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1 **TITLE: Wild rats as urban detectives for latent sources of asbestos contamination**

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20 **Keywords: asbestos contamination, sentinel animals, SEM-EDS analysis, environmental risk**
21 **assessment, exposure assessment.**

22 **HIGHLIGHTS**

23

24 **Abstract**

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26

27

28 1. INTRODUCTION

29

30 According to international laws ‘asbestos’ is a set of six asbestiform silicates, five belonging to the
31 mineralogical group of amphiboles (actinolite, amosite or grunerite asbestos, anthophyllite,
32 crocidolite and tremolite) and one to serpentine group (chrysotile) (Dir. 2009/148/EC).

33 In the past, nearly all over the world, asbestos have been widely employed in many composite
34 materials for their chemical-physical and technological properties: resistance to abrasion, heat (non-
35 flammable even at high temperatures) and chemicals, low sound-transmission coefficients, low
36 thermal conductivity and low density, flexibility (Gualtieri, 2012). Chrysotile, crocidolite and amosite
37 were broadly employed in the building field to produce asbestos-cement.

38 The asbestiform habit and surface activity are responsible of adverse effects on human health, when
39 the fibres from asbestos and asbestos containing material (ACM) are inhaled, especially at high doses.
40 ACM may potentially release respirable fibres (Stanton, 1981) especially if the ACM is deteriorated
41 or constituted by a friable matrix. At present, there is a large body of scientific literature on the
42 presence of asbestos fibres in human lungs (and other tissues and organs) and its association with
43 pathological changes (*e.g.*, lung cancer, pleural and peritoneal mesothelioma) both in humans and in
44 animals (IARC, 2012). In Italy there were from 2001 to 2007 about 1,000 cases of deaths per year for
45 pleural mesothelioma, 3,000 estimated cases of lung cancer and 560 asbestosis per year from 2003 to
46 2007 (Marinaccio *et al.*, 2008); between 1993 and 2015 the Italian National Register of Malignant
47 Mesotheliomas collected 27,356 cases (Marinaccio *et al.*, 2018).

48 From 1907 to 1986 the most important Italian manufacturing plant of asbestos-cement (Eternit S.p.A.)
49 was active in the municipality of Casale Monferrato (Piedmont Region, north-western Italy).
50 Corrugated and plane sheets, pipes and pressure pipes and other artefacts were produced, reaching a
51 peak consumption of 15,000 metric tons of raw asbestos in 1981. Chrysotile represented 90% of all
52 asbestos used that year and crocidolite the remaining 10% (Maule *et al.*, 2007). Chrysotile was
53 provided by the Italian mine in Balangero but was also imported, mainly from Russia. It is worth
54 noting that Russian chrysotile was contaminated by tremolite asbestos (REF?). Today Casale
55 Monferrato is known for the great impact that occupational exposure to asbestos had on the health of
56 its citizens, in terms of incidence of asbestos related diseases (*i.e.*, asbestosis, mesothelioma, lung
57 cancer, etc.) (Magnani *et al.*, 2001; Magnani *et al.*, 2007; Comba *et al.*, 2018). However, most
58 mesothelioma cases occurring among residents of Casale Monferrato had never been employed at the
59 asbestos-cement factory. Epidemiological studies carried out in Casale Monferrato have shown that
60 the asbestos related diseases can not only depend on occupational and para-occupational exposure
61 but also on passive exposure in asbestos containing buildings, such as public offices or schools, where

62 the people involved have no awareness of direct physical contact with asbestos-containing material
63 (Mirabelli *et al.*, 2010; Comba *et al.*, 2018). The main sources of exposure were traced to residential
64 exposure to fall-out from the factory, sharing home with Eternit workers, and reuse of waste materials
65 that were made freely available to the local population. These materials included broken products,
66 reduced by people to the dimension of small pebbles and used as substitutes of gravel in road and
67 courtyard pavements, and fine dust resulting from the grinding of asbestos-cement pipes extremities,
68 used as thermal insulation in attics or remixed with cement to produce pavements (Magnani *et al.*,
69 2001; Maule *et al.*, 2007). In 1992 the Italian legislation forbade the extraction, import, processing,
70 marketing, use and sales of asbestos and ACM and required the issue of information about
71 remediation measures and controlled disposal. The widespread uses of asbestos-cement waste
72 materials were the target of remediation projects promoted over the years by the Casale Monferrato
73 municipality, but no census existed and there is concern that potential sources of asbestos pollution
74 still exist in the city, unrecognised because either undeclared or forgotten, perhaps decades after their
75 installation.

