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Abrasion Testing of Graphene Enhanced 3 Layer TNT Heat Sealed Texile

Project Code: P5118

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Revision

2

Report for:

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Third party disclaimer

Document revisions

No	Details	Date
2	Directa Plus requested updates	7 Oct 21

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1. Introduction

At the request of Carolina Volonte at Directa Plus Spa, IOM undertook a pilot study to determine the release of free graphene platelets (as a High Aspect Ratio Nanomaterial) from 3 Layer TNT heat sealed textile samples when an abrasion force is applied.

As indicated within the proposal currently there is no specific standard to measure particulate release for textiles during wearing. This testing is an adaptation of a textile industry ISO standard for abrasion testing,[1] in combination with characterisation and measurement being determined with reference to the ISO/TC 229 Nanotechnology. This work is carried out as a pilot study to determine which measurements are suitable based on the sample matrix and results required by Directa Plus.

Following previous experimentation and discussion with Directa Plus, the testing exposure time of this fabric was reduced due to delicate nature of the fabric. In Directa Plus opinion the testing conducted is more aggressive than the intended wear as the observed fabric destruction is greater than expected from their experience and as such the testing represents a worse case scenario in regards to emission and release

2. Methodology

2.1. Abrasion Testing

Determination of particle release when an abrasion force is applied was carried out by following the ISO 12947 standard for "Determination of the abrasion resistance of fabrics".[1] A Martindale abrasion tester was used to subject the textile to a defined load and number of rubs. This was carried out against an abrasive medium (i.e. standard abradant fabric) in a translational movement tracing a Lissajous figure. The rotational frequency of the outer drive units was 47.5 min^{-1}, 9kPa nominal pressure was applied and the number of rubs was set at 500.

In deviation from the standard, we did not measure the endpoints of mass loss. Instead, we identified and characterised particle release by real-time and static monitoring methods, as outlined in the proposal (ref. Q1161). Further to this, appearance change and specimen breakdown was analysed by SEM/EDXS to determine the potential for graphene release.

Note: IOM has identified during the proposal steps with Directa Plus that the number of test rubs would be set to 500, due to delicate nature of the test fabric and proposed end application, this is a deviation from the test

[1] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method (ISO 12947)

standard, which is already being adapted to determine exposure/release of graphene in place of visible ware for the abrasion testing. The final decision to conduct exposure/release determination was set by Directa Plus.

Two test materials were provided by Directa Plus; a) the 3 Layer TNT heat sealed base textile containing no graphene coating (subsequently referred to as "reference sample") and b) the 3 Layer TNT heat sealed textile with graphene coating (subsequently referred to as "graphene-enhanced fabric"). Four replicates of each textile were tested on the Martindale at one time in an attempt to enhance any possible graphene release. The samples and foam supports were cut to a diameter of 38 mm before mounting into the sample holder (as shown in Figure 2.1). The standard abradant material (flat woven wool fabric, 140 mm diameter) and felt underlay materials were used as received from suppliers.

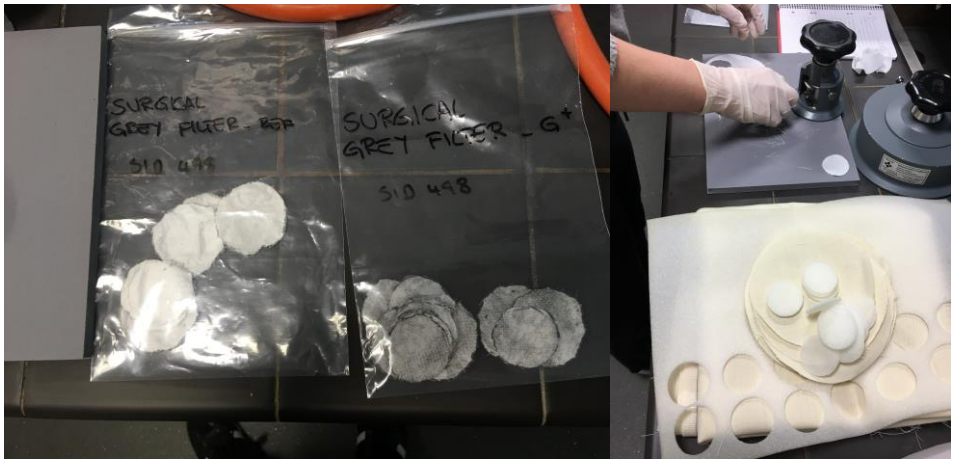


Figure 2.1 3 Layer TNT heat sealed textile samples (left) and polyetherurethane foam inserts cut to correct size (right)

Figure 2.2 outlines the position of the real-time sampling tubes and the static filter sampling pumps.

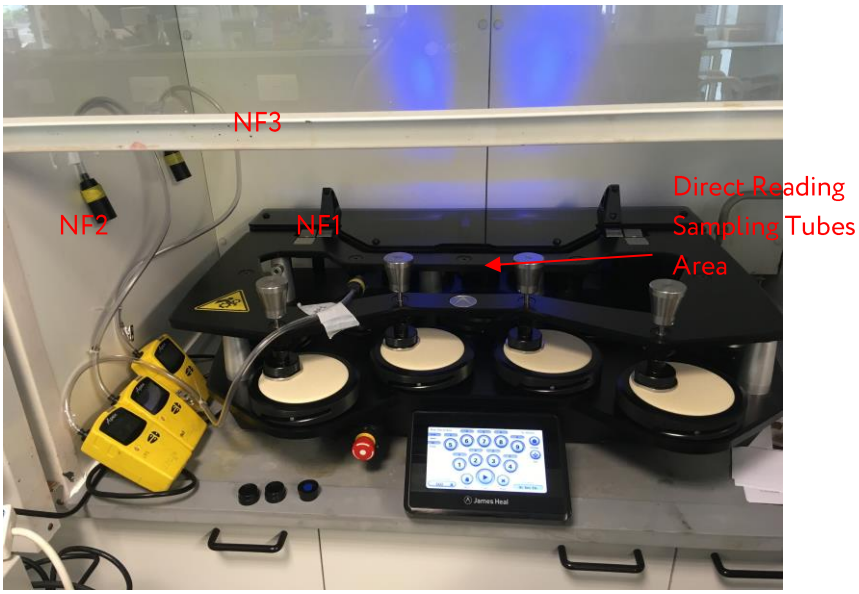


Figure 2.2 Image of Martindale instrument with static sampling and real-time sampling positions outlined

2.2. Real-Time Particle Monitoring

IOM established practices for determining particle release are consistent with BSI recommendations[2,3,4] for the identification and assessment of emissions of airborne manufactured nanomaterials in the workplace. Multiple instruments, suited for the detection of both nano- and micron-sized particles, acknowledged as good practice by BSI, were deployed for real-time characterisation of particle release (number, mass, size distribution) to give an informed assessment of the potential for release.

Condensation Particle Counter – CPC (TSI, Model 3007)

The CPC instrument is a hand held portable instrument which detects particle concentration within the 5 nm – 1 µm size range as a function of time, making it ideal for the primary identification of sources of particle emission during workplace activities. Particles present in the sample stream serve as condensation sites for the alcohol vapour in the instrument. Once condensation begins, particles grow quickly into larger alcohol droplets which pass through an optical detector and are counted. This instrument does not classify according to particle size.

Aerodynamic Particle Sizer Spectrometer - APS (TSI, Model 3321)

The APS sizes particles in the range from 500 nm to 20 microns using a time-of-flight light-scattering technique that measures aerodynamic diameter in real time. Particle classification results from differences in the mobility of particles based on their size, density and charge as they travel through an optical detector. Results are presented as aerodynamic equivalent diameter. This is defined as the physical diameter of a unity density sphere that settles through the air with a velocity equal to that of the particle in question.

Aerodynamic diameter is a significant aerosol size parameter as it determines the particles behaviour while airborne. Particles that have the same aerodynamic diameter will exhibit the same airborne behaviour, regardless of their physical size, shape, density or composition. Knowledge of the aerodynamic diameter subsequently allows determination of where the particle will be deposited in the human respiratory tract[5] and whether the particle will penetrate a filter, cyclone or other particle-removing device.

DustTrak DRX (TSI, Model 8533)

The DustTrak DRX air sampler is a laser photometer that simultaneously measures both mass and size fraction of airborne particulates. The instrument simultaneously measures PM1, PM2.5, PM4, PM10 and TPM (total particulate matter).

[2] Workplace atmospheres – Ultrafine, nanoparticle and nano-structured aerosols – inhalation exposure characterisation and assessment (PD ISO/TR 27628:2007)

[3] Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies (PD ISO/TR 12885:2018)

[4] Nanotechnologies – Part 3: Guide to assessing airborne exposure in occupational settings relevant to nanomaterials (PD 6699-3:2010)

[5] Workplace atmospheres – Size fraction definitions for measurement of airborne particles. CEN, European Committee for Standardisation. European Standard EN 481:1993.

2.3. Static Aerosol Particle Sampling

To facilitate the specific identification of materials released from the textiles, localised particle sampling was carried out.

Cowl Sampling Head for SEM and Elemental Analysis

An open cowl sampling head (25 mm) connected to a battery operated pump was used for particle collection onto polycarbonate filter (25 mm x 0.4 µm) for subsequent analyses by Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy (SEM/EDXS) to image and identify any particles sampled.

Apex sample pumps were calibrated to a flow rate of 2.2 L/min; flow rates were checked at the start and end of the sampling activities.

Surface Tape Samples, Sampling and Analysis

To facilitate the specific identification of materials released from the textile during abrasion, surface samples were taken, where considered appropriate, with proprietary Stick-to-It® sampling tapes. After sampling, the tape was placed in its sealed holder to avoid contamination.

2.4. Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy (SEM/EDXS)

The filter, textile and tape samples collected were analysed by image and elemental profiling using a modification of the following method:

SOP-009_V2: "Scanning Electron Microscopy – sample preparation, EDXS analysis and systematic filter analysis".

The technique for systematic filter analysis is adopted from ISO 14966:2002: "Ambient Air – Determination of numerical concentration of inorganic fibrous particles – Scanning electron microscopy method".

In preparation for SEM/EDXS analysis, a portion of each filter, tape or textile sample was excised and mounted onto a 13mm diameter aluminium SEM stub and coated with a thin layer of gold to enhance the conductivity of the surface and the imaging resolution. Images were recorded at various magnifications to best represent the distribution, size and shape of particles captured from the testing process and elemental analysis was carried out for chemical composition.

3. Results

3.1. Visual observations of abrasion testing

Photographs were taken of the textiles before and after abrasion testing to determine if there had been any visible signs of wear during the testing. As observed in Figure 3.1 and Figure 3.2, notable visible wear occurred for each material tested, especially the graphene enhanced material. Both surface bobbling and material gathering was observed, and some rips or tears were noted in the graphene enhanced fabric. Fibrous strands of the abradant material (beige flat woven wool fabric) were visible on the Martindale instrument, sample holder and test material following abrasion testing after 500 rubs.

Close visual inspection of the abradant material following testing of the graphene-enhanced material, showed no visible evidence of graphene transfer to the abradant material, despite the damage to the test fabric (Figure 3.2). If transfer had occurred in significant amounts we would expect to observe black residue on the abradant material.

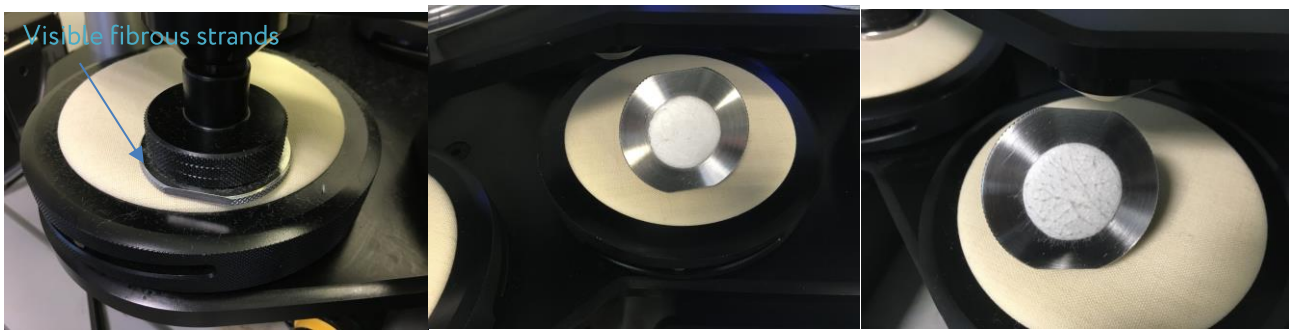


Figure 3.1 Image showing visible fibrous strands (left), wear to baseline fabric (middle) and abradant material (right).

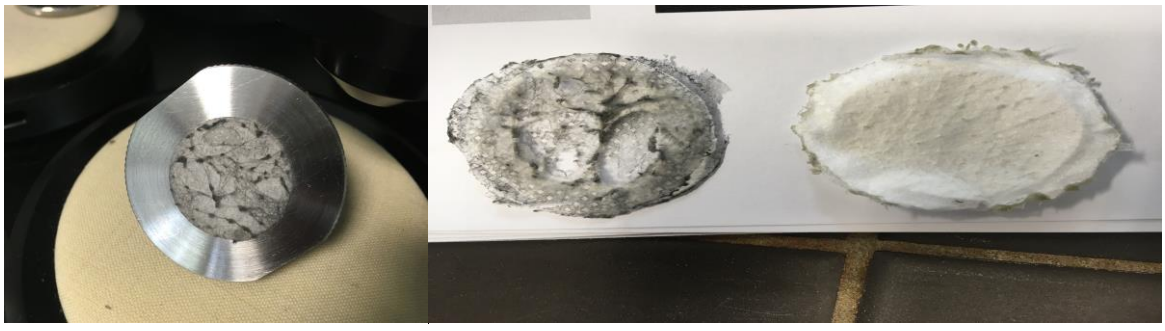


Figure 3.2 Image showing wear to graphene-enhanced fabric post-test (left) and post testing abradant materials (right).

3.2. Real-Time Monitoring

An overview of the real time monitoring is given in Figure 3.3, presenting how the measured particle concentration varied over time during abrasion testing of both the reference material and the graphene-enhanced material. The instrument sampling tubes were positioned on the top of the Martindale instrument and directly beside the site of abrasion (**Figure 2.2**). The monitoring was conducted over approximately 95 minutes to allow repeat monitoring and a description of activities carried out during this time is outlined in Table 3.1.

Note: Due to onsite instrument failure DustTrak data only available for repeat monitoring.

Table 3.1 Activity Information

Elapsed Time (s)	Testing Material	Description
10:25:05	Background measurement – no fabric	Start background - Run 1 reference & enhanced 3 Layer TNT heat sealed
10:37:53	Reference sample	Start Martindale running
10:47:17	Reference sample	Stop Martindale running
11:04:38	Graphene enhanced fabric	Start Martindale running
11:14:08	Graphene enhanced fabric	Stop Martindale running
11:30:04	Background measurement – no fabric	Start background - Run 2 reference & enhanced 3 Layer TNT heat sealed
11:35:38	Reference sample	Start Martindale running - Rpt
11:46:26	Reference sample	Stop Martindale running - Rpt
11:51:19	Graphene enhanced fabric	Start Martindale running - Rpt
12:01:49	Graphene enhanced fabric	Stop Martindale running - Rpt

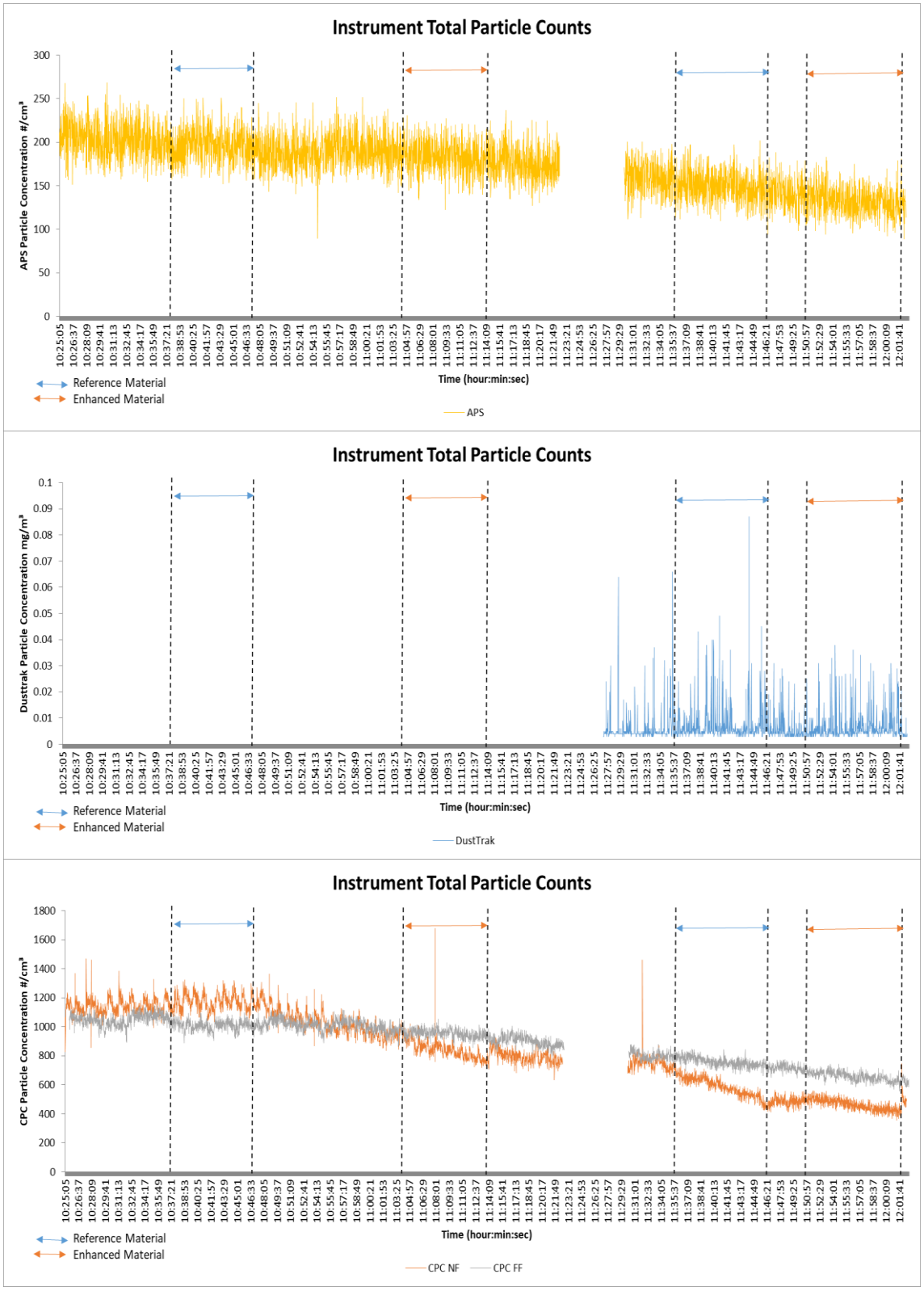


Figure 3.3 Top – APS; Middle – DustTrak particle concentration-time series (Rpt only); Bottom CPC, Abrasion testing. Instrument detection limits outlined in Section 2.2.

3.2.1. Reference material

The CPC, DustTrak and APS concentrations, collectively covering a size range of 5 nm to 20 µm, remained low and stable while abrasion occurred during all testing. A slight decrease in the baseline levels were detected by the CPC and APS over the period of the sampling, however this was observed on both the near and far field instruments suggesting decreasing particle disturbance due to lack of movement within the laboratory building through the day.

3.2.2. Graphene-enhanced material

The CPC particle concentration (size range of 5 nm to 1 µm) was seen to steadily decrease over the monitoring of abrasion testing of the graphene-enhanced material and the repeat monitoring. Over the testing, the concentration was seen to decrease gradually from 1200 to 600 particles/cm^3. It should however be noted that for this type of testing environment these are very low total counts and the trend observed is not significant. The most likely explanation for this observation is a general reduction in heat/activity as the day progressed (lab was not occupied due to COVID restrictions and building sparsely populated), leading to an decrease in natural and anthropogenic particles and dusts.

Overall, no significant difference is noted for the total particle counts recorded on any of the direct reading equipment for either fabric when compared with the background or each other; and therefore we do not associate this change to the tested sample itself.

With no significant change from the initial background monitoring during all the fabric testing conducted, and the same data trend was indicated on both the near- and far-field CPC instruments this suggests the decreasing particle total trend was typical for the day of sampling. Analysis of samples for SEM collected both in the near- and far-field during the abrasion testing of the graphene enhance fabric is presented below in Section 3.3.

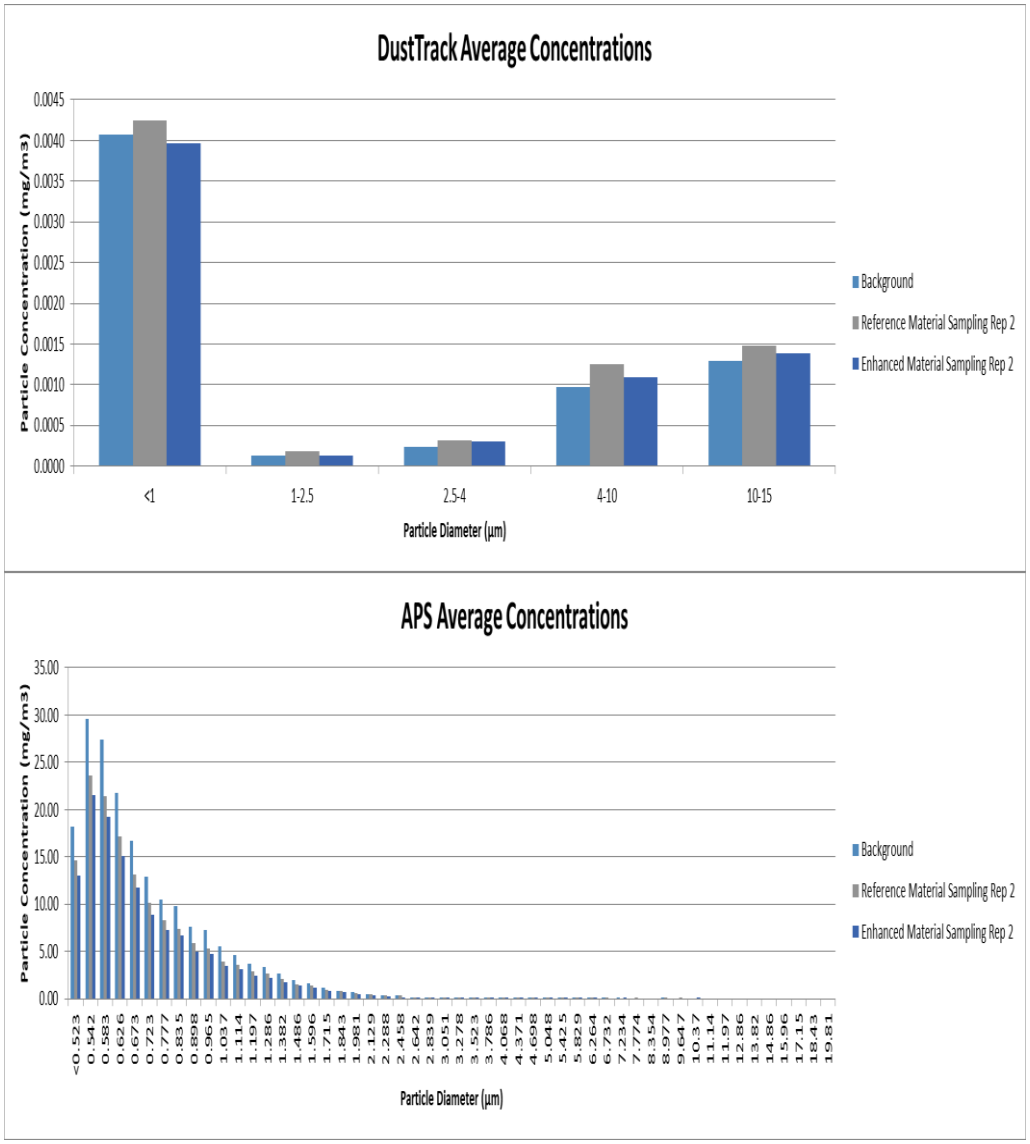


Figure 3.4. DustTrak and APS particle size distribution (PSD) graphs of the background period before the start of abrasion testing and for graphene-enhanced textile testing. DustTrak, size range <1 – 15 µm. APS, size range 500 nm - 1 µm.

Overall, the real-time data suggests no significant airborne release from abrasion of the graphene-enhanced materials. However, analysis of the static filter samples and SEM imaging (Section 3.3) of the material before and after abrasion is necessary to confirm this hypothesis.

3.3. SEM/EDXS Analysis

3.3.1. Air filter samples

The air filter samples collected during the abrasion testing of the materials were analysed by SEM imaging and elemental profiling to identify the presence and identity of any released particles. **Figure 2.2** indicates the positioning of the static sampling beside the Martindale instrument. A summary of the collected samples is given in Table 3.2 and the associated analysis in Table 3.3 to Table 3.5.

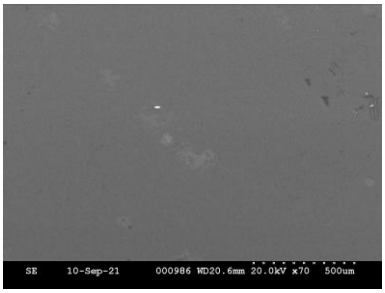
Table 3.2 Air filter samples collected

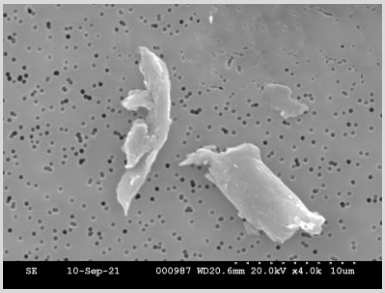
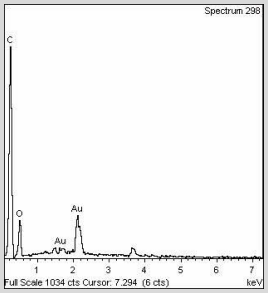
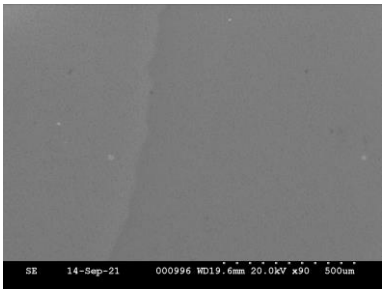
Sample ID	Description (see Figure 2.2)	Sampling time (min)
(a) Far Field Sampling		
DP01	Far-field position outside of fume hood, refernce material testing	17
DP05	Far-field position outside of fume hood, graphene enhanced fabric testing	12
*(b) During abrasion testing of **reference material***		
DP02	Near-field position NF_1	17
DP03	Near-field position NF_2	17
DP04	Near-field position NF_3	17
*(c) During abrasion testing of **graphene-enhanced material***		
DP06	Near-field position NF_1	17
DP07	Near-field position NF_2	17
DP08	Near-field position NF_3	17

(a) Far field sampling

Analysis of the SEM images obtained from the far-field position outside of the fume hood when the abrasion was occurring is given in Table 3.3.

Table 3.3 SEM/EDXS Analysis results for background filter samples

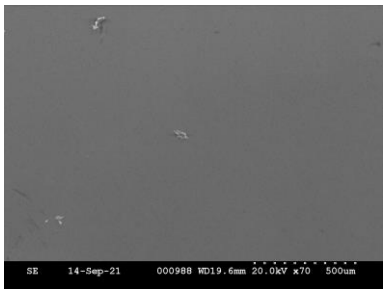
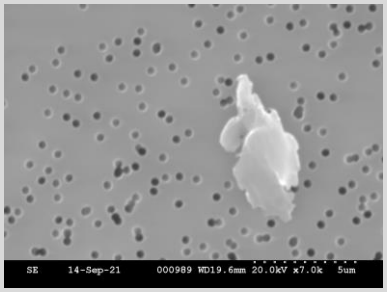
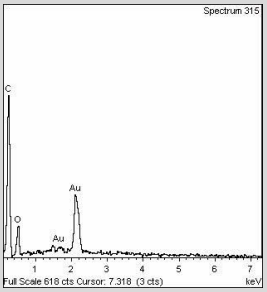
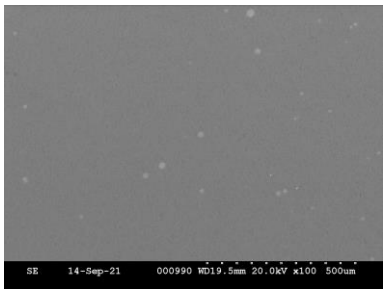
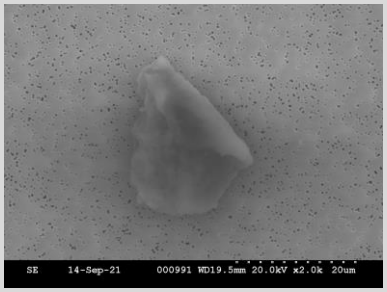
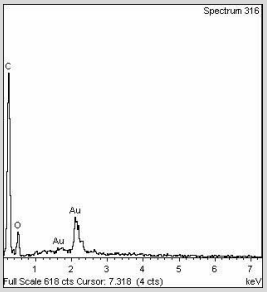
Sample ID		SEM Images	EDXS Spectra	Comment
DP01	A		N/A	Low magnification image showing very light loading of filter.

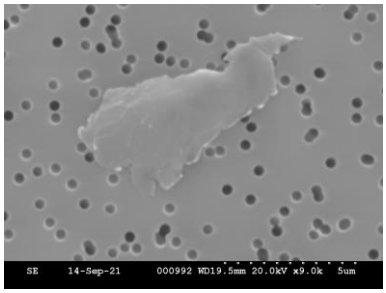
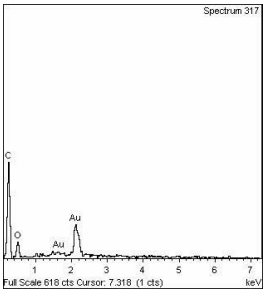
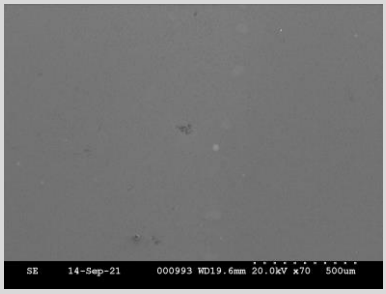
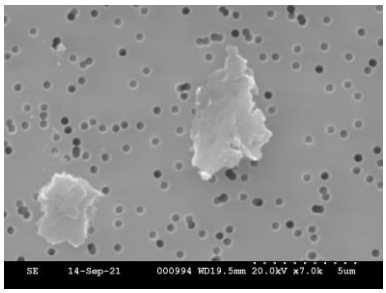
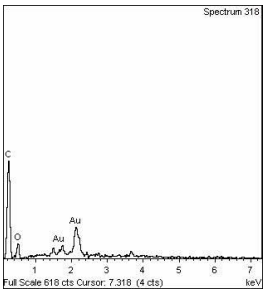
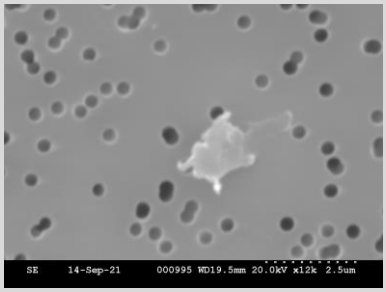
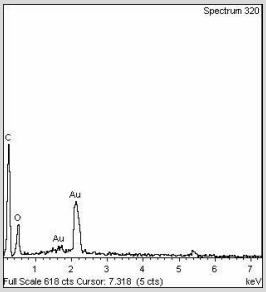
Sample ID		SEM Images	EDXS Spectra	Comment
	B			Platelets of dimensions 4.1 x 13.8 µm (left) and 4.1 x 12.1 µm (right), exhibiting C and O in EDXS spectrum.
DP05	A		N/A	Low magnification image showing very light loading of filter. No particles of interest were observed.

(b) Reference material

Analysis of the SEM images obtained during abrasion testing of the reference material is given in Table 3.4.

Table 3.4 SEM/EDXS Analysis results for filter samples from abrasion testing of the reference material

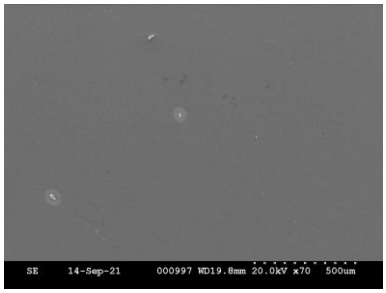
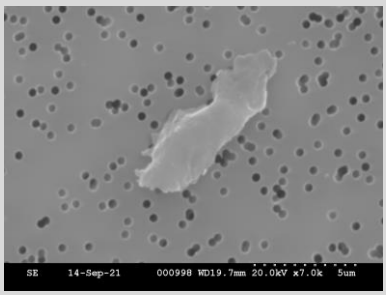
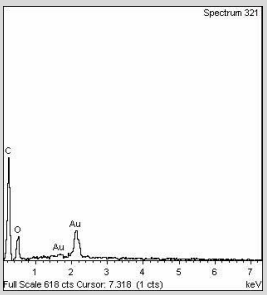
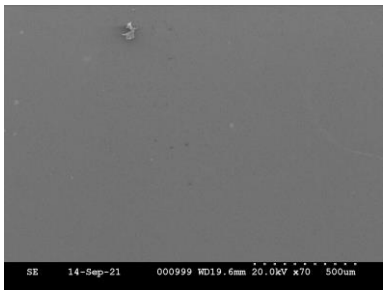
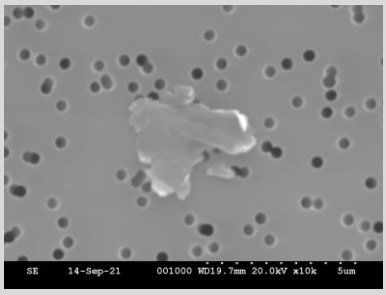
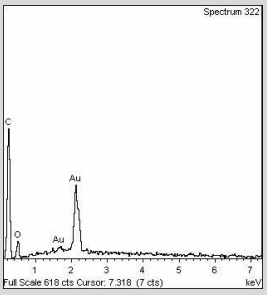
Sample ID		SEM Images	EDXS Spectra	Comment
DP02	A		N/A	Low magnification image showing very light loading of filter.
	B			Particle of size 3.4 x 7.4 µm exhibiting C and O in EDXS spectrum.
DP03	A		N/A	Low magnification image showing very light loading of filter.
	B			Particle of size 20.7 x 27.0 µm exhibiting C and O in EDXS spectrum.

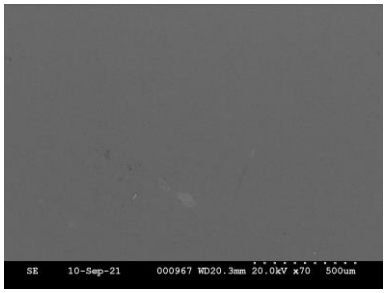
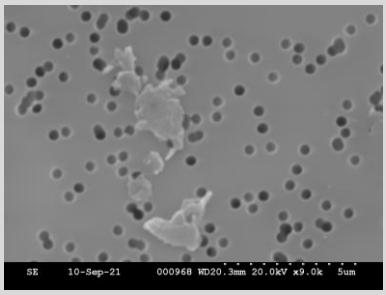
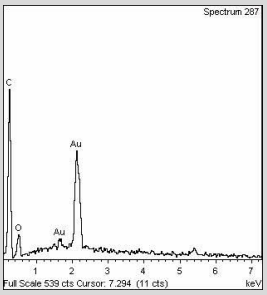
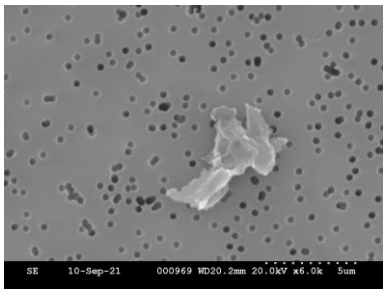
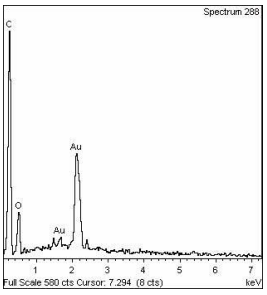
Sample ID		SEM Images	EDXS Spectra	Comment
	C			Platelet of size 4.3 x 8.8 µm, EDXS exhibiting C and O.
DP04	A		N/A	Low magnification image showing very light loading of filter.
	B			Particles of size 3.5 x 3.6 µm (bottom left) and 3.9 x 6.5 µm (middle) exhibiting C and O in EDXS spectrum.
	C			Particle of size approx. 1.6 x 2.0 µm exhibiting C and O in EDXS spectrum.

(c) Graphene-enhanced material

Analysis of the SEM images obtained during abrasion testing of the graphene-enhanced material is given in Table 3.5.

Table 3.5 SEM/EDXS Analysis results for filter samples from abrasion testing of graphene-enhanced material

Sample ID		SEM Images	EDXS Spectra	Comment
DP06	A		N/A	Low magnification image showing very light loading of filter.
	B			Platelet of dimensions 3.2 x 8.8 µm exhibiting C and O in EDXS spectrum.
DP07	A		N/A	Low magnification image showing very light loading of filter.
	B			Platelet of dimensions 3.7 x 4.4 µm exhibiting C and O in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
DP08	A		N/A	Low magnification image showing very light loading of filter.
	B			Aggregated platelets of lengths approximately 0.8 – 2.6 µm exhibiting C and O in EDXS spectrum.
	C			Platelet of approximate dimensions 4.6 x 6.7 µm, exhibiting C and O in EDXS spectrum.

Very few particles were observed on the filters collected during abrasion sampling of the graphene-enhanced fabric. This is most likely due to the very short sampling time due to the reduced number of rubs to the fabric.

The particles on these filters exhibited a morphology and composition similar to what could be expected for graphene. However, material with a similar morphology was found on filters collected during testing of the reference 3 Layer TNT heat sealed fabric, i.e. with no graphene coating (Table 3.4). It is therefore not possible to conclusively identify these platelets as graphene.

The use of free graphene reference material for our own SEM analysis would be useful to confirm or refute these observations. Additionally, the use of TEM analysis in place of SEM would allow distinction between graphene platelets and other carbonaceous material.

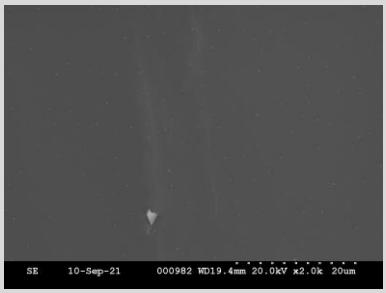
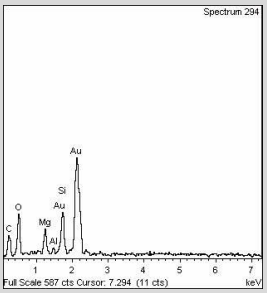
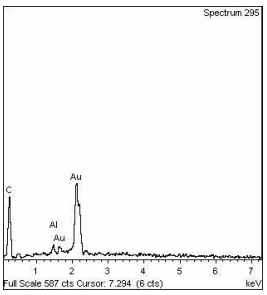
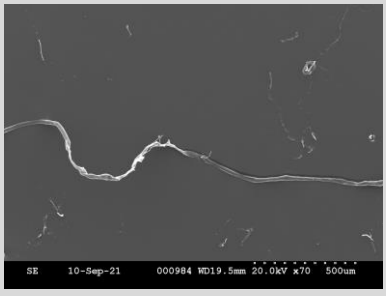
3.3.2. Stick-to-it® tape samples

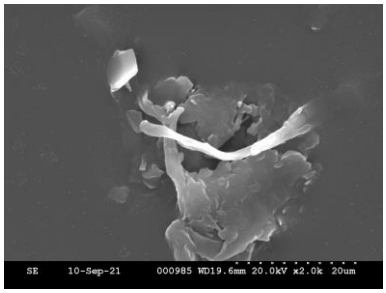
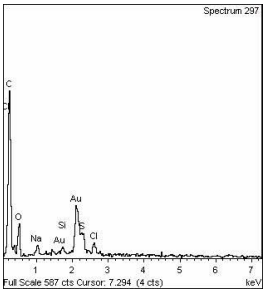
The Stick-to-it® samples were analysed by SEM and EDXS to identify the presence and identity of any released particles. These samples were taken from various positions on the Martindale instrument and sample holder after the abrasion testing had stopped. A summary of the collected samples is given in Table 3.6 and the associated analysis in Table 3.7.

Table 3.6 Stick-to-it® tapes collected

Sample ID	Description
DP09	Material (including visible abradant fibres) collected from the edges of the sample holder on Martindale instrument after abrasion test of reference material
DP10	Material (including visible abradant fibres) collected from the edges of the sample holder on Martindale instrument after abrasion test of graphene-enhanced material
DP11	Material collected from the surface of the abradant material after abrasion test of graphene-enhanced material

Table 3.7 SEM/EDXS Analysis results for Stick-to-it® tapes

Sample ID		SEM Images	EDXS Spectra	Comment
Reference material (no graphene)				
DP09				No significant particles observed on sample
Graphene-enhanced material				
DP10	A			Very few particles observed on sample. Inorganic particle present showing C, O, Mg, and Si in EDXS spectrum.
	B			Organic material observed measuring approximately 6.8 µm in length.
DP11	A		N/A	Low magnification view of sample with some large fibres visible.

Sample ID		SEM Images	EDXS Spectra	Comment
	B			Example image showing some platelet-like material exhibiting C, O, Na and Cl in EDXS spectrum.

