National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Mesh implants intended to treat patients with pelvic organ prolapse

Laboratory analysis for market surveillance

RIVM report 2022-0177
N.R.M. Beijer et al.

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Synopsis

Mesh implants intended to treat patients with pelvic organ prolapse

Laboratory analysis for market surveillance

Patients suffering from pelvic organ prolapse (POP) can be treated by placing mesh implants in the body. The Health and Youth Care Inspectorate (IGJ) asked RIVM to conduct a laboratory analysis on mesh implants used to treat POP in the Netherlands in 2018. The request was to analyse various properties of these products. The results of this laboratory analysis do not indicate that the tested products are unsafe for patients.

The laboratory study consisted of various types of tests. From six mesh implants from different manufacturers we analysed the chemical composition, as well as the structure and dimensions of various components of the products. The results of these tests were in line with the descriptions in the technical documentation for these products.

Furthermore, we tested whether the mesh implants are harmful to laboratory-grown biological cells. The results of this test were also in line with the test results described by the manufacturers in the technical documentation. Five of the six products triggered no response in the cells used in the test. One product triggered a mild cellular response. This particular product is designed to be partially absorbed in the body. In comparison, the other tested mesh products are non-absorbable. This difference might play a role in the observed mild cellular response with this product.

The test with biological cells is part of the standard set of tests prescribed for the biological evaluation of implants. Based on this broader set of tests, the manufacturer tested this product more extensively before it was authorised to be placed on the market. The combined results from these laboratory tests gave no indication of harmful effects.

RIVM puts up for debate whether the test using lab-grown cells is suitable to assess the safety of partially absorbable mesh implants. It therefore recommends taking this consideration into account in the periodic review of the international document that describes this test method.

Keywords: mesh implant, pelvic organ prolapse, market research, biocompatibility, product composition, product safety

Publiekssamenvatting

Bekkenbodemmatjes

Laboratoriumanalyse voor markttoezicht

Bekkenbodemmatjes zijn implantaten die in het lichaam kunnen worden geplaatst om verzakkingen van organen in het gebied van de bekkenbodem te behandelen. De Inspectie Gezondheidszorg en Jeugd (IGJ) heeft het RIVM gevraagd om laboratoriumonderzoek te doen naar bekkenbodemmatjes die artsen in 2018 in Nederland hebben geplaatst. De vraag was om verschillende eigenschappen van de producten te onderzoeken. In dit laboratoriumonderzoek zijn geen resultaten gevonden die aangeven dat de producten onveilig zijn voor patiënten.

Het laboratoriumonderzoek bestond uit verschillende typen analyses. Zo is van zes bekkenbodemmatjes van verschillende fabrikanten onderzocht van welke materialen ze gemaakt zijn. Ook is gekeken naar de opbouw en afmetingen van verschillende onderdelen van de matjes. De resultaten hiervan kloppen met wat de fabrikanten beschrijven in de technische documentatie over hun producten.

Met een ander soort analyse is gekeken of bekkenbodemmatjes een schadelijke reactie veroorzaken in cellen die in het laboratorium zijn gekweekt. Ook deze resultaten komen overeen met wat de fabrikanten in de technische documentatie vermelden. Bij vijf van de zes producten was er geen reactie te zien in de cellen waarmee ze werden getest. Bij één product was een milde reactie in de cellen te zien. Dit product is zo gemaakt dat het voor een deel in het lichaam wordt afgebroken, terwijl dit bij de andere producten niet het geval is. Dat kan een verklaring zijn voor de milde reactie in de cellen bij dit ene product.

De test die is gedaan, is onderdeel van een standaardpakket van testen die worden voorgeschreven om te testen of implantaten mogelijk nadelige effecten op het lichaam hebben. Voor de toelating op de markt heeft de fabrikant dit product veel uitgebreider getest. De gecombineerde resultaten van het hele pakket aan laboratoriumtesten gaven geen aanwijzingen voor schadelijke effecten.

Het RIVM vraagt zich wel af of de test met cellen in het laboratorium geschikt is voor materiaal van bekkenbodemmatjes dat voor een deel wordt afgebroken in het lichaam. Het RIVM adviseert om dit punt mee te nemen bij de periodieke herziening van het internationale document dat deze testmethode beschrijft.

Kernwoorden: bekkenbodemmatjes, verzakking organen bekkenbodemgebied; marktonderzoek; biocompatibiliteit; productsamenstelling, productveiligheid

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Summary

Previously, RIVM provided an overview of mesh implants to treat pelvic organ prolapse (POP) that were used in the Netherlands in 2018, and assessed the technical documentation of these products (RIVM 2020-0154). In this report, again commissioned by the Dutch Health and Youth Care Inspectorate, RIVM verified selected material specifications included in the technical documentation of the products by performing tests at the RIVM laboratories. Experimental data on physicochemical characteristics and biocompatibility were obtained from the product samples of six types of mesh implants. The selected analyses do not represent a full physicochemical characterisation and biological product evaluation. We used two spectroscopic techniques to determine the identity of the materials, and assessed relevant product dimensions using bright field microscopy. As a first screen for potential alarm signals for biocompatibility, we selected an assay to quantify *in vitro* cytotoxicity of material extracts. This widely used *in vitro* cytotoxicity assay provides a relatively quick screening to determine the presence and potential toxicity of substances that can leach from a medical device, and therefore allows us to crudely screen for alarm signals in implant safety.

Physicochemical analyses showed that all samples consisted of polypropylene. One product contained an additional material, which matched the information in the technical documentation of the manufacturer. Pore sizes, monofilament diameters, and chain diameters were also consistent with those reported by the manufacturers.

Also, our results on *in vitro* cytotoxicity testing were in line with the results of the manufacturers, as reported in their technical documentation. Five out of the six tested mesh products were not cytotoxic *in vitro*, whereas one mesh product showed mild *in vitro* cytotoxicity when tested in line with applicable standards at the time. Mild *in vitro* cytotoxicity was observed in the only partially absorbable product in this study. When more stringent protocols were applied, in line with current insights, stronger effects were observed. However, we have to debate whether the applied test system is appropriate for absorbable materials. In contrast with the situation in a living organism, the degradation products that are formed when hydrolysis of the absorbable material already starts during the test remain present and can strongly influence the test results. We recommend that testing of absorbable materials is specifically considered during the ongoing revision of the relevant standard, EN ISO 10993-5.

For the biological evaluation of a product, manufacturers perform multiple types of testing, including *in vivo* animal studies. Animal studies do not fully represent the human situation, but do measure potential adverse effects in the intact organism. The results of such studies can outweigh the results of the *in vitro* cytotoxicity screening assay in the product evaluation. For the products we tested, the manufacturers performed *in vivo* studies from which they concluded there was no

evidence of toxicity. As a consequence, our experimental results do not lead to a potential impact on patient safety for these products.

1 Introduction

1.1 Background

A mesh implant is a medical device that is permanently implanted into the body to provide extra support to weakened tissue. Surgeons have been using mesh implants to treat hernia repairs for over fifty years [1]. Mesh implants can be used to treat patients for various indications, such as stress urine incontinence, hernia repair and pelvic organ prolapse (POP). In 2020, RIVM provided an overview with seven of these indications [2]. Mesh material can be synthetic or biological. It can be non-absorbable, partially absorbable or absorbable [3, 4]. Synthetic mesh implants are usually made from a woven plastic. Over the years, various materials have been used, such as polyester and Gore-Tex. Nowadays, mesh implants are often made of the plastic polypropylene [5-7]. In the current report, we focus on synthetic, non-absorbable or partially absorbable surgical mesh implants for the treatment of POP.

POP is the descent of one or more of the pelvic organs, for example, uterus, vagina, bladder or bowel into or out of the vagina. It may affect the anterior (bladder), middle (uterus) or posterior (rectum, or back wall of the vagina) compartment. Although POP can affect women of all ages, it more commonly occurs in older women and affects 30-40% of women worldwide [8, 9]. The etiology of POP is complex and multifactorial, and is linked to childbearing, obesity and advancing age [10]. POP is not life-threatening, but it reduces the quality of life for women [11].

There are several treatment options available for POP, depending on the severity of the symptoms and the severity of the prolapse, in combination with the patient's age and health. For women with a mild degree of POP, conservative treatment options are lifestyle changes, pelvic floor physiotherapy and vaginal pessaries [12, 13]. If these treatment options do not work, or if the prolapse and symptoms are very severe, surgery is a treatment option. A variety of reconstructive surgical procedures is available for these women, for instance native tissue repair and surgical mesh implantation. Surgical mesh can be implanted via two surgical approaches: via the abdomen (transabdominal) or via the vagina (transvaginal). Scientific studies showed that native tissue repair has a failure rate of recurrent prolapse of 17-20% [14]. This resulted in embracing surgical mesh as a treatment option for POP in the beginning of the 20th century [3, 4, 8-10].

Given that the use of surgical mesh implants in the treatment of POP had been shown to be associated with various adverse events, the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) was asked to provide a scientific opinion on the health risks of mesh implants used in urogynaecological surgery. They outlined that clinical outcome following mesh implantation is influenced by material properties, product design, overall mesh size, route of implantation, patient characteristics, associated procedures (for example, hysterectomy) and the surgeon's experience [15]. They also indicated that the implantation of any mesh for the treatment of POP via the

vaginal route should only be considered in complex cases, particularly after failed primary repair surgery [15]. In 2018, RIVM reviewed international literature on long-term complications of transvaginal mesh implants. In the reviewed literature, the range of long-term complications varied from 0 to 48%. The identified complications were primarily associated with products that were not available on the Dutch market in 2018 [16]. In 2022, RIVM published a report on long-term health complaints and possibilities for care after mesh implantation. In the reviewed literature, the type of long-term health complaints described in the literature are similar comparing patients with transabdominal and transvaginal mesh implants. The long-term health complaints are more commonly reported after transvaginal mesh implantation (0-33.8%) than after transabdominal mesh implantation (1-16%) [17].

The Dutch Health and Youth Care Inspectorate (hereinafter referred to as 'Inspectorate') is entrusted with market surveillance and law enforcement of medical devices and their use, in order to address the greatest risks for patient safety and their early identification. To gain more insight in the state of affairs for mesh implants used to treat POP in the Netherlands, the Inspectorate commissioned RIVM to perform a study. This study consisted of two parts: assessment of technical documentation and laboratory testing. In 2022, RIVM published a report on a market survey and an assessment of the quality of technical documentation of mesh implants used to treat POP in the Netherlands in 2018 [18]. Nine mesh implants produced by six different manufacturers were included. In parallel to this, the Inspectorate commissioned laboratory testing of mesh products. The results of this work are described in the current report.

1.2 Aim of the study

The aim of this study on mesh implants used to treat POP in the Netherlands in 2018 is to verify selected material specifications included in the technical documentation of the products by performing tests at the RIVM laboratories. The potential impact of the test results on patient safety will be discussed.

We performed a physicochemical analysis using FT(N)IR and RAMAN spectroscopy to determine the identity of the materials, and bright field microscopy to assess relevant dimensions. Furthermore, we performed a specifically selected assay to quantify *in vitro* cytotoxicity of mesh material extracts.

A full physicochemical characterisation and biological evaluation was outside the scope of this study. Therefore, the selected analyses do not represent a full laboratory evaluation of the tested mesh products. All experimental work was performed in ISO-9001 certified laboratories, according to the at that time valid standard.

1.3 Guide to reading the report

The mesh products used in this study are described in Chapter 2. In Chapter 3, the results of the physicochemical analyses are described. The *in vitro* cytotoxicity data are presented in Chapter 4. In Chapter 5, we presented general conclusions.

2 Product sample information

At the request of the Inspectorate, four manufacturers supplied us with samples of a total of six types of mesh implants (Table 1). These types of mesh implants were used to treat POP in the Netherlands in 2018. Two of the samples were used for the development of our experimental procedures. These two samples were therefore not included in the main study. Of the remaining eight samples, six samples differed in either product size or production batch. All samples received a unique sample code upon receipt in order to be able to discriminate between these variations later on, if needed.

The two pre-study and six of the main study samples were indicated to consist of non-absorbable polypropylene filaments, while two samples were indicated to consist of a combination of polypropylene filaments and absorbable fibres. More information about the mesh products can be found in the report on the products' technical documentation [18].

Not all products included in the technical documentation report [18] were included in the laboratory study. The products from Johnson & Johnson, Ethicon, Promedon and A.M.I. were used in both studies. From Coloplast, both studies included the Restorelle DirectFix and a product from the M/L/XL series. The Y version of this product line was included in the technical documentation report, but not in the laboratory study because the mesh material is identical to the material used in the M/L/XL series. Both BD products were not included in this study because no samples were available to us. BD had removed these products from the European market in March 2019 before the Inspectorate requested the products. In the following sections of this report, products will be designated via their unique RIVM sample code provided for all samples in Table 1.