76 In recent decades, it was found that the identification and monitoring of a wide variety of hazardous
77 environmental pollutants on human health can be done through surveys of animal populations defined
78 as “sentinel animals” systems (Reif, 2011). Specific experiences have been gained on the animal
79 exposure to asbestos (Dumortier *et al.*, 2002; De Nardo *et al.*, 2004; Bellis *et al.*, 2005; Belluso *et al.*,
80 2006; Fornero *et al.*, 2009; Ben-Shlomo *et al.*, 2011; Capella *et al.* 2017).

81 In 2011, our research team has conducted a pilot study to develop and assess procedures to capture
82 wild rats and analyse them to make possible the monitoring of an urban area (Ardizzone *et al.*, 2014).
83 Casale Monferrato had been selected as study area based on the known historical and diffuse asbestos
84 contamination and the choice of the rats was based on their adaptation in urbanized environments,
85 the extensive and widespread presence and the demonstrated susceptibility to asbestos fibres.

86 The aim of this study was to uncover sites with the greatest potential of non-occupational exposure
87 to asbestos within the urban area of Casale Monferrato by quantifying the amount of asbestos fibres
88 in lung tissue of captured rats used as sentinel animals.

89

90 **2. MATERIAL AND METHODS**

91

92 **2.1. Design of the study and sampling**

93 Rats (*i.e.*, *Rattus rattus* and *R. norvegicus*) have been chosen as sentinel animals to uncover asbestos
94 contamination sites in the urban area of Casale Monferrato.

95 The sample design was initially set as follows:

96 a) A regular grid of squares of 200 meters per side was placed on the map of the city (Fig. 1,
97 OpenStreetMap® cartographic image processed with QGIS System). The length of the side of the
98 squares was defined considering the rat behaviour and the home range usually covered around a
99 permanent den (Gardner-Santana *et al.*, 2009). Initially the priority was given to areas with buildings
100 built in 60's and 70's ("60/70 area"), perimeter area located S and SW of the old town, *i.e.* dating
101 back to the period of greater asbestos production and high densities of population. A control location,
102 to check the ability of rat capturing of the research team, has been added in a suburban area (~2.5 km
103 from Casale Monferrato) where the presence of rats had been reported by private citizens.

104 b) Multiple capture traps (Ekomille, Ekocommerce SRL, Atessa, Italy) were selected as sampling tools.
105 These devices allow multiple and continuous catches, until 10-12 wild rats are captured discouraging
106 other animal species to enter the trap. Traps were used following the instructions of the construction
107 companies using the appropriate personal protective equipment. It was planned that the available
108 traps (1 to 3) should have been placed as close as possible to the centre of squares for about 3 weeks
109 or less if the number of captured rats had been satisfactory (initially a target of 5 rats per trap had
110 been fixed). After the maximum 3-week period the traps were moved to other squares in the grid.
111 Each sampling point was localized using a GPS. The traps were named with the name of the quadrant
112 (letter-number, Fig. 1) plus a consecutive number per site and activation period (mid-April 2013 to
113 end-June 2015).

114 c) The rats had to be individually subjected to necropsy and histopathology. Later, the lung material
115 at each trapping sites was to be pooled and analysed, keeping separate the material from rats weighing
116 100 g or more (*heavy rats*: H) from that of smaller rats (*light rats*: L), regardless of sex or species.
117 The "100 g" cut-off was then used as a proxy for age at exposure to asbestos fibres: the assumption
118 was that younger animals (body-weight < 100 g), given their shorter lifespan, would have had fewer
119 opportunities to inhale and accumulate asbestos.

