HEALTH RISKS ASSOCIATED WITH THE MANUFACTURE AND PROCESSING OF NANOFIBRE MATERIALS IN INDUSTRIAL ACTIVITIES

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INTRODUCTION

The rapid proliferation of many different engineered nanomaterials presents a dilemma to regulators regarding hazard identification.

This work is dedicated to nanofibre fly-off testing during their manufacture using Nanospider™ technology, and the test results of biocompatibility of nanofibres in an organism, taking into consideration potential health risks associated with their production and subsequent processing.

The respiratory system, skin and mucous membrane [1] were identified as one possible method of nanofibre entry into an organism.

This study is devoted to, in our opinion, the most probable risk, the risk associated with potential inhalation of polymer and anorganic nanofibres.

Negative health effects of respirable fibres are caused mainly by physical factors, primarily by the fact that fibres penetrate deeper into the respiratory system than would be expected given their geometric dimensions. This is mainly caused by the fibres often directing themselves in the direction of air flow lines and their resting place is therefore affected more by their radius rather than length. The fibres can become wedged in the thin capillaries of the lower respiratory tract and cause long term respiratory system irritation (i.e. asbestosis, fibrosis).

Nanofibre fly-off was tested during operation of different types of Nanospider™ technologies and polymers PA6 and PEA. Measuring method used – definition of concentration variability of aerosol particles in production by local concentration mapping using a mobile condensation particle meter P-trak. Values reached were compared to concentrations in the outside air and concentrations reached in a resting state. Leakage measuring and results evaluation was carried out by personnel of the Institute of Chemical Processes ČR v.v.i..

The biocompatibility of polymer and anorganic nanofibres was tested using the method of inserting a sterile implant of the tested nanofibre material into the subcutaneous tissue of a laboratory rat. Within the testing of biocompatibility nanofibre materials, nanoparticles TiO2 were also tested in order to get a nanofibrous TiO2 comparison. The testing and results evaluation were carried out by a certified BIOPHARM a.s. workplace. The output and summary formulation was compiled with the kind assistance of Prof. Václav Mandys from the Institute of Pathology, 3rd Medical Faculty of the Charles University in Prague.
METHODOLOGY OF NANOFIBRE FLY-OFF TESTING

MEASURING OF AEROSOL NANOFIBRES AT THE ELMARCO PLANT

For the measurement of a numerical concentration of nanofibres produced during individual production operations the following measuring devices were used:

• Mobile particle detector (P-TRAK) – mapping possible local sources of aerosol particles
• Scanning sorter of moving particles (SMPS) – concentration and numerical division of aerosol particle sizes in a submicron part of the size spectrum, i.e. particles smaller than 1 micrometer
• Aerodynamic particle counter – division of particle sizes in the range of 0,5 – 10 micrometers
• Low volume sampler Leckel (LVS) – aerosol sample collection for electron microscopy and determination of weight concentration

The measuring was carried out during an electrospinning process on the following machines, alternatively during the following operations with defined polymers:

• laboratory machine for spinning from Nanospider™ solutions – polymer PA6 (polyamid)
• laboratory machine for spinning down from Nanospider™ solutions – polymer PVA (polyvinylalcohol)
• industrial Nanospider™ lines – polymer PA6 (polyamid)
• laboratory machine Nanospider™ NS Melt for melt spinning – polymer PEA (polyesteramid)

Fig. 1. Total aerosol particle concentration as measured for each machine and operation using condensation counter P-TRAK. The concentrations are shown in particles detected per cubic centimeter of analysed air.
For each operation a background value (bkg) and concentration by the equipment output were measured, usually in the exhaust (exh) or output (out). It is apparent from Fig. 1, that for the majority of machines and operations (except from an operation carried out on lab equipment for melt spinning) the background concentrations were on the level as the concentrations at the machine outlet. Possible slight variances between the two values reflected well the continuous change in the total aerosol concentration in the area. Thus it can be concluded that no aerosol particle leakage occurs on these machines and operations, as measurable with the instruments used.

For laboratory equipment producing nanofibres from melt, concentrations measured were a severalfold increase of the background concentration measured prior to machine start-up. Assuming this result is caused by the fact that this is a high-temperature process during which the material is evaporated, subsequently condensing back to aerosol particles inside the cooler exhaust. The final particle concentration will therefore be influenced by the surface temperature of the evaporating material, exhaust temperature, air through flow, etc. Given the nature of the evaporation – condensation process, it may be assumed that the detected particles are round in shape and they are almost certainly not nanofibres.

The process of production and processing of anorganic nanofibre materials created by the burning of a nanofibre precursor, was not monitored for outlet.
RESULTS OF NANOFIBRE FLY-OFF MEASUREMENTS

In the production area, using available aerosol instrumentation, no nanofibre outlets were detected during the production process on Nanospider™ technology. At the same time, no nanofibre outlets were detected during subsequent technologic operation of processing the material into the final product (filter) stage.

