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**Elemental fingerprint of human amniotic fluids and relationship with potential sources  
of maternal exposure**

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**Short title**

*Elemental fingerprint of human amniotic fluids*

## **Abstract**

*Background* - The impact of nanoparticles we are increasingly exposed to remains largely unknown. Of particular concern is the exposure of pregnant women and potential impact on fetal development. Indeed, many *in vitro* and *in vivo* animal studies have shown that nanoparticles are able to cross the placental barrier and induce toxic effects to the fetus. However, little is known in humans. *Objective* - The aim and originality of this study were to investigate the nanoparticle burden of amniotic fluids in pregnant women. *Methods* - To that purpose, 100 amniotic fluids collected for clinical purposes were used to determine the nanoparticle quantity and nature by inductively coupled plasma atomic emission spectroscopy (NAMIOTIC, ClinicalTrials.gov Identifier: NCT02720887). *Results* - The prevalence of patients with a substantial concentration for the essential trace elements Cu, Fe and Zn was high, while that of patients with a substantial concentration of Al, Ag, Be, Co, Cr, Ni, Si, Ti and W was relatively low (under 20%). It was generally higher in the fraction containing nanoparticles and ions than in the fraction containing micro- and submicroparticles. No correlation was found between the nanoparticle burden and the different potential sources of exposure to nanoparticles (smoking status of the patient, living area, heating source, mode of transport, leisure, use of hygiene products and cosmetics and occupational activities). *Conclusion* - Our results showing low concentrations and low prevalence of most of the assessed elements in amniotic fluids are reassuring. Further research is needed to draw firm conclusions on the developmental toxicity of engineered nanoparticles in humans but the present paper can provide a useful basis for further evaluation of the fetal toxicity of nanoparticles.

## **Key-words**

Nanoparticles; amniotic fluids; mineralogical analyses; developmental toxicity.

## Introduction

In addition to environmental ultrafine particles (such as dust from fires, volcano eruptions, sand, or particles from industrial or car pollution) we are increasingly exposed to engineered nanoparticles. These latter can be found in a wide range of daily products: cosmetics, toothpaste, sunscreen, for automobile, informatics or health applications, etc. [1–4]. However, their impact on the environment and human health is not fully elucidated yet, raising potential concerns. A better understanding of the interactions between nanoparticles and the human body and their biological consequences requires careful attention. A way to tackle with this issue could be by performing mineralogical analyses of biological tissues or fluids [5]. This kind of analyses consisting of monitoring nanoparticles in human biological samples can bring very informative data. It was for instance widely used in clinical practice to identify the causal link between exposure to asbestosis and pulmonary diseases [6]. Indeed, to investigate potential relationships between an exposure to inhaled manufactured nanoparticles and biological effects (development of a disease for instance), it is crucial to quantify the internal dose of nanoparticles in a tissue and not only an external dose measured by atmospheric metrology or surface sampling. Indeed, following an exposure to nanoparticles we can distinguish the external dose from the internal dose that is also different from the biologically active dose able to induce biological effects [7]. The assessment of the internal dose is a first step toward the characterization of persistent nanoparticles in tissues and the understanding of this potential source of adverse effects. With methodologies we specifically developed for that purpose, we have previously applied successfully this approach to the analysis of the nanoparticle burden in broncho-alveolar lavages [8–10].

Similarly, we propose to use this approach to better understand if maternal exposure to nanoparticles during pregnancy may have consequences on fetal development. Indeed, *in vivo* animal studies have shown that inhaled nanoparticles can translocate from the respiratory tract

to the placenta and fetus, potentially affecting it [11]. The placenta is a key organ in fetal growth as it controls maternal-to-fetal exchanges [12]. Multiple *in vivo* and *ex vivo* studies have demonstrated that nanoparticles are able to cross the placental barrier [13–22]. This trans-placental passage is highly dependent on the physico-chemical features of the nanoparticles such as their size, chemical nature and surface [14,16,17,20]. A recent study has shown that inhaled silver nanoparticles could cross the mouse placenta and were able to induce adverse effects such as an increased number of resorbed fetuses associated with reduced estrogen plasma levels and an increase of inflammatory cytokines [23]. These results suggest that precaution should be taken, especially regarding pregnant women, toward exposure to silver nanoparticles which are widely used in consumer products or biomedical applications. Recently, another study has investigated the impact of titanium dioxide nanoparticles on amniotic fluid-derived cells [24]. It resulted in disrupted cell adhesion, decreased proliferation, increased mortality rates and reduced mitotic index. Once again these results argue for the fact that these nanoparticles, largely included in cosmetics and hygiene products, should be used with care, especially for pregnant women.

