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1 **Colloidal gel as an efficient process to treat CBR and prion**
2 **contaminated solid surfaces**

3
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24 **Abstract**

25 Chemical, biological, radiological and nuclear (CBRN) accidents or attacks typically disseminate
26 contaminants inside and outside buildings and structures whose surfaces have to be decontaminated.
27 This paper describes an innovative colloidal gel formulation and its effects against CBR threat agents
28 and also against prion contaminations. The gel's rheological properties ensure it remains attached even
29 to vertical surfaces. The gel then dries with the contaminants trapped inside, leaving behind non-dusting
30 solid residues that are easy to recover by brushing or vacuum cleaning. The results obtained here on
31 different formulations show that decontamination proceeds in two steps. Contaminants are first
32 solubilized or absorbed into the gel thanks to the efficient wetting of the surface by the gel. Chemical
33 and biological agents are then neutralized by the NaOH and hypochlorite ions in the gel. This article
34 demonstrates the efficiency of this CBR gel against *Bacillus anthracis*, the chemical agents sulfur
35 mustard, VX, and soman, ¹³⁷Cs, and prion proteins.

36 **Keywords**

37 *Bacillus anthracis*, VX, soman, sulfur mustard, Sodium hypochlorite, shear thinning

38 **List of abbreviations and symbols**

39 *Ba*: *Bacillus anthracis*

40 *Bt*: *Bacillus thuringiensis*

41 CFU: colony-forming unit

42 CWAs: Chemical Warfare Agents

43 LB: Lysogeny broth

44 PrP: Prion Proteins

45 **1. Introduction**

46 Chemical, biological, radiological and nuclear (CBRN) threats and the use of CBRN weapons
47 in the Tokyo subway sarin attack in 1995, the 2001 anthrax attacks in the USA in 2001 [1] and most
48 recently, the chlorine attacks in Syria in 2013 and 2017 [2, 3] have led to the investigation of surface
49 decontamination methods in several countries [4, 5]. Furthermore, in post accidental situation, the
50 contamination can be easily disseminated inside and outside the civilian infrastructure [6, 7], making
51 the development of specific, effective decontamination products and processes crucial. Currently
52 available CBRN decontamination technologies include physical, chemical and biological processes [4,
53 8, 9]. Chemical decontamination products often contain an oxidant such as peroxides or hypochlorite
54 ions [8-10], which are effective against biological and chemical contaminations [11, 12], with
55 hypochlorite also being particularly effective against a large variety of spores and microorganisms [1,
56 13-15]. Against chemical agents, while a basic medium effectively hydrolyzes (and thus neutralizes)
57 sulfur mustard and nerve agents such as soman [11, 16, 17], an oxidant is required to neutralize other
58 nerve agents such as VX. Various commercial CBRN decontamination products containing oxidative
59 compounds have been developed over the years in liquid, gas (fumigant), foam, gel forms [8, 12, 18].

60 The decontamination of solid surfaces is also an important issue in the nuclear industry, notably
61 to make decommissioning waste easier to handle by reducing its radioactivity. In this case, the aim of
62 the decontamination process is to concentrate the radioactivity and maximally reduce the final waste
63 volume. Colloidal gels have been developed over the past 15 years for this purpose [19-21]. These gels,
64 also named “vacuumable gels”, consist of a surface-adapted decontaminating solution in which
65 inorganic particles (typically silica or alumina) are used as a thickening agent and dispersed in a
66 decontaminating solution to form a gel-like concentrated colloidal suspension. The gel’s viscosity
67 decreases under shearing but returns almost immediately, making them easy to implement at large scale
68 and at a distance from the contamination by spraying [19, 22, 23]. The gels remain attached even to
69 vertical surfaces, increasing the contact time between the decontaminating solution contained in the gel
70 and the contaminated surface. The gels crack as they dry to form non-dusting solid flakes in which the

71 contaminants are trapped and which are easily to remove by brushing or vacuum cleaning. Vacuumable
72 gels have recently been applied to treat other surfaces, such as bitumen stains [22], and other
73 contaminants, such as heavy metals [24]. Based on our experience in the formulation of active colloidal
74 gels, we have been investigating the possibility of using vacuumable gels to treat building materials
75 contaminated by CBR agents. We propose then here to add hypochlorite ions and sodium hydroxide in
76 the decontaminating solution to obtain an innovative CBR gel that neutralizes both biological and
77 chemical contaminations [23, 25]. As vacuumable gels, they should be effective against radiological
78 contaminations. In this study, we also investigated their decontamination efficiency against prions,
79 which are problematic in healthcare and veterinary settings because of their transmissibility and wide
80 spectrum of resistance [26].

81 After characterizing the physicochemical properties of the CBR gel, we tested the decontamination
82 efficiency of fresh and aged gel samples, to evaluate its efficiency in time, against a radiological agent
83 (^{137}Cs), the chemical agents soman, sulfur mustard, and VX, spores of *Bacillus thuringiensis* (*Bt*) and of
84 *Bacillus anthracis* (*Ba*) as biological agents, and hamster prion protein (PrP) as a model disease-
85 associated prion contamination. The decontamination mechanisms involved in each case are discussed.

86 **2. Experimental section**

87 *2.1. Materials and sample preparation*

88 Alumina was purchased as Aeroxide Alu C (specific surface area, $100 \text{ m}^2\cdot\text{g}^{-1}$) from Evonik
89 Industries. Pluronic PE 6200 was purchased from BASF and iron oxide (Ferroxide 212M) was
90 purchased from Rockwood Pigments. Nitric acid, sodium hydroxide, acetone, heptane, potassium iodide
91 and starch were purchased from Sigma Aldrich; sulfuric acid from VWR; bleach (sodium hypochlorite)
92 from Fisher, and sodium thiosulfate from Fluka. The chemical agents sulfur mustard (HD), VX, and
93 soman (GD) were provided by the French Directorate General of Armaments (*Direction Générale de*
94 *l'Armement*). *Bt* DSM 5815 was provided by the Leibniz-Institute DSMZ (Braunschweig, Germany)
95 and *Ba* (Sterne strain NCTC-08234 Weybridge Lot 100816) was provided by NCTC (National

96 Collection of Type Cultures) and stored at 4 °C. ¹³⁷CsCl was purchased from Orano's Radioactive
97 Standard Laboratory (*Laboratoire d'Étalons d'Activité*). Prion studies were performed using brain
98 homogenate from Syrian hamsters infected with hamster-adapted scrapie strain 263 K [27].