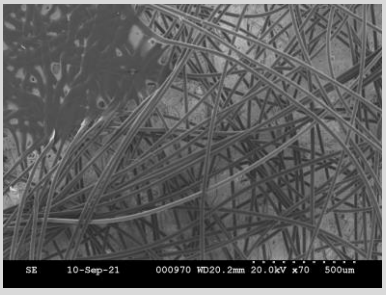
3.3.3. Textile material

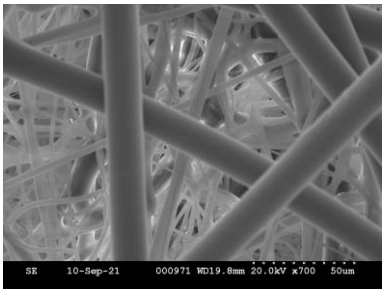
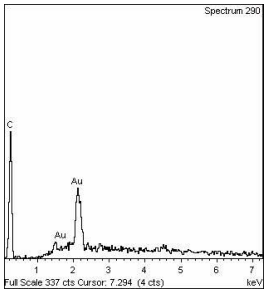
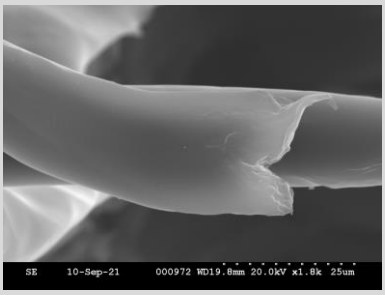
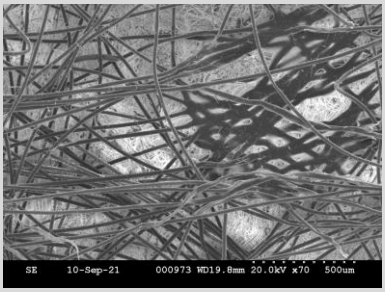
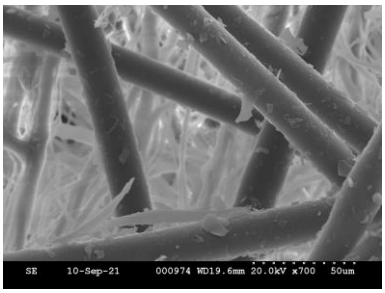
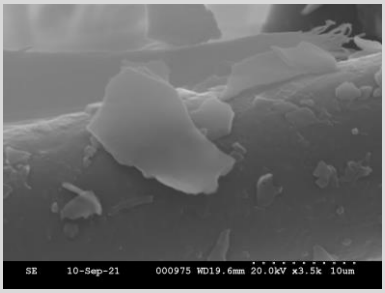
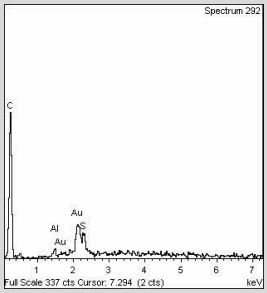
In addition to the collected filter samples, SEM and EDXS imaging was conducted on the two textile materials before and after the abrasion testing to see if any difference was evident. A summary of the samples is given in Table 3.8 and Table 3.9.

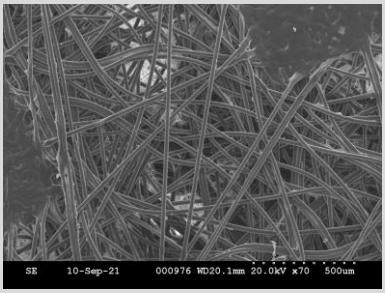
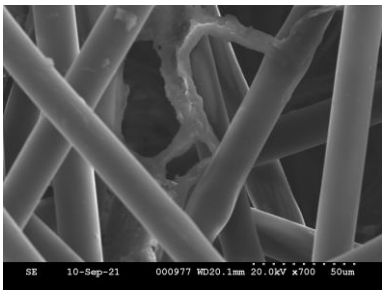
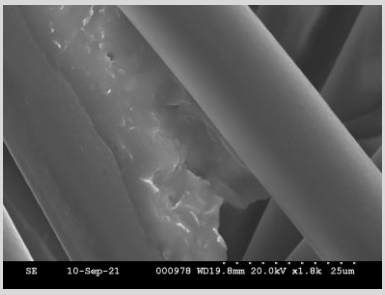
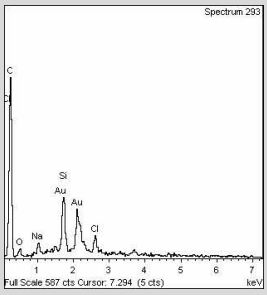
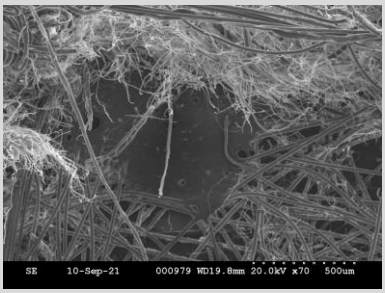
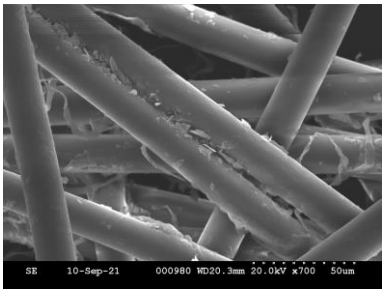
Table 3.8 Textile materials

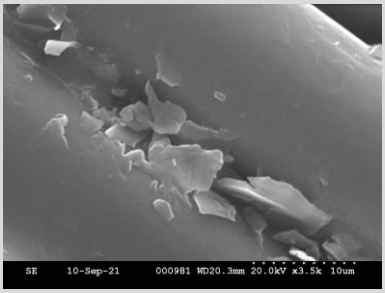
Sample ID	Description
Reference material	
DP14	Reference textile material **prior to** abrasion testing
DP12	Reference textile material **after** abrasion testing
Graphene-enhanced material	
DP15	Graphene coated textile material **prior to** abrasion testing
DP13	Graphene coated textile material **after** abrasion testing

Table 3.9 SEM/EDXS Images of fabrics

Sample ID	SEM Images		EDXS Spectra	Comment
Reference material (no graphene) – Before Abrasion				
DP14	A		N/A	Overview of reference fabric prior to abrasion testing.

Sample ID		SEM Images	EDXS Spectra	Comment
	B			Closer view of threads showing distinction between the top layer threads (thick) and middle layer threads (thin).
	C		N/A	Magnified view showing end of fibrous strand.
Reference material (no graphene) – After Abrasion				
DP12	A		N/A	Low magnification image showing the reference fabric following abrasion testing. Top layer fibres show much more disorder than image DP14 –A.
	B		N/A	Magnified view of threads showing some collection of small particles on the surface of the threads. Thin fibres of inner layer show breakages, likely the cause of the small particles on larger fibres.
	C			Closer view of platelet material deposited on large fibres of fabric top layer. Large platelet in the centre of the image measures approximately 14 µm in length, other smaller platelets are <4 µm and hence would be in the respirable range.
Graphene-enhanced material – Before Abrasion				

Sample ID		SEM Images	EDXS Spectra	Comment
DP15	A		N/A	Low magnification image showing the graphene coated fabric prior to abrasion testing.
	B		N/A	Closer view of fibrous top layer, showing the graphene coating on surface of textile.
	C			Graphene coating adhering to threads of the top layer of the textile. Surface is slightly rough in texture due to graphene platelets.
Graphene-enhanced material – After Abrasion				
DP13	A		N/A	Low magnification image showing the graphene-enhanced fabric following abrasion testing. Higher degree of breakages to the fibrous strands than was observed for the reference material.
	B		N/A	Higher magnification of thick fibres from top layer of fabric. Deposition of platelets on the fibres can be noted. Deposition of these platelets appears to be concentrated between fibres where the graphene coating was noted in image DP15-C.

Sample ID	SEM Images	EDXS Spectra	Comment
C		N/A	Closer view between threads shows the platelets in more detail. Width of platelets vary, with some measuring <4 µm and hence these would be in the respirable range.

4. Discussion and Recommendations

4.1. Visual inspection of abrasion testing

Following agreed abrasion testing protocol set with Directa Plus, both materials showed obvious wear by visual inspection even after 500 rubs. As was identified in the previous study (P2590), fibrous strands were visible on the Martindale equipment once the abrasion test had finished, and due to their colour and appearance these were attributed to the abradant material (beige flat woven wool fabric). As seen in Figure 3.2, no visible transfer of graphene to the abradant material was observed following abrasive testing of the graphene-enhanced textile.

The abradant material used was a woven wool fabric as recommended in the ISO standard (ISO 12947-2).[6] It is suggested that further assessment may involve using test material as the abradant material on the base pad of the Martindale instrument to reduce the presence of any contaminants from other materials and this would also give an indication of how the graphene-enhanced material wears in contact with itself.

4.2. Real-time Monitoring

Data collected by real-time monitoring showed, in general, steady particle concentration values throughout sampling at relatively low levels of measurement. Additionally, very low level of deposition on the air filters were detected at all sampling positions and during all material testing.

A gradual decrease in particle concentration was observed on the CPC during sampling and repeat sampling of the abrasion test on the reference and graphene-enhanced fabric conducted later in the day. The likely reasons why this gradual decrease may have occurred is a general reduction in heat/activity as the day progressed and reduced particle disturbance in sampling area due to limited access to the sampling facility during current COVID restriction, although the total counts are very low throughout sampling and the changes are not significant for the testing environment or suggesting delayed particle deposition from earlier testing. SEM analysis of the air filters collected during abrasion showed no notable difference in deposition between the reference and graphene-enhanced materials in both the near and far field sampling. Additionally no significant peaks or baseline changes were observed on the APS or DustTrak instruments during the activities monitored.

[6] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method - Part 2: Determination of specimen breakdown (ISO 12947-2:2016)

4.3. SEM/EDXS Analysis

All filter samples collected showed very light loading, with very few particles of interest observed. This may be a result of the very short sampling time of this test due to the reduced number of rubs to the fabric set by Directa plus during the proposal stage.

Filter samples collected during the abrasion testing of the graphene-enhanced fabric showed presence of carbon-based platelets (images DP07-B and DP08-B). However, platelets with similar morphology and elemental profile were also identified on the filters collected during abrasion testing of the reference material (images DP02-B, DP04-B and DP04-C). These platelets also have similar morphology to those observed in the images of the fabrics after abrasion testing (images DP12-C and DP13-C), but again it is difficult to distinguish between those observed on the reference fabric and those in the graphene-enhanced fabric. In this case, carbon-based platelets do not exactly match the images of graphene platelets provided by Directa Plus, and therefore it is not possible to conclusively identify these platelets as graphene. As suggested previously (P4627), the use of a free graphene reference material for our own SEM analysis would be useful to confirm or refute these observations.

Analysis of the Stick-to-it® samples collected from the edges of the sample holder on the Martindale instrument after abrasion testing (DP09 and DP10) found very few carbon-based platelets, and those observed did not match the morphology of those found on the filter samples, hence were not believed to be derived from the graphene coating of the Directa Plus material.

For sample DP11 (Stick-to-it® sample collected from the surface of the abradant material after abrasion test of graphene-enhanced material), a mixture of inorganic particles, fibres and carbonaceous platelets were observed. Platelets-like morphologies were observed in image DP11-B and appear to be similar to carbon-based platelets noted on the filter samples. However, as morphology is not clear and carbonaceous material is observed in reference samples the presence of graphene platelets cannot be conclusively determined.

When analysed by SEM, wear to the fabric fibres of the graphene-enhanced material was greater than the reference material (Table 3.9). As discussed above, both the graphene-coated fabric (DP13) and the reference material (DP12) showed presence of platelet-like material deposited on the larger fibrous strands on the top-layer of the fabric (images DP12-C and DP13-C). As both materials are carbon-based it is very difficult to determine if the platelets observed in DP13 originate from the graphene coating or as a result of fibrous breakages as in DP12. However, it would be reasonable to expect that due to the level of wear observed on the fabric following abrasion testing, potentially graphene platelets could become free from the fabric. Additionally, the platelets observed in image DP13-C were in the respirable range and would therefore be a concern if released in high volumes, however testing indicated very low levels of release and could not definitively be identified as graphene.

4.4. Recommendations

Following previous studies to establish methodology we have shown the suitability of this experimental setup for the questions Directa Plus wished to answer comparing the emission/release through simulated wear testing of their fabrics. From the obtained results during these test it can be concluded that i) the adaptions to the ISO 12947 method to include real-time and static monitoring was successful and informative, and ii) under the conditions used, the release of graphene was, at best, not observed, and at worst, at low levels which will however have been

influenced by the short sampling time due to the 500 rub testing requested by Directa Plus during the proposal stage due to fabric fragility and intended use of the fabric.

Following on from these results, we recommend carrying out follow-up tests, including:

- Abrasion testing using the reference or the graphene-enhanced fabric as the abrasive material, or an alternative suitable material;
- Abrasion testing using both a higher number of rubs to simulate harder and longer-term wear (although 500 rubs showed fabric damage);
- Provision of reference materials: free graphene and graphene held within application medium (e.g. paste). This will allow accurate comparisons to be made between the reference and collected samples after any specific testing.

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Abrasion Testing of Graphene Enhanced CottonTexile

Project Code: P4627

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Abrasion Testing of Graphene Enhanced Cotton Texile

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Document revisions

No	Details	Date

Contents

1. Introduction

At the request of Carolina Volonte at Directa Plus Spa, IOM undertook a pilot study to determine the release of free graphene platelets (as a High Aspect Ratio Nanomaterial) from cotton based textile samples when an abrasion force is applied.

At this time, there is no standard to measure particulate release for textiles, for this reason textile industry ISO standard for abrasion testing,[1] in combination with characterisation and measurement being determined with reference to the ISO/TC 229 Nanotechnology standard where applicable. This work is carried out as a pilot study to determine which measurements are suitable based on the sample matrix and results required by Directa Plus.

2. Methodology

2.1. Abrasion Testing

Determination of particle release when an abrasion force is applied was carried out by following the ISO 12947 standard for "Determination of the abrasion resistance of fabrics".[1] A Martindale abrasion tester was used to subject the textile to a defined load and number of rubs. This was carried out against an abrasive medium (i.e. standard abradant fabric) in a translational movement tracing a Lissajous figure. The rotational frequency of the outer drive units was 47.5 min^{-1}, 9kPa nominal pressure was applied and the number of rubs was set at 5000.

In deviation from the standard, we did not measure the endpoints mass loss. Instead, we identified and characterised particle release by real-time and static monitoring methods, as outlined in the proposal (ref. Q1161). Further to this, appearance change and specimen breakdown was analysed by SEM/EDXS to determine the potential for graphene release.

Two test materials were provided by Directa Plus; a) the cotton base textile containing no graphene coating and b) the cotton textile with graphene coating. Four replicates of each textile were tested on the Martindale at one time in an attempt to enhance any possible graphene release. The samples and foam supports were cut to a diameter of 38 mm before mounting into the sample holder (as shown in Figure 2.1). The standard abradant material (flat woven wool fabric, 140 mm diameter) and felt underlay materials were used as received from suppliers.

[1] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method (ISO 12947)

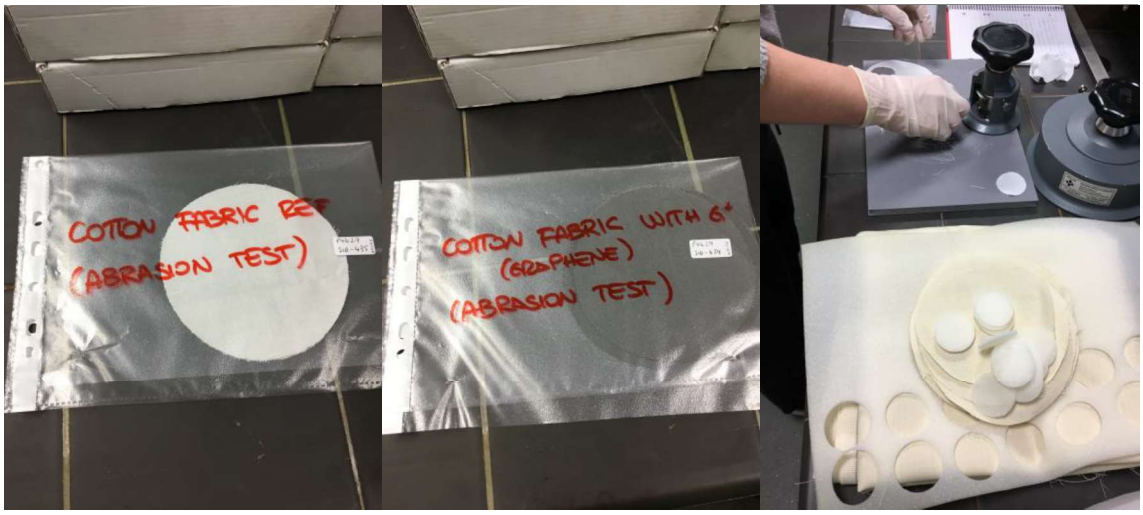

Figure 2.1 Cotton textile samples (left and middle) and polyetherurethane foam inserts cut to correct size (right)

Figure 2.2 outlines the position of the real-time sampling tubes and the static filter sampling pumps.

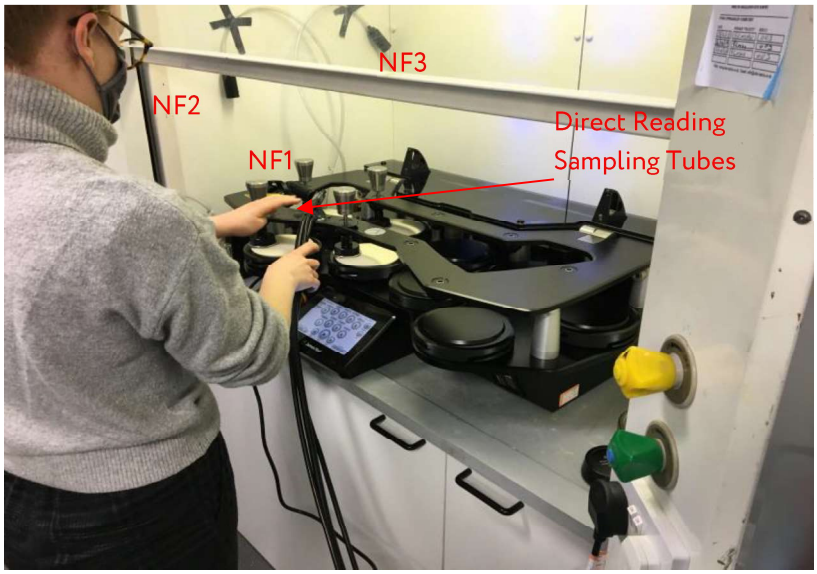

Figure 2.2 Image of Martindale instrument with static sampling and real-time sampling positions outlined

2.2. Real-Time Particle Monitoring

IOM established practices for determining particle release are consistent with BSI recommendations[2,3,4] for the identification and assessment of emissions of airborne manufactured nanomaterials in the workplace. Multiple instruments, suited for the detection of both nano- and micron-sized particles, acknowledged as good practice by BSI, were deployed for real-time characterisation of particle release (number, mass, size distribution) to give an informed assessment of the potential for release.

Condensation Particle Counter – CPC (TSI, Model 3007)

The CPC instrument is a hand held portable instrument which detects particle concentration within the 5 nm – 1 µm size range as a function of time, making it ideal for the primary identification of sources of particle emission during workplace activities. Particles present in the sample stream serve as condensation sites for the alcohol vapour in the instrument. Once condensation begins, particles grow quickly into larger alcohol droplets which pass through an optical detector and are counted. This instrument does not classify according to particle size.

Aerodynamic Particle Sizer Spectrometer - APS (TSI, Model 3321)

The APS sizes particles in the range from 500 nm to 20 microns using a time-of-flight light-scattering technique that measures aerodynamic diameter in real time. Particle classification results from differences in the mobility of particles based on their size, density and charge as they travel through an optical detector. Results are presented as aerodynamic equivalent diameter. This is defined as the physical diameter of a unity density sphere that settles through the air with a velocity equal to that of the particle in question.

Aerodynamic diameter is a significant aerosol size parameter as it determines the particles behaviour while airborne. Particles that have the same aerodynamic diameter will exhibit the same airborne behaviour, regardless of their physical size, shape, density or composition. Knowledge of the aerodynamic diameter subsequently allows determination of where the particle will be deposited in the human respiratory tract[5] and whether the particle will penetrate a filter, cyclone or other particle-removing device.

DustTrak DRX (TSI, Model 8533)

The DustTrak DRX air sampler is a laser photometer that simultaneously measures both mass and size fraction of airborne particulates. The instrument simultaneously measures PM1, PM2.5, PM4, PM10 and TPM (total particulate matter).

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[2] Workplace atmospheres – Ultrafine, nanoparticle and nano-structured aerosols – inhalation exposure characterisation and assessment (PD ISO/TR 27628:2007)

[3] Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies (PD ISO/TR 12885)

[4] Nanotechnologies – Part 3: Guide to assessing airborne exposure in occupational settings relevant to nanomaterials (PD 6699-3:2010)

[5] Workplace atmospheres – Size fraction definitions for measurement of airborne particles. CEN, European Committee for Standardisation. European Standard EN 481:1993.

2.3. Static Aerosol Particle Sampling

To facilitate the specific identification of materials released from the textiles, localised particle sampling was carried out.

Cowl Sampling Head for SEM and Elemental Analysis

An open cowl sampling head (25 mm) connected to a battery operated pump was used for particle collection onto polycarbonate filter (25 mm x 0.4 µm) for subsequent analyses by Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy (SEM/EDXS) to image and identify any particles sampled.

Sample pumps were calibrated to a flow rate of 2.2 L/min; flow rates were checked at the start and end of the sampling activities.

Surface Tape Samples, Sampling and Analysis

To facilitate the specific identification of materials released from the textile during abrasion, surface samples were taken, where considered appropriate, with proprietary Stick-to-It® sampling tapes. After sampling, the tape was placed in its sealed holder to avoid contamination.

2.4. Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy (SEM/EDXS)

The filter, textile and tape samples collected were analysed by image and elemental profiling using a modification of the following method:

SOP-009_V2: "Scanning Electron Microscopy – sample preparation, EDXS analysis and systematic filter analysis".

The technique for systematic filter analysis is adopted from ISO 14966:2002: "Ambient Air – Determination of numerical concentration of inorganic fibrous particles – Scanning electron microscopy method".

In preparation for SEM/EDXS analysis, a portion of each filter, tape or textile sample was excised and mounted onto a 13mm diameter aluminium SEM stub and coated with a thin layer of gold to enhance the conductivity of the surface and the imaging resolution. Images were recorded at various magnifications to best represent the distribution, size and shape of particles captured from the testing process and elemental analysis was carried out for chemical composition.

3. Results

3.1. Visual observations of abrasion testing

Photographs were taken of the textiles before and after abrasion testing to determine if there had been any visible signs of wear. As observed in Figure 3.1 and Figure 3.2, minimal visible wear occurred for each material tested and no breakages, rips or tears were noted in the fabric. Fibrous strands of the abradant material (beige flat woven wool fabric) were visible on the Martindale instrument, sample holder and test material following abrasion testing after 5000 rubs.

Close visual inspection of the abradant material following testing of the graphene-enhanced material, showed no visible evidence of graphene transfer, which would be expected to blacken the material if significant transfer had occurred (Figure 3.2).

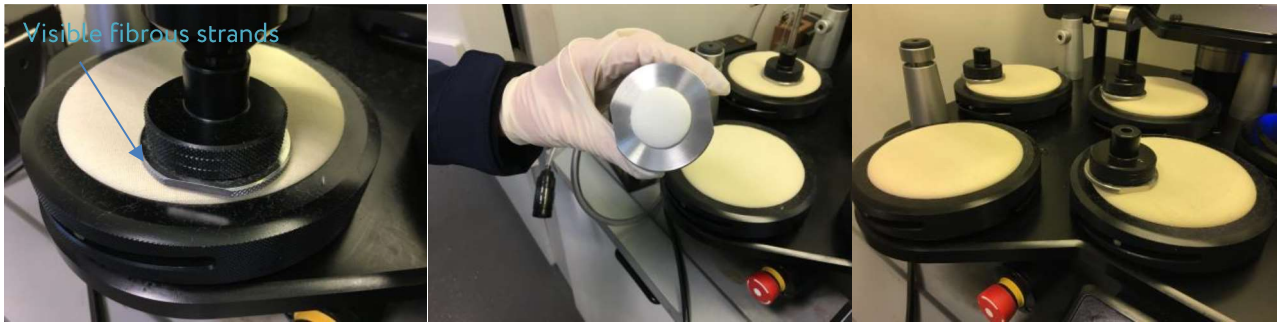


Figure 3.1 Image showing visible fibrous strands (left), wear to baseline fabric (middle) and abradant material (right).

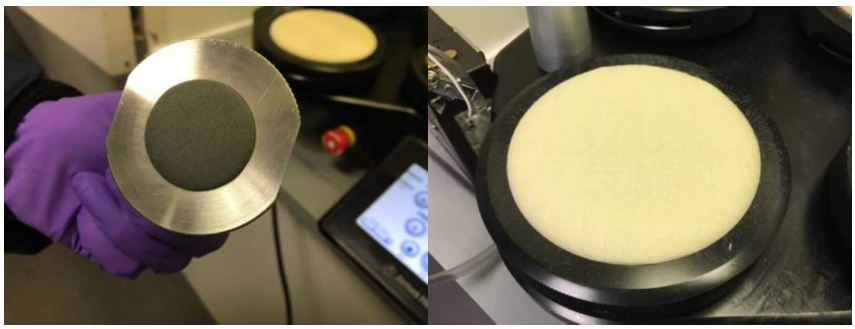


Figure 3.2 Image showing wear to graphene-enhanced fabric post-test (left) and abradant material (right).

3.2. Real-Time Monitoring

An overview of the real time monitoring is given in Figure 3.3, presenting how the measured particle concentration varied over time during abrasion testing of both the reference material and the graphene-enhanced material. The instrument sampling tubes were positioned on the top of the Martindale instrument and directly beside the site of abrasion (Figure 2.2). The monitoring was conducted for approximately 255 minutes and a description of activities carried out during this time is outlined in Table 3.1.

Table 3.1 Activity Information

Elapsed Time (s)	Description
09:50:37	Start background - Run 1 reference Cotton (DT = +20s, CPC NF + 30s, CPC FF +50s)
10:10:57	Start Martindale running (with ref sample)
11:58:54	Stop Martindale running (with ref sample)
12:16:45	Start background - Run 1 Cotton + Graphene (DT = +10s, CPC NF + 20s, CPC FF +30s)
12:19:42	Start Martindale running (with cotton + graphene sample)
14:05:32	Stop Martindale running (with cotton + graphene sample)

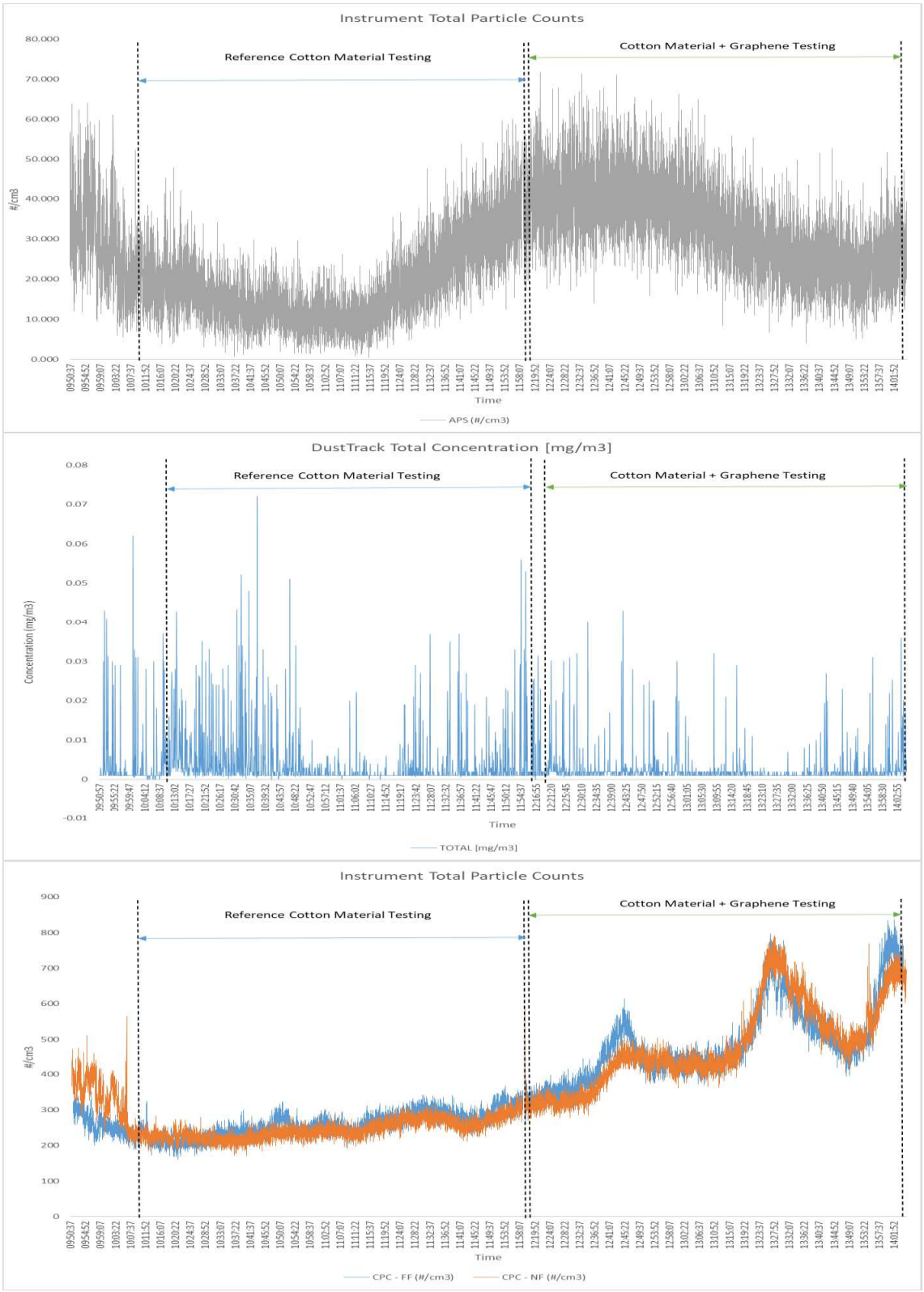


Figure 3.3 Top – APS; Middle – DustTrak particle concentration-time series; Bottom CPC, Abrasion testing. Instrument detection limits outlined in Section 0.

3.2.1. Reference material (no graphene)

The CPC, DustTrak and APS concentrations, collectively covering a size range of 5 nm to 20 µm, remained low and stable while abrasion occurred. A slight increase in the baseline levels were detected by the CPC and APS over the period of the sampling, however this was observed on both the near and far field instruments suggesting increasing particle disturbance through movement within the laboratory building through the day but absolute particle numbers were still at very low levels. Peaks or increasing counts can be a result of perturbed air flow from nearby movement (for example closing/opening of the fume cupboard or laboratory doors), variable temperatures in the room or due to external sources (e.g. ventilation systems).

3.2.2. Graphene-enhanced material

The CPC particle concentration (size range of 5 nm to 1 µm) was seen to steadily increase over the monitoring of abrasion testing of the graphene-enhanced material. Over the testing, the concentration was seen to increase gradually from 300 to 800 particles/cm^3. There are a number of possible reasons why this gradual increase may have occurred including:

- a general increase in heat/activity as the day progressed, leading to an increase in natural and anthropogenic particles and dusts;
- an increase in the number of process-related nanomaterials present as the Martindale instrument was used for a significant time period; or
- the presence of material specific particle release (potentially graphene).

No analogous increase was observed on the APS or DustTrak, indicating the particles being detected by the CPC are sized between 5-500 nm. It is important to note that the real-time instruments measure **total particle concentration** and therefore do not provide any information on the identity of the particles. For example, the instruments do not distinguish between free graphene, abradant fibres or background dust. It is for this reason that static samples are taken for SEM analysis, and these have been analysed in Section 3.3.

This therefore indicates a change from the initial background, however the same data trend was indicated on both the near- and far-field CPC instruments which outlines the release was not likely due to the process or material being monitored but a build up from movement and activity within the laboratory over the day. Analysis of samples for SEM collected both in the near- and far-field during the abrasion testing of the graphene enhance fabric is presented below in Section 3.3.

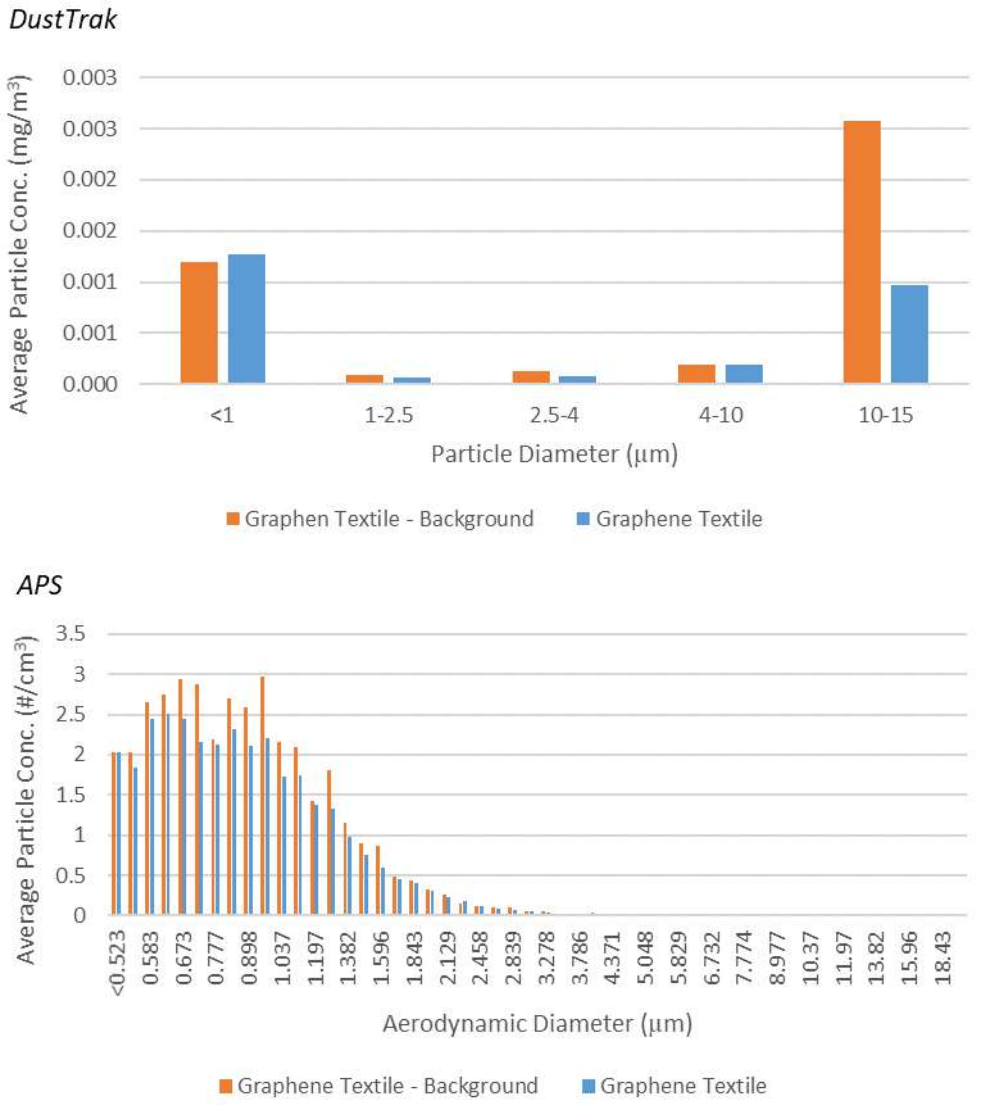


Figure 3.4. DustTrak and APS particle size distribution (PSD) graphs of the background period before the start of abrasion testing and for graphene-enhanced textile testing. DustTrak, size range <1 – 15 µm. APS, size range 500 nm - 1 µm.

Overall, the real-time data suggests no significant airborne release from abrasion of the graphene-enhanced materials. However, analysis of the static filter samples and SEM imaging (Section 3.3) of the material before and after abrasion is necessary to confirm this hypothesis.

3.3. SEM/EDXS Analysis

3.3.1. Air filter samples

The air filter samples collected during the abrasion testing of the materials were analysed by SEM imaging and elemental profiling to identify the presence and identity of any released particles. Figure 2.2 indicates the positioning of the static sampling beside the Martindale instrument. A summary of the collected samples is given in Table 3.2 and the associated analysis in Table 3.3 to Table 3.5.

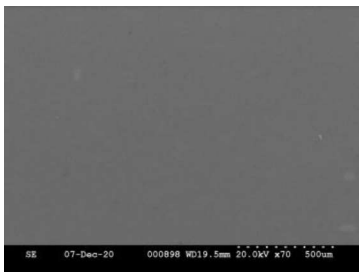
Table 3.2 Air filter samples collected

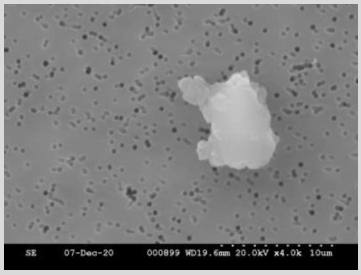
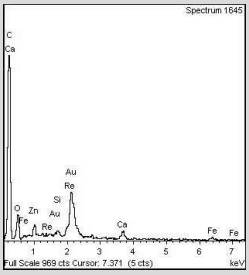
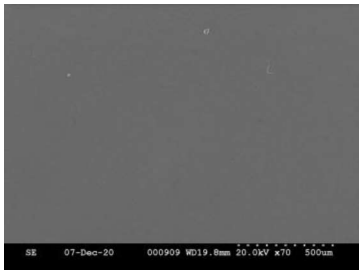
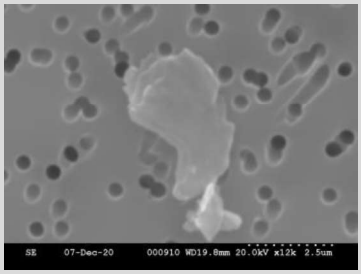
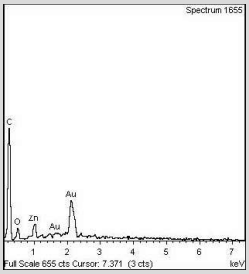
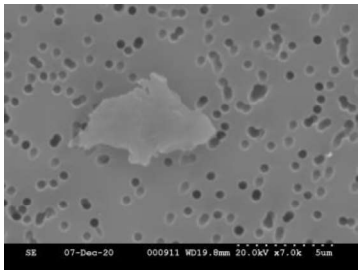
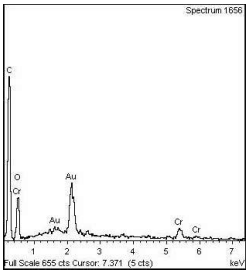
Sample ID	Description (see Figure 2.2)	Sampling time (min)
(a) Background Sampling		
DP01	Far-field position outside of fume hood, cotton reference sample	115
DP05	Far-field position outside of fume hood, cotton graphene enhanced sample	108
*(b) During abrasion testing of **reference material***		
DP02	Near-field position NF_1	104
DP03	Near-field position NF_2	104
DP04	Near-field position NF_3	104
*(c) During abrasion testing of **graphene-enhanced material***		
DP06	Near-field position NF_1	106
DP07	Near-field position NF_2	106
DP08	Near-field position NF_3	106

(a) Background sampling

Analysis of the SEM images obtained from the far-field position outside of the fume hood when the abrasion was occurring is given in Table 3.3.

Table 3.3 SEM/EDXS Analysis results for background filter samples

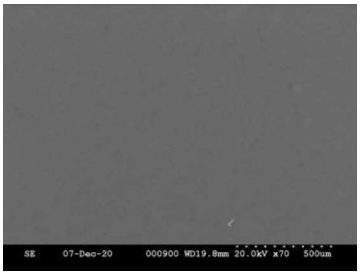
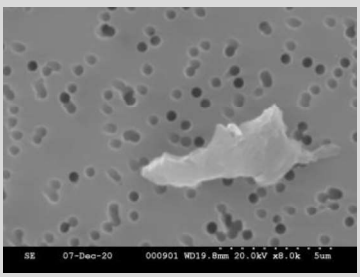
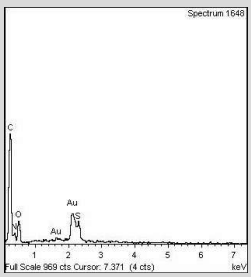
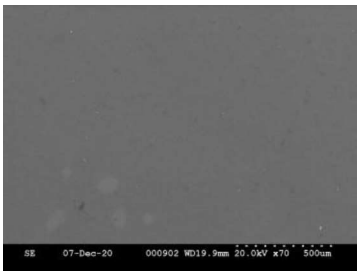
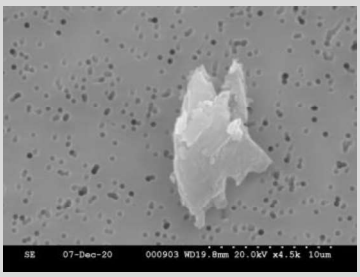
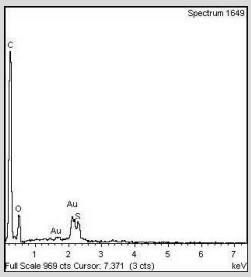
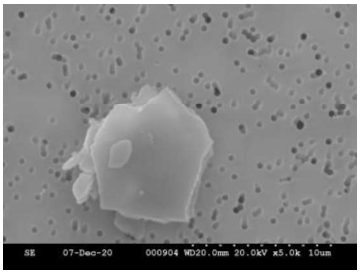
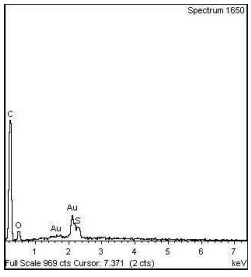
Sample ID		SEM Images	EDXS Spectra	Comment
DP01	A		N/A	Low magnification image showing very light loading of filter.

