Nanoparticle exposure biomonitoring: exposure/effect indicator development approaches

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Abstract. The use of engineered nanoparticles (NP) is more and more widespread in various industrial sectors. The inhalation route of exposure is a matter of concern (adverse effects of air pollution by ultrafine particles and asbestos). No NP biomonitoring recommendations or standards are available so far. The LBM laboratory is currently studying several approaches to develop bioindicators for occupational health applications. As regards exposure indicators, new tools are being implemented to assess potentially inhaled NP in non-invasive respiratory sampling (nasal sampling and exhaled breath condensates (EBC)). Diverse NP analytical characterization methods are used (ICP-MS, dynamic light scattering and electron microscopy coupled to energy-dispersive X-ray analysis). As regards effect indicators, a methodology has been developed to assess a range of 29 cytokines in EBCs (potential respiratory inflammation due to NP exposure). Secondly, collaboration between the LBM laboratory and the EDyp team has allowed the EBC proteome to be characterized by means of an LC-MS/MS process. These projects are expected to facilitate the development of individual NP exposure biomonitoring tools and the analysis of early potential impacts on health. Innovative techniques such as field-flow fractionation combined with ICP-MS and single particle-ICPMS are currently being explored. These tools are directly intended to assist occupational physicians in the identification of exposure situations.

1. Introduction

The use of engineered nanoparticles (NP) is in constant growth in international research and industry. However their toxicity has not yet clearly been identified and the precautionary principle is currently recommended in France by the Public Health Council (HCSP, "Haut Conseil de la Santé Publique") owing to alert signals, such as a similar toxic action between the asbestos fibres and certain carbon nanotubes (CNT) as evidenced in animals [1, 2] or the known health effects of ultrafine particle pollution. With respect to these data, the inhalation exposure route now seems the most preoccupying for NPs.

Besides, carbon nanotubes and, by way of example, titanium dioxide (TiO2) widely used as a pigment and opacifier in the composition of many everyday consumer products (sunscreens, tooth paste, confectionery, paints, drugs…) is assumed to feature certain toxic effects similar to those of asbestos. The tests conducted on mice and on human cells show that in nanometric form, titanium dioxide produces a pro-inflammatory activity on the lungs and the peritoneum, like asbestos and silica [3].
addition, titanium dioxide was classified as a possible carcinogenic agent in humans (2B) by the International Agency for Research on Cancer (IARC) in 2006.

Although uncertainties remain in the field of occupational risk assessment, strict measures for protection of workers potentially exposed to NPs must be implemented. For this purpose, an exposure monitoring procedure is of the essence. In addition to atmospheric monitoring, biological monitoring is a suitable approach to optimise the determination of individual exposure to occupational toxic substances. In fact, work atmosphere monitoring (atmospheric metrology) versus limit values to be observed is an indispensable process for risk assessment and prevention. It also allows the external dose to be measured. However, it is affected by certain limitations notably due to the integration of the sole respiratory tract, as well as the application of limit values to pure substances and not to the preparations, or to multiple exposures, and the failure to integrate individual absorption, metabolism and susceptibility factors.

Biological monitoring includes the use of exposure bioindicators and effect biomarkers. Exposure biomarkers are informative of the subjects’ exposure level. They allow the assessment of the toxic substance quantities which actually penetrated into the body (internal dose) through the respiratory tract, but also through dermal or even digestive routes. Therefore, it is possible to determine the substance itself or its metabolites in the biological fluids, and this is constitutive of exposure biomarkers. Effect biomarkers are intended to highlight potential biological alterations linked with exposure.

Figure 1 illustrates the exposure and effect biomarkers in the continuum between exposure and pathology.

![Figure 1. Continuum between exposure and pathology and exposure monitoring.](image)

Generally, biomonitoring (which regroups biometrology and biotoxicology) offers the interest of integrating the various absorption routes, as well as multi-exposures (either occupational or not), with their possible synergy. Besides, it integrates the individual susceptibility factors and the actual exposure conditions such as physical activity, amongst others, which causes a hyperventilation and the resulting increase in lung absorption of toxic substances, as well as the use of individual protective gear (breathing mask, gloves, protection garments).

