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Opinion and expert microbiological assessment of two test reports issued by the Fraunhofer Institut für Bauphysik (Fraunhofer IBP) (Frauenhofer Institute for Building Physics), Emissions, Environment

Test report No. 330241-177: Qualification of a mobile air purification device to reduce aerosol concentration in enclosed indoor spaces (September 18, 2021)

Date: August 30, 2022

Outline of the issue and content

Test report No. 330241-177 outlining the afore-mentioned issue presents inactivation test results for microbiologically active aerosols and volatile organic compounds (VOCs) in indoor air.

The object being tested is the "CUBUSAN CP 120" device produced by Wintersteiger AG, A-4910 Ried im Innkreis, Austria. The device has an electrical plasma generator which produces physical atmospheric low-temperature plasma from gaseous components of ambient air. This plasma reaction primarily converts oxygen and water vapor in indoor air to hydroxyl radicals, which are the effective plasma reaction products. Hydroxyl radicals lead to oxidation of small-molecule organic compounds. Hydroxyl radicals can also destroy micro-organisms (primarily viruses, bacteria, and fungi), thus generating active disinfectant properties. Hydroxyl radicals do not have an adverse effect on cell components (tissues) and therefore on higher organisms (plants, animals, and humans) as the oxidation potential is immediately inactivated on contact with tissues and both plant and animal tissues have enzymatic mechanisms that directly inactivate the oxidation potential.

This constellation allows hydroxyl radicals to be used to disinfect indoor air.

Test report No. 330241-177 investigates the inactivation of aerogenic virus particles that act as surrogates for viruses with an affinity for the respiratory tract, such as the SARS-CoV-2 virus. These surrogate viruses in the form of bacteriophage viruses (in this case, Phi6 phages) have a biochemical structure similar to the SARS-CoV-2 virus but are not harmful to humans. The presence or absence of Phi6 phages over time using the CUBUSAN device is tested and quantified with the bacterial lawn culture method and PCR (polymerase chain reaction).

Furthermore, the change in concentration of a selection of 13 volatile organic compounds (VOCs) over time is investigated using the CUBUSAN device in the test facility.

Description of the test procedures:

A defined testing facility is used (IPA Cape Testcenter ICT: 75 m³), which can be exposed to the aerosol and in which the effect of the "CUBUSAN CP120" device can be observed to document changes in aerosol composition over time.

The test report is drafted in accordance with "good scientific practice" in terms of methodology and from an expert and technical perspective. The facts investigated are set out on the basis of sound scientific knowledge.

Examination of the microbiological aerosol composition over time has been slightly modified and applied to aerogenic viruses based on DIN ISO 16000-36 for the testing of aerogenic bacteria.

Changes in aerosol composition are investigated in three phases: Phase 1:
Atomization and concentration of phage viruses as air-borne aerosol without CUBUSAN.

Phase 2:
Atomization of phages and air purification, i.e., aerosols are supplied whilst the CUBUSAN device is operational.

Phase 3:
After previous concentration and possibly partial depletion during phase 2, the change in aerosol composition over time, with the CUBUSAN device in operation, is investigated following inactivation of the aerosol source.

At this point it should be noted that Phase 3 follows on from Phase 2 directly. This means that supersaturation can occur in phase 2. This leads to the formation of particles > 20 µm (condensation nucleus, increase in droplet size with increase in volume and decrease in surface area). Such droplets may contain active viruses that cannot be inactivated by air-borne hydroxyl radicals as the latter are unable to penetrate droplets > 20 µm at depth. Hydroxyl radicals are inactivated at the surface and the solubility and persistence in this type of droplet cannot be reproduced. Hydroxyl radicals may well be capable of penetrating deeper liquid layers but, in the worst case scenario, it must be assumed that the oxidants (hydroxyl radicals) are inactivated on the droplet surface – even in the presence of organic components in such droplets.

These droplets form sediment or are adsorbed on surfaces and escape particle size distribution analysis.

One recommendation for methodological optimization would therefore be to apply phase 1 before each aerosol adjustment (i.e., phase 2 and then phase 3).

Presentation of results – Microbiology:

The results show that there is a depletion of microbiologically active aerosol particles as soon as the CUBUSAN device becomes operational.