METHODOLOGY FOR TESTING OF BIOCOMPATIBILITY OF POLYMER AND ANORGANIC NANOFIBRES

The biocompatibility of selected nanofibrous materials was tested using the method of inserting a sterile implant of the tested nanofibre material into the subcutaneous tissue of a laboratory rat. The surgery was carried out under sterile conditions on an animal under total anaesthesia. The aim of the test was to consider biological reaction of the subcutaneous tissue to the implanted material. The biological test was not carried out in an SLP/SVP system.

![Tested polymer materials: a) PCL, b) PLA, c) gelatine, d) PA6](image1)

![Tested anorganic materials: a) TiO2, b) SiO2](image2)
Testing

For each test, 15 laboratory rats (9 males, 6 females) from the Sprague – Dawley (Charles River) species were used. The following procedures were used during testing:

- **brief total anaesthesia** – once prior to the implant surgery
- **surgical procedure** – implantation on the dorsal part of the body, in the subcutaneous tissue pockets of cca 15 x 10 mm were created, sterile nanofibre samples weighing 20 mg insterted on each side of the body, surgery woud closed.
- **clinical observation** – was done once a day during the whole biological test, after implant surgery it focussed on a local reaction at the implant site.
- **sample collection** – at intervals of 7,22 and 91 days following surgery, 5 animals were destroyed each time and a samples collected (skin, subcutaneous tissue and adjoining soft tissue)
- **histological processing** – preparation and section tinting, viewing under a light microscope, photodocumentation of a representative pathohistology findings

**Result of a pathohistological exam 7 days after the implant surgery**

Skin and subcutaneous tissue did not appear painful at implant sites, and there were no clinical signs of infection (except for SiO2 sample). Microscopically all implant sites exhibited inflammatory reaction with swelling spreading to the surrounding connecting tissue with a mixed inflammatory cellularisation with neutrofile granulocites and small lymphoid cells present. The likely cause of an inflammatory reaction is the thinner residue left on nanofibres following the spinning process.

At the implant sites and their immediate surroundings a significant giant-cell granulomatous reaction with signs of phagocytosis of the implant material was visible.

**Result of a pathohistological exam 22 days after the implant surgery**

Inflammatory swelling receded at all implant sites except SiO2. In the subcutaneous tissue surrounding the implant site the appearance of an unspecific granulous tissue was noted, completely demarcating the
Implant, mixed inflammatory infiltration mainly with small lymphoid cells still present. At all implant sites a giant-cell granulomatous reaction still present. Peripheral implant sites show signs of phagocytosis. The gelatine implants show degradation (mass reduction).

**Result of a pathohistological exam 91 days after the implant surgery**

None of the implant sites show signs of inflammatory swelling.

Implants PLA, and PA 6 are seated in a mature granulous tissue, giant-cell granulomatous reaction still present, surrounding tissue without pathological changes.

Implants of TiO2 nanofibre, SiO2 nanofibre and TiO2 nanoparticles, are spread at at the implant site and seated in an mature granulous tissue. Giant-cell granulomatous reaction still present, surrounding tissue without pathological changes.

Implants PCL are partly degraded, fibre residue surrounded by a thin layer of granulous tissue.

Gelatine implants completely degraded.

**Results of the subcutaneous implant surgery**

- Clinical examination of the laboratory rats carried out during the biological test did not show any fundamental changes of the overall health condition. Irritation of the surrounding subcutaneous tissue was macroscopically visible only after the SiO2 implant and was only temporary (to the end of week 4 after implant).

- The process of polymer implant healing in (PCL, PLA PA-6, gelatine) is accompanied by a long-term (chronic) giant-cell granulomatous reaction and fibreproductive changes.

- The gelatine implants were fully resorbed 91 days after the implantation and in their place only a minute seat of newlyground tissue could be found.

- Implants TiO2 nanofibre and TiO2 nanoparticle, SIO2 are not subject to biodegradation and are surrounded by a persistent granulomatous reaction. Moreover, material of these implants exhibited a tendency toward dispersion spreading from the innital implant site into its surrounding area.

**Summary and recommendations**

- Subcutaneous implantation of polymer and anorganic nanofibres into the hypodermis of a rat showed the possible health risks associated with their potential massive inhalation.

- Measuring of the aerosol nanofibres PA6 fly-off during production and processing proved that practically no fly-off occurs. The operator of NANOSPIDER™ technology is not subjected to any risk of polymer nanofibre inhalation during production of surface nanofibrous layers. When processing these materials in a technological step causing destruction of such layer whilst allowing nanofibre cluster release and their potential fly-off, it is necessary to protect the operator’s respiratory system with an FFP2 class respirator.
• In production of anorganic nanofibrous materials in technological steps following on nanofibre burning (crushing, adjustment, final product processing,...) to protect the respiratory system of operators using a class FFP2 respirator, alternatively a full face mask (for eye protection).

• Nanofibrous and nanoparticle form TiO2 accordingly create an inflammatory and giant-cell granulomatous reaction and fibreproductive changes to the hypodermal tissue surrounding the implant.

• TiO2 nanofibrous materials compared with the nanoparticle ones exhibited lesser tendency to disseminate into the surrounding tissue.

• Following the implant, a long-term swelling of the connecting tissue was present with the nanoparticle form implant.

REFERENCE