: 2-15) and 9 (range: 3-16) years for Susa and Lanzo  
120 samples respectively. The details of the cattle – sex, age and origin – are reported  
121 in Table 1. The distribution map of the 21 localities of where the cattle come from  
122 is shown in Figure 2.

123 Lung samples were examined for count asbestos bodies (Ab) by Optical  
124 Microscope (OM, Leica DMLB at the Pathological Service of Giovanni Bosco  
125 Hospital – Torino). The identification and quantification of inorganic fibres was  
126 carried out by Scanning Electron Microscope (SEM, Cambridge Stereoscan S-  
127 360) equipped with an Energy Dispersive Spectrometer (EDS, Link-Oxford  
128 Pentafet ATW2, Si(Li) detector), located in the Mineralogical and Petrological  
129 Department of the University of Turin.

130 To quantify Ab by OM and/or inorganic fibres by SEM-EDS, a mass of 1500  
131 mg of lung tissues previously preserved in formalin to 10 %, was used. Two  
132 portions of 500 mg were digested each in 30 mL of NaClO in order to eliminate  
133 the organic matrix and to produce a suspension of inorganic material. The first  
134 portion was filtered on a mixed cellulose esters membrane with a diameter of 25  
135 mm and pore size of 3  $\mu\text{m}$  for OM examination. The second one was filtered on a  
136 mixed cellulose esters membrane with a diameter of 25 mm with pore size of 0.45  
137  $\mu\text{m}$  for SEM-EDS observations. During the filtering step, all membranes were  
138 washed with warm, distilled water to accelerate the dissolution of micrometric

139 crystals of NaCl, grown during the chemical digestion. This step is necessary  
140 since NaCl precipitated on the membranes could hide the inorganic particles  
141 and/or it could be included in the analyzed volume thus disturbing the chemical  
142 analyses. The filters were then dried.

143 All membranes prepared for analysis of Ab by OM were attached to a glass  
144 slide using acetone vapour (clarification); the filters used for SEM-EDS  
145 observation were glued onto the SEM aluminium pin stub by adhesive type. These  
146 last filters were also made conductive by carbon sputter coating prior to the SEM-  
147 EDS study.

148 The third portion of 500 mg was dehydrated in a drying oven at 60° C, in order  
149 to measure its dry weight, which was used to determine the concentration of fibres  
150 expressed as the number of fibres per gram of dry lung tissue (Belluso et al.,  
151 2006).

152 Ab counting by OM was carried out observing the whole membrane at 400  
153 magnifications (Karjalainen et al., 1996).

154 Identification and quantification of inorganic fibres was carried out by SEM-  
155 EDS at 2000 magnification and, to minimize both time and costs, observing a  
156 portion of filter according to Belluso et al. (2006). Each inorganic fibre found was  
157 measured in length and diameter and only the particles with an aspect ratio  $\geq 3$   
158 were considered. A chemical analysis was conducted after the observations were  
159 completed. The number of Ab and the inorganic fibres was normalized to 1 gram  
160 of dry weight, according to the international standard (De Vuyst et al., 1998).

161

## 162 **Results**

163 OM observation shows a rare presence of Ab in cattle lungs. In fact, they have  
164 been found in only 7 of the 39 samples - 4 from the Susa Valley and 3 from the

165 Lanzo Valleys as shown in Table 2, where the age of cattle is reported as well. In  
166 all samples Ab concentrations were always below 200 Ab/g<sub>dw</sub>, a value clearly less  
167 than 1000 Ab/g<sub>dw</sub> usually considered as indicative of occupational exposure (De  
168 Vuyst et al., 1998).

169 In the cattle lung samples from Susa Valley 16 different groups of inorganic  
170 fibrous species have been determined by SEM-EDS investigation (Fig. 3). Among  
171 these, 5 were identified as asbestos: asbestos actinolite, asbestos tremolite,  
172 chrysotile s.s., asbestos grunerite and crocidolite. Tremolite and actinolite were  
173 grouped together because their chemical characterization can not be determined  
174 by qualitative EDS-SEM analyses. For these minerals we distinguished between  
175 fibres with asbestos morphology (called ASBESTOS) and fibrous particles  
176 without these parameters (with length < 5 µm and aspect ratio > 3:1, reported as  
177 NAC: Not Asbestos Classified). We included chrysotile in the cluster of asbestos  
178 (chrysotile s.s.) when the fibres presented the elemental analyses of serpentine  
179 and, at the same time, a flexible morphology, typically of chrysotile only (Fig 4).  
180 Alternatively, we put together chrysotile (an asbestos) and antigorite (a not  
181 asbestos) under the label “fibrous serpentine” (Fig 5) because of the difficulty in  
182 distinguishing them correctly using EDS-SEM technique.

183 Among the asbestos species found, asbestos tremolite/actinolite comes from  
184 natural sources in the studied area, asbestos grunerite and crocidolite may come  
185 from anthropogenic sources only - because they are not present in the local  
186 outcropping rocks (Compagnoni et al., 1983); chrysotile may have both origins.

