

Opinion on Effectiveness of Antiviral Personal Protective Equipment and Materials for Air Filtering

Project Antiviral NanOmaterials for Personal SAfety

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Abbreviations :

1. Abstract

Antiviral activity could be addressed using MNs due to their large surface-to-volume ratio, surface charge, size and shape as well as their specific optical, electronic, biological and other functional properties. Furthermore, NM-based approaches are feasible, cost effective, potentially non-toxic, biocompatible and a convenient strategy to deal with various types of viral infections, particularly SARS CoV-2/COVID-19. Important types of MNs include Ag MNs, Au MNs, ZnO MNs, QDs, organic MNs, liposomes, and polymers like dendrimers revealing various types of viral activity, not only for use as an antiviral agent for PPE and FMs, but also for therapeutic use, the **specific Questions** covered by the report include:

- 1. Assessment regarding risks for workers and their employers when using such PPE including how to address them in workplace risk assessment as well as
- 2. Risk assessment at all phases of the product life cycle (manufacturing, use in industrial or clinical applications, recycling and disposal) in relation to environmental aspects
- 3. Guidelines for the use of MN activated PPEs and FM.

2. Scope and goal of the report

Assessment regarding risks for workers and their employers when using such PPE including how to address them in workplace risk assessment as well as risk assessment at all phases of the product life cycle (manufacturing, use in industrial or clinical applications, recycling and disposal) in respect to environmental risks.

Furthermore, recommendations and guidelines will be determined concerning the correct use, cleaning and reuse of filtration devices for use in workplaces, schools and public transport.

Figure 2.1 - Flow of information from all activities towards 'Risk assessment and Guidelines' which is accompanying the entire project.

3. Description of the objectives

Objective 1:

Assessment regarding risks for workers and their employers when using PPE activated with MNs offering antiviral activity, including how to address them in workplace risk assessment.

Objective 2:

Risk assessment at all phases of the product life cycle (manufacturing, use in industrial or clinical applications, recycling and disposal) in particular with increasing the use of MNs in PPE.

Objective 3:

Recommendations for guidelines concerning the correct use, cleaning and reuse of filtration devices (FM) and personnel protective equipment (PPE) for use in workplaces, schools and public transport

4. Methods:

4.1 Selection, design and manufacture of MNs with antiviral properties:

ZnO MNs have been selected for the study along with Ag and other materials. We concluded that ZnO MNs are the most widely used MNs for the antiviral activation of PPE and FM. In their powder form, they are ideal for both further immuno-assays as well as for the immobilization process on PPE and FM samples. Fig. 4.2.1 shows ZnO MNs providing antiviral activity due to their material composition and their size distribution. Their size distribution has a significant impact on their antiviral activity.

Fig. 4.2.1: Transmission microscopy image of ZnO MNs with antiviral activity.

4.2 Application of selected MNs for PPE and FMs

Plasma activated textile and/or nonwoven have been utilized to immobilize nanomaterial onto the surface.

The main activities concentrated on:

- Formulation of the NMs for coating
- Antiviral nonwoven for FMs
- Antiviral textile PPE

NMs immobilization onto facemasks were found significantly affects the air permeability, salt and oil efficiency and assumed the risk of inhalation. Therefore, the research focused on optimizing the NMs formulation and coating for air filter and textile PPE. Figure 4.2.1 shows the preparation of nonwoven materials for FMs

Figure 4.2.1: Nonwoven materials activated with antiviral MNs before and after their mounting in filter frames.

4.3 Characterization of MNs, PPEs and FMs

Optical characterization:

IR-spectroscopy

- Concentration series of ZnO/Ag-doped ZnO
- IR-spectra of filter-material to check spectral compatibility
- ATR-IR spectroscopy of textile and filter samples with ZnO/Ag-doped ZnO
- DMAS/SMPS aerosol tests

Fluorescence scanning

- Design of sample holder for filter material
- Measurements of virus-like particles (VLPs)
- Scans of filter and textile samples with ZnO/Ag-doped ZnO at two different wavelengths

Figure 4.3.1 shows textile samples (preferable for PPE) as well as nonwoven materials (preferable for FM) under UV illumination. ZnO exhibits luminescence under UV illumination, and therefore a specific scanner system for the investigated samples has been developed. The UV scans were performed with a flexible, home-built setup which is specifically adjusted for the detection of the sample autofluorescence at an excitation wavelength of 365 nm excitation.

