eNOTICE-2
EU Network of Training Centres for preparedness to CBRN Events

D5.2: Set up of experimental studies incl. definition of devices to be analysed

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Executive Summary

In Task 5.2, tests are to be carried out to safely decontaminate assistive devices for people with disabilities if they have been exposed to a hazardous substance. It is intended that the owners can continue to use the assistive devices immediately after the decontamination procedure. Currently, items for which no suitable decontamination procedures are available are disposed of and must be replaced. For items with low replacement costs and high availability, this is a viable way of eliminating any residual risk of persistent contamination. Clothing, for example, can easily be replaced at short notice. Assistive devices, however, are often custom-made for their owners by specialised manufacturers. Artificial limbs in particular are highly complex devices that can cost tens of thousands of euros, depending on the choice of materials and utilised technologies. As these are customised devices, manufacturing and delivery times are correspondingly long. This means a significant and prolonged restriction of autonomy and personal freedom of the affected persons. Affected persons can suffer from multiple impairments which are not only motor-related, but may also affect cognition, sensory functions or vital functions. If the assistive device is required to preserve vital functions, e.g. breathing, it must be replaced immediately. Intensive individual care may be required in such cases. All aspects concerning the health of the affected persons must be considered when selecting the actions to be taken. Here, the well-being of the affected persons is the top priority. This must be taken into account when the question arises as to whether an assistive device is to be disposed of or whether a decontamination is to be carried out. The prerequisite is that a suitable decontamination procedure is available. The suitability of decontamination procedures must be experimentally verified.

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1. General Considerations

1.1 General Considerations

The effort required for decontamination depends on various factors, which are examined experimentally in WP5.2. Decisive factors are, for example, the materials from which the assistive devices are made, the geometry of the devices, whether electronic parts are installed, and which hazardous substance is to be neutralised, i.e. which decontamination process is to be used under which conditions. Complex geometries with gaps, cavities and moving parts make decontamination difficult. Greases and oils for lubrication may also pose a problem, for example, if they prevent pathogens from being wetted by a disinfectant.

The parameters that must be considered as variables in the design of experiments can be roughly divided into intrinsic and extrinsic factors. The intrinsic factors are related to the object to be decontaminated and the applied decontamination procedure. The extrinsic factors result from the general conditions to be applied when carrying out the decontamination procedure at the site of operation. Intrinsic and extrinsic factors result in a set of requirements for decontamination. Each factor can be regarded as an independent variable in an experimental design. It is therefore extremely challenging to systematically analyse the application of different decontamination methods to various assistive devices, especially if a possible operator influence is also to be investigated. A comprehensive review of the state of the art is therefore required.

1.2 Determination of extrinsic and intrinsic factors

In the research project DEFERM (Decontamination Measures to Restore Facilities and the Environment after a Natural or Deliberate Release of Pathogenic Microorganisms), the intrinsic and extrinsic factors for a reliable decontamination of emergency responder's equipment were investigated. Table 1 lists intrinsic factors and the effects they were expected to have on decontamination. The final section discusses the extrinsic factors affecting the validation of the procedures analysed in the project. In terms of materials and geometric complexity, the emergency responder's equipment differs little from assistive devices. The factors can also be used for planning the tests to be carried out in project eNOTICE-2.

Table 1. Intrinsic factors / intrinsic variables (source: FDDO)

factor / variable	impact	response	other
materials	decontamination agent causes physicochemical changes to the material; material causes	compatibility chart	

	physicochemical changes to the decontamination agent		
size	size of compartment required for decontamination	adaption of decontamination equipment	
complexity of geometry (microscopic)	microscopic gaps, cavities (e.g. porous materials, fabrics); difficulties applying the decontamination agent	selection of a different decontamination procedure	
complexity of geometry (macroscopic)	macroscopic gaps, cavities, moving parts; difficulties applying the decontamination agent	selection of a different decontamination procedure; maybe partial disassembly of an item	
electronic components	decontamination agent causes short circuits and/or corrosion	selection of a different decontamination procedure; maybe partial disassembly of an item (only electronic components are to be replaced)	cross sensitivity to geometry: decontamination procedure suitable for electronics, but unsuitable for geometry
decontamination procedure	general requirements for decontamination; different use cases depending on application and chemical composition; long treatment times	selection of decontamination procedure suitable for the material, electronics and general requirements	chemical resistance may be affected by the materials: e.g. catalytic decomposition due to metals = no decontamination effect; limited applicability for electronics; dew point must not be exceeded

The active decontamination procedures listed in Figure 17 of D5.1 must be tested for their applicability under operational conditions. Extrinsic factors might include weather conditions such as ambient temperature, humidity and air pressure, as well as the effects of wind and precipitation. A basic prerequisite for mobile application is compliance with the general

requirements specified for the process to ensure reliable decontamination. Deviations are not permitted without re-validation of the procedures according to standard testing methods. The reproducibility of a decontamination procedure is essential and must be maintained regardless of extrinsic factors dictated by the operational conditions. For mobile application of a decontamination procedure, the general requirements and the time required must also be assessed. As envisaged in D5.1, a parallel decontamination of the assistive device and its owner (utilizing different decontamination procedures) is considered the best solution if the decontaminations are completed at the same time and the person can continue to use the device immediately. This results in requirements for the decontamination procedures and the actions of first responders in general.

2. Findings

2.1 DEFERM project

In the following - as a supplementary investigation - the topic of decontamination of the emergency services' equipment is also considered. There are parallels here to the aids used by people with disabilities (e.g. materials used). The following results provide a summary of a Franco-German project. Further details can be found on the project website[1].

Three established disinfection processes were analysed in the DEFERM research project to assess their suitability for use under operational conditions. First, applicable process parameters were determined under laboratory conditions in accordance with the factors/variables listed in table 1. The process parameters were then validated in realistic deployment scenarios. Below results are given for the disinfection procedures hydrogen peroxide in gaseous form, peracetic acid as an aerosol and sodium hypochlorite as a foam. Additional studies on prosthesis parts as part of the eNotice-2 project were conducted in a master's thesis (Imgrund, L. [2024]: Decontamination of medical equipment in biohazard situations, Masterthesis, Hamburg). Information on other disinfection methods such as the vacuum method or cold plasma was compiled based on a literature search.