187 Among the fibres not classified as asbestos we determined both mineral phases  
188 (not asbestos tremolite/actinolite, clay and micaceous phyllosilicates, fibrous  
189 serpentine, feldspars, edenite, diopside, balangeroite), and artificial fibrous  
190 products: TiO<sub>2</sub>, silicatic man-made, Fe-Cr rich (Dodis et al., 1982; Uljanova et al.,

191 1999). Fibres of SiO<sub>2</sub> were also found. A very low quantity of fibres was not  
192 identified (N.R. in Fig. 3).

193 The average concentration of total fibres was 100347 ff/g<sub>dw</sub> (0.1 x 10<sup>6</sup> ff/g<sub>dw</sub>)  
194 and considering only the asbestos fibres the average concentration is 36623 ff/g<sub>dw</sub>  
195 (0.04 x 10<sup>6</sup> ff/g<sub>dw</sub>). The most abundant fibrous species found were TiO<sub>2</sub> (33%) and  
196 asbestos tremolite/actinolite (32%); altogether asbestos represents 36% of the  
197 fibres.

198 Inorganic fibres were found in most samples from the Lanzo Valleys. In these  
199 samples we found 4 asbestos species: asbestos tremolite/actinolite, asbestos  
200 grunerite and chrysotile s.s.. Fibres not classified as asbestos included the  
201 minerals fibrous serpentinite, clay/micaceous phyllosilicates, not asbestos  
202 tremolite/actinolite and edenite, whereas TiO<sub>2</sub>, silicatic man-made and Fe-Cr rich  
203 were classified as artificial fibrous products. The Lanzo Valleys samples also  
204 contained fibres of SiO<sub>2</sub> (Fig. 6).

205 The average concentration of all inorganic fibres was 92626 ff/g<sub>dw</sub> (0.1 x 10<sup>6</sup>  
206 ff/g<sub>dw</sub>) with the average concentration of asbestos fibres of 34543 ff/g<sub>dw</sub> (0.03 x  
207 10<sup>6</sup> ff/g<sub>dw</sub>). The most abundant fibrous species are asbestos tremolite/actinolite  
208 (35%) and fibrous serpentine group (21%); the asbestos group represents 37% of  
209 all fibres. A very low quantity of fibres was not identified (N.R. in Fig. 6).

210 Inorganic fibres were detected in all samples apart within one from the Lanzo  
211 Valleys (VL09). At the moment it is not possible to give an exhaustive explanation  
212 for this sample because the lack of fibres might depend on cattle age, its location  
213 or perhaps both.

214 Examining the average concentrations in the two valleys, we found similar  
215 amounts of total inorganic fibres: 100347 ff/g<sub>dw</sub> (0.1 x 10<sup>6</sup> ff/g<sub>dw</sub>) in Susa Valley  
216 and 92626 ff/g<sub>dw</sub> (0.1 x 10<sup>6</sup> ff/g<sub>dw</sub>) in Lanzo ones.

217 Asbestos fibres were found in 29 of the 39 samples: 14 from Susa Valley and  
218 15 from the Lanzo Valleys. We detected the presence of 5 asbestos types in Susa  
219 Valley samples (asbestos tremolite/actinolite, asbestos grunerite, crocidolite and  
220 chrysotile s.s.) and 4 in those from the Lanzo Valleys (asbestos  
221 tremolite/actinolite, asbestos grunerite and chrysotile s.s.). Asbestos coming from  
222 natural sources (tremolite/actinolite), was present in both groups. Asbestos with  
223 anthropogenic origins was rare, detected only in 3 samples (2 from Susa Valley  
224 and 1 from Lanzo); asbestos grunerite was found in both valleys, crocidolite only  
225 in Susa Valley. Fibrous serpentine was found in both valleys.

226 Asbestos tremolite/actinolite always represented the most abundant asbestos  
227 found, with similar average concentrations: 32076 ff/g<sub>dw</sub> in samples from Susa  
228 and 32667 ff/g<sub>dw</sub> in Lanzo samples. On the whole, asbestos made up 36% of the  
229 total fibres detected in Susa samples and the 37% in Lanzo Valleys.

230 Of the fibres not classified as asbestos, the most frequent specie was TiO<sub>2</sub> in  
231 the Susa Valley and “fibrous serpentine” in the Lanzo Valleys.