Figure 4.3.1: Fluorescence scans of the provided ZnO and ZnO/Ag samples on textile and filter materials obtained with the dedicated scanner developed at BAM, measured at 365 nm excitation. For the detection of the emission, a bandpass filter was used (detection window from 525-560 nm). Samples 2-5 were plasma-pretreated before particle deposition, while samples 7-10 were not pretreated.

The suitability of fluorescence measurements performed with a custom designed laser scanner has proven very valuable. It can be employed to evaluate and screen the homogeneity of the particle coating procedures which were used for the filter and textile samples to coat them with ZnO and ZnO/Ag particles using different coating and treatment procedures. This underlines the applicability of the scanner for the quality control of coating processes on different substrates utilizing fluorescent functional materials such as antibacterial and most likely also antiviral, luminescent ZnO and ZnO/Ag particles. It was, however, not possible to quantify the number of particles deposited on the different substrates based upon measurements of the fluorescence intensity.

4.4 Antiviral tests of MNs, PPEs and FMs

The tests were performed with the following steps / means:

Cell culture:

Calu-3 cells have been used for all in vitro experiments

Dispersion of MNs:

The MNs have been suspended in MEM medium without FCS

Preparation of SARS-CoV2- virus stock:

All work with infectious SARS-CoV-2 was performed under biosafety level (BSL)-3 conditions by using two SARS-CoV-2 variants, the Delta strain(isolated at Institute of Pathology, Graz, Austria, GISAID accession number EPI ISL 4847176 delta like variant) and Omicron strain (hCoV-19/Netherlands/NH-EMC-1720/2021, Omicron variant).

SARS-CoV-2 neutralization assays:

To determine the antiviral activity of ZnO-NPs and the ZnO-coating against SARS-CoV-2, cells were seeded into 48-well cell culture plates 48 hr prior to infection. The following ZnO-NP concentrations were chosen: 2 %, 1 % and 0.5 %. SARS-CoV-2 virus was added to the prepared NP suspension and incubated for 1 hour at 37 °C under continuous shaking. Afterwards the samples were centrifuged and supernatants were collected to determine the virus input (VI) for cell infection. Cells were infected in replicates with the supernatant of each NP concentration for 1 hour.. Virus without NP-treatment was used as positive infection control, non-infected cells served as negative control for the assay. After infection, the cells were washed with MEM (without FCS) and fresh MEM (10% FCS) was added to the cells. After 48 hours of incubation at 37 °C and 5 % $CO₂$ the supernatant of each well was collected to determine virus concentrations (t=48) via RNA isolation and RT-qPCR.

Figure 4.4.1.: SARS-CoV-2 virus adsorption and neutralization by NPs. A fixed volume of SARS-CoV-2 and NP suspension was incubated with decreasing concentrations of NPs. The unbound virus particles in the supernatant were quantified by RT-qPCR either after incubation with NP (left bar of the named NP) or after infection and cultivation on Calu3 cells (right bar). In some cases the concentration of the NP was too high and there was no intact cell monolayer left to measure t=48.

Over all experiments, it was possible to find a concentration which affects SARS-CoV-2 infection in-vitro. The area where the NP shows an antiviral activity but no harm to the cell monolayer is narrow. Similar results were measured with the coated materials. Specimens made out of woven could also absorb more virus suspension than plastic

based ones. With all types of coated specimen, NP could be washed out of the material during the preparation phase of the neutralization assay.

More details can be found in the publication: <https://pubmed.ncbi.nlm.nih.gov/37176131/>

Wolfgruber S, Rieger J, Cardozo O, Punz B, Himly M, Stingl A, Farias PMA, Abuja PM, Zatloukal K. *Antiviral Activity of Zinc Oxide Nanoparticles against SARS-CoV-2.* Int J Mol Sci. 2023 May 8;24(9):8425. doi: 10.3390/ijms24098425. PMID: 37176131; PMCID: PMC10179150.

4.5 Virus - like particles (VLPs)

VLPs have been designed with the aim to provide specimens for test purposes that do not impose any risks and do not require BSL 3 laboratory infrastructure. The synthesis is schematically represented in Figure 4.5.1, based on polystyrene particles (PSPs) functionalized with COOH groups. 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), dioleoylphosphatidylethanolamine (DOPE) and cholesterol in ethanol were added to the dye-stained polymer beads.

Dynamic light scattering (DLS), and zeta potential measurements were performed for the initial particles, after the dye incorporation, and after the lipid coating step.

Figure 4.5.1: Schematic representation of the synthesis of VLPs from 50/100 nm PSPs, with a) encapsulation of NR 1 , and b) coating with a lipid layer consisting of DOPE, DOTAP and cholesterol.