2.1.1 Pros and cons of the investigated disinfection procedures

An advantage of hydrogen peroxide in gaseous form is that it can be used to disinfect electronic equipment, provided the dew point is not exceeded. Otherwise, a continuous layer of water will form on the components, which can lead to short circuits. Hydrogen peroxide has good material compatibility but has a tendency for catalytic decomposition on contact with metals, which nullifies the disinfecting effect. Compliance with the general conditions is challenging: a gas-tight compartment; level 2 protective clothing (according to FwDV500); technical equipment for generating hydrogen peroxide gas; technical equipment for

[1] https://www.sifo.de/sifo/de/projekte/schutz-und-rettung-von-menschen/praevention-und-schnelle-hilfe-bei-biologischen-gefahren/deferm-dekontaminationsmassnah-von-pathogenen-mikroorganismen/deferm-dekontaminationsmassnah-von-pathogenen-mikroorganismen.html (21.01.2025)

monitoring the concentration of hydrogen peroxide gas; guidance of gas flow in a circulation process; a relatively long exposure time depending on the gas concentration; air must be dried before disinfection; catalytic air purification of excess hydrogen peroxide must take place after exposure time. An advantage is the option to disinfect a large quantity of items in batch mode, depending on the compartment space. Mobile disinfection systems are commercially available.

An aerosol of peracetic acid is used in a similar way to hydrogen peroxide in gaseous form and therefore requires comparable preconditions: a gas-tight compartment; level 2 protective clothing (according to FwDV500). Batch operation is possible. An aerosol generator is of a much simpler in design compared to a gas generator and is easier to handle. The material compatibility of the aerosol is limited. Metals corrode due to acidic properties of the aerosol. In the case of non-acid-resistant ferrous metals, this leads to rust formation despite buffering. Galvanised surfaces are corroded, and hydrogen is formed. The chemical reaction produces acetic acid with a pungent odour.

The disinfectant foam based on sodium hypochlorite also adheres to smooth vertical surfaces and thus achieves sufficient exposure times. The foam is applied under level 2 protective clothing. A device was developed for simple application. It consists, among other things, of three compressed air cylinders. One that supplies the user with breathing air and two that provide compressed air for foam generation and application. The foam has an intense odour of chlorine. Application in confined spaces requires intensive ventilation afterwards. The foam can also be applied outdoors if weather conditions are favourable.

2.1.2 Experimental design and implementation

(a) The DEFERM project:

The DEFREM project focussed on the task of disinfecting equipment contaminated with pathogens (bacteria, viruses) at the site of deployment. The aim is to prevent the spread of contamination and to be able to return the equipment to service. The effectiveness of the three disinfection methods used in DEFERM project was assessed in the laboratory and then verified in field tests. Laboratory tests were carried out by the Robert Koch Institute. Biomarkers were used to test the procedures. Also, colour indicators were deployed in the field trials to provide a quick first impression on contamination.
Samples consisting of materials typically used for first responder equipment were prepared for the laboratory tests. The material samples were exposed to bacillus thuringiensis spores in the laboratory. Bacillus thuringiensis is related to the anthrax pathogen bacillus anthracis and reacts similarly to disinfection processes. In contrast to bacillus anthracis, bacillus thuringiensis is harmless to humans. On average approx. 1.37×10^6 spores (concentration defined as the content of a tested sample containing Bacillus spores in solution) were detected on the sample surfaces. An additional positive sample was used to confirm the result.

Disinfection was considered successful if the germinable spores were reduced by a level of 5log10. Spores like bacillus thuringiensis are regarded as very resilient. Therefore, their successful disinfection is an indicator for high process reliability.

The material samples analysed included metals, plastics, wood, glass and rubber. Fabric samples from car seats and a tent were also tested (Table 2). In the master's thesis assistive devices such as rollators, wheelchairs and lower leg prostheses were analysed in the laboratory to determine their ability to be disinfected.

Table 2. Material Compatibility and Effects of Decontamination Processes

Material group	**Material**	**Disinfection Process**	**Conspicuous features**
Metals	Stainless steel	H_2O_2	Material is easily attacked
	Brass	H_2O_2	Catalytic decomposition
	Copper	H_2O_2	Catalytic decomposition
	Iron	Peracetic acid	Rusting
	Aluminium	-	-
Plastic	Linoleum	-	-
	Plastics	-	-
Gum	Door seal	-	-
	Tyres	-	-
	Mat	-	-
Glas		-	-
	Tent Inside	-	-
	Tent outside	-	-
	Safety belt	-	-
	Vehicle seat fabric cover	-	-

(b) Decontamination Research at CTMA-UCLouvain: Objectives and Scope Preliminary Findings from a Literature Review

CTMA-UCLouvain has been conducting experimental research on new decontamination methods. This work extends beyond eNOTICE-2, contributing to ongoing projects such as Horizon-TeamUP and Horizon-eNOVATION—both focused on technologies that can be used on-site by first responders. It also aligns with the upcoming EU4Health-Easy2ReUse project, launching in September 2025, which aims to develop electronic- and material-friendly decontamination methods for protective masks, enabling their reuse for at least 100 cycles (Table 3). Some of these experimental observations have also been validated under real operational conditions during the cleaning and decontamination of electronic-sensitive satellite communication tools from SES TechCom, Luxembourg, in an open-air environment.

Table 3. Assessment of Emerging Decontamination Methods (CTMA-UCLouvain)

International Projects Acrocnym	Research Funding	Type of Project	Project Title	CTMA_UCLouvain Coordination / partnership	Duration (months)
eNOTICE-2	UCPM-2023-KAPP	UCPM	EU Network of Training Centres for preparedness to CBRN Events	**COORD**	24
TeamUp	HORIZON-CL3-2022-DRS-01	IA	HolisTic capability and tEchnology evAluation and co-creation fraMework for Upskilled first responders and enhanced CBRN-E resPonse	P	36
eNOVATION	HORIZON-CL3-2023-DRS-01-04	IA	Extended Network of CBRN Training Centers for Innovation	**Technical COORD**	36
Easy2reUse	EU4H-2024-PJ-01-2 HERA Action Grants	EU4H-PJG	Reusable Easy to Breath and Use Masks – Elastomeric half-mask	P	48

(c) Decontamination Research at CTMA-UCLouvain: Preliminary Findings from a Literature Review

The decontamination of biological agents and toxins, along with the monitoring of residual contamination, is a key focus of CTMA's research. To identify the most effective methods, we conduct a comprehensive literature review on these topics. The selected decontamination methods must meet two key criteria:

- *Requirement 1 (**R-1**):* Effectiveness against a broad range of biological agents and toxins

- *Requirement 2 (**R-2**):* Compatibility with electronic components.

NATO unclassified documents were also examined in this context (Figure 1).