In the case of nanoparticles, no biological monitoring standards or recommendations are currently in effect, although this monitoring process is indispensable in the general approach of nanoparticle health impact prevention [4]. The remaining question is the choice of biological matrices to be used. Occupational monitoring refers to non- or minimally invasive matrices (conventionally urine and blood) but in the case of nanoparticles, the toxicokinetic data acquired in animals tend to prove that only very few nanoparticles could be found in these matrices after inhalation, owing to a translocation process which is demonstrated but however remains quantitatively low, as well as a quick internalisation in different organs after passage into the blood stream [5]. Therefore, we are studying other biological
matrices, and the development of non-invasive exposure monitoring tools specific to the inhalation exposure route would be particularly useful.

A first non-invasive approach consists of sampling nasal secretions on a tissue flag for the analysis of potentially inhaled nanoparticles, in order to provide a “yes/no” type inhalation indicator at the workstations. This method is already in use for this type of indication in the case of radioactive particles [6]. Besides, concentrations of nickel dose-dependents with atmospheric exposure to nickel particles were found in workers’ nasal mucous membrane [7], indicative of a significant retention of inhaled particles at nose level. In the case of nanoparticles, the deposit curves for particles inhaled in the respiratory tract proposed by the ICRP (International Commission on Radiological Protection) confirm that nanoparticles are especially retained at nose level, all the more so as they are smaller.

A second non-invasive approach consists of sampling exhaled breath condensates (EBC) which are a biological environment of interest for respiratory tract in connection with exposure by inhalation. In fact, the exhaled breath is in equilibrium with the water vapour at body temperature. Owing to the large lung exchange surface, 400 mL water are daily lost by evaporation. The exhaled aerosol is formed of microscopic droplets which appear at the respiratory mucus surface which covers the respiratory tract, due to turbulence phenomena between inhaled and exhaled air in the bronchi and alveoli. The exhaled air contains more than 99% water and a small fraction of volatile and non-volatile compounds from the respiratory tract. The exhaled air can be condensed in the form of liquid (the EBC) in which various molecules may be searched. The exhaled molecules are rather small, not exceeding 100 kDa, amongst which certain oxidative stress markers or inflammation markers may be found [8, 9]. Exposure biomarkers such as metals were also studied in EBCs in connection with smoking habits [10] or with occupational exposure to metal particles [11, 12]. The remaining question is to determine whether NPs could be detected in EBCs further to exposure by inhalation.

Whereas the development of exposure biomarkers is intended to determine the nanoparticles themselves (or their derivatives) in the biological matrices, the development of precautious effect biomarkers firstly requires the study of the toxicity mechanisms and the associated markers. Inflammation notably seems to result from toxicity linked with exposure to nanoparticles [13]. By way of example, certain bronchoalveolar liquid cytokines are induced by CNT inhalation, notably TNF-α, IL-6 and TGF-β in an inhalation study in mice concerning single-walled CNT, [14]. In another inhalation study on mice the TiO₂ causes the secretion of interleukines IL-1α and IL-1β [3]. More generally, cytokines IL-3, II-4, IL-5, IL-6, IL-10, IL-12, TNF-α and IFN-γ are pointed out in the immunotoxicity of nanoparticles [15].

For its part, exposure to nanoparticles emitted by photocopiiers causes an increase in proteins IL-6, IL-8, TNF-α, IL-1β, G-CSF, EGF, IL-10, MCP1, fractalkine, and VEGF in nasal lavage [16]. The analysis of the induced expectoration from patients diagnosed with silicosis or asbestosis by comparison with des control subjects indicates that interleukines IL-1β, IL-6, the TNF-α, and the metalloprotease inhibitor TIMP-1 are associated with the impairment of respiratory function in the sick patients [17]. Finally, a significant increase in interleukines IL-1β, IL-6, IL-8, IL-10 and IL-12p70 in EBCs was underlined in smokers versus non-smokers. The same observation was made in patients suffering from a chronic acute obstructive respiratory disease versus a stable pathology [18].

The search for biomarkers targeted on a toxicity mechanism such as inflammation is a possible approach. However, it would be interesting to combine it with non-targeted approaches, allowing the discovery of new bioindicators. Proteomics, for example, consists of describing all proteins present in specific cells or tissues. Contrary to the genome, the proteome is very variable in the course of time and incurs evolutions dictated by pathological and environmental changes [19]. Over the past 15 years, the quick progress of proteomics based on quantitative mass spectrometry allowed the discovery of many potential biomarkers for different cancers and other diseases [20, 21]. In the field of environmental and occupational health, although the data are sparser, a change in protein expression due to inhaled toxic substances was noted by proteomic approaches. Concerning NPs, the change in protein expression was found in the bronchoalveolar liquid (BAL) after carbon black NP intratracheal instillation in mice, and ZnO NP inhalation in rats [22, 23]. Concerning EBCs: the proteomic study showed that the proteins
found in the EBCs featured 83% similarity with the proteins found in the BAL [24], thereby confirming the interest of this matrix to represent the deep lung. Different studies were consequently focused on the search for a protein signature in EBCs for determination of biomarkers according to different conditions (smoking, chronic obstructive bronchopneumopathy, pulmonary emphysema) [24, 25].