¹ Nirmalananthan-Budau, Nithiya. *Synthesis, Characterization and Surface Group Quantification of Functionalized Polymer Particles for Signal Amplification Strategies*. Diss. 2019. doi: 10.17169/refubium-26041. ANOPSA Grant Agreement# 888037 9/16

Overall, the synthesis and characterization of the VLPs proved to be very challenging. One problem is the very low concentration (about 1 mL with 1 mg/mL maximum, with several simultaneously prepared batches being combined) of particles yielded by the synthesis, and the tendency of the particles to aggregate. The results also suggest an incomplete formation of the lipid coating.

5. Takeaway messages:

5.1. Objective 1: Risk assessment with regard to the Users

Report on risk assessment in respect to users:

a. Changes in the Risk for the user due to the antiviral activity of selected MNs:

The findings of this study clearly demonstrate a high antiviral activity of ZnO MNs against the two SARS-CoV-2 variants Delta and Omicron, which is based on adsorption and an additional not yet defined antiviral effect².

Conclusion for ZnO - based PPE and FM

Textile and nonwoven both can be coated using pad-dry-cure method at 7 kP/cm² padding pressure, 20 meter/min and 3 min drying, resulting in a very resistant coating, avoiding leaving of MNs during use.

Smaller ZnO MNs are more active than larger ZnO MNs. They could be a promising material for future usage as a surface coating for antiviral PPE, especially coverall suits, face masks, and antiviral filters that could be used for air-conditioning and room ventilation system

Both salt and Oil efficiency performance may be affected by the ZnO coating.

Commonly used face masks against COVID‐*19 pandemic:*

Without having antibacterial/antiviral activities, the current masks act only as filtering materials of the aerosols containing microorganisms. Meanwhile, in surgical masks, the viral and bacterial filtration highly depends on the electrostatic charges of masks. These electrostatic charges disappear after 8 hours, which leads to a significant decline in filtration efficiency.

Functionalizing agents which can endow four important functions in the masks including boosting the antimicrobial and self‐disinfectant characteristics via incorporating metal MNs or photosensitizers.

The filtration effectiveness of 0.3 um particles by FFP1, FFP2, and FFP3 are around 80%, 94%, and 99%, respectively. Practically, the filtration efficiency of FFP2 and FFP3 is almost equal to N95 and N99, respectively (Figure 2:).

² Wolfgruber S, Rieger J, Cardozo O, Punz B, Himly M, Stingl A, Farias PMA, Abuja PM, Zatloukal K. Antiviral Activity of Zinc Oxide Nanoparticles against SARS-CoV-2. Int J Mol Sci. 2023 May 8;24(9):8425. doi: 10.3390/ijms24098425. PMID: 37176131; PMCID: PMC10179150.

Figure 5.2: Commonly used face masks against COVID‐*19 pandemic 3*

Antiviral face masks based on MNs have drastically diminished close to zero on the EU-market within the course of the pandemic. The majority of providers of antiviral face-masks can be divided into the following categories:

a) A generally serious provider offers the product outside of the EU. Due to information of a manufacturer, the certification process required for the EU, which is considered lengthy, and expensive, in relation to the potential market, motivates this. Once the pandemic turns endemic and emergency rules are no longer valid, manufacturers are pulling out of the EU-market.

b) A provider offers antiviral face masks - presumably CE-certified - with a very dubious description. This may be based on insufficient knowledge or on intentional misleading.

b. Changes in the Risk for the user due to the adverse effects of selected MNs:

Size-related properties of nanomaterials are linked with toxicity and exposure, focusing chemical modification on surface by doping 4 , coating, or functionalization

³ Seidi F, Deng C, Zhong Y, Liu Y, Huang Y, Li C, Xiao H. Functionalized Masks: Powerful Materials against COVID-19 and Future Pandemics. Small. 2021 Oct;17(42):e2102453. doi: 10.1002/smll.202102453. Epub 2021 Jul 28. PMID: 34319644; PMCID: PMC8420174.

⁴ Naatz, H., Lin, S., Li, R., Jiang, W., Ji, Z., Chang, C. H., Pokhrel, S. Safe-by-Design CuO Nanoparticles via Fe Doping, Cu–O Bond Length Variation, and Biological Assessment in Cells and Zebrafish Embryos. *ACS Nano, 2017, 11(1), 501–515*. doi:10.1021/acsnano.6b06495

Example for a **dubious Product description, containing a number of severe errors and contradiction:**

> **Citation from a manufacturer: 'Face mask FFP2 with silver ANTI-COVID19:** FFP2 respirators are made of nanofibers, which are enriched with **silver molecules**. This ensures that the **bacteria dies in a very short time**. The nanofiber filter ensures high **virus detection - more than 94%'**.