120 After the first year of sampling, the number of sampling points with successful captures and the
121 number of captured rats were much lower than expected. To improve capture performance, advice
122 from rodent control companies was obtained and an enhanced cooperation was searched from citizens
123 reporting rats. Therefore, the revised procedures about the traps' management were: site selection
124 based on information from residents, new types of bait, multiple traps per sampling point. Additional
125 30 individual-capture traps (hereinafter called as "snap traps") were incorporated into the sampling.
126 These traps (Trapper® T-Rex, Bell Laboratories Inc., Madison, US) differed in the working features
127 (snap spring), in the number of catchable rats (one animal at time) and in the management of the
128 captured animals. Moreover, as a result of citizen reporting, the area was extended to the Casale's old
129 town (Fig. 1) where inhabitants were complaining about the presence of many rodents.

130

131 **2.2. Necropsy**

132 The captured rats were sexed and weighed. A necropsy was performed according to a standardised
133 protocol in order to detect the presence of any lesions referable to zoonotic diseases (as post-capture
134 safeguarding of the health of the research team) and to collect tissue portions for further examination.
135 Spleen, liver and kidneys were collected and tested for *Francisella tularensis* and *Leptospira* spp. by
136 PCR (Forsman *et al.*, 1994; ...). Lungs were sampled and fixed in 4% buffered formaldehyde
137 solution. The left lung was subjected to histopathology whereas the right lung was subjected to
138 electron microscopy. Heart, spleen, liver and kidneys were also sampled for histopathological
139 examination to evaluate the general health status of the animals.

140

141 **2.3. Histopathology**

142 Histological examination of the lung tissue was performed in order to highlight the so-called asbestos
143 corpuscles, golden-brown rounded or handlebar formations with a thin and translucent fibre in the
144 core, or any histopathological changes related to the inhalation of asbestos fibres. The corpuscles are
145 typically described around fibres of amphiboles, while rarely around those of chrysotile and are
146 usually located within the fibrous tissue or can be placed within the alveolar spaces or
147 intracytoplasmic in macrophages or in multinucleated giant cells.

148 Each sampled organ was fixed in 10% neutral buffered formalin and routinely processed: paraffin
149 inclusion and cut into 3-5 µm sections and stained with haematoxylin-eosin (HE).

150 Serial sections of the lung were also stained by two histochemical stainings: Perls (method for ferric
151 iron, Bio Optica Milano S.p.A, Milan, Italy) and Masson (Masson trichrome with aniline blue, Bio
152 Optica Milano S.p.A, Milan, Italy), in order to easily detect, respectively, the presence of ferruginous
153 corpuscles due to asbestos and the presence and the extent of pulmonary fibrosis.

154 The Perls staining is suitable to stain in blue iron-ferric on tissue sections but is not able to stain the
155 iron-ferrous and the iron bound to haemoglobin, ferritin and pigments due to use of acid formalin. As
156 positive control for the Perls staining was used a sample of lung tissue from rats subjected to
157 inhalation of amosite. The result of Perls staining was expressed as positive or negative.

158 The Masson staining allows the visualization of muscular fibres in red, collagenous tissue in blue and
159 erythrocytes in yellow. An *ad hoc* grading score was used for the evaluation of presence/absence of
160 pulmonary fibrosis and its extent, performing a semi-quantitative evaluation: Negative (absence of
161 appreciable pulmonary fibrosis), Positive with grade I (minor fibrosis), Positive with grade II
162 (moderate fibrosis), and Positive with grade III (severe fibrosis).

163

164 **2.4. Scanning electron microscopy**

165 The pools of lung samples were prepared for scanning electron microscopy with energy dispersive
166 spectrometry (SEM-EDS) and examined to evaluate the presence of asbestos fibres and their
167 concentration according the protocol described by Belluso *et al.* (2006).

168 Each preparation was investigated by SEM (StereoScan-360, Cambridge Instruments, Cambridge,
169 UK). Only inorganic particles having aspect ratio $> 3:1$ have been considered. Their chemical
170 composition was measured by EDS (INCA Energy 2000, Oxford Instruments, Abingdon, UK). The
171 detected inorganic fibres were identified by comparing their EDS spectrum with those collected in
172 the available laboratory database. The inorganic fibres detected in rat lungs have been classified in
173 ‘asbestos fibres’ and ‘non-asbestos fibres’, and in turn the ‘asbestos fibres’ were divided based on
174 dimension into ‘short asbestos fibres’ (SAF: $L < 5 \mu\text{m}$, $d < 3 \mu\text{m}$ and $L/d > 3$) or ‘long asbestos fibres’
175 (LAF: $L > 5 \mu\text{m}$, $d < 3 \mu\text{m}$ and $L/d > 3$) according to the criteria defined in the European directive
176 (Dir. 2009/148/EC).