99 Three kinds of substrates were used: stainless steel (for chemical, biological and radiological
100 experiments), ceramic tiles (9 × 2 cm, identical to the ones used in the Paris metro, for the biological
101 experiments) and glass (prion experiments). Stainless steel and ceramic tiles are reference materials for
102 CBRN experiments. The chemical and radiological experiments, which are very complicated to conduct
103 safely, were only performed on stainless steel because of financial constraints. The radiological
104 decontamination experiments were performed on 7 cm² 316L stainless steel discs and the chemical
105 decontamination experiments were performed on 5 x 5 cm coupons of 304L stainless steel according to
106 NATO standards [28]. The prion decontamination experiments were performed on 7.6 × 2.6 cm pieces
107 of glass as proof of concept.

108 2.2. *Gel formulation and aging*

109 The different operations and reactions were performed under ambient conditions. The CBR gel
110 is a patented formulation of 14 wt.% Aeroxide Alu C alumina as a thickening agent, 1 wt.% Pluronic
111 PE 6200 as a surfactant, and 0.1 wt. % iron oxide as a pigment and the remainder (84.9 wt. %) of a
112 decontaminating solution of 0.5 M NaOH containing hypochlorite ions, which is equivalent to the active
113 chlorine amount [23]. Typically, for 100 g of CBR gel, 1 g Pluronic PE 6200 was first mixed with 84.9
114 g of a 0.5 M NaOH and active chlorine solution using a three-blade stirrer at 200 rpm for 5 min. 0.1 g
115 of iron oxide and 14 g of alumina particles were then gradually added, while increasing the stirring speed
116 up to 500 rpm as the viscosity increased. Stirring was maintained for 5 min after all the compounds had
117 been added to homogenize the gel. Samples of a second type of gel (referred to as NaOH-gel) were
118 prepared in the same manner but without presence of hypochlorite ions. For the prion decontamination
119 experiments, a third gel was tested, which was prepared with a 1 M (rather than a 0.5 M) solution of
120 NaOH.

121 The gels were either studied within 3 months of synthesis (fresh), after storing for 15–27 months
 122 at room temperature, or after storing for 8 weeks at 40 °C in an environmental chamber (Table 1). The
 123 active chlorine content of the gels was determined using a specific in-house titration method (see
 124 Supplementary Material). In this study, we express the amount of active chlorine in the gels using the
 125 ratio $R_{a.c.}$ defined as followed:

$$R_{a.c.} (\%) = \left(\frac{\text{Active chlorine measured in the gel after aging}}{\text{Initial active chlorine in the fresh gel}} \right) \cdot 100 \quad (1)$$

126

127 **Table 1**

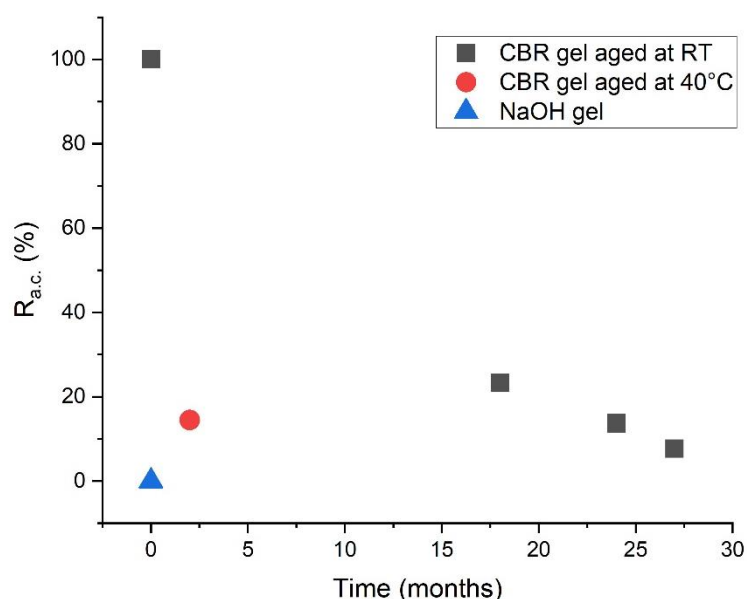
128 Compositions of the gels used in the study

Name	Aging	$R_{a.c.}$ (%)	NaOH (M)	pH	Decontamination tests
CBR gel	Fresh	100	0.5	13	R, B, C, prion
	18 months, RT	23.3	NM	13	B
	24 months, RT	13.7	0.16	12	B
	27 months, RT	7.7	NM	12	B
	8 weeks, 40 °C	14.5	0.23	12	C
	Fresh for Prion	100	1	13	Prion
NaOH-gel	Fresh	0	0.5	NM	R, B, C, prion

129 R, radiological; B, biological; C, chemical; NM, not measured; RT, room temperature

130

131 The Fig. 1 presents the evolution of the $R_{a.c.}$ ratio as a function of the time and depending on the storing
 132 conditions.



133

134 **Fig. 1:** Evolution of the active chlorine percentage in the gels as a function of the storage time. Black squares,
 135 red circle and blue triangle represent the CBR gel aged at RT and 40°C and the NaOH aged at RT respectively.

136

137 Note that, as the $R_{ac.}$ ratios of the gels stored at 40 °C for 8 weeks and aged for 24 months at room
 138 temperature were similar (14.5 and 13.7 respectively), we decided to use the gels stored at 40 °C for 8
 139 weeks as more rapidly prepared surrogates for the gels aged for 24 months to facilitate the evaluation of
 140 the chemical decontamination experiments.

141 2.3. CBR decontamination experiments and analytical methods

142 2.3.1. Preliminary gel characterization

143 The pH of the gels was measured using a glass combination electrode connected to a pH-meter
 144 calibrated at pH = 4.01, 7.00 and 9.18, in a suspension of 1 g of gel in 10 mL of water. NaOH
 145 concentrations were then specifically measured in this diluted solution by acid-base titration using a 0.1
 146 M solution of HCl. The in-house titration method used to measure the active chlorine percentage in the
 147 gels is described in the Supplementary Material. Rheological measurements were performed with a
 148 Discovery HR-1 plate-plate rheometer (TA Instruments; diameter, 40 mm; gap between the plates, 1

149 mm). The viscosity of the gel was measured using stepped flow tests at shear rates ranging from 0.01
150 s^{-1} to 100 s^{-1} . A 1 mm thick layer of gel was deposited on a vertical stainless steel substrates and left to
151 dry at room temperature to observe the formation of flakes. In the rest of the study, the gel will be
152 considered as dried as soon as the formation of flakes.