Conclusion for Ag-MN based PE:

Thirteen of 20 selected masks contained detectable amounts of silver ranging from 3 μg to 235 mg. Four of these masks contained Ag MNs, and in one of these masks, the MNs were present in a coating⁵.

> Application of this information in the approach, which compares the Ag content in the mask with limit values derived from existing inhalation exposure limits for a specific silver biocide making a minimum of assumptions, allows identifying face masks containing Ag-based biocide that can be considered intrinsically safe (safe-by-design),

⁵ Mast J, Van Miert E, Siciliani L, Cheyns K, Blaude MN, Wouters C, Waegeneers N, Bernsen R, Vleminckx C, Van Loco J, Verleysen E. Application of silver-based biocides in face masks intended for general use requires regulatory control. Sci Total Environ. 2023 Apr 20;870:161889. doi: 10.1016/j.scitotenv.2023.161889. Epub 2023 Jan 31. PMID: 36731552; PMCID: PMC9886386[.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9886386/#bb0025](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9886386/#bb0025)

and also face masks that require a more extensive safety assessment.

Furthermore, these results raise questions about the safety of some face masks and their intended use. In line with ANSES's recommendations⁶ to limit the use of Ag MNs (production, processing, use) to applications whose usefulness has been clearly demonstrated, more data and methodologies should become available to assess the balance of benefits of Ag-based biocide on face masks for human health in relation to their health and environmental risks.

5.2. Objective 2: Risk assessment with regard to the Environment

Report on risk assessment in respect to environment

a) **Risk due to the MNs in use for antiviral activity of the PPE and FM**

Micro- and Nanoplastics:

Besides the MN added to provide antiviral activity, the multiple **plastic fibers** content per se, mainly polypropylene, in the face mask will persist in the environment for decades and possibly centuries while disintegrating into smaller micro- and nanoplastics⁷.

Ecotoxicity of ZnO MNs is specifically eminent in the marine environment 8 :

Several potential routes need to be investigated, evaluated and considered. Examples for understanding on the ecotoxicity of ZnO-MNs for environmental risk assessment and management for this group of highly popular, commercialized nanoparticles are the consideration of the following effects e.g.:

1. Molecular toxic mechanisms to marine organisms to differentiate different modes of toxic action between the nanoparticles of ZnO-MNs and their associated dissolved zinc ions and between waterborne and dietary exposure.

2. Given that ZnO-MNs are highly photoactive, it is important to consider the photoinduced toxicity under environmentally relevant UV radiation to an array of different marine species from different taxonomic groups.

3. Chronic effects of ZnO-MNs to marine organisms, and thus more chronic toxicity studies such as life-cycle studies to improve our understanding on the long-term and low-dose effect of these MNs on selected marine species. ZnO-MNs have a high potential to aggregate and settle on the sediment in marine environments and bottom filter feeders and deposit feeders have a great potential to consume the nanoparticles associated with organic matter.

4. Toxicity data from marine fish species so as to reveal the sublethal toxic effects and associated toxic mechanisms of ZnO-MNs in this important group of higher-level organisms in the marine ecosystem.

⁶ ANSES . ANSES; Maisons-Alfort, France: 2020. ANSES Opinion on the Estimation of Potential Risks Associated With Wearing Masks Treated With Silver Zeolite and Silver-Copper Zeolite (no. 2020- SA- 0134) [Google [Scholar\]](https://scholar.google.com/scholar?q=ANSES+ANSES+Opinion+on+the+Estimation+of+Potential+Risks+Associated+With+Wearing+Masks+Treated+With+Silver+Zeolite+and+Silver-Copper+Zeolite+(no.+2020-+SA-+0134)+2020+ANSES+Maisons-Alfort,+France+)

⁷ Hui Li AS, Sathishkumar P, Selahuddeen ML, Asyraf Wan Mahmood WM, Zainal Abidin MH, Wahab RA, Mohamed Huri MA, Abdullah F. Adverse environmental effects of disposable face masks due to the excess usage. Environ Pollut. 2022 Sep 1;308:119674. doi: 10.1016/j.envpol.2022.119674. Epub 2022 Jun 27. PMID: 35772616; PMCID: PMC9233961.

⁸ Yung, M.M.N., Mouneyrac, C., Leung, K.M.Y. (2014). Ecotoxicity of Zinc Oxide Nanoparticles in the Marine Environment. In: Bhushan, B. (eds) Encyclopedia of Nanotechnology. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6178-0_100970-1

5. Bioaccumulation, cellular localization or tissue distribution, biotransformation, and trophic transfer of the nanoparticles in selected, typical marine food chains.