177 By the SEM-EDS it is difficult to distinguish correctly between chrysotile and asbestiform antigorite
178 (a non-asbestos), both species belonging to the serpentine group (Fornero *et al.*, 2009). When it was
179 not possible to classify the fibre as chrysotile *sensu stricto*, in conservative mode and considering that
180 the final objective is to locate residual sources of asbestos to sanitize the area, for our analysis we
181 assumed that these fibres belonging to the asbestos group chrysotile. Besides, our assumption is
182 supported by the fact that there is no deposit or natural outcrops of these minerals were reported near
183 Casale Monferrato; therefore, it was reasonable to assume that these fibres derive from anthropogenic
184 manufacturing linked to asbestos.

185 Since mineral species tremolite and actinolite (both asbestos) do differ only in their chemical
186 composition (amount of Mg and Fe), it is not possible to distinguish them by SEM-EDS, are hereafter
187 considered one group (indicated tremolite/actinolite) (Capella *et al.*, 2017). The number of detected
188 inorganic fibres was normalized to 1 g of dry tissue as indicated by international guidelines (De Vuyst
189 *et al.*, 1998) reporting the concentration in terms of load of asbestos fibres per gram of dry lung tissue
190 weight: ff/gdw.

191

192 **2.5. Statistical analysis**

193 The concentration of inorganic fibres (ff/gdw) detected in each pool was described by sampling point,
194 fibre category and weight group (*i.e.*, L: $< 100 \text{ g}$ or H: $\geq 100 \text{ g}$) of sampled animals.

195 A statistical analysis was aimed at exploring the existence of geographical differences in the
196 concentration of asbestos fibres and to detect the potential for outliers associated to specific sites. The
197 outliers identified were considered as probable hot-spot points. For this purpose, since the fibre

198 concentration in the different pools analysed did not fit a Gaussian distribution, a MAD (median
199 absolute deviation) method was used (Leys *et al.*, 2013; Iglewicz and Hoaglin, 1993). Iglewicz and
200 Hoaglin (1993) suggested that observations could be labelled outliers when $|MAD\text{-score}| > 3.5$.
201 Statistical analysis was performed using Stata 14.1 (StataCorp, 2015).

202 Furthermore, to identify the most likely area where putative unrecognized local sources of asbestos
203 contamination were present, we delimited a circular area with a 100 m radius (“buffer area”) around
204 sampling points with outliers in the lung fibre burden. One hundred meters is a plausible distance
205 covered by wild rats around a permanent den (Byers *et al.*, 2019). The buffer area was processed with
206 QGIS System (GNU) and drawn on an OpenStreetMap[®] cartographic image (CC BY-SA).

207

208 **3. RESULTS**

209

210 **3.1. Rodent trapping**

211 Based on the operational capacity of the research team and the reporting of murine presence by the
212 citizens, over the study period, the traps have been placed in (or just outside) 29 squares of the grid
213 (17 from the 60/70 area and 12 from the old town, Fig. 1). It was not possible to obtain captures in
214 all the sampled squares, although the presence of rodents was demonstrated by the large consumption
215 of bait in all traps. In total 40 rats (37 *R. norvegicus* and 3 *R. rattus*) were caught from 15 sampling
216 points (11 and 4 respectively from the old town and the 60/70 area; Fig. 1, Table 1) and from the
217 control location outside urban area. Thirty animals were trapped by multiple capture traps (20, 7 and
218 3 from respectively the old town, the 60/70 area, and the control location), 8 by snap traps and 2
219 caught by a cat near a multiple capture trap placed in the old town. Both H and L rats were captured
220 in five sampling points.

221

222 **3.2. Necropsy and PCR**

223 During the necropsy, no macroscopic lesions were found in any captured rat. All PCR analyses
224 conducted on the samples were negative for *F. tularensis* and *Leptospira spp.* It was not possible to
225 perform any laboratory analysis on two of the captured rats since, at a first macroscopic evaluation,
226 they were in very poor conditions of conservation.