153 *2.3.2. Radiological decontamination experiments*

154 The contaminant for these experiments was a certified acidic solution of CsCl. A volume of
155 CsCl corresponding to 5000 Bq of ^{137}Cs was deposited on the surface of the substrates prior to
156 evaporation under a fume hood. The initial activity (Bq) of each substrate was measured by gamma
157 spectrometry using a Eurisys Mesures coaxial germanium detector. This was connected to an Itech
158 measurement chain controlled using the Interwinner 7.0 software program. ^{137}Cs activity was measured
159 at an energy of 661.66 keV. A 1 mm thick layer of the studied gel was then applied with a spatula on
160 each substrate. After drying for a few hours, the flakes were removed from the substrate with a
161 paintbrush. Finally, the activity of the substrates was measured again to calculate the decontamination
162 factor (the ratio of the initial and final activities).

163 *2.3.3. Chemical decontamination experiments*

164 Chemical decontamination experiments were performed with both the CBR gel and the NaOH-
165 gel to highlight the effect of active chlorine on the decontamination efficiency. For each of the three
166 agents (sulfur mustard, VX and soman, see chemical structures Fig. SI 1), eight stainless steel coupons
167 were contaminated at $10 \text{ g}\cdot\text{m}^{-2}$ by dropwise deposition of these CWAs. To confirm the level of
168 contamination, three of the substrates were mixed in a 90/10 vol.% heptane/acetone mixture for 1.5 h to
169 extract the chemical agents and analyze their concentration by gas chromatography with helium as
170 carrier gas. The five remaining samples and a non-contaminated coupon used as a blank were placed
171 inside a closed dispersion chamber before the gels were applied with a spray gun through a slot. The
172 thickness of the gel layers was measured by gravimetry for each sample based on the gel density

173 (calculated as $1.11 \text{ g}\cdot\text{cm}^{-3}$, see detailed in Supplementary Material) and estimated at $1 \pm 0.2 \text{ mm}$. After
174 applying the gel, the samples were dried overnight at room temperature under a fume hood. The
175 millimeter-scale flakes that had formed were retrieved using a spatula. Residual levels of contamination
176 were determined by dipping the substrate samples in a 90/10 vol.% heptane/acetone mixture for 1.5 h.
177 In parallel, the flakes were also stirred (without dissolution) in a 90/10 vol.% heptane/acetone mixture
178 for 1.5 h, to extract and quantify any remaining chemical agents. The chemical agents solubilized into
179 the heptane/acetone mixture were analyzed by gas chromatography. The samples were injected in
180 splitless mode. A flame ionization detector (Perkin Clarus 680) was used for HD and a flame photometry
181 detector (Shimadzu GC2010) was used for VX and GD. The decontamination efficiency was calculated
182 by determining the two following decontamination factors:

$$\text{Substrate decontamination (\%)} = \left(1 - \frac{\text{Residual contamination level}}{\text{Initial contamination level}}\right) \cdot 100 \quad (2)$$

$$\text{Residual contamination in flakes (\%)} = \left(\frac{\text{Contamination extracted from residual flakes}}{\text{Initial contamination level} \times A}\right) \cdot 100 \quad (3)$$

183 In Eq. (2), the initial and residual contamination levels (in $\mu\text{g}\cdot\text{cm}^{-2}$) are respectively the amounts of
184 chemical agent on the substrate before and after the decontamination step. In Eq. (3), the mass of
185 chemical agent extracted from the flakes (μg) is divided by the initial contamination level multiplied by
186 the contaminated area (A , cm^2). The results of the decontamination tests are expressed as the mean and
187 standard deviation of five repeats.

188 2.3.4. *Biological decontamination experiments*

189 Preliminary experiments were performed with *Bt*, which is typically used as a surrogate for *Ba*
190 [29]. Experiments were repeated with *Ba* if the log reduction in colony forming units (CFU) was greater
191 than 6. DSM 5815 was prepared as described by Wood et al. [30]. The *Bt* concentration and the protocol
192 used for deposition on the substrates were as described by Le Toquin et al. [31]. A bacterial suspension
193 of *Bt* spores was prepared in distilled water at a concentration of $2 \times 10^8 \text{ CFU}\cdot\text{mL}^{-1}$. The experiments

194 with *Ba* were performed at biosafety level 3. An aliquot of 100 μ L (equivalent to an initial amount of 2
195 $\times 10^7$ CFU onto the coupons) was inoculated on the surface of each substrate using a micropipette. After
196 inoculation, the substrates were air dried for 1 h in a 20 $^{\circ}$ C biosafety cabinet and the gel was applied
197 with a sterilized spatula (layer around 1 mm thick). The different substrates covered with a layer of gel
198 were then placed inside open Falcon tubes lying on their sides and left to dry for a few hours. The dried
199 flakes were recovered and mixed in 10 mL of lysogeny broth (LB) medium (Becton Dickinson Co.,
200 Sparks, MD, United States) in Falcon tubes. In parallel, the treated substrates were also placed in Falcon
201 tubes, in 20 mL of LB medium. All these Falcon tubes were incubated for 1 h at 30 $^{\circ}$ C. To isolate the
202 spores from the residual flakes in suspension in LB, each tube was centrifuged at 4500 rpm for 3 min at
203 4 $^{\circ}$ C and the supernatant was separated. Several serial dilutions in LB medium were plated for each
204 Falcon tube and covered with LB agar. After drying, the substrates were incubated for 24 h at 30 $^{\circ}$ C for
205 *Bt* and 24 h at 37 $^{\circ}$ C for *Ba* and, finally, the number of viable CFU formed in the LB agar were counted
206 visually in the plates containing 30–300 CFU. The gel decontamination efficiency was finally calculated
207 as the log reduction in the number of microorganisms after decontamination:

$$LR = -\log_{10} \left(\frac{CFU_f}{CFU_i} \right) \quad (3)$$

208 where CFU_i and CFU_f are the counts respectively before and after the decontamination process
209 recovered from the substrates.