232

### 233 Morphological data

234 In Figures 7 and 8 the length and diameter of total fibres are plotted for the  
235 Susa and Lanzo valleys. In the Susa Valley samples the average length (L) is 13.2  
236 µm and the average diameter (d) is 1 µm. Most of fibres (84%) fall within the  
237 breathable fibres definition (L > 5 µm and d < 3 µm; WHO, 1986), whilst 16 % of  
238 fibres did not meet these parameters, mainly because they were shorter than 5 µm  
239 (only one fibre had a diameter greater than 3 µm). One fibre satisfied the Stanton'  
240 definition of carcinogenic fibre (L > 8 µm and d < 0.25 µm; Stanton et al., 1981).  
241 With regard to the Lanzo samples the plot is less diverse: the average length is  
242 12.6 µm and the average diameter is 1.1 µm. For this group, most of fibres (82%)

243 have dimensions which fall within the breathable definition; the 18% of fibres  
244 with dimensions outside this definition had a length less than 5 µm. None of the  
245 fibres met the Stanton definition parameters.

246 The data for only the asbestos tremolite/actinolite fibres in the Susa and Lanzo  
247 samples are shown in Figures 9-10.

248 For both groups, these fibres fall within the breathable fibres definition and  
249 none of them is in agreement with the Stanton parameters. The two valley samples  
250 had different average length: in the Susa samples the asbestos tremolite/actinolite  
251 fibres have lengths with an average value of 19.6 µm, whereas in the Lanzo  
252 samples the average length is 10.5 µm. The average diameter of fibres was 1.1 µm  
253 for the Susa samples and 1.2 µm for Lanzo samples.

254

## 255 **Discussion**

256 The study examined 39 lung samples, 20 from the Susa Valley and 19 from the  
257 Lanzo Valleys.

258 Asbestos bodies were found in only 7 samples (4 from Susa and 3 from Lanzo  
259 respectively) and always in very low concentrations with respect the limit of 1000  
260 Ab/g<sub>dw</sub>, the threshold indicating professional exposure (De Vuyst et al., 1998). The  
261 inorganic fibre concentrations detected in the two clusters are compared in Figure  
262 11. For a better comprehension the following groups are reported: “natural source  
263 asbestos”, “anthropogenic source asbestos”, “chrysotile s.s.”, “fibrous serpentine”  
264 and “artificial fibrous products” (TiO<sub>2</sub>, silicatic man-made, Fe-Cr rich), in order to  
265 show differences between natural and anthropogenic fibres found in the two  
266 clusters.

267 Similar concentrations of asbestos from natural sources (tremolite/actinolite)  
268 and asbestos from anthropogenic sources (crocidolite, asbestos grunerite) were



269 found. Fibres of chrysotile s.s. are almost absent in both valleys. It is very  
270 interesting to note that instead, a significant difference between the concentrations  
271 of the fibrous serpentine group and artificial fibrous products (TiO<sub>2</sub>, silicatic man-  
272 made, Fe-Cr rich). The fibrous serpentine is much more abundant in the Lanzo  
273 Valleys (21%) with respect to the Susa Valley (3%), whereas artificial fibrous  
274 products are higher in Susa samples (45%) than in Lanzo ones (20%). This result  
275 emphasizes the different anthropization of two valleys, showing clearly the higher  
276 human impact in Susa Valley. A correlation between natural source mineral phases  
277 (asbestos tremolite/actinolite, non asbestos tremolite/actinolite) and outcropping  
278 serpentine rocks was also found. For this, the average concentrations of these two  
279 mineral groups were split between the upper and lower valleys (see Table 3)  
280 because the outcropping rocks (matrix of tremolite/actinolite fibres) are more  
281 widespread in the Lower Susa Valley (LSV) and in the Upper Lanzo Valleys  
282 (ULV). By these comparisons, we can see that animals from Upper Susa Valley  
283 (USV) and Lower Lanzo Valleys (LLV) show less amounts ( $0.01 \times 10^6$  ff/g<sub>dw</sub> and  
284  $0.01 \times 10^6$  ff/g<sub>dw</sub> respectively) than Lower Susa Valley and Upper Lanzo Valleys  
285 ( $0.06 \times 10^6$  ff/g<sub>dw</sub> and  $0.13 \times 10^6$  ff/g<sub>dw</sub> respectively), in agreement with the lower  
286 quantities of serpentinite outcrops.

287 When we compare only the concentration of asbestos tremolite/actinolite from  
288 natural sources in the Susa and Lanzo samples with similar studies (i. e. Dumortier  
289 et al. 2002; Abraham et al., 2005) we note that in comparison with Corsican goats  
290 (Dumortier et al. 2002) we found lower concentrations in USV and LLV, similar  
291 amounts of LSV and higher quantities of ULV. Our concentrations were all lower  
292 than the California data (Abraham et al., 2005) - Table 3. Nevertheless, these  
293 papers don't report precisely the same research conditions because the animals are  
294 different and the environmental context as well, but they are the only data

295 available in the scientific literature.

296 We found a similar morphology distribution when comparing the average  
297 dimensions for the whole inorganic fibres (length 13.2  $\mu\text{m}$  and diameter 1  $\mu\text{m}$  for  
298 Susa Valley and length 12.6  $\mu\text{m}$  and diameter 1.1  $\mu\text{m}$  for Lanzo ones).