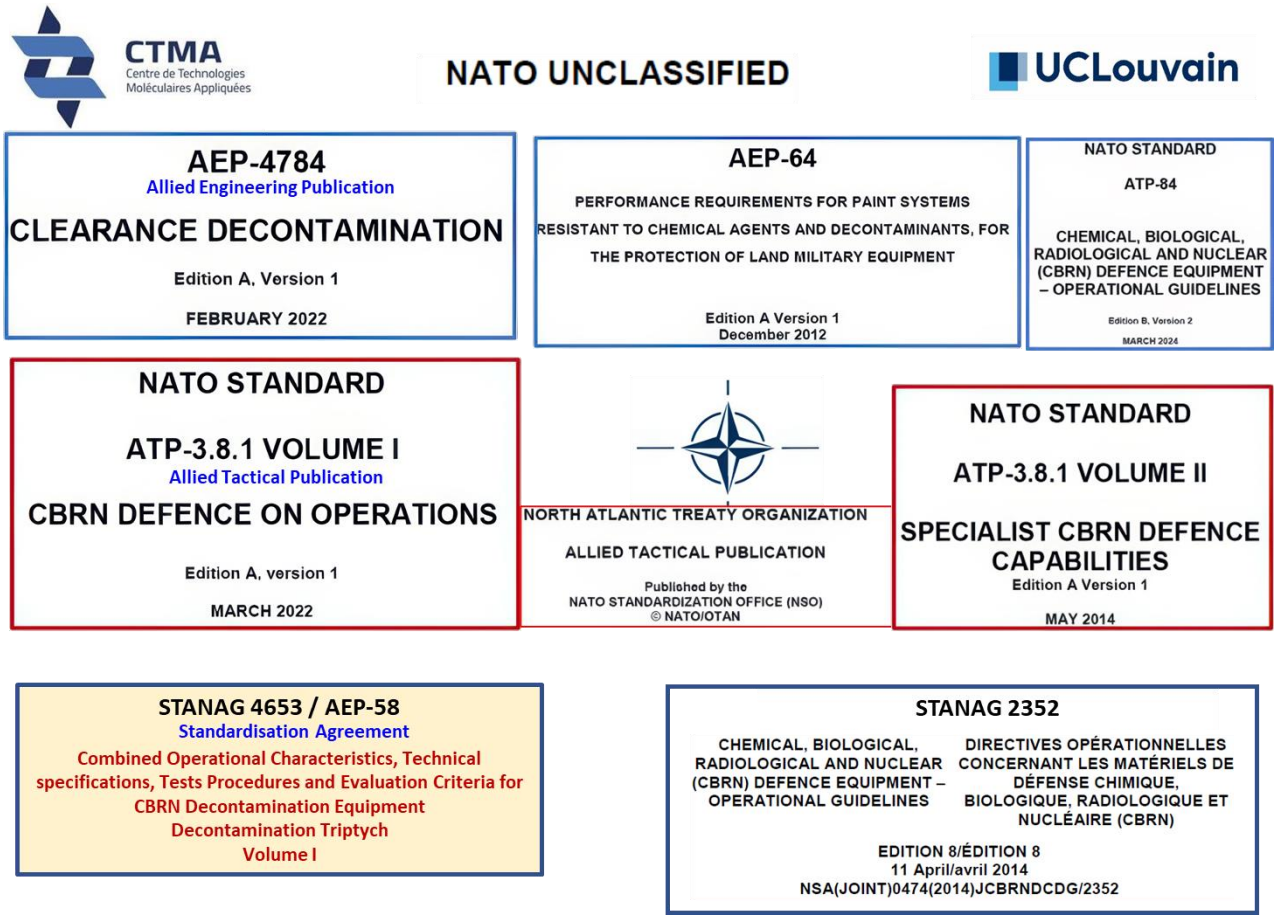

Figure 1. Unclassified NATO Standards (STANAG, ATP) and Guidelines on Decontamination

Particular attention was given to the updated version of the Allied Engineering Publication '*Chemical, Biological, Radiological, and Nuclear (CBRN) Decontamination Equipment – Combined Operational Characteristics, Technical Specifications, Test Procedures, and Evaluation Criteria'*, Edition 3, Study Draft 1, AEP-58, October 2024 (NATO), which is highlighted in orange in Fig. 1.

The main preliminary conclusions from the review of current literature and NATO standards and guidelines are as follows:

1. *Lack of a Unified Standard for Decontaminating Assistive Devices*: While we reviewed existing methods for each separate requirement (R-1 and -2), we could not find any method fulfilling both requirements together. There were no EU or international standards complying with both requirements in an integrated way.

2. *Decontamination Mechanisms and Key Factors Influencing Their Effectiveness*: Each method leverages distinct mechanisms such as oxidative damage or photochemical disruption. While their effectiveness varies depending on microbial targets—influenced by factors such as concentration, exposure time, and experimental conditions, as well as extrinsic factors like temperature and relative humidity—all three methods demonstrate strong efficacy against bacteria and viruses, with varying degrees of effectiveness against spores.

3. *Operational Strengths and Limitations of Decontamination Methods*: Despite their advantages, each method presents operational challenges, including the need for specialised equipment, controlled exposure conditions, and potential material compatibility concerns. The impact on materials ranges from corrosion and oxidation risks for metals and electronics to surface degradation in certain polymers and elastomers. Additionally, some methods require precise environmental control, such as humidity regulation or shielding against secondary by-products (e.g., ozone or reactive residues).

(d) Experimental and On-Site Decontamination Research at CTMA-UCLouvain: Selected Methods and Comparative Analysis

While focusing on both requirements R-1 and R-2, we selected the three methods (Table 4): vaporised hydrogen peroxide (VHP, dry HP), cold plasma (non-thermal plasma), and UV-C - Pulsed krypton fluoride excimer laser. Table 4 provides a comparative evaluation of the three selected decontamination methods analysing their mechanisms, effectiveness, advantages, limitations, and material impact.

Table 4. Experimental Evaluation of Multiple Decontamination Methods at CTMA-UCLouvain

Decontamination Method	Working mechanism	Effectiveness against biological agents	Pros	Cons	Impact on materials
Vaporised Hydrogen Peroxide (VHP, Dry HP)	Vaporised hydrogen peroxide generates reactive oxygen species (ROS), which cause oxidative damage to microorganisms by disrupting their genetic material and proteins	**Highly effective against most pathogens, including spores** The effectiveness against *Bacillus thuringiensis* and *Geobacillus stearothermophilus* spores depends on the VHP concentration, exposure time, temperature and relative humidity Note: Spore conditioning in powder form can reduce VHP decontamination efficiency	- Highly effective against various pathogens - Can reach hard-to-access areas	- Requires specialised equipment - Requires careful handling to avoid overexposure (exceeding the dew point)	**The impact varies with VHP concentration and duration of exposure**: - Generally compatible with a wide range of materials, including plastics and metals if condensation on the surface is prevented - May not be suitable for electronics due to its corrosiveness and moisture; some materials used in electronic devices are incompatible - Suitable for devices that are well-sealed, moisture-resistant, or have protective coatings, with only temporary or controlled exposure
Cold plasma (Non-thermal plasma)	Cold plasma generates a highly ionised gas, producing reactive oxygen species (ROS) that induce oxidative damage to pathogens' genetic material and proteins	**Highly effective against most pathogens** However, efficacy against Bacillus thuringiensis *and* Geobacillus stearothermophilus spores is reduced *NB-1: The effectiveness of cold plasma against Bacillus thuringiensis and Geobacillus stearothermophilus endospores depends on several factors, including the type of cold plasma used (atmospheric or low-pressure), the exposure time, plasma power, and the presence of any additional treatment conditions*	- Non-chemical, environmentally friendly - Compatible with electronics and metals - Operates at low temperatures - Cold plasma can generate hazardous by-products (like ozone), but risks are typically managed through strict safety protocols.	- Can modify surface properties of polymers and rubbers - Corrosive to some materials (e.g., rubbers, certain metals) : potential material degradation with prolonged exposure - Requires specialised equipment that hampers rapid and easy on-site use - Limited penetration in complex geometries	- Polymers (Polypropylene, PETG, Polyethylene, etc.) may experience surface modifications - Metals (Aluminium, Tungsten) generally compatible - May degrade elastomers, some polymers, and sensitive metals like silver - Electronics generally stable but avoid prolonged exposure; certain materials might be more reactive or sensitive to the plasma-generated species than others - Risk of oxidation for electronic components; suitable only for well-sealed, moisture-resistant devices with protective coatings, and if exposure is temporary or controlled

Table 4 (Continued). Experimental Evaluation of Multiple Decontamination Methods at CTMA-UCLouvain

Decontamination Method	Working mechanism	Effectiveness against biological agents	Pros	Cons	Impact on materials
UV-C (Pulsed krypton fluoride excimer laser)	UV-C induces direct photochemical damage to living organisms by forming thymine dimers in DNA, thereby inhibiting microbial replication	**Highly effective against viruses (particularly enveloped viruses), but classically less effective against spores** Conventional UV-C has a reduced efficacy against Bacillus spores. The efficacy of pulsed krypton fluoride excimer laser UV-C against *Bacillus thuringiensis* and *Geobacillus stearothermophilus* spores is currently being assessed *NB: CTMA is now assessing high energy UV-C (pulsed krypton fluoride excimer laser: see ref) to assess efficiency of this*	- Safe for metals and most electronics when exposure is short	- Heat generation: Some UV-C lamps produce heat as a byproduct, which may harm heat-sensitive electronic components - Limited penetration: Reduced efficacy in shadowed areas - Surface damage: Prolonged exposure can degrade polymers and rubbers, causing discoloration, brittleness, and other forms of surface degradation that may compromise the structural integrity of sensitive electronic devices	- Polymers (PP, PETG, Polycarbonate) may yellow or become brittle - Tungsten and aluminium are generally unaffected - Not suitable for light-sensitive electronic components - Plastic or rubber casings of electronics may degrade over time, potentially compromising their integrity and functionality

(e) Experimental Evaluation of VHP and Cold Plasma DECON for R-1 and R-2

The effectiveness of VHP and cold plasma decontamination was assessed in a laboratory room where we placed a range of representative electronically sensitive equipment alongside the following biological simulants: *Geobacillus stearothermophilus* (Apex disk and spore solution), *Bacillus thuringiensis* powder (Turex), *Escherichia coli* (MS2 host), MS2 bacteriophage, *Cladosporium spp*., ricin chain A (RCA), and ricin extract obtained directly from seeds.
While DECON effectiveness was assessed by culturing the simulants post-treatment, toxins were tested using a functional assay.

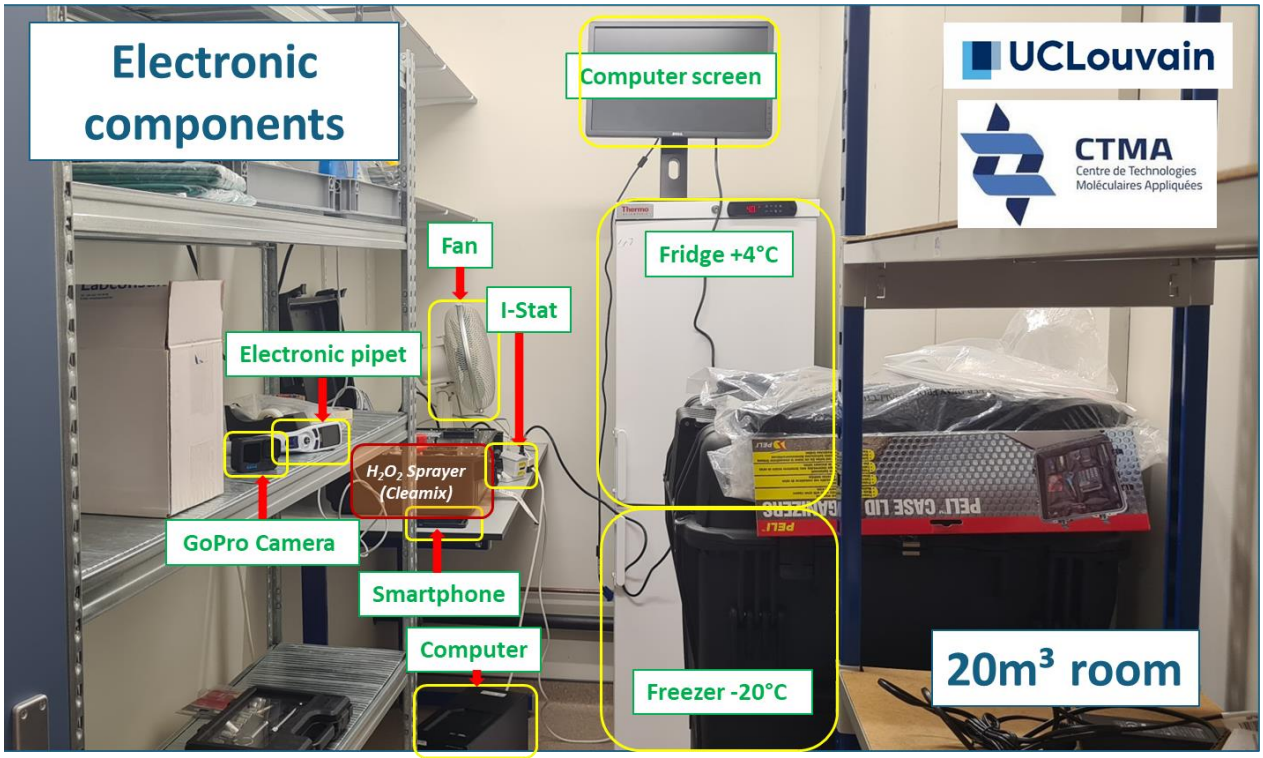

Figure 2. Laboratory room containing electronic-sensitive equipment undergoing VHP decontamination

Figure 2. Decontamination devices used at CTMA-UCLouvain

(a) VHP generator (Cleamix 100) spreading HP vapour on a controlled way according to the continuous monitoring of ambient temperature, relative humidity and VHP concentration.

Regarding R-1, the results reflect the differential level of resistance of agent's biological agent to decontamination methods (Figure ").

Figure 3. Hierarchy of decontamination susceptibility: Differential Resistance of Biological Agents to decontamination

Given that the DECON process depends on the type of pathogen involved—which cannot be predicted in advance—it is essential to consider a broad spectrum of agents for decontamination assessment. However, such extensive testing would be highly demanding in terms of time, resources, and financial costs. Moreover, this approach extends beyond the current scope of our experimental assessment, as no NATO or EU standard integrates R-1 and R-2 requirements in a unified framework.

To validate the DECON process, it is crucial to test the most resistant agents—endospores (Figure 2)—eliminating the need for exhaustive testing across all biological agents. However, unlike small viral particles, endospores are relatively large, which may limit their penetration into complex equipment and assistive devices with intricate geometries. While testing endospores provides a strong indicator of biological inactivation, assessing a broader range of biological agents within sealed or complex structures remains informative.

This rationale led us to test not only exposed surfaces but also the internal compartments of sealed devices—such as printers, computers, smartphones, and GoPro cameras—to better evaluate decontamination effectiveness in real-world conditions.

Table 5. Bacillus Endospores Used as Simulants for Bacillus anthracis Spores

Feature	Bacillus anthracis	*B. subtilis*	*B. cereus*	*B. atropheus*	*B. thuringiensis*	*G. stearothermophilus*
Genus	Bacillus	Bacillus	*Bacillus*	Bacillus	Bacillus	Geobacillus
Gram Stain	Positive	Positive	Positive	Positive	Positive	Positive
Shape	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped
Spore Formation	Yes	Yes	Yes	Yes	Yes	Yes
Temperature	Mesophilic (~37°C)	Mesophilic (~30–37°C)	Mesophilic (~4–50°C)	Mesophilic (~30–37°C)	Mesophilic (~25–37°C)	**Thermophilic** (~50–65°C)
Habitat	Soil (spores can persist for long periods); often associated with grazing areas	Soil; frequently found on plant surfaces and in decaying matter	Soil, food, dairy products	Soil; commonly found in dust, air, and dry environments	Soil	**Hot environments** (e.g., compost, autoclaves)
Main Function	Pathogenic; causes anthrax in humans and animals	Model organism for research; robust spores assist in survival	Foodborne pathogen; produces diarrheal and emetic toxins	Used as a biological indicator for sterilisation validation	Produces insecticidal toxins	Used in sterilisation control
Application	Vaccine development, biodefence research, and study of anthrax pathology	Industrial enzyme production, probiotics, biotechnological research	Food safety research, microbial contamination control, and toxin studies	Sterilization process validation (e.g., for ethylene oxide and dry heat sterilisation)	Biopesticides, genetically modified crops protected against specifc insect pests	Sterilisation validation

Note: Key features of endospores selected by CTMA for experimental and operational assessment of decontamination efficacy are highlighted in pale blue.

To test *Bacillus* endospores as surrogates for *Bacillus anthracis* spores, we used:

- **TUREX WG 1KG (50% w/w *Bacillus thuringiensis* aizawai strain GC-91**) (Certis Belchim). This strain product, certified for use in organic farming, is a transjugate of two *Bacillus thuringiensis* subspecies (*aizawai* and *kurstaki*), strain GC-91, which provides a mixture of CRY toxins from both groups within the same product. The spore content is 25,000 UI/mg, corresponding to 3.05×10^{13} CFU/kg**.**
  - *For the experiments, we used 100 mg of powder, corresponding to 3×10^9 CFU.*
- ***Geobacillus stearothermophilus* spore solution, strain ATCC 7953** (Merck) at a concentration of 5 x 10^7 to 5 x 10^8 CFU/ml.
  - *For the experiments, we used 100 µl of solution, corresponding to 5×10^6 to 5 x 10^5 CFU, on a Petri dish.*
- ***Geobacillus stearothermophilus APEX disc, strain ATCC 12980*** (MESALABS).
  - *For the experiments, we used a disc containing $10E^6$ spores / disc.*

The following table (Table 6) presents the preliminary results obtained under experimental conditions.

- VHP effectively decontaminated all tested biological agents, except for B. thuringiensis spores in powder form. While no growth was observed within the first 24 hours (Figure

4), occasional Bacillus colonies appeared during extended culturing, which continued for seven days post-treatment. Notably, both decontamination methods produced identical results in this regard.

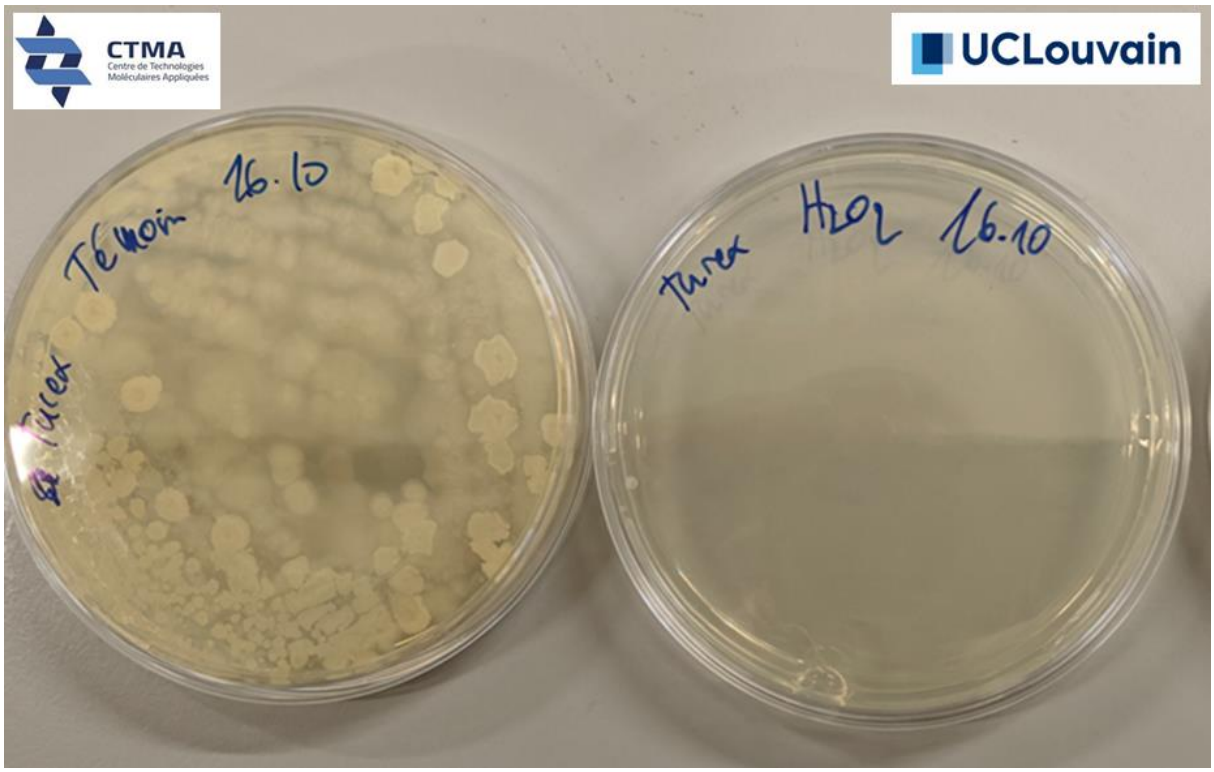

Figure 4. Bacillus thuringiensis control (left) and decontaminated sample (right). Decontamination was performed using VHP with an exposure time of 4 hours at 160 ppm in a room with a volume of 20 m³.

- The toxin simulant and toxin extract remained functionally active under the tested cold plasma conditions, suggesting the need for further optimization if feasible. RCA (ricin simulant) was functionally inactivated by VHP, whereas the ricin extract remained active.

- Electronic compatibility was confirmed for all tested devices. However, due to the small volume of the cold plasma chamber, testing was limited to compact devices, such as smartphones.

Further evaluations will explore the impact of key parameters, including concentration, exposure time, and energy input, to enhance decontamination efficiency.

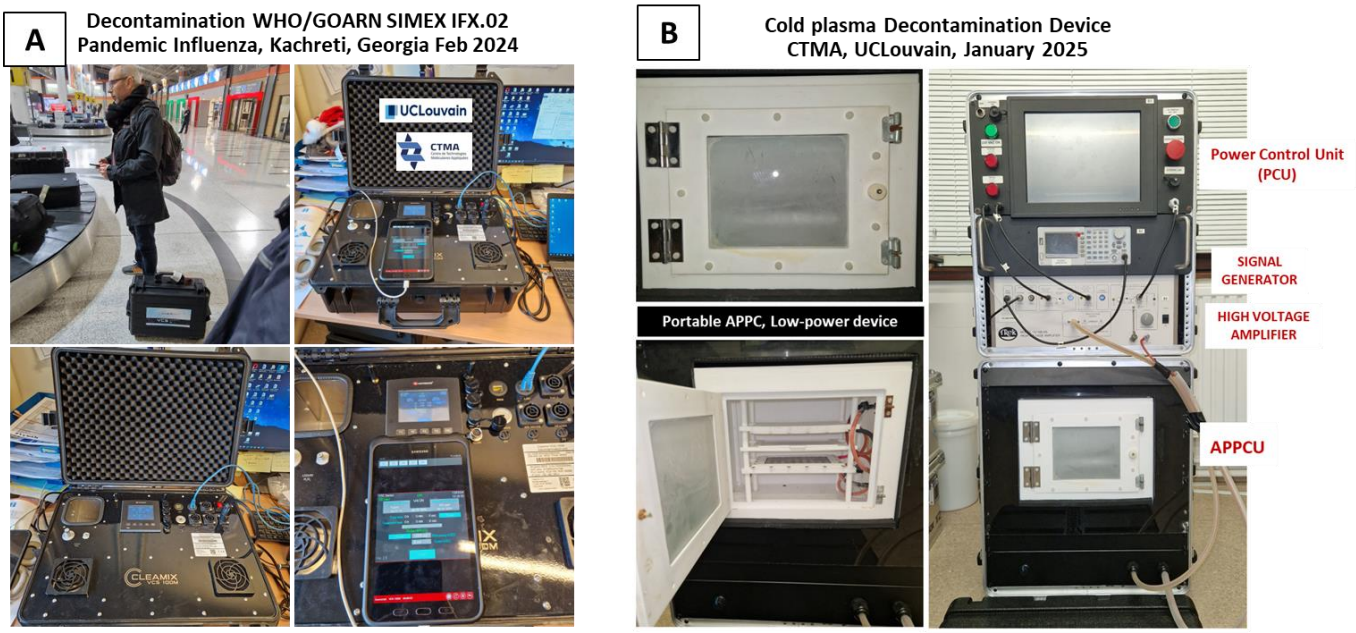

Figure 5. VHP spreading device (portable CLEAMIX 100 suitcase) with its standalone monitoring tablet control (Panel A); Cold plasma device with the Atmospheric Pressure Plasma Chamber (Panel B)."

Table 6. Experimental and On-Site Decontamination Research at CTMA-UCLouvain: Methods and Key Findings

Targets	Methods	H2O2 vapors		Cold Plasma	
	Sealed Containment Area (m^3)	**3**	**3**	**0,015**	**0,015**
	Exposure time (h)	**1**	**2**	**3**	**6**
	Voltage_Electric Potential (kV)			**19**	**19**
	Frequency (kHz)			**1**	**1**
	Concentration (ppm)	**600**	**600**		
Bacteria	*Geobacillus stearothermophilus* (Solution)				
	Geobacillus stearothermophilus (Apex disk)				
	Bacillus thuringiensis powder (Turex)	**Residual growth**	**To evaluate**	**Growth**	To evaluate
	Escherichia coli (MS2 host)				
Virus	MS2 bacteriophage				
	JVE	To evaluate		To evaluate	
Toxin	Ricin (RCA)			**Functional**	To evaluate
	Ricin Extract	**Functional**	**Functional**	**Functional**	To evaluate
Fungi	*Cladosporium species*			To evaluate	To evaluate
	Candida auris	To evaluate		To evaluate	
Electronic equipment	*Smartphone*	**Functional**		**Functional**	
	Telecommunication electronic devices	**Functional**		To evaluate	
	Laboratory electronic devices	**Functional**		To evaluate	

Note: Green cells indicate effective decontamination for biological agents or the absence of detrimental effects on electronically sensitive equipment.

(f) Operational Evaluation of VHP Decontamination for R-1 and R-2: On-Site Testing at the Luxembourg Civil Protection Facility

We evaluated the use and efficiency of VHP decontamination under operational conditions during two successive decontamination missions at the Luxembourg Civil Protection Facility in Lintgen, conducted in December 2024 and January 2025 (Figure 6).

During these tests, we successfully decontaminated biological simulants (*Geobacillus stearothermophilus* and *Bacillus thuringiensis*), selected as representative test cases. The results were consistent with those previously presented and summarised in Table 6.