6. Combined effects of environmental stressors such as temperature, salinity, pH, UV radiation, and presence of DOM and combined toxic effects of ZnO-MNs and other pollutants to marine organisms should be investigated to better understand the behavior and toxicity of ZnO-MNs under environmen-tally realistic scenarios.

b) **Risk reduction due to longer use time, compared to single use PPE & traditional FM**

Figure 5.3 'The Guardian': More masks than jellyfish': coronavirus waste ends up in ocean

Improvements to increase the use time of PPEs and FMs without increasing the risk for the user, potentially has a very positive impact on the environmental effect in particular in comparison to disposable face masks, which constitute a significant risk for the environment.

Among the most often used tools and approaches are Risk Assessment, *Life Cycle Assessment* (LCA) and Socio-Economic Analysis. In the area of nanotechnology, such analysis tools are increasingly applied despite current limitations due to the (still) rather limited knowledge in terms of toxicity, environmental fate and distribution, and environmental and human exposure of nanoparticles and their release along the life cycle⁹.

The conventional masks only act as filtrating materials or barriers without deactivating viruses and bacteria. Consequently, the masks might become the sources for spreading viruses or microorganisms after disposal. Therefore, safe discarding methods are required to eliminate the spreading of this contaminated source. The most reliable method for discarding these masks is incineration. However, due to the production of over 250 000 tons of mask waste per day, incineration generates a massive amount of toxic gases and $CO₂$, which raises pressing environmental concerns including the increase of greenhouse gases in the atmosphere. Accordingly, fabrication and development of functionalized masks with reusability, self-sterilization, self-cleaning, antiviral, and antibacterial properties is in high demand to not only provide better protection against the pandemic diseases but also benefit the environment by reducing the excessive consumption of mask 10 .

⁹ Beatrice Salieri, Leire Barruetabeña, Isabel Rodríguez-Llopis, Nicklas Raun Jacobsen, Nicolas Manier, Bénédicte Trouiller, Valentin Chapon, Niels Hadrup, Araceli Sánchez Jiménez, Christian Micheletti, Blanca Suarez Merino, Jean-Marc Brignon, Jacques Bouillard, Roland Hischier, Integrative approach in a safe by design context combining risk, life cycle and socio-economic assessment for safer and sustainable nanomaterials, *NanoImpact, Vol. 23, 2021, 100335*. doi.org/10.1016/j.impact.2021.100335.

¹⁰ Seidi F, Deng C, Zhong Y, Liu Y, Huang Y, Li C, Xiao H. Functionalized Masks: Powerful Materials against COVID-19 and Future Pandemics. Small. 2021 Oct;17(42):e2102453. doi: 10.1002/smll.202102453. Epub 2021 Jul 28. PMID: 34319644; PMCID: PMC8420174.

6. Conclusion

Objective 3: Recommendations for guidelines concerning the correct use, cleaning and reuse of filtration devices (FM) (and personnel protective equipment - PPE) for use in workplaces, schools and public transport

Although this deliverable focuses primarily on filtration devices and filter materials (FM), parallels regarding PPEs are highlighted as well.

6.1. Security aspect of FMs and PPEs with MNs with antiviral activity

Improving the efficacy of PPE and FM and fabrication of synergistic antiviral coatings and surfaces is of critical importance in reducing the spreading of this virus.

The MN coating of textile and nonwoven obtained by **pad-dry-cure method** can provide an **extended utilization period** due to its self-decontaminating surface for air filtration application in environments like workplace, schools and public transports.

Spray coating might lead to leaching of the MNs from the nonwoven surface and is therefore not recommended for being used as a filter for mask application.

6.2. The environmental impact:

The largest impact to the environment stems from disposable face masks, regardless of the presence of MNs.

The safe **extension of the use time** of PPE and FM by the application of MNs has a **positive impact on the environment.** Doubling the use time of such disposable PPE or FM cuts such environmental impact in half.

Conventional disposable PPEs without the use of MNs will eventually degrade to **micro- and nanoplastics** without proper recycling and thus impose an environmental risk.

Considerations re. the washing cycle In case of face masks (not recommended in case of filtration materials):

Disposable masks and washable masks generally constitute different structures and materials. Washable masks generally do not show classifications like FFP2, FFP3, etc. With more and more washing-cycles, it is likely that their antiviral activity diminishes. At the same time, every extension of their life time simultaneously reduces the volume of litter.