299 When the sizes of only asbestos tremolite/actinolite was compared, we found  
300 similar average values for the diameters (1.1  $\mu\text{m}$  and 1.2  $\mu\text{m}$  respectively for Susa  
301 and Lanzo Valleys), whereas for the lengths we found different average values. In  
302 fact, in the Susa Valley the average length was 19.6  $\mu\text{m}$  and in the Lanzo Valleys  
303 it was only 10.5  $\mu\text{m}$ . This difference may be correlated to the size of fibres in the  
304 natural source (i.e. into matrix rocks), because the same animal species with  
305 similar range-ages were sampled.

306

## 307 **Conclusions**

308 Analyzing cattle lung samples for the research of asbestos and inorganic fibres  
309 can be useful in order to shed light on the typology and amounts of inorganic  
310 breathable airborne fibres in the environment. The presence of asbestos and  
311 inorganic fibres in sentinel animals can provide information about “environmental  
312 background”, a term used here to indicate the average exposure to airborne fibres,  
313 according to the topographic, geological, anemometric, and anthropogenic  
314 characteristics in specific areas. In this experimental model it has been possible to  
315 evaluate the environmental background for natural and anthropogenic inorganic  
316 fibres in the two valleys.

317 A very low concentration of asbestos bodies were detected in only 7 samples (4  
318 from Susa Valley and 3 from Lanzo Valleys respectively) and no correlation with  
319 the age or localities is found.

320 Moreover, results show higher concentrations of artificial fibrous products

321 (45%) in the Susa Valley, which is more industrialized than the Lanzo Valleys  
322 (20% of artificial fibrous products). In addition, we found a correlation between  
323 the concentration of natural source mineral phases (asbestos tremolite/actinolite,  
324 non asbestos tremolite/actinolite) in lungs and the higher distribution of  
325 outcropping serpentine rocks in Upper Lanzo Valleys and the Lower Susa Valley.

326 The low concentration of chrysotile s.s., also abundantly present in the  
327 outcropping serpentine rocks, probably depends upon its low biopersistence in the  
328 lungs (Bernstein and Hoskins, 2006).

329 The limited number of samples does not allow a correlation between age of  
330 animals and inorganic fibres burden. Moreover for this purpose, it would be better  
331 to analyses older animals – which more closely simulates human exposure– but  
332 for commercial reasons the cattle are slaughtered while young.

333 These results show that there is a significant local environmental exposure for  
334 the human population living in the studied areas. And that the study of inorganic  
335 fibres in animals is important in order to better understand the morphological  
336 characteristics of breathable inorganic fibrous particles and correlate this data to  
337 potential carcinogenic effects for humans.

338 The sentinel animals are an excellent model to assess breathable environmental  
339 background because it is possible to eliminate some variables, such as unknown  
340 occupational exposure.

341

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395 **Tables**

396 Table 1: List of the samples analysed both in Susa and Lanzo valleys. For each

397 of them the sex, the age and the locality are reported.

SUSA Valley				LANZO Valleys			
SAMPLE code	SEX	AGE (years)	LOCALITY	SAMPLE code	SEX	AGE (years)	LOCALITY
VS65	♀	2	Bardonecchia	VL05	♀	9	Ala di Stura
VS66	♀	4		VL07	♀	16	
VS08	♀	6	Bruzolo	VL02	♀	4	Balangero
VS22	♀	12	Bussoleno	VL16	♀	12	
VS67	♀	7	Cesana	VL17	♀	9	
VS36	♀	4	Chiomonte	VL18	♀	10	
VS40	♀	3		VL12	♀	8	Cafasse
VS64	♀	10	Exilles	VL09	♀	3	Ceres
VS15	♀	11	Mompantero	VL14	♀	7	Coassolo
VS11	♀	4	Novalesa	VL03	♀	7	
VS34	♀	5	Oulx	VL06	♀	12	Corio
VS39	♀	4		VL04	♀	7	
VS58	♀	8		VL13	♀	5	
VS62	♀	7		VL19	♀	3	
VS45	♀	5	Salbertrand	VL20	♀	15	Groscavallo
VS01	♀	11	Susa	VL21	♀	12	
VS14	♂	2		VL11	♀	13	
VS02	♀	15	Venaus	VL15	♀	9	Monastero
VS21	♂	3		VL01	♀	12	S. Gillio
VS72	♀	9					

398

399

400 Table 2: Concentrations of Ab found in 7 samples (4 from Susa and 3 from  
 401 Lanzo valleys).

SUSA Valley			LANZO Valleys		
SAMPLE and LOCALITY	AGE (years)	Ab/g <sub>dw</sub>	SAMPLE and LOCALITY	AGE (years)	Ab/g <sub>dw</sub>
VS66 Bardonecchia	4	43	VL03 Coassolo	7	13
VS39 Oulx	5	50	VL16 Balangero	12	183
VS45 Salbertrand	5	25	VL07 Ala di Stura	16	33
VS64 Exilles	10	50			

402

403



404 **Table 3:** Comparison between the average concentration and the range of fibres  
 405 from natural source found in animals from Susa and Lanzo Valleys

	SUSA Valley		LANZO Valleys		Corsica <sup>1</sup>	California <sup>2</sup>	
	cattle		cattle		goats	cats	dogs
	Upper	Lower	Upper	Lower			
Asb tr/act +	0.01	0.06	0.13	0.01			
non Asb tr/act							
range	0-0.06	0-0.3	0-0.5	0.04			
Asb tr/act	0.01	0.06	0.09	0.01	0.04	0.12	2.98
range	0-0.05	0-0.3	0-0.4	0-0.4	0-0.15		

406 Corsica<sup>1</sup> (Dumortier et al., 2002) and California<sup>2</sup> (Abraham et al., 2005). tr:

407 tremolite, act: actinolite

## 408 Figure captions

409 Figure.1: Lithological map of Piedmont Region (Regione Piemonte, 1990).

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1. Terraced, recent and present day fluvial deposits, mainly consisting of coarse-grained sand bodies with clayey intercalations (Olocene-Middle Pleistocene)
  2. Complexes of morainic ridges and intermorainic depressions (Upper Pleistocene-Middle Pleistocene)
  3. Yellow sands with gravel lenses or silty-clayey coastal sediments; sandy, sandy-gravelly or loamy-clayey alluvial deposits ("Villafranchian", Middle Pliocene-Lower Pleistocene)
  4. Sands richly marine molluscs bearing with sandstones lenses (Middle Pliocene)
  5. Pelites, clayey marls, marls, gypsarenites and evaporites (Middle Pliocene-Messinian)
  6. Marls with interbedded sand or sandstone levels (Miocene)
  7. Marly siltstones, with locally interbedded sandstone levels or conglomerate lenses (Miocene-Upper Oligocene)
  8. Sandstone and conglomerate levels, with interbedded marls and sandy marls (Oligocene)
  9. Clays, marls, limestones and chaotic clayey complex (Cretaceous-Eocene)
  10. Ophiolites: peridotites, gabbros, basalts, serpentinites and ophiolitic breccias, with various grade metamorphism (Piedmont-type units, Jurassic-Cretaceous)
  11. Quartzites, schists, marbles, phyllites ("Schistes Lustrés" Auctt., Piedmont-type units, Jurassic-Cretaceous)
  12. Crystalline dolostones and limestones, dolomitic limestones, sandy-marly limestones (Cretaceous-Triassic)
  13. Micaschists and gneisses, with subordinate phyllites, quartzites, eclogites, slates and marbles (Hercynian and Pre-Hercynian crystalline massifs)
  14. Gneisses and migmatites, with subordinate schists, porphyries and amphibolitic lenses (Hercynian and Pre-Hercynian crystalline massifs)
  15. Plutonic and volcanic Alpine or Pre-Alpine rocks

431

432 Figure 2: Distribution map of the 21 localities of where the cattle come from; ● localities  
433 in Lanzo Valleys, \* localities in Susa Valley.

434

435 Figure 3: Concentrations of fibres in cattle from Susa Valley, expressed both as ff/g<sub>dw</sub> and  
436 percent. ASBESTOS and Not Asbestos Classified (NAC). Tremolite/actinolite are divided  
437 according to dimension of breathability; tr: tremolite, act: actinolite. N.R. = Not  
438 Recognized.

439

440 Figure 4: Secondary electron image SEM of a chrysotile fibre (2000M).

441

442 Figure 5: Backscattered electron SEM image of serpentine fibres: chrysotile and/or  
443 antigorite (2000M).

444

445 Figure 6: Concentrations of fibres in cattle from Lanzo Valleys, expressed both as ff/g<sub>dw</sub>  
446 and percent. ASBESTOS and Not Asbestos Classified (NAC). Tremolite/actinolite are  
447 divided according to dimension of breathability; tr: tremolite, act: actinolite. N.R. = Not  
448 Recognized.

449

450 Figure 7: Plot of length and diameter of total inorganic fibres found in Susa samples.

451           - - - - - mineralogical fibres;  
452           — area ascribed to breathable fibres;  
453           ..... area for fibres according to Stanton definition  
454

455 Figure 8: Plot of length and diameter of total inorganic fibres found in Lanzo samples;

456           - - - - - mineralogical fibres  
457           — area ascribed to breathable fibres  
458           ..... area for fibres according to Stanton definition  
459

460 Figure 9: Plot of length and diameter of asbestos tremolite/actinolite fibres found in Susa  
461 samples;

462           - - - - - mineralogical fibres  
463           — area ascribed to breathable fibres  
464           ..... area for fibres according to Stanton definition  
465

466 Figure 10: Plot of length and diameter of asbestos tremolite/actinolite fibres found in  
467 Lanzo samples;

468           - - - - - mineralogical fibres;  
469           — area ascribed to breathable fibres;  
470           ..... area for fibres according to Stanton definition  
471

472 Figure 11: Comparison between average concentrations of inorganic fibres found in cattle  
473 lung tissue from Susa and Lanzo Valleys; N.S.= natural sources (asbestos  
474 tremolite/actinolite); A.S. = anthropogenic sources (for crocidolite and asbestos  
475 grunerite).