All these data come from *in vitro*, *ex vivo* or *in vivo* animal experiments but to the best of our knowledge, previous work has not described the trans-placental passage of nanoparticles in human. To assess if indeed nanoparticles could cross the placental barrier we investigated their presence in the amniotic fluid of pregnant women. We thus performed mineralogical analyses of 100 samples from patients who underwent an amniocentesis for medical reasons. Samples were pre-treated to isolate the nanoparticle fraction which was then analyzed by inductively coupled plasma atomic emission spectroscopy. Finally, correlations with potential exposure to nanoparticles (occupational activities, smoking status, living area, type of transport, heating, use of hygiene products, leisure, etc.) were also investigated.

## **Materials and methods**

### *Patients*

During 2 years, 100 patients from the Department of Gynecology and Obstetrics from the University Hospital of Saint-Etienne were included in a prospective, single-center and observational study (NAMIOTIC, ClinicalTrials.gov Identifier: NCT02720887). The patients were in need of an amniocentesis for medical reasons (serum marker of the first quarter with an increased risk of trisomy 21 higher than 1/250, amniodrainage, sonographic sign of call). All patients were informed about this study and gave their written consent to participate. The protocol was in accordance with ethical principles defined by the World Medical Association declaration of Helsinki and subsequent amendments and was approved by an ethics committee (Comité de Protection des Personnes, Sud-Est I) as well as by the French agency regulating biomedical research (Agence Nationale de Sécurité du Médicament et des produits de santé, ANSM).

Criteria for the inclusion in the study were: i) patients in need of an amniocentesis for medical reasons other than the present study, ii) patients older than 18, iii) patients who had given their voluntary, informed and written consent, iv) patients having a social insurance or beneficiary (mandatory for any French clinical study). Exclusion criteria were: i) patients who had not given their consent, ii) when removal of amniotic fluid was not possible, iii) patients under 18.

All patients also replied to a questionnaire on their potential exposure to nanoparticles, their occupational activities, their life habits (smoking, drinking, leisure, place of residence, transports, use of cosmetics, etc.). Details on the cohort are reported in Tables 1 and 2.

### *Sample analysis*

Amniotic fluids were collected by following regular practice in the Gynecology and Obstetrics Department of the University Hospital of Saint-Etienne. 10 mL of sample were dedicated to the present study and stored at 4°C until use.

Particles were extracted based on a size-fractionation protocol consisting of a centrifugation on a glycerol cushion, for more details please see references [8,10]. Briefly, to extract the fraction containing the nanoparticles, samples were vortexed, 1 mL was taken and added with 0.5 mL dispersion buffer consisting of 50 mM Tris-base, 0.1% w/v sodium dodecyl sulfate, 150 mM NaCl, 0.5% w/v Na-deoxycholate, 5M urea, 2M thiourea and 0.8% v/v Triton X-100. After a 20 min incubation at 37°C, 0.5 mL of glycerol at 75% was added to each tube. Samples were then centrifuged at 2500g for 3.5h at 4°C. The supernatant containing the nanoparticles (*i.e.* particles <100 nm) and ions was transferred into a new tube and stored at 4°C until analysis. The pellet (250 µL) containing the micron and submicron-sized particles (*i.e.* particles which size ranges from 100 nm to several µm) was suspended in 750 µL of distilled water and stored at 4°C until analysis.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES, Jobin-Yvon JY138 Ultrace) was used to determine the concentration of the following metals in the two fractions (so-called micro/submicro and nano/ion fractions) of each sample: aluminum (Al), silver (Ag), beryllium (Be), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), silicon (Si), titanium (Ti), tungsten (W), and zinc (Zn). 500µL of sample (either from the micro/submicro fraction or from the nano/ion fraction) were added with 9.5 mL PBS and was analyzed by ICP-AES. To assess the background noise and potential matrix effects, “blank” samples consisting of 250 µL of glycerol (*i.e.* the medium in which the nanoparticles were extracted) + 750 µL of distilled water were analyzed.