Table 1 Sample information

Manufacturer	Product name	Batch number	Exp. Date	Sample code RIVM	Use
Coloplast A/S	Restorelle® DirectFix™ Posterior[1]	6398792	28 Sept 2021	A151401	Pre-study
Coloplast A/S	Restorelle® Flat Mesh XL	6416268	17 Oct 2021	A151402	Pre-study
Coloplast A/S	Restorelle® Flat Mesh XL	6596844	12 Feb 2022	A165201	Study
Coloplast A/S	Restorelle® DirectFix™ Posterior[1]	5992580	27 Feb 2021	A165202	Study
Johnson & Johnson Int	ARTISYN™ Y-Shaped Mesh	PABBZGC0	31 Jan 2021	A165203	Study
Johnson & Johnson Int	ARTISYN™ Y-Shaped Mesh	PBBDRZC0	31 Jan 2021	A165204	Study
Ethicon LLC[2]	GYNECARE GYNEMESH™ PS Nonabsorbable PROLENE™ Soft Mesh 10x15	PGB783	31 May 2024	A165205	Study
Ethicon LLC[2]	GYNECARE GYNEMESH™ PS Nonabsorbable PROLENE™ Soft Mesh 25x25	PEJ694	30 April 2024	A165206	Study
Promedon SA	Calistar S	50110	April 2022	A165207	Study
A.M.I. GmbH	BSC Mesh PP 0	191116	30 June 2024	A165208	Study

[1] In May 2019, Coloplast A/S stopped selling Restorelle® DirectFix™.
[2] Ethicon LLC is a Johnson & Johnson subsidiary.

3 Physicochemical analysis

Chemical characterisation of products is an important element of the EN ISO 10993 series. We analysed the products described in Chapter 2 for multiple physicochemical parameters. Using various spectroscopy and microscopy techniques, we identified the chemical identity of the products as well as their material structure and dimensions. The details of the analytical methods are described in Annex 1.

3.1 Identity of mesh product materials

The identity of the used materials was assessed using FTIR (Fourier Transformed InfraRed spectroscopy), FTNIR (Fourier Transformed Near-InfraRed spectroscopy) and RAMAN spectroscopy. Spectra are displayed in Annexes 2, 3 and 4. All physicochemical studies were performed in 2019.

All techniques show that the mesh implants are made from polypropylene as declared by the manufacturers (Table 2).

The FTIR spectra of A165203 and A165204 indicate the presence of an unidentified additional material besides polypropylene. It is known that the product is partially made from absorbable fibres (polyglecaprone-25) [19]. The presence of this component could explain the additional peak around 1740 cm^{-1} in the spectra. This peak is indicative of a C=O stretch vibration, and a C=O group is present in this component.

The FTNIR spectra of all meshes are very similar, although spectra of A165203, A165204 and A165206 differ slightly from those from the other samples. In the samples with blue fibres (see Figure 1), an additional peak in the FTNIR spectrum was observed at 9300 cm^{-1}, which might be explained by the presence of the used dye. Only the white part of A165205 was analysed. On top of this, spectra of A165203 and A162504 are slightly different from those of the other samples. This is probably caused by the presence of the resolvable component.

The Raman spectra of all meshes are very similar too, although spectra from A165203, A165204, A165205 and A165206 differ from those of the other samples. The spectra indicate the presence of additional components beside the polypropylene. The spectra of the blue fibres were different from those of the white fibres and could not be identified.

Table 2 Identity of mesh product materials as determined by FT(N)IR and RAMAN spectroscopy

Sample code	FTIR	FTNIR	RAMAN
A165201	Polypropylene	Polypropylene	Polypropylene (isotactic)
A165202	Polypropylene	Polypropylene	Polypropylene (isotactic)
A165203	Polypropylene + unidentified[1]	Polypropylene *slightly different from other spectra*	White material: Polypropylene (isotactic) + unidentified[1]
A165204	Polypropylene + unidentified[1]	Polypropylene *slightly different from other spectra*	White material: Polypropylene (isotactic) + unidentified[1]
A165205	Polypropylene	Polypropylene[2]	Polypropylene (isotactic) + unidentified[1]
A165206	Polypropylene	Polypropylene *slightly different from other spectra*	Polypropylene (isotactic) + unidentified[1]
A165207	Polypropylene	Polypropylene	Polypropylene (isotactic)
A165208	Polypropylene	Polypropylene	Polypropylene (isotactic)

[1] The unidentified additional material is attributed to the presence of polyglecaprone-25 in the product as indicated by the manufacturer

[2] Only the white fibres were analysed.

3.2 Dimensions and structure of mesh products

The structure of the mesh products was investigated in terms of pore size, and monofilament and chain diameter, using bright field microscopy. Representative microscopy images of the samples to capture their specific structures are displayed in Figure 1. All types of mesh implants have their unique configuration, varying in fibre diameter and knitting pattern. Typically, the pore sizes are in the single millimetre range, and fibre diameter around 100 micrometres (Table 3). The data obtained for these samples are in line with what the manufacturers report in their technical documentation.

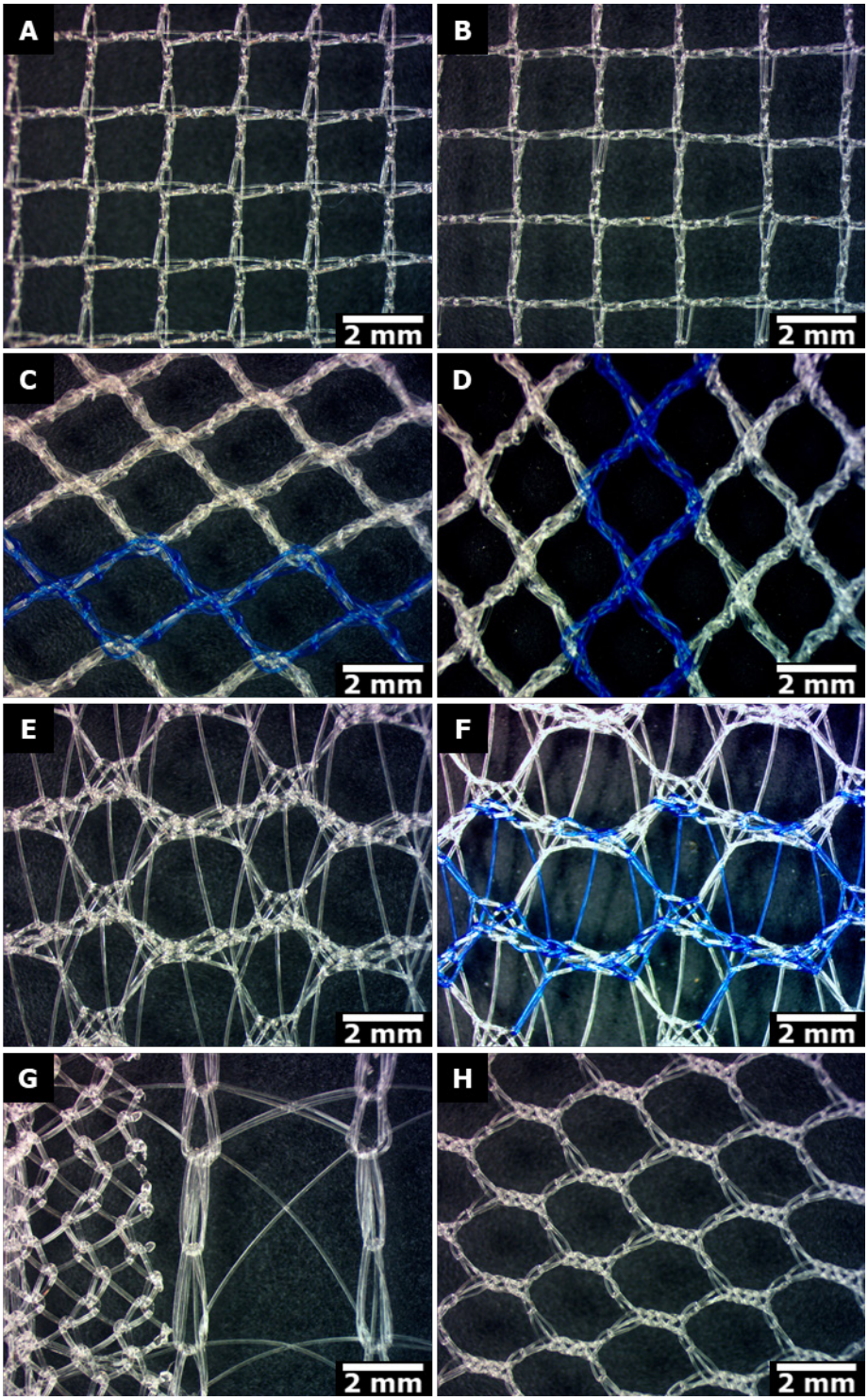

Figure 1 Microscopy images of the meshes. A) A165201, B) A165202, C) A165203, D) A165204, E) A165205, F) A165206, G) A165207, H) A165208. Scale bar representing 2 mm.

Table 3 Pore dimensions, monofilament dimensions and chain diameters of the mesh products.

Sample	Description	Pore dimensions [mm]	Monofilament diameter [mm]	Chain diameter [mm]
A165201	Square pores, white fibres	1.5 – 1.7	0.1	0.4
A165202	Square pores, white fibres	1.8 – 2.0	0.1	0.3 – 0.4
A165203	Diamond-shaped pores, combination of white and blue fibres	1.5 – 2.1	0.1	0.5
A165204	Diamond-shaped pores, combination of white and blue fibres	1.6 – 2.1	0.1	0.4
A165205	Hexagonal pores and cross-linking fibres, combination of white and blue fibres (blue fibres not visible in displayed picture)	0.8 – 2.7	0.1	0.7
A165206	Hexagonal pores and cross-linking fibres, combination of white and blue fibres	0.2 – 2.4	0.1	0.5
A165207	Combination of smaller and larger pores, including thinner fibres crossing over the larger pores, white fibre	0.4 – 3.8	0.1 – 0.2	0.7
A165208	Hexagonal pores, white fibres	0.3 – 2.6	0.1	0.4

3.3 Conclusions of physicochemical analysis

In all samples, the material was confirmed to be polypropylene. Spectral differences in two samples can be explained by the presence of an additional, absorbable material, as indicated by the manufacturer. An additional unidentified component in spectra of four samples can be explained by the presence of a blue dye in these samples. Pore sizes, monofilament diameters, and chain diameters were consistent with those reported by the manufacturers in their technical documentation. All results obtained by the physicochemical analysis are in accordance with the technical documentation of the products.

4 *In vitro* cytotoxicity

For a complete evaluation of biocompatibility, a combination of a variety of data and testing is necessary. According to EN ISO 10993-1:2020 [20], to assess biocompatibility as part of the biological evaluation of medical devices, one should consider a wide range of assays: for example, *in vitro* testing on genotoxicity, haemotoxicity and cytotoxicity, as well as *in vivo* testing on sensitization, repeated dose toxicity and effects after implantation. However, this is beyond the scope of the current study. In addition, many of the biocompatibility assays are *in vivo* studies in rodents which we will not repeat for ethical reasons. From the afore-listed *in vitro* assays, we selected a widely used *in vitro* cytotoxicity assay for this study. This assay provides a relatively quick screening to determine the presence and potential toxicity of substances that can leach from a medical device, and therefore allows us to crudely screen for alarm signals in implant safety. When toxic compounds are present in the extracts prepared from medical devices, they can be detected in an *in vitro* cell culture system by their toxic activity, inducing cell death or affecting cell functionality [21]. The *in vitro* cytotoxicity has also been measured by the manufacturers for all products as part of the biological evaluation included in their technical documentation.

To assess *in vitro* cell cytotoxicity, typically cell viability is measured. If cell viability below 70% is measured, this is considered a sign of cytotoxicity. The cell viability assay and the sample preparation applied in this study were performed according to the relevant European standards [22, 23]. The methods used are described in Annex 5. Similar to the physicochemical analysis, we evaluated the samples described in Chapter 2.

4.1 Results *in vitro* cytotoxicity

4.1.1 Experimental design

The cytotoxicity studies were performed between 2019 and 2021, when the 2009 version of 'EN ISO 10993-5 Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity' [22] and the 2012 version of 'EN ISO 10993-12 Biological evaluation of medical devices Part 12: Sample preparation and reference materials' [23] were applicable. These standards describe protocols to test for, among others, *in vitro* cytotoxicity and the corresponding sample preparations. In these versions of the standards, extraction of the testing materials is carried out during 24 hours at 37 °C, and exposure of the cell culture to this material extract for 24 hours at 37 °C as well.

In 2021, EN ISO 10993-12 was revised [24] and now requires a 72-hour extraction period, describing that "for medical devices which are in prolonged (>24 hours to 30 days) or long-term contact (>30 days), extraction times of 72 hours are recommended for cytotoxicity testing because extraction for 24 hours may not be sufficient to obtain an extract that represents the chemicals released beyond 24 hours of device use". Furthermore, extension of the exposure duration of 24 hours towards 72 hours is currently under investigation by

ISO/TC194/WG5 to gather data for the upcoming revision of EN ISO 10993-5.

Being aware of these developments, we decided to perform two rounds of experiments. One using the versions of the standards applicable at the time when manufacturers submitted their products, and one using the more stringent extraction and exposure durations included in the revised EN ISO 10993-12, respectively, which is considered for inclusion in an upcoming revision of EN ISO 10993-5. However, considering material and resource efficiency, we designed the experiments in such a way that we did not need to perform both experiments using all materials.

We first ran the experiment with the most stringent conditions, 72 hours extraction and 72 hours exposure, in order to get a worst-case scenario in assessing the product samples' *in vitro* cytotoxicity. The product extracts that affected cell survival under these conditions were subsequently tested, using protocols applicable when the manufacturers supplied mesh samples upon request of the Inspectorate in 2019 – for example, extraction and exposure durations of 24 hours each. In case a product was selected for the second round of experiments, any other product from the same manufacturer was included as well. Products that did not induce cytotoxicity using the stringent conditions of 72 hours extraction and 72 hours exposure were excluded from further testing as it is to be expected that these products will not induce cytotoxicity after shorter extraction and exposures times either. Details of the experimental methods are included in Annex 5.

4.1.2 Results 72-hour extraction and exposure

For cytotoxicity testing with quantitative read-out systems like the ones we used, a sample is considered to induce cytotoxicity when cell survival is below 70% of the control cells [22]. After 72 hours of extraction and exposure, A165201, A165205 and A165206, A165207, and A165208 showed no signs of *in vitro* cytotoxicity (Figure 2) in comparison with the medium negative control. Note: more details on the included positive and negative controls can be found in Annex 5. In this 72-hour extraction and exposure model, A165202 was found to approximate the border of mild *in vitro* cytotoxicity, and A165203 and A165204 extracts showed strong signs of *in vitro* cytotoxicity (Figure 2). No significant differences were observed between the different batches of a similar product.

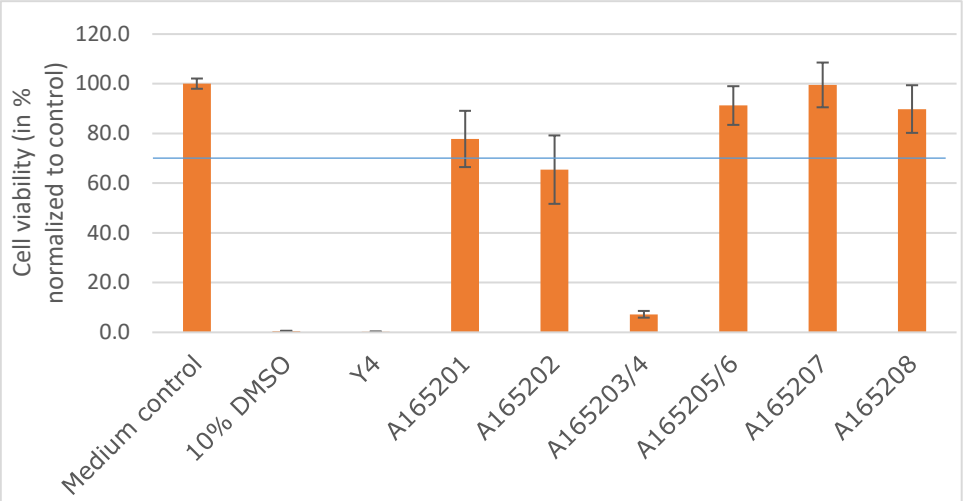

Figure 2 In vitro cytotoxicity results, measured by cell viability, of the test items and control samples (10% DMSO and Y4 are the cytotoxic positive control samples) after exposure of L929 cells to mesh-product extracts measured using the WST-1 assay. All experimental groups included four technical replicates from four independent biological replicate experiments. Test items were extracted for 72 hours, and cells were exposed for 72 hours. Error bars represent the 95% confidence interval. The horizontal blue line at 70% marks the threshold for signs of cytotoxicity. A165205/6 and A165203/4 are the average value of both samples plotted together due to product similarity as described in Chapter 2

4.1.3 Results 24-hour extraction and exposure

Given the signs for *in vitro* cytotoxicity of the A165202 and A165203/4 extracts, we tested these products again, now using the 24 hour protocols. Since A165201 is a product from the same manufacturer as A165202, both products were taken for further assessment. The legal manufacturers of A165203/4 and A165205/6 are both part of the same multinational company. Therefore, we decided to include both these products in the 24-hour protocol.

We tested the above four types of products and similar positive and negative controls using a 24 hour extraction and 24 hour exposure period as described in the relevant standards applicable in 2018 (Figure 3). Both A165201 and A165202 showed no signs of *in vitro* cytotoxicity at this time point. A165205 and A165206 are samples from one type of product, each coming from a different production batch, which also holds true for A165203 and A165204. The 24-hour experiments were executed using one sample per product type: specifically A165205 and A165203. A165205 showed no signs of *in vitro* cytotoxicity at the 24-hour protocol either. For A165203, we observed mild *in vitro* cytotoxicity after 24 hours of extraction and 24 hours of exposure (Figure 3).

At the time of the 24 hours extraction and 24 hours exposure, A165202 and A165203 were approximately 5 and 6 months past their expiry date. The results at 24 hours exposure are in line with the data from the manufacturer (see 4.2.) Therefore, it is assumed that despite the fact that A165202 and A165203 were tested after the expiry date, this did not affect the results of the cytotoxicity tests.

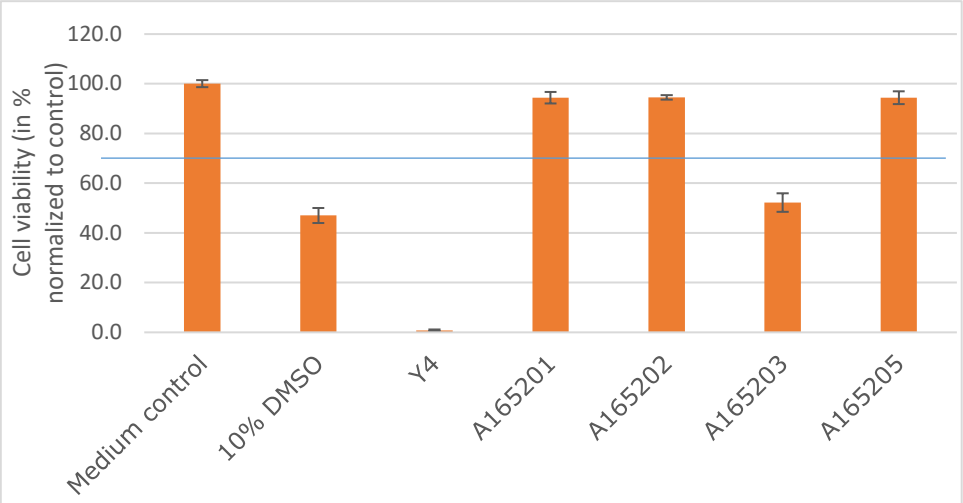

Figure 3 In vitro cytotoxicity results, measured by cell viability, of the test items and control samples (10% DMSO and Y4 are the cytotoxic positive control samples) after exposure of L929 cells to mesh product extracts measured using the WST-1 assay. All experimental groups included four technical replicates from four independent biological replicate experiments. Test items were extracted for 24 hours, and cells were exposed for 24 hours. Error bars represent the 95% confidence interval. The horizontal blue line at 70% marks the threshold for signs of cytotoxicity.

4.2 Comparison with technical documentation

We verified whether our findings on *in vitro* cytotoxicity were in line with those reported in the technical documentation of the mesh products.

From the technical documentation we obtained data on the tested products (1), material extraction procedure (2), *in vitro* cell exposure model (3), read-out system used to assess *in vitro* cytotoxicity (4), and interpretation of the obtained results (5). General observations from these data were the following:

1. tests were performed by either using the product itself, or by using a comparable product based on a description of similarity;
2. proper amounts of test item material were used for the extraction procedure, and the extraction duration and temperature were as required at the time: 24 hours at 37 °C;
3. approved cell types were correctly exposed to the test item extracts and relevant controls for at least 24 hours as required. Note: in most cases, tests with prolonged exposure periods of 48 or 72 hours were included as well;
4. most tests were assessed using qualitative, and some using quantitative read-out systems. Since EN ISO 10993-5:2009 became available, quantitative read-out systems are preferred, qualitative read-out systems were used more commonly in earlier times, and some products have been on the market for a long time;
5. all test items passed the *in vitro* cytotoxicity assessment according to the scoring and grading system applicable to the applied read-out system.

A165207 and A165208 were tested using 72 hours of extraction and exposure, at which they showed no signs of cytotoxicity. Results are in line with the results as described in the technical documentation of the products.

A165205 and A165206 showed no signs of *in vitro* cytotoxicity at either time point, which is in line with the data presented in its technical documentation.

For A165201, an average of 78%, and for A165202 an average of 65%, cell viability was measured after 72 hours of exposure to the material extracts. This would mean that one sample is above and one sample is below the 70% cell viability threshold of *in vitro* cytotoxicity. According to the technical files, A165201 and A165202 are made from the same monofilament polypropylene material. Therefore, a difference in *in vitro* cytotoxicity observed after exposure for 72 hours to extracts from A165201 and A165202 would not be expected. In fact, for both samples, the 70% cell viability threshold lies within the 95% confidence interval of the measured data. Furthermore, there was no statistical difference between the test results for A165201 and those for A165202 (t-test $p<0.05$). The results from the 24 hour extraction and 24 hour exposure experiments were comparable for A165201 and A165202. Here we observed a low spread between data points from the independent experiments. Furthermore, our findings using the 24 hour protocol are in line with the data presented in the technical documentation of the products.

The level of *in vitro* cytotoxicity following 24 hours of exposure to 24 hour extracts of A165203 was in a similar range as the one found by the manufacturer. According to ISO 10993-5 [22], the reduction of cell viability found in this study is graded as a 'cytotoxic effect' when applying a quantitative read-out system (cell viability is <70%). However, when applying a qualitative read-out system according to ISO 10993-5 [22] these levels would be graded as 'mild cytotoxicity' (not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable) and as such would pass the *in vitro* cytotoxicity assay.

4.3 Potential impact of *in vitro* cytotoxicity results on patient safety

In order to assess the potential impact of the experimental *in vitro* cytotoxicity results on patient safety, we should first look at the impact of the results on the overall biological evaluation of the product. In order to help us determine this, we needed more context for several products tested. We looked at the broader set of studies for the biological evaluation as performed by the manufacturer. In addition, we provided the test results to the manufacturers, and invited them to reflect on the results, as well as on the impact of the results on the overall biological evaluation. Since A165207 and A165208 showed no *in vitro* cytotoxicity in the stringent test protocol, we provided the results to their manufacturers without asking for their reflection. The responses from the manufacturers of the other products were in line with our considerations and were taken into account in the discussion below.

Earlier, we identified shortcomings in – among others – the biological evaluation file items of technical documentation of mesh products [18]. This was also the case for the partially absorbable mesh product. The Inspectorate has informed us that the manufacturer addressed the identified shortcomings in their technical documentation after the finalisation of our studies. The manufacturer indicated that the improved technical documentation passed audits by their notified body and stated that their technical documentation is now compliant with European regulatory requirements.

Since A165205 and A165206, A165207 and A165208 showed no *in vitro* cytotoxicity in our experiments, no further discussion is needed on the impact of our results on the overall biological evaluation of the products or on a potential impact on patient safety.

As discussed in the previous paragraph, the threshold for *in vitro* cytotoxicity lies within the 95% confidence interval of both A165201 and A165202 results from 72 hours of extraction and 72 hours exposure. This could be considered a mild cytotoxic effect *in vitro*. This result does not translate into a toxicological impact in *in vivo* studies as shown in other studies reported in the technical documentation of this product. As part of the biological evaluation of these products, multiple *in vivo* studies were performed. These studies showed no evidence of *in vivo* toxicity, results that outweigh the *in vitro* cytotoxicity data in the overall biological evaluation. As a consequence, our experimental results do not lead to a potential impact on patient safety for these products.

For A165203, we found a cytotoxic effect *in vitro* after 24 hours of extraction and 24 hours of exposure, similar to the manufacturer's results when performing the assay in this way, in line with standards applicable at the time. When testing A165203 and A165204 after 72 hours of extraction and 72 hours of exposure, a strong cytotoxic response was observed. These results can be due to the combination of the partially absorbable nature of this mesh material and the chosen experimental procedures. The cellular exposure model as described in EN ISO 10993-5:2009 is a sensitive assay. During the extraction procedure, the hydrolysis of the absorbable polymer component of the device may already start, possibly leading to the presence of degradation products in the extract, which could lead to cytotoxic effects. Obviously, such effects can be expected to be stronger in the 72-hour protocol.

This raises the question whether the 72-hour results or even the 24-hour results should be considered as such in the overall biological evaluation, or whether the test system as described in the standard should be amended when testing (partially) absorbable materials. In the current test system, the extract is presented to a static cell culture in which the cells are potentially exposed to a concentration of degradation products that is higher than expected to be found in experimental animals or in patients, where degradation products are directly spread within the host tissue and potentially removed via standard mechanisms in the body. The biological and clinical relevance of this test system for (partially) absorbable materials can thus be debated. The test results from A165203/4 can, therefore, not be used in the overall biological

evaluation without taking these considerations into account. Furthermore, as part of the biological evaluation of this product, multiple *in vivo* studies were performed. These studies showed no evidence of *in vivo* toxicity, results that outweigh the mild *in vitro* cytotoxicity data after 24 hours in the overall biological evaluation. As a consequence, our experimental results do not lead to a potential impact on patient safety for these products.

4.4 Conclusions of *in vitro* cytotoxicity

The selected *in vitro cytotoxicity* assay is typically used as a first screen for alarm signals for biological safety due to toxic leachables. Our results were in line with those described by the manufacturers in their technical documentation. The results from five out of six tested products did not raise concerns, and after more elaborate analysis, we have to debate the suitability of this assay for the sixth product, which was the only partially absorbable product in this study. Especially when using current insights on the test protocol with regard to longer extraction and exposure duration, this became apparent. We recommend that this question is considered during the ongoing revision of the relevant standard, EN ISO 10993-5.

Manufacturers gather both *in vitro* and *in vivo* data on the safety of their product, often starting by screening for *in vitro* cytotoxicity. Animal studies do not fully represent the human situation, but do measure potential adverse effects in the intact organism. Results of, for example, *in vivo* implantation studies in animals are included in the products' technical documentation as well and can outweigh the results of an *in vitro* cytotoxicity assay in a full biological evaluation. For the products we tested, *in vivo* studies performed by the manufacturers showed no evidence of toxicity.

In conclusion, our results do not raise concerns with regard to a potential impact on patient safety. However, they do lead to a recommendation to ISO to consider whether the applied test method is suitable for absorbable materials.

5 Overall conclusion

In this study, experimental data on physicochemical characteristics and biocompatibility were obtained to study six different mesh products that were used to treat POP in the Netherlands in 2018. We performed selected types of analysis to evaluate the products, which do not represent a full physicochemical characterisation nor a full biological evaluation. As a physicochemical analysis, we performed FT(N)IR and RAMAN spectroscopy to determine the identity of the materials, and bright field microscopy to assess relevant dimensions. Furthermore, we selected an assay to quantify *in vitro* cytotoxicity of material extracts.

The physicochemical analyses showed that most of the tested mesh products consisted of polypropylene, which matched the information in the technical documentation. One mesh product consisted of a combination of polypropylene and the absorbable material polyglecaprone-25, as indicated by the manufacturer. Also pore sizes, monofilament diameters, and chain diameters were consistent with those reported by the manufacturers in their technical documentation.

In five out of the six tested mesh products, no *in vitro* cytotoxicity was observed in our experiments. The partially absorbable mesh product showed mild *in vitro* cytotoxicity when tested in line with standards that were applicable at the time. These results were in line with those described by the manufacturers in their technical documentation. Stronger effects were observed with more stringent protocols in line with current insights. When considering these results, however, we have to debate whether this static *in vitro* cellular model is in fact appropriate to test (partially) absorbable materials for *in vitro* cytotoxicity. Degradation products created when hydrolysis of the absorbable material already starts during the extraction procedure can strongly influence cell viability, and it should be realised that the degradation product concentrations can be relatively high in this model, compared to the dynamic situation during clinical application, when the device is implanted in living tissue with active mechanisms to remove such substances. We recommend that this question is considered during the ongoing revision of the relevant standard, EN ISO 10993-5.

Besides multiple *in vitro* tests as the start of the biological evaluation process of a product, manufacturers gather *in vivo* data in animal studies as well. Animal studies do not fully represent the human situation, but do measure potential adverse effects in the intact organism. The results of such studies can outweigh the results of the *in vitro* cytotoxicity screening assay when appropriately substantiated. For the tested products, the manufacturers performed *in vivo* studies from which they concluded there was no evidence of toxicity. As a consequence, our experimental results do not lead to a potential impact on patient safety for these products.

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Annex 1 Physicochemical methods

Physicochemical analysis using FT(N)IR and RAMAN spectroscopy to determine the identity of the materials and bright field microscopy to assess relevant dimensions was performed in ISO-9001 accredited laboratories. All measurements were performed in 2019.

FTIR

FTIR IR spectra were recorded using a Bruker Alpha spectrometer (Bruker, Massachusetts, USA) in ATR mode. Unprepared samples were clamped directly under the ATR crystal in such a way that at least a chain of the mesh product would be in the IR beam. The spectral range was 3750 – 400 cm^{-1} and resolution 2 cm^{-1}. Background spectra were recorded before each sample measurement. The spectrometer was calibrated before measurements and the correct performance of the spectrometer was verified by a verification measurement of a polystyrene filter.

FTNIR

NIR measurements were performed using an Antaris II FTNIR spectrometer (Thermo Scientific, Madison USA) and TQ-Analyst software vs 8.4 (Thermo Scientific, Madison USA). Small pieces of unprepared mesh products with a diameter of about 1 cm were placed on the detector in cardboard holders. Five spectra of 32 scans were collected per sample in the transmission mode, with a resolution of 8 cm^{-1}, at a spectral range of 12000 – 3000 cm^{-1}. The spectrometer was calibrated before measurements and the correct performance of the spectrometer was verified before measurements by measuring a caffeine sample and a polystyrene filter.

Raman spectroscopy

Raman measurements were performed using a DXR Raman microscope (Thermo Scientific, Madison USA). Small pieces of unprepared mesh products were placed on the microscope stage and focus was put on threads and wires. Measurements were carried out using a 10x and a 20x objective, a 780 nm laser with a laser power of 20 mW, a collection time of 10 seconds and a slit width of 25 µm. The spectrometer was calibrated on the parameters wavelength, white light and laser and was aligned before measurements. The correct performance was subsequently verified by measuring a certified polystyrene standard (internal number: FREF0097).

Bright field microscopy

Microscopy was performed using an Olympus CHS microscope (Olympus, Tokio Japan). Correct size measurements were ensured by the use of a certified micrometer slide (internal number: FREF0026).

Annex 2 FTIR spectra

Representative FTIR spectra per mesh sample are displayed below. Peaks in FTIR spectra are indicative of certain vibrations in molecules and can be used for identification of materials.

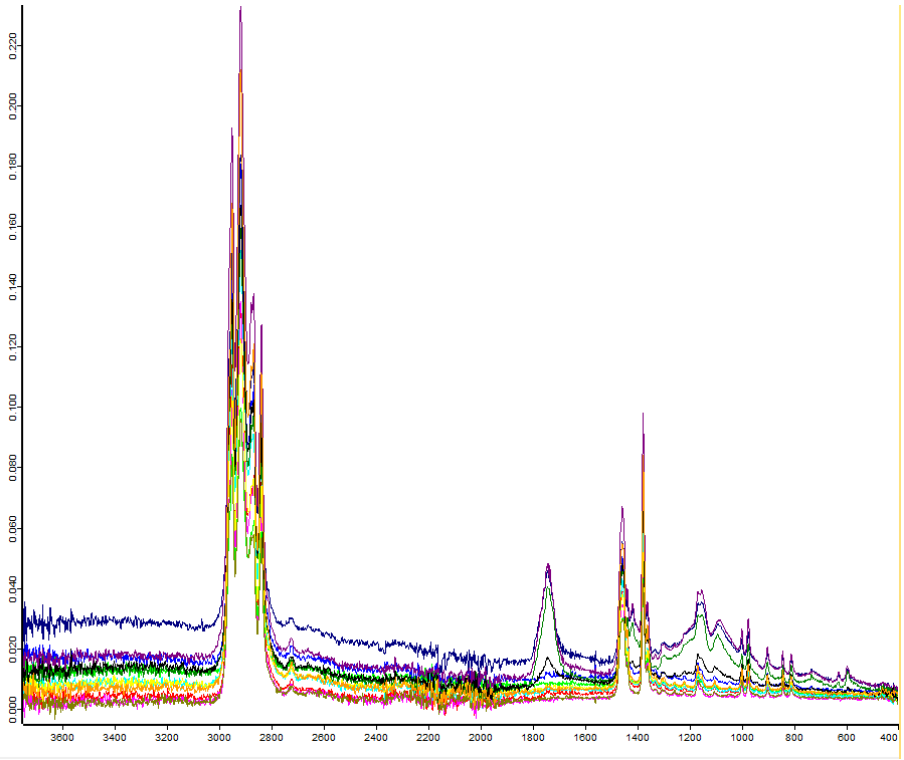

Figure 4 Overlay of FTIR spectra of the samples. A165201 (yellow line), A165202 (golden line), A165203 blue fiber (dark blue line), A165203 white fiber (purple line), A165204 blue fiber (dark green line), A165204 white fiber (black line), A165205 (orange line), A165206 blue fiber (blue line), A165206 white fiber (red line), A165207 fiber around small pore (light green line), A165207 fiber around large pore (magenta line), A165208 (cyan line).

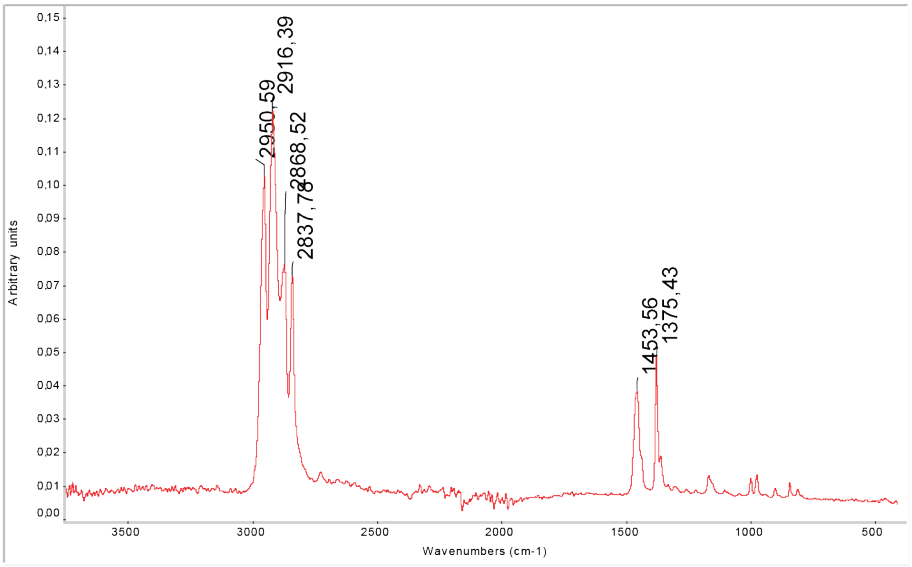

Figure 5 FTIR spectrum of sample A165201.