### Fornero\_Figures

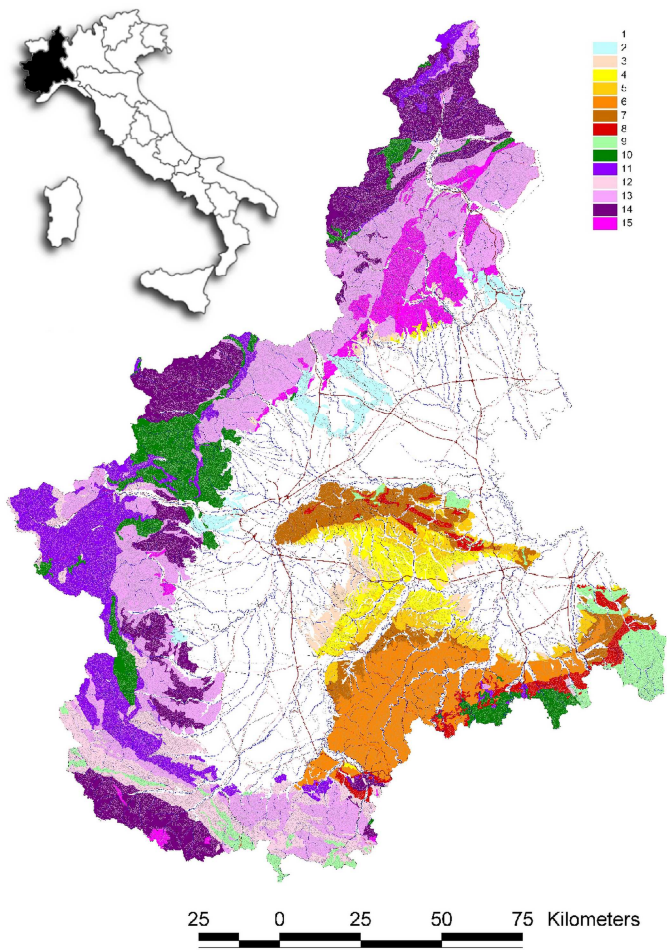


Fig. 1

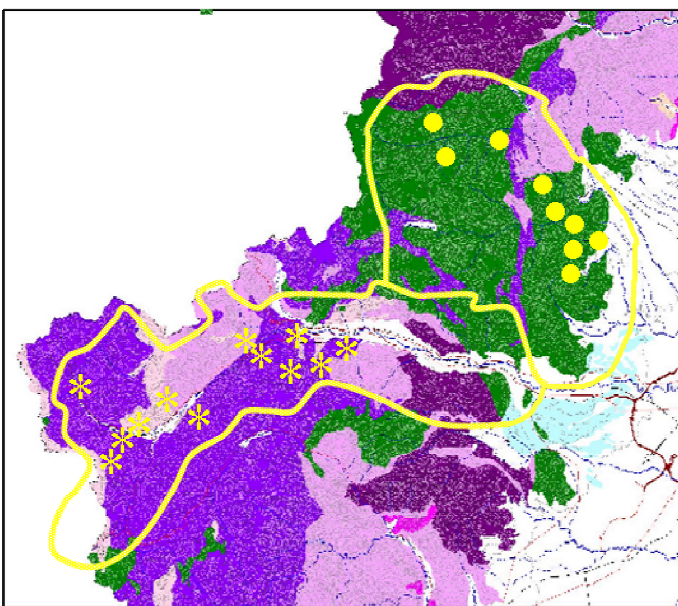


Fig. 2

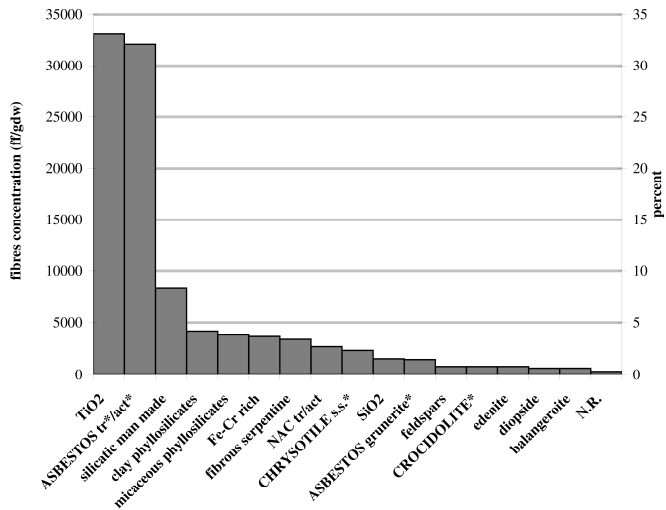


Fig. 3

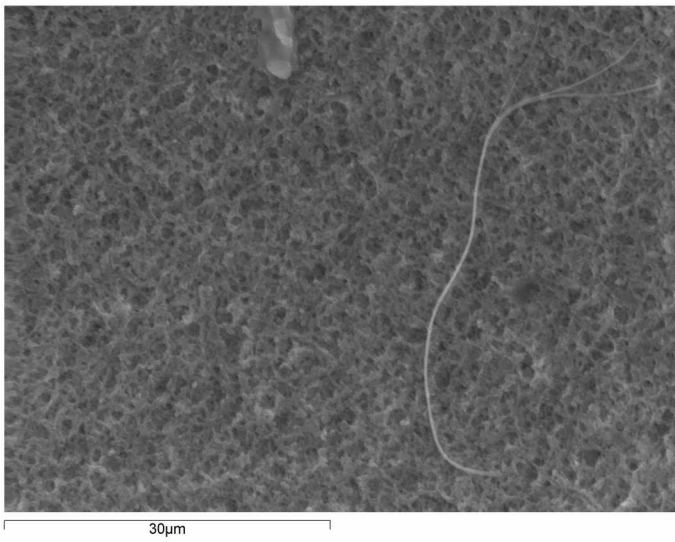