### *Statistical analysis*

The ICP-AES analysis allowed quantifying Al, Ag, Be, Co, Cr, Cu, Fe, Ni, Si, Ti, W, and Zn in parts-per-billion (ppb), *i.e.*, as  $\mu\text{g/L}$ . Means, standard deviations (SD) and medians were calculated taking into account only concentrations higher than the LoB. Minimal (min) and maximal (max) values were also reported. For each element, the limit of blank (LoB) was calculated based on literature reference [25] as follows:  $\text{LoB} = \text{mean}_{\text{blank}} + 1.645 (\text{SD}_{\text{blank}})$ . Values lower than the LoB were considered as not detectable. We then assessed the number of patients for which the value was higher than the LoB, defining the prevalence.

### *Complementary analyses*

To confirm the size and the chemical nature of the particles present in the two fractions of amniotic fluids, some samples were analyzed using scanning electron microscopy coupled to energy dispersive X-ray (EDX) spectrometry. To that purpose, specimen holders were coated with carbon tape and sample droplets ( $\sim 4\text{-}6 \mu\text{L}$ ) were deposited and left to dry at  $200^\circ\text{C}$  for about 12 min protected from dust. Images were obtained using the angular selective back-scatter detector operated between 10 and 20 keV and the in-lens secondary electron detector operated at 1 keV, at a working distance of  $\sim 4 \text{ mm}$  (Zeiss SUPRA<sup>TM</sup>55VP equipped with a Gemini column and an EDX Oxford X-Max<sup>N</sup>80 detector). Acquisition of EDX spectra were performed for the qualitative analysis of the particles.

## **Results**

### *Description of the cohort*

The cohort consisted of 100 patients whose clinical data are reported in Table 1. The mean age was 33.4 years. Most of the patients have already had a child. 14 patients (14.7%) smoked with a mean of 5.8 cigarettes per day. None of the patients drank alcohol. The majority of the patients (66%) have not taken medicine during their pregnancy and the most frequent treatments were



simple analgesics or antibiotics. The most common obstetrical antecedent was spontaneous miscarriage occurring before 14 weeks (20.4%, this rate is similar to that of the general population). The amniocentesis was mainly performed between 15 and 17 weeks (67.4%) because of the indication which was mainly high maternal serum markers (52%).

Regarding the potential sources of exposure to nanoparticles, as reported in Table 2, we found that patients mainly lived in an urban area (44.4%). Patients mostly used diesel cars (62%). The population was quite homogeneous regarding their use of hygiene and cosmetic products. The main heating source was found to be gas heating (35.1%). The most frequent occupational activity was office work/secretariat (28%).

### ***Particle burden in amniotic fluids***

Table 3 reports the results from the ICP-AES analysis. For each element the limit of blank (LoB) was calculated as detailed in the Methods section. The LoB corresponds to the smallest concentration of a mesurand that can be reliably measured by an analytical procedure [25]. Therefore, values lower than LoB were considered as not detectable. We then determined the prevalence of patients exhibiting a positive signal (*i.e.* the number of patients with an element concentration higher than the LoB).

We first observed that except for Ni and Co, the prevalence was higher in the nano/ion fraction than in the micro/submicro fraction. In the nano/ion fraction, the highest prevalences were observed for Cu, Fe and Zn (100%, 100% and 32% respectively), which are essential trace elements. Medium prevalences (ranging between 10 and 20%) were reported for Ni, Al and Cr, while low prevalence (<10%) were recorded for Ag, Be, Co, Si, Ti, and W.

Regarding the micro/submicro fraction, the highest prevalence was observed for Ni (12%), followed by Fe (8%). The prevalence of the other assessed elements ranged between 1 and 2%. Cr, Si and W were not detected at all.

In terms of mean concentration, it ranged between 2 and 4590 ppb, *i.e.*  $\mu\text{g/L}$  for Be and Si respectively in the nano/ion fraction. In the micro/submicro fraction it ranged from 1 to 16940 ppb for Be and Ag respectively.

The presence and nature of nanoparticles in the amniotic fluids were confirmed using scanning electron microscopy and EDX analysis as illustrated by Figure 1 for the nano/ion fraction.

## **Discussion**

The aim and the originality of this paper were to investigate the nanoparticle burden of amniotic fluids in pregnant women. A limitation of this study however is that we are not able to discriminate nanoparticles from ions. Thus, a detected quantity of chemical species in the nano/ion fraction does not necessarily mean the presence of nanoparticles. This comment is particularly relevant when we consider the 3 elements for which the prevalence in the nano/ion fraction is high (Fe, Cu and Zn). This observation is quite logical in view of the function of these trace elements which are indeed critical to the good functioning of the body. It is thus reasonable to assume that a major part of these elements are present under an ionic form. This assumption was confirmed by SEM observations. In addition, this assumption is consistent when we compare the concentrations found for these elements in amniotic fluids reported in the literature to our data, as detailed below.

Although we particularly focused our analysis on nanoparticles, we first observed that our data were usually consistent with those available from the literature, especially when we considered the micro/submicro fraction. Indeed, we found a mean Fe concentration of 434 ppb while it ranged from 466 to 536 ppb in different studies [26–28]. Similarly, Al concentration in amniotic fluids is reported to be between 131 and 159 ppb in the literature [26,28–30], while we found a concentration of 141 ppb. For Cu [26,28,30], Ni [28,30] and Zn [26,28,30,31], although we did not find exactly the same concentration as in the literature, they were in the same order of

magnitude. Regarding Co, while it is reported that its concentration in amniotic fluid is about 0.2-0.5 ppb [26,28–31] we found a patient with a concentration of 318 ppb. Obviously, this result should be taken with care as it is only from one patient but it is consistent with data from Jalali and Koski [27] who found a Co concentration in amniotic fluid ranging from 26 to 366 ppb.

Similarly, although we found no Cr in our samples, this finding could be in agreement with literature data where it was reported to be very low, between 2 and 3 ppb [26–30]. Interestingly, while no Cr was detected in the micro/submicro fraction, 13% of the patients had a concentration higher than the limit of blank in the nano/ion fraction. This results appears as quite logical in view of the Cr quite high solubility.

To the best of our knowledge, no data was available for Be, Si, Ti and W concentrations for comparison.

Taken together, our results are consistent and it should be kept in mind that an inter-individual variability could be explained to some extent by the fact that element concentrations may vary during pregnancy [29,32] and the gestational age is not the same for all the patients from this cohort.

We also observed that some samples had a red to brown color, suggesting a potential contamination by blood. We therefore investigated if there was any correlation between hemorrhagic samples and iron concentration. Indeed, iron contained in blood could be brought by a contamination during amniocentesis from the placenta or maternal blood. In this case a high iron content of the amniotic fluid would rather be due to a technical defect. Out of the 8 samples exhibiting an iron concentration higher than the LoB in the micro/submicro fraction, only 3 were hemorrhagic, suggesting that in the 5 other samples the presence of iron was not due to an artifact. Regarding the 3 hemorrhagic samples, 1 is a particular case: the amniocentesis was performed after 27 weeks because of an important *in utero* growth

restriction, oligohydramnios and brain and heart defects. The patient has thus undergone a therapeutic abortion with a fetus autopsy that revealed metabolic abnormalities (pyruvate dehydrogenase deficiency or mitochondrial disease which usually lead to an accumulation of toxics within fetal tissues).

As a high Ni concentration could be related to tobacco or to an environmental exposure, we investigated potential correlation with the smoking status of the patient as well as with the living area, the heating source, the transport, leisure, use of hygiene products and cosmetics and the *curriculum laboris*. No correlation was found (Supplementary information). Similarly, no correