# TOXIC EFFECT OF NANOTEXTILES AS WASTE OF RESPIRATORS AND FACE MASKS

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**Abstract**

The article deals with the toxic effect of nanotextiles as waste from respirators and face masks. The article is focused on ecotoxicology and basic ecotoxicological tests according to available European standards and Czech legislation. Article describes the production and other characteristics of the nonwovens that were tested. Part of article concludes with a review of foreign studies that have also dealt with the testing of microplastics and nanoplastics on various organisms. The experimental results presents specific experimental characteristics of the textiles and then presents individual tests. Tests on seeds of *Sinapis alba* L. plants growing and *Lemna minor* L. were performed. In all cases, the tested non-woven fabrics had a non-toxic effect on the organisms, but it cannot be confirmed that there is no reaction. Further tests will be carried out to clarify the effects of the nonwovens on the test organisms.

**Keywords:** Nanotextiles, non-woven fabrics, respirators, ecotoxicity

## 1. INTRODUCTION

Nonwovens are formations of textile fibres, filaments or yarns which are interconnected by techniques other than weaving, knitting, bobbin-making, plaiting and tufting. However, the production and handling of nanofibres cannot completely eliminate their release into the environment or the working environment. This can occur in four ways, namely during the manufacturing process of the nanofibres that make up the textiles, during the processing of the materials containing the nanofibres (which also results in release to the working environment and exposure of workers), during the use of the nanofibres (as the fibres are mechanically stressed and thus further released to the environment) or during the disposal of the textiles already used, when the nonwovens are discarded freely into the environment and its components, water and soil, where the undesirable effect of adsorbed pollutants trapped during use or absorbed into the layers of the nonwovens may be manifested, which may lead to a change in toxicological properties [1]. Three types of materials were selected to be tested in the tests carried out. These are personal protective equipment used for respiratory protection, which were widely used during the spread of the Covid-19 viral disease. Specifically, they are unlaminated PP-PVDF (polyvinylidene fluoride laminated on one side with a polypropylene layer), laminated PP-PVDF-PP, which is similar to the first material mentioned, but this time laminated on both sides, and the last material is a specific type of Nanovia mask (all of them are shown in **Figure 1**), respectivelly. The ecotoxicity of these three materials was tested on two different organisms - mustard seeds in a range of concentrations of aqueous leachates, following the OEDC methodology, and perch leaves, also in a range of concentrations of aqueous leachates, following the standard EN ISO 20079 (2001) Water quality - Determination of toxic effects of water and wastewater constituents on perch (*Lemna minor* L.) - Perch growth inhibition test. [2].

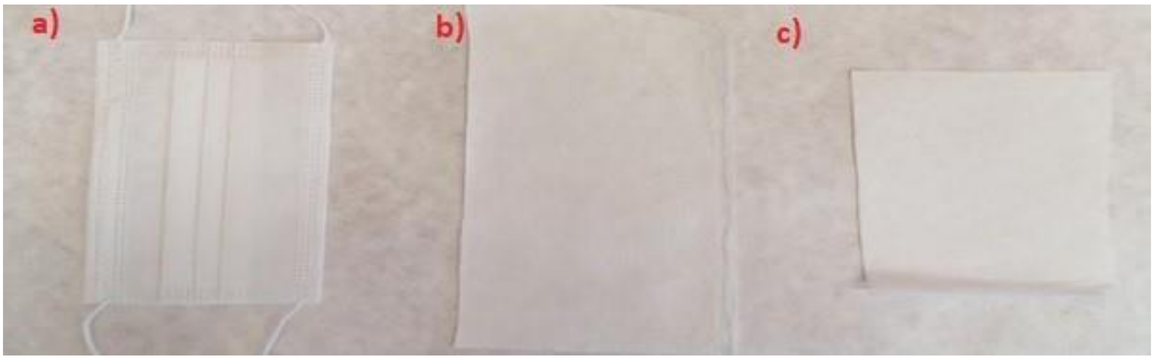


**Figure 1** Photographs of materials before preparation of aqueous leachates (from the left Nanovia facemask, unlaminated PP-PVDF, laminated PP-PVDF-PP)

## 2. EXPERIMENTAL PART AND RESULTS

The basis of the experimental part is the production of polymer nanofibres - these can be prepared in several different ways, some of which can only be discussed in the laboratory for the time being. Others, however, have already been improved to such a level that they can be used in large-scale production - for example, electrospinning or nanospider$^{TM}$. Other methods include centrifugal force spinning (Force Spinning) or the Melt - Blown method, which is based on the melt blowing principle. The requirements for product properties and production speed are then a decisive factor in the production of the technology. However, the most widely-used production method is undoubtedly electrospinning, the essence of which is the use of the effect of an electrostatic field on a charged viscoelastic liquid (usually a polymer solution), whereby thin filaments of different lengths are formed when given conditions are met [3-6].

### 2.1. Specification of nonwovens and aqueous leachates

Aqueous leachates were prepared using 100 g of material (nanotextile) and 1000 mL of distilled water. The prepared leachates were shaken on a shaker for 24 hours, then filtered through a 0.40 µm pore membrane. The stock solution of the test substance and dilution solution were also diluted according to ISO 7346 [7,8]. The first two materials used were laminated PP-PVDF and unlaminated PP-PVDF, being multilayered polypropylene fabrics produced either by the spunbond (S) method, where a small volume of ambient temperature air is used, thus achieving coarser textiles with larger diameters and higher tensile and compressive strengths, or by the meltblown (M) method, where high velocity (100-500 m/s) hot air is used to produce smaller fibres with more diameter options. The fibres produced in this way are not very strong, but have excellent filtration properties, which is why both methods are most often combined: a multi-layer structure is used (SM for PP-PVDF, SMS for PP-PVDF-PP). Another option is to combine both methods with nanotextiles to achieve better filtration properties [1,9]. The production itself is then carried out as follows - PVDF nanofibers, which were obtained from a dimethylacetamide solution, are subsequently deposited on the multilayered structure using the electrospinning method. If the aim is to obtain a laminated PP-PVDF-PP material, another non-woven PP fabric (S) is laminated onto the multi-layer structure to form a sandwich trilayer [1,9]. The fabrication of Nanovia Mask 99.97 nanofibres is similar to the two materials mentioned above, i.e. by joining polypropylene fabrics produced by spunbond technology, on top of which lies another layer of fabric produced by the meltblown method, and finally a PVDF nanofibrous structure. Another polypropylene spunbond layer is bonded to the bottom of the PVDF layer using an ethylene vinyl acetate adhesive [9].

### 2.2. Ecotoxicological test procedure

The first test was to test *Sinapis alba* L. seeds using a concentration range of aqueous leachates of all selected materials to observe the effect of increasing concentrations on the resulting root length compared to the control. The procedure is based on the methodology of the Ministry of Environment for the determination of ecotoxicity of wastes. The basis of this test is to pipette a specified amount of solution (2.5 mL) at different concentrations (10, 20, 50, 100, 200, 500 and 1000 mL/L) and to place 20 seeds (4 rows of 5 seeds = 20 seeds) on a soaked filter paper. The procedure described above, including the formula for calculating root growth inhibition, is then followed. Potassium dichromate at concentrations of 1.25; 2.5; 5; 10; 20; 40; 80; 160 and 320 mL/L was used as a reference substance, which gave results corresponding to the standard results - with increasing concentration, the root length of *Sinapis alba* L. decreased [2]. First, Petri dishes and filter papers are prepared, then the solution is pipetted, always 2.5 mL, (the 1st concentration is always for the blank solution) and a number of seeds (20 in this case) are sown. Finally, the petri dishes are placed in an unlit place where the temperature is constant at (20±1) °C. After 72 hours, a root growth reading is taken and the values obtained are entered into the formula. The percentage of root inhibition can then be calculated. The second test carried out was a semichronic toxicity test on lesser perch, a monocotyledonous plant that often covers the surface of standing water [10,12]. The test procedure is based on the standard EN ISO 20079 (2001) Water quality - Determination of toxic effects of water and wastewater constituents on perch (*Lemna minor* L.) - Perch growth inhibition test and lasts for 7 days, during which the perch grows in a nutrient solution to which different concentrations of the substances whose ecotoxicity is to be determined are added. The result of this semi-chronic toxicity test is the effect of the substance on the growth rate, which varies based on how the number of leaves changes with different concentrations of the test substance. 8 stock solutions of precisely defined concentrations are to be prepared to serve as stock solutions and these 8 solutions are to be mixed in a 2-litre flask. The solution and its pH must be stabilised before the test begins. The procedure for the preparation of the aqueous leachates is given in Section 2.1. The concentrations of the filtrates of each material were then adjusted so that the resulting concentrations of the aqueous leachates were 200 ; 500 and 1000 mL/L (100 g/L), all concentrations being prepared in parallel determinations A and B. However, prior to the actual deployment of the perch, the pH of all filtrates had to be adjusted so that the pH was between 5 and 6. Once the filtrates were adjusted and prepared, four two-leaf and four three-leaf perch colonies were carefully transferred into these beakers. In total, there were therefore 20 leaves. Tweezers, a spoon and possibly a glass rod were used for the transfer. Once the perch had been moved into all the beakers, the entire tray was covered with clear food-grade foil and placed in a culture chamber for 168 hours. During this time, the humidity, temperature, and amount of light were set constant [11].

Total growth inhibition for *Lemna minor* L. was calculated according to the following formulas:

**a) growth rate $\mu$**

The growth rate indicates the final number of petals per unit time the test was run, calculated for each single concentration and also for blanks according to the following formula, where $N_0$ is number of petals at the beginning of the test, $N_k$ is number of petals at the end of the test and $t_k$ is duration of the test [11]:

$$\mu = \frac{\ln N_k - \ln N_0}{t_k} \tag{1}$$

**b) inhibition (stimulation) of $I_{\mu i}$ growth**

The inhibition or stimulation of growth indicates the relative comparison in percent of the growth rate of the blank (number of leaves, per unit time the test was run) to the growth rate of each concentration tested. It is calculated according to the following formula, where $\mu_c$ is growth rate of the blank (number of leaves per unit time of test) and $\mu_i$ is growth rate of each concentration (number of leaves per unit time of test) [11]:

$$I_{\mu i} = \frac{\mu_c - \mu_i}{\mu_c} \times 100 \tag{2}$$

### 2.3. Evaluation and discussion of results

A semichronic toxicity test was conducted on *Sinapis alba* L. seeds using a concentration series of aqueous leachates of Nanovia, PP-PVDF-PP and PP-PVDF materials. The calculation of root growth inhibition is in the measurement of root length (root elongation) after the test according to the calculation relationship:

$$IC = \frac{L_c - L_v}{L_c} \times 100 \qquad (3)$$

where *IC* is the root growth inhibition concentration in %, (in case of negative inhibition it is stimulation), $L_c$ is the arithmetic mean of the root length in control in mm, $L_v$ is the arithmetic mean of the root length in the test solution in mm [2,7,8].

**Table 1** Resulting inhibition of mustard seeds for a concentration range of aqueous leachates of all materials

| Nanovia | | PP-PVDF-PP | | PP-PVDF | |
|---|---|---|---|---|---|
| **Leachates concentration [mL/L]** | **IC [%]** | **Leachates concentration [mL/L]** | **IC [%]** | **Leachates concentration [mL/L]** | **IC [%]** |
| 10 | 8.48 | 10 | 22.51 | 10 | 6.92 |
| 20 | -4.56 | 20 | 8.19 | 20 | 1.91 |
| 50 | -9.96 | 50 | 13.78 | 50 | 2.89 |
| 100 | -1.62 | 100 | 3.19 | 100 | -16.72 |
| 200 | -5.74 | 200 | 20.26 | 200 | 7.41 |
| 500 | 5.74 | 500 | 23.31 | 500 | 0.15 |
| 1000 | 25.55 | 1000 | -2.01 | 1000 | -3.09 |

All concentration series results shown in the tables are averages of several parallel determinations. The obtained inhibition results can be easily summarised as they are almost completely different from each other. In all cases there were very low inhibitions or very low stimulations, with none of the inhibitions reaching even 30 % (the highest was 25.55 %). Thus, no $EC_{50}$ ($IC_{50}$) value can be determined for any of the materials, and it can be reasonably stated that these materials do not appear to be ecotoxic in this type of test, and do not meet the HP 14 property.The results of the semichronic toxicity tests on lesser spotted bass are shown in **Table 2**.

**Table 2** Inhibition of periwinkle growth after 168 hours for all materials in parallel determination

| **Material** | **Leachates concentration [mL/L]** | **Number of leaves for parallel determination (A/B)** | **Average number of leaves** | **Inhibition [%]** |
|---|---|---|---|---|
| | 0 | 28/34/44 | 35.3 | 0.0 |
| **Nanovia** | 200 | 30/32 | 31.0 | 23.0 |
| | 500 | 31/34 | 32.5 | 14.6 |
| | 1000 | 32/36 | 34.0 | 6.8 |
| **PP-PVDF-PP** | 200 | 38/32 | 35.0 | 1.7 |
| | 500 | 41/40 | 40.5 | -24.0 |
| | 1000 | 40/39 | 39.5 | -19.6 |
| **PP-PVDF** | 0 | 51/51/55/51 | 52.0 | 0 |
| | 200 | 74/58 | 66.0 | -24.8 |
| | 500 | 70/56 | 63.0 | -19.7 |
| | 1000 | 55/54 | 54.5 | -4.4 |

Throughout the experiment, necrosis often appeared on the leaves, and chlorosis was rarely seen. For the first two materials, the growth rate was equal to 0.0813 petals per day, and for the PP-PVDF material was equal to 0.1365 petals per day. It was not possible to trace a trend among the results to confirm that inhibition also increased with the increasing leachate concentration, and in neither case could $IC_{80}$ or $IC_{50}$ be determined. It was only possible to determine $IC_{20}$ for the Nanovia drape sample, where inhibition was exactly 23.0 % for a leachate concentration of 200 mL/L. Negative numbers in the inhibition column then indicate that more than half of the samples had leaf growth stimulation, indicating that the concentrated leachate from the materials used created a more acceptable growth environment for the periwinkle than the standardised nutrient solution alone. The highest stimulation occurred in the unlaminated PP-PVDF sample at a concentration of 200 mL/L, where the value reached -24.8 %, while another high stimulation was observed in the laminated PP-PVDF-PP sample, where the stimulation was -24.0 %.Thus, for the selected concentration range of the selected materials Nanovia, PP-PVDF-PP and PP-PVDF, it can be stated that the leachates were not toxic to lesser celandine leaves, thus not possessing the hazardous property HP 14. However, although the results did not exceed the limit values required to label the tested materials as ecotoxic, organism reactions to the toxic substance did occur, as the leaves were abundantly covered with necrosis and chlorosis. Similar results were obtained in foreign studies [13,14], where, for example, the effects of polyethylene on leaf growth, chlorophyll content, number and length of roots and root cell condition were investigated, with no significant negative or positive effects on any of the above parameters. The second study also tested microplastics and their effect on perch; this time 6 different sources of microplastics were chosen. Attention was focused on the growth rate, root length and chlorophyll a content, which was measured at wavelengths of 662.4 and 648.6 nm. The experimental results showed that growth rate was not negatively affected by microplastics. The same was true for chlorophyll a content. As in the first study, the only parameter affected was root length, which was reduced by 10-40 %. However, this inhibition is explained by the mechanical action of microplastic particles on the test organisms, namely their rough surface, sharp edges and particle size. [13,14]

## 3. CONCLUSION

Based on the ecotoxicity tests performed on mustard seeds and the aquatic perch plant, it was shown that the test substances (Nanovia drape, laminated PP-PVDF and unlaminated PP-PVDF) do not meet the limit values for being toxic to the selected organisms. However, in no case can the effects of the test materials on the selected organisms be completely excluded. Testing a concentration range of aqueous leachate materials on seeds gave good results, with none of the materials tested acting as an overly large inhibitor or stimulant on mustard seeds. The maximum inhibition achieved was around 25.0 %, while the highest stimulation was 15.0 %. This was the first test on the basis of the results of which it can be stated that aqueous leachates from the materials tested are not ecotoxic, whether the leachate is dilute or maximally concentrated. The semichronic toxicity test on lesser knapweed was aimed at monitoring inhibition (or stimulation) of leaf growth per unit. The results were as follows - the inhibition of leaf growth of the Eurasian watermilfoil occurred essentially only in the Nanovia sample, with the highest inhibition (23 %) occurring when using a 200 mL/L aqueous leachate concentration. From this value, $IC_{20}$ = 276.5 mL/L was subsequently calculated. Which means that at this concentration just 20 % of the test specimens will be inhibited ($IC_{50}$ and $IC_{80}$ could not be determined because such inhibition values were not achieved). The other inhibitions were then only lower (14.6 and 6.8 % for Nanovia, 1.7 % for PP-PVDF-PP). Across the other samples, there was always stimulation, with the highest stimulation achieved in the PP-PVDF sample, when it was close to -25.0 %.The summary of all the experiments performed can be made relatively simply, since in none of the cases were the threshold values reached to make it possible to label the tested materials Nanovia, laminated PP-PVDF-PP and unlaminated PP-PVDF as ecotoxic. At the same time, however, the results show that interactions between organisms and materials are there - both positive and negative. Further tests will be carried out in the future in order to specify and explain this interaction; at present, even the literature outputs do not agree on explanations in all cases.

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