210 2.3.5. Prion decontamination experiments

211 Brain homogenate from Syrian hamsters infected with scrapie strain 263 K were first dispersed
212 in a PBS solution at 10 wt/vol %. Then, 25 μ L of this solution is deposited on glass substrates, to make
213 them contaminated with 2.5 mg of brain homogenate, and the liquid solution is let to evaporate [27].
214 After drying and rinsing in deionized water, the contaminated substrates were covered with a layer
215 (around 1 mm thick) of one of three gels (the CBR gels with 0.5 and 1 M NaOH and the NaOH-gel) and
216 left to dry for a few hours. The solid flakes were retrieved and there were no visible residues on the

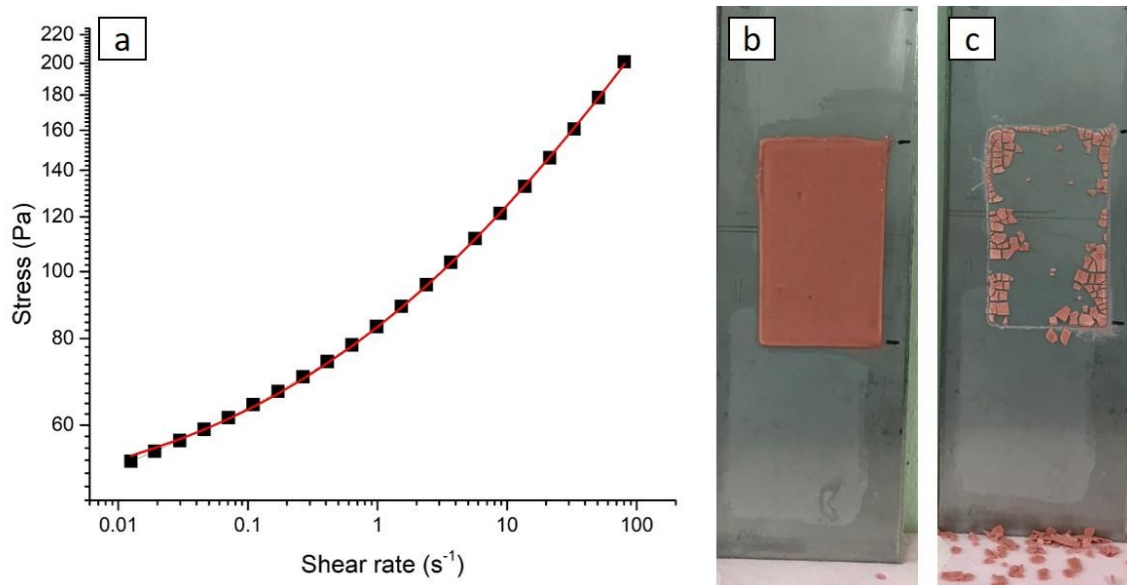
217 substrates. The substrates were nonetheless scraped and the presence of residual PrP was analyzed by
218 western blotting as previously described [27]. Briefly, the samples are purified according to the Bio-Rad
219 TeSeE protocol with or without proteinase K treatment (4 μ g of enzyme per mg of protein) to detect
220 either abnormal protease-resistant PrP (PrP-res), or total PrP, respectively. The purified samples were
221 loaded on acrylamide gel, transferred onto a PVDF membrane and the presence of PrP was evidenced
222 using Sha-31 anti-PrP monoclonal antibody [32].

223 In parallel, four control contaminated substrates were respectively immersed in different
224 solutions (ineffective or effective treatments) to confirm the relevance of the protocol: (i) in water for
225 24 h at room temperature (ineffective treatment); (ii) in water for 10 min at 55°C (ineffective treatment);
226 (iii) in 1 M sodium hydroxide, pH = 14 for 10 min at 55°C (the reference effective treatment) and (iv)
227 in 0.1 mM sodium hydroxide, pH = 10 for 10 min at 55°C (a second, partially effective reference
228 treatment). The substrates were then rinsed, dried and the residues were dissolved in 100 μ L of 10 wt/vol
229 % homogenate of brains from PrP-deficient mice [33]. The presence of PrP in the samples was detected
230 by western blotting as described above.

231 **3. Results and discussion**

232 *3.1. Physicochemical properties of the CBR gel*

233 In the different experiment presented in this study, the gel was applied manually using a spatula
234 because tests were performed on small surface areas. However, for an industrial use of this process,
235 these gels have to be easily sprayable on large surfaces such as walls or floors. These properties have
236 been confirmed using rheological analyses. The flow curve of the CBR gel, representing the stress as a
237 function of an applied shear, is shown in Fig. 2a.



238
 239 **Fig. 2.** (a) Stress in the CBR gel as a function of the applied shear rate. The black squares are the experimental
 240 data and the red line is a fit using the Herschel-Bulkley model. (b, c) Photographs of a 1 mm thick layer of CBR
 241 gel deposited on a vertical stainless steel substrate (b) before and (c) after drying.

242
 243 The fact that the experimental data are well fitted by a Herschel-Bulkley model (described in the
 244 Supplementary Material) indicates that the gel is a shear thinning yield stress fluid, meaning that its
 245 viscosity decreases drastically under high shearing forces. This makes the gel easy to spray, the shearing
 246 imparted by the spraying device ensuring that the gel is fluid enough to pass through the nozzle without
 247 sticking. The gel's yield stress of 44 Pa (obtained from the Herschel-Bulkley fit, see Supplementary
 248 Material) means that it only begins to flow above this threshold and thus that a layer of gel sprayed on
 249 a vertical surface remains attached under its own weight (assuming perfect adhesion of the gel to the
 250 surface).

251 These rheological properties are highlighted by the photographs of a 1 mm thick layer of the
 252 CBR gel straight after spraying (Fig. 2b) and after drying (Fig. 2c), the two black marks drawn on the
 253 substrate showing that it remains rigidly attached throughout the drying process. This ensures prolonged
 254 contact between the surface and the gel, which increases the efficiency of the decontamination process.
 255 Note that the red color of the gel comes from the iron oxide pigment that was added to facilitate visual
 256 inspection [25]. Fig. 2c shows the millimeter-sized solid flakes that form during the drying process, with

257 some having fallen off under the effect of gravity. The remaining flakes were easily removed by manual
258 brushing.

259 In summary, the CBR gel is well suited to be used as a vacuumable gel. It could be easily sprayed
260 and remains attached to vertical surfaces in layers 1 mm thick, and forms solid flakes after drying, which
261 can be removed effortlessly from the substrate.