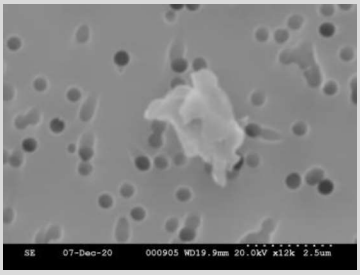
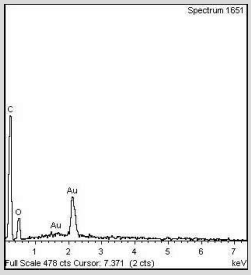
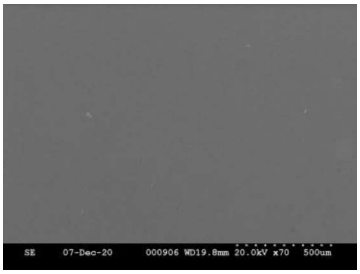
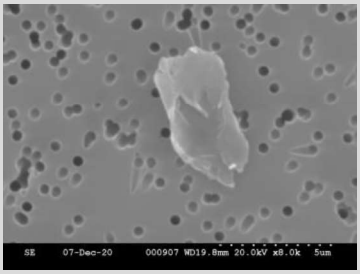
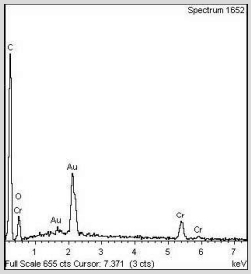
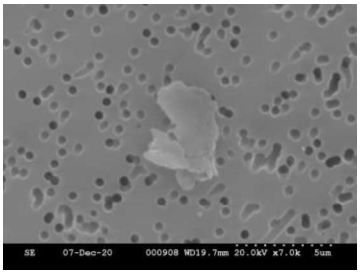
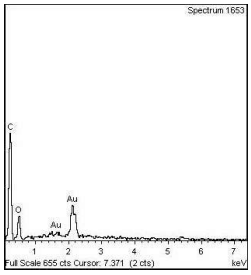
Sample ID		SEM Images	EDXS Spectra	Comment
	B			Platelet of dimensions 8 x 10.8 µm exhibiting C, Ca, Zn and O in EDXS spectrum.
DP05	A		N/A	Low magnification image showing very light loading of filter.
	B			Platelet of dimensions 2.8 x 4.1 µm exhibiting C, Zn and O in EDXS spectrum.
	C			Platelet of dimensions 4.8 x 7.6 µm exhibiting C, Cr and O in EDXS spectrum.

(b) Reference material

Analysis of the SEM images obtained during abrasion testing of the reference material is given in Table 3.4.

Table 3.4 SEM/EDXS Analysis results for filter samples from abrasion testing of the reference material

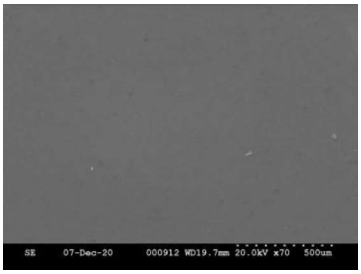
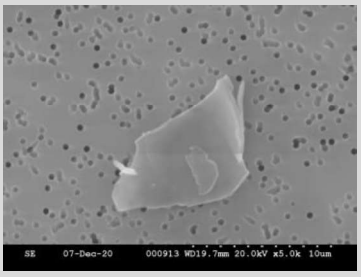
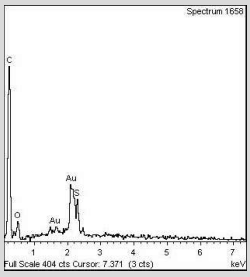
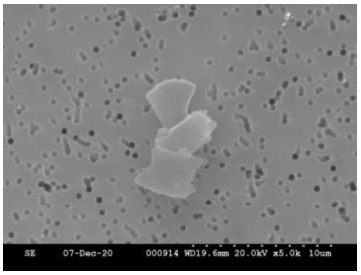
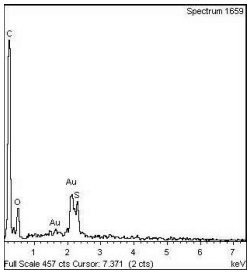
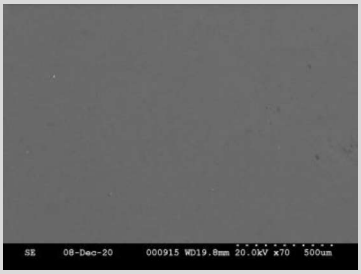
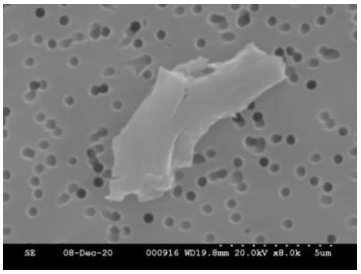
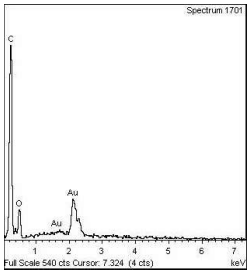
Sample ID		SEM Images	EDXS Spectra	Comment
DP02	A		N/A	Low magnification image showing very light loading of filter.
	B			Particle of size 3.7 x 8.9 µm exhibiting C and O in EDXS spectrum.
DP03	A		N/A	Low magnification image showing very light loading of filter.
	B			Particle of size 8 x 13.2 µm exhibiting C, O and S in EDXS spectrum.
	C			Platelet of size 8 x 9.6 µm, EDXS exhibiting C, O and S.

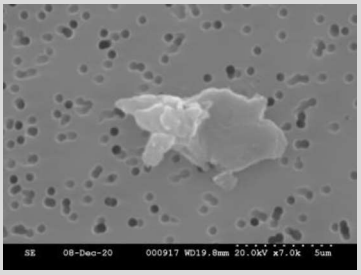
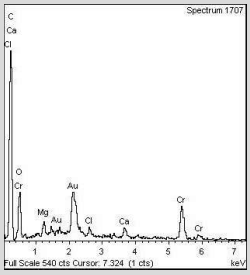
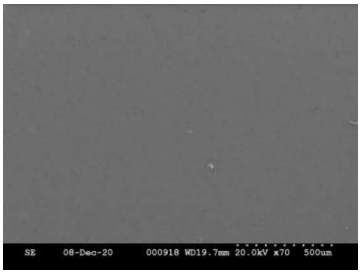
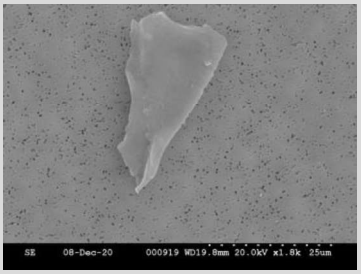
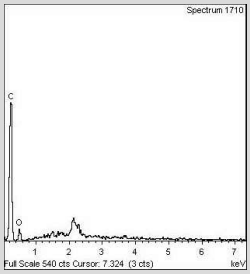
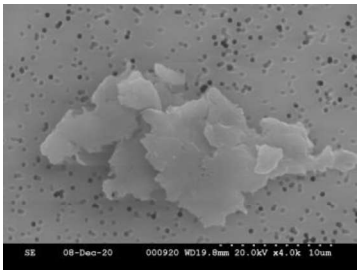
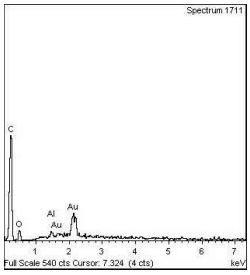
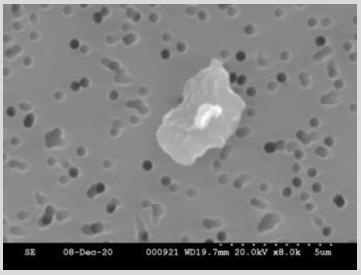
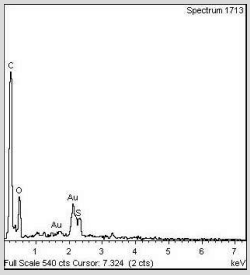
Sample ID		SEM Images	EDXS Spectra	Comment
	D			Particle of size 2.5 x 3.3 µm exhibiting C and O in EDXS spectrum.
DP04	A		N/A	Low magnification image showing very light loading of filter.
	B			Particle of size 3.3 x 6.6 µm exhibiting C, Cr and O in EDXS spectrum.
	C			Particle of size approx. 4.4 x 5.3 µm exhibiting C and O in EDXS spectrum.

(c) Graphene-enhanced material

Analysis of the SEM images obtained during abrasion testing of the graphene-enhanced material is given in Table 3.5.

Table 3.5 SEM/EDXS Analysis results for filter samples from abrasion testing of graphene-enhanced material

Sample ID		SEM Images	EDXS Spectra	Comment
DP06	A		N/A	Low magnification image showing very light loading of filter.
	B			Platelet of dimensions 7.4 x 11.2 µm exhibiting C, O and S in EDXS spectrum.
	C			Agglomerated platelets. Approx. 3 – 4 µm in width individually, length of agglomerate is 8.6 µm. EDXS spectrum identified C and O.
DP07	A		N/A	Low magnification image showing very light loading of filter.
	B			Platelet of dimensions 4.6 x 10 µm exhibiting C and O in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
	C			Platelet of dimensions 5.2 x 9 µm exhibiting C, O, Ca, Mg, Cl and Cr in EDXS spectrum.
DP08	A		N/A	Low magnification image showing very light loading of filter.
	B			Large platelet of dimensions 19.3 x 36.5 µm exhibiting C and O in EDXS spectrum.
	C			Agglomerated platelets approx. 28.4 µm in length. EDXS spectrum identified C and O. Particle size is 5.85 x 3.20 µm.
	D			Particle of dimensions 3.2 x 5 µm exhibiting C, O and S in EDXS spectrum.

The particles on these filters exhibited a morphology and composition similar to what could be expected for graphene. However, material with a similar morphology was found on filters collected during testing of the reference cotton fabric, i.e. with no graphene coating (Table 3.4). It is therefore not possible to conclusively identify these platelets as graphene. The use of free graphene reference material for our own SEM analysis would

be useful to confirm or refute these observations. Additionally, the use of TEM analysis in place of SEM would allow distinction between graphene platelets and other carbonaceous material.

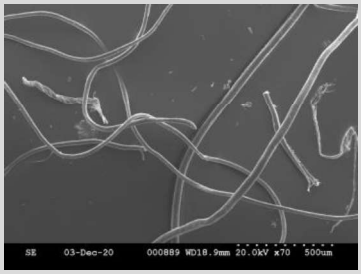
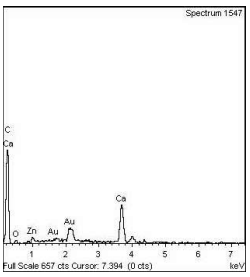
3.3.2. Stick-to-it® tape samples

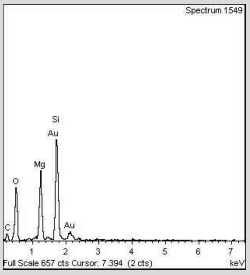
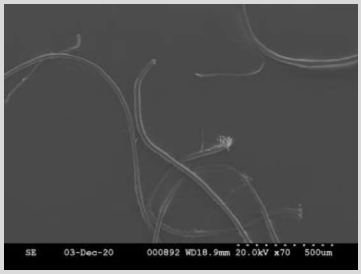
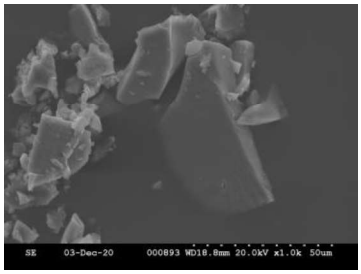
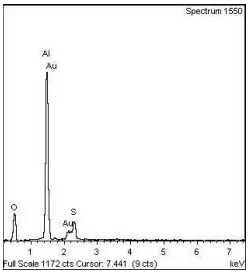
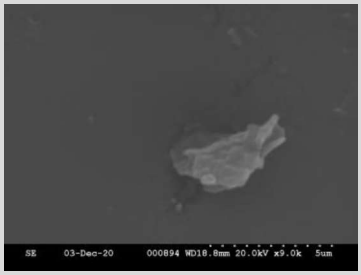
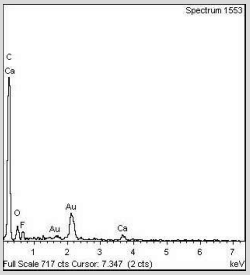
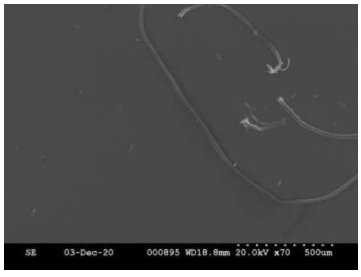
The Stick-to-it® samples were analysed by SEM and EDXS to identify the presence and identity of any released particles. These samples were taken from various positions on the Martindale instrument and sample holder *after* the abrasion testing had stopped. A summary of the collected samples is given in Table 3.6 and the associated analysis in Table 3.7.

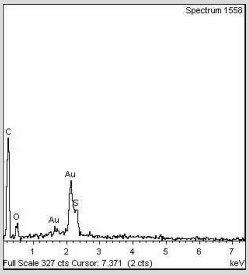
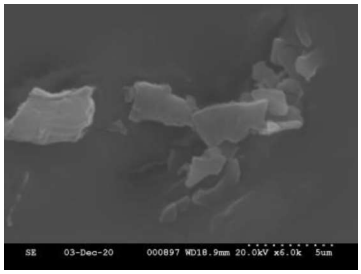
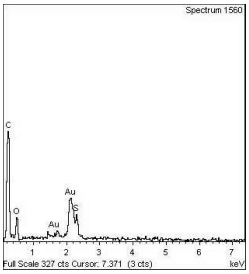
Table 3.6 Stick-to-it® tapes collected

Sample ID	Description
DP09	Material (including visible abradant fibres) collected from the edges of the sample holder on Martindale instrument after abrasion test of reference material
DP10	Material (including visible abradant fibres) collected from the edges of the sample holder on Martindale instrument after abrasion test of graphene-enhanced material
DP11	Material collected from the surface of the abradant material after abrasion test of graphene-enhanced material

Table 3.7 SEM/EDXS Analysis results for Stick-to-it® tapes

Sample ID		SEM Images	EDXS Spectra	Comment
Reference material (no graphene)				
DP09	A		NA	Low magnification view of Stick-to-it ® sample. Large fibres observed likely to be from the abradant material (also identified in project P2590).
	B			Calcium-based background particles of widths 5 µm (top) and 2.4 µm (bottom).

Sample ID		SEM Images	EDXS Spectra	Comment
	C			Material with platelet morphology of dimensions 6.9 x 9.1 µm. EDXS spectra identified C, O, Mg and Si.
Graphene-enhanced material				
	A		N/A	Low magnification view of sample with large fibres visible. Again, these are likely to be from the abradant material.
DP10	B			Inorganic material observed exhibiting Al, O and S in EDXS spectrum. Larger platelets measured between 27.2 and 62.2 µm in length.
	C			Organic platelet observed of length 4.6 µm, exhibiting C, O, F and Ca in EDXS spectrum.
DP11	A		N/A	Low magnification view of sample with some large fibres visible.

Sample ID		SEM Images	EDXS Spectra	Comment
	B			Platelet identified of length 4.6 µm exhibiting C, O and S in EDXS spectrum.
	C			Various platelets identified, some of them agglomerated. Length of platelets vary from approx. 3 – 5 µm exhibiting C, O and S in EDXS spectrum.

3.3.3. Textile material

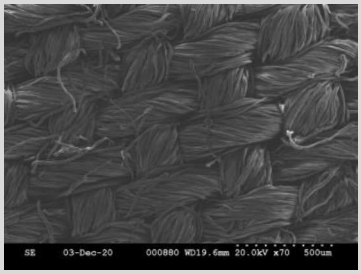
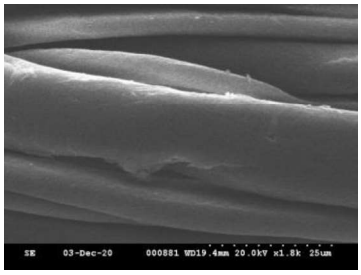
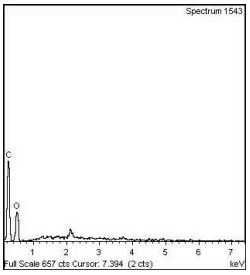
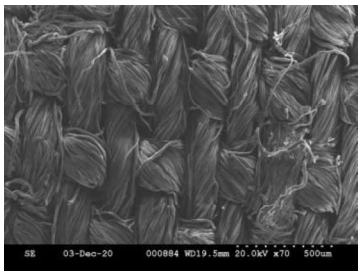
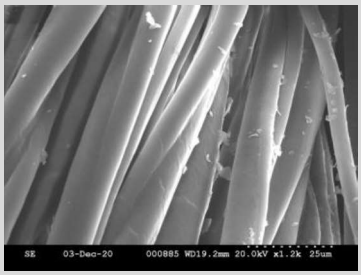
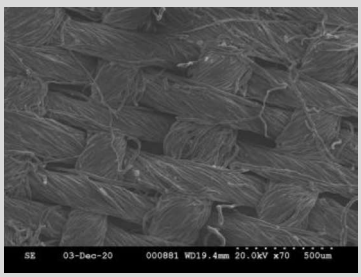
In addition to the collected filter samples, SEM and EDXS imaging was conducted on the two textile materials before and after the abrasion testing to see if any difference was evident. A summary of the samples is given in Table 3.8 and

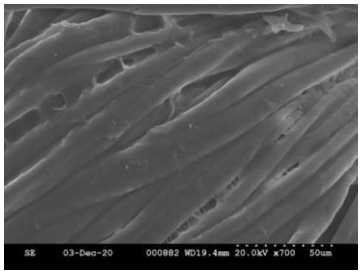
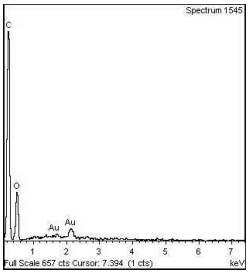
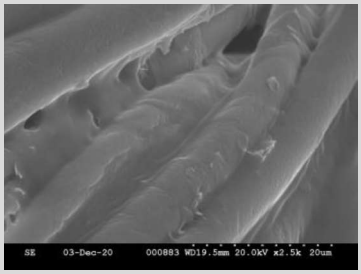
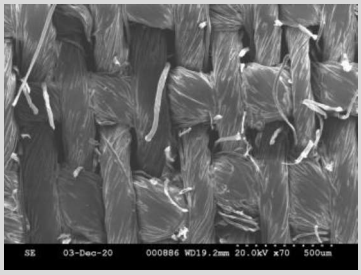
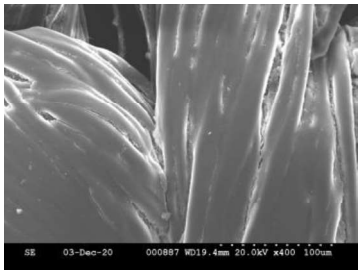
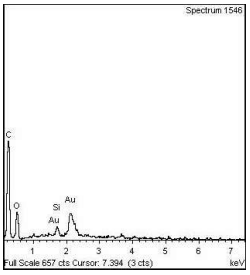
Table 3.9.

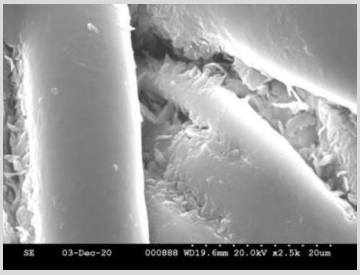
Table 3.8 Textile materials

Sample ID	Description
Reference material	
DP14	Reference textile material **prior to** abrasion testing
DP12	Reference textile material **after** abrasion testing
Graphene-enhanced material	
DP15	Graphene coated textile material **prior to** abrasion testing
DP13	Graphene coated textile material **after** abrasion testing

Table 3.9 SEM/EDXS Images of fabrics

Sample ID		SEM Images	EDXS Spectra	Comment
Reference material (no graphene) – Before Abrasion				
DP14	A		N/A	Overview of woven reference fabric prior to abrasion testing.
	B			Closer view of threads.
Reference material (no graphene) – After Abrasion				
DP12	A		N/A	Low magnification image showing the reference fabric following abrasion testing. Visible breakages to threads. Slightly more wear noted than for graphene-coated fabric.
	B		N/A	Magnified view of threads showing some collection of small particles on the surface of the threads.
Graphene-enhanced material – Before Abrasion				
DP15	A		N/A	Low magnification image showing the graphene coated fabric prior to abrasion testing.

Sample ID		SEM Images	EDXS Spectra	Comment
	B			Higher magnification, showing the graphene coating on surface of textile
	C		N/A	Graphene coating adhering to multiple threads of the textile. Surface is slightly rough in texture due to graphene platelets.
Graphene-enhanced material – After Abrasion				
DP13	A		N/A	Low magnification image showing the graphene coating following abrasion testing. Overall, the surface has been smoothed and threads appear to be free from the weave.
	B			Higher magnification, texture of surface is smoother than before testing.

Sample ID	SEM Images	EDXS Spectra	Comment
C		N/A	Closer view between threads shows higher degree of roughness due to the graphene coating than observed prior to testing.

4. Discussion and Recommendations

4.1. Visual inspection of abrasion testing

Following abrasion testing, both materials showed minimal wear by visual inspection. As was identified in the previous study (P2590), fibrous strands were visible on the Martindale equipment once the abrasion test had finished, and due to their colour and appearance these were attributed to the abradant material (beige flat woven wool fabric). As seen in Figure 3.2, no visible transfer of graphene to the abradant material was observed following abrasive testing of the graphene-enhanced textile.

The abradant material used was a woven wool fabric as recommended in the ISO standard (ISO 12947-2).[6] It is suggested that further assessment may involve using test material as the abradant material on the base pad of the Martindale instrument to reduce the presence of any contaminants from other materials and this would also give an indication of how the graphene-enhanced material wears in contact with itself.

4.2. Real-time Monitoring

Data collected by real-time monitoring showed, in general, steady particle concentration values throughout sampling at relatively low levels of measurement. Additionally, low level of deposition on the air filters were detected at all sampling positions and during all material testing.

A gradual rise in particle concentration was observed on the CPC during sampling of the abrasion test on the graphene-enhanced fabric conducted later in the day. There are a number of reasons why this gradual increase may have occurred; including a general increase in heat/activity as the day progressed, increase in process related nanomaterials present, or the presence of material specific nanomaterial (i.e. graphene). SEM analysis of the air filters collected during abrasion showed no difference in deposition between the reference and graphene-enhanced material. This indicates that this increase in CPC concentration is likely related to the laboratory environment or process and not indicative of graphene release, this was supported by the FF CPC readings matching those taken in the NF. No significant peaks or baseline increase were observed on the APS or DustTrak instruments from the activities monitored. If further tests were performed this could be investigated further, for example by running the graphene-enhanced fabric on the Martindale before the reference material or running each material at the same time on different days.

[6] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method - Part 2: Determination of specimen breakdown (ISO 12947-2:2016)

4.3. SEM/EDXS Analysis

All filter samples collected showed very light loading, despite noting some visible fibres released from the abradant material on to the Martindale after the test was complete, which demonstrates the low level of release during the short sampling time of this test and the low abrasive nature of the ISO recommended baseline material.

Filter samples collected during the abrasion testing of the graphene-enhanced fabric showed presence of carbon-based platelets. However, platelets with similar morphology and elemental profile were also identified on the filters collected during abrasion testing of the reference material (images DP03-B, DP04-C). In this case, carbon-based platelets do not exactly match the images of graphene platelets provided by Directa Plus, and therefore it is not possible to conclusively identify these platelets as graphene. The use of a free graphene reference material for our own SEM analysis would be useful to confirm or refute these observations.

Analysis of the Stick-to-it® samples collected from the edges of the sample holder on the Martindale instrument after abrasion testing (DP09 and DP10) found a mixture of inorganic particles (images DP09-B, DP10-B) and fibres (images DP09-A, DP10-A). In both samples, very few carbon-based platelets observed and those observed did not match the morphology of those found on the filter samples, hence were not believed to be derived from the graphene coating of the Directa Plus material.

For sample DP11 (Stick-to-it® sample collected from the surface of the abradant material after abrasion test of graphene-enhanced material), again a mixture of inorganic particles, fibres and carbonaceous platelets were observed. Platelets-like morphologies were observed in images DP11-B and DP11-C and appear to be similar to carbon-based platelets noted on the filter samples. However, as morphology is not clear and carbonaceous material is observed in reference samples the presence of graphene platelets cannot be conclusively determined.

When analysed by SEM, wear to the fabric fibres of the reference material was slightly greater than the graphene-coated material (

Table 3.9, DP12). The graphene-coated fabric (DP13) showed a general smoothing of the fabric surface texture, with enhanced roughness between the fibres than was noted in the fabric sample prior to testing (DP13-C and DP15-C, respectively). Overall, these SEM images show abrasion has caused the surface of the graphene coating to become smooth but rough between fibre strands, which may have led to a release of graphene platelets, however this has not been confirmed by the current sampling.

4.4. Recommendations

As described in the original quote, this work was a pilot study to (partly) determine the feasibility of including the proposed adaption of an ISO standard used in the textiles industry. In addition, to determine which measurements were suitable based on the sample matrix and results required by Directa Plus. From the obtained results it can be concluded that i) the adaptions to the ISO 12947 method to include real-time and static monitoring was successful and informative, and ii) under the conditions used, the release of graphene was, at best, not observed, and at worst, at low levels.

Following on from these results, we recommend carrying out follow-up tests, including:

- Abrasion testing using the reference or the graphene-enhanced fabric as the abrasive material, or an alternative suitable material;
- Abrasion testing using both a higher applied nominal pressure and a higher number of rubs to simulate harder and longer-term wear;
- Provision of reference materials: free graphene and graphene held within application medium (e.g. paste). This will allow accurate comparisons to be made between the reference and collected samples after any specific testing.

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Document revisions

No	Details	Date

Contents

1. Introduction

At the request of Carolina Volonte at Directa Plus Spa, IOM undertook a pilot study to determine the release of free graphene platelets (as a High Aspect Ratio Nanomaterial) from textile samples when an abrasion force is applied.

At this time, there is no standard to measure particulate release for textiles, for this reason textile industry ISO standard for abrasion testing,[1] in combination with characterisation and measurement being determined with reference to the ISO/TC 229 Nanotechnology standard where applicable. This work is carried out as a pilot study to determine which measurements are suitable based on the sample matrix and results required by Directa Plus.

2. Methodology

2.1. Abrasion Testing

Determination of particle release when an abrasion force is applied was carried out by following the ISO 12947 standard for "Determination of the abrasion resistance of fabrics".[1] A Martindale abrasion tester was used to subject the textile to a defined load and number of rubs. This was carried out against an abrasive medium (ie. standard abradant fabric) in a translational movement tracing a Lissajous figure. The rotational frequency of the outer drive units was 47.5 min^{-1}, 9kPa nominal pressure was applied and the number of rubs was set at 5000.

In deviation from the standard, we did not measure the endpoints mass loss. Instead, we identified and characterised particle release by real-time and static monitoring methods, as outlined in the proposal (ref. Q317). Further to this, appearance change and specimen breakdown was analysed by SEM/EDXS to determine the potential for graphene release.

Two test materials were provided by Directa Plus; a) the base textile containing no graphene coating and b) the textile with graphene coating applied in a hexagonal shaped pattern. Four replicates of each textile were tested on the Martindale at one time in an attempt to enhance any possible graphene release. The samples and foam supports were cut to a diameter of 38 mm before mounting into the sample holder (as shown in Figure 2.1). The standard abradant material (flat woven wool fabric, 140 mm diameter) and felt underlay materials were used as received from suppliers.

[1] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method (ISO 12947)

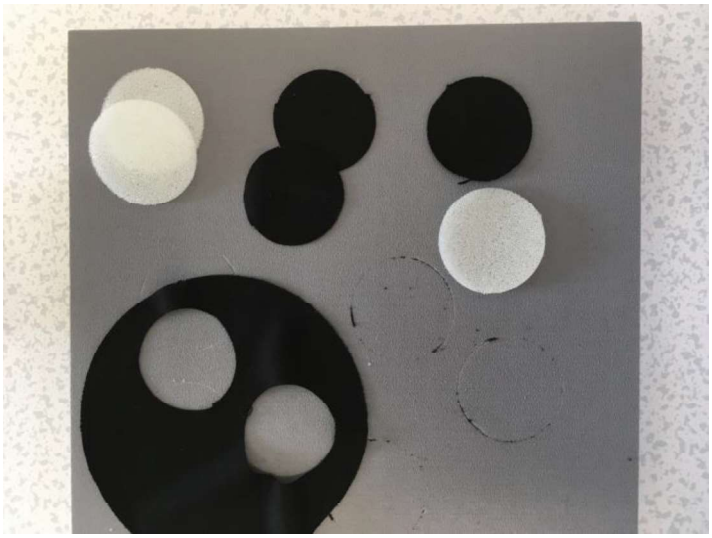


Figure 2.1 Textile samples and polyetherurethane foam inserts cut to correct size

Figure 2.2 outlines the position of the real-time sampling tubes and the static filter sampling pumps.

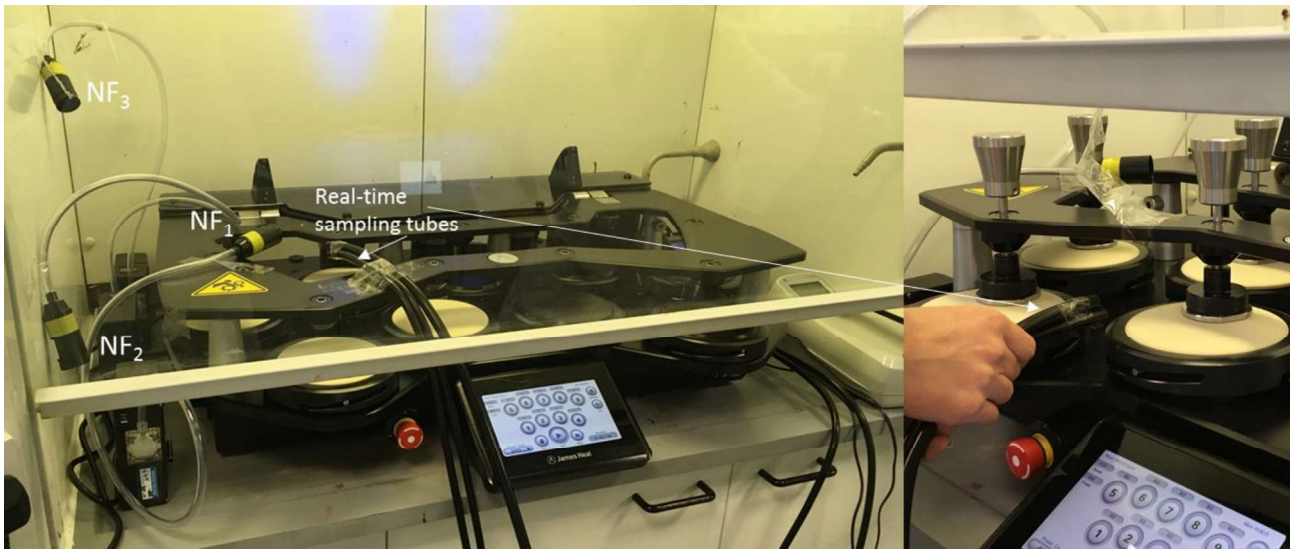


Figure 2.2 Image of Martindale instrument with static sampling and real-time sampling positions outlined

2.2. Real-Time Particle Monitoring

IOM established practices for determining particle release are consistent with BSI recommendations[2,3,4] for the identification and assessment of emissions of airborne manufactured nanomaterials in the workplace. Multiple

[2] Workplace atmospheres – Ultrafine, nanoparticle and nano-structured aerosols – inhalation exposure characterisation and assessment (PD ISO/TR 27628:2007)

[3] Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies (PD ISO/TR 12885)

[4] Nanotechnologies – Part 3: Guide to assessing airborne exposure in occupational settings relevant to nanomaterials (PD 6699-3:2010)

instruments, suited for the detection of both nano- and micron-sized particles, acknowledged as good practice by BSI, were deployed for real-time characterisation of particle release (number, mass, size distribution) to give an informed assessment of the potential for release.

Condensation Particle Counter – CPC (TSI, Model 3007)

The CPC instrument is a hand held portable instrument which detects particle concentration within the 5 nm – 1 µm size range as a function of time, making it ideal for the primary identification of sources of particle emission during workplace activities. Particles present in the sample stream serve as condensation sites for the alcohol vapour in the instrument. Once condensation begins, particles grow quickly into larger alcohol droplets which pass through an optical detector and are counted. This instrument does not classify according to particle size.

Aerodynamic Particle Sizer Spectrometer - APS (TSI, Model 3321)

The APS sizes particles in the range from 500 nm to 20 microns using a time-of-flight light-scattering technique that measures aerodynamic diameter in real time. Particle classification results from differences in the mobility of particles based on their size, density and charge as they travel through an optical detector. Results are presented as aerodynamic equivalent diameter. This is defined as the physical diameter of a unity density sphere that settles through the air with a velocity equal to that of the particle in question.

Aerodynamic diameter is a significant aerosol size parameter as it determines the particles behaviour while airborne. Particles that have the same aerodynamic diameter will exhibit the same airborne behaviour, regardless of their physical size, shape, density or composition. Knowledge of the aerodynamic diameter subsequently allows determination of where the particle will be deposited in the human respiratory tract[5] and whether the particle will penetrate a filter, cyclone or other particle-removing device.

DustTrak DRX (TSI, Model 8534)

The DustTrak DRX air sampler is a laser photometer that simultaneously measures both mass and size fraction of airborne particulates. The instrument simultaneously measures PM1, PM2.5, PM4, PM10 and TPM (total particulate matter).

[5] Workplace atmospheres – Size fraction definitions for measurement of airborne particles. CEN, European Committee for Standardisation. European Standard EN 481:1993.

2.3. Static Aerosol Particle Sampling

To facilitate the specific identification of materials released from the textiles, localised particle sampling was carried out.

Cowl Sampling Head for SEM and Elemental Analysis
An open cowl sampling head (25 mm) connected to a battery operated pump was used for particle collection onto polycarbonate filter (25 mm x 0.4 µm) for subsequent analyses by Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy (SEM/EDXS) to image and identify any particles sampled.

Sample pumps were calibrated to a flow rate of 2.2 L/min; flow rates were checked at the start and end of the sampling activities.

Surface Tape Samples, Sampling and Analysis
To facilitate the specific identification of materials released from the textile during abrasion, surface samples were taken, where considered appropriate, with proprietary Stick-to-It® sampling tapes. After sampling, the tape was placed in its sealed holder to avoid contamination.

2.4. Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy (SEM/EDXS)

The filter and tape samples collected were analysed by image and elemental profiling using a modification of the following method:

SOP-009_V2: "Scanning Electron Microscopy – sample preparation, EDXS analysis and systematic filter analysis".

The technique for systematic filter analysis is adopted from ISO 14966:2002: "Ambient Air – Determination of numerical concentration of inorganic fibrous particles – Scanning electron microscopy method".

In preparation for SEM/EDXS analysis, a portion of each filter, tape or textile sample was excised and mounted onto a 13mm or 25mm diameter aluminium SEM stub, respectively, and coated with a thin layer of gold to enhance the conductivity of the surface and the imaging resolution. Images were recorded at various magnifications to best represent the distribution, size and shape of particles captured from the testing process and elemental analysis was carried out for chemical composition.

3. Results

3.1. Visual observations of abrasion testing

Photographs were taken of the textiles before and after abrasion testing to determine if there had been any visible signs of wear. As observed in Figure 3.1 and Figure 3.2, minimal visible wear occurred for each material tested and no breakages, rips or tears were noted in the fabric. Fibrous strands of the abradant material (beige flat woven wool fabric) were visible on the Martindale instrument, sample holder and test material following abrasion testing after 5000 rubs.

Close visual inspection of the abradant material following testing of the graphene-enhanced material, showed no visible evidence of graphene transfer, which would be expected to blacken the material if significant transfer had occurred (Figure 3.2).

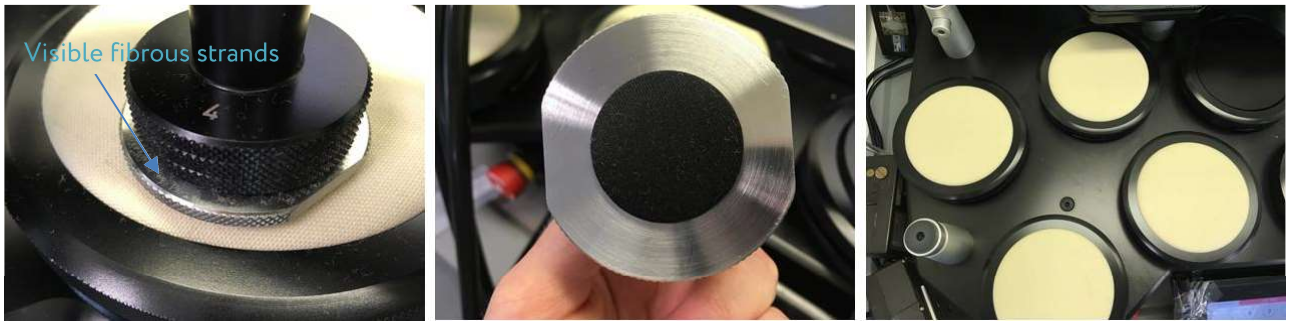


Figure 3.1 Image showing visible fibrous strands (left), wear to baseline fabric (middle) and abradant material (right).

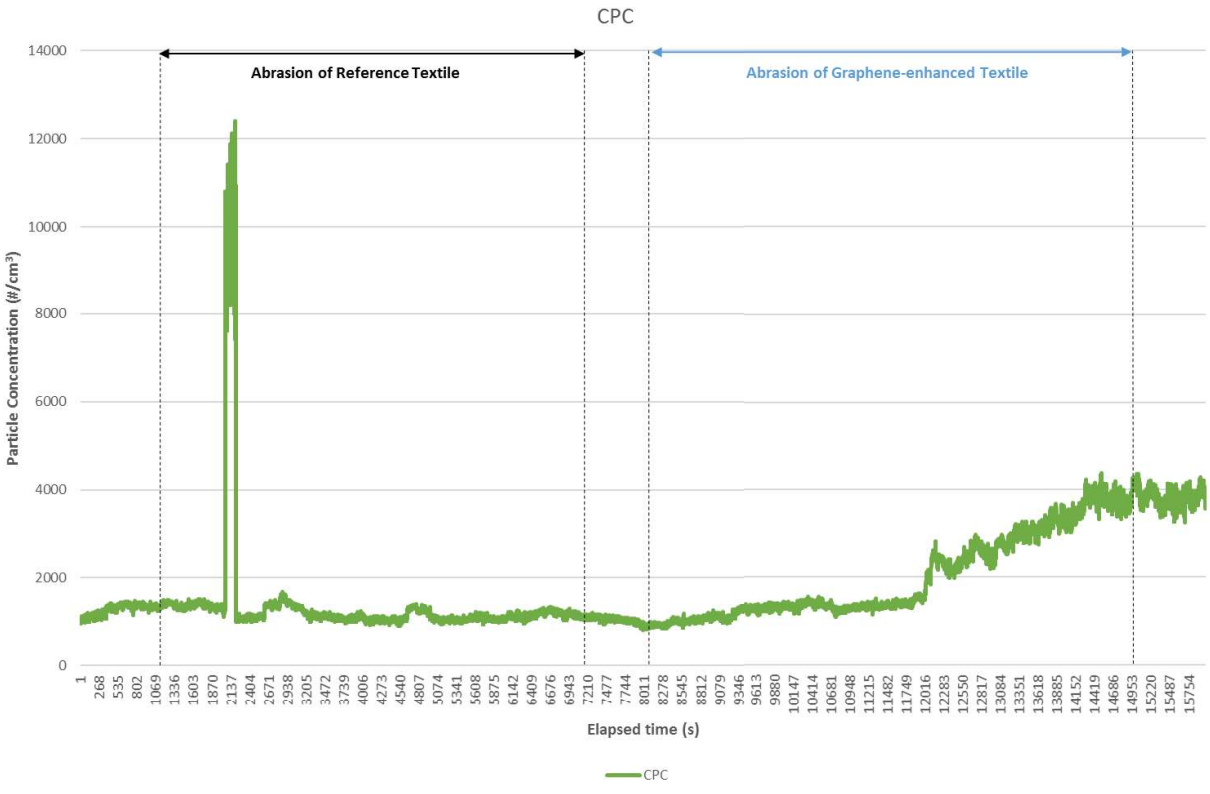
Figure 3.2 Image showing graphene-enhanced fabric before test (left), wear to graphene-enhanced fabric (middle) and abradant material (right).

3.2. Real-Time Monitoring

An overview of the real time monitoring is given in Figure 3.3, presenting how the measured particle concentration varied over time during abrasion testing of both the reference material and the graphene-enhanced material. The instrument sampling tubes were positioned on the top of the Martindale instrument and directly beside the site of abrasion (Figure 2.2). The monitoring was conducted for approximately 267 minutes and a description of activities carried out during this time is outlined in Table 3.1.

Table 3.1 Activity Information

Elapsed Time (s)	Description
622 – 951	Martindale running with no material present
1170 – 7449	Martindale running with reference material present
8080 – 14980	Martindale running with graphene-enhanced material present



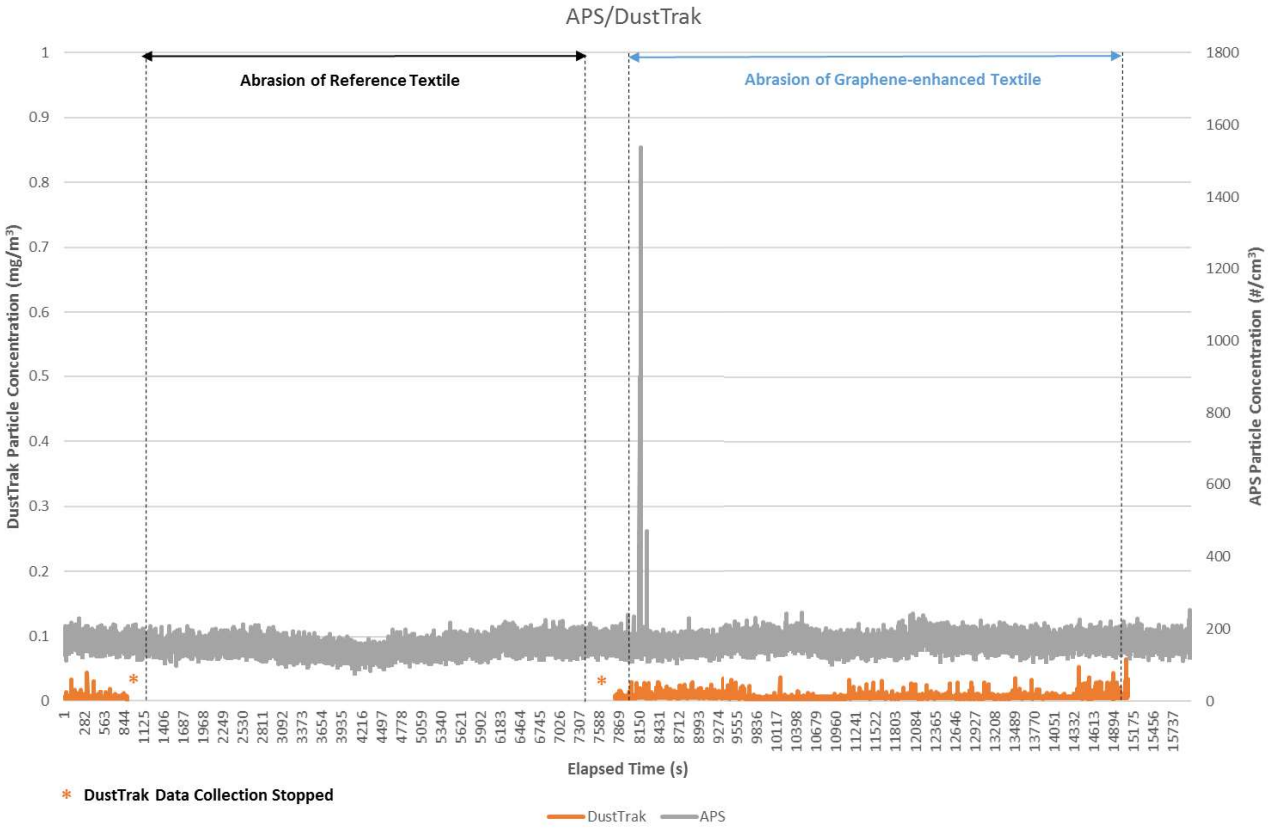


Figure 3.3 Top – CPC; Bottom – APS and DustTrak particle concentration-time series, Abrasion testing. Instrument detection limits outlined in Section 2.2.

3.2.1. Reference material (no graphene)

The CPC and APS concentrations, collectively covering a size range of 5 nm to 20 µm, remained low and stable while abrasion occurred. A distinct peak was noted on the CPC trace between 2040 and 2240s, however no analogous peak was observed on the APS or DustTrak at this time, indicating the particles being detected by the CPC are sized between 5-500 nm. As no noted activity occurred at this time, it is not possible to assign this peak to a specific release, however due to the sensitivity of the CPC this is likely an artefact and is not thought to be a true particle release from the abrasion being monitored. Peaks can be a results of perturbed air flow from nearby movement, for example closing/opening of the fume cupboard or relocation the sampling tubes. This is further confirmed by the SEM analysis outlined in Section 3.3. As outlined in the graph, data collection for the DustTrak stopped during the abrasion testing of the reference material.

3.2.2. Graphene-enhanced material

The CPC particle concentration (size range of 5 nm to 1 µm) was seen to remain steady over the first hour of abrasion testing of the graphene-enhanced material. In the following 54 minutes, the concentration was seen to increase gradually from 1500 to 3900 particles/cm^3. There are a number of possible reasons why this gradual increase may have occurred including:

- a general increase in heat/activity as the day progressed, leading to an increase in natural and anthropogenic particles and dusts;
- An increase in the number of process-related nanomaterials present as the Martindale instrument was used for a significant time period; or
- the presence of material specific particle release (potentially graphene).

No analogous increase was observed on the APS or DustTrak, indicating the particles being detected by the CPC are sized between 5-500 nm. It is important to note that the real-time instruments measure **total particle concentration** and therefore do not provide any information on the identity of the particles. For example, the instruments do not distinguish between free graphene, abradant fibres or background dust. It is for this reason that static samples are taken for SEM analysis, and these have been analysed in Section 3.3.

In addition, a short-lived peak appeared in the APS trace between 8155 and 8182s that did not correlate with any notable activity from the contextual information gathered during the testing. Characterisation of how the size distribution of this peak compared to the background distribution is shown in Figure 3.4. The DustTrak and APS show an increase in particles sized 4-10 µm, and <0.523 – 11.97 µm, respectively, during the elapsed time period 8155 – 8182s. This explains why no peak is seen on the CPC as these particles are out with the size range of CPC operation (<1 µm). This therefore indicates a change from background, however it is not possible for the available data to accurately assign this peak to a specific event or to indicate its significance. While this is the case, as is presented below in Section 3.3, SEM analysis of the near-field air samples did not indicate a significant particle release from the graphene-enhanced material and showed no distinction from the SEM samples collected during the abrasion of the reference material.

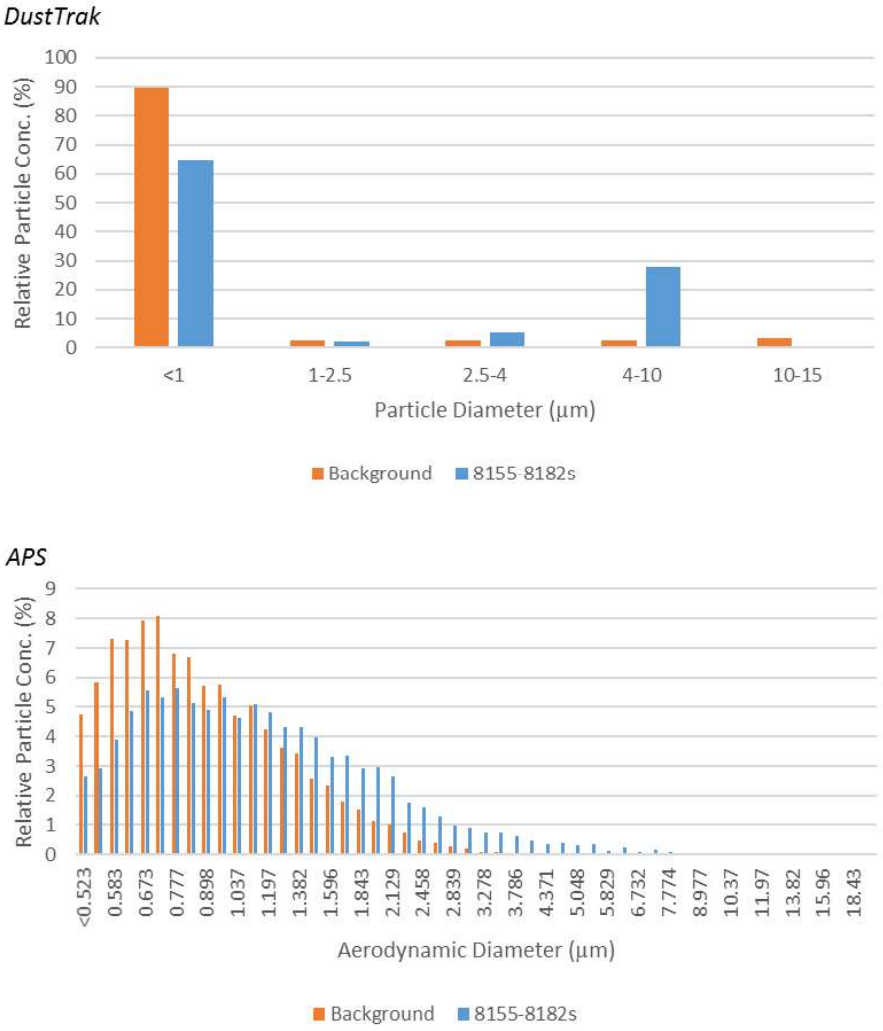


Figure 3.4. DustTrak and APS particle size distribution (PSD) graphs of the peak observed at the start of abrasion testing of graphene-enhanced textile (Figure 2.3, 8155 and 8182 s). DustTrak, size range <1 – 15 µm. APS, size range 500 nm - 1 µm.

Overall, the real-time data indicates no significant airborne release from abrasion of the reference material. For the graphene-enhanced materials, further analysis of the static filter samples and SEM imaging of the material before and after abrasion is necessary to provide further information on the graphene behaviour.

3.3. SEM/EDXS Analysis

3.3.1. Air filter samples

The air filter samples collected during the abrasion testing of the materials were analysed by SEM imaging and elemental profiling to identify the presence and identity of any released particles. Figure 2.2 indicates the positioning of the static sampling beside the Martindale instrument. A summary of the collected samples is given in Table 3.2 and the associated analysis in Table 3.3 to Table 3.5.

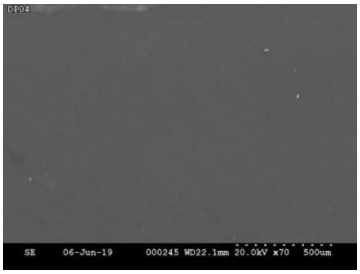
Table 3.2 Air filter samples collected

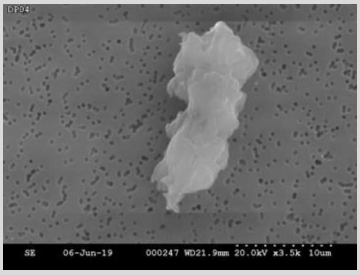
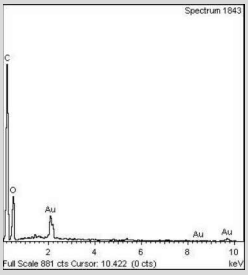
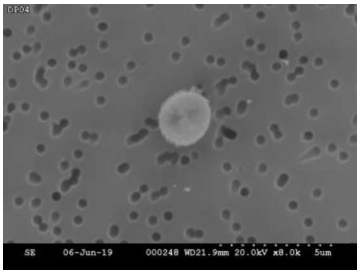
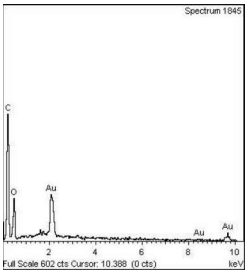
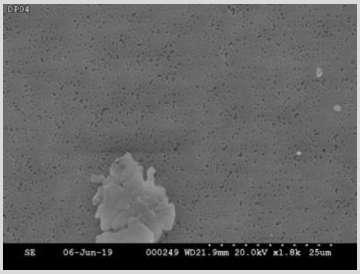
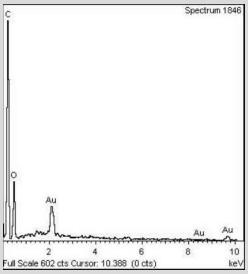
Sample ID	Description (see Figure 2.2)	Sampling time (min)
(a) Background Sampling		
DP04	Far-field position outside of fume hood, full day of sampling	244
*(b) During abrasion testing of **reference material***		
DP01	Near-field position NF_1	108
DP02	Near-field position NF_2	108
DP03	Near-field position NF_3	108
*(c) During abrasion testing of **graphene-enhanced material***		
DP05	Near-field position NF_1	120
DP06	Near-field position NF_2	120
DP07	Near-field position NF_3	120

(a) Background sampling

Analysis of the SEM images obtained from the far-field position outside of the fume hood when the abrasion was occurring is given in Table 3.3.

Table 3.3 SEM/EDXS Analysis results for background filter samples

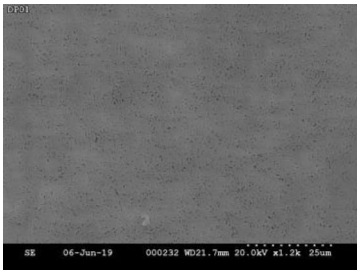
Sample ID		SEM Images	EDXS Spectra	Comment
DP04	A		N/A	Low magnification image showing light loading of filter.

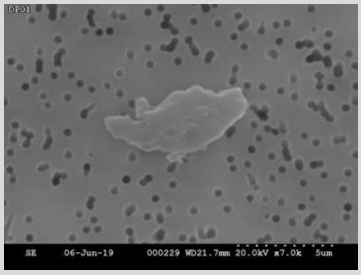
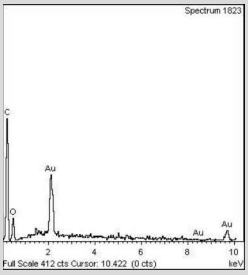
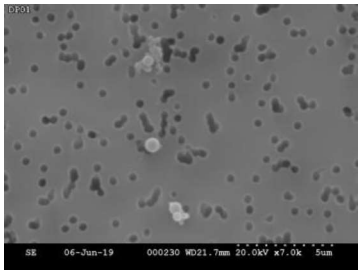
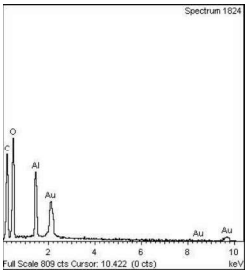
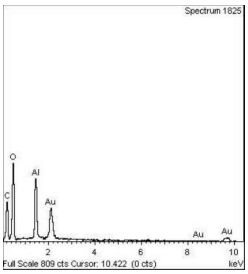
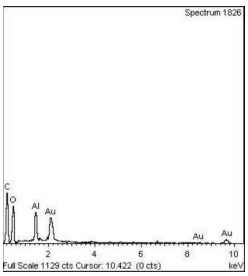
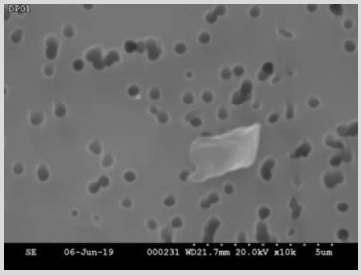
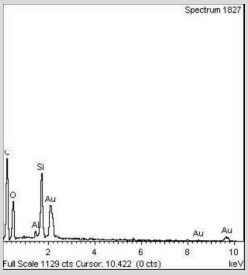
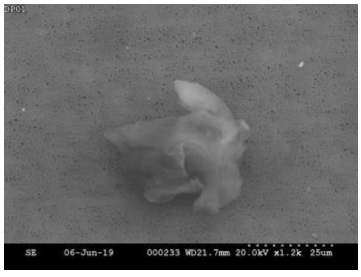
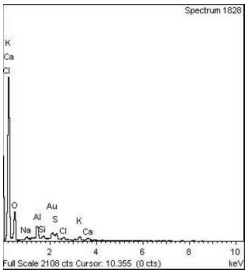
Sample ID	SEM Images	EDXS Spectra	Comment
	B			Platelet of dimensions 19.25 x 7.33 µm exhibiting C and O in EDXS spectrum.
	C			Particle of diameter 2.12 µm exhibiting C and O in EDXS spectrum.
	D			Platelets of various dimensions exhibiting C and O in EDXS spectrum.

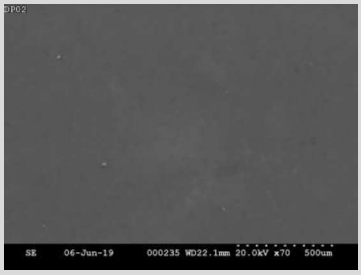
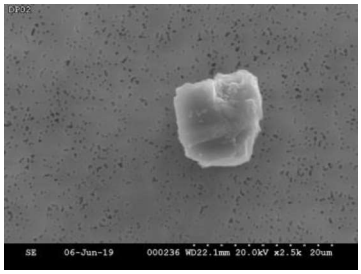
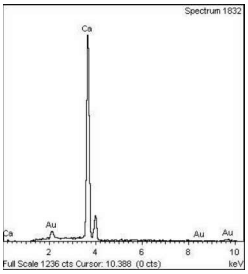
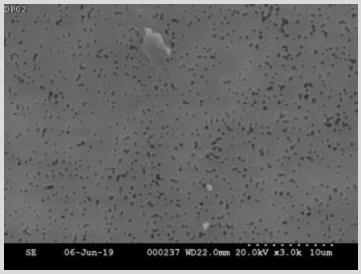
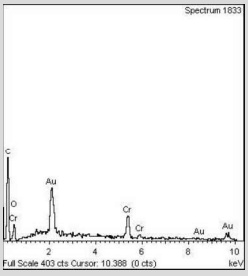
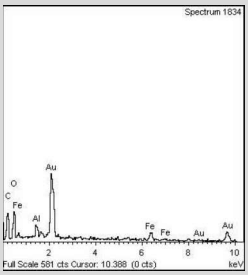
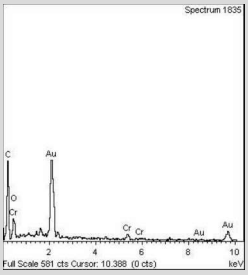
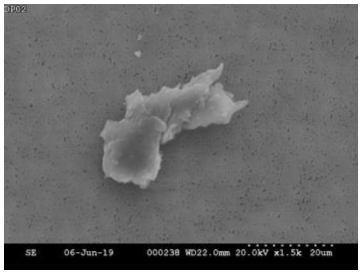
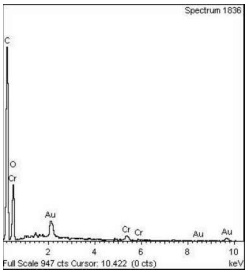
(b) Reference material

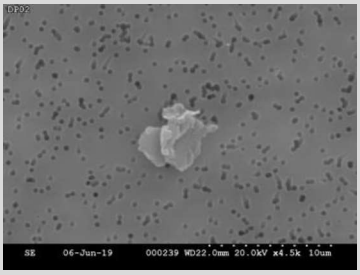
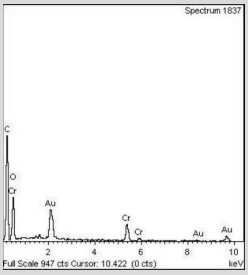
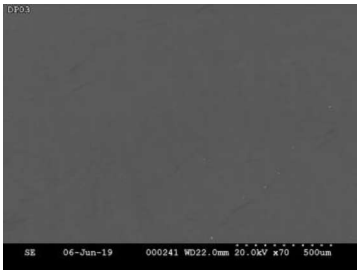
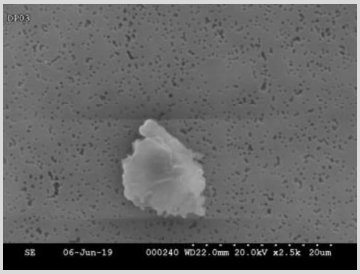
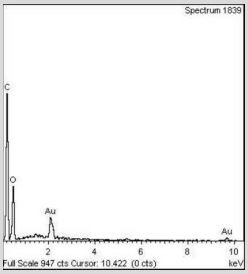
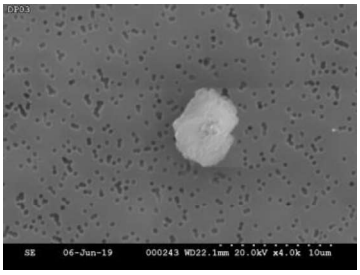
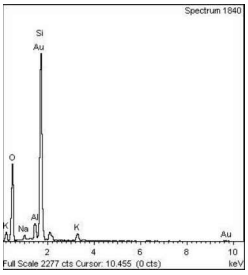
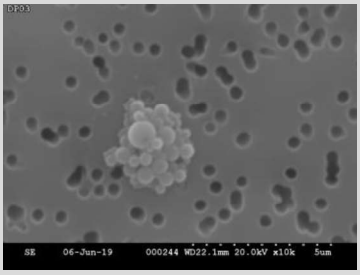
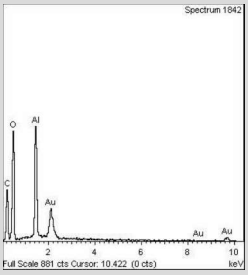
Analysis of the SEM images obtained during abrasion testing of the reference material is given in Table 3.4.

Table 3.4 SEM/EDXS Analysis results for filter samples from abrasion testing of the reference material

Sample ID	SEM Images	EDXS Spectra	Comment
DP01	A		N/A	Low magnification image showing light loading of filter.

Sample ID	SEM Images	EDXS Spectra	Comment
B			Particle of size 7.08 x 3.57 µm exhibiting C and O in EDXS spectrum.
C			Top – Top aggregate of spherical particles, ~100-515 nm diameter, EDXS exhibiting C, O and Al;
			Middle – Spherical particle in middle, 670 nm diameter, EDXS exhibiting C, O and Al;
			Bottom – Bottom aggregate, 210 – 570 nm diameters of individual spheres, EDXS exhibiting C, O and Al.
D			Particle of size 2.19 x 1.25 µm exhibiting C, O, Al and Si in EDXS spectrum.
E			Particle of size 39.63 x 35.69 µm exhibiting a range of elements including Ca, K, Na and O in EDXS spectrum.

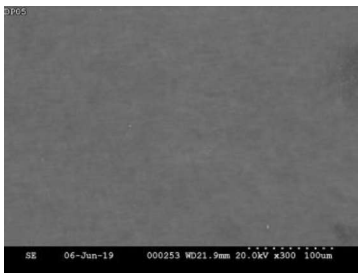
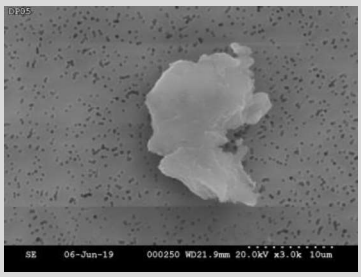
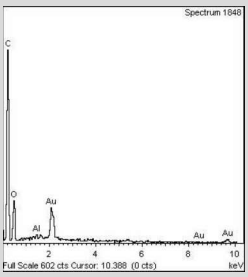
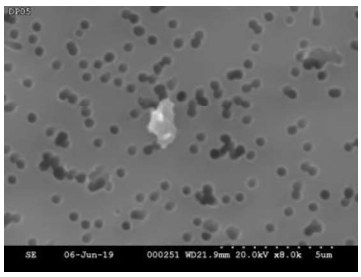
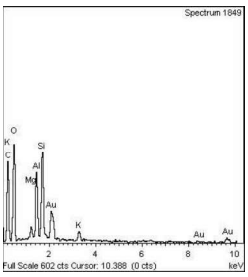
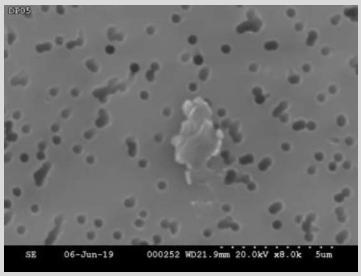
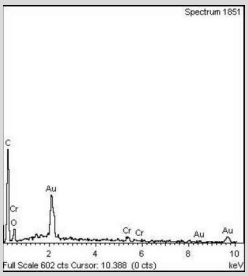
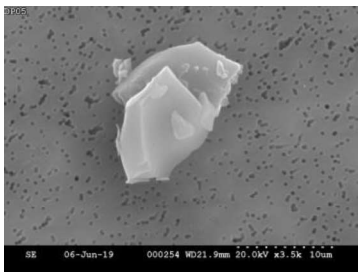
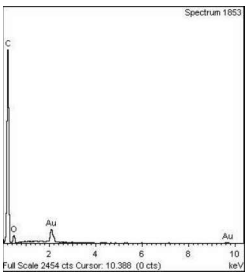
Sample ID		SEM Images	EDXS Spectra	Comment
DP02	A		N/A	Low magnification image showing light loading of filter.
	B			Particle of size 13.06 x 11.94 µm exhibiting Ca in EDXS spectrum.
	C		  	Top – Top platelet, 5.34 x 3.25 µm, EDXS exhibiting C, O and Cr; Middle – Small particle in middle near bottom, 759 nm diameter, EDXS exhibiting C, O, Fe and Al; Bottom – Bottom middle particle, 790 nm diameter, EDXS exhibiting C, O and Cr.
	D			Large particle of size 36.69 x 16.85 µm exhibiting C, O and Cr in EDXS spectrum.

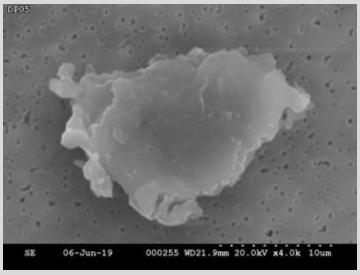
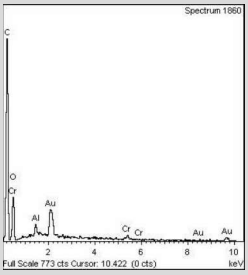
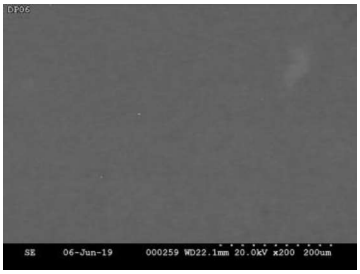
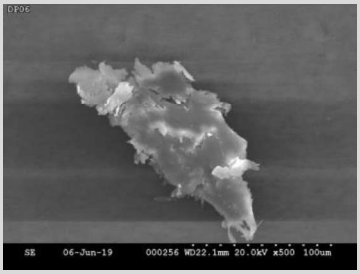
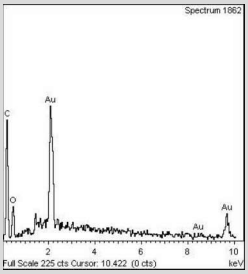
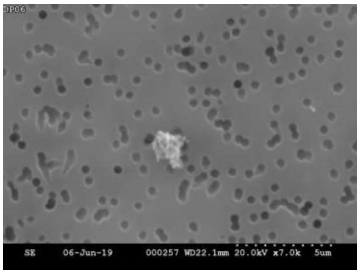
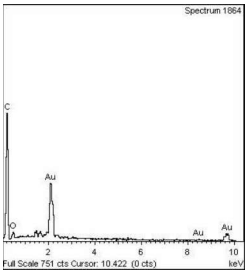
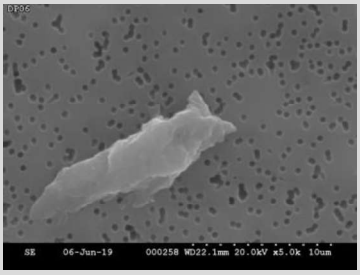
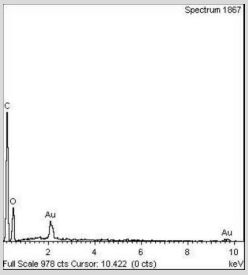
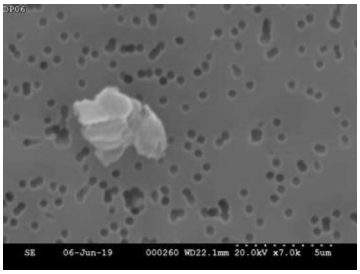
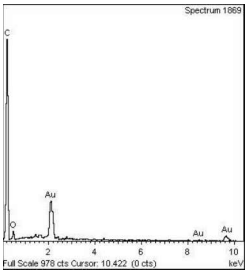
Sample ID		SEM Images	EDXS Spectra	Comment
	E			Particle of size 5.0 x 5.9 µm exhibiting C, O and Cr in EDXS spectrum.
DP03	A		N/A	Low magnification image showing light loading of filter.
	B			Particle of size 14.59 x 11.65 µm exhibiting C and O in EDXS spectrum.
	D			Particle of size 6.77 x 5.29 µm exhibiting K, O, Na, Al and Si in EDXS spectrum.
	E			Aggregation of spherical particles which exhibit C, O and Al in EDXS spectrum.

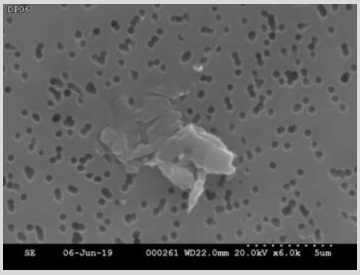
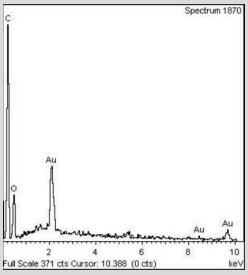
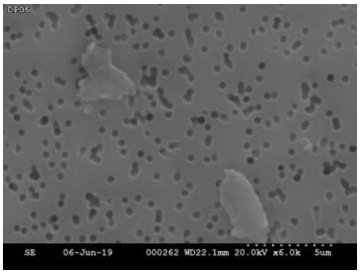
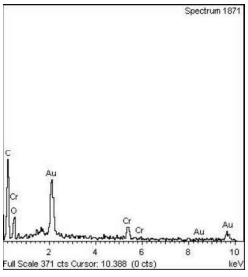
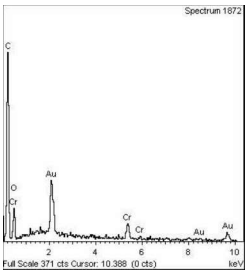
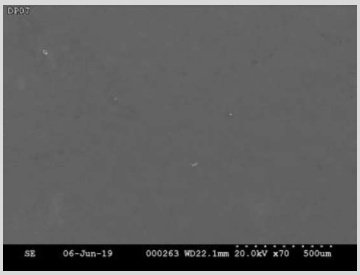
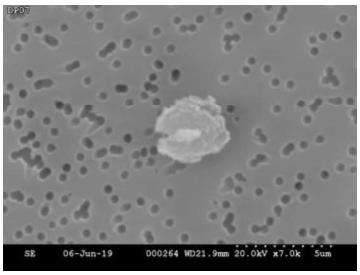
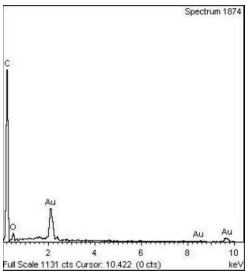
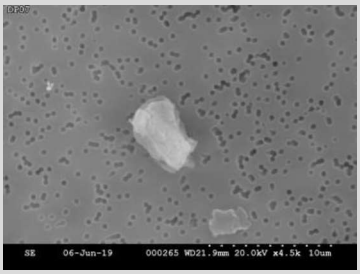
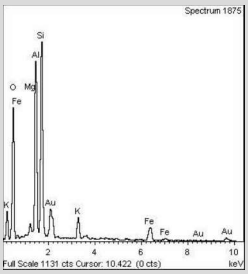
(c) Graphene-enhanced material

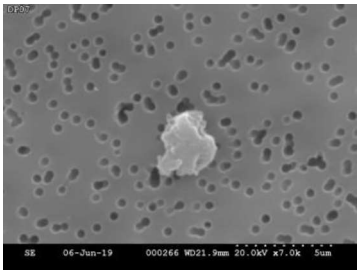
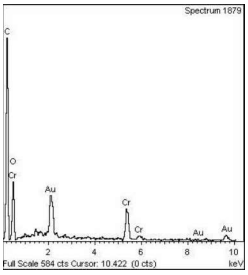
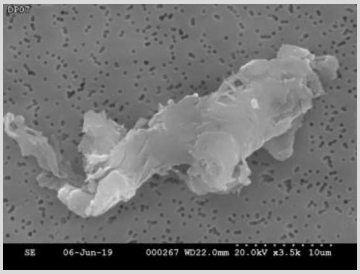
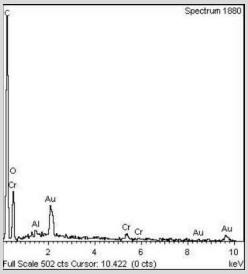
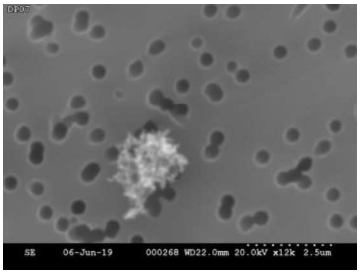
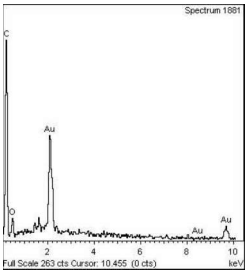
Analysis of the SEM images obtained during abrasion testing of the graphene-enhanced material is given in Table 3.5.

Table 3.5 SEM/EDXS Analysis results for filter samples from abrasion testing of graphene-enhanced material

Sample ID		SEM Images	EDXS Spectra	Comment
DP05	A		N/A	Low magnification image showing light loading of filter.
	B			Platelet of dimensions 17 x 14 µm exhibiting C and O in EDXS spectrum.
	C			Inorganic background material exhibiting K, Fe, Mg, Al, and Si in EDXS spectrum. Particle size is 2.25 x 1.26 µm.
	D			Platelet of dimensions 3.64 x 2.11 µm exhibiting C and O in EDXS spectrum.
	E			Platelet of dimensions 15.40 x 9.88 µm exhibiting mainly C with a small proportion of O in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
	F			Large particle of dimensions 21.22 x 14.36 µm exhibiting C, O, Al and Cr in EDXS.
DP06	A		N/A	Low magnification image showing light loading of filter.
	B			Large platelet of dimensions 167.73 x 65.87 µm exhibiting C and O in EDXS spectrum.
	C			Platelet of dimensions 2.06 x 1.54 µm exhibiting mainly C with a small proportion of O in EDXS spectrum.
	D			Platelet of dimensions 15.14 x 4.64 µm exhibiting C and O in EDXS spectrum.
	E			Platelet of dimensions 4.98 x 3.43 µm exhibiting C and O in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
	F			Aggregation of platelets exhibiting C and O in EDXS spectrum.
	G		 	Platelets exhibiting C, O and Cr peaks in EDXS spectra. EDXS Spectra: Top – Top left platelet, 3.22 x 3.44 µm; Bottom – Bottom right platelet, 5.09 x 2.30 µm.
DP07	A		N/A	Low magnification image showing light loading of filter.
	B			Platelet of dimensions 3.73 x 3.34 µm exhibiting mainly C with a small proportion of O in EDXS spectrum.
	C			Background material exhibiting K, Fe, Al, Si and O in EDXS spectrum. Particle size is 5.85 x 3.20 µm.

Sample ID	SEM Images	EDXS Spectra	Comment
D			Platelet of dimensions 3.06 x 2.95 µm exhibiting C, O and Cr in EDXS spectrum.
E			Large particle of dimensions 29.33 x 10.86 µm exhibiting C, O, Al and Cr in EDXS.
F			C based material of dimension 2.09 x 1.73 µm.

The particles on these filters exhibited a morphology and composition similar to what could be expected for graphene, in particular on DP06 B-F. While this is the case, material with a similar morphology was found in absence of the graphene coating when testing the reference material (Table 3.4). It is therefore not possible to conclusively identify these platelets as graphene. The use of free graphene reference material for our own SEM analysis would be useful to confirm or refute these observations.

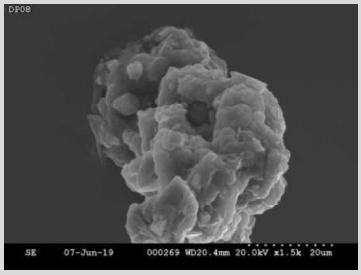
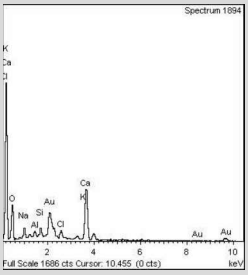
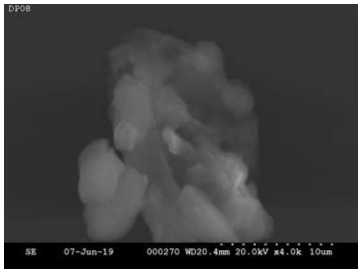
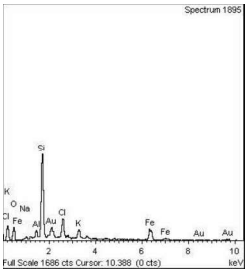
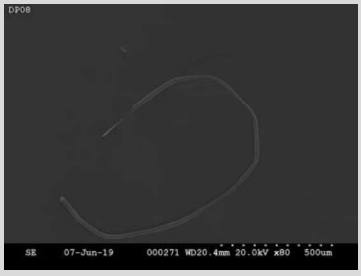
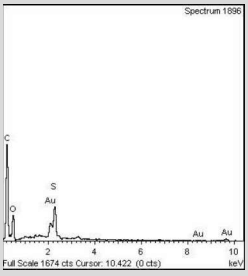
3.3.2. Stick-to-it® tape samples

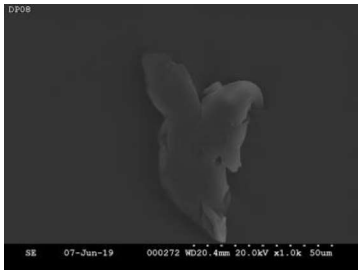
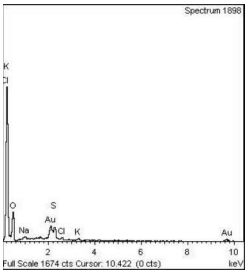
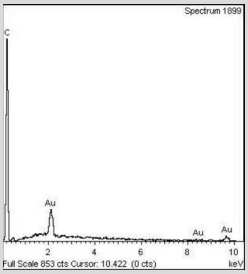
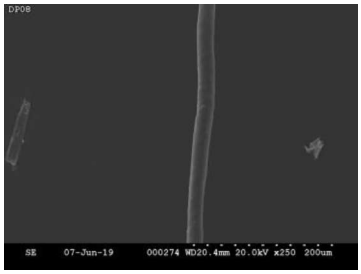
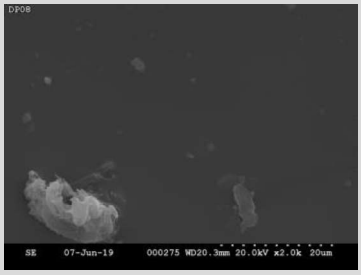
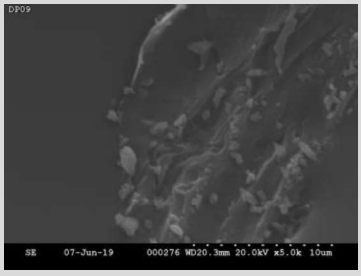
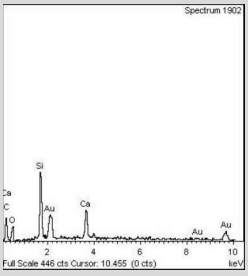
The Stick-to-it® samples were analysed by SEM EDXS to identify the presence and identity of any released particles. These were taken from various positions on the Martindale instrument and sample holder *after* the abrasion testing had stopped. A summary of the collected samples is given in Table 3.6 and the associated analysis in Table 3.7Table 3.2.

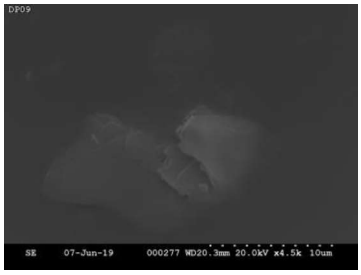
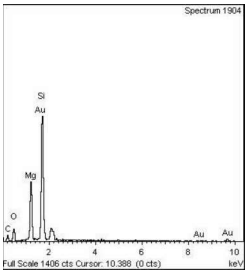
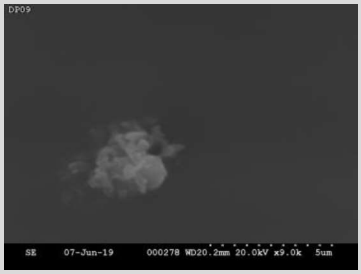
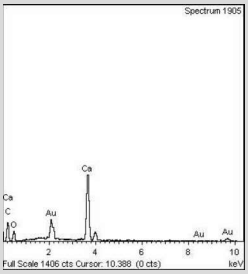
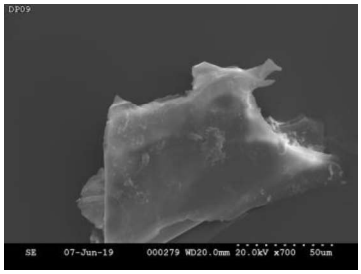
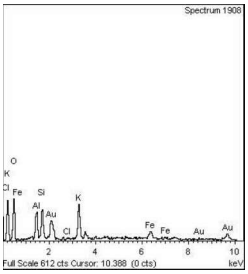
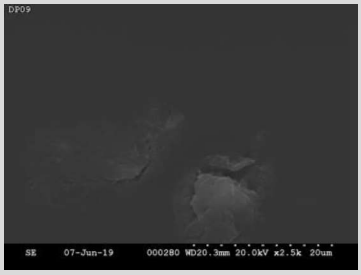
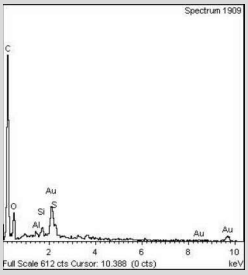
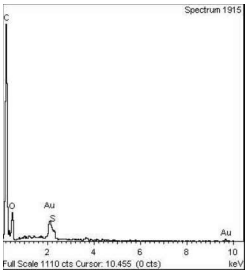
Table 3.6 Stick-to-it® tapes collected

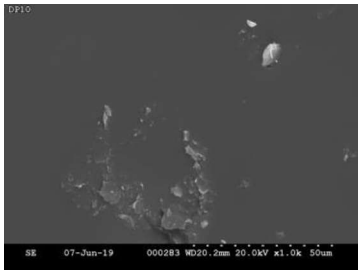
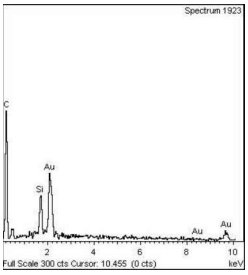
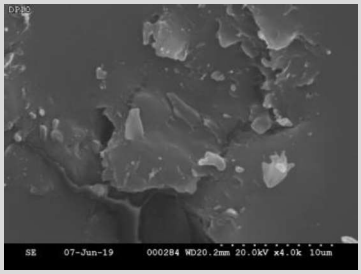
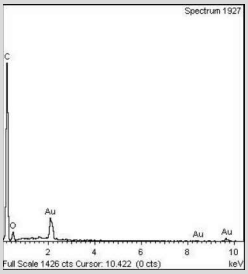
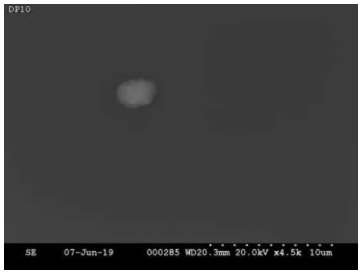
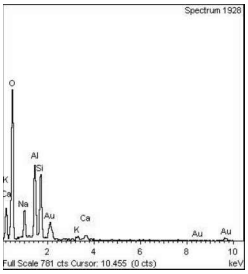
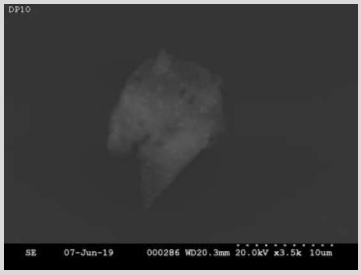
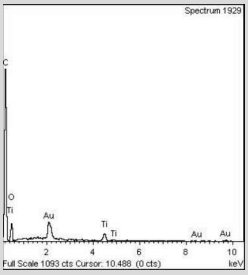
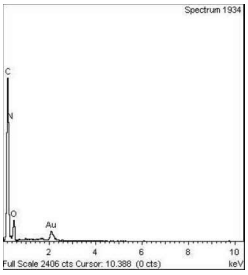
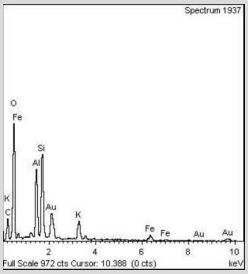
Sample ID	Description
DP08	Material (including visible abradant fibres) collected from the edges of the sample holder on Martindale instrument after abrasion test of reference material
DP09	Material (including visible abradant fibres) collected from the edges of the sample holder on Martindale instrument after abrasion test of graphene-enhanced material
DP10	Material collected from the surface of the abradant material after abrasion test of graphene-enhanced material

Table 3.7 SEM/EDXS Analysis results for Stick-to-it® tapes

Sample ID		SEM Images	EDXS Spectra	Comment
Reference material (no graphene)				
DP08	A			Large inorganic particle of dimensions 53.0 x 43.5 µm exhibiting Ca, O, Na, Al, Si and Cl in EDXS spectrum.
	B			Silicon based particle of dimensions 19.8 x 16.4 µm exhibiting K, Fe, Al, Si and Cl in EDXS spectrum.
	C			Large fibre, likely from abradant material

Sample ID		SEM Images	EDXS Spectra	Comment
	D			Large platelet of dimensions 71.3 x 46.9 µm exhibiting C, O, and S in EDXS spectrum.
	E			Range of carbon based particles, likely to be general dust.
	F		N/A	Overview showing fibre and range of sizes of background dust particles.
	G		N/A	Overview image showing a range of background dust particles. Image is of low resolution so morphology of smallest particles not visible.
Graphene-enhanced material				
DP09	A			Overview of deposition showing the range of sizes of particles observed. EDXS spectrum shows presences of C, O, Si and Ca.

Sample ID	SEM Images	EDXS Spectra	Comment
B			Platelet observed exhibiting C, O, Mg and Si in EDXS spectrum.
C			Agglomerated particle observed of diameter 3.2 µm, exhibiting C, O, and Ca in EDXS spectrum.
D			Inorganic platelet observed of dimensions 132.3 x 78.8 µm exhibiting Cl, K, O, Fe, Al and Si in EDXS spectrum.
E			Platelet observed of dimensions 10.6 x 9.0 µm exhibiting C, O, Si, Al and S in EDXS spectrum.
F			Platelet observed of dimensions 10.6 x 9.0 µm exhibiting C and O in EDXS spectrum.
G		N/A	Image showing fibres, likely to be from the abradant material.

Sample ID		SEM Images	EDXS Spectra	Comment
DP10	A			Range of particles observed majority of which were <3 µm. EDXS spectrum exhibits C, O and Si.
	B			Higher magnification view of platelets, exhibiting C and O in EDXS spectrum.
	C			Poor resolution image showing inorganic particle of dimensions 3.16 x 2.49 µm exhibiting Ca, O, Na, Al and Si in EDXS spectrum.
	D			Poor resolution image showing agglomerated particle of dimensions 15.6 x 10.9 µm exhibiting C, O and Ti in EDXS spectrum.
	E			Poor resolution image showing agglomerated particle of dimensions 9.5 x 6.7 µm exhibiting C and O in EDXS spectrum. Other particles observed ~3.2 – 1.8 µm in size.
	F			Poor resolution image showing inorganic particle of dimensions 3.90 x 3.15 µm exhibiting C, O, Al, K, Fe and Si in EDXS spectrum.

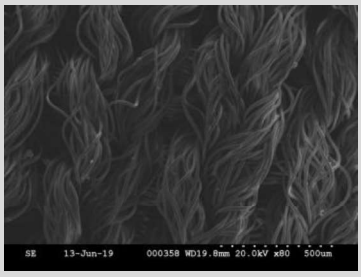
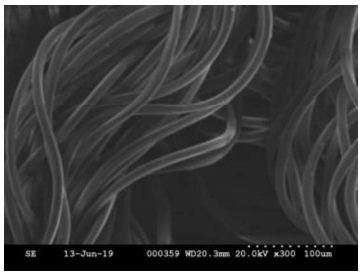
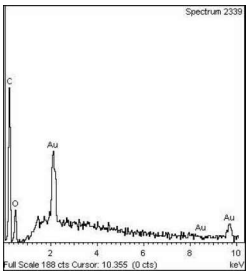
3.3.3. Textile material

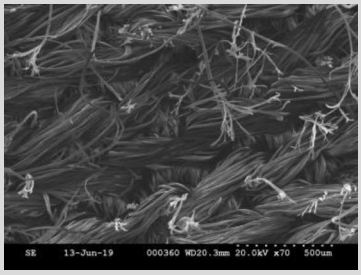
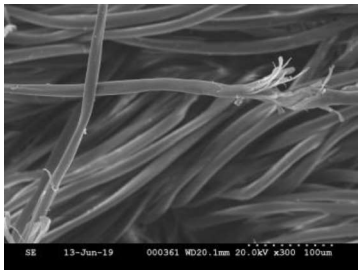
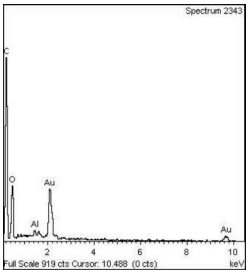
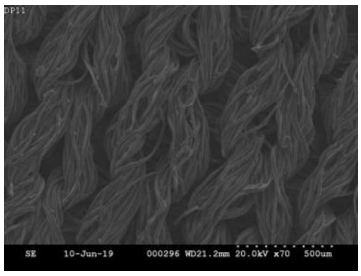
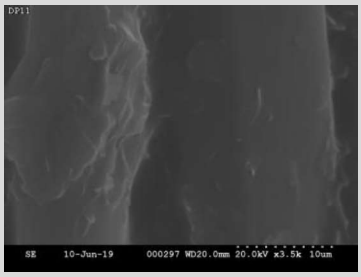
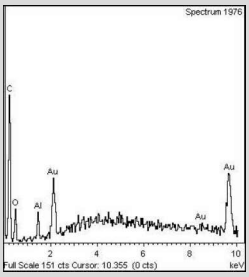
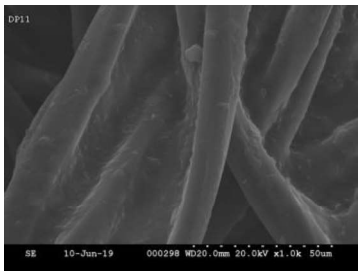
In addition to the collected filter samples, SEM and EDXS imaging was conducted on the two textile materials before and after the abrasion testing to see if any difference was evident. A summary of the samples is given in Table 3.8 and Table 3.9.

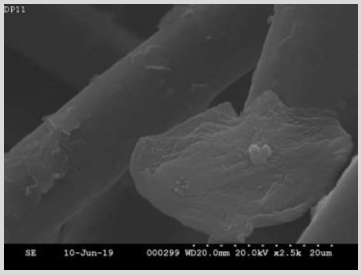
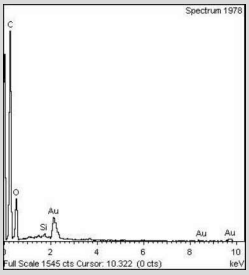
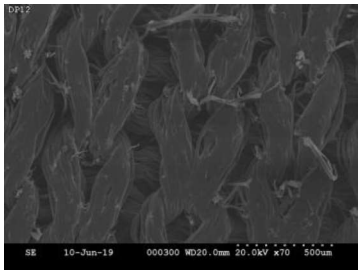
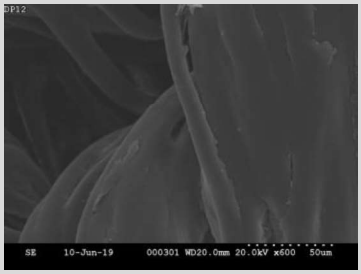
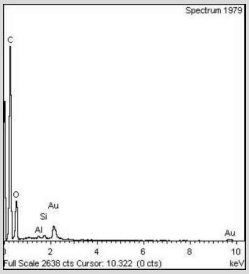
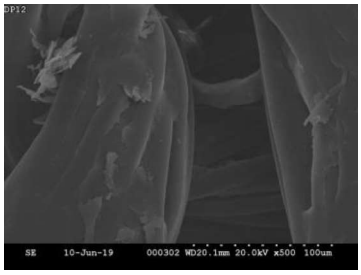
Table 3.8 Textile materials

Sample ID	Description
Reference material	
DP13	Reference textile material **prior to** abrasion testing
DP14	Reference textile material **after** abrasion testing
Graphene-enhanced material	
DP11	Graphene coated textile material **prior to** abrasion testing
DP12	Graphene coated textile material **after** abrasion testing

Table 3.9 SEM/EDXS Images of fabrics

Sample ID		SEM Images	EDXS Spectra	Comment
Reference material (no graphene)				
DP13	A		N/A	Overview of woven reference fabric prior to abrasion testing.
	B			Closer view of threads.

Sample ID		SEM Images	EDXS Spectra	Comment
DP14	A		N/A	Low magnification image showing the reference fabric following abrasion testing. Visible breakages to threads. Greater wear noted than for graphene-coated fabric.
	B			Magnified view showing breakage of threads.
Graphene-enhanced material				
DP11	A		N/A	Low magnification image showing the graphene coating prior to abrasion testing
	B			Higher magnification, graphene platelets observed on surface of textile
	C		N/A	Graphene coating adhering to multiple threads of the textile. Surface is rough in texture due to graphene platelets.

Sample ID		SEM Images	EDXS Spectra	Comment
	D			Large graphene platelet observed between threads of dimensions 30.6 x 19.7 µm.
DP12	A		N/A	Low magnification image showing the graphene coating following abrasion testing. Overall, the surface has been smoothed and threads appear to be free from the weave.
	B			Higher magnification, very few graphene platelets observed on surface of textile. Texture of surface is smooth.
	C		N/A	Some graphene platelets are still visible on individual threads.

4. Discussion and Recommendations

4.1. Visual inspection of abrasion testing

Following abrasion testing, both materials showed minimal wear by visual inspection. Fibrous strands were visible on the Martindale equipment once the abrasion test had finished, however due to their colour and appearance these were attributed to the abradant material (beige flat woven wool fabric) used rather than the textile materials under test. As seen in Figure 3.2, no visible transfer of graphene to the abradant material was observed following abrasive testing of the graphene-enhanced textile. It was noted however, that the graphene-enhanced material was coated in a hexagonal "honeycomb" pattern and the sample diameter was small and therefore the amount of graphene present on the textile is likely to be low. Despite running multiple samples, it is likely that the level of graphene released would be too low to be visible to the naked eye.

The abradant material used was a woven wool fabric as recommended in the ISO standard (ISO 12947-2).[6] However, as a follow-up test, it is suggested that further test material is supplied to allow this to be used as the abradant material on the base pad of the Martindale instrument. This will prevent any contaminants from other materials and would also give an indication of how the graphene-enhanced material wears in contact with itself.

4.2. Real-time Monitoring

Data collected by real-time monitoring showed, in general, steady particle concentration values throughout sampling. The peaks detected (CPC trace 2040-2240s, APS 8155-8182s) did not correlate with any notable activity from the contextual information gathered and when viewed alongside the low level of deposition on the air filters were concluded to be artefacts.

A gradual rise in particle concentration was observed on the CPC during sampling of the abrasion test on the graphene-enhanced fabric. There are a number of reasons why this gradual increase may have occurred; including a general increase in heat/activity as the day progressed, increase in process related nanomaterials present, or the presence of material specific nanomaterial (i.e. graphene). SEM analysis of the air filters collected during abrasion showed no difference in deposition between the reference and graphene-enhanced material. This indicates that this increase in CPC concentration is likely related to the laboratory environment or process and not indicative of graphene release. As no analogous peak was observed on the APS or DustTrak the particles being detected are

[6] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method - Part 2: Determination of specimen breakdown (ISO 12947-2:2016)

likely sized between 5-500 nm. If further tests were performed this could be investigated further, for example by running the graphene-enhanced fabric on the Martindale before the reference material or running each material at the same time on different days.

4.3. SEM/EDXS Analysis

All filter samples collected showed very light loading, despite noting some visible fibres released from the abradant material on to the Martindale after the test was complete, which demonstrates the low level of release during the short sampling time of this test and the low abrasive nature of the ISO recommended baseline material.

Filter samples collected during the abrasion testing of the graphene-enhanced fabric showed presence of carbon-based platelets. However, the morphology of these materials do not fully correspond with example images supplied by Directa Plus. Furthermore, as carbonaceous platelets of a similar morphology and elemental profile were also identified on the filters collected during abrasion testing of the reference material (images DP01-B, DP02-E, DP03-B) and in the far-field location (image DP04-B), it is not possible to conclusively identify these platelets as graphene. The use of free graphene reference material for our own SEM analysis would be useful to confirm or refute these observations.

Analysis of the Stick-to-it® samples collected from the edges of the sample holder on the Martindale instrument after abrasion testing (DP08 and DP09) found a mixture of inorganic particles (images DP08-A, DP09-D), fibres (images DP08-C, DP09-G) and carbonaceous platelets (images DP08-E, DP09-F). In both samples, the carbon-based platelets observed were similar in morphology to those found on the filter samples taken during abrasion testing of the reference material, hence are unlikely to have derived from the graphene coating of the Directa Plus material.

For sample DP10 (Stick-to-it® sample collected from the surface of the abradant material after abrasion test of graphene-enhanced material), again a mixture of inorganic particles, fibres and carbonaceous platelets were observed. Platelets-like morphologies were observed in images DP08, DP09, DP10-A and DP10-B. Due to the poor resolution obtained from Stick-to-it® tapes, the morphology of these individual platelets is not detailed (images DP10-C – DP10-F). Again, as morphology is not clear and carbonaceous material is observed in reference samples the presence of graphene platelets cannot be conclusively determined.

When analysed by SEM, obvious wear to the fabric fibres was noted for the reference material which was not observed for the graphene-coated material (Table 3.9, DP11-14). Samples DP13 and DP14 showed visible breakages to the threads of the reference material. This was not the case for the graphene-coated fabric (DP11 and DP12) which instead showed a general smoothing of the fabric surface texture. In sample DP11, graphene platelets can be observed on the surface of the fibres, giving an overall rough texture to the coating. Furthermore, larger free platelets were observed between fibres (image DP11-D). Sample DP12 shows that abrasion has caused some threads to become free from the fabric weave (image DP12-A) and the overall texture of the coating is smooth compared with sample DP11 (image DP12-B). However, some platelets were still visible on the surface of the coating (image DP12-C). Overall, these SEM images show abrasion has caused the graphene coating to become smooth, which may have led to a release of graphene platelets, however this has not been confirmed by the current

sampling. It is likely that the level of graphene release would be very low for the amount of material tested in this case.

4.4. Recommendations

As described in the original quote, this work was a pilot study to (partly) determine the feasibility of including the proposed adaption of an ISO standard used in the textiles industry. In addition, to determine which measurements were suitable based on the sample matrix and results required by Directa Plus. From the obtained results it can be concluded that i) the adaptions to the ISO 12947 method to include real-time and static monitoring was successful and informative, and ii) under the conditions used, the release of graphene was, at best, not observed, and at worst, at low levels.

Following on from these results, we recommend carrying out follow-up tests, including:

- Abrasion testing using the reference or the graphene-enhanced fabric as the abrasive material, or an alternative suitable material;
- Abrasion testing using both a higher applied nominal pressure and a higher number of rubs to simulate harder and longer-term wear;
- Bioelution testing of the base textile with and without graphene in simulated sweat (SSW) solution to determine if any free graphene may be released upon dermal contact with the enhanced material. This information would be relevant for both occupational exposure and consequent consumer exposure;
- Elution testing of the base textile with and without graphene in detergent following the ISO standard test for "colour fastness to commercial and domestic laundering" (ISO 105-C06:2010). This will allow assessment of the release of graphene upon washing the fabric. Additionally, this test could be used in combination with the abrasion testing procedure to determine the effect of abrasion on laundered graphene-enhanced textiles.
- Provision of reference materials: free graphene and graphene held within application medium (e.g. paste). This will allow accurate comparisons to be made between the reference and collected samples after any specific testing.

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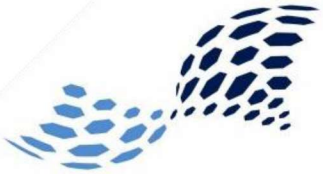

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Bioelution of Graphene-Containing Cotton Textiles

Project Code: P4627

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Bioelution of Graphene-Containing Textiles

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Revision

1

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Document revisions

No	Details	Date
01	Draft with client	09/02/2021

Contents

1. Introduction

At the request of Carolina Volonte at Directa Plus Spa, IOM undertook a study to determine the release of free graphene platelets (as a High Aspect Ratio Nanomaterial) from cotton based textile samples during general wear.

At this time, there is no standard to measure particulate release for textiles. Therefore, IOM have previously devised a testing strategy to determine graphene release from textiles using PES-based textiles, including measurements of particle release when an abrasion force is applied, as an adaption of the ISO 12947 standard for "Determination of the abrasion resistance of fabrics",[1] and particle release in simulated biological fluids relating to dermal exposure (P2590 and P2965, abrasion and bioelution studies, respectively). This information was deemed relevant for both occupational exposure and subsequent consumer use.

Here we conduct the same bioelution testing, using the base cotton textile with and without graphene in simulated sweat (SSW) solution to determine if any free graphene may be released from the enhanced material when in an environment used to simulate dermal exposure and dermal contact, i.e. sweat. The above mentioned abrasion testing method has already been conducted for cotton-based textiles, and is reported in P4627.

IOM's standard procedure for bioelution testing was followed in combination with SEM/EDXS analysis and total and elemental carbon analysis. These would allow assessment of the potential release of free graphene from the enhanced material during general wear.

[1] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method (ISO 12947)

2. Methodology

2.1. Bioelution Study

Material samples provided by Directa Plus (diameter of 10 cm) were added to a volume of 50 mL of simulated fluid in a 250 mL Erlenmeyer flask. Three replicates and a blank were produced to correspond with each sampling point. The flasks were covered with Parafilm and placed in an orbital shaker at 37°C with an agitation rate of 100 rpm. Sampling occurred at 0, 2, 6 and 24 hours.

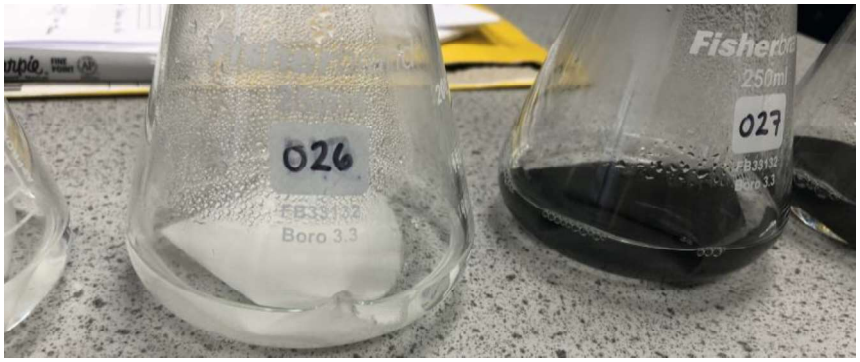


Figure 1 Cotton material samples were folded into the 250mL flasks (as provided at 10cm)

Table 2.1 Testing Matrix per Test Material per Simulated Fluid

	Sample Points (hours)			
Sample	0	2	6	24
Replicate 1	✓	✓	✓	✓
Replicate 2	✓	✓	✓	✓
Replicate 3	✓	✓	✓	✓
Blank	✓	✓	✓	✓
Simulated Fluid	✓	-	-	-

Table 2.2 General Details

Condition	Details
Test Duration (hours)	24
Number of replicates	3
Sampling time points (hours)	0, 2, 6 and 24
Temperature ^{0}C	37

Condition	Details
Simulated biological fluids	Artificial sweat (SSW(3))
Flask size (mL)	250 (Erlenmeyer, covered with Parafilm)
Sample volume (mL)	50
Agitation method	Environmentally controlled orbital shaker at 100 rpm
Samples assessed	2 samples: Reference cotton material and graphene coated cotton material

In accordance with the Bioaccessibility SOP[2], after the appropriate extraction time, the test vessels were removed from the shaker. They were left to settle for a standardised period of 5 minutes and the Parafilm top removed. The fabric was removed from the fluid and then fluid samples were transferred to a uniquely labelled sample tube and stored at room temperature in the dark until filtered for subsequent total carbon analysis and SEM/EDXS. Prior to filtration, the three replicate samples were combined. Samples from each time point were filtered through 25mm polycarbonate filters (pore size 0.2 µm) and 25mm tissue quartz filters for SEM/EDXS and total carbon analysis, respectively. The polycarbonate filter sample was rinsed with deionised water prior to analysis to remove excess salts from SSW solution. It was hypothesised that we may be able to use total carbon as a proxy for graphene, in the same fashion NIOSH recommend for carbon nanotubes.[3] Note that carbon analysis was outsourced to Marchwood Scientific Services (Gtr Manchester, UK).

Table 2.3 Simulated Biological Fluids[4]

Compositions in g/L	Artificial Sweat Fluid (SSW(3))
Sodium Chloride	2.92
Calcium Chloride	0.166
Magnesium Sulphate	0.12
Potassium Phosphate Monobasic	1.02
pH	5.4

The solution was prepared and left to equilibrate overnight. The pH of the solution was then checked and adjusted accordingly with either concentrated HCl or 10N NaOH.

[2] The Standard Operating Procedure for the Bioaccesibility Testing Program. Version 2.0. Revision by Tony Brouwers.

[3] NIOSH Current Intelligent Bulleting 65 "*Occupational Exposure to Carbon Nanotubes and Nanofibers*" advocates the use of elemental carbon determination as a practical method for the measurement of carbon nanotubes (CNT) and carbon nanofibres (CNF) in the workplace. Document available: https://www.cdc.gov/niosh/docs/2013-145/pdfs/2013-145.pdf?id=10.26616/NIOSHPUB2013145 Accessed 03/10/2019.

[4] Margareth R.C Marques, Raimar Loebenberg, and May Almukainzi. Simulated Biological Fluids with Possible Application in Dissolution Testing.Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

2.2. Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy (SEM/EDXS)

The filter samples prepared were analysed by image and elemental profiling using a Hitachi: S-2600N Scanning Electron Microscope, INCA analyser software, and a modification of the following method:

SOP-009_V2: "Scanning Electron Microscopy – sample preparation, EDXS analysis and systematic filter analysis".

The technique for systematic filter analysis is adopted from ISO 14966:2019: "Ambient Air – Determination of numerical concentration of inorganic fibrous particles – Scanning electron microscopy method".

In preparation for SEM/EDXS analysis, a portion of each filter or textile sample was excised and mounted onto a 13 mm diameter aluminium SEM stub and sputter-coated with gold to enhance the conductivity of the surface and therefore imaging resolution. Images were recorded at various magnifications to best represent the distribution, size and shape of particles captured from the testing process and elemental analysis was carried out for chemical composition.

3. Results

3.1. Bioelution Results

Following 24 hour incubation in SSW(3) fluid, no visible change in colour of the fluid was noted (Figure 2). If a high concentration of graphene was released, it may be expected that the fluid would blacken due to the presence of graphene.

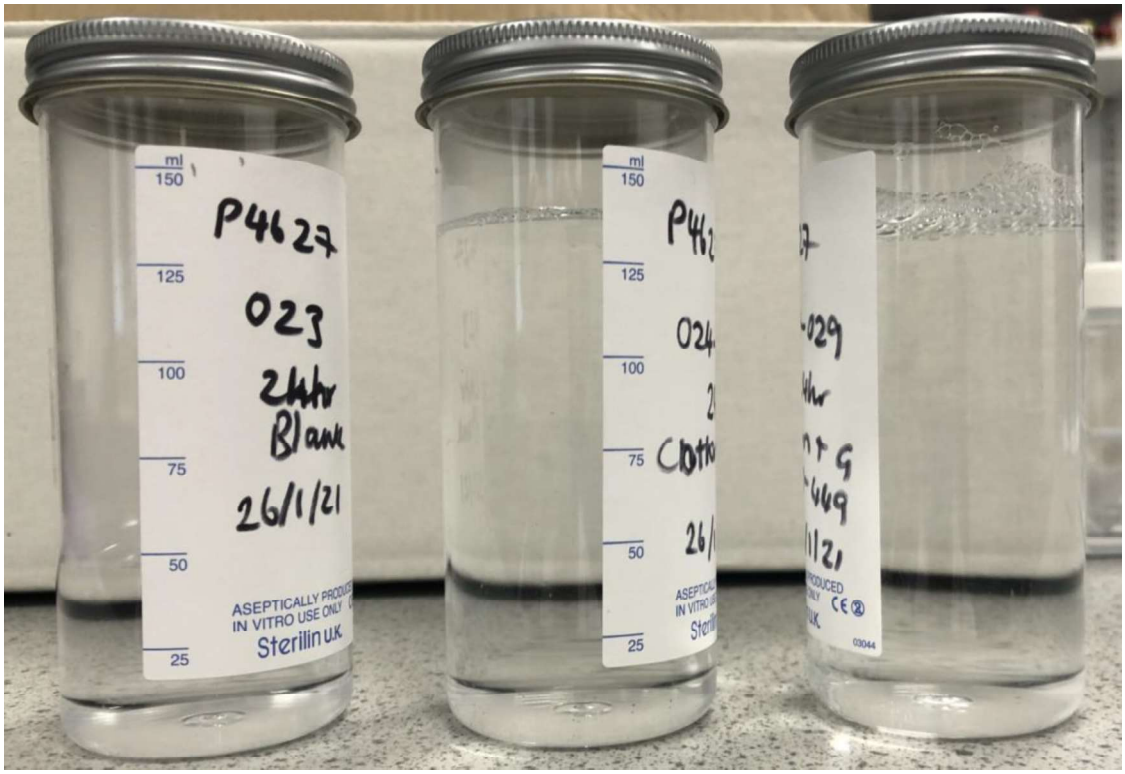


Figure 2 Fluid samples following 24 hour incubation with no material (left), cotton reference material (middle), and graphene-enhanced cotton material (right).

Table 3.1 Consolidated results of total carbon quantification in Simulated Sweat Fluid (SSW(3))

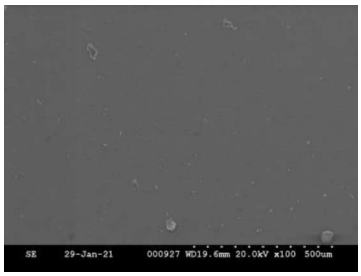
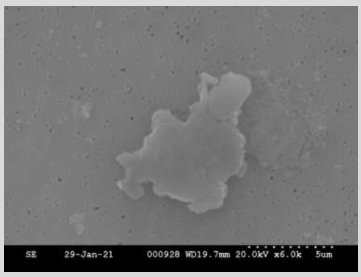
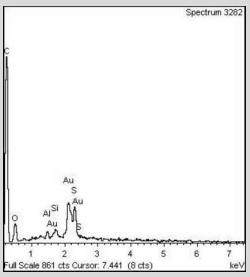
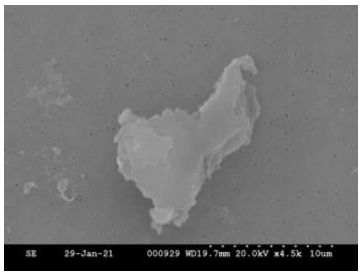
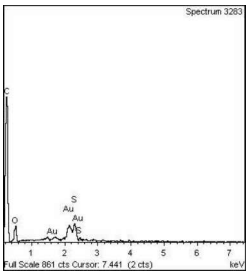
Total Carbon Analysis	Volume filtered (mL)	Elemental Carbon (µg)				Organic Carbon (µg)				Total Carbon (µg)			
		0	2	6	24	0	2	6	24	0	2	6	24
		Sampling Point (hours)											
Reference Fabric	20	8.6	2.7	9.3	2.6	100	67	64	34	110	70	73	36
Enhanced Fabric	20	6.5	17	12	5.9	68	46	54	37	74	63	66	43
Fluid Blank					<2				24				24

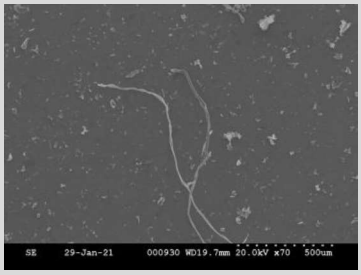
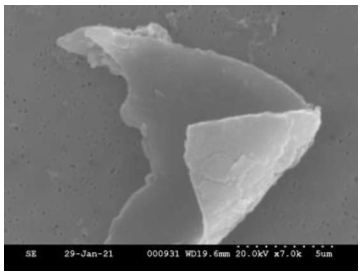
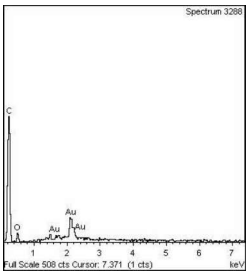
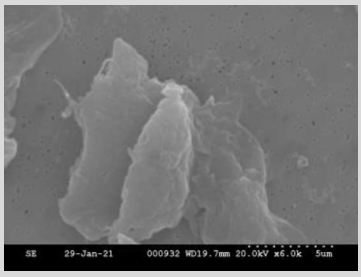
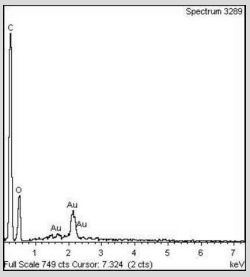
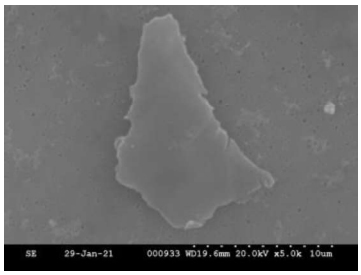
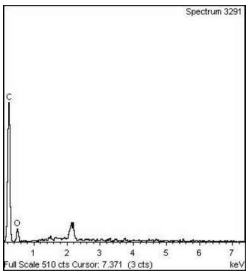
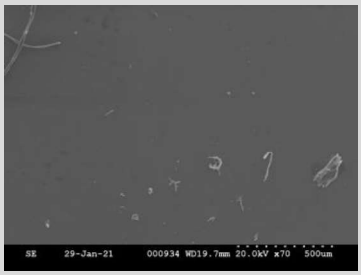
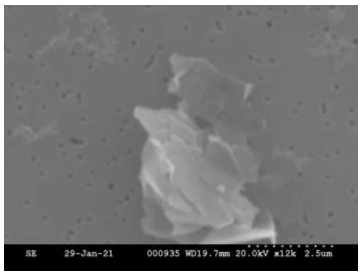
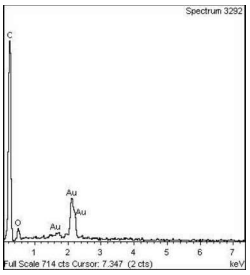
The limit of detection (LOD) for this method is 2µg for elemental carbon and 10 µg for organic and total carbon.

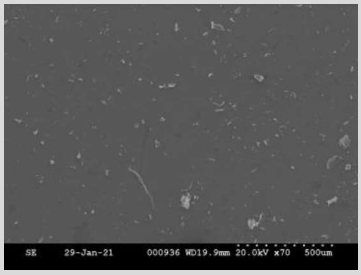
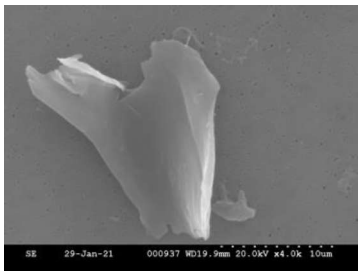
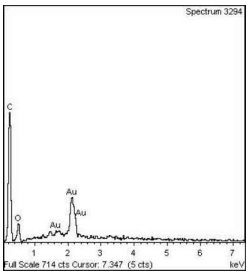
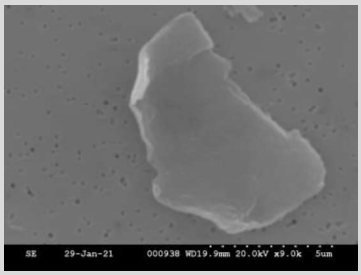
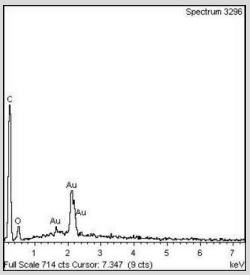
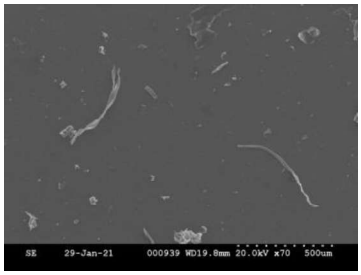
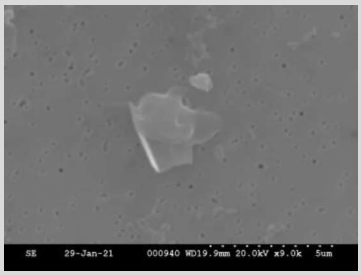
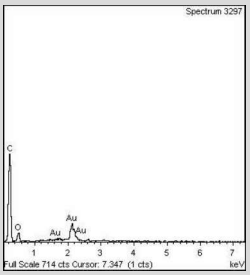
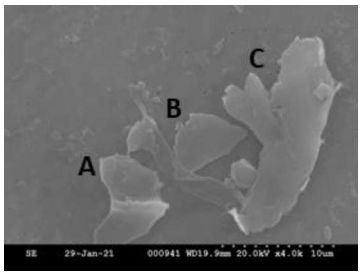
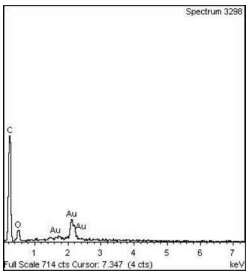
3.2. SEM/EDXS Analysis

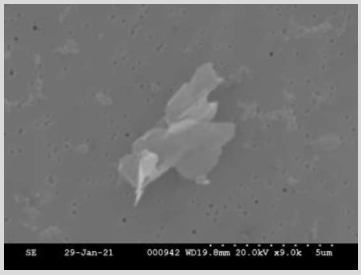
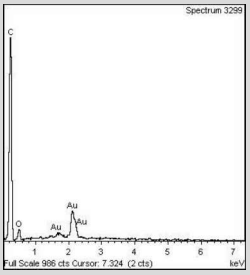
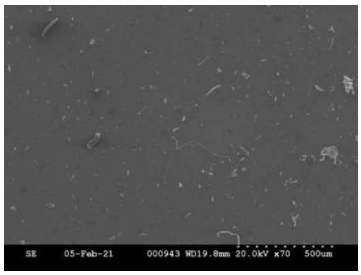
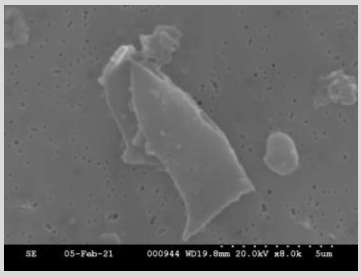
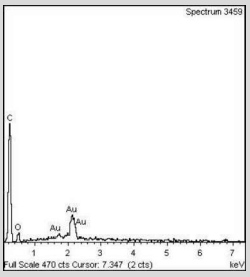
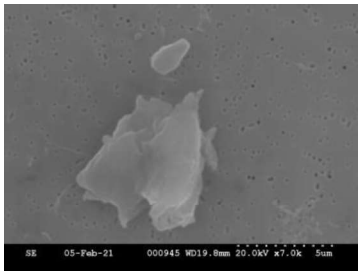
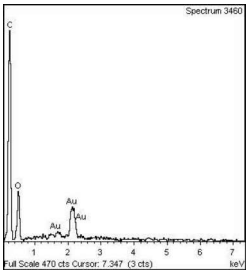
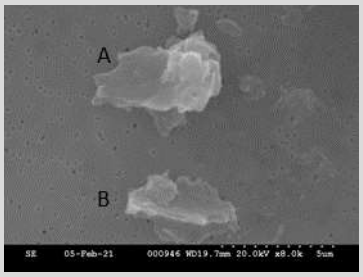
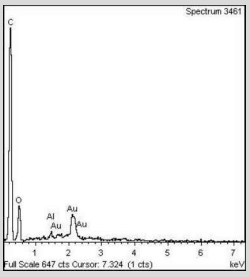
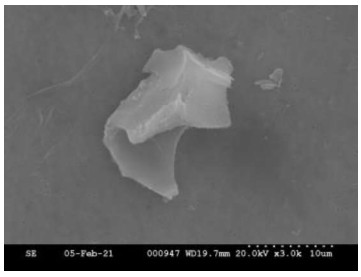
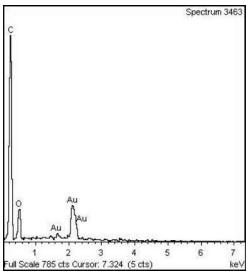
Sample ID	Volume filtered (mL)	Fabric	Time point (hours)
Blank	20	N/A	N/A
DP01	20	Reference	0
DP02	20	Enhanced	0
DP03	20	Reference	2
DP04	20	Enhanced	2
DP05	20	Reference	6
DP06	20	Enhanced	6
DP07	20	Reference	24
DP08	20	Enhanced	24

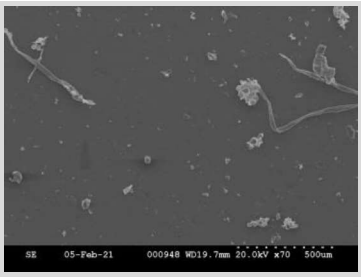
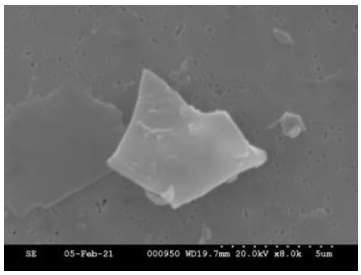
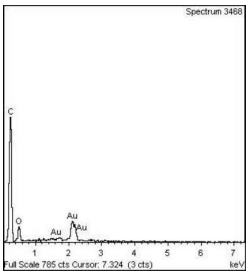
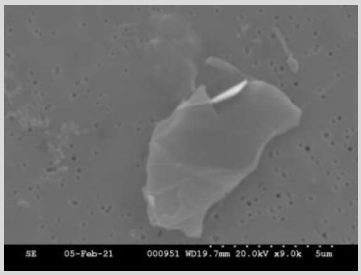
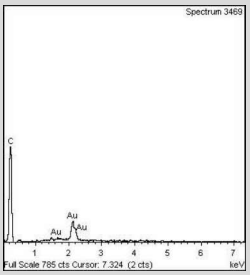
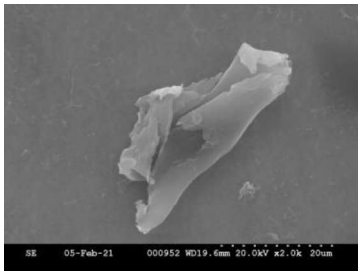
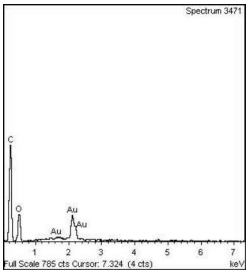
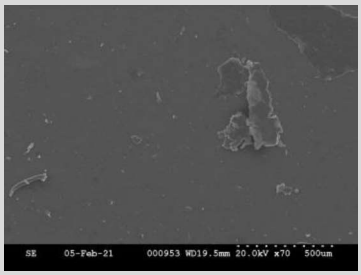
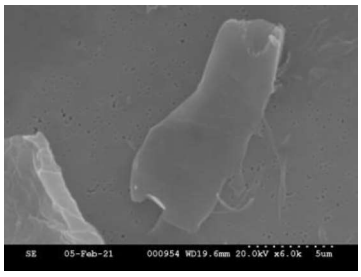
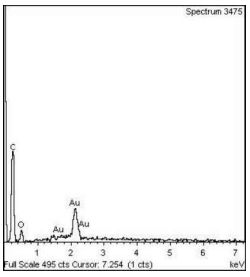
Table 3.2 SEM/EDXS Analysis results

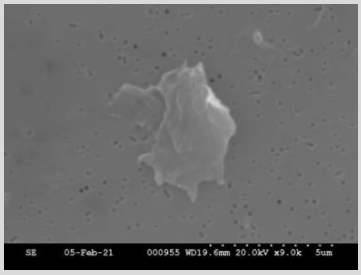
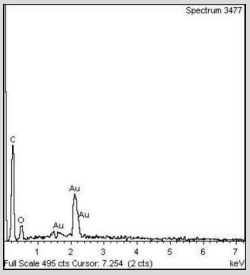
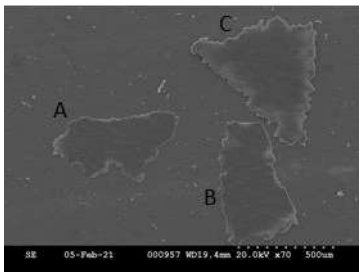
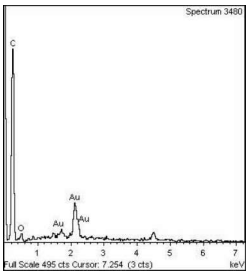
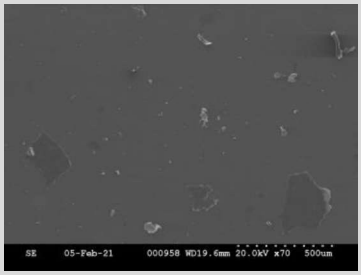
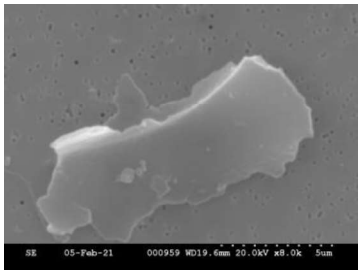
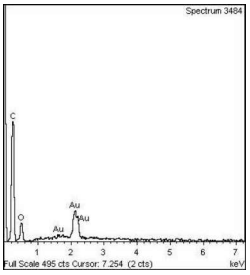
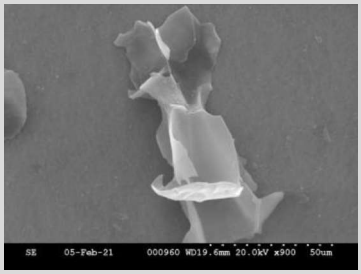
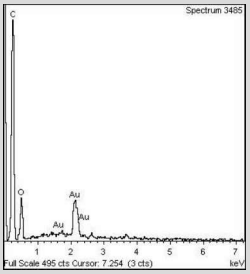
Sample ID		SEM Images	EDXS Spectra	Comment
Blank	A		N/A	Overview of filter, showing a light coverage of particles.
	B			Particle of dimensions 9.8 x 6.6 µm exhibiting C, O and S in EDXS spectrum.
	C			Particle of dimensions 15.7 x 8.0 µm exhibiting C, O and S in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
DP01	A		N/A	Overview of filter, showing a moderate coverage of particles with fibres present.
	B			Particle present of approximate dimensions 14 x 12 µm. Particle is folded over itself, and surface is rough in texture. EDXS spectrum exhibits C and O.
	C			Carbonaceous material of width 11.2 µm.
	D			Carbon-based platelet of dimensions 13.9 x 7.9 µm exhibiting C and O in EDXS spectrum.
DP02	A		N/A	Overview of filter, showing a light coverage of particles with fibres present.
	B			Collection of carbon-based platelets. Width of agglomerate is 4 µm.

Sample ID		SEM Images	EDXS Spectra	Comment
DP03	A		N/A	Overview of filter, showing a moderate coverage of particles with some fibres present.
	B			Carbon-based platelet of width 17.8 µm, exhibiting C and O in EDXS spectrum.
	C			Carbon-based platelet of dimensions 8.9 x 6.2 µm exhibiting C and O in EDXS spectrum.
DP04	A		N/A	Overview of filter, showing a moderate coverage of particles with fibres present.
	B			Particle of dimensions 3.2 x 3.0 µm exhibiting C and O in EDXS spectrum.
	C			Collection of carbon-based platelets. Dimensions of (A) 9.5 x 6.1 µm, (B) 5.9 µm width, and (C) 18.8 x 9.0 µm.

Sample ID		SEM Images	EDXS Spectra	Comment
	D			Particle of dimensions 5.8 x 2.9 µm exhibiting C and O in EDXS spectrum.
DP05	A		N/A	Overview of filter, showing a moderate coverage of particles with fibres present.
	B			Particle of dimensions 4.0 x 8.6 µm exhibiting C and O in EDXS spectrum.
	C			Particle of dimensions 6.4 x 8.3 µm exhibiting C and O in EDXS spectrum.
	D			Two carbon-based platelets of dimensions: A – 5.0 x 5.6 µm; B – 3.1 x 5.1 µm.
	E			Larger carbon-based particle of dimensions 11.7 x 17.6 µm.

Sample ID		SEM Images	EDXS Spectra	Comment
DP06	A		N/A	Overview of filter, showing a moderate coverage of particles with fibres present.
	B			Particle of dimensions 6.1 x 6.3 µm exhibiting C and O in EDXS spectrum.
	C			Platelet of dimensions 4.5 x 7.2 µm exhibiting C in EDXS spectrum.
	D			Large carbon-based material of dimensions 17.7 x 41.5 µm.
DP07	A		N/A	Overview of filter, showing moderate loading with large platelets visible (top right hand side of image).
	B			Larger carbon-based particle of dimensions 5.7 x 13.3 µm exhibiting C and O in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
	C			Particle of dimensions 4.0 x 5.5 µm exhibiting C and O in EDXS spectrum.
	D			Large carbon-based platelets of dimensions: A – 311 x 656 µm; B – 371 x 628 µm; C – 520 x 756 µm.
DP08	A		N/A	Overview of filter, showing a light coverage of particles and platelets. Fewer small platelets observed on this sample compared with previous time points.
	B			Platelet of dimensions 5.7 x 12.9 µm exhibiting C and O in EDXS spectrum.
	C			Large carbon-based particle of dimensions 45.4 x 95.7 µm.

The particles on these filters exhibited a morphology and composition similar to what could be expected for graphene, in particular DP02 B, DP04 B, DP04 D, and DP06 C. However, as has been highlighted in the previous reports with this fabric (abrasion testing, P4627), material with a similar morphology was found in the assessment of control material, in this case on filters from bioelution tests in absence of the graphene coating (e.g. DP03 B, DP05 C, DP07 C). In both the reference and enhanced samples, large carbon-based material was observed on the filter and could be the origin of the smaller platelets (e.g. DP04 C, DP06 D, DP08 C). Furthermore, carbon-based material was also identified on the fluid blank sample analysed. It is therefore not possible to conclusively identify these platelets as graphene.

4. Discussion and Recommendations

4.1. Bioelution Testing

Carbon analysis of the fluid extracted at each time point had a varied response for elemental, organic or total carbon. No clear trend could be detected for total or organic carbon, while elemental carbon appeared raised in all incubated (2, 6 and 24 hr) samples of the graphene-enhanced fabric. However, only one sample (enhanced after 2-hour incubation) showed a substantial difference in elemental carbon compared with the reference sample, with 2.7 µg for the reference fabric and 17 µg for the enhanced fabric. However, to fully interpret whether these values have a substantial meaning, a calibration of this technique would be required using suitable reference materials; to better understand the limitations of this method and provide more robust interpretation, it is recommended that the free graphene is used to better define reliable detection values which would indicate a significant change.

4.2. SEM/EDXS Analysis

Carbon-based particles were observed on all filters prepared. The morphology of carbon particles observed on filters DP04, DP06 and DP08 are similar to what could be expected for graphene, in particular the particle imaged in DP04 B and DP06 C where the visible layers can be seen on the surface of the particle, similar to images provided by Directa Plus for graphene coating. However, as carbonaceous platelets of a similar morphology and elemental profile were also identified on the filters collected from the reference material (DP03, DP05 and DP07), it is not possible to conclusively identify these platelets as graphene. As shown in 0, the fibre end of the reference textile also shows layers on the surface and therefore, any fibre breakages may show morphology such as that seen in DP04 and DP06.

4.3. Conclusions

From the obtained results, the following conclusions can be made:

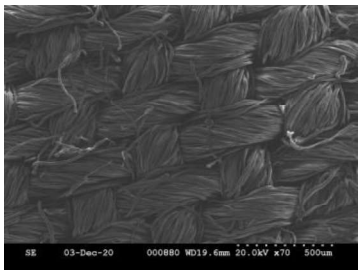
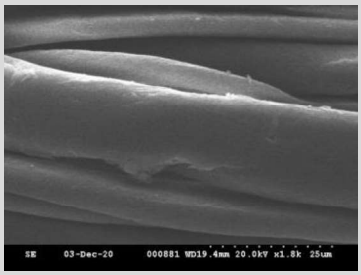
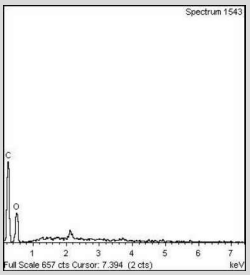
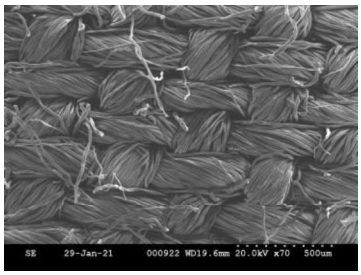
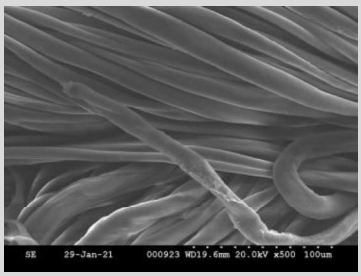
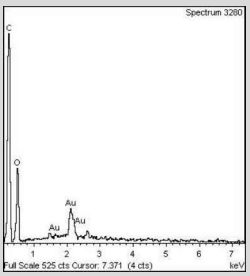
i) Adaptations to our in-house bioelution method was successful and informative.

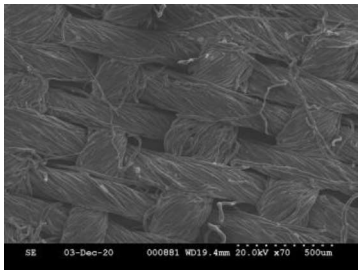
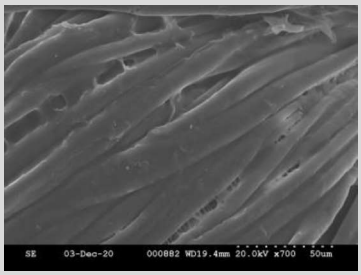
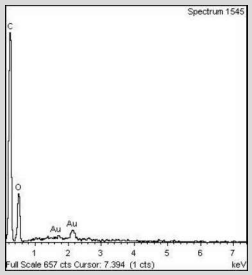
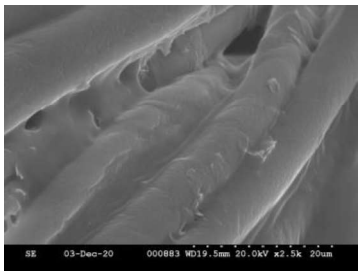
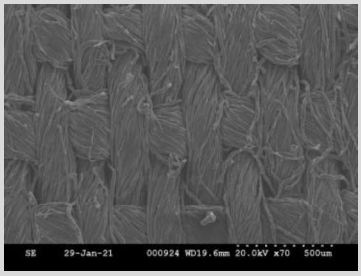
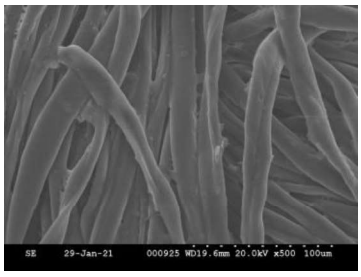
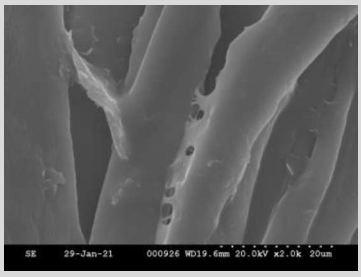
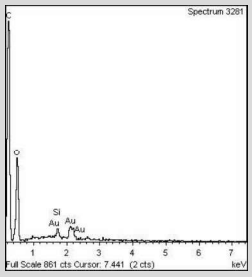
ii) Release of graphene in simulated sweat fluid (SSW(3)) following bioleution testing for 24 hours was, at best, not observed, and at worst, at low levels.

iii) The use of various reference materials, such as free graphene and graphene-containing paste (prior to application to fabrics), would be crucial to confirm or refute these observations, as well as allowing us to validate the analysis techniques employed in this pilot study.

Appendix 1. SEM/EDXS Images of Textiles

Table 0.1 SEM/EDXS Images of Textiles

Sample ID		SEM Images	EDXS Spectra	Comment
Reference Material Before Bioelution	A		N/A	No visible change has been identified with the reference textile following bioelution testing.
	B			Rough edges can be seen on the cotton fibres, which may fragment during bioelution and could be misinterpreted as graphene platelets.
Reference Material After Bioelution (T = 24 hours)	A		N/A	
	B			

Sample ID		SEM Images	EDXS Spectra	Comment
Graphene Coated Material Before Bioelution	A		N/A	No significant visible change has been identified with the graphene coated textile following bioelution testing. Images B show that the graphene coating has remained intact following exposure to simulated sweat fluid for 24 hours. Some thinning of the coating between fibres may be suggested when comparing images C of the material before and after bioelution testing, however this may also reflect varying coating concentration on individual fibres across the fabric.
	B			
	C		N/A	
Graphene Coated Material After Bioelution (T = 24 hours)	A		N/A	
	B		N/A	
	C			

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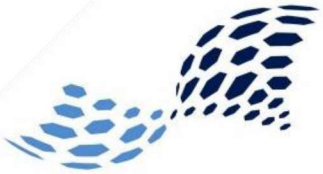

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Bioelution of Graphene-Containing Textiles

Project Code: P2965

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Document revisions

No	Details	Date
01	Draft with client	03/10/2019

Contents

1. Introduction

At the request of Carolina Volonte at Directa Plus Spa, IOM undertook a pilot study to determine the release of free graphene platelets (as a High Aspect Ratio Nanomaterial) from textile samples during general wear.

At this time, there is no standard to measure particulate release for textiles. Previous work carried out by IOM to determine particle release when an abrasion force is applied was carried out by following the ISO 12947 standard for "Determination of the abrasion resistance of fabrics" (P2590).[1,2]

Following on from this study, it was recommended for bioelution testing to be conducted of the base textile with and without graphene in simulated sweat (SSW) solution to determine if any free graphene may be released from the enhanced material when in an environment used to simulate components of dermal exposure and dermal contact, i.e. sweat. This information was deemed relevant for both occupational exposure and subsequent consumer use.

IOM's standard procedure for bioelution testing was followed in combination with SEM/EDXS analysis and total carbon analysis, these would allow assessment of the potential release of free graphene from the enhanced material during general wear. This work is carried out as a pilot study to determine which measurements are suitable for this form of sample matrix.

[1] Textiles — Determination of the abrasion resistance of fabrics by the Martindale method (ISO 12947)
[2] IOM Report P2590 - Abrasion Testing of Graphene Textile

2. Methodology

2.1. Bioelution Study

Material samples provided by Directa Plus (diameter of 6cm) were added to a volume of 50 mL of simulated fluid in a 250 mL Erlenmeyer flask. Three replicates and a blank were produced to correspond with each sampling point. The flasks were covered with Parafilm and placed in an orbital shaker at 37°C with an agitation rate of 100 rpm. Sampling occurred at 0, 2, 6 and 24 hours.

Table 2.1 Testing Matrix per Test Material per Simulated Fluid

	Sample Points (hours)			
Sample	0	2	6	24
Replicate 1	✓	✓	✓	✓
Replicate 2	✓	✓	✓	✓
Replicate 3	✓	✓	✓	✓
Blank	✓	✓	✓	✓
Simulated Fluid	✓	-	-	-

Table 2.2 General Details

Condition	Details
Test Duration (hours)	24
Number of replicates	3
Sampling time points (hours)	0, 2, 6 and 24
Temperature ^{0}C	37
Simulated biological fluids	Artificial sweat (SSW(3))
Flask size (mL)	250 (Erlenmeyer, covered with Parafilm)
Sample volume (mL)	50
Agitation method	Environmentally controlled orbital shaker at 100 rpm
Samples assessed	2 samples: Reference material and graphene coated material

In accordance with the Bioaccessibility SOP[3], after the appropriate extraction time, the test vessels were removed from the shaker. They were left to settle for a standardised period of 5 minutes and the Parafilm top removed. The samples were then transferred to a uniquely labelled sample tube and stored at room temperature in the dark until filtered for subsequent total carbon analysis and SEM/EDXS. Prior to filtration, the three replicate samples were combined. Samples from each time point were filtered through 25mm polycarbonate filters (pore size 0.2 µm). The

[3] The Standard Operating Procedure for the Bioaccesibility Testing Program. Version 2.0. Revision by Tony Brouwers.

filtered sample was rinsed with deionised water prior to analysis to remove excess salts from SSW solution. It was hypothesised that we may be able to use total carbon as a proxy for graphene, in the same fashion NIOSH recommend for carbon nanotubes.[4]

Table 2.3 Simulated Biological Fluids[5]

Compositions in g/L	Artificial Sweat Fluid (SSW(3))
Sodium Chloride	2.92
Calcium Chloride	0.166
Magnesium Sulphate	0.12
Potassium Phosphate Monobasic	1.02
pH	5.4

The solution was prepared and left to equilibrate overnight. The pH of the solution was then checked and adjusted accordingly with either concentrated HCl or 10N NaOH.

2.2. Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy (SEM/EDXS)

The filter samples prepared were analysed by image and elemental profiling using a Hitachi: S-2600N Scanning Electron Microscope, INCA analyser software, and a modification of the following method:

SOP-009_V2: "Scanning Electron Microscopy – sample preparation, EDXS analysis and systematic filter analysis".

The technique for systematic filter analysis is adopted from ISO 14966:2002: "Ambient Air – Determination of numerical concentration of inorganic fibrous particles – Scanning electron microscopy method".

In preparation for SEM/EDXS analysis, a portion of each filter, tape or textile sample was excised and mounted onto a 13 mm or 25 mm diameter aluminium SEM stub, respectively, and sputter-coated with gold to enhance the conductivity of the surface and therefore imaging resolution. Images were recorded at various magnifications to best represent the distribution, size and shape of particles captured from the testing process and elemental analysis was carried out for chemical composition.

[4] NIOSH Current Intelligent Bulleting 65 "*Occupational Exposure to Carbon Nanotubes and Nanofibers*" advocates the use of elemental carbon determination as a practical method for the measurement of carbon nanotubes (CNT) and carbon nanofibres (CNF) in the workplace. Document available: https://www.cdc.gov/niosh/docs/2013-145/pdfs/2013-145.pdf?id=10.26616/NIOSHPUB2013145 Accessed 03/10/2019.
[5] Margareth R.C Marques, Raimar Loebenberg, and May Almukainzi. Simulated Biological Fluids with Possible Application in Dissolution Testing.Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

3. Results

3.1. Bioelution Results

Table 3.1 Consolidated results of fabrics in Simulated Sweat Fluid (SSW(3))

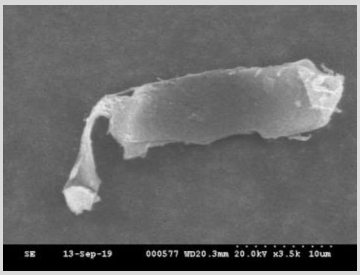
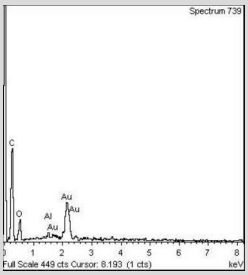
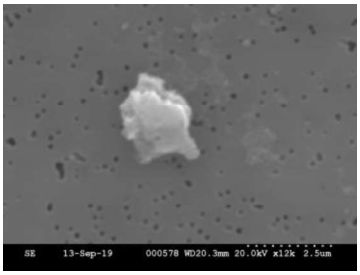
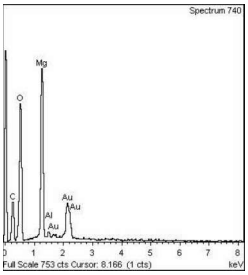
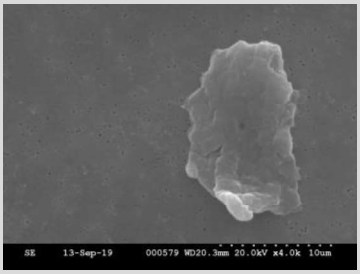
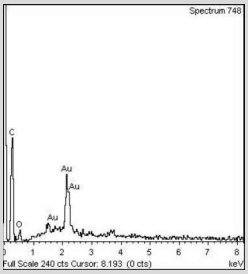
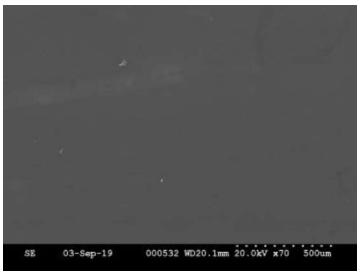
Total Carbon Analysis	Volume filtered (mL)	Sampling Point (hours)			
		0	2	6	24
		Total Carbon (µg)			
Reference Fabric	20	400	<LOD	<LOD	460
Enhanced Fabric	20	170	440	300	240
Fluid Blank	20	<LOD	N/A	N/A	250

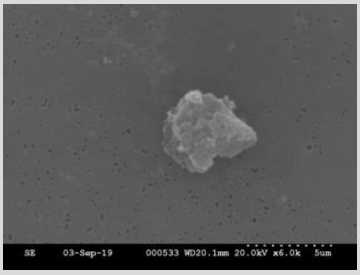
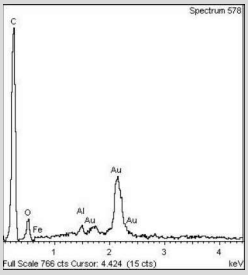
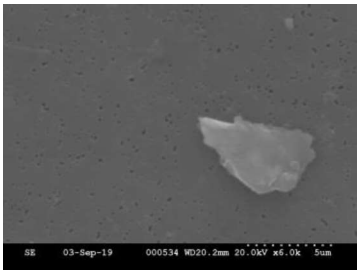
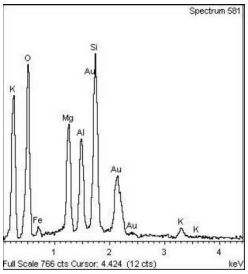
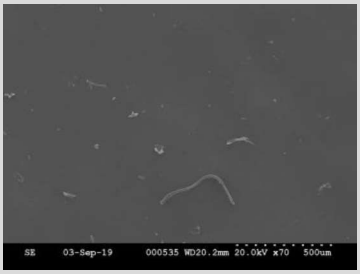
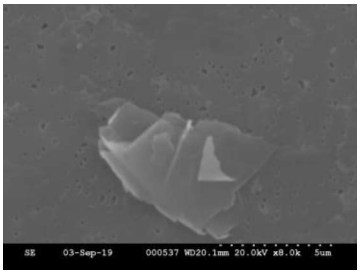
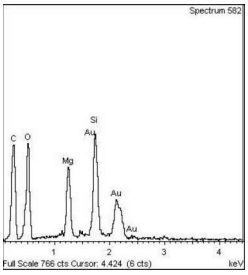
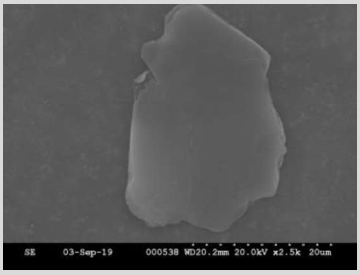
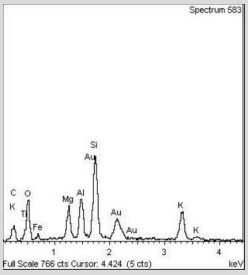
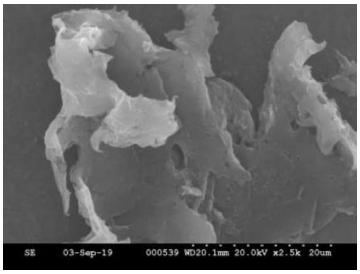
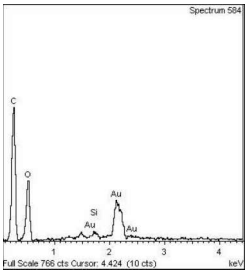
The limit of detection (LOD) for this method is 10 µg. It was noted that the fluid blank sample taken after 24 hours gave a value of 250 µg. It is unlikely that the simulated sweat fluid (SSW(3)) is responsible for this, therefore variations in the sample media (25mm polycarbonate filter, pore size 0.2 µm) are responsible for the variation in carbon content when samples were blank-corrected. This is considered when assessing the values observed for both the reference and enhanced fabrics.

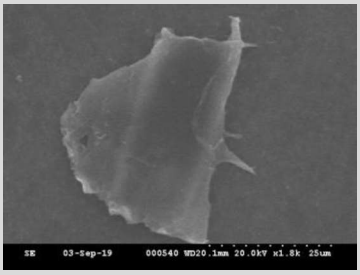
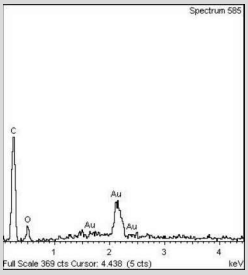
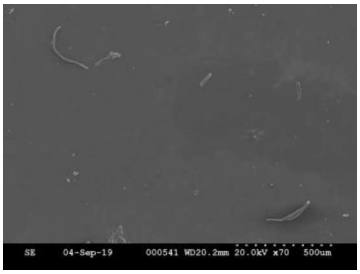
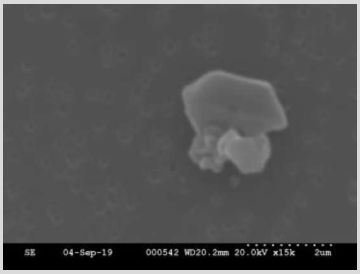
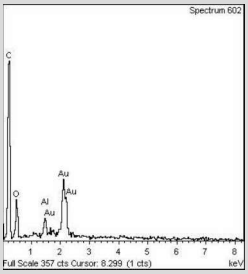
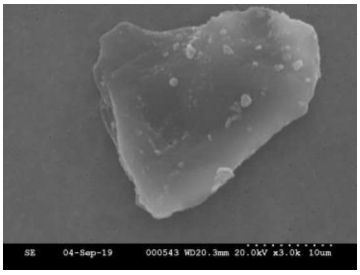
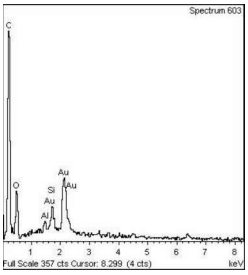
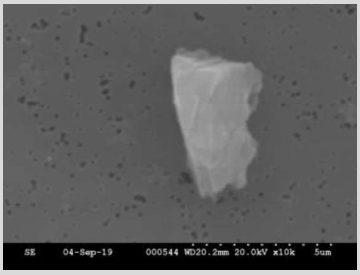
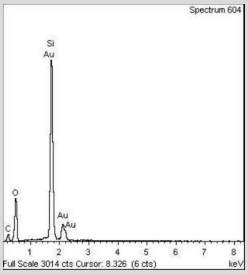
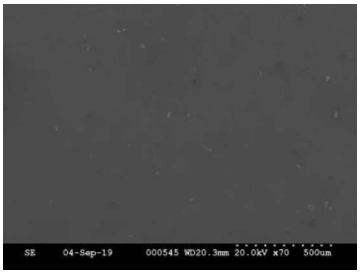
3.2. SEM/EDXS Analysis

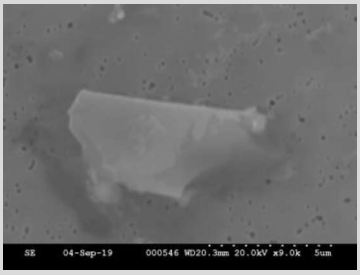
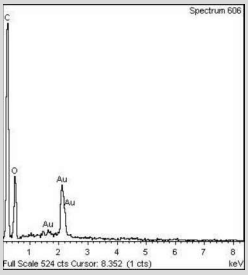
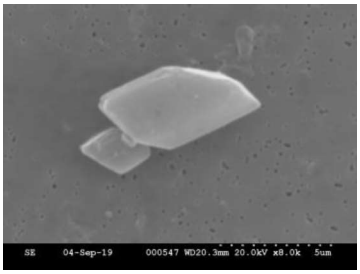
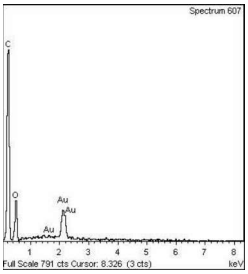
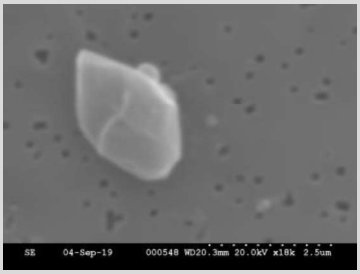
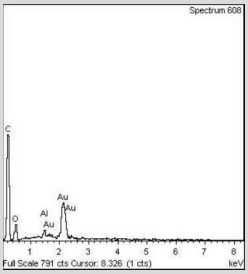
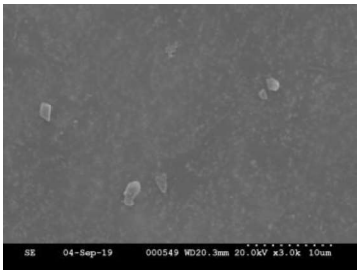
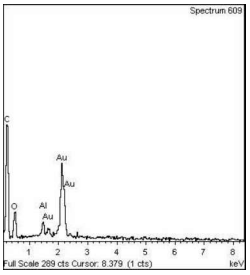
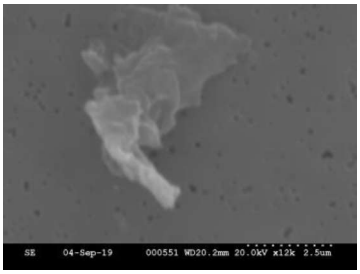
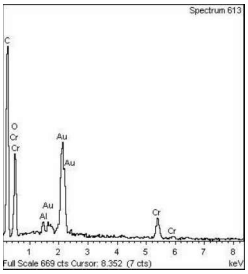
Sample ID	Fabric	Time point (hours)
Blank	N/A	N/A
DP11	Reference	0
DP12	Enhanced	0
DP13	Reference	2
DP14	Enhanced	2
DP15	Reference	6
DP16	Enhanced	6
DP17	Reference	24
DP18	Enhanced	24

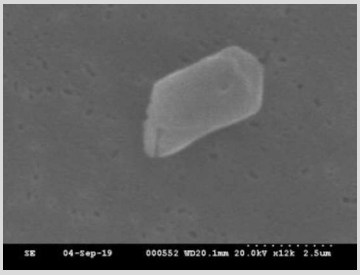
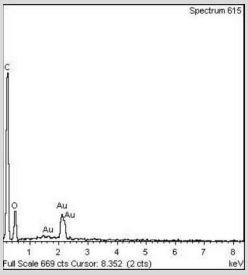
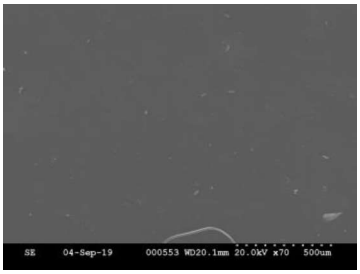
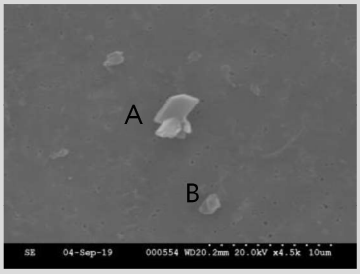
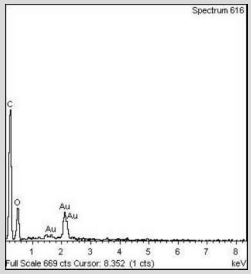
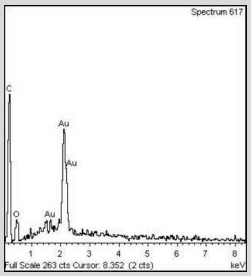
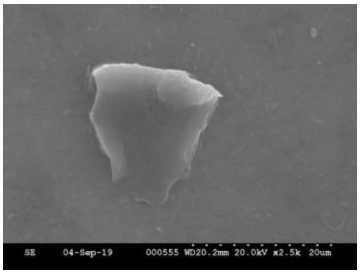
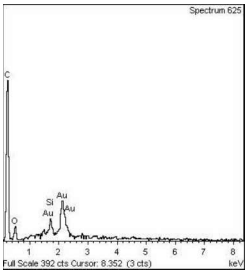
Table 3.2 SEM/EDXS Analysis results

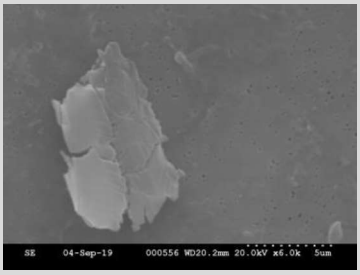
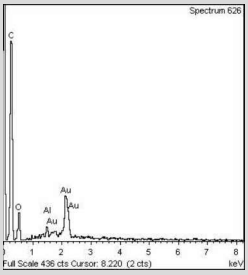
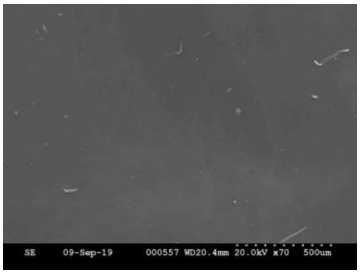
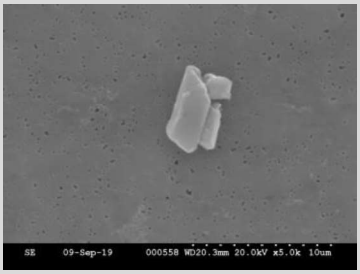
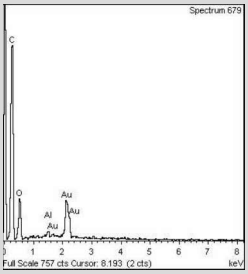
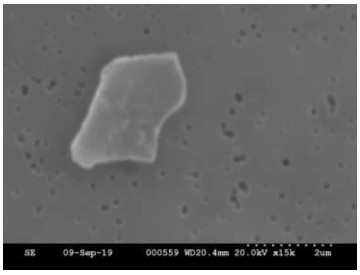
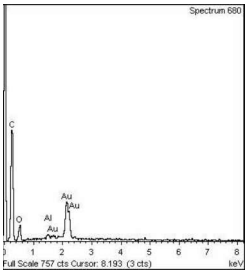
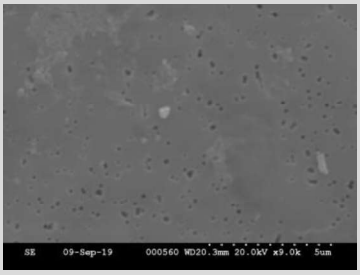
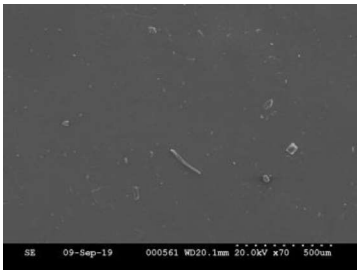
Sample ID		SEM Images	EDXS Spectra	Comment
Blank	A		N/A	Overview of filter, showing very little presence of particles.
	B			Particle of dimensions 6.9 x 25.7 µm exhibiting C and O in EDXS spectrum.
	C			Particle of dimensions 2.6 x 2.8 µm exhibiting C, O and Mg in EDXS spectrum.
	D			Particle of dimensions 8.7 x 15.7 µm exhibiting C and O in EDXS spectrum.
DP11	A		N/A	Low magnification image showing light loading of filter.

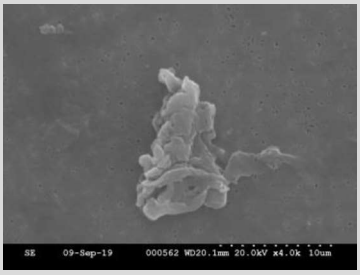
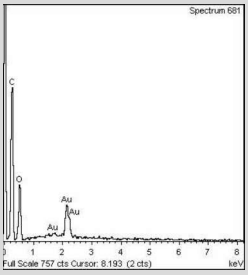
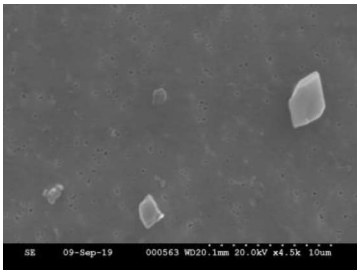
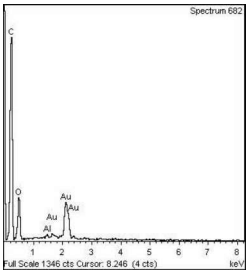
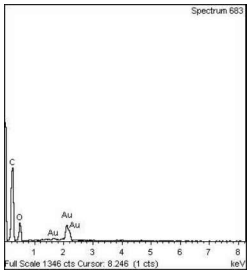
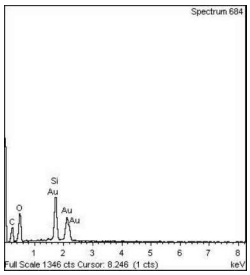
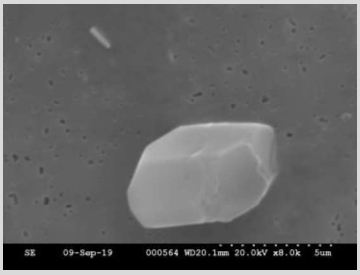
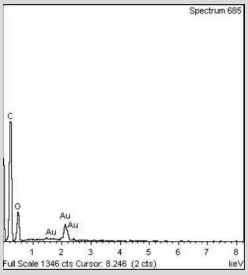
Sample ID		SEM Images	EDXS Spectra	Comment
	B			Particle of dimensions 4.8 x 5.4 µm exhibiting C and O in EDXS spectrum.
	C			Inorganic particle of dimensions 3.9 x 6.6 µm.
DP12	A		N/A	Low magnification image showing loading of filter. Range of particles and fibrous strands visible. Heavier loading noted compared with DP11.
	B			Particle of diameter 7.5 µm exhibiting C, O, Mg and Si in EDXS spectrum.
	C			Inorganic particle of dimensions 21 x 31 µm.
	D			Large, globular carbon based particle.

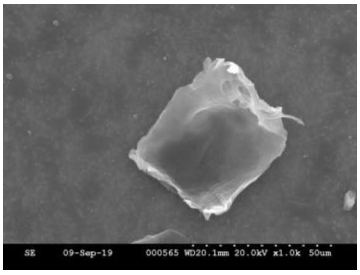
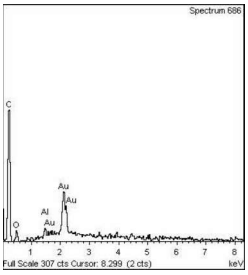
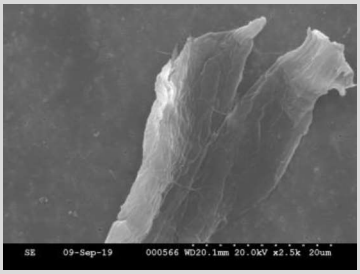
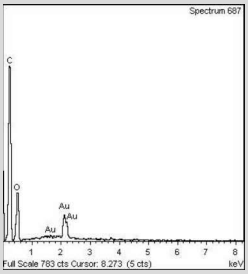
Sample ID		SEM Images	EDXS Spectra	Comment
	E			Large carbon based particle of dimensions 4.8 x 5.4 µm exhibiting C and O in EDXS spectrum.
DP13	A		N/A	Low magnification image showing loading of filter. Range of particles and fibrous strands visible.
	B			Particle of dimensions 2.3 x 2.5 µm exhibiting C and O in EDXS spectrum.
	C			Particle of dimensions 20.9 x 28.1 µm exhibiting C, O and Si in EDXS spectrum.
	D			Particle of dimensions 3.1 x 5.2 µm exhibiting C, O and Si in EDXS spectrum.
DP14	A		N/A	Low magnification image showing light loading of filter.

Sample ID		SEM Images	EDXS Spectra	Comment
	B			Particle of dimensions 3.5 x 8.4 µm exhibiting C and O in EDXS spectrum.
	C			Particle of dimensions 3.4 x 5.5 µm exhibiting C and O in EDXS spectrum.
	D			Particle of dimensions 1.8 x 2.9 µm exhibiting C and O in EDXS spectrum.
	E			Range of carbon-based particles detected, some observed to be <1 µm.
DP15	A		N/A	Low magnification image showing very light loading of filter.
	B			Particle of dimensions ~3.1 x 5.2 µm exhibiting C and O in EDXS spectrum.

Sample ID		SEM Images	EDXS Spectra	Comment
	C			Particle of dimensions 2.1 x 4.3 µm exhibiting C and O in EDXS spectrum.
DP16	A		N/A	Low magnification image showing light loading of filter. Loading noted to be heavier than DP15.
	B		A  B 	Range of carbon-based particles detected. A- Dimensions 2.2 x 3.7 µm; B- Dimensions 1.2 x 1.8 µm.
	C			Carbon-based particle of dimensions 17.9 x 20.4 µm.

Sample ID		SEM Images	EDXS Spectra	Comment
	D			Carbon-based particle of dimensions 6.0 x 10.9 µm.
DP17	A		N/A	Low magnification image showing light loading of filter.
	B			Particle of dimensions 3.6 x 6.1 µm exhibiting C and O in EDXS spectrum.
	C			Particle of dimensions 2.4 x 3.2 µm exhibiting C and O in EDXS spectrum.
	D		N/A	Particles observed <1 µm.
DP18	A		N/A	Low magnification image showing loading of filter. Range of particles and fibrous strands visible.

Sample ID	SEM Images	EDXS Spectra	Comment
B			Globular carbon-based particle observed.
C		A  B  C 	Range of particles observed. A- Dimensions 2.7 x 3.5 µm exhibiting C and O in EDXS spectrum; B- Dimensions 1.6 x 2.0 µm exhibiting C and O in EDXS spectrum; C- Dimensions 850 nm x 1.5 µm exhibiting C, O and Si in EDXS spectrum.
D			Particle of dimensions 4.6 x 6.7 µm exhibiting C and O in EDXS spectrum.

Sample ID	SEM Images	EDXS Spectra	Comment
E			Particle of dimensions 41.4 x 46.7 µm exhibiting C and O in EDXS spectrum.
F			Carbon based particle observed. Surface of particle shows visible layers, similar to images of graphene coating provided by Directa Plus.

The particles on these filters exhibited a morphology and composition similar to what could be expected for graphene, in particular DP14 B, DP16 D, and DP18 F. However, as highlighted in the previous report on abrasion testing (P2590), material with a similar morphology was found in assessment of control material, in this case on filters from bioelution tests in absence of the graphene coating (e.g. DP13 C, DP15 B, DP17 C). Furthermore, carbon-based material was also identified on the fluid blank sample analysed. It is therefore not possible to conclusively identify these platelets as graphene.

4. Discussion and Recommendations

4.1. Bioelution Testing

Results from total carbon analysis of the fluid extracted at each time point show no clear trend. Mass of carbon detected varied greatly at each time point for both the reference and enhanced material. Furthermore, results obtained from the fluid blank samples at 0 and 24 hours were not in agreement. This suggests the variation may be due to the sampling media and not the samples themselves. Using this method, we were unable to confirm any release of graphene into the simulated sweat fluid.

4.2. SEM/EDXS Analysis

Carbon-based particles were observed on all filters prepared. The morphology of carbon particles observed on filters DP14, DP16 and DP18 are similar to what could be expected for graphene, in particular the particle imaged in DP18 F where the visible layers can be seen on the surface of the particle, similar to images provided by Directa Plus for graphene coating. However, as carbonaceous platelets of a similar morphology and elemental profile were also identified on the filters collected from the reference material (DP13, DP15 and DP17), it is not possible to conclusively identify these platelets as graphene. As shown in 5, the fibre end of the reference textile also shows layers on the surface and therefore, any fibre breakages may show morphology such as that seen in DP18 F.

4.3. Recommendations

As described in the original quote, this work was a pilot study to (partly) determine which measurements are suitable based on the sample matrix and results required by Directa Plus. From the obtained results, the following conclusions can be made:

i) Adaptations to our in-house bioelution method was successful and informative.

ii) Release of graphene in simulated sweat fluid (SSW(3)) following bioleution testing for 24 hours was, at best, not observed, and at worst, at low levels.

iii) The use of various reference materials, such as free graphene and graphene-containing paste (prior to application to fabrics), would be crucial to confirm or refute these observations, as well as allowing us to validate the analysis techniques employed in this pilot study.

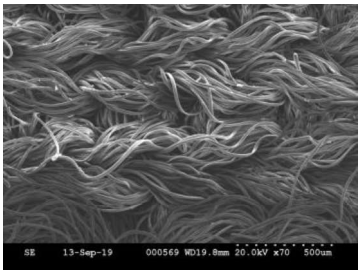
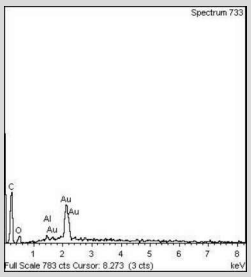
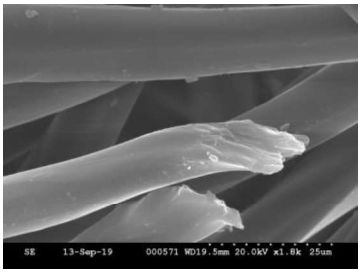
Following on from these results, we recommend carrying out follow-up tests, including:

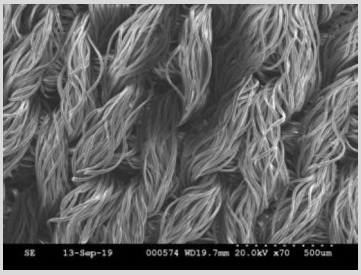
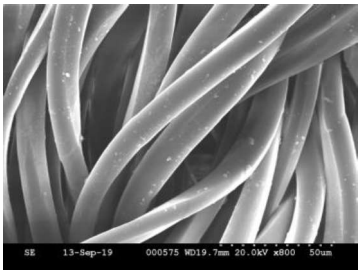
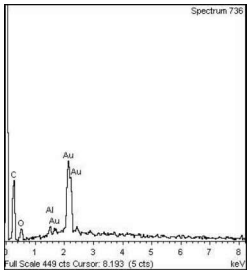
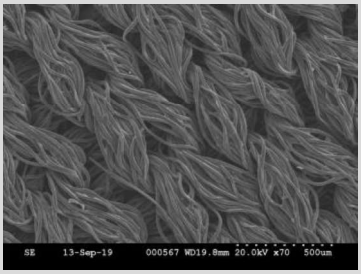
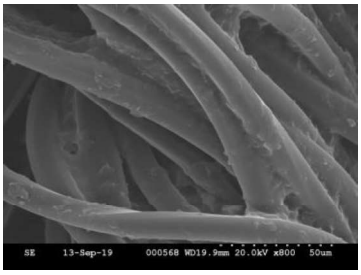
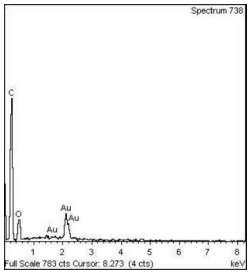
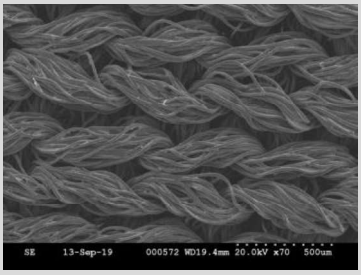
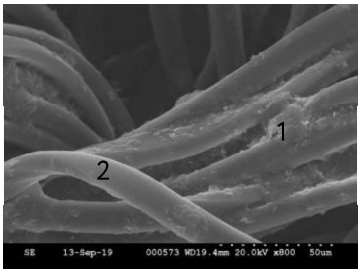
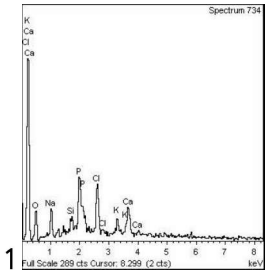
- TEM (preferably HR-TEM) analysis to obtain a more robust identification of graphene presence;

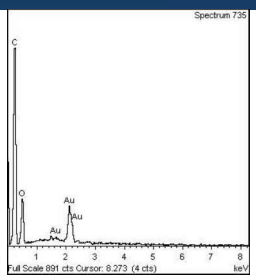
- Calibration of the total carbon analysis method using polycarbonate filters, including creating a calibration curve using the coating with graphene present, the coating without graphene present and free graphene in simulated sweat fluid (SSW(3)) to allow for better comparison of the results obtained;
- Elution testing of the base textile with and without graphene in detergent following an adaption of the ISO standard test for "colour fastness to commercial and domestic laundering" (ISO 105-C06:2010). This will allow assessment of the release of graphene upon washing the fabric. Additionally, this test could be used in combination with the abrasion testing procedure to determine the effect of abrasion on laundered graphene-enhanced textiles.

5. Appendix 1. SEM/EDXS Images of Textiles

Table 5.1 SEM/EDXS Images of Textiles

Sample ID		SEM Images	EDXS Spectra	Comment
Reference Material Before Bioelution	A		N/A	No visible change has been identified with the reference textile following bioelution testing. Image C from before bioelution testing shows a magnified view of a fibre end. The layers visible on the surface could be mistakenly identified as the graphene coating.
	B			
	C		N/A	

Sample ID		SEM Images	EDXS Spectra	Comment
Reference Material After Bioelution (T = 24 hours)	A		N/A	
	B			
Graphene Coated Material Before Bioelution	A			No visible change has been identified with the graphene coated textile following bioelution testing.
	B			Images B show that the graphene coating has remained intact following exposure to simulated sweat fluid for 24 hours.
Graphene Coated Material After Bioelution (T = 24 hours)	A			EDXS spectra taken of the graphene coating after bioelution testing showed presence of inorganic material, likely to be salt residue from the SSW(3) fluid.
	B			

Sample ID	SEM Images	EDXS Spectra	Comment
2			

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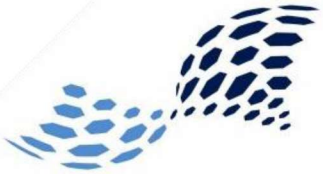

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REPORT ON A HUMAN PATCH TEST

48 hours closed patch test under Occlusion

Skin test to evaluate potential skin irritation after contact with a woven fabric

DIRECTA PLUS S.P.A.

COTTON IMPREGNATED WITH GRAFYPAD G+

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STUDY DESIGN

Title

REPORT ON A HUMAN PATCH TEST
Skin test to evaluate potential skin irritation after contact with a woven fabric.

Aim of the study

This study assesses the potential side effects (skin erythema and oedema reactions) that may occur after applying a woven fabric to evaluate whether the topical product is safe for consumer use.

Tested Product

Information provided by the Customer

- Product name:

COTTON IMPREGNATED WITH GRAFYPAD G+

- Fabric Composition:
  97% cotton + 3% span

Product image

Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
- All of the subjects participating in the study are selected under the supervision of a dermatologist according to inclusion/non inclusion criteria (see respective paragraph “Inclusion criteria” and “Non inclusion Criteria”).
- Volunteer participation in the study is totally free.
- All of the subjects participating in the study are informed of the aim and the nature of the study.
- All of the subjects participating in the study are informed of the potential risks involved.
- All of the subjects participating in the study give their informed consent signed at the beginning of the study.
- Before volunteers were exposed to the product to be tested, all relevant safety information about the product itself and each ingredient were collected and evaluated.
- All of the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments)
- All necessary precautions were taken to avoid adverse skin reactions.
- If unexpected/adverse skin reactions occur, the dermatologist evaluates the severity of the reaction (and report it in the data collecting sheet) and if necessary proceed with the appropriate therapy.

Subjects selection

Volunteers recruitment

25 volunteers were recruited to take a part in the test in accordance with the following inclusion and non inclusion criteria:

Inclusion criteria

- ☑ Male and female subjects
- ☑ Subjects between 18 and 70 years old
- ☑ Healthy subjects
- ☑ Subjects informed about test purposes

Non inclusion criteria

- ✘ Subjects who do not fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Subjects with marks (for example tattoos, scars, burns) in the tested skin region, which might interfere with clinical evaluation
- ✘ Subjects with dermatological problems in the test area
- ✘ Subjects with medication that may affect skin response
- ✘ Subjects undergoing pharmacological treatment (both locally or systemically)
- ✘ Subjects with history for contact dermatitis
- ✘ Positive anamnesis for atopy

Withdrawal criteria

Partecipants are withdrawan if:

- ✘ They do not follow the conditions of the Study Information Sheet that they receive after the recruitment
- ✘ They suffer any illness or accident or develop any condition during the study which could affect the out come of the study
- ✘ They no longer wish to participate in the study.

Behaviour of volunteers during the test

Through patch application and 24 hours after patch removal volunteers must avoid situations or activity that could interfere with clinical evaluations:

- ✘ Exposition to sun or solarium
- ✘ Sport activity
- ✘ Immersion in water or steam bath
- ✘ Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Materials and Methods

Tested product and concentration

name	COTTON IMPREGNATED WITH GRAFYPAD G+
sponsor	DIRECTA PLUS S.P.A.
Study start (first enrolment date)	20/07/2020
Study end (last evaluation date)	23/07/2020
concentration	as it is
application method	Occlusive

Sample preparation and application

The product is applied as it is by using the Finn Chamber, an 8 mm diameter aluminium disk. The woven fabric was cut and placed in the Finn Chamber. The Finn Chamber is fixed to the skin with a tape already been tested for its safety that ensure the occlusive application of the product. Applied quantity is sufficient to fill the chamber without overflowing from it when applied on the skin. The product is left in contact with the skin surface for 48 hours. The cutaneous reactions are analysed 15 minutes, one hour and 24 hours after Finn Chamber removal. A Finn Chamber containing a blotting paper disk soaked with demineralized water is applied and used as a negative control.

Clinical examination and scoring

Skin reactions are evaluated at 15 minutes, 1 hour and 24 hours after patch removal according to the scores reported in Table 1, that describes the severity of erythema, oedema or other types of skin irritation. The results are collected in a table and represented graphically. For each experimental time Mean Irritation Index (IIM) is calculated by adding erythema mean value and oedema mean value. The tested product is then classified following Table 2 which is based on the Mean Irritation Index.

Table 1 - *Clinical score of skin reactions*

No erythema	0
Light erythema (hardly visible)	1
Clearly visible erythema	2
Moderate erythema	3
Serious erythema (dark red with possible formation of light scars)	4

No oedema	0
Very Light oedema (hardly visible)	1
Light oedema	2
Moderate oedema (about 1 mm raised skin)	3
Strong oedema (extended swelling even beyond the application area)	4

Table 2 - *Classification of the medium irritation index (according to the amended Draize classification).*

Mean Irritation Index (IIM)	**Product classification**
< 0,5	non irritating
0.5 ≤ IIM < 2.0	slightly irritating
2.0 ≤ IIM < 5.0	moderately irritating
5.0 ≤ IIM ≤ 8.0	highly irritating

RESULTS

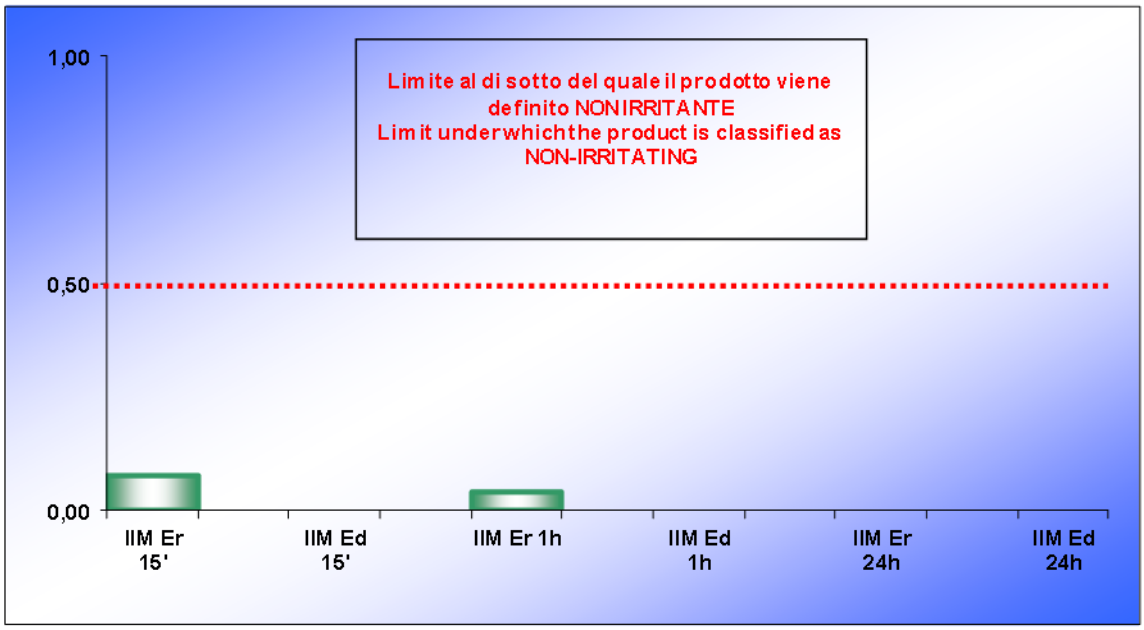
Summary of the data obtained and evaluation of the product irritation potential

OEDEMA AND ERYTHEMA REACTIONS

ID Vol		Sex	ERITEMA ERYTHEMA 15'	EDEMA OEDEMA 15'	ERITEMA ERYTHEMA 1h	EDEMA OEDEMA 1h	ERITEMA ERYTHEMA 24h	EDEMA OEDEMA 24h
1	C0059A	M	0	0	0	0	0	0
2	G4288O	F	0	0	0	0	0	0
3	P4241M	F	0	0	0	0	0	0
4	B4523A	F	0	0	0	0	0	0
5	Q2899C	F	0	0	0	0	0	0
6	C4071V	F	0	0	0	0	0	0
7	Z2981F	F	0	0	0	0	0	0
8	C0085N	F	0	0	0	0	0	0
9	B0526A	F	0	0	0	0	0	0
10	R1810M	F	2	0	1	0	0	0
11	F0122M	F	0	0	0	0	0	0
12	B4128L	F	0	0	0	0	0	0
13	D0668A	F	0	0	0	0	0	0
14	T3581R	F	0	0	0	0	0	0
15	O1477V	F	0	0	0	0	0	0
16	D0102G	M	0	0	0	0	0	0
17	B2742E	M	0	0	0	0	0	0
18	C0843M	F	0	0	0	0	0	0
19	F2070C	F	0	0	0	0	0	0
20	Y4277V	F	0	0	0	0	0	0
21	Y4276N	F	0	0	0	0	0	0
22	A4003S	F	0	0	0	0	0	0
23	Z2121M	F	0	0	0	0	0	0
24	G2688F	M	0	0	0	0	0	0
25	P2591C	F	0	0	0	0	0	0

MEAN VALUES FOR OEDEMA AND ERYTHEMA

IIM Er 15'	IIM Ed 15'	IIM Er 1h	IIM Ed 1h	IIM Er 24h	IIM Ed 24h
0,08	0,00	0,04	0,00	0,00	0,00



IRRITATION INDEX MEAN VALUES

IIM 15'	IIM 1h	IIM 24h
0,08	0,04	0,00

CONCLUSIONS

The table and the graphs listed above contain the values of the erythema and oedema indices recorded for each volunteer. Potential skin irritation of the product has been assessed according to the amended Draize classification.

On the basis of the data obtained we deem the product:

DIRECTA PLUS S.P.A.

COTTON IMPREGNATED WITH GRAFYPAD G+

NON IRRITATING

"DERMATOLOGICALLY TESTED"

Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
0	10/08/20	First release

Experimenter

Dr. Enza Cestone

Data analysis and Report

Dr. Carmen Palumbo

BIBLIOGRAPHY

Berger, R.S., and J.P. Bowman, 1982, "A Reappraisal of the 21-day Cumulative Irritation Test in Man," *J. Toxicol. - Ot. & Ocular Toxicol.,* 1(2);109-115.

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Complife Italia S.r.l. Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, | P.I./C.F. 11093320155 | Capitale Sociale € 161.834 i.v., C.C.I.A.A. MI 1437929 - Reg. Soc. N. 342215.

REPORT ON A HUMAN PATCH TEST

48 hours closed patch test under Semi Occlusion

Skin test to evaluate potential skin irritation after contact with a fabric

DIRECTA PLUS S.P.A.

G+ PRINTED FABRIC

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Capitale Sociale € 161.834 i.v., C.C.I.A.A. MI 1437929 - Registro Società N. 342215.
Centro di Saggio operante in BPL - Sistema di gestione qualità certificato ISO 9001

INDEX

KEY PERSONNEL

Customer

DIRECTA PLUS S.P.A.
Via Cavour 2
22074 Lomazzo (CO)
Italia

Experimenter

Dr. **Enza Cestone**
Degree in Medicine and Surgery, Specialist in Dermatology and Venereology
Consultant Complife Italia S.r.l.

Quality Control

Dr. **Cristina Scilironi**
Biologist
Complife Italia S.r.l.

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Location
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Mail: info@complifegroup.com

STUDY DESIGN

Title

REPORT ON A HUMAN PATCH TEST
Skin test to evaluate potential skin irritation after contact with a fabric.

Aim of the study

This study assesses the potential side effects (skin erythema and oedema reactions) that may occur after applying a fabric to evaluate whether the product is safe for consumer use.

Tested Product

Information provided by the Customer

- Product name:

G+ PRINTED FABRIC

- Qualitative INCI formula:
  Fabric composition: 100% polyester;
  Print composition: 95% polyurethane+5% PURE G+

Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
- All of the subjects participating in the study are selected under the supervision of a dermatologist according to inclusion/non inclusion criteria (see respective paragraph “Inclusion criteria” and “Non inclusion Criteria”).
- Volunteer participation in the study is totally free.
- All of the subjects participating in the study are informed of the aim and the nature of the study.
- All of the subjects participating in the study are informed of the potential risks involved.
- All of the subjects participating in the study give their informed consent signed at the beginning of the study.
- Before volunteers were exposed to the product to be tested, all relevant safety information about the product itself and each ingredient were collected and evaluated.
- All of the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments)
- All necessary precautions were taken to avoid adverse skin reactions.
- If unexpected/adverse skin reactions occur, the dermatologist evaluates the severity of the reaction (and report it in the data collecting sheet) and if necessary proceed with the appropriate therapy.

Subjects selection

Volunteers recruitment

25 volunteers were recruited to take a part in the test in accordance with the following inclusion and non inclusion criteria:

Inclusion criteria

- ☑ Male and female subjects
- ☑ Subjects between 18 and 70 years old
- ☑ Healthy subjects
- ☑ Subjects informed about test purposes

Non inclusion criteria

- ✘ Subjects who do not fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Subjects with marks (for example tattoos, scars, burns) in the tested skin region, which might interfere with clinical evaluation
- ✘ Subjects with dermatological problems in the test area
- ✘ Subjects with medication that may affect skin response
- ✘ Subjects undergoing pharmacological treatment (both locally or systemically)
- ✘ Subjects with history for contact dermatitis
- ✘ Positive anamnesis for atopy

Withdrawal criteria

Partecipants are withdrawan if:

- ✘ They do not follow the conditions of the Study Information Sheet that they receive after the recruitment
- ✘ They suffer any illness or accident or develop any condition during the study which could affect the out come of the study
- ✘ They no longer wish to participate in the study.

Behaviour of volunteers during the test

Through patch application and 24 hours after patch removal volunteers must avoid situations or activity that could interfere with clinical evaluations:

- Exposition to sun or solarium
- Sport activity
- Immersion in water or steam bath
- Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Materials and Methods

Tested product and concentration

name	G+ PRINTED FABRIC
sponsor	DIRECTA PLUS S.P.A.
Study start (first enrolment date)	23/10/17
Study end (last evaluation date)	26/10/17
concentration	as it is
application method	Semi-occlusive

Sample preparation and application

The product is applied as it is directly on the skin's back (a clipping of 2x2 cm) fixed to the skin with a tape already been tested for its safety.

The product is left in contact with the skin surface for 48 hours. The cutaneous reactions are analysed 15 minutes, one hour and 24 hours after patch removal. A Finn Chamber containing a blotting paper disk soaked with demineralized water is applied and used as a negative control.

Clinical examination and scoring

Skin reactions are evaluated at 15 minutes, 1 hour and 24 hours after patch removal according to the scores reported in Table 1, that describes the severity of erythema, oedema or other types of skin irritation. The results are collected in a table and represented graphically. For each experimental time Mean Irritation Index (IIM) is calculated by adding erythema mean value and oedema mean value. The tested product is then classified following Table 2 which is based on the Mean Irritation Index.

Table 1 - *Clinical score of skin reactions*

No erythema	0
Light erythema (hardly visible)	1
Clearly visible erythema	2
Moderate erythema	3
Serious erythema (dark red with possible formation of light scars)	4

No oedema	0
Very Light oedema (hardly visible)	1
Light oedema	2
Moderate oedema (about 1 mm raised skin)	3
Strong oedema (extended swelling even beyond the application area)	4

Table 2 -
Classification of the medium irritation index (according to the amended Draize classification).

Mean Irritation Index (IIM)	**Product classification**
< 0,5	non irritating
0.5 ≤ IIM < 2.0	slightly irritating
2.0 ≤ IIM < 5.0	moderately irritating
5.0 ≤ IIM ≤ 8.0	highly irritating

RESULTS

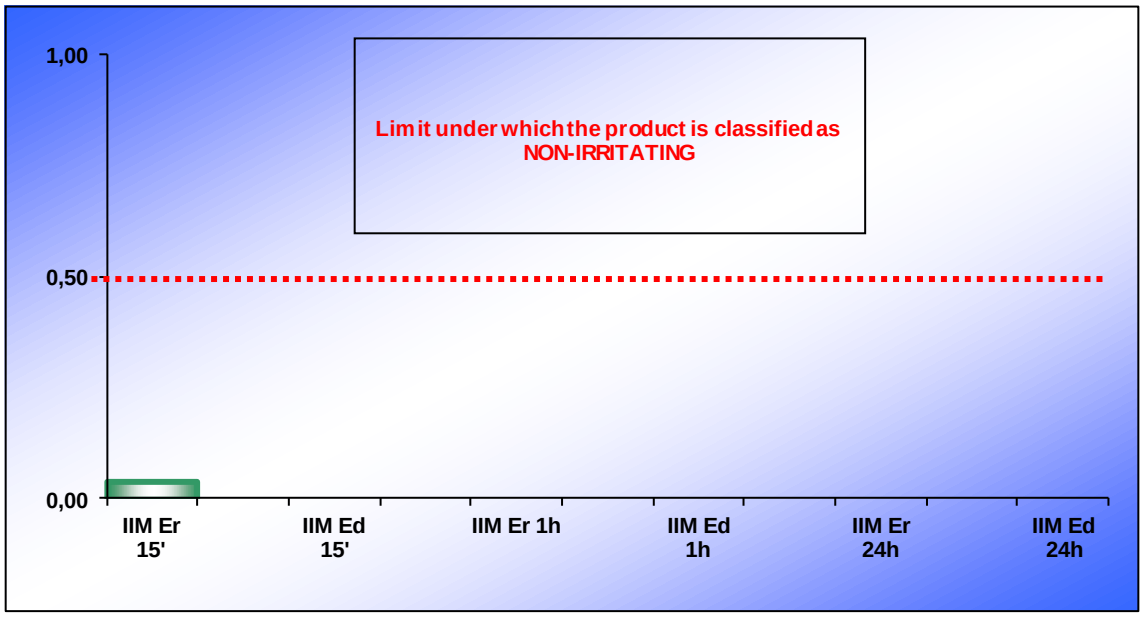
Summary of the data obtained and evaluation of the product irritation potential

OEDEMA AND ERYTHEMA REACTIONS

	ID Vol	Sex	ERYTHEMA 15'	OEDEMA 15'	ERYTHEMA 1h	OEDEMA 1h	ERYTHEMA 24h	OEDEMA 24h
1	S0502J	F	0	0	0	0	0	0
2	G0656C	F	0	0	0	0	0	0
3	P3503G	F	0	0	0	0	0	0
4	M3661M	F	0	0	0	0	0	0
5	D0106M	F	0	0	0	0	0	0
6	V2222D	F	0	0	0	0	0	0
7	B0012G	F	0	0	0	0	0	0
8	E3159L	F	0	0	0	0	0	0
9	I0170M	F	0	0	0	0	0	0
10	D1174B	F	0	0	0	0	0	0
11	T1779G	F	0	0	0	0	0	0
12	D1355R	F	1	0	0	0	0	0
13	G3721M	M	0	0	0	0	0	0
14	C0053D	F	0	0	0	0	0	0
15	C2741G	M	0	0	0	0	0	0
16	C3443V	F	0	0	0	0	0	0
17	C2336L	F	0	0	0	0	0	0
18	P2126A	F	0	0	0	0	0	0
19	D2313T	F	0	0	0	0	0	0
20	D0102G	M	0	0	0	0	0	0
21	C1167R	F	0	0	0	0	0	0
22	D3635R	M	0	0	0	0	0	0
23	P0284N	M	0	0	0	0	0	0
24	V3024C	F	0	0	0	0	0	0
25	P2591C	F	0	0	0	0	0	0

MEAN VALUES FOR OEDEMA AND ERYTHEMA

IIM Er 15'	IIM Ed 15'	IIM Er 1h	IIM Ed 1h	IIM Er 24h	IIM Ed 24h
0,04	0,00	0,00	0,00	0,00	0,00



IRRITATION INDEX MEAN VALUES

IIM 15'	IIM 1h	IIM 24h
0,04	0,00	0,00

CONCLUSIONS

The table and the graphs listed above contain the values of the erythema and oedema indices recorded for each volunteer. Potential skin irritation of the fabric has been assessed according to the amended Draize classification.

On the basis of the data obtained we deem the product:

DIRECTA PLUS S.P.A.

G+ PRINTED FABRIC

NON IRRITATING

"DERMATOLOGICALLY TESTED"

Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
0	30/10/2017	First release

Experimenter	Quality control
Dr. Enza Cestone	Dr. Cristina Scilironi

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REPORT ON A HUMAN PATCH TEST

48 hours closed patch test under Semi Occlusion

Skin test to evaluate potential skin irritation after contact with a fabric

DIRECTA PLUS S.P.A.

MONOLAYER PU G+

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Centro di Saggio operante in BPL - Sistema di gestione qualità certificato ISO 9001

INDEX

KEY PERSONNEL

Customer

DIRECTA PLUS S.P.A.
Via Cavour, 2
22074 Lomazzo (CO)
Italia

Experimenter

Dr. **Enza Cestone**
Degree in Medicine and Surgery, Specialist in Dermatology and Venereology
Consultant Complife Italia S.r.l.

Quality Control

Dr. **Cristina Scilironi**
Biologist
Complife Italia S.r.l.

Complife Italia Srl

Location
Via Angelini, 21
27028 San Martino Siccomario (PV)
Italy
tel. +39-0382 25504 - fax +39-0382 536006
Mail: info@complifegroup.com

STUDY DESIGN

Title

REPORT ON A HUMAN PATCH TEST
Skin test to evaluate potential skin irritation after contact with a fabric.

Aim of the study

This study assesses the potential side effects (skin erythema and oedema reactions) that may occur after applying a fabric to evaluate whether the topical product is safe for consumer use.

Tested Product

Information provided by the Customer

- Product name:

MONOLAYER PU G+

- Qualitative INCI formula:
  Composition: 95% polyurethane+5% PURE G+

Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
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- Exposition to sun or solarium
- Sport activity
- Immersion in water or steam bath
- Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Materials and Methods

Tested product and concentration

name	MONOLAYER PU G+
sponsor	DIRECTA PLUS S.P.A.
Study start (first enrolment date)	23/10/17
Study end (last evaluation date)	26/10/17
concentration	as it is
application method	Semi-occlusive

Sample preparation and application

The product is applied as it is directly on the skin's back (a clipping of 2x2 cm) fixed to the skin with a tape already been tested for its safety.

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Summary of the data obtained and evaluation of the product irritation potential

OEDEMA AND ERYTHEMA REACTIONS

	ID Vol	Sex	ERYTHEMA 15'	OEDEMA 15'	ERYTHEMA 1h	OEDEMA 1h	ERYTHEMA 24h	OEDEMA 24h
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21	C1167R	F	0	0	0	0	0	0
22	D3635R	M	0	0	0	0	0	0
23	P0284N	M	0	0	0	0	0	0
24	V3024C	F	1	0	0	0	0	0
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MEAN VALUES FOR OEDEMA AND ERYTHEMA

IIM Er 15'	IIM Ed 15'	IIM Er 1h	IIM Ed 1h	IIM Er 24h	IIM Ed 24h
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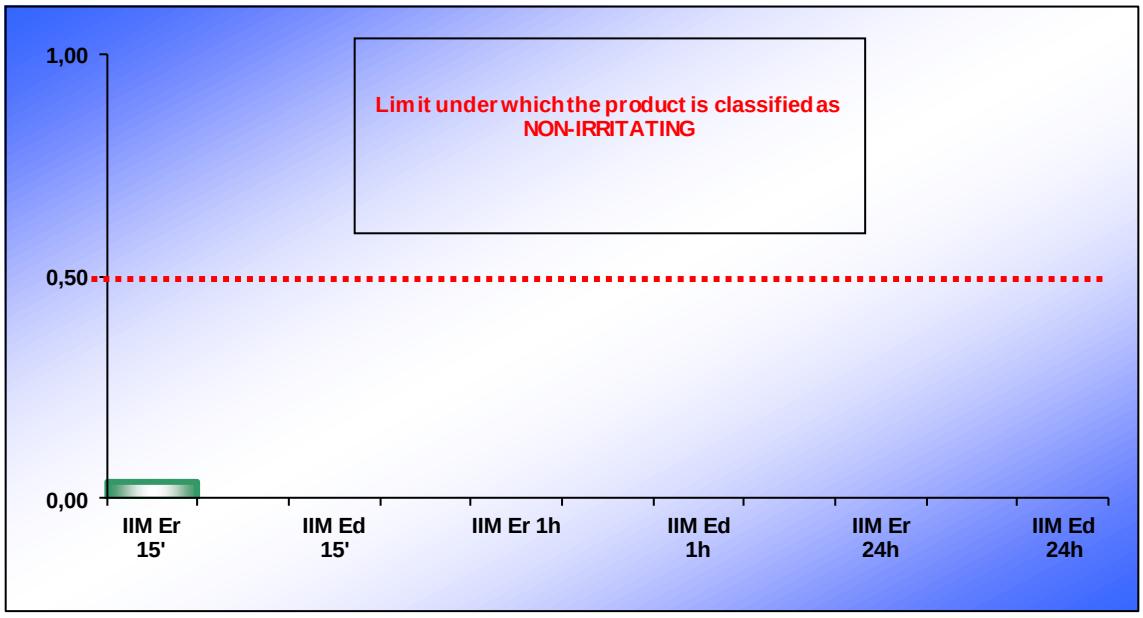

IRRITATION INDEX MEAN VALUES

IIM 15'	IIM 1h	IIM 24h
0,04	0,00	0,00

CONCLUSIONS

The table and the graphs listed above contain the values of the erythema and oedema indices recorded for each volunteer. Potential skin irritation of the fabric has been assessed according to the amended Draize classification.

On the basis of the data obtained we deem the product:

DIRECTA PLUS S.P.A.

MONOLAYER PU G+

NON IRRITATING

"DERMATOLOGICALLY TESTED"

Report change record

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Rev. no	Date	Description
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Dr. Enza Cestone	Dr. Cristina Scilironi

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SCIENTISTS BEHIND WELLNESS

REPORT ON A HUMAN PATCH TEST

48 hours closed patch test under Occlusion

Skin test to evaluate potential skin irritation after contact with a fabric

DIRECTA PLUS S.P.A.

COATED SAMPLE - NEW FORMULATION - TEST 2B 3% -G+ SIDE

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Iscritto al Registro Regione Lombardia ai fini dell'autocontrollo alimentare (N 030015309008)
Laboratorio di Prova Accreditato ACCREDIA LAB N 1318L (UNI CEI EN ISO/IEC 17025:2018)
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INDEX

KEY PERSONNEL

Customer

DIRECTA PLUS S.P.A.
Via Cavour 2
22074 Lomazzo (CO)
Italia

Experimenter

Dr. **Gloria Roveda**
Degree in Medicine and Surgery, Specialist in Dermatology and Venereology
Consultant Complife Italia S.r.l.

Data analysis and Report

Dr. **Cristina Scilironi**
Biologist
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Complife Italia Srl

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STUDY DESIGN

Title

REPORT ON A HUMAN PATCH TEST
Skin test to evaluate potential skin irritation after contact with a fabric.

Aim of the study

This study assesses the potential side effects (skin erythema and oedema reactions) that may occur after applying a product to evaluate whether the product is safe for consumer use.

Tested Product

Information provided by the Customer

- Product name:

COATED SAMPLE - NEW FORMULATION - TEST 2B 3% -G+ SIDE

- Composition:
  filed

Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
- All of the subjects participating in the study are selected under the supervision of a dermatologist according to inclusion/non inclusion criteria (see respective paragraph “Inclusion criteria” and “Non inclusion Criteria”).
- Volunteer participation in the study is totally free.
- All of the subjects participating in the study are informed of the aim and the nature of the study.
- All of the subjects participating in the study are informed of the potential risks involved.
- All of the subjects participating in the study give their informed consent signed at the beginning of the study.
- Before volunteers were exposed to the product to be tested, all relevant safety information about the product itself and each ingredient were collected and evaluated.
- All of the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments)
- All necessary precautions were taken to avoid adverse skin reactions.
- If unexpected/adverse skin reactions occur, the dermatologist evaluates the severity of the reaction (and report it in the data collecting sheet) and if necessary proceed with the appropriate therapy.

Subjects selection

Volunteers recruitment

25 volunteers were recruited to take a part in the test in accordance with the following inclusion and non inclusion criteria:

Inclusion criteria

- ☑ Male and female subjects
- ☑ Subjects between 18 and 70 years old
- ☑ Healthy subjects
- ☑ Subjects informed about test purposes

Non inclusion criteria

- ✘ Subjects who do not fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Subjects with marks (for example tattoos, scars, burns) in the tested skin region, which might interfere with clinical evaluation
- ✘ Subjects with dermatological problems in the test area
- ✘ Subjects with medication that may affect skin response
- ✘ Subjects undergoing pharmacological treatment (both locally or systemically)
- ✘ Subjects with history for contact dermatitis
- ✘ Positive anamnesis for atopy

Withdrawal criteria

Partecipants are withdrawan if:

- ✘ They do not follow the conditions of the Study Information Sheet that they receive after the recruitment
- ✘ They suffer any illness or accident or develop any condition during the study which could affect the out come of the study
- ✘ They no longer wish to participate in the study.

Behaviour of volunteers during the test

Through patch application and 24 hours after patch removal volunteers must avoid situations or activity that could interfere with clinical evaluations:

- ✘ Exposition to sun or solarium
- ✘ Sport activity
- ✘ Immersion in water or steam bath
- ✘ Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Materials and Methods

Tested product and concentration

nome I name	COATED SAMPLE - NEW FORMULATION - TEST 2B 3% -G+ SIDE
sponsor	DIRECTA PLUS S.P.A.
Study start (first enrolment date)	29/03/2021
Study end (last evaluation date)	01/04/2021
concentration	as it is
application method	Occlusive

Sample preparation and application

The product is applied as it is by using the Finn Chamber, an 8 mm diameter aluminium disk. The fabric was cut and placed in the Finn Chamber. The Finn Chamber is fixed to the skin with a tape already been tested for its safety that ensure the occlusive application of the product. Applied quantity is sufficient to fill the chamber without overflowing from it when applied on the skin. The product is left in contact with the skin surface for 48 hours. The cutaneous reactions are analysed 15 minutes, one hour and 24 hours after Finn Chamber removal. A Finn Chamber containing a blotting paper disk soaked with demineralized water is applied and used as a negative control.

Clinical examination and scoring

Skin reactions are evaluated at 15 minutes, 1 hour and 24 hours after patch removal according to the scores reported in Table 1, that describes the severity of erythema, oedema or other types of skin irritation. The results are collected in a table and represented graphically. For each experimental time Mean Irritation Index (IIM) is calculated by adding erythema mean value and oedema mean value. The tested product is then classified following Table 2 which is based on the Mean Irritation Index.

Table 1 - *Clinical score of skin reactions*

No erythema	0
Light erythema (hardly visible)	1
Clearly visible erythema	2
Moderate erythema	3
Serious erythema (dark red with possible formation of light scars)	4

No oedema	0
Very Light oedema (hardly visible)	1
Light oedema	2
Moderate oedema (about 1 mm raised skin)	3
Strong oedema (extended swelling even beyond the application area)	4

Table 2 - *Classification of the medium irritation index (according to the amended Draize classification)*.

Mean Irritation Index (IIM)	**Product classification**
< 0,5	non irritating
0.5 ≤ IIM < 2.0	slightly irritating
2.0 ≤ IIM < 5.0	moderately irritating
5.0 ≤ IIM ≤ 8.0	highly irritating

Customer	DIRECTA PLUS S.P.A.
Record no	H.S.HU.MP.NTC00.025.00.00_IT0001436/21
Date	21/04/2021

RESULTS

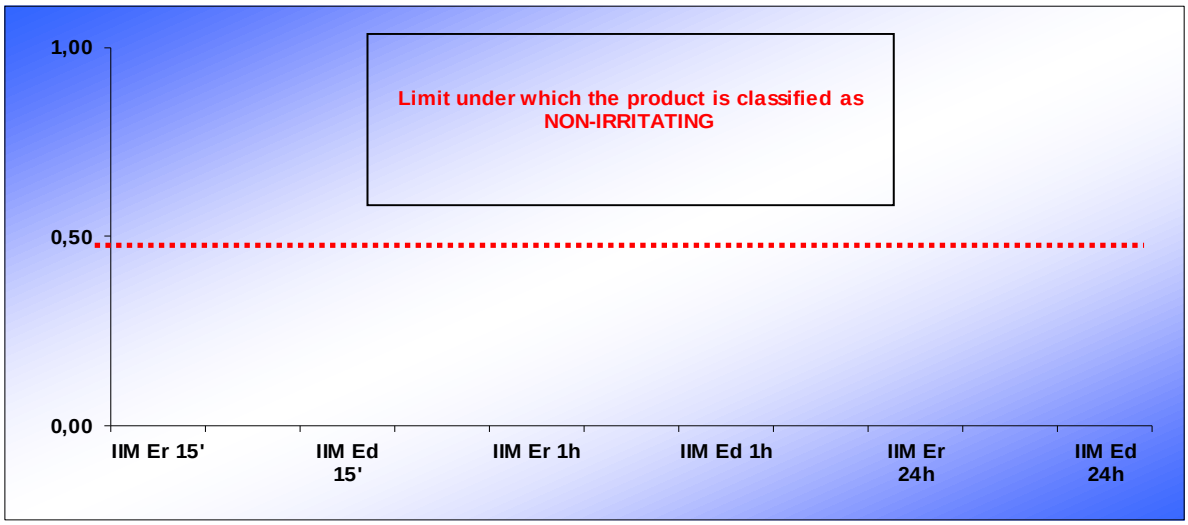
Summary of the data obtained and evaluation of the product irritation potential

OEDEMA AND ERYTHEMA REACTIONS

ID Vol		Sex	ERYTHEMA 15'	OEDEMA 15'	ERYTHEMA 1h	OEDEMA 1h	ERYTHEMA 24h	OEDEMA 24h
1	M3265G	F	0	0	0	0	0	0
2	A2458S	F	0	0	0	0	0	0
3	V1341A	F	0	0	0	0	0	0
4	C1167R	F	0	0	0	0	0	0
5	D3635R	M	0	0	0	0	0	0
6	Z3554A	F	0	0	0	0	0	0
7	A3836A	F	0	0	0	0	0	0
8	M3661M	F	0	0	0	0	0	0
9	M5010M	F	0	0	0	0	0	0
10	M2123M	F	0	0	0	0	0	0
11	G4259A	F	0	0	0	0	0	0
12	M4289S	F	0	0	0	0	0	0
13	S4526P	M	0	0	0	0	0	0
14	R3160L	F	0	0	0	0	0	0
15	P1492V	F	0	0	0	0	0	0
16	B4168M	M	0	0	0	0	0	0
17	S4278A	F	0	0	0	0	0	0
18	N4388M	F	0	0	0	0	0	0
19	T4004E	F	0	0	0	0	0	0
20	F4631M	F	0	0	0	0	0	0
21	M4290G	F	0	0	0	0	0	0
22	P2505M	F	0	0	0	0	0	0
23	M3758E	F	0	0	0	0	0	0
24	D1353D	F	0	0	0	0	0	0
25	H3063L	F	0	0	0	0	0	0

MEAN VALUES FOR OEDEMA AND ERYTHEMA

IIM Er 15'	IIM Ed 15'	IIM Er 1h	IIM Ed 1h	IIM Er 24h	IIM Ed 24h
0,00	0,00	0,00	0,00	0,00	0,00



IRRITATION INDEX MEAN VALUES

IIM 15'	IIM 1h	IIM 24h
0,00	0,00	0,00

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CONCLUSIONS

The table and the graphs listed above contain the values of the erythema and oedema indices recorded for each volunteer. Potential skin irritation of the product has been assessed according to the amended Draize classification.

On the basis of the data obtained we deem the product:

DIRECTA PLUS S.P.A.

COATED SAMPLE - NEW FORMULATION - TEST 2B 3% -G+ SIDE

NON IRRITATING

"DERMATOLOGICALLY TESTED"

Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
0	21/04/2021	First release

Experimenter

Dr. Gloria Roveda

Data analysis and Report

Dr. Cristina Scilironi

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HUMAN DERMATOLOGICAL INVESTIGATION

Repeated patch test to detect the skin sensitization potency of a fabric by using the protocol of Marzulli-Maibach

DIRECTA PLUS S.P.A.

COTTON IMPREGNATED WITH G+

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Farcoderm TESTED WELLNESS
FARCOS

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STUDY DESIGN

Title

HUMAN DERMATOLOGICAL INVESTIGATION
Repeated patch test to detect the skin sensitization potency of a fabric by using the protocol of Marzulli-Maibach.

Aim of the study

The assessment of adverse effects (erythema and edematous skin lesions, dryness and vesicles) occurred, as a result of repeated application of the product under test is useful to check if that product can cause allergic sensitization reactions.

Tested Product

Information provided by the Customer

- Product name:

DIRECTA PLUS S.P.A.

COTTON IMPREGNATED WITH G+

- Composition:
  Fabric composition (110 gsm): 97% COTTON, 3%SPAN;
  fabric impregnated with Grafypad G+.

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Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
- All of the subjects participating in the study are selected under the supervision of a dermatologist according to inclusion/non inclusion criteria (see respective paragraph “Inclusion criteria” and “Non inclusion Criteria”).
- Volunteer participation in the study is totally free.
- All of the subjects participating in the study are informed of the aim and the nature of the study.
- All of the subjects participating in the study are informed of the potential risks involved.
- All of the subjects participating in the study give their informed consent signed at the beginning of the study.
- Before volunteers were exposed to the product to be tested, all relevant safety information about the product itself and each ingredient were collected and evaluated.
- All of the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments)
- All necessary precautions were taken to avoid adverse skin reactions.
- If unexpected/adverse skin reactions occur, the dermatologist evaluates the severity of the reaction (and report it in the data collecting sheet) and if necessary proceed with the appropriate therapy.

Subjects selection

Volunteers recruitment

50 volunteers were recruited to take a part in the test in accordance with the following inclusion and non inclusion criteria:

Inclusion criteria

- ☑ Male and female subjects
- ☑ Subjects between 18 and 70 years old
- ☑ Subjects with sensitive skin*
- ☑ Healthy subjects
- ☑ Caucasian people
- ☑ Subjects informed about test purposes

Non - inclusion criteria

- ✘ Subjects who do not fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Subjects with marks (for example tattoos, scars, burns) in the tested skin region, which might interfere with clinical evaluation
- ✘ Subjects who exposed themselves to sunlight or tanning lamps in the 15 days before the start of the test
- ✘ Subjects with dermatological problems in the test area
- ✘ Subjects with medication which may affect skin response
- ✘ Subjects undergoing pharmacological treatment (both locally or systemically)
- ✘ Subjects with past history for contact dermatitis

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- Positive anamnesis for atopy

Withdrawal criteria

Partecipants are withdrawan if

- They do not follow the conditions of the Study Information Sheet that they receive after the recruitment
- They suffer any illnes or accident or develop any condition during the study which could affect the out come of the study
- They no longer wish to participate in the study.

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Behaviour of volunteers during the test

Through patch application and 48 hours after the last patch removal volunteers must avoid situations or activity that could interfere with clinical evaluations:

- ✘ Exposition to sun or solarium
- ✘ Sport activity
- ✘ Immersion in water or steam bath
- ✘ Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Volunteers with sensitive skin are confirmed according to stinging* test with lactic acid.

***Stinging test**

A 10% water solution of lactic acid is applied by means of a cotton pad along the furrow which runs between the nose and the superior lip; an especially rich zone of thin nervous fibres which determine the hypersensitivity problem. The diagnosis results positive when, after some minutes, volunteer reports an itching or burning sensation

Materials and Methods

Tested product and concentration

name	COTTON IMPREGNATED WITH G+
sponsor	DIRECTA PLUS S.P.A.
Study start (first enrolment date)	24/08/2020
Study end (last evaluation date)	02/10/2020
concentration	as it is
application method	occlusive

Sample preparation and application

The product is applied as it is directly on the skin's back (a clipping of 2x2 cm) fixed to the skin with a tape already been tested for its safety.

The Finn Chamber is fixed to the skin with a tape already been tested for its safety that ensure the occlusive application of the product. Applied quantity is sufficient to fill the chamber without overflowing from it when applied on the skin. A patch without product is applied at the same conditions. This last is the control "not treated skin". The test includes three phases:

- INDUCTION PHASE, These patches are applied in the same skin area (same side scapular area). The products under test are applied using the patch test under occlusion in number equal to nine (9): three (3) patches a week for 3 weeks (W1, W2, W3) for forty-eight (48) hours or seventy-two (72) hours for patches applied on Friday. The patches are applied on Monday, Wednesday and Friday. The patches are removed in the laboratory, skin reactions are assessed according to the scale shown in Table 1 and the new patch is applied on the same site. The procedure is repeated until the 9th (nine) induction patches are applied in the same site.

W1:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Day of study	D1	D2	D3	D4	D5	D6	D7
Application of the product	✓		✓		✓		
Check			**C**		**C**		

W2:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Day of study	D8	D9	D10	D11	D12	D13	D14

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Application of the product	✓		✓		✓		
Check	C		C		C		

W3:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Day of study	D15	D16	D17	D18	D19	D20	D21
Application of the product	✓		✓		✓		
Check	C		C		C		

- REST PERIOD, A rest period of two (2) weeks (W4, W5) will follow the removal of the last induction patches; no test materials is applied during this rest period

W4:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Day of study	D22	D23	D24	D25	D26	D27	D28
Application of the product							
Check	C						

W5:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Day of study	D29	D30	D31	D32	D33	D34	D35
Application of the product							
Check							

- INDICATOR PATCH (W6) Following the rest period, a forty eight (48) hours challenge patch of each test material will be applied under occlusion to a new site on the back different from which of the induction phase (contralateral scapular area). After its removal at the laboratory, the skin reactions will be scored by experimenter at 30 minutes and forty eight (48) hours according to table 3 score.

W6:

Day of the week	Mo	Tu	We	Th	Fr
Day of study	D36	D37	D38	D39	D40
Application of the product	✓				
Check			C		C

Legend: W: Week, D: Day, C: Check

Clinical examination and scoring

After patches removal, volunteers are subjected to clinical evaluation of the possible erythematic and oedema reactions, dryness and vesicles.

INDUCTIVE PHASE (CLASSIFICATION OF THE IRRITATING POWER): clinical evaluation is performed 30 minutes after patch removal. The final reading is made on day of study D22.

Skin reactions are evaluated according to the scores reported in table 1, that describes the severity of erythema, oedema and other types of skin irritations clinically observed. The results are collected in a table. For each experimental time Irritation Index is calculated by adding erythema mean value, oedema, dryness and vesicles mean value. Medium Irritation Index (IIM) is calculated by adding the irritation indices of each experimental time and dividing by the number of checks. The tested product is then classified following table 2.

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INDICATOR PATCH (CLASSIFICATION OF THE SENSITIZING POWER): clinical evaluation is carried out 30 minutes (D38) and 48 hours (D40) after indicator patch removal.
A possible allergic reaction during the inductive phase or indicator patch is evaluated with a score ranging from 0 to 3 according to the criteria of the ICDRG (International Contact Dermatitis Research Group) - see table 3.
A ++ reaction (2) or + + + reaction (3) must be re-evaluated the next day to see if the reaction is in decline or increasing. A rapid improvement of the reaction indicates that it is irritating, its persistence or worsening show that it is allergic type.
The occurrence of a single case of reaction (reaction with a score greater than or equal to + + (2)) in the contralateral area leads to the conclusion: "potentially sensitizing product." While, the product is defined “hypoallergenic” if no subject shows allergic reactions.

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Table 1 - *Clinical score of skin reactions*

Score	ERYTHEMA	EDEMA	DRYNESS	VESICLES
0 (No reaction)	Normal appearance	Normal appearance	Normal appearance	Normal appearance
1 (SLight reaction)	Slight pink color over the tested area or visible pigmentation on a part of the area	Edema more palpable than visible	Light desquamation, cracked skin	Vesicles more palpable than visible
2 (Evident reaction)	Clear and visible Erythema on the whole tested area	Evident edema	Evident desquamation, scaly appearance	Visible vesicles
3 (Serious reaction)	Intense erythema over the entire surface tested, or diffuse erythema outside the area	Edema extended outside the tested area	Serious desquamation and fissuring	Vesicles outside the tested area or bullae

Table 2 - *Classification of the Irritancy*

Mean Irritation Index (IIM)	Product classification
< 0.080	Not irritant
0,080 ≤score < 0,160	Very slightly irritating
0,160 ≤score < 0,560	Slightly irritating
0,560 ≤score < 1,000	Moderately irritating
1,000 ≤score < 1,600	Strongly irritating
≥1,600	Very strong irritating

Table 3 –*Evaluation of the sensitizing potential power*

Criteria	ICDRG score	Assigned score
No reaction	0	0
Doubtful reaction: mild redness only	?	?
Irritant reaction	IR	-
Weak, positive reaction: Erythema and Edema	+	1
Strong positive reaction: Erythema, Edema and vesicles	++	2
Extreme positive reaction: intense redness and swelling with coalesced large blisters or spreading reaction	+++	3

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RESULTS

Summary of the data obtained and evaluation of the product irritation potential

N	ID subject	Sex	1° patch test				2° patch test				3° patch test				4° patch test				5° patch test				6° patch test				7° patch test				8° patch test				9° patch test			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	C4463I	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	M3768G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	G4466E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	O4227L	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	F4631M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	L4516G	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	M3131M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	B0015R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	A2458S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	D0102G	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	P2591C	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	C3858I	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	C0085N	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	C1421F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	Y4276N	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Y4277V	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	P2126A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	M0204B	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	G2051C	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	B4168M	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	P1492V	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
22	R1784M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	F2070C	F	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	F3716A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	G0656C	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	R4295G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	S4671E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	R3791R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	S2856N	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	P3428G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	M4556S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	P4682J	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	A2533P	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	S2978A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	S4307R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	Z3430E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	M3754L	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	F4063A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	M4193I	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	B4412P	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	A3485P	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	G4312M	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	M3825A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	M4196E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	B3976A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	D4638M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	D3727F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	M3945D	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	M4135S	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	R1478E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend

1: Erythema, 2: Oedema, 3: Dryness, 4: Vesicles

Irritation Index

D3	D5	D8	D10	D12	D15	D17	D19	D22
0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,02	0,00

Mean Irritation Index (MII)

0,00

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Summary of the data obtained and evaluation of the product sensitizing potential

N	Codice Soggetto	Sesso	Indicator patch test	
			30'	48h
1	C4463I	F	0	0
2	M3768G	F	0	0
3	G4466E	F	0	0
4	O4227L	F	0	0
5	F4631M	F	0	0
6	L4516G	M	0	0
7	M3131M	F	0	0
8	B0015R	F	0	0
9	A2458S	F	0	0
10	D0102G	M	0	0
11	P2591C	F	0	0
12	C3858I	M	0	0
13	C0085N	F	0	0
14	C1421F	F	0	0
15	Y4276N	F	0	0
16	Y4277V	F	0	0
17	P2126A	F	0	0
18	M0204B	F	0	0
19	G2051C	M	0	0
20	B4168M	M	0	0
21	P1492V	F	0	0
22	R1784M	F	0	0
23	F2070C	F	0	0
24	F3716A	F	0	0
25	G0656C	F	0	0
26	R4295G	F	0	0
27	S4671E	F	0	0
28	R3791R	F	0	0
29	S2856N	F	0	0
30	P3428G	F	0	0
31	M4556S	F	0	0
32	P4682J	F	0	0
33	A2533P	M	0	0
34	S2978A	F	0	0
35	S4307R	F	0	0
36	Z3430E	F	0	0
37	M3754L	F	0	0
38	F4063A	F	0	0
39	M4193I	F	0	0
40	B4412P	F	0	0
41	A3485P	F	0	0
42	G4312M	M	0	0
43	M3825A	F	0	0
44	M4196E	F	0	0
45	B3976A	F	0	0
46	D4638M	F	0	0
47	D3727F	F	0	0
48	M3945D	M	0	0
49	M4135S	M	0	0
50	R1478E	F	0	0

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CONCLUSIONS

On the basis of the data obtained we asses that the application on the skin of tested product:

DIRECTA PLUS S.P.A.

COTTON IMPREGNATED WITH G+

is

"CLINICALLY TESTED"

NON IRRITATING, HYPOALLERGENIC*

(*LOW SENSITIZATING POTENTIAL)

Report change record

The table here below reports the change log of all approved changes made to the document.

Rev. no	Date	Description
0	15/10/2020	First release

Experimenter

Dr. Gloria Roveda

Data Analysis and Report

Dr. Cristina Scilironi

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HUMAN DERMATOLOGICAL INVESTIGATION

Repeated patch test to detect the skin sensitization potency of a fabric

DIRECTA PLUS S.P.A.

G+ PRINTED FABRIC

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INDEX

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STUDY DESIGN

Title

HUMAN DERMATOLOGICAL INVESTIGATION
Repeated patch test to detect the skin sensitization potency of a fabric

Aim of the study

The assessment of adverse effects (erythema and edematous skin lesions, dryness and vesicles) occurred as a result of repeated application of the product under test is useful to check if that product can cause allergic sensitization reactions.

Tested Product

Information provided by the Customer

- Product name:

G+ PRINTED FABRIC

- Qualitative INCI formula:
  Fabric composition: 100% polyester;
  Print composition: 95% polyurethane+5% PURE G+

Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
- All of the subjects participating in the study are selected under the supervision of a dermatologist according to inclusion/non inclusion criteria (see respective paragraph “Inclusion criteria” and “Non inclusion Criteria”).
- Volunteer participation in the study is totally free.
- All of the subjects participating in the study are informed of the aim and the nature of the study.
- All of the subjects participating in the study are informed of the potential risks involved.
- All of the subjects participating in the study give their informed consent signed at the beginning of the study.
- Before volunteers were exposed to the product to be tested, all relevant safety information about the product itself and each ingredient were collected and evaluated.
- All of the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments)
- All necessary precautions were taken to avoid adverse skin reactions.
- If unexpected/adverse skin reactions occur, the dermatologist evaluates the severity of the reaction (and report it in the data collecting sheet) and if necessary proceed with the appropriate therapy.

Subjects selection

Volunteers recruitment

50 volunteers were recruited to take a part in the test in accordance with the following inclusion and non inclusion criteria:

Inclusion criteria

- ☑ Male and female subjects
- ☑ Subjects between 18 and 70 years old
- ☑ Healthy subjects
- ☑ Subjects with sensitive skin*
- ☑ Caucasian people
- ☑ Subjects informed about test purposes

Non - inclusion criteria

- ✘ Subjects who do not fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Subjects with marks (for example tattoos, scars, burns) in the tested skin region, which might interfere with clinical evaluation
- ✘ Subjects who exposed themselves to sunlight or tanning lamps in the 15 days before the start of the test
- ✘ Subjects with dermatological problems in the test area
- ✘ Subjects with medication which may affect skin response
- ✘ Subjects undergoing pharmacological treatment (both locally or systemically)
- ✘ Subjects with past history for contact dermatitis
- ✘ Positive anamnesis for atopy

Withdrawal criteria

Partecipants are withdrawan if

- ✘ They do not follow the conditions of the Study Information Sheet that they receive after the recruitment
- ✘ They suffer any illnes or accident or develop any condition during the study which could affect the out come of the study
- ✘ They no longer wish to participate in the study.

Behaviour of volunteers during the test

Through patch application and 48 hours after the last patch removal volunteers must avoid situations or activity that could interfere with clinical evaluations:

- ✘ Exposition to sun or solarium
- ✘ Sport activity
- ✘ Immersion in water or steam bath
- ✘ Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Volunteers with sensitive skin are confirmed according to stinging* test with lactic acid.

***Stinging test**

A 10% water solution of lactic acid is applied by means of a cotton pad along the furrow which runs between the nose and the superior lip; an especially rich zone of thin nervous fibres which determine the hypersensitivity problem. The diagnosis results positive when, after some minutes, volunteer reports an itching or burning sensation.

Materials and Methods

Tested product and concentration

name	G+ PRINTED FABRIC
sponsor	DIRECTA PLUS S.P.A.
product description	Fabric
Study start (first enrolment date)	06/11/2017
Study end (last evaluation date)	15/12/2017
concentration	as it is
application method	semi- occlusive

Sample preparation and application

The product is applied as it is on the back (clipping of 2X2 cm) fixed with a tape already been tested for its safety.
A Finn Chamber containing a blotting paper disk soaked with demineralized water is applied and used as a negative control. The test includes three phases:

- INDUCTION PHASE. These patches are applied in the same skin area (same side scapular area). The products under test are applied using the patch test under semi occlusion in number equal to nine (9): three (3) patches a week for 3 weeks (W1, W2, W3) for forty-eight (48) hours or seventy-two (72) hours for patches applied on Friday. The patches are applied on Monday, Wednesday and Friday. The patches are removed in the laboratory, skin reactions are assessed according to the scale shown in Table 1 and the new patch is applied on the same site. The procedure is repeated until the 9 (nine) induction patches are applied in the same site.

W1:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D1	D2	D3	D4	D5	D6	D7
Application of the product	✓		✓		✓		
Check			C		C		

W2:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D8	D9	D10	D11	D12	D13	D14
Application of the product	✓		✓		✓		
Check	C		C		C		

W3:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D15	D16	D17	D18	D19	D20	D21
Application of the product	✓		✓		✓		
Check	C		C		C		

- REST PERIOD, A rest period of two (2) weeks (W4, W5) will follow the removal of the last induction patches; no test materials is applied during this rest period

W4:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D22	D23	D24	D25	D26	D27	D28
Application of the product							
Check	C						

W5:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D29	D30	D31	D32	D33	D34	D35
Application of the product							
Check							

- INDICATOR PATCH (W6) Following the rest period, a forty eight (48) hours challenge patch of each test material will be applied under semi occlusion to a new site on the back different from which of the induction phase (contralateral scapular area). After its removal at the laboratory, the skin reactions will be scored by experimenter at 30 minutes and forty eight (48) hours according to table 3 score.

W6:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr
Day of study	D36	D37	D38	D39	D40
Application of the product	✓				
Check			C		C

Legend:
W: Week
D: Day
C: Check

Clinical examination and scoring

After patches removal, volunteers are subjected to clinical evaluation of the possible erythematic and oedema reactions, dryness and vesicles.

INDUCTIVE PHASE (CLASSIFICATION OF THE IRRITATING POWER): clinical evaluation is performed 30 minutes after patch removal. The final reading is made on day of study D22.

Skin reactions are evaluated according to the scores reported in table 1,that describes the severity of erythema, oedema and other types of skin irritations clinically observed. The results are collected in a table. For each experimental time Irritation Index is calculated by adding erythema mean value, oedema, dryness and vesicles mean value. Medium Irritation Index (IIM) is calculated by adding the irritation indices of each experimental time and dividing by the number of checks. The tested product is then classified following table 2.

INDICATOR PATCH (CLASSIFICATION OF THE SENSITIZING POWER): clinical evaluation is carried out 30 minutes (D38) and 48 hours (D40) after indicator patch removal.

A possible allergic reaction during the inductive phase or indicator patch is evaluated with a score ranging from 0 to 3 according to the criteria of the ICDRG (International Contact Dermatitis Research Group) - see table 3.

A ++ reaction (2) or + + + reaction (3) must be re-evaluated the next day to see if the reaction is in decline or increasing. A rapid improvement of the reaction indicates that it is irritating, its persistence or worsening show that it is allergic type.

The occurrence of a single case of reaction (reaction with a score greater than or equal to + + (2)) in the contralateral area leads to the conclusion: "potentially sensitizing product." While, the product is defined “hypoallergenic” if no subject shows allergic reactions.

Table 1 - *Clinical score of skin reactions*

Score	ERYTHEMA	EDEMA	DRYNESS	VESICLES
0 (No reaction)	Normal appearance	Normal appearance	Normal appearance	Normal appearance
1 (Light reaction)	Slight pink color over the tested area or visible pigmentation on a part of the area	Edema more palpable than visible	Light desquamation, cracked skin	Vesicles more palpable than visible
2 (Evident reaction)	Clear and visible Erythema on the whole tested area	Evident edema	Evident desquamation, scaly appearance	Visible vesicles
3 (Serious reaction)	Intense erythema over the entire surface tested, or diffuse erythema outside the area	Edema extended outside the tested area	Serious desquamation and fissuring	Vesicles outside the tested area or bullae

Table 2 - *Classification of the Irritancy*

Mean Irritation Index (IIM)	Product classification
< 0.080	Not irritant
0,080 ≤score < 0,160	Very slightly irritating
0,160 ≤score < 0,560	Slightly irritating
0,560 ≤score < 1,000	Moderately irritating
1,000 ≤score < 1,600	Strongly irritating
≥1,600	Very strong irritating

Table 3 –*Evaluation of the sensitizing potential power*

Criteria	ICDRG score	Assigned score
No reaction	0	0
Doubtful reaction: mild redness only	?	?
Weak, positive reaction: Erythema and Edema	+	1
Strong positive reaction: Erythema, Edema and vesicles	++	2
Extreme positive reaction: intense redness and swelling with coalesced large blisters or spreading reaction	+++	3

Record no°: S.HU.003-0050.02.39SL_2017/3461

date: 21/12/2017

RESULTS

Summary of the data obtained and evaluation of the product irritation potential

N	Subject's code	Sex	1ST patch test				2ND patch test				3RD patch test				4TH patch test				5TH patch test				6TH patch test				7TH patch test				8TH patch test				9TH patch test			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	T3581R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	P2820M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Z2121M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	G2688F	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	B0015R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	M2000D	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	M3762S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	B2947R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	P1778A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	D0668A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	I0172A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	N0265D	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	R0333S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	G2051C	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	R0790F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	B2059G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	R3623F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	B3258A	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	A1462R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	F2070C	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	M2006A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	P0290M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	B0014L	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	O3319M	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	M3768G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	O0957S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	C1934S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	C1620T	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	S1738A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	S1739A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	T1434E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	L1086A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	B1081G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	A0677G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	S1258E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	D0946G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	C1267R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	S0869S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	S2140I	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	C2155P	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	A0871F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	C0771F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	C3690S	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	C3147F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	C1250V	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	C1049V	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	C1052D	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	B1438P	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	F0548M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	C3658M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend:
1: Erythema
2: Edema
3: Dryness
4: Vesicles

Irritation Index

D3	D5	D8	D10	D12	D15	D17	D19	D22
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Mean Irritation Index (MII)

0,00

Summary of the data obtained and evaluation of the product sensitizing potential

N	Subject's code	Sex	Indicator patch test	
			30'	48h
1	T3581R	F	0	0
2	P2820M	F	0	0
3	Z2121M	F	0	0
4	G2688F	M	0	0
5	B0015R	F	0	0
6	M2000D	F	0	0
7	M3762S	F	0	0
8	B2947R	F	0	0
9	P1778A	F	0	0
10	D0668A	F	0	0
11	I0172A	F	0	0
12	N0265D	M	0	0
13	R0333S	F	0	0
14	G2051C	M	0	0
15	R0790F	F	0	0
16	B2059G	F	0	0
17	R3623F	F	0	0
18	B3258A	M	0	0
19	A1462R	F	0	0
20	F2070C	F	0	0
21	M2006A	F	0	0
22	P0290M	F	0	0
23	B0014L	F	0	0
24	O3319M	M	0	0
25	M3768G	F	0	0
26	O0957S	F	0	0
27	C1934S	F	0	0
28	C1620T	F	0	0
29	S1738A	F	0	0
30	S1739A	F	0	0
31	T1434E	F	0	0
32	L1086A	F	0	0
33	B1081G	F	0	0
34	A0677G	F	0	0
35	S1258E	F	0	0
36	D0946G	F	0	0
37	C1267R	F	0	0
38	S0869S	F	0	0
39	S2140I	F	0	0
40	C2155P	F	0	0
41	A0871F	F	0	0
42	C0771F	F	0	0
43	C3690S	M	0	0
44	C3147F	F	0	0
45	C1250V	M	0	0
46	C1049V	F	0	0
47	C1052D	M	0	0
48	B1438P	M	0	0
49	F0548M	F	0	0
50	C3658M	F	0	0

CONCLUSIONS

On the basis of the data obtained we asses that the application on the skin of tested product do not cause any skin irritation or sensitization.
It is concluded that this product should be well tolerated and that the risk of sensitization is exceedingly small.

DIRECTA PLUS S.P.A.

G+ PRINTED FABRIC

is

"CLINICALLY TESTED"

NON IRRITATING, HYPOALLERGENIC*

(*LOW SENSITIZATING POTENTIAL)

Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
0	21/12/2017	First release

Experimenter	Quality control
Dr. Enza Cestone	**Dr. Cristina Scilironi**

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Patch Testing and Prick Testing A Practical Guide Official Publication of the ICDRG Lachapelle, Jean-Marie, Maibach, Howard I. 2nd ed., 2009.

Complife Italia S.r.l. Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, | P.I./C.F. 11093320155 | Capitale Sociale € 161.834 i.v., C.C.I.A.A. MI 1437929 - Reg. Soc. N. 342215.

HUMAN DERMATOLOGICAL INVESTIGATION

Repeated patch test to detect the skin sensitization potency of a fabric

DIRECTA PLUS S.P.A.

MONOLAYER PU G+

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Centro di Saggio operante in BPL - Sistema di gestione qualità certificato ISO 9001

INDEX

KEY PERSONNEL

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STUDY DESIGN

Title

HUMAN DERMATOLOGICAL INVESTIGATION
Repeated patch test to detect the skin sensitization potency of a fabric

Aim of the study

The assessment of adverse effects (erythema and edematous skin lesions, dryness and vesicles) occurred as a result of repeated application of the product under test is useful to check if that product can cause allergic sensitization reactions.

Tested Product

Information provided by the Customer

- Product name:

MONOLAYER PU G+

- Qualitative INCI formula:
  Composition: 95% polyurethane+5% PURE G+

Ethical requirements

The study was carried out in compliance with the following ethical requirements:

- All of the subjects participating in the study are healthy volunteers at least 18 years old.
- All of the subjects participating in the study are selected under the supervision of a dermatologist according to inclusion/non inclusion criteria (see respective paragraph “Inclusion criteria” and “Non inclusion Criteria”).
- Volunteer participation in the study is totally free.
- All of the subjects participating in the study are informed of the aim and the nature of the study.
- All of the subjects participating in the study are informed of the potential risks involved.
- All of the subjects participating in the study give their informed consent signed at the beginning of the study.
- Before volunteers were exposed to the product to be tested, all relevant safety information about the product itself and each ingredient were collected and evaluated.
- All of the study procedures are carried out in accordance with the ethical principles for the medical research (Ethical Principles for Medical Research Involving Human Subjects, adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and successive amendments)
- All necessary precautions were taken to avoid adverse skin reactions.
- If unexpected/adverse skin reactions occur, the dermatologist evaluates the severity of the reaction (and report it in the data collecting sheet) and if necessary proceed with the appropriate therapy.

Subjects selection

Volunteers recruitment

50 volunteers were recruited to take a part in the test in accordance with the following inclusion and non inclusion criteria:

Inclusion criteria

- ☑ Male and female subjects
- ☑ Subjects between 18 and 70 years old
- ☑ Healthy subjects
- ☑ Subjects with sensitive skin*
- ☑ Caucasian people
- ☑ Subjects informed about test purposes

Non - inclusion criteria

- ✘ Subjects who do not fit the inclusion criteria
- ✘ Pregnant or breastfeeding women
- ✘ Subjects with marks (for example tattoos, scars, burns) in the tested skin region, which might interfere with clinical evaluation
- ✘ Subjects who exposed themselves to sunlight or tanning lamps in the 15 days before the start of the test
- ✘ Subjects with dermatological problems in the test area
- ✘ Subjects with medication which may affect skin response
- ✘ Subjects undergoing pharmacological treatment (both locally or systemically)
- ✘ Subjects with past history for contact dermatitis
- ✘ Positive anamnesis for atopy

Withdrawal criteria

Partecipants are withdrawan if

- ✘ They do not follow the conditions of the Study Information Sheet that they receive after the recruitment
- ✘ They suffer any illnes or accident or develop any condition during the study which could affect the out come of the study
- ✘ They no longer wish to participate in the study.

Behaviour of volunteers during the test

Through patch application and 48 hours after the last patch removal volunteers must avoid situations or activity that could interfere with clinical evaluations:

- ✘ Exposition to sun or solarium
- ✘ Sport activity
- ✘ Immersion in water or steam bath
- ✘ Chafing and mechanical or thermal stress in the area in which patch is/has been applied.

Volunteers with sensitive skin are confirmed according to stinging* test with lactic acid.

***Stinging test**

A 10% water solution of lactic acid is applied by means of a cotton pad along the furrow which runs between the nose and the superior lip; an especially rich zone of thin nervous fibres which determine the hypersensitivity problem. The diagnosis results positive when, after some minutes, volunteer reports an itching or burning sensation.

Materials and Methods

Tested product and concentration

name	MONOLAYER PU G+
sponsor	DIRECTA PLUS S.P.A.
product description	Fabric
Study start (first enrolment date)	06/11/2017
Study end (last evaluation date)	15/12/2017
concentration	as it is
application method	semi- occlusive

Sample preparation and application

The product is applied as it is on the back (a clipping of 2X2 cm) fixed with a tape already been tested for its safety.
A Finn Chamber containing a blotting paper disk soaked with demineralized water is applied and used as a negative control. The test includes three phases:

- INDUCTION PHASE. These patches are applied in the same skin area (same side scapular area). The products under test are applied using the patch test under semi occlusion in number equal to nine (9): three (3) patches a week for 3 weeks (W1, W2, W3) for forty-eight (48) hours or seventy-two (72) hours for patches applied on Friday. The patches are applied on Monday, Wednesday and Friday. The patches are removed in the laboratory, skin reactions are assessed according to the scale shown in Table 1 and the new patch is applied on the same site. The procedure is repeated until the 9 (nine) induction patches are applied in the same site.

W1:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D1	D2	D3	D4	D5	D6	D7
Application of the product	✓		✓		✓		
Check			C		C		

W2:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D8	D9	D10	D11	D12	D13	D14
Application of the product	✓		✓		✓		
Check	C		C		C		

W3:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D15	D16	D17	D18	D19	D20	D21
Application of the product	✓		✓		✓		
Check	C		C		C		

- REST PERIOD, A rest period of two (2) weeks (W4, W5) will follow the removal of the last induction patches; no test materials is applied during this rest period

W4:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D22	D23	D24	D25	D26	D27	D28
Application of the product							
Check	C						

W5:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr	Sa/Sa	Do/Su
Day of study	D29	D30	D31	D32	D33	D34	D35
Application of the product							
Check							

- INDICATOR PATCH (W6) Following the rest period, a forty eight (48) hours challenge patch of each test material will be applied under semi occlusion to a new site on the back different from which of the induction phase (contralateral scapular area). After its removal at the laboratory, the skin reactions will be scored by experimenter at 30 minutes and forty eight (48) hours according to table 3 score.

W6:

Day of the week	Lu/Mo	Ma/Tu	Me/We	Gi/Th	Ve/Fr
Day of study	D36	D37	D38	D39	D40
Application of the product	✓				
Check			C		C

Legend:
W: Week
D: Day
C: Check

Clinical examination and scoring

After patches removal, volunteers are subjected to clinical evaluation of the possible erythematic and oedema reactions, dryness and vesicles.

INDUCTIVE PHASE (CLASSIFICATION OF THE IRRITATING POWER): clinical evaluation is performed 30 minutes after patch removal. The final reading is made on day of study D22.

Skin reactions are evaluated according to the scores reported in table 1,that describes the severity of erythema, oedema and other types of skin irritations clinically observed. The results are collected in a table. For each experimental time Irritation Index is calculated by adding erythema mean value, oedema, dryness and vesicles mean value. Medium Irritation Index (IIM) is calculated by adding the irritation indices of each experimental time and dividing by the number of checks. The tested product is then classified following table 2.

INDICATOR PATCH (CLASSIFICATION OF THE SENSITIZING POWER): clinical evaluation is carried out 30 minutes (D38) and 48 hours (D40) after indicator patch removal.

A possible allergic reaction during the inductive phase or indicator patch is evaluated with a score ranging from 0 to 3 according to the criteria of the ICDRG (International Contact Dermatitis Research Group) - see table 3.

A ++ reaction (2) or + + + reaction (3) must be re-evaluated the next day to see if the reaction is in decline or increasing. A rapid improvement of the reaction indicates that it is irritating, its persistence or worsening show that it is allergic type.

The occurrence of a single case of reaction (reaction with a score greater than or equal to + + (2)) in the contralateral area leads to the conclusion: "potentially sensitizing product." While, the product is defined “hypoallergenic” if no subject shows allergic reactions.

Table 1 - *Clinical score of skin reactions*

Score	ERYTHEMA	EDEMA	DRYNESS	VESICLES
0 (No reaction)	Normal appearance	Normal appearance	Normal appearance	Normal appearance
1 (Light reaction)	Slight pink color over the tested area or visible pigmentation on a part of the area	Edema more palpable than visible	Light desquamation, cracked skin	Vesicles more palpable than visible
2 (Evident reaction)	Clear and visible Erythema on the whole tested area	Evident edema	Evident desquamation, scaly appearance	Visible vesicles
3 (Serious reaction)	Intense erythema over the entire surface tested, or diffuse erythema outside the area	Edema extended outside the tested area	Serious desquamation and fissuring	Vesicles outside the tested area or bullae

Table 2 - *Classification of the Irritancy*

Mean Irritation Index (IIM)	Product classification
< 0.080	Not irritant
0,080 ≤score < 0,160	Very slightly irritating
0,160 ≤score < 0,560	Slightly irritating
0,560 ≤score < 1,000	Moderately irritating
1,000 ≤score < 1,600	Strongly irritating
≥1,600	Very strong irritating

Table 3 –*Evaluation of the sensitizing potential power*

Criteria	ICDRG score	Assigned score
No reaction	0	0
Doubtful reaction: mild redness only	?	?
Weak, positive reaction: Erythema and Edema	+	1
Strong positive reaction: Erythema, Edema and vesicles	++	2
Extreme positive reaction: intense redness and swelling with coalesced large blisters or spreading reaction	+++	3

Record no°: S.HU.003-0050.02.39SL_2017/3462

date: 21/12/2017

RESULTS

Summary of the data obtained and evaluation of the product irritation potential

N	Subject's code	Sex	1ST patch test				2nd patch test				3rd patch test				4th patch test				5th patch test				6th patch test				7th patch test				8th patch test				9th patch test			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	T3581R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	P2820M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Z2121M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	G2688F	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	B0015R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	M2000D	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	M3762S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	B2947R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	P1778A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	D0668A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	I0172A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	N0265D	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	R0333S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	G2051C	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	R0790F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	B2059G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	R3623F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	B3258A	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	A1462R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	F2070C	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	M2006A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	P0290M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	B0014L	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
24	O3319M	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	M3768G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	O0957S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	C1934S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	C1620T	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	S1738A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	S1739A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	T1434E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	L1086A	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	B1081G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	A0677G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	S1258E	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	D0946G	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	C1267R	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	S0869S	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	S2140I	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	C2155P	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	A0871F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	C0771F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	C3690S	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	C3147F	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	C1250V	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	C1049V	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	C1052D	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	B1438P	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	F0548M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	C3658M	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend:
1: Erythema
2: Edema
3: Dryness
4: Vesicles

Irritation Index

D3	D5	D8	D10	D12	D15	D17	D19	D22
0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00

Mean Irritation Index (MII)

0,00

Summary of the data obtained and evaluation of the product sensitizing potential

N	Subject's code	Sex	Indicator patch test	
			30'	48h
1	T3581R	F	0	0
2	P2820M	F	0	0
3	Z2121M	F	0	0
4	G2688F	M	0	0
5	B0015R	F	0	0
6	M2000D	F	0	0
7	M3762S	F	0	0
8	B2947R	F	0	0
9	P1778A	F	0	0
10	D0668A	F	0	0
11	I0172A	F	0	0
12	N0265D	M	0	0
13	R0333S	F	0	0
14	G2051C	M	0	0
15	R0790F	F	0	0
16	B2059G	F	0	0
17	R3623F	F	0	0
18	B3258A	M	0	0
19	A1462R	F	0	0
20	F2070C	F	0	0
21	M2006A	F	0	0
22	P0290M	F	0	0
23	B0014L	F	0	0
24	O3319M	M	0	0
25	M3768G	F	0	0
26	O0957S	F	0	0
27	C1934S	F	0	0
28	C1620T	F	0	0
29	S1738A	F	0	0
30	S1739A	F	0	0
31	T1434E	F	0	0
32	L1086A	F	0	0
33	B1081G	F	0	0
34	A0677G	F	0	0
35	S1258E	F	0	0
36	D0946G	F	0	0
37	C1267R	F	0	0
38	S0869S	F	0	0
39	S2140I	F	0	0
40	C2155P	F	0	0
41	A0871F	F	0	0
42	C0771F	F	0	0
43	C3690S	M	0	0
44	C3147F	F	0	0
45	C1250V	M	0	0
46	C1049V	F	0	0
47	C1052D	M	0	0
48	B1438P	M	0	0
49	F0548M	F	0	0
50	C3658M	F	0	0

CONCLUSIONS

On the basis of the data obtained we asses that the application on the skin of tested product do not cause any skin irritation or sensitization.
It is concluded that this product should be well tolerated and that the risk of sensitization is exceedingly small.

DIRECTA PLUS S.P.A.

MONOLAYER PU G+

is

"CLINICALLY TESTED"

NON IRRITATING, HYPOALLERGENIC*

(*LOW SENSITIZATING POTENTIAL)

Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
0	21/12/2017	First release

Experimenter	Quality control
Dr. Enza Cestone	**Dr. Cristina Scilironi**

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Dermatotoxicology, Seventh Edition Edited by Hongbo Zhai , Klaus-Peter Wilhelm , and Howard I. Maibach Informa Healthcare 2008.

Patch Testing and Prick Testing A Practical Guide Official Publication of the ICDRG Lachapelle, Jean-Marie, Maibach, Howard I. 2nd ed., 2009.

Complife Italia S.r.l. Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, | P.I./C.F. 11093320155 | Capitale Sociale € 161.834 i.v., C.C.I.A.A. MI 1437929 - Reg. Soc. N. 342215.

CENTROCOT
Innovation experience

DIRECTA PLUS S.P.A

Via Cavour, 2
22074 LOMAZZO (CO)

CERTIFICATE N. 21CC0007_21RA01190

Emission date	26/02/2021
Valid until	25/02/2022
ZDHC MRSL	Version 2.0 Level 1

SAMPLE IDENTIFICATION

Code	PST11
Name	GRAFYPAD G PLUS
Batch number	001/2020
MRSL code	1.7.12
Application	Disperse dye -Additive for foulard impregnation
Attachments	SDS GRAFYPAD G PLUS_IT

RESULT	**ZDHC MRSL V2.0 LEVEL 1**
	COMPLIANT

Laboratory Manager

Dr. Letizia Bregola

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CENTROCOT
Innovation experience

DIRECTA PLUS S.P.A

Via Cavour, 2
22074 LOMAZZO (CO)

CERTIFICATE N. 21CC0008_21RA01191

Emission date	25/02/2021
Valid until	24/02/2022
ZDHC MRSL	Version 2.0 Level 1

SAMPLE IDENTIFICATION

Code	PST03
Name	GRAFYTEX SP9
Batch number	013/2020
MRSL code	1.3.25.2
Application	Water based ready to use printing paste - Paste for screen printing and rotary printing
Attachments	SDS SDS_GrafyTex G+ SP9 rev 2 ita

RESULT	**ZDHC MRSL V2.0 LEVEL 1**
	COMPLIANT

Laboratory Manager

Dr. Letizia Bregola

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OEKO-TEX®
INSPIRING CONFIDENCE

ECO PASSPORT SCREENING REPORT

The Global RSL and MRSL Search and Screen Tool

ECO PASSPORT Screening is used to screen chemical products – at the ingredient level – against the world's leading restricted substance lists and manufacturing restricted substance lists:

- STANDARD 100 by OEKO-TEX® Restricted Substance List (RSL)
- ECO PASSPORT by OEKO-TEX® Restricted Substance List (RSL)
- STeP by OEKO-TEX® Manufacturer Restricted Substance List (MRSL)
- LEATHER STANDARD by OEKO-TEX® Restricted Substance List (RSL)
- REACH Substances of Very High Concern (SVHC)

The product components, main constituents and impurities declared by the applicant are not mentioned on the MRSL 2.0 of ZDHC.

Company

Achitex Minerva Spa
Via degli Artigiani 6
26010 Vaiano Cremasco CR, ITALY

Category: 1.3 Textile auxiliaries for dyeing and printing, 2.5 Pigments

Certificate No: 20EP00001

Issued by: CENTRO TESSILE COTONIERO E ABBIGLIAMENTO S.p.A.

ECO PASSPORT SCREENING SYNOPSIS

The following product(s) are suitable for ECO PASSPORT analytical verification:

GRAFYPAD G PLUS

GRAFYTEX BIANCO SP11

GRAFYTEX SP9

The following product(s) contain(s) at least one substance restricted by OEKO-TEX® and may therefore not be suitable for certification according to ECO PASSPORT:

None

Information disclosure level: MINIMAL

The screening has been conducted against the above mentioned Restricted Substances Lists valid on 01.03.2022

CENTRO TESSILE COTONIERO E ABBIGLIAMENTO S.p.A.
Piazza Sant' Anna 2
21052 Busto Arsizio VA, Italy

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CERTIFICATE

Achitex Minerva Spa
Via degli Artigiani 6
26010 Vaiano Cremasco CR, ITALY

is granted ECO PASSPORT by OEKO-TEX® certification and the right to use its trademark for the certified products listed below. This is based on the test report **22RA01339**.

OEKO-TEX®
INSPIRING CONFIDENCE
ECO PASSPORT
20EP00001 CENTROCOT
Textile and leather chemicals. Tested and verified.
www.oeko-tex.com/ecopass

Scope

ECO PASSPORT by OEKO-TEX®

Products: See attached enclosure
Product Category: Multiple

The above captioned products can be used for the production of human-ecological optimized textiles & leathers. The combined results of the reports mentioned above reveal that there is no harmful effect on the human and environmental health of the textiles & leathers treated/finished with the certified products. The products fulfill requirements of Annex XVII of REACH (incl. the use of azo colourants, chromium (VI), nickel release, etc.). The evaluation was based on the test methods and requirements of the ECO PASSPORT by OEKO-TEX® that were in force at the time of evaluation.
ZDHC MRSL 2.0 Conformance Level 1 is achieved for certified products without restrictions.

Supporting Documents

- Declaration of conformity in accordance with EN ISO 17050-1 included in ECO PASSPORT by OEKO-TEX® Terms of Use.
- Test Report Number: 22RA01339
- RSL Screening Report
- Detailed information about the components and safety data sheets of the certified chemical products.

The certificate 20EP00001 is valid until 28.02.2023

Busto Arsizio, 01.03.2022

Chiara Salmoiraghi
OEKO-TEX® Certification Scheme Manager
CENTROCOT

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Enclosure for Certificate No. 20EP00001

Achitex Minerva Spa
Via degli Artigiani 6
26010 Vaiano Cremasco CR, ITALY

ECO PASSPORT

Certificate Number: 20EP00001

Test Report Number: 22RA01339

Certified Products

No.	Product Name	Trade Name	Restriction(s) [1]	ZDHC LEVEL	Product Category
1	GRAFYPAD G PLUS	GRAFYPAD G PLUS	none	Level 1	2.5 Pigments
2	GRAFYTEX BIANCO SP11	GRAFYTEX BIANCO SP11	none	Level 1	1.3 Textile auxiliaries for dyeing and printing
3	GRAFYTEX SP9	GRAFYTEX SP9	none	Level 1	1.3 Textile auxiliaries for dyeing and printing

Issue Date 01.03.2022

[1] Restriction(s): The parameter(s) mentioned under Restriction(s) have to be checked on the treated textile for compliance with the regulations of STANDARD 100 by OEKO-TEX® / LEATHER STANDARD by OEKO-TEX®.