227

228 **3.3. Histopathology**

229 Out of the 38 rats (22 classified as H) available for histopathological analysis, in 12 it was possible
230 to detect pneumonia or bronchopneumonia with different degree of inflammatory lesions, and in one
231 rat an area of fibrotic pleural thickening was apparent. No pathological lesions directly attributable to

232 the inhalation of asbestos fibres were identified in hearts, spleens, livers, and kidneys. Perls staining
233 was positive in 17 animals, even if only in 5 of them it was possible to identify the presence of
234 ferruginous bodies compatible with asbestos corpuscles. Masson staining allowed the detection of
235 severe, moderate, and minor fibrosis in 5, 14 and 3 samples respectively; no appreciable fibrosis was
236 found in 16 samples.

237 A histopathological diagnosis of asbestosis could be done in 2 animals from one sampling point
238 (F1_1), where both corpuscles and severe and moderate fibrosis were simultaneously detected. In 9
239 rats (7 sampling points), asbestosis could be hypothesized since Perls staining was positive and
240 associated with fibrosis detected by Masson staining; however, they didn't show any ferruginous
241 body.

242

243 **3.4. Scanning electron microscopy**

244 In total lung tissues from 36 wild rats were suitable for SEM-EDS investigation and have been
245 analysed in 19 pools (7 from L rats); the remaining four rats were excluded due to very poor lungs
246 condition.

247 Inorganic fibres have been detected in 15 pools. In total, 13 types of fibrous inorganic species have
248 been identified, among which asbestos tremolite *s.s.* (or tremolite/actinolite), amosite, and chrysotile
249 *s.s.* (or chrysotile/antigorite). Eleven positive pools (8 pools from H rats) contained asbestos fibres;
250 in one case only SAF were detectable whereas in 6 only LAF.

251 Among the 11 pools positive to asbestos, one contained the 2 rats with histopathological diagnosis
252 for asbestosis; one rat with probable asbestosis (*i.e.*, Perls and Masson stains positive but without
253 identifying ferruginous bodies) was present in each of other 3 pools.

254 The mean concentration of asbestos fibres was 30,136 ff/gdw (n = 11 pools, sd = 27,126 ff/gdw).
255 LAF showed higher concentration (n = 10, mean = 30,600, sd = 26,336 ff/gdw) compared with SAF
256 (n = 5, mean = 5,100, sd = 0 ff/gdw).

257 Regardless of the length of the asbestos fibres, tremolite/actinolite (including tremolite *s.s.*) was the
258 most common species of asbestos found in the lungs of rats, both in absolute number of fibres (43
259 fibres in 9 pools, 16 rats) and per gram of dry tissue (mean = 24,367 ff/gdw), followed by amosite (9
260 fibres in 2 pools, 7 rats; 22,950 ff/gdw) and chrysotile/asbestiform antigorite (including those
261 properly identified as chrysotile: 9 fibres in 6 pools, 12 rats; 11,050 ff/gdw).

262 The highest asbestos load has been detected in the pools of the sampling points F2_1 (2 pools, 1 H
263 and 1 L, mean = 58,650 ff/gdw) and F2_3 (81,600 ff/gdw) (Fig. 2).

264 Outlier concentrations was detected at the sampling point F2_3 (MAD-score = 4.38); a high MAD-
265 score (2.87), but not significant, was obtained for the sampling point F2_1. Furthermore, was

266 observed an overlap of the respective buffer areas (*i.e.*, hypothetical home-range around the sampling
267 point), consistent with the hypothesis of an asbestos hot-spot in this area (Fig. 3).

268

269 4. DISCUSSION AND CONCLUSIONS

270 Our study confirms the rat (*R. norvegicus* and *R. rattus*) as a helpful sentinel of remaining asbestos;
271 moreover, our results suggest that at least one geographically defined location in the urban area of
272 Casale Monferrato could represent a probable hot-spot. Our approach is based on the observation that
273 the murine lung tissue acts as a filter for mineral fibres, among which the asbestos fibres are
274 distinguishable and can be also classified by species.

275 The heterogeneity of the mineral species found and the marked differences between the
276 concentrations detected make it possible to process the data and search for the causes (sources) of
277 these differences, valid for territorial realities even diverse from Casale Monferrato.

278 Three types of asbestos were detected in the lung tissue of the captured rats: chrysotile (the most used
279 asbestos at the Eternit plant), tremolite/actinolite, and amosite. Interesting to note is the high load of
280 tremolite/actinolite asbestos, which were not commercially used in ACM to a significant extent (*e.g.*,
281 in the preparation of fibre cement), and the absence of crocidolite fibres.

282 The high load of tremolite/actinolite asbestos in the rat lungs could be explained by the following
283 reasons: the presence of natural sources (Capella *et al.*, 2017), their high persistence in biological
284 tissues (Ref?), and the contamination of crocidolite imported by Eternit from Russia by tremolite
285 asbestos (Ref?). On the other hand, given the great distance from the hypothetical home-ranges of
286 asbestos-positive rats to the railway track (Fig. 3B), it would not seem likely that the railway ballast
287 are sources of asbestos tremolite/actinolite.

288 The non-detection of crocidolite (fibres that were commonly used at the plant) could be the result of
289 the remediation effort carried out in recent decades in our study area. On the contrary, amosite was
290 rarely used in the productive process of Eternit, but it was widely used for the insulation of the boiler
291 bodies, in the central heating pipes and for the spray coating of walls and ceilings of electrical control
292 units. Then the quite unexpected finding of high load of amosite in two close sites could be explained
293 by a specific source of asbestos.

294 Significant rat capture difficulties arose during the study: despite of large consumption of bait in all
295 traps, the number of caught rats was less than expected. The reasons for this can be found in the innate
296 ability of rats to warn of the danger of traps, in the periodic rodent control activities carried out in the
297 urban area, and in the low effectiveness of traps. Multiple capture traps allow for long-term
298 monitoring with acceptable management costs (*i.e.*, personnel effort to check the traps) and it is very
299 effective when the spread of rats is epidemic. However, given that periodic rodent control activities

300 have reduced the murine population in the urban area, it is reasonable to assume that the presence of
301 wild rats is widespread but without very large colonies, except in certain areas.

302 In contrast, snap traps appeared to be more effective, but these require higher management costs to
303 ensure daily, or twice-daily, controls.

304 Therefore, the skill and the effectiveness in the capture are crucial to successfully complete the study
305 and were a main stone in the planning of the study. Our experience suggests to considering all the
306 information about the territory, the density of murine population and to place several and different
307 kind of trap devices. For example, we suggest using the snap traps for short intensive periods and the
308 multiple capture traps for long periods, especially with high murine population density.

309 The methodology proposed by this study represents a preliminary screening step that, in situations
310 where the presence of latent sources of asbestos is suspected, significantly reduces the geographic
311 magnitude of the area to be investigated. Given a radius of 100 m around the sampling point, the
312 exact identification of the latent source requires a further and more detailed search. At this stage,
313 sentinel animals may still be useful, *e.g.*, other traps could be placed at a shorter distance and
314 additional research strategies applied in the suspect area, such as the administration to local
315 inhabitants of an *ad hoc* questionnaire to help in the detection of the putative latent source.
316 Furthermore, the search could be guided by the type of asbestos found in the lungs of locally captured
317 rats, that is, particularly by looking at the ACM that might contain them or the sites where they were
318 known to be located. Finally, to improve the search, the assistance of rodent experts could help to
319 track the footpaths and burrows of rats, which would make it possible to better determinate the
320 rodents' home-range and thus the search area.

321 As conclusion, we can say that the approach presented in this study can be used in the framework of
322 public health campaigns to address asbestos removal activity.

323

324

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328 Monferrato for their kind contribution in signalling sites infested by rats. We also thank the Hannover
329 Fraunhofer Institute (Germany) for providing us with positive control for Perls staining. Map data
330 copyrighted OpenStreetMap contributors and available from <https://www.openstreetmap.org>. This
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332 Table 1: number of rats captured and analysed by sampling point, capture method, species, weight class (L: < 100 g or H: ≥ 100 g).