262 *3.2. Gel efficiency against radiological agents and associated decontamination* 263 *mechanisms*

264 The ^{137}Cs γ activities measured on the stainless plates, before and after application of fresh
265 samples of CBR gel and NaOH gel are listed in Table 2 along with the calculated decontamination
266 factors. The decontamination factors are similar to those obtained with a vacuumable gel specifically
267 designed for nuclear applications [19, 20, 22-24], where decontamination factors of 15–20 are
268 considered satisfactory. The CBR gel is therefore suitable for radiological decontamination. The
269 proposed mechanism is that the aqueous solution in the gel solubilizes the radioelement (here ^{137}Cs) on
270 the substrate. This is enhanced by the presence of surfactants (Pluronic PE 6200) in the gel, which
271 improve its degree of wetting on the surface [19]. The solubilized radiological contamination is then
272 trapped in the flakes obtained after drying. The similar results obtained with the NaOH gel show that
273 the absence of hypochlorite ions did not significantly affect the decontamination efficiency, which is
274 consistent with the proposed solubilization mechanism.

275 **Table 2**

276 Radioactivity and measurement accuracy of substrates contaminated with ^{137}Cs before and after gel treatment.

Gel	Initial ^{137}Cs activity (Bq)	Final ^{137}Cs activity (Bq)	Decontamination factor ^a
NaOH gel	5000 ± 12%	160 ± 12%	31
CBR gel	5200 ± 12%	270 ± 12%	19

277 ^aInitial activity / final activity

278 3.3. Gel efficiency against chemical agents and associated decontamination
 279 mechanisms

280 **Erreur ! Source du renvoi introuvable. Erreur ! Source du renvoi introuvable.**lists the
 281 initial and residual contamination levels of CWAs on stainless steel substrates before and after the
 282 decontamination step and the associated substrate decontamination rates with fresh CBR gel, fresh
 283 NaOH gel, and a sample of CBR gel aged for 8 weeks at 40 °C (mimicking 24 months' aging at room
 284 temperature, see Table 1). Table 3 lists also the residual contamination in flakes rates. Furthermore, the
 285 evolution of the substrate decontamination percentage and residual contamination in flakes associated
 286 to these results are represented on Fig. 3.

287 **Fig. 3**

289 **Table 3**

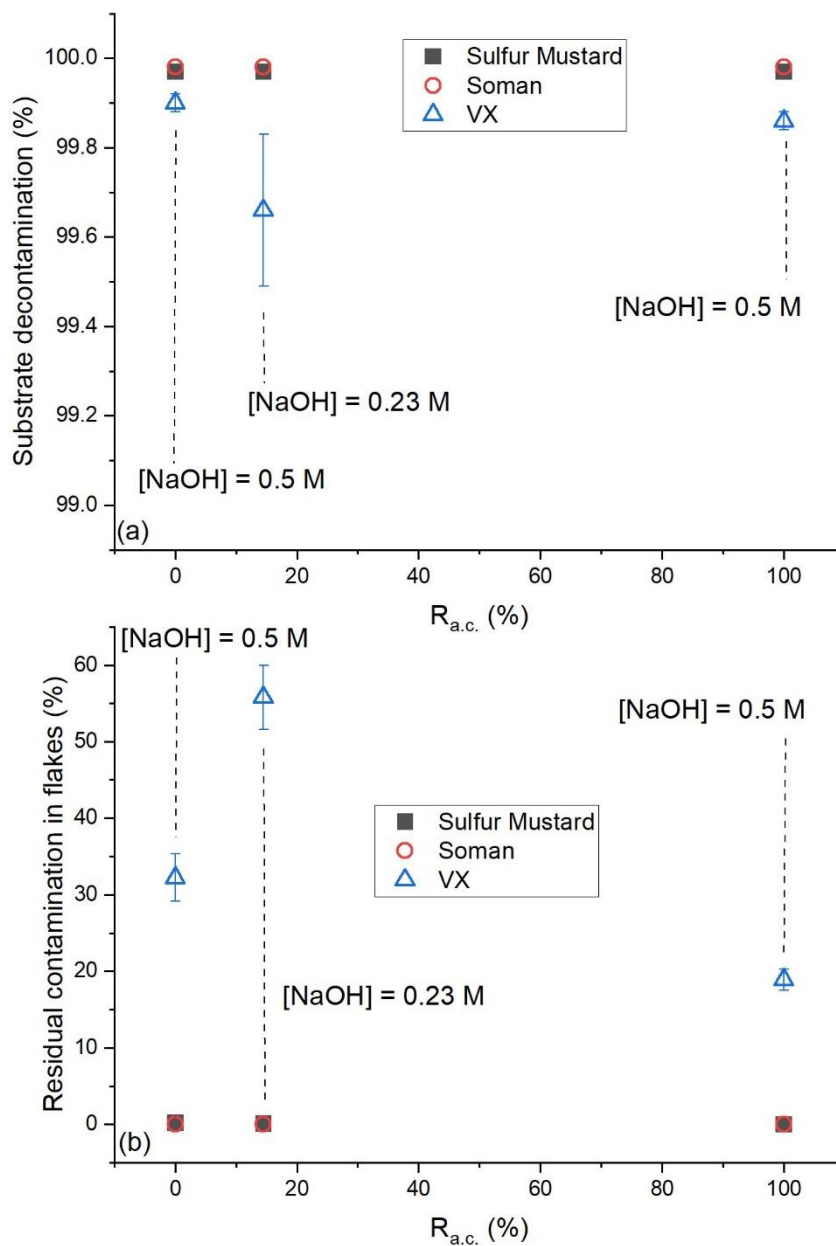
290 Initial and residual contamination levels of chemical warfare agents deposited on the stainless steel substrates
 291 before and after the decontamination step, substrate decontamination rates, contamination extracted from the flakes
 292 and residual contamination in flakes rates.