Fig 4

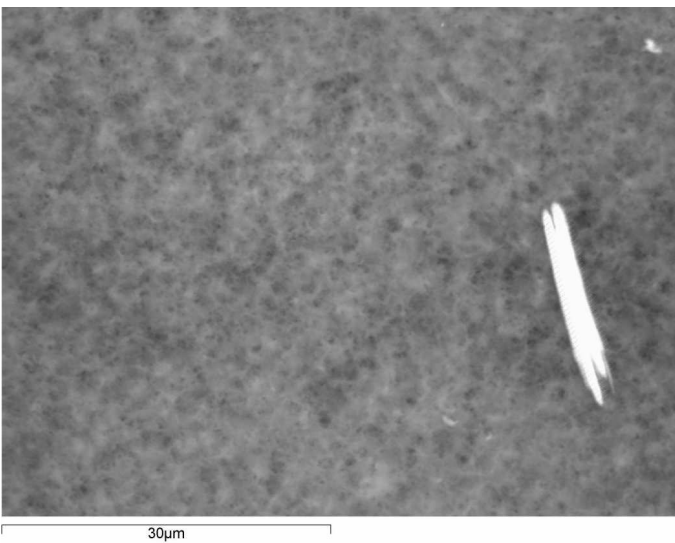


Fig.5

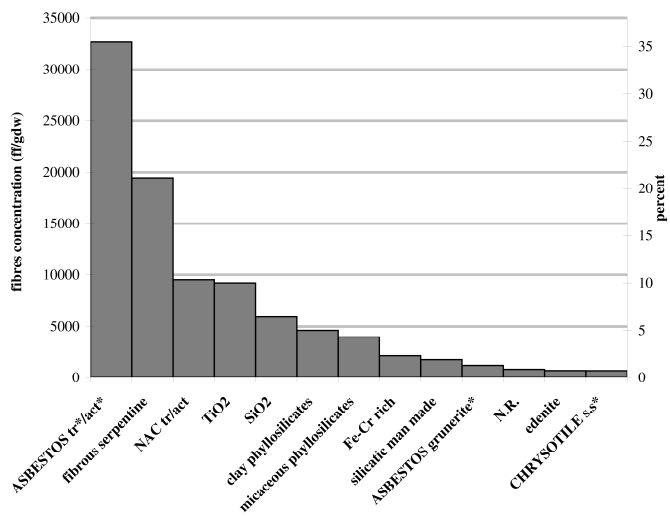


Fig. 6

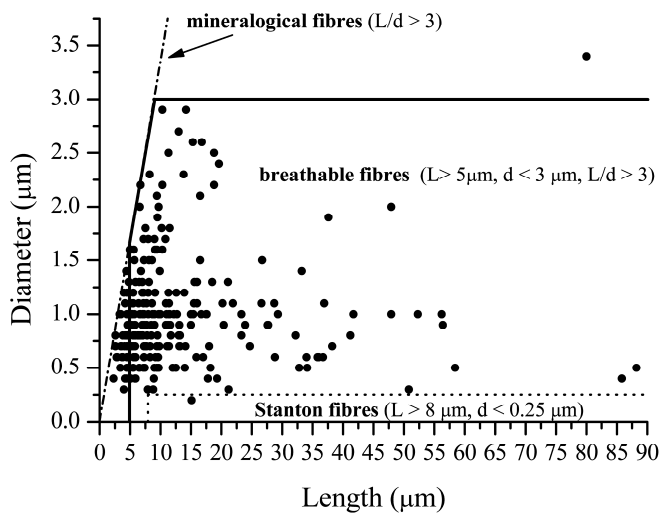
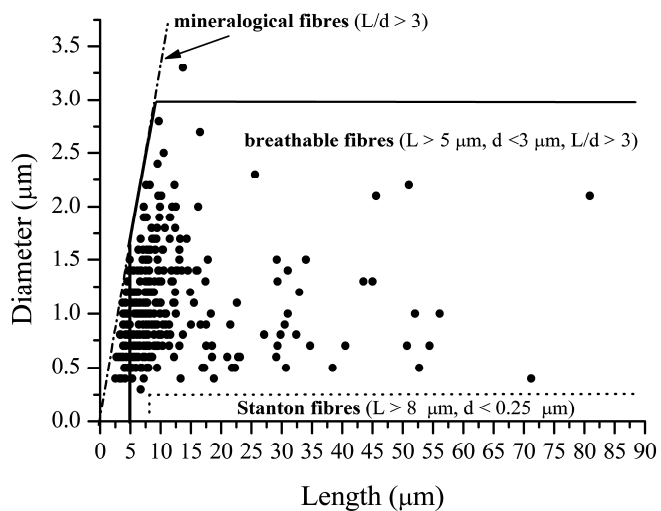