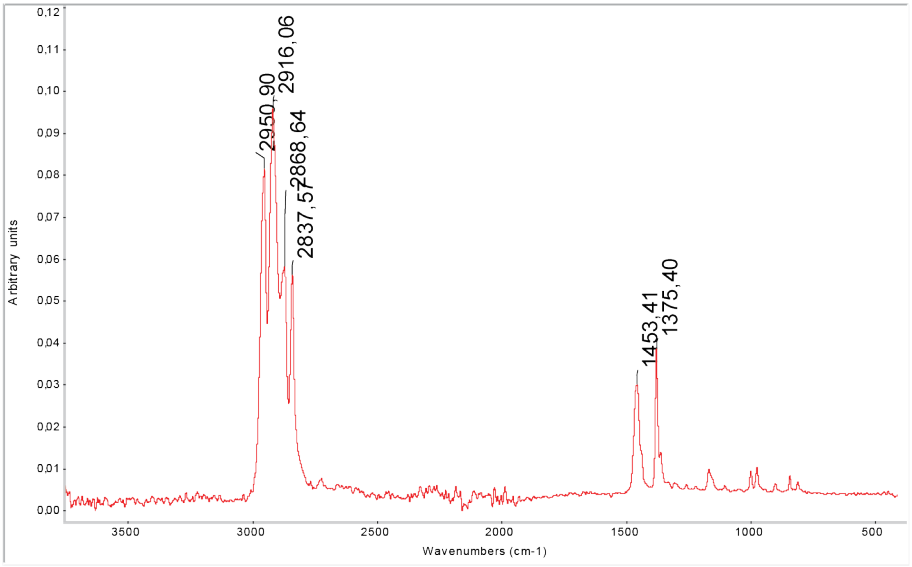

Figure 6 FTIR spectrum of sample A165202.

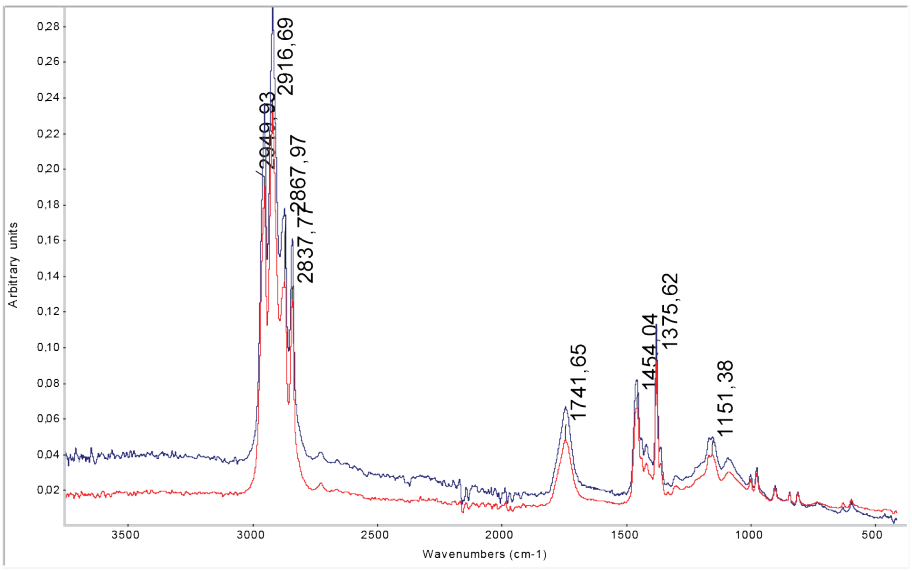

Figure 7 FTIR spectra of the white fibers (red line) and blue fibers (blue line) of sample A165203.

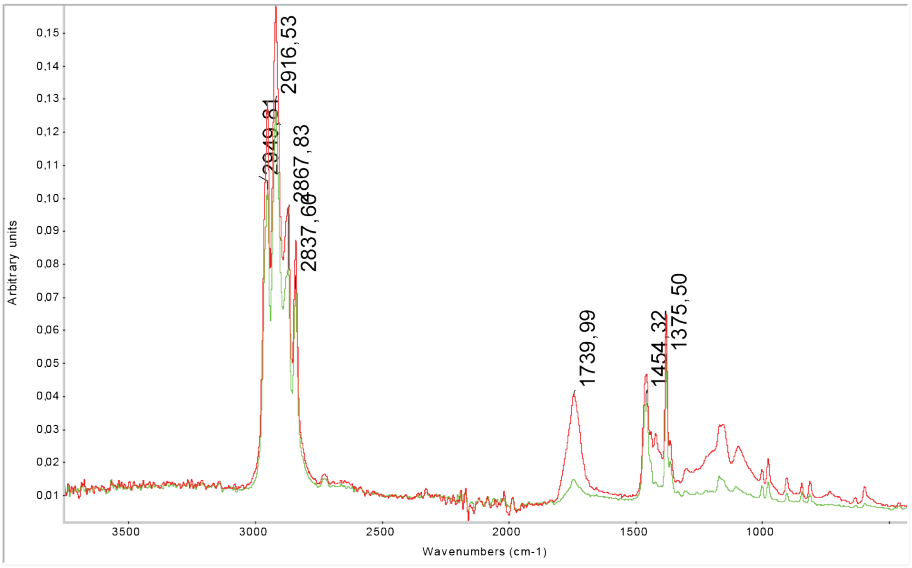

Figure 8 FTIR spectra of the white fibers (green line) and blue fibers (blue line) of sample A165204.

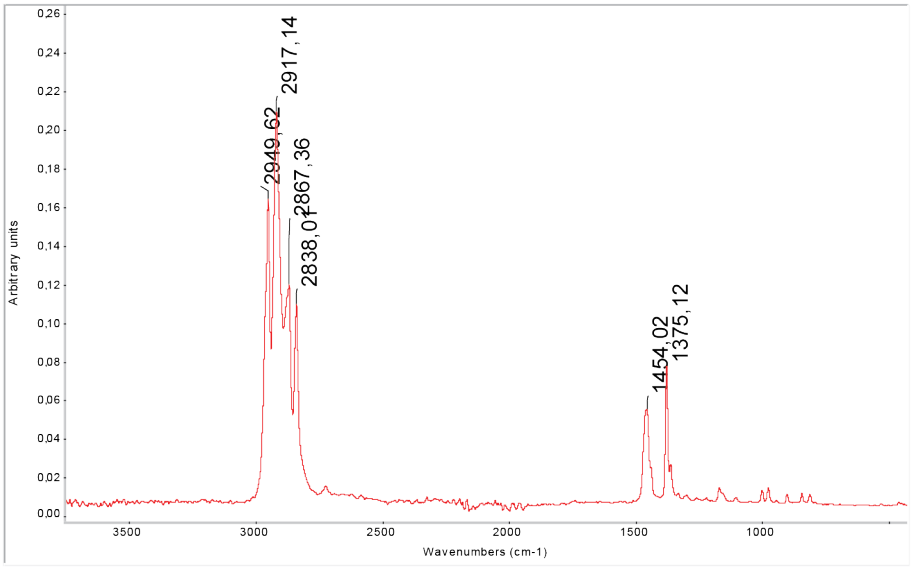

Figure 9 FTIR spectrum of sample A165205.

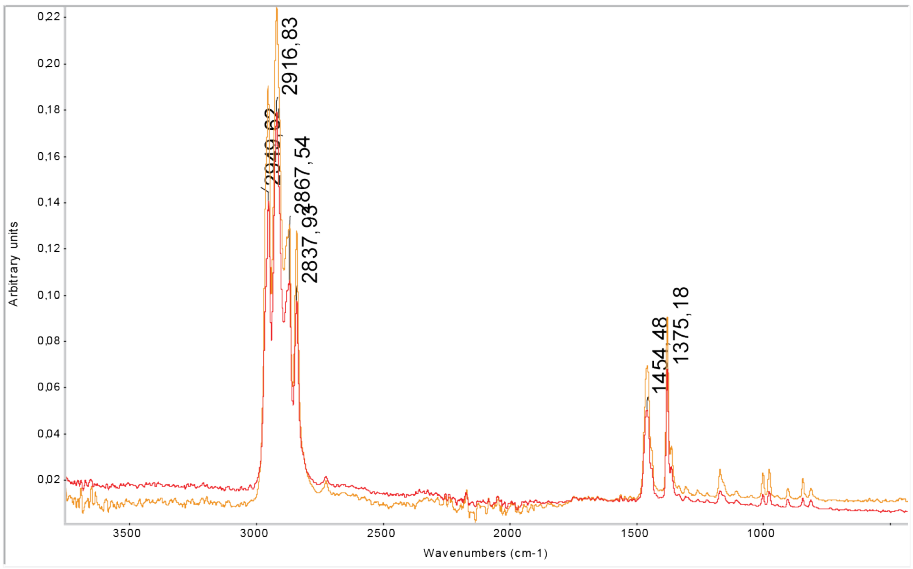

Figure 10 FTIR spectra of the white fibers (orange line) and blue fibers (red line) of sample A165206.

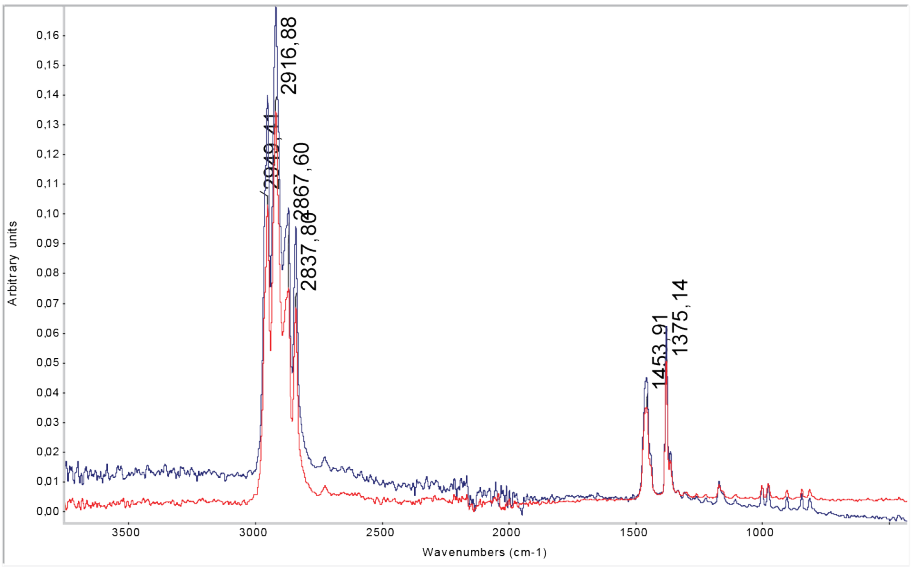

Figure 11 FTIR spectra of the fibers around the small (blue line) and large pores (red line) of sample A165207.

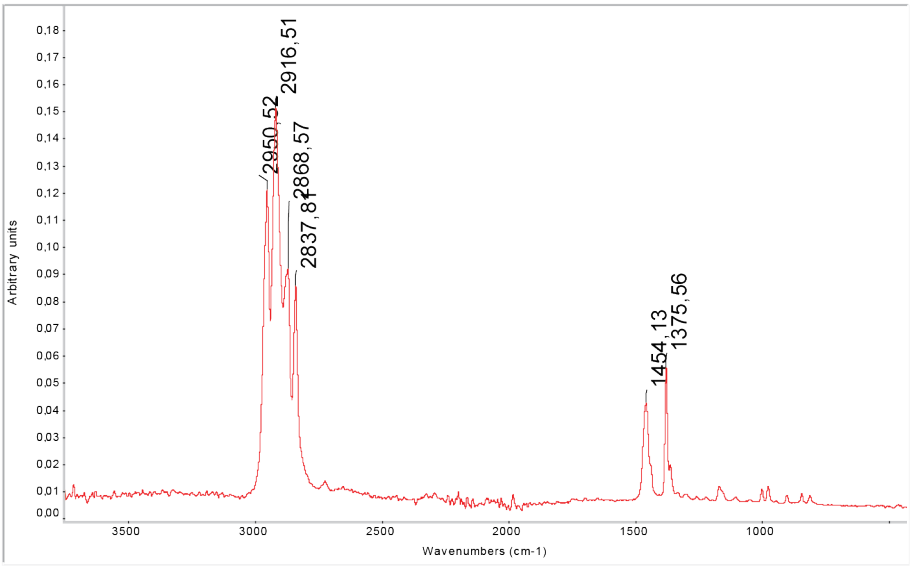

Figure 12 FTIR spectrum of sample A165208.

Annex 3 FTNIR spectra

Representative FTNIR spectra of the meshes are displayed below. FTNIR spectra can used for the comparison of materials.

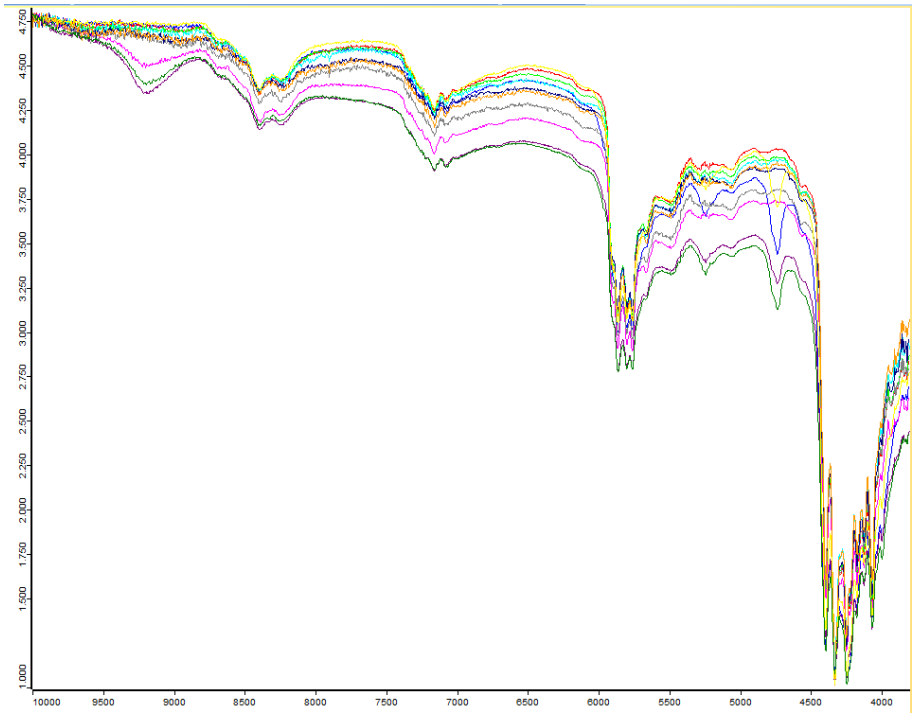

Figure 13 Overlay of FTNIR spectra of the samples. A165201 (blue line), A165202 (orange line), A165203 blue fiber (purple line), A165203 white fiber (yellow line), A165204 blue fiber (dark green line), A165204 white fiber (blue line), A165205 (red line), A165206 blue fiber (magenta line), A165206 white fiber (light green line), A165207 (cyan line), A165208 (dark blue line).

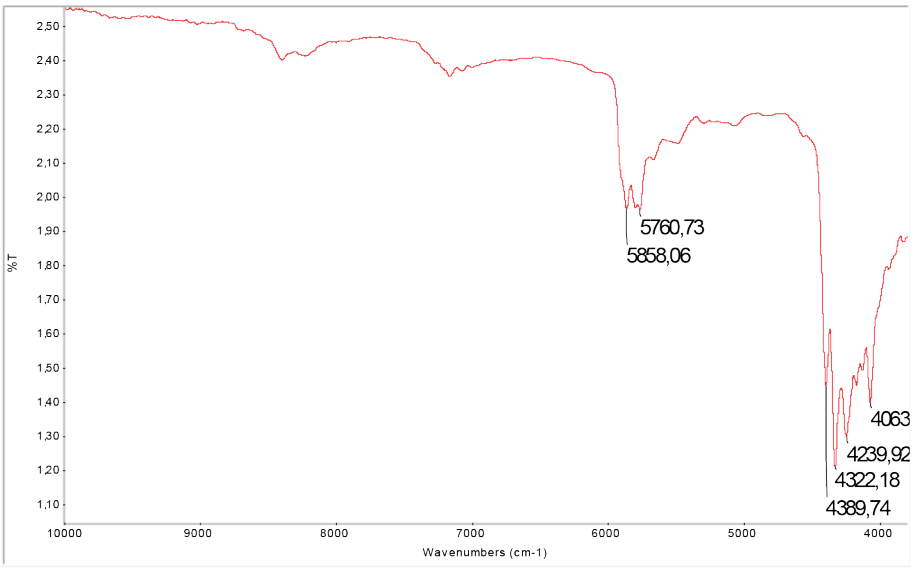

Figure 14 FTNIR spectrum of sample A165201.

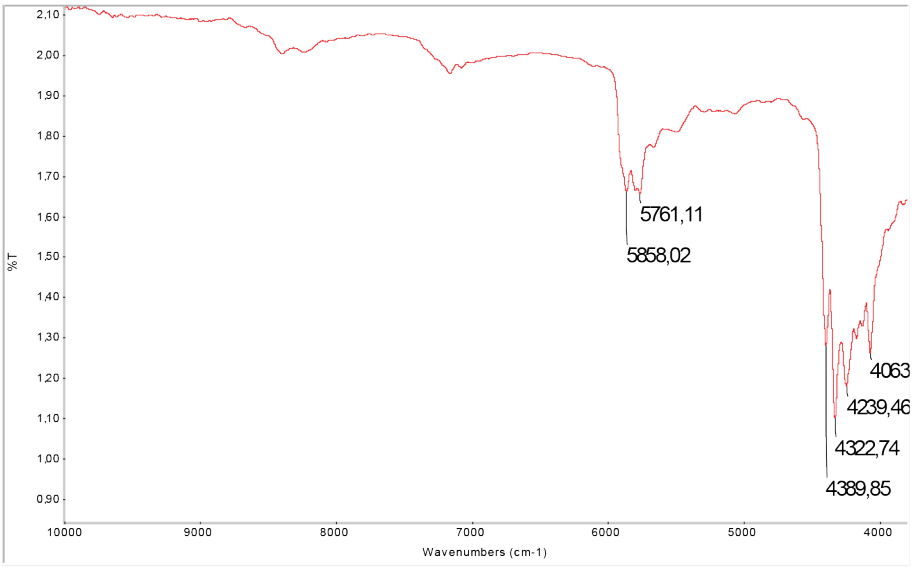

Figure 15 FTNIR spectrum of sample A165202.

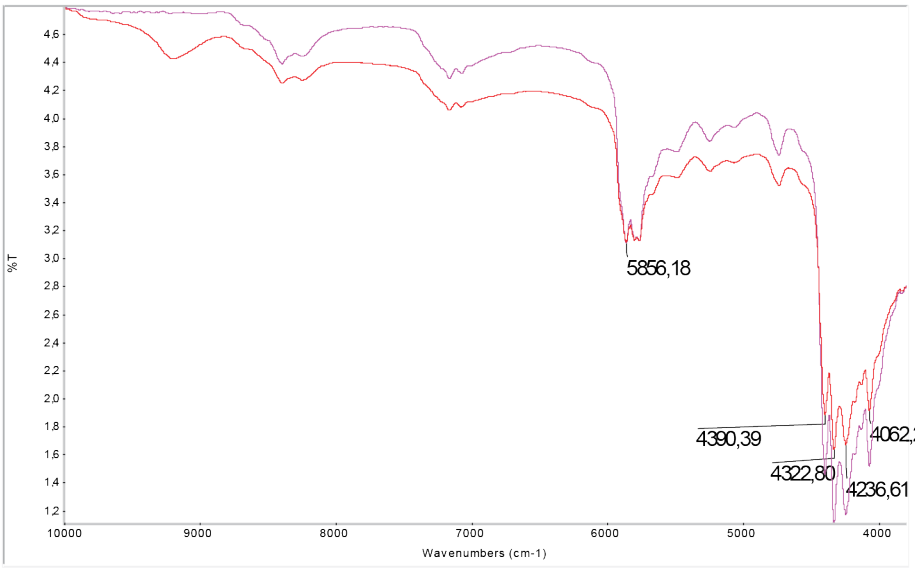

Figure 16 FTNIR spectra of the white fibers (pink line) and blue fibers (red line) of sample A165203.

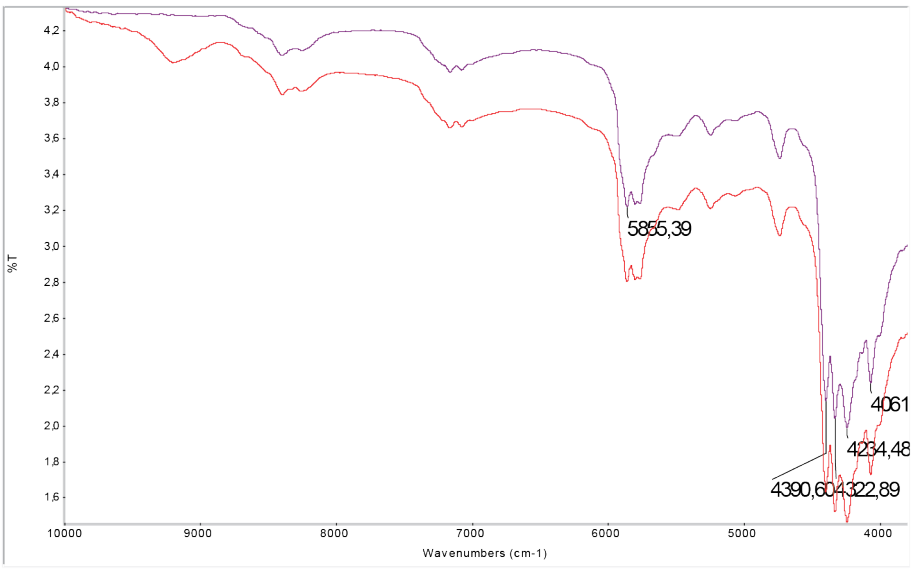

Figure 17 FTNIR spectra of the white fibers (purple line) and blue fibers (red line) of sample A165204.

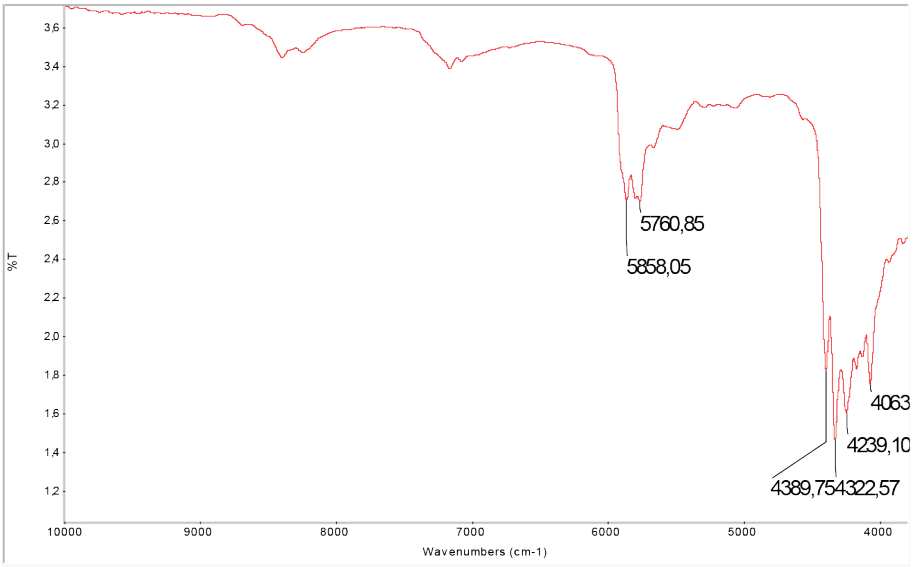

Figure 18 FTNIR spectrum of sample A165205.

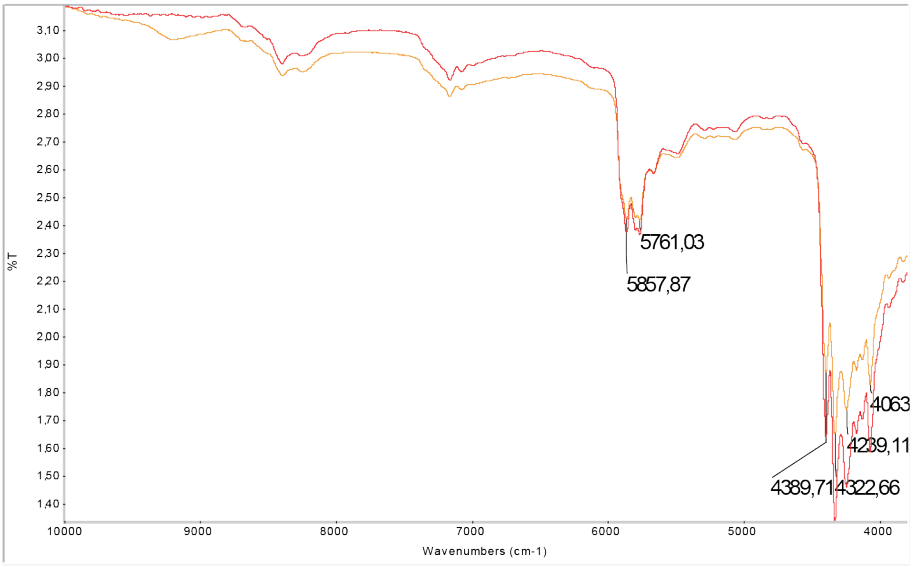

Figure 19 FTNIR spectra of the white fibers (red line) and blue fibers (orange line) of sample A165206.

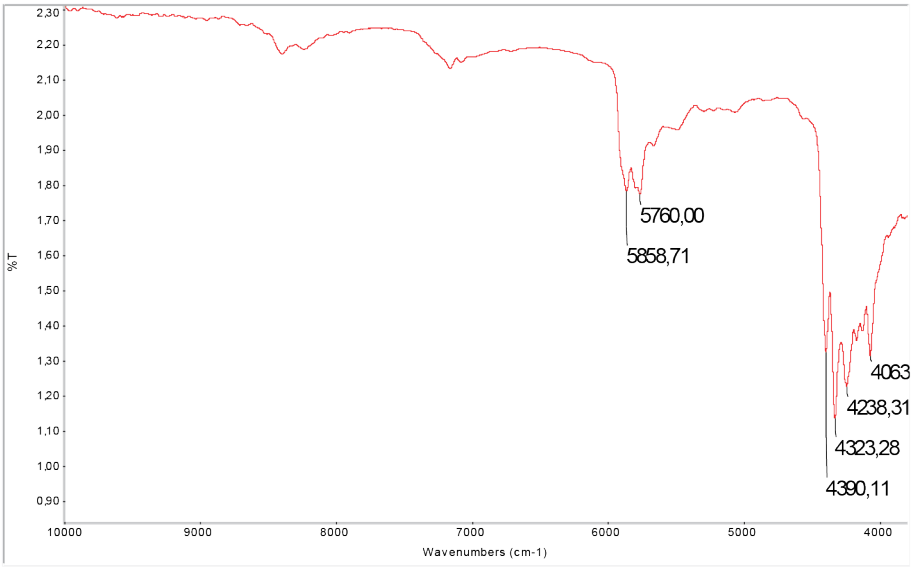

Figure 20 FTNIR spectra of sample A165207.

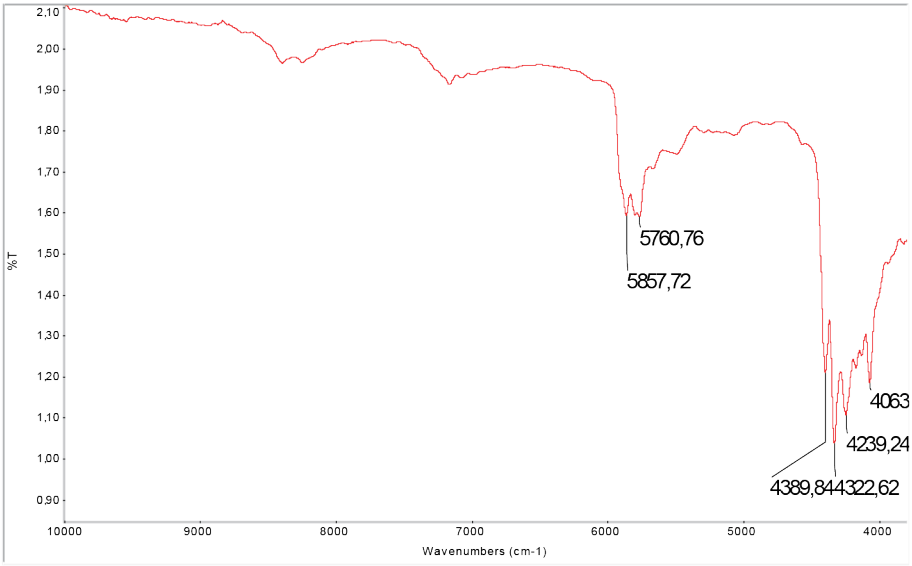

Figure 21 FTNIR spectra of the white fibers (purple line) and blue fibers (red line) of sample A165208.

Annex 4 Raman spectra

Representative Raman spectra of the meshes are displayed below. Raman spectra can used for the identification of materials.

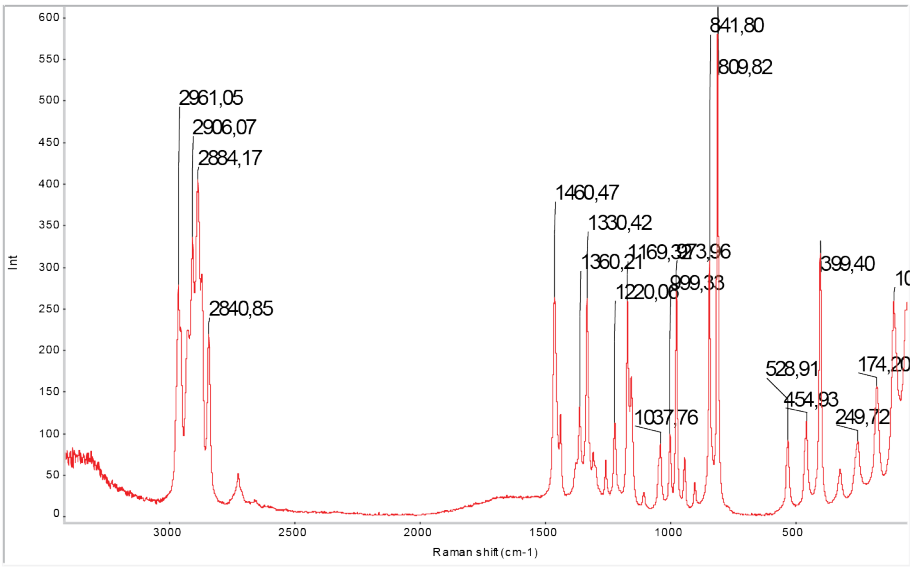

Figure 22 Raman spectrum of sample A165201.

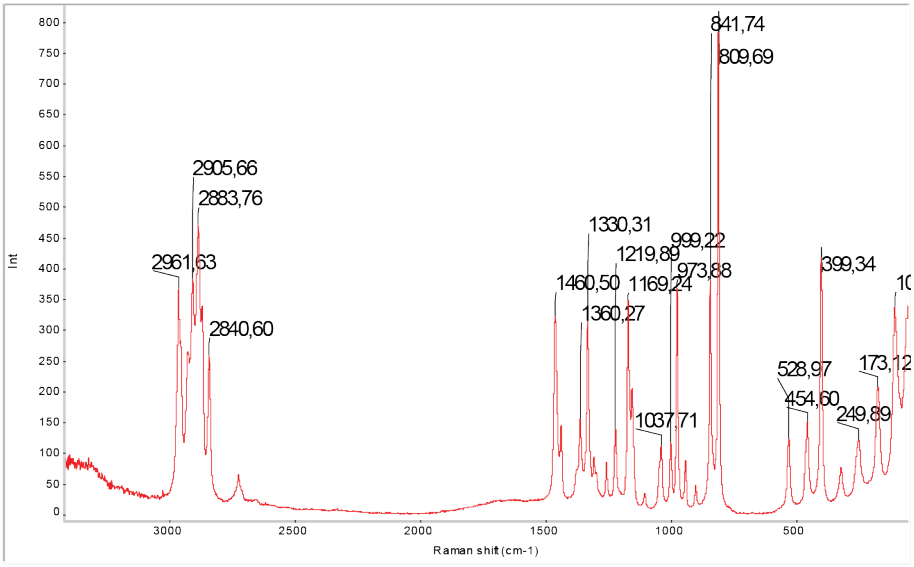

Figure 23 Raman spectrum of sample A165202.

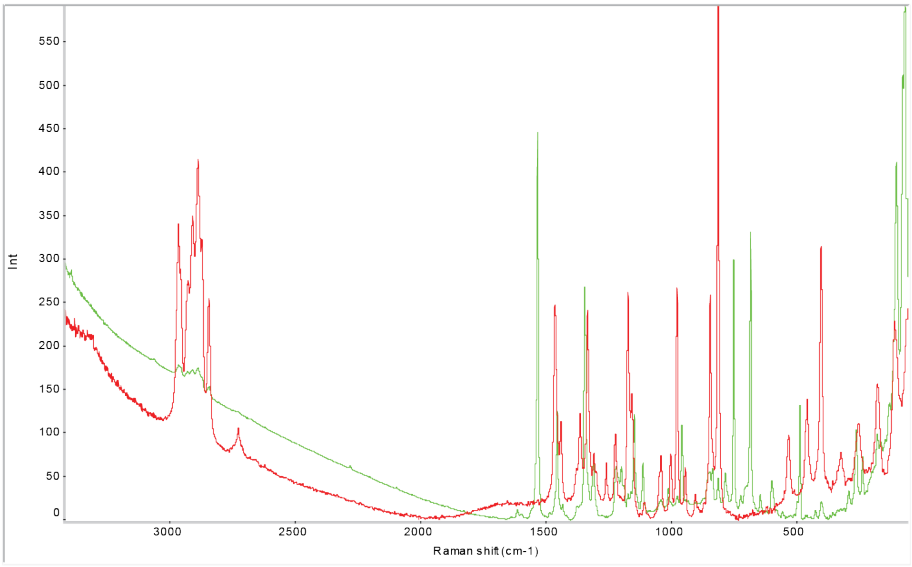

Figure 24 Raman spectra of the white fibers (red line) and blue fibers (green line) of sample A165203.

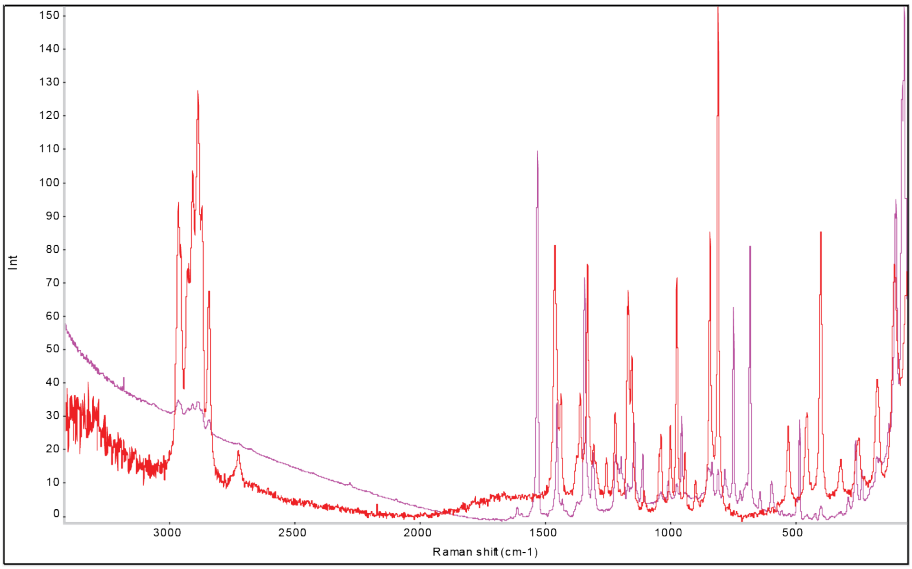

Figure 25 Raman spectra of the white fibers (red line) and blue fibers (purple line) of sample A165204.

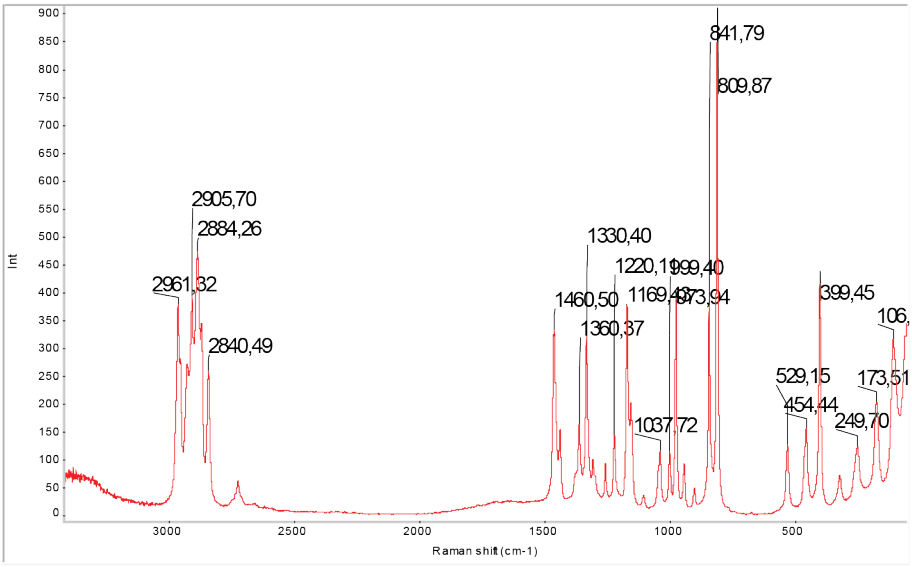

Figure 26 Raman spectrum of sample A165205.