A bacterial count of $9.4*10^6$ pfu/m³ is initially present (in the form of Phi6 phage viruses introduced via a compressed air aerosol generator) (P1 page 23).

This is already reduced by 0.8 log levels to $1.53*10^6$ pfu/m³ when the plasma generator is activated (P2 page 23).

With a constant aerosol feed, a steady-state is reached within one log level (the results alter within a power of ten of 10^6: $9.46*10^6$ pfu/m3 in P1 and $5.67*10^6$ pfu/m³ in P3).
There is no concentration here, which means that at least as many virus-laden aerosol particles are eliminated as are added.
When comparing P1, P3 is lower, which means that when aerosol is added, the virus-laden aerosol particle count is somewhat lower than at P1 initial maximum saturation.
This observation shows that, when the CUBUSAN device is used, more virus-laden particles are eliminated than are constantly added over time (60 min).

The continuous supply of virus particles is a maximum load that does not occur in reality.

The excretion of pathogens from humans occurs in isolation. While an infected person eliminates virtually no virus particles at all when breathing at rest without speaking or during other additional breathing exercises, a bout of coughing, for instance, will result in the isolated release of a high pathogen count.
Therefore, an assessment of the decrease in pathogen-containing aerosol particles during constant supply (in this case, over 60 min) can be viewed as a release that does not occur in reality. It can be interpreted here as a maximum or worst-case scenario.

Following saturation or supersaturation (aerosol supply active from P1 and remaining active over time up to P3), the microbial count decreases by 1 to 1.5 log levels ($5.67*10^6$ to $1.53*10^5$ pfu/m³ at P4). Here, the aerosol feed is inactive and the CUBUSAN unit remains active.

The test report only presents the analytical results of the microbiological testing of ambient air in the results section. No assessment is carried out, which is correct in this context.

Assessment of the results – Microbiology:

These results can be assessed based on the general definition of disinfection.

Disinfection means: The transformation of an object or a medium into a condition that no longer poses a risk of infection.
Disinfection is therefore intended to reduce or eliminate the risk of infection. This is achieved by reducing the micro-organisms triggering infection on the object or in the medium (here: ambient air).

The absence of any risk of infection is referred to as asepsis. The pathogen-reducing methods leading to this status (disinfection in this context) are known as aseptic measures.

In the case of point contamination, surface contamination, or airborne contamination by a pathogen eliminator, asepsis is generally achieved as per the specifications of professional hygiene and microbiology societies when the initial microbial load is reduced by 3 to 5 powers of ten (log levels). Between 99.9 and 99.999% of the pathogens have then been eliminated and the remaining pathogens usually no longer pose a risk of infection (given the low count).
The number of log levels to be achieved in terms of microbial reduction depends on each

individual case and must be partly determined for the individual case in question. In the case of hand disinfection, a reduction of at least 3 log levels in terms of microbial count should be attempted compared to a reduction of over 5 log levels for disinfecting instruments. Since the 2000s, effectiveness has been described by an additional disinfection-specific parameter, the A_0 value, which will not be discussed in any more detail in this report.

This definition cannot be applied if a pathogen-containing aerosol is constantly tracked (P2 to P3).
Here, elimination of the infection potential is apparent when the added pathogens are also constantly destroyed – hence no further saturation occurs. P2 and P3 are < P1 (initial microbial load; Table 3 Page 23).
The bacterial count in P2 and P3 is significantly lower than in P1, which is why the CUBUSAN technique eliminates more micro-organisms than are added.

This observation also describes the elimination of infectivity, since the pathogens constantly added are also constantly destroyed.

The definition of disinfection, i.e., the elimination of infectivity, is given here specifically because more pathogens are destroyed by CUBUSAN than are added.

The difference between P3 and P4 is described approximately (depending on the recovery compensation calculation) as 1 to 1.5 log levels.
After more than 120 minutes of aerosol feed, it is acceptable to no longer refer to the homogeneity of the aerosol in the > 20 µm size range.
As described above, the aerosol particles aggregate to form droplets of increasing size, which are no longer detected by particle size distribution but which appear in the sample due to impingement.

In these aerosol particles, the effect of the active disinfectant plasma reaction product (hydroxyl radicals) is questionable.
However, from a medical perspective, such large particles in the form of droplets cannot penetrate the bronchi or lungs and are therefore of secondary relevance in terms of the aerogenic transmission of infection.