Figure 6. On-site decontamination was conducted inside a small container and a tent deployed at the Luxembourg Civil Protection Facility. Colorimetric indicators were placed at various locations to monitor VHP exposure. As indicated by the red arrows in the top-left panel, a small heater was placed beside the VHP spreading device to enhance ppm levels, while a fan ensured homogeneous hydrogen peroxide distribution within both structures

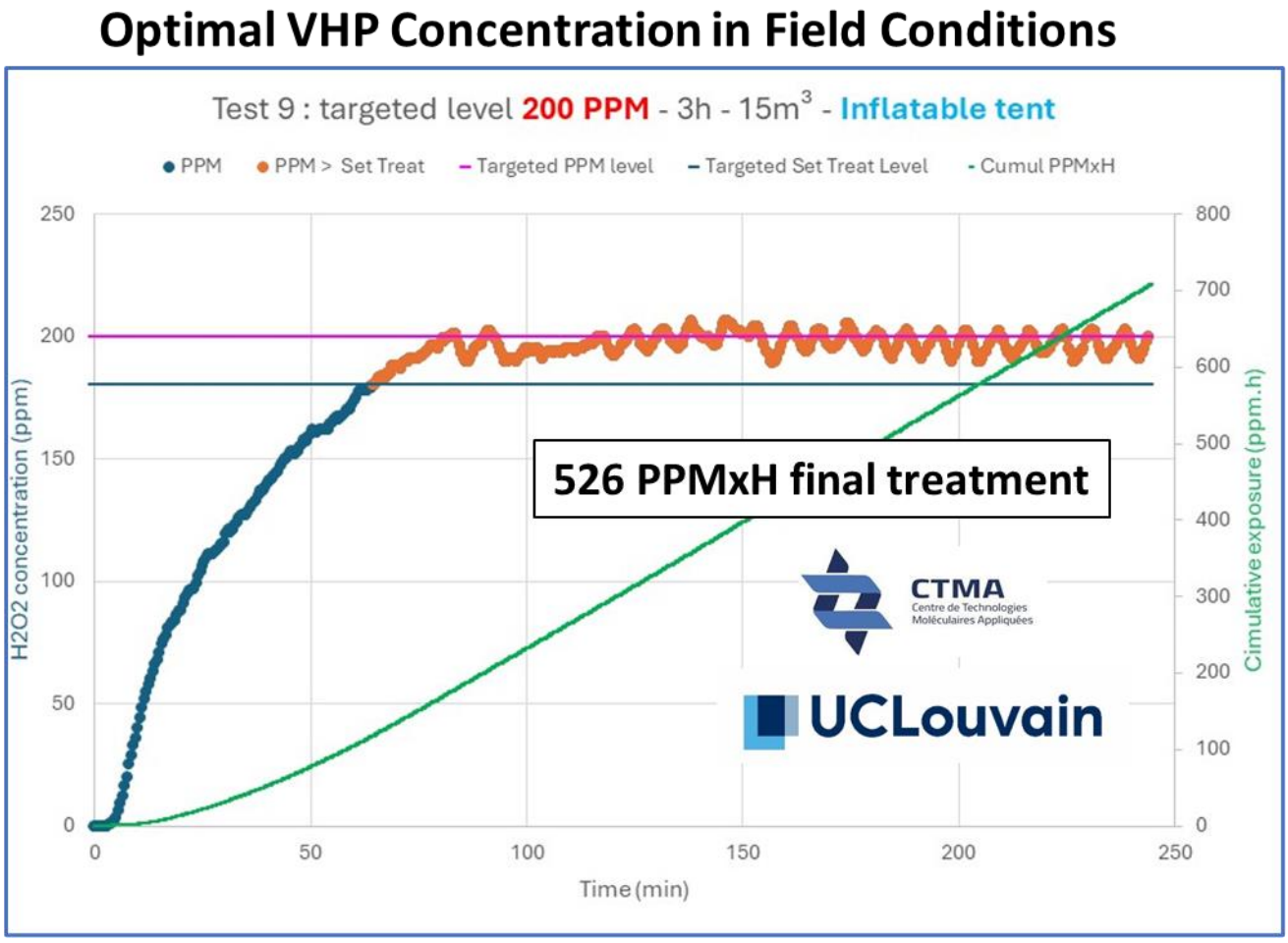

Figure 7. Example of a real-time VHP concentration curve. On-site decontamination was conducted inside a small container and a tent. Colorimetric indicators were placed at various locations to monitor VHP exposure. The target concentration was 200 ppm. The device began recording cumulative VHP exposure once the Targeted Set Treatment Level was reached and continued monitoring throughout the 3-hour exposure period, resulting in a total cumulative exposure of 526 ppmxh.

(g) Comparison between DEFERM, the master thesis and CTMA experimental and operational work decontamination for R-1 and R-2

We compared the methodologies and results of DEFERM and Master thesis, as presented in this deliverable, with those recorded by CTMA In Table 7.

Table 7. Experimental Comparison of Decontamination Methods: Impact on Endospores (R-1) and Sensitive Equipment (R-2)

Decontamination method		DEFERM Master thesis (Imgrund, L.[2024]	CTMA (eNOTICE-2)
	Type of exposure		
Hydrogen peroxide	• Aerosol (*20 µm droplet size*)		Not done
	• DryFog generator (*7.5 µm droplet size*)		Not done
	• Dry Vapour (*Gaseous phase*)		CLEAMIX 100 is designed to generate a true gaseous state of hydrogen peroxide
Peracetic acid	Aerosol	• Limited material compatibility (corrosion of metal and galvanised surfaces -Rust on non-acid -resistant ferrous metals); • Pungent odour	Not Done
Sodium hypochlorite	Foam	• Intensive ventilation needed • Odour of Chlorine	Not done
UV-C mercury vapor lamp (16 UV)			Not done
Cold Plasma		Not Done (Literature review)	Not done
Vaccum method		Not Done (Literature review)	Not done
	Material exposed		
Equipment	Medical; leg prosthesis (made of metals, plastics, wood, glass, rubber, fabrics)		Range of electronic-sensitive equipment (lab tools; computer; smartphone; motherboard…)
	Reagents exposed		
Reagents	Endospores	*Bacillus thuringiensis* *(1.37 X 10^6 spores)*	*Bacillus thuringiensis* *(3 x 10^9 CFU / 100 mg de poudre)* *Geobacillus stearothermophicus* *(APEX Disc: 10^6 / disc)*
	Decontamination efficiency		
Results	Endospores	Reduction by 5 $\log_{10}$	• No growth after 24 hours • Late occasional growth (test ended at day 7

When comparing the same decontamination method (i.e., hydrogen peroxide), some differences were observed between the DEFERM and UCLouvain groups (Table 8), particularly regarding the viability of *B. thuringiensis* endospores. While the concentration of *B. thuringiensis* in the liquid sample tested by DEFERM and the Apex disc tested by UCLouvain were comparable (~10^6 spores per sample), it remains to be determined whether these differences stem from variations in decontamination efficacy due to the mode of hydrogen

peroxide exposure—either gaseous phase (dry vapor, as used by UCLouvain) or aerosol (droplets of different sizes, as used by DEFERM).