Sampling point	Species	Capture method	Captures			SEM-EDS	
			Total	L	H	L	H
00_1	<i>R. norvegicus</i>	Multiple capture trap	3		3		2**
D9_1			1		1		1
F7_1			4	1	3	1	3
F6_2			1		1		1
E6_2			1		1		1
H3_1			2	2		2	
E3_1			7	6	1	4*	1
F2_1			6	5	1	5	1
F1_1			3		3		3
H3_1		Snap trap	1		1		1
E3_4			1		1		**
H2_1			2	1	1	1	1 [#]
E1_2			2	1	1	1	1 [§]
E1_3			1		1		1 [§]
E2_3		Preyed by cat	2	2		2	
F2_3	<i>R. rattus</i>	Multiple capture trap	2		2		2
H2_1		Snap trap	1		1		1 [#]
Total			40	18	22	16	20

333

334 **SEM numbers in bold were positive pools for asbestos fibres.**

335 *: two rats have been excluded by the histopathologic and SEM-EDS investigations since they were in poor conditions of conservation at a first
336 macroscopic evaluation.

337 **: one rat has been excluded by the SEM-EDS investigations because the quality of the sample did not allow it.

338 [#] and [§]: analysed in the same pool.

339 **Figure in altro file**

340 **REFERENCES**

- 341 Ardizzone M, Vizio C, Bozzetta E, Pezzolato M, Meistro S, Dondo A, Giorgi I, Seghesio A, Mirabelli
342 D, Capella S, Vigliaturo R, Belluso E; the wild rat as sentinel animal in the environmental risk
343 assessment of asbestos pollution: a pilot study; Science of the Total Environment; 2014; 479-480; 31-
344 38
- 345
- 346 Bellis D, Belluso E, Capella S, Coverlizza S, Ferraris G, Graziano E, Colzani A, Gobetto M, Curti
347 PC, Dermateis A; Dati preliminari sul carico mineralogico nei polmoni di differenti specie animali
348 per la mappatura delle aree a rischio ambientale da amianto; Il progresso veterinario, 2005, anno LX,
349 1, 9-15.
- 350
- 351 Belluso E., Bellis D., Fornero E., Capella S., Ferraris G., Coverlizza S.; Assessment of inorganic fibre
352 burden in biological samples by SEM-EDS; Microchimica Acta, 2006; 155: 95-100.
- 353
- 354 Ben-Shlomo R, Shanas U, Env. sci and Poll.Res., Genetic ecotoxicology of asbestos pollution in the
355 house mouse *Mus musculus domesticus*, Environ Sci Pollut Int; 2011, Sep; 18 (8): 1264-9
- 356
- 357 Byers, K. A., Lee, M. J., Patrick, D. M., & Himsworth, C. G. (2019). Rats About Town: A Systematic
358 Review of Rat Movement in Urban Ecosystems. *Frontiers in Ecology and Evolution*, 7, 13.
- 359
- 360 Capella, S., Fornero, E., Bellis, D., & Belluso, E. (2017). Analysis of respired amphibole fibers
361 (asbestos and non-asbestos classified): discrimination between natural and anthropogenic sources
362 using sentinel animals. *Air Quality, Atmosphere & Health*, 10(5), 533-542.
- 363
- 364 Comba, P., D'Angelo, M., Fazzo, L., Magnani, C., Marinaccio, A., Mirabelli, D., & Terracini, B.
365 Mesothelioma in Italy: the Casale Monferrato model to a national epidemiological surveillance
366 system. *Annali dell'Istituto superiore di sanita*, 2018. 54(2), 139-148.
- 367
- 368 De Nardo P, Bruni B, Paoletti L, Pasetto R, Sirianni B, Pulmonary fibre burden in sheep living in the
369 Biancavilla area (Sicily): preliminary results, Sci Tot Env, 2004, 325, 51-5
- 370
- 371 De Vuyst P., Karjalainen A., Dumortier P., Pairen P.C., Monsò E., Brochard P., Teschler H.,
372 Tossavainen A., Gibbs A., Guidelines for mineral fibre analyses in biological samples: report for ERS
373 Working Group. *Eur. Resp. J.* 1998; 11:1416-1426

374

375 Directive 2009/148/EC of the European Parliament and of the Council of 30 November 2009 on the
376 protection of workers from the risks related to exposure to asbestos at work. Official Journal of the
377 European Union, L 330, Vol. 52, 16/12/2009, 52: 28-36.