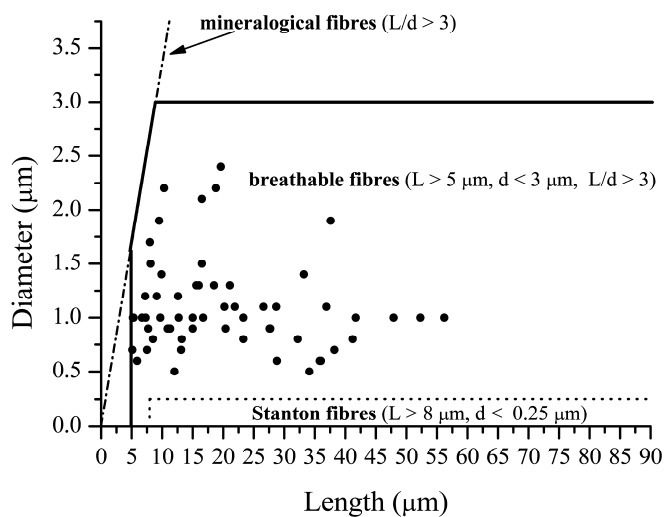


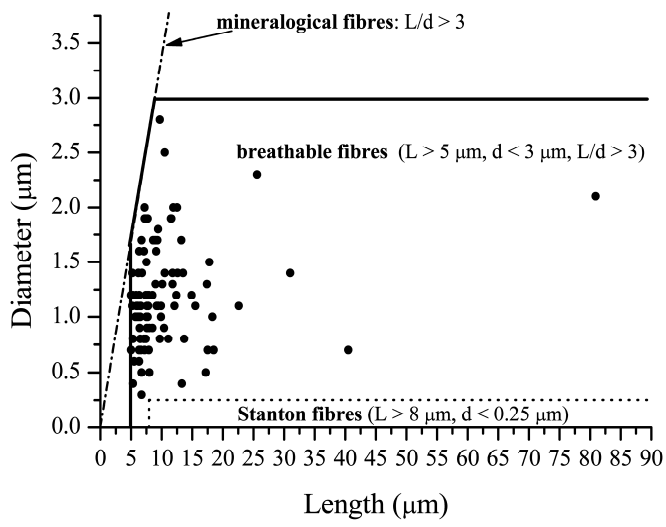
Fig. 7



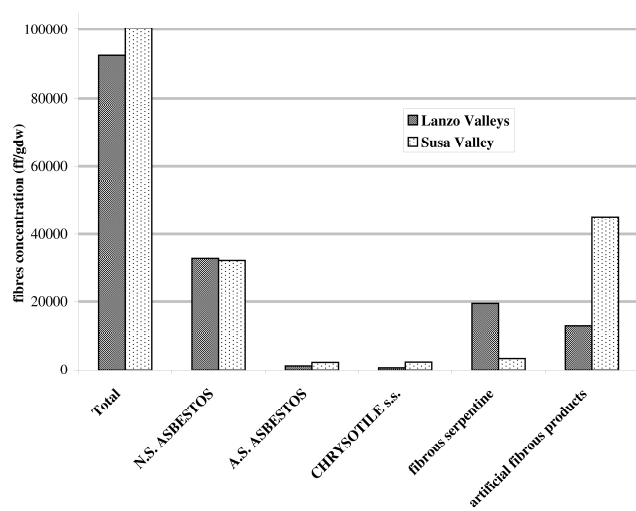
**Fig. 8**



**Fig. 9**



**Fig. 10**



**Fig. 11**