Chemical agent		CBR gel (fresh)	CBR gel (Ra.c. = 14.5 %)	NaOH-gel (fresh)
Sulfur mustard	Initial contamination level ($\mu\text{g.cm}^{-2}$)	1035.3 \pm 6.3		
	Residual contamination level ($\mu\text{g.cm}^{-2}$)	< 0.30	< 0.30	< 0.30
	Substrate decontamination (%)	> 99.97	> 99.97	> 99.97
	Contamination extracted form flakes (μg)	8.9 \pm 1.4	33.6 \pm 24.6	54.6 \pm 47.0
	Residual contamination in flakes (%)	0.03 \pm 0.01	0.13 \pm 0.10	0.21 \pm 0.18
Soman	Initial contamination level ($\mu\text{g.cm}^{-2}$)	891.9 \pm 6.5		
	Residual contamination level ($\mu\text{g.cm}^{-2}$)	< 0.14	< 0.14	< 0.14
	Substrate decontamination (%)	> 99.98	> 99.98	> 99.98
	Contamination extracted form flakes (μg)	< 3.50	< 3.50	< 3.50

	Residual contamination in flakes (%)	< 0.02	< 0.02	< 0.02
VX	Initial contamination level ($\mu\text{g}\cdot\text{cm}^{-2}$)	1099.3 \pm 79.7		
	Residual contamination level ($\mu\text{g}\cdot\text{cm}^{-2}$)	1.54 \pm 0.23	3.90 \pm 1.74	1.11 \pm 0.18
	Substrate decontamination (%)	99.86 \pm 0.02	99.66 \pm 0.17	99.90 \pm 0.02
	Contamination extracted from flakes (μg)	5193.3 \pm 386.5	15327.9 \pm 1152.4	8850.2 \pm 849.1
	Residual contamination in flakes (%)	18.90 \pm 1.41	55.77 \pm 4.19	32.20 \pm 3.09

293

294



295
 296 **Fig. 3:** (a) Substrate decontamination efficiencies and (b) Residual contamination in flakes calculated after the
 297 decontamination step using fresh CBR gel ($R_{a.c.} = 100\%$), fresh NaOH gel ($R_{a.c.} = 0\%$) and CBR gel aged for 8
 298 weeks at $40\text{ }^{\circ}\text{C}$ ($R_{a.c.} = 14.5\%$).

299
 300 All the gels had substrate decontamination efficiency higher than 99.97% for HD, 99.98% for GD and
 301 99.66% for VX (Fig. 3a). **Fig. 3**The decontamination efficiency depends little therefore on the active
 302 compounds present in the gel. Only the NaOH concentration has a slight influence on VX because the

303 presence of NaOH makes the solubilization of CWA, which are organic compounds easier. Regarding
304 the degradation of the chemical agents, while HD and GD are almost completely neutralized in the flakes
305 (residue contamination in flakes efficiencies respectively lower than 0.2 % and 0.02 %), a significant
306 amount of residual VX is still detected in the flakes for all gels (18.9 to 32.2 % of the initial
307 contamination, depending on the nature of gel) (Fig. 3b). These results suggest a two-step
308 decontamination mechanism: first, solubilization of the chemical contaminant on the substrate (in the
309 similar manner as for radiological contamination), and second, chemical degradation of the contaminant
310 by active compounds in the gel.

311 Under basic conditions, HD is hydrolyzed into unchlorinated and thiodiglycol compounds [11,
312 17]. These two degradation compounds are probably produced in the gels because of the presence of
313 NaOH. In further possibility is that HD is oxidized by active chlorine, producing the corresponding
314 sulfoxide (HDO) [34]. In this case however, since the NaOH gel and both CBR gel flakes had similarly
315 low residual contamination in the flakes efficiencies, this suggests that only NaOH-related degradation
316 occurred. Soman is hydrolyzed into phosphonic acid in alkaline media ($\text{pH} > 10$) [16]. The high pH of
317 the tested gels (Table 1) thus makes them highly effective at neutralizing GD and the resulting flake
318 residues have insignificant residual contamination efficiencies. This point is of high interest because no
319 further treatment are required and the waste can be directly conditioned. In the case of VX, the higher
320 residue contamination efficiencies indicate that the active compounds in the gels are less effective,
321 meaning that the second, “chemical” step of the decontamination process (degradation of chemical
322 agent) is incomplete. VX is slowly hydrolyzed by NaOH, leading mainly to an acid after breakage of
323 the P-S bond [16], which explains why the least alkaline gel (the aged CBR gel, Table 1) had the lowest
324 degradation efficiency. However, since sulfur is oxidized in the presence of hypochlorite ions, this
325 should facilitate cleavage of the P-S bond [16]. Recently indeed, VX has successfully been degraded in
326 hypochlorite solutions into non-toxic *N,N*-diisopropylformamide and *N,N*-diisopropylamine [35]. This
327 mechanism is consistent with the higher degradation efficiency obtained with fresh CBR gel compared
328 to fresh NaOH-gel, which have the same NaOH concentration, because of the presence of hypochlorite

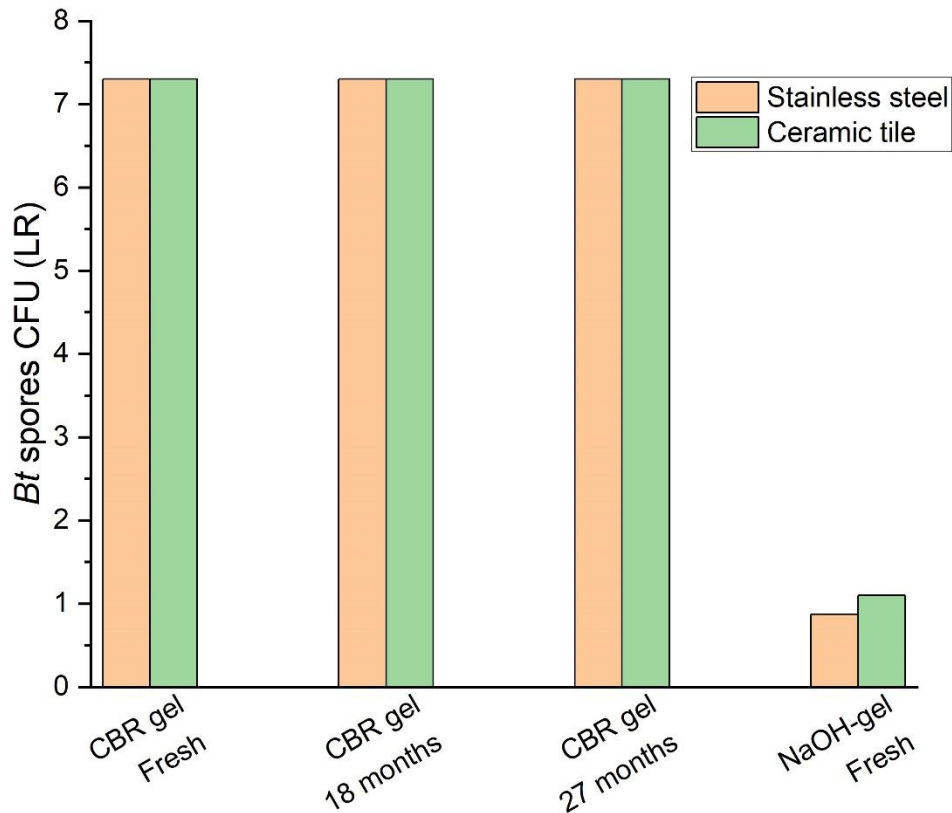
329 ions in the fresh CBR gel. Furthermore, the highest degradation efficiency obtained here (81%)
330 compares favorably with the results of a previous study in which a degradation efficiency of 67% was
331 measured after applying a solution at 0.5 wt.% sodium hypochlorite for 24 h on stainless steel surfaces
332 contaminated with VX [36].