Table 8. Comparison of Hydrogen Peroxide Decontamination Methods: DryFog vs. Vapour (Controlled Spread).

Comparative parameters for using HP for decontamination	Hydrogen peroxide	
	DryFog Generator	Vapour (controlled spread)
Working Under Dew Point	Requirement	Requirement
Extrinsic Factors		
Temperature	Requirement	Requirement
Humidity	Requirement	Requirement
Air pressure	Requirement	Requirement
Wind - Precipitation	Requirement	Requirement
Catalytic Decomposition	Requirement	**Not required**
Gas-Tight Compartment	Requirement	Requirement
Controlled Gas Flow	Requirement	Requirement
Equipment to Generate Gas	Requirement	Requirement
Monitoring of Concentration	Requirement	Requirement
Time of Exposure	Relatively long (9h at 150 ppm vs. 1 h at 500 ppm)	**Can be significantly reduced in experimental and customised field setting**
Dried Air Before Disinfection	Requirement	**Not required**
Catalytic Air Purification	Requirement	**Not required**
Turnaround Time	Time-consuming	**Manageable in a customised field setting**
Efficiency	Low efficiency (Short dwell time)	**Efficient even on spores (current limitation: powder can be an issue**

(h) Assessing VHP and Cold Plasma DECON by UCLouvain group: Initial Conclusions on R-1 and R-2 Compliance

Selecting an appropriate DECON method depends on the intended application, targeted pathogens, and material constraints, requiring a balance between efficacy, operational complexity, and compatibility with sensitive equipment. While a universal solution that fully meets both R-1 and R-2 requirements is currently unrealistic, a combination of electronic-friendly methods can achieve high decontamination efficiency across a broad range of biological agents.

By implementing validated decontamination approaches, first responders can effectively and safely decontaminate assistive devices within a short operational timeframe, ensuring both biological safety and equipment integrity.

3. Results and discussion

Taking into account the various tests (both as part of the eNOTICE-2 and Deferm projects as well as the aforementioned master's thesis), the following results can be summarised.
Assistive devices are made of similar materials to the equipment used by first responders. From the perspective of FDDO, the results from the DEFERM project are therefore also relevant for eNOTICE-2. In particular, the results from the field tests provide empirical values regarding the intended parallel disinfection of owners and their assistive devices. In the laboratory tests, the concentrations of the disinfecting agent were compared to the exposure times. According to Sinner's circle, higher concentrations correspond to shorter exposure times, which was confirmed by the test results. Prerequisites for the field tests were derived from this if successful disinfection is to be achieved under field conditions.

Disinfection with hydrogen peroxide gas is time-consuming and material-intensive. Depending on the H_2O_2 concentration, processing times of 9 hours at 150 ppm or 1 hour at 500 ppm were required. Fumigation was the only method capable of disinfecting electronic components without causing damage. UCLouvain's experimental work further confirmed these observations, demonstrating that dry vapor hydrogen peroxide (VHP) could effectively decontaminate electronic-sensitive equipment while ensuring functional integrity. Although sporicidal efficiency was challenged when dealing with powdered *Bacillus thuringiensis* spores, only occasional regrowth was detected at later culturing stages, result which seems to differ from the DEFEEM's results.

Cold plasma decontamination, as assessed by UCLouvain, was tested under controlled laboratory conditions using an atmospheric pressure plasma chamber. Results showed strong decontamination potential against *Geobacillus stearothermophilus* and *Bacillus thuringiensis* simulants, yet its scalability for field use remains a key challenge due to chamber size limitations and power requirements. Cold plasma exposure did not compromise electronic functionality, making it a promising alternative for sensitive equipment.

Disinfection with peracetic acid aerosol was performed using a nebulized 10% solution, while surface wiping typically uses a 2% solution. Samples were analysed after a 1-hour exposure. Peracetic acid is effective on non-porous materials but showed disinfection gaps on fabrics such as seat belts, likely due to aerosol droplet size and surface wetting properties (e.g., contact angle). Further investigation is needed. A major drawback of this method is corrosion of non-acid-resistant metals.

Sodium hypochlorite foam successfully disinfected all tested materials, requiring a relatively short contact time of 10-20 minutes depending on the material. While aerosol and gas methods are limited to enclosed spaces, foam can also be used outdoors. However, tests revealed a limitation in spore disinfection – even with extended exposure times, not all *Bacillus thuringiensis* spores were effectively inactivated.

Evaluation of the Master thesis

In the Master thesis, the disinfecting effect of various disinfection methods on lower leg prostheses was investigated under laboratory conditions. The requirement was complete disinfection within 20 minutes. The time span was derived from an estimate of the time required to decontaminate the prosthesis wearer. Apart from the material compatibility, the penetration behaviour into gaps and cavities was of interest. A 12 vol% hydrogen peroxide solution and a 10 vol% peracetic acid solution were used. Both solutions were aerosolized, once with a typical droplet spectrum of approx. 20µm and once with a dry fog generator and 7.5µm droplet size. The assumption was, analogous to the gas, that a smaller droplet size should have better penetration behaviour in gaps and cavities. In addition, tests were carried out with a wet peracetic foam and a UV-C mercury vapor lamp with 16W UV power.

The results with the DryFog generator were contradictory. Aerosolized peracetic acid showed a complete positive result with a room load of 60 ml/m3 and an exposure time of 5 minutes, while the hydrogen peroxide solution did not provide any positive results and is therefore unsuitable as a disinfectant in the DryFog process. The reason for this is the very short dwell time of the aerosol in the air and the associated decrease in concentration, which could not be increased any further. An attempt to keep the aerosol in the air for longer by means of coarser aerosolization also failed. Further investigations were continued with peracetic acid. Conclusion: disinfection of metal and plastic parts is possible with the two aerosol processes and peracetic acid as a disinfectant agent. No residues remained on the surfaces after the end of the procedures. However, the methods show their weaknesses with complex geometries. Structures preloaded with spores were not reliably disinfected, while environmental germs were consistently disinfected. This suggests that although the aerosol can penetrate the complex structures, it does not achieve a sufficient concentration to disinfect the spores. No disinfecting effect could be achieved with the UV-C lamp. Only the foam achieved positive disinfection everywhere. Under the microscope, electronic components were found to show corrosion after disinfection.