378

379 Dumortier, F Rey, J R Viallat, I Broucke, C Boutin, P De Vuyst, Chrysotile and tremolite asbestos
380 fibres in the lungs and parietal pleura of Corsican goats; *Occup Environ Med*, 2002;59:643–646

381

382 Fornero E, Belluso E, Capella S, Bellis D; Environmental exposure to asbestos and other inorganic
383 fibres using animal lung model, *Sci Tot Env*,2009, 407,:1010-1018

384

385 Forsman M, Sandström G, Sjöstedt A. 1994. Analysis of 16S ribosomal DNA sequences of
386 *Francisella* strains and utilization for determination of the phylogeny of the genus and for
387 identification of strains by PCR; *International journal of systematic bacteriology (Int J Syst*
388 *Bacteriol)*; 44(1):38-46.

389

390 Gardner-Santana, L. C., Norris, D. E., Fornadel, C. M., Hinson, E. R., Klein, S. L., & Glass, G. E.
391 (2009). Commensal ecology, urban landscapes, and their influence on the genetic characteristics of
392 city-dwelling Norway rats (*Rattus norvegicus*). *Molecular ecology*, 18(13), 2766-2778.

393

394 Gualtieri A.F. (2012). Cap. 7: “Mineral fibre-based building materials and their health hazards.” pp
395 166-195 In: *Toxicity of Building Materials*. Editors: F. Pacheco-Torgal, S. Jalali, A. Fucic. Woodhead
396 Publishing, Cambridge, UK, pp: 166-195.

397

398 Iglewicz B., Hoaglin D. (1993), "Volume 16: How to Detect and Handle Outliers", *The ASQC Basic*
399 *References in Quality Control: Statistical Techniques*, Edward F. Mykytka, Ph.D., Editor

400

401 IARC, 2012. *Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 100C,*
402 *Asbestos (Chrysotile, Amosite, Crocidolite, Tremolite, Actinolite and Anthophyllite)*, pp: 219-309.

403

404

405 Leys, C., *et al.*, Detecting outliers: Do not use standard deviation around the mean, use absolute
406 deviation around the median, *Journal of Experimental Social Psychology* (2013),
407 <http://dx.doi.org/10.1016/j.jesp.2013.03.013>

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410
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435
436
437
438
439
440
441

Magnani C, Dalmaso P, Biggeri A et al, Increased risk of malignant Mesothelioma of the pleura after residential or domestic exposure to asbestos. A case-control study in Casale Monferrato- Italy; Environ Health Perspect. 2001;109: 915-9.

Magnani C, Ferrante D, Barone-Adesi F, Bertolotti M, Todesco A, Mirabelli D, Terracini B. Cancer risk after cessation of asbestos exposure: a cohort study of Italian asbestos cement workers. Occup Environ Med 2007; 65:164–170.

Marinaccio, A., Scarselli, A., Binazzi, A., Mastrantonio, M., Ferrante, P., & Iavicoli, S. (2008). Magnitude of asbestos-related lung cancer mortality in Italy. British journal of cancer, 99(1), 173.

Marinaccio, A., Binazzi, A., Bonafede, M., Di Marzio, D., & Scarselli, A. (2018). Epidemiology of malignant mesothelioma in Italy: surveillance systems, territorial clusters and occupations involved. Journal of thoracic disease, 10(Suppl 2), S221.

Maule MM, Magnani C, Dalmaso P, Mirabelli D, Merletti F, Biggeri A. Modeling mesothelioma risk associated with environmental asbestos exposure. Environ Health Perspect 2007;115:1066–1071

Mirabelli D, Cavone D, Merler E, *et al.* Non-occupational exposure to asbestos and malignant mesothelioma in the Italian National Registry of Mesotheliomas. Occup Environ Med 2010; 67:792–4.

QGIS Development Team (2019). QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>.

Reif JS. 2011. Animal sentinels for environmental and public health. Public Health Rep. 126(Suppl 1): 50–57.

Stanton, M.F., Layard, M., Tegello, A., Miller, E., May, M., Morgan, E. & Smith, A. (1981) Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. *Journal of the National Cancer Institute*, 67(5), 965-975.

442 StataCorp. 2015. Stata Statistical Software: Release 14.1. College Station, TX: StataCorp LP.

443

444

445