333 In summary, the CBR gel fully decontaminated the stainless steel surfaces onto which chemical
334 warfare agents (HD, GD and VX) had been deposited and the extracted agents were neutralized by the
335 active compounds in the gel, reducing the toxicity of the waste. NaOH plays an important role in the
336 degradation process, especially for HD and GD. Although VX was not fully neutralized, the presence
337 of hypochlorite ions increases the efficiency of the gel in degrading VX and VX-OH, a highly toxic
338 product of VX hydrolysis. Furthermore, one of the main benefits of the vacuumable gel process is that
339 the final waste is produced in the form of solid flakes, which entrap any residual contaminants and
340 prevent their release into the environment.

341 *3.4. Gel efficiency against biological agents and associated decontamination* 342 *mechanisms*

343 *3.4.1. Gel efficiency on Bacillus thuringiensis and Bacillus anthracis spores*

344 Fig. 4 shows the spore neutralization efficiency (log reduction in CFU) for *Bt* on stainless steel
345 and ceramic tile substrates after application of differently aged CBR gels and fresh NaOH-gel.



346

347 **Fig. 4.** Log reduction of *Bacillus thuringiensis* colony forming units on stainless steel and ceramic tile surfaces
 348 after treatment with different gels.

349

350 The removal of *Bt* spores was complete for all the CBR gels, even the one stored for 27 months, with
 351 no CFU counted on any of the substrates or in any of the flakes after decontamination (

352

353 Table 4). The CBR gel therefore remains effective against *Bt* even after important reductions in its active
 354 chlorine percentage (Table 1). This is probably because of the long contact time (few hours) between
 355 the gel and the spores on the surfaces, as discussed in the following paragraph. The biocidal activity of
 356 the gel thus involves two steps spore absorption-spore inactivation. First, the *Bt* spores are adsorbed into
 357 the gel and, as the gel dries, the known biocidal activity of the hypochlorite ions [1, 13, 15] ensures that
 358 the *Bt* spores are neutralized. This mechanism is consistent with the fact that the NaOH-gel is ineffective

359 against *Bt* spores, with a log reduction in CFU of less than 1.1 obtained with a fresh sample (Fig. 4).

360 Furthermore,

361
362 Table 4 shows that the number of CFU in the flakes is higher than the number remaining on the surface.
363 The spores are thus absorbed into the gel but NaOH has no neutralizing effect on them.

364 In liquid decontamination solutions, results in the literature indicate that the higher the active
365 chlorine percentage, the shorter the decontamination time is. Muhammad et al. have notably studied the
366 decontamination efficiency of chlorine bleach solutions against *B. atrophaus* spores as a function of the
367 active chlorine percentage and the contact time [37]. At room temperature, the log reductions obtained
368 were 6.96 in 30 min for a solution with 2 % liquid bleach and up to 7 in 10 min for solutions with 5 %
369 liquid bleach. Another study focusing on *Geobacillus stearothermophilus* decontamination found that
370 5.8 % bleach was necessary to obtain a log reduction greater than 6 in 20 min [38]. The higher log
371 reduction values we obtained in this work can therefore be explained by the much longer contact time
372 between the CBR gel and the contaminants, highlighting once again the value of its specific rheological
373 properties. The volume of decontaminating solution is also much lower than with liquid
374 decontamination, and the residues can be disposed of without further treatment.

375
376 **Table 4.** Bacterial colony forming units after decontamination by CBR and NaOH-gels of different substrates.

Gel	<i>Bacillus thuringiensis</i>				<i>Bacillus anthracis</i>			
	Stainless steel		Ceramic tile		Stainless steel		Ceramic tile	
	On surface	In flakes	On surface	In flakes	On surface	In flakes	On surface	In flakes
CBR gel (fresh)	0	0	0	0	NM	NM	NM	NM
CBR gel (18 months)	0	0	0	0	NM	NM	NM	NM
CBR gel (27 months)	0	0	0	0	0	0	0	0

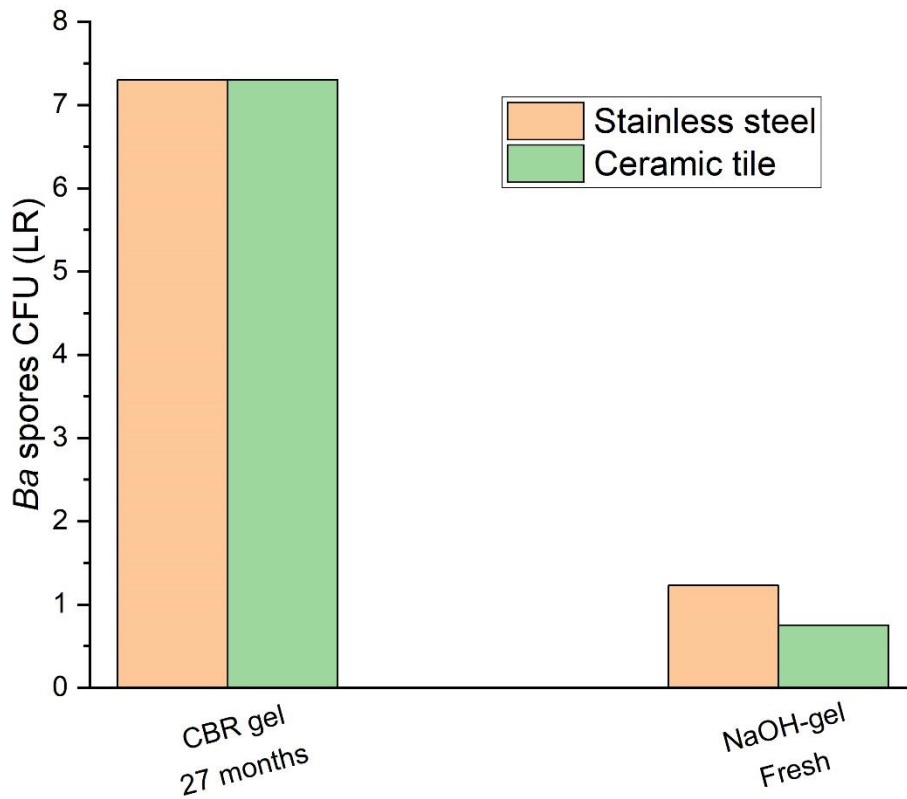
NaOH-gel (fresh)	1.3×10 ⁵	2.5×10 ⁶	8.0×10 ⁵	7.9×10 ⁵	5.3×10 ⁵	6.5×10 ⁵	5.5×10 ⁵	3.1×10 ⁶
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377 The initial number of colony forming units on the substrates obtained from the positive control was 2×10^7