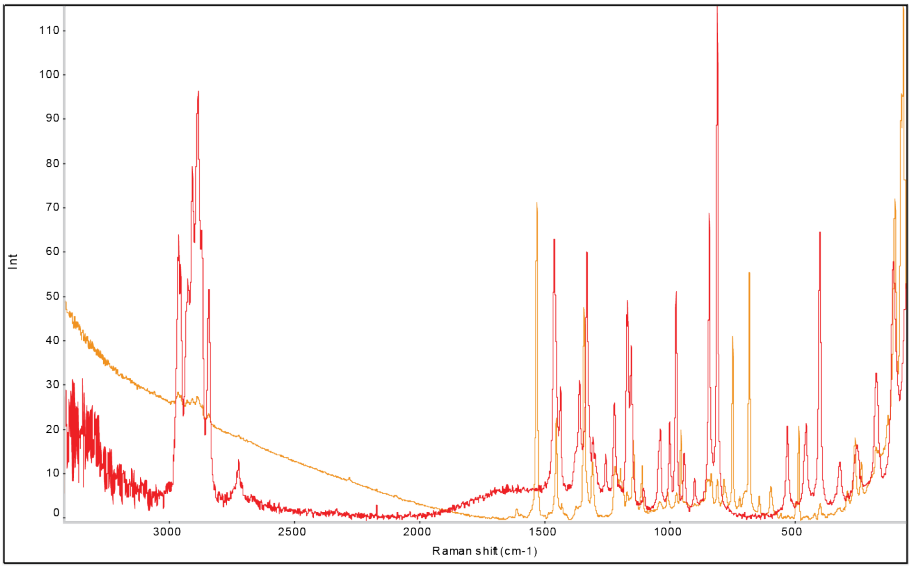

Figure 27 Raman spectra of the white fibers (red line) and blue fibers (orange line) of sample A165206.

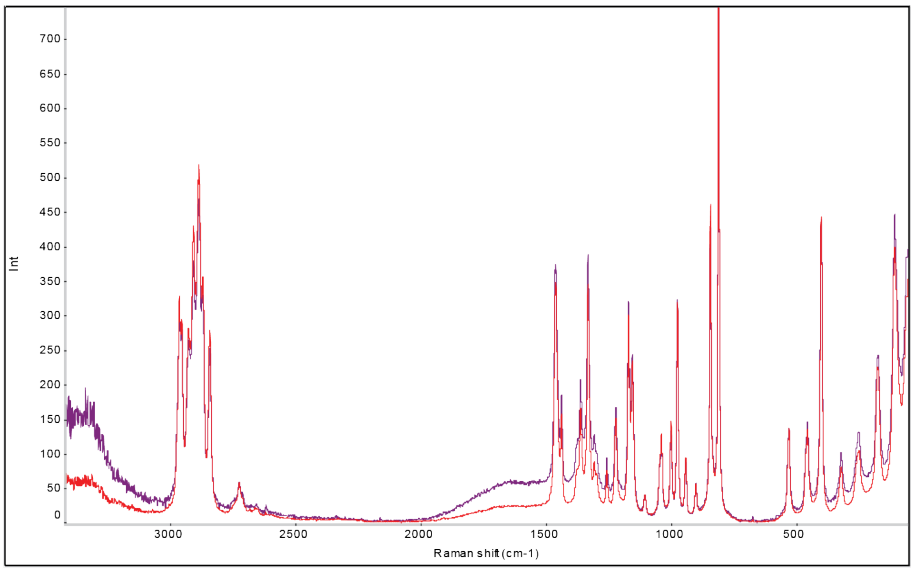

Figure 28 Raman spectra of the fibers around the small (red line) and large pores (purple line) of sample A165207.

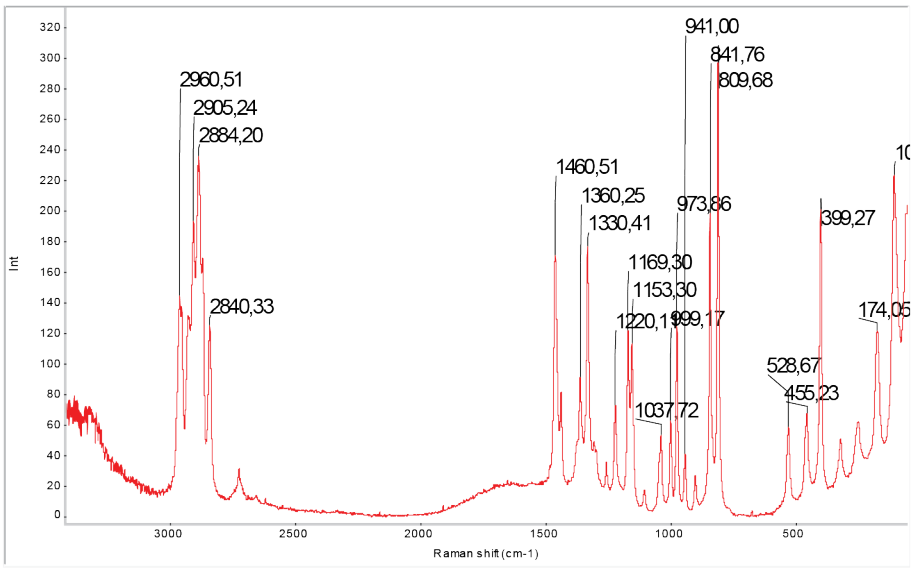

Figure 29 Raman spectrum of sample A165208.

Annex 5 *In vitro* cytotoxicity methods

Study design

The assay to quantify *in vitro* cytotoxicity of material extracts was based on EN ISO 10993-5:2009 [22] and EN ISO 10993-12:2012 [23], and performed in ISO-9001 certified laboratories. In short, we used the mesh product samples described in chapter 2 to prepare extracts for 24 or 72 hours at 37 °C, exposed L929 cells to these extracts for 24 or 72 hours at 37 °C, and subsequently quantitatively assessed *in vitro* cytotoxicity using a WST-1 assay. L929 cells were exposed to the extracts, fresh complete culture medium, 24 and 72 hours incubated in complete medium (to correct for potential nutritional deficiencies in the culture medium during the extraction period), Y4 extracts as positive extraction control, and 10% DMSO as positive assay control. All cytotoxicity experiments were performed between 2019 and 2021. At the time of the 24 hours extraction and 24 hours exposure, A165202 and A165203 were approximately 5 and 6 months past their expiry date. Since the results of their analysis did not deviate from the expected results, it is safe to assume that the cytotoxicity was not affected in the 5 or 6 months after the expiry date.

Results from three independent experiments (biological replicates) all including at least 4 technical replicates (within one individual experiment) were combined into the final data set. The WST-1 assay was used to quantify the metabolic activity at the optimum time point for read-out of the cell cultures representing the amount of viable cells. The average value of the 'Medium control' group was set as 100% and used to normalize the other control and experimental groups. A sample is considered to induce cytotoxicity when cell viability is below 70% of the control cells.

Cell culture

Mycoplasma-free L929 mouse fibroblasts (ATCC CCL-1, Lotnr. 70001022) were grown in DMEM + GlutaMAX (Gibco 61965-026) containing 10% FBS (Greiner Bio-One 758093), 1% MEM NEAA (Gibco 11140-035) and 1% Pen Strep (Gibco 15140-122). Cells were grown in T75 culture flasks (Greiner Bio-One 661 175) in a IFIX-logged incubator at 37 °C and 5% CO_2 in a humidified environment. Cells were passaged at approximately 80% confluency by washing twice in warm PBS (Gibco 14190-094) and detached using 0.05% trypsin/EDTA (Gibco 25300-054). Cells were passaged generally every three to four days, generally split 1:10, and used for experiments between passage 6 and 14.

Extraction process and exposure of cells

Extraction was performed in borosilicate glass vials (ThermoFisher 60180-508) using complete cell culture medium, without the use of any vehicle for extraction. Representative pieces of mesh of 6 cm^2 fiber surface area/ml extraction medium were cut using autoclaved scissors and tweezers in a laminar flow cabinet. As a positive control to the extraction process, 6 cm^2 surface area pieces of PVC 5.8% Genapol X-80

(Y4; previously used at the RIVM[1]) were cut using sterile surgical blades (Swann-Morton No. 23) under aseptic conditions. Mesh and Y4 pieces were then rolled up and put into extraction vials, using separate sets of autoclaved tweezers for each material. Complete medium was added, and extraction vials containing only complete medium were added as negative controls. Extraction was performed at 37°C for 72 hours at continuous rotation in a HulaMixer sample mixer (ThermoFisher 15920D). After 72 hours, the extract was separated from material by pipetting it into new sterile extraction vials and stored at room temperature in the dark for <24 hours before use.

Cells were seeded in 96-well plates (Greiner bio-one 655-180) in 100 µl cell culture medium per well using a multichannel pipet, four wells at a time. No cells were seeded at the border-wells of the plates and generally any row of four seeded wells was surrounded by empty wells. Plates were seeded with 2×10^3 L929 per well, and grown for 24 hours before starting exposure. Individual wells were assessed for aberrations, and wells that deviated from the norm were replaced by back-up wells. Complete medium was replaced by extraction medium four wells at a time, and two extract interference controls were added in wells without cells for each condition. Fresh medium and fresh medium containing 10% DMSO were added as additional negative and positive controls, respectively. Positive control Y4 was placed on separate plates for each time point. Negative controls were also added to these plates to be able to check for plate differences. All wells at plate borders were filled with 200 µl PBS to counter evaporation. After incubation cell cultures were again inspected visually for aberrations, including e.g. cell distribution throughout the wells, cell morphology and color changes of the pH-indicator in the pH buffered culture medium. Subsequently, cell viability was assessed using the WST-1 assay, as described below.

Metabolism-based cell viability assessment

EN ISO 10993-5: 2009 describes the use of MTT/XTT assays to quantify *in vitro* cytotoxicity. MTT, XTT, and WST-1 are all tetrazolium salts used to quantify cell viability, based on the cleavage of a formazan dye in metabolically active cells. The manufacturer states at the product protocol website: "*MTT assay and XTT assay can also be used for measuring cell viability and proliferation*".[2] Such types of assays are described to be applicable in a wide range of experimental set-ups to quantify the viability of mammalian cells. Similar to MTT/XTT [3,4] is the more stable WST-1 assay that we used to quantify cell viability. Metabolism and viability assessments were performed according to the manufacturer using the WST-1 assay (Roche 11 644 807 001). In addition, control samples without cells were included to check for any interference of the samples with the assay reagents or read-outs. No interference was observed.

[1] Coleman, K. P. et al. (2018). Preparation of irritant polymer samples for an in vitro round robin study. *Toxicology in vitro.* https://doi.org/10.1016/j.tiv.2018.01.018
[2] Protocol Guide: WST-1 Assay for Cell Proliferation and Viability (sigmaaldrich.com) last visited 24-06-2022.
[3] Kim, K.M., et al., *Comparison of Validity between WST-1 and MTT Test in Bioceramic Materials.* Key Engineering Materials, 2005. **284-286**: p. 585-588.
[4] Ngamwongsatit, P., et al., *WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic Bacillus species using CHO cell line.* Journal of Microbiological Methods, 2008. **73**(3): p. 211-215.

WST-1 was pre-warmed and homogenized before 1:10 addition of WST-1 to complete medium. In 96-well plates, WST-1 was added using a multichannel pipet. One to three interference controls were always included in order to create in-experiment reference measurements. After the start of the incubation of the cell culture with the WST-1-medium starts, cleavage of the reagent happens resulting in a change of color of the supernatant. We measured the experimental plates multiple times over the course of 3 hours in order to find the optimum optical density. Taking the equipment sensitivity into consideration, as well as the spread in signal through-out the different samples included in the study. Based on the values measured we picked the optimum time-point for data analysis. For the 4 independent experimental runs executed for the 72 hours study, data were used of the quantification after respectively 2, 1, 2 hours, and 50 minutes of incubating WST-1 with the cell culture. The runs for the 24 hour study were quantified after 2 hours of incubation. We measured absorbance at 440 nm using a SpectraMax M2 plate reader (Molecular Devices). 620 nm background absorbance was subtracted from all values, and subsequently averaged treatment interference values were subtracted from sample values before further analysis. All experiments were independently executed four times on different days in order to obtain experimental replicates.

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