In summary, the combined search for exposure and effect bioindicators, bearing on biological matrices such as nasal secretions and the exhaled breath condensates, seems a promising approach for the development of biological NP exposure monitoring tools representative of the respiratory tract, like biomarkers measured in blood and urine which are more informative on a systemic rather than a local illness.

2. Equipment and methods

2.1. Nasal secretion sampling.
The device is comprised of a small absorbent tissue strip secured to a wooden stem (shaped as a flag) to collect nasal secretions by smear test in each nostril. The tissue is slightly moistened with ultrapure water before sampling. One flag is used for each nostril and each individual.

2.2. Exhaled breath condensate sampling.
The LBM laboratory uses the RTube device (Respiratory Research) according to the recommendations proposed by the "American Thoracic Society" and the "European Respiratory Society" [9]. Before using these devices, the LBM laboratory implements an intensive flushing protocol with ultrapure water and a check for absence of background noise by dynamic light scattering.

In brief, the sampling with nose blocked lasts for 15 minutes during which the subject breathes normally through the device. Water rinsing of the mouth was carried out and the subject was asked to abstain from drinking or eating for an hour before the sampling. A survey is also associated and allows these elements to be clarified. After collection, the exhaled breath condensates are immediately stored at -80°C in low absorption tubes. The EBC quantity collected generally varies between 1 and 2 mL in healthy adults.

2.3. Analysis techniques

2.3.1 Exposure biomarkers. The various techniques used by the LBM laboratory belong to the arsenal of the processes of the LBM and the Nanosafety Platform of CEA Grenoble (PNS, "Plateforme NanoSécurité"). They provide data concerning size, shape and chemical composition. In fact, there is no universal technique for nanoparticle analysis, allowing the determination in one single analysis of the different physico-chemical parameters of the particles required for their characterization. By priority, we combined three analyses, namely: inductively coupled plasma mass spectrometry (ICP-MS) for elemental composition determination, dynamic light scattering (DLS) for granulometric distribution determination, and scanning electron microscopy (SEM) coupled to X-ray fluorescence (EDX) for the determination of particle size/morphology and elemental composition.

The ICP-MS is the reference technique for a multi-element quantitative determination, both specific and sensitive. The LBM laboratory is equipped with a Nexion 300X device from the Perkin Elmer company, with methane reaction cell for the interfered elements such as iron and selenium. The LBM laboratory developed and validated a multi-element analysis technique allowing the simultaneous determination of 17 elements (Zn, Al, Ti, Co, Cu, Zr, Ni, Cr, Ga, In, Pl, Mn, Fe, Se, Cd, Ge, Be). The LBM laboratory determined the analytical LD and LQ as well as the LDm and LQm methods (LDm and LQm), taking into account the background noise associated with sampling. Considering the very low concentrations found in EBCs, the analysis by ICP-MS is performed at this stage without prior mineralization. However, for the nasal smear test flag, a mineralization step is required in order to
dissolve the flag before analysis by ICP-MS. This soft mineralization is performed in HNO3 in a microwave oven (MW3000 Anton Paar).

The DLS allows the granulometry measurement of particles in suspension in liquids. The LBM laboratory uses a Zetasizer Nano ZS device from the Malvern company (results not provided). Electron microscopy coupled to X-ray elemental analysis is the reference technique for the qualitative confirmation of the size, morphology and chemical composition of the particles. The SEM produces high resolution images of a sample surface, up to a few nanometres. For its part, the EDX X-photon energy scattering analysis system, associated with the SEM, allows the chemical characterization of constitutive elements in the observed materials.

A SEM model 5500 unit (Scanning Electron Microscope) from the Hitachi company associated with a Noran model EDX system (X-ray analysis system) from the Thermo company was used. Analyses with the TEM-EDX (Hitachi 5500.) were also performed on certain samplings.

2.3.2. Effect biomarkers. For analysis of cytokines in EBCs we used a multiplex technique (Magpix technology Biorad) allowing the analysis of a range of 29 inflammatory markers. The EBC samples were concentrated by freeze-drying (concentration by a factor 12) and the results are expressed in U/ml EBC, where U is a technical unit of the laboratory.

The inflammatory markers sought were classified by family: interleukines (Il 1b, Il 1ra, Il2, Il 4, Il5, Il 6, Il 7, Il 9, Il 10, Il 12, Il-13 , Il-15, Il-17a), chemokines (Il 8, eotaxin, RANTES, IP-10, MCP-1, MIP-1a, MIP-1b) and others (TNF alpha, IFN-g, G-CSF, GM-CSF, PDGF, FGF, VEGF, ICAM-1, VCAM-1).

The proteomic analysis required a previous biochemical treatment of the sample in order to extract the proteins while eliminating the phospholipids. In fact, the droplets of exhaled biological material in the EBCs are comprised of 90% lipids from the surfactant [24], which may impair the proteomic analysis quality. The EBC proteome was characterized by mass spectrometry (LC-MS/MS analyses on a mass spectrometer of OrbiTrap Velos type (Thermo). The analyses were firstly performed on EBC pools (triplicate of 10 different EBCs in each triplicate) owing to the very low concentrations.

2.4. Studied populations

For EBCs, for analysis of metals and particle content, the LBM laboratory firstly took part in a large-scale study in an airport environment whose purpose was to assess the respiratory health of this type of population. This study offered the interest of testing the feasibility of EBC sampling in occupational health care, as more than 200 volunteer employees benefited from this type of RTube sampling. This sampling was associated with a wide range of clinical examinations and a detailed survey. The analysis by ICP-MS and DLS (results not provided) was performed on all samplings.

For the analysis on cytokines, we worked on a population de 16 healthy volunteers representative of the working population (aged between 28 and 56, 15 men, 1 woman, 12 non-smokers, and 1 smoker), and on a professional population of 32 employees in the scope of a confidential study. For the proteomic analysis, a population de 30 healthy volunteers representative of the working population was studied.

For each one of these studies, prior informed consent from the subjects had been obtained either in the scope of a method development process on anonymised samples, or in that of a study approved by the concerned company’s Management and occupational physicians.
3. Results

3.1 Nostril smear test flags

For the flags, the results are essentially of analytical order. The use of the ICP-MS allowed an analytical sensitivity improvement from 1 µg to 25 ng by flag. The LBM laboratory proceeded with analytical developments on other types of nanoparticles. A first confidential feasibility study was performed on workers in contact with metal oxide nanoparticles. This study produced first significant results through smear test flags on subjects after a workshift on a potentially exposed workstation and after a holiday period.

3.2 Metals in the exhaled breath condensate

The elemental concentrations obtained in the EBCs from 211 airport office employees for 17 elements are shown in Table 1.

Table 1. Elemental concentrations in EBCs (µg/L) shown as mean, median and range for concentrations above LDm or LQm. Aberrant values characterized by several orders of magnitude above other data were discarded.