Thus, the decrease in pathogen count is lower at this point compared to testing that looks at the decrease in a one-off aerosol concentration.
Investigations into the decrease of a single, pathogen-laden aerosol dose have already been carried out several times by the author of this document (Dr. Schmelz GmbH). A decrease ranging from 3 to 4 log levels was recorded.

Therefore, the reduction of 1 to 1.5 log levels after prior implementation of P1, P2, and P3 is to be explained. It provides adequate evidence of disinfecting properties from a microbiological perspective.

When interpreting the results, it is very important not to see an increase in the microbial count on constantly adding pathogen-laden aerosol particles. In fact, the microbial count decreases compared to P1, which shows that more particles are eliminated than are added.

It can therefore be established at this point that, considering the test set-up and procedure, the CUBUSAN device disinfects ambient air under the test conditions as infectivity is eliminated and the constant feed of aerogenic micro-organisms is reliably destroyed. A significant reduction in microbial count is also evident during continued operation and supersaturation conditions.

Summary:

Summary from a medical perspective of the assessment of the disinfection efficacy of the Sterex process in the Cubusan device based on the results of the aerosol investigations conducted by the Fraunhofer Institute:
It is acknowledged that continued operation of the "Cubusan" device, as per its intended use, eliminates infectivity due to aerogenic micro-organisms through disinfection. No concentration of airborne micro-organisms is observed with the permanent feed of aerogenic micro-organisms. A decrease in microbial count by approximately 1 log level is observed. This means that the device eliminates more aerogenic micro-organisms than are continuously supplied.
It can therefore be concluded that microorganisms supplied selectively (for instance, by a pathogen infection eliminator during a bout of coughing) are eliminated immediately post-release by the plasma reaction products generated by the Sterex process.
This leads to an immediate break in the aerogenic infection chains, thus achieving the target stated initially, namely to eliminate infectivity.
Furthermore, the tests were carried out under worst-case scenario conditions. The microbial count around the room was clearly set to > 10^6 cfu/m³ and the tests were carried out in this atmosphere. In comparison, infection eliminators of aerogen-transmitted diseases release up to 10^4 cfu/m³ at certain points.
It can therefore be deduced that the anticipated efficacy in terms of breaking aerogenic infection chains will be even higher when used in a real setting.

Assessment of related issues: VOCs and ozone:

With regard to VOCs, the expert report highlights a reduction in concentration due to oxidation effects on the compounds, which mainly comprise a selection of alcohols and ketones.
The VOCs are still not reformulated.
Overall, this accounts for the deodorizing effect observed when CUBUSAN is operated indoors.

The expert report also shows that ozone formation does not occur when the CUBUSAN device is operational. This is relevant as ozone is considered "mutagenic" according to REACH legislation.

Overall assessment:

Overall, the test report compiled by the Fraunhofer Institute describes the disinfectant properties of the CUBUSAN device in terms of eliminating the infection load by applying the test procedure, which is set out in detail in the test report.

In this respect, it is important to note that the influx of micro-organisms into ambient air in the form of an aerosol is reliably eliminated by operating the CUBUSAN device.

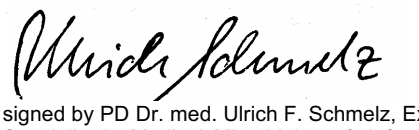
It is hereby proven that the CUBUSAN device, similar to the contamination tests conducted in rooms with a bacterial aerosol load, which take into consideration the reduction in a one-off microbial load over time, also achieves sufficient disinfection with regard to SARS-CoV-2 equivalent surrogate viruses (Phi6 bacteriophages), resulting in aseptic status.

The VOC load in ambient air is reduced whilst the CUBUSAN device is operational.
Furthermore, ozone is not formed as a by-product of the plasma reaction.

Therefore, there are no health risks when using the CUBUSAN device. Moreover, cytotoxicological tests and mutagenicity tests (Ames test) have demonstrated that the CUBUSAN device cannot trigger acute or chronic toxicity. The corresponding reports are available to Wintersteiger AG. This remark should be regarded as an additional comment in the context of this expert report.

Contact the expert on +49 (0) 5661 / 4875 or +49 (0) 175 / 9150334 if you have any further questions.

With kind regards,



signed by PD Dr. med. Ulrich F. Schmelz, Expert
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