378 NM, not measured.

379 *3.4.2. Gel efficiency on Bacillus anthracis spores*

380 Similar results were obtained with *Ba* (Fig. 5), namely complete removal with CBR gel aged
 381 for 27 months on both stainless steel and ceramic tile and very low decontamination efficiency with
 382 fresh NaOH-gel, confirming the tendency observed with *Bt*.



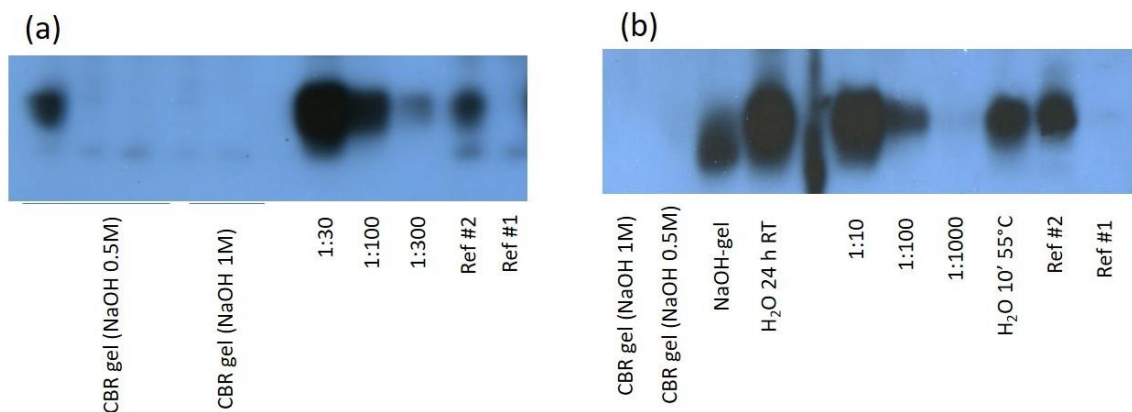
383

384 **Fig. 5.** Log reduction of *Bacillus anthracis* colony forming units on stainless steel and ceramic tile surfaces after
 385 treatment with different vacuumable gels.

386

387 *3.5. Gel efficiency against prion proteins and associated decontamination mechanisms*

388 Western blot analyses of PrP after treatments with different gels and control inefficient
389 treatments are shown in Fig. 6. The CBR gel prepared with 1 M NaOH was as effective as the first
390 reference treatment (Ref #1, effective, 1 M NaOH for 10 min at 55°C), with no PrP detected on the
391 substrate, suggesting a greater than 3-log reduction in PrP concentration given the detection limits of
392 the technique. The CBR gel with 0.5 M NaOH was less effective, with one of the three triplicates
393 showing just a ~2-log reduction in concentration (Fig. 6a), similar to the second reference treatment
394 (Ref #2, partially effective, NaOH 0.1 mM for 10 minutes at 55°C). However, no PrPres was detected
395 for the CBR gels after proteolysis (Fig. 6b) indicating that a 0.5 M NaOH concentration in the gel is
396 sufficient to inactivate PrP, in keeping with the known effects of millimolar NaOH solutions on PrP
397 [39].



398
399 **Fig. 6.** Western blot analysis of the efficacy of different vacuumable gels and reference treatments against prion
400 protein (PrP) contamination. (a) Total remaining PrP (no proteolysis with proteinase K) and (b) total remaining
401 protease-resistant PrP (after proteolysis with proteinase K) after treatment with CBR gels prepared with 0.5 and
402 1 M NaOH and two reference treatments (Ref #1, 1 M NaOH for 10 min at 55°C; Ref #2, 0.1 M NaOH for 10
403 min at 55°C), and in serial dilutions of non-treated contaminated substrate.

404

405 These results indicate that the CBR gel acts against PrP contamination with the same two-step
406 mechanism as against biological agents, namely that PrP is first absorbed into the gel, then destabilized
407 by NaOH and hypochlorite during the drying process.

408 **4. Conclusions**

409 This paper presents an efficient process to decontaminate solid surfaces after CBRN
410 contamination using an inorganic CBR gel. This CBR gel can be sprayed at a distance across large
411 surfaces and remain attached, even on vertical walls, without flowing. Once in contact with the
412 contaminated surface, the CBR gel acts with different mechanisms depending on the nature of the
413 contamination. First, the gel wets the surface and either solubilizes (radiological and chemical agents)
414 or absorbs (biological agents and prion proteins) the contaminants. The contaminants remain trapped in
415 the gel as it dries. Chemical agents, bacteria and prions are neutralized by NaOH and hypochlorite in
416 the process. The resulting waste in the form of non-dusting solid flake is easily removed from the surface
417 by brushing or vacuuming. In this way, no liquid effluent is produced and no secondary treatment
418 required, although the solid waste should be treated as toxic if any residual contamination remains. The
419 efficacy of the CBR gel was tested here on actual radiological (^{137}Cs), chemical (sulfur mustard, VX
420 and soman) and biological (*B. thuringiensis* and *B. anthracis*) contaminations with satisfactory
421 decontamination factors, even after two years' storage. The preliminary results presented here on prion
422 decontamination are also promising. The wide spectrum of action of the gel should make it an attractive
423 option to decontaminate CBRN agents but also in more conventional health and safety applications
424 against various pathogens. In the context of the ongoing COVID-19 pandemic, it would be particularly
425 interesting to investigate the efficiency of this CBR gel against viral particles.

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431

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