| µg/L EBC | Mean | Median | Range^a | n  | Mean | Median | Range^a | n  |
|----------|------|--------|---------|----|------|--------|---------|----|
| Zn⁶⁶     | 63   | 41     | 25-303  | 110| 180  | 168    | 85-303  | 18 |
| Al¹²⁷    | 6.9  | 5.2    | 3.2-46  | 121| 21   | 17     | 11-46   | 13 |
| Ti⁴⁸      | 0.8  | 0.6    | 0.3-3.6 | 88 | 1.8  | 1.5    | 1.1-3.6 | 12 |
| Co⁵⁹      | 0.3  | 0.2    | 0.1-1.1 | 58 | 0.7  | 0.5    | 0.4-1.1 | 16 |
| Cu⁶³      | 1.6  | 1.0    | 0.6-16  | 101| 3.4  | 2.6    | 1.9-16  | 24 |
| Zr⁹⁰      | 0.7  | 0.6    | 0.2-9.7 | 211| 0.7  | 0.6    | 0.1-4.3 | 211|
| Ni¹⁰⁰     | 1.0  | 0.5    | 0.9-2.1 | 135| 2.4  | 1.1    | 0.8-10  | 37 |
| Cr³²      | 0.8  | 0.6    | 0.1-8.7 | 193| 0.9  | 0.7    | 0.4-8.7 | 157|
| Ga⁶⁹      | 0.2  | 0.1    | 0.04-0.9| 70 | 0.3  | 0.2    | 0.1-0.9 | 22 |
| In¹¹⁵     | 0.1  | 0.1    | 0.01-0.3| 60 | 0.1  | 0.1    | 0.02-0.3| 49 |
| Pt¹⁹⁵     | 0.4  | 0.4    | 0.2-1.2 | 170| 0.8  | 0.7    | 0.5-1.2 | 35 |
| Mn⁸⁵      | 0.8  | 0.4    | 0.3-7.2 | 75 | 3.2  | 2.4    | 1.1-7.2 | 10 |
| Fe⁵⁶      | 12   | 8      | 3.8-36  | 20 | 22   | 20     | 13-36   | 7  |
| Se⁸⁰      | 0.7  | 0.5    | 0.4-1.2 | 4  | —    | —      | —       | 0  |
| Cd¹¹¹     | 0.2  | 0.1    | 0.1-0.7 | 56 | 0.4  | 0.3    | 0.3-0.7 | 17 |
| Ge⁷²      | 0.1  | 0.1    | 0.01-0.5| 78 | 0.1  | 0.1    | 0.03-0.5| 65 |
| Be⁹       | 0.1  | 0.1    | 0.01-0.6| 82 | 0.1  | 0.1    | 0.02-0.6| 63 |

^a Range (min-max)
Elemental concentration distribution is shown by way of example for 2 elements in Figure 2.

**Figure 2.** Elemental concentration dispersion in EBC for Pt (A) and Ti (B)

TEM-EDX images were shot on certain EBC samples to highlight a carbon nanoparticle within the EBC shown by its Na content (Figure 3).

**Figure 3.** TEM picture of a particle of about 500 nm identified in EBC (A). C EDX mapping (B), Na EDX mapping (C), combination of C and Na EDX mappings.
3.3 Cytokines in EBCs

Our first results on volunteers indicate that cytokine levels are very low despite the concentration step by freeze-drying. We expressed our results in technical units instead of mass units before confirmation. An example of the results obtained on the interleukines is shown in Table 2.

The cytokines analyses were also carried out on workers' EBCs, but in strict confidence.

Table 2: result examples for cytokines on a volunteer population

| Volunteer | Smoking habit | Interleukines (U/mL EBC) |
|-----------|---------------|--------------------------|
|           |               | II-1b II-1ra II-2 II-4 II-5 II-6 II-7 II-9 II-10 II-12 II-13 II-15 II-17A |
| 1         | NS            | 0.0024 0.52 0.050 0.012 0.021 0.074 0.020 0.0072 |
| 3         | NS            | 0.045 0.95 0.096 0.026 0.035 0.041 0.014 0.1152 0.050 0.033 0.26 |
| 4         | NS            | 0.0024 0.95 0.018 0.009 0.050 |
|           |               | 0.015 |
| 6         | NS            | 0.31 0.037 0.028 |
| 8         | NS            | 2.1 0.050 0.012 0.038 0.032 0.017 |
| 9         | NS            | 0.11 |
| 10        | NS            | 0.012 0.81 0.014 0.067 0.015 0.21 |
| 12        | NS            | 0.0040 0.66 0.10 0.006 0.039 0.49 0.034 0.0016 0.39 |
| 14        | S             | 0.0016 0.13 0.020 0.023 |
| 15        | S             | 0.73 0.019 0.012 |
| 2         | S             | 0.0024 0.38 0.050 0.0096 0.036 |
| 5         | S             | 0.0016 0.52 0.12 0.009 0.023 0.033 |

3.4 Proteomics in EBCs

The last study consists in the proteomic analysis of EBCs that has been conducted in collaboration with the EDYP team. The EBC proteome from healthy volunteers has been determined based on a pooled EBC analysis. Three replicates have been performed on a pool of about 10 EBCs and more than 100 proteins have been described. The data analysis is in progress but some proteins of interest are emerging. In fact, some proteins originate from the deep lung, such as surfactant proteins, and others from macrophages. The next step will consist of improving the method sensitivity for the purpose of individual analysis.
4. Discussion

Through this paper, the LBM laboratory proposes various approaches to efficiently develop NP exposure and effect bioindicators. The interest of the search for NPs in biological mediums is the availability of an exposure-specific bioindicator, provided that the NPs can be characterized specifically. This is an analytical challenge, strongly linked with the nature of the NPs. For example, carbonaceous NPs are not easy to characterize specifically as, in fact, the elemental analyses of ICP-MS type fail to allow their detection and as these NPs may be ubiquitously present in the environment. Consequently, it is often necessary to combine different techniques such as ICP-MS, DLS and electron microscopy. The object is then to ensure that the results provided by the ICP-MS and the DLS comply with SEM-EDX or TEM-EDX observations for the purpose of possibly proposing, in the long term, a simplified analysis protocol in which the electron microscopy observation could be avoided, as this technique is heavy to implement owing to a long analysis time for each sample.

For the effect bioindicators, the search for inflammatory markers in the tested range offers the interest of highlighting, not only one marker (which, when isolated, would largely be non-specific) but several markers which, when simultaneously analysed, would provide potentially specific signatures of an exposure. Finally, proteomic analysis allows the exploration of the possible existence of new effect bioindicators as, in principle, it is not focused on suspected targeted mechanisms in the toxicity of NPs.

However, difficulties arose in the development of such engineered nanoparticle exposure and effect biomarkers. At analytical level, one of the main issues that the laboratory is currently addressing is that the EBC is strongly diluted biological medium. Consequently, it requires perfectly optimised and highly sensitive techniques. For example, the levels obtained for cytokines in EBCs are very close to the quantification or detection limits of the technique, despite the freeze-drying step, and this causes severe uncertainty factors. Therefore, for the time being, we are not in a position to conduct relevant comparisons between different types of populations. These comparisons will be planned in the longer term, when this barrier is lifted. This is why at this stage, the LBM laboratory prefers to provide the obtained results in technical unit rather than in concentration. This sensitivity issue is faced with, both for cytokine measurement and for the proteomic analysis which could only be conducted so far on EBC pools, as was already described in the literature [24, 25]. Once the technique is optimised, our objective is to proceed with individual analyses, failing which the use of the identified bioindicators would be compromised. For the elemental analysis by ICP-MS, the very low concentrations dictated the choice not to mineralize the sample firstly, in order to avoid the introduction of an additional sample contamination and dilution source.

As regards the nasal smear test flag, the LBM laboratory had initially performed early analytical developments on ZnO nanoparticles through the TXRF Total X-Ray Fluorescence technique [26]. The technique sensitivity was improved by a factor 40 for ZnO NPs, thanks to the use of the ICP-MS. This result is encouraging and the process is currently being developed for other NPs. In addition to analytical improvements, an also critical issue is the small number of studies on volunteers conducted so far. Before proceeding with a wider scale study, the LBM laboratory wishes to optimise all these techniques, in order to further obtain reliable reference value on a general population, for comparison with populations of workers exposed to NPs.

The EBC standardisation issue also forms part of the subject to be looked at. On all EBC samples in airport environment, the laboratory performed analyses in µg/L EBC for all metals measured, but also in µg/100 µg sodium in EBCs (results not provided), as sodium is proposed as a standardisation tool by several teams [9]. However, the statistic result analysis is not different, whether it uses the results in µg by litre or in µg/100 µg sodium. The determination of total proteins by µBCA is currently being assessed at the laboratory with a view to standardisation by the total proteins present in the EBC samples. Proteomic analysis may also allow the identification of a protein which could be seen as the relevant candidate for a standardisation of the analyses in EBCs.
In parallel, it is also important to develop techniques for nanoparticle characterization improvement in the biological matrices, which remain complex even when they are diluted, as is the case of EBCs. The behaviour of nanoparticles in the biological fluids will depend both on the nanoparticle properties (solubility, zeta potential, etc.) and on the biological fluid nature itself, notably the presence of more or less large quantities of proteins and/or phospholipids likely to form coronas, thereby making their characterization more complex.

In conclusion, the studies presented in this paper are of interest insofar as they are directly conducted on humans: consequently, they reflect reality, whereas extrapolations from in vitro and in vivo studies are not always relevant as the behaviour of the nanoparticle is closely linked with its medium or environment. Consequently, the combination of several characterization techniques is of the essence. The LBM laboratory is currently working on the examination of biological mediums by innovative techniques such as ICP-MS Field-Flow Fractionation (FFF-ICP MS) or single-particle ICP MS (SP-ICP MS).

All these tools are intended to be used by the occupational physicians in order to ensure biological monitoring of employees working on engineered nanoparticles, as a complement to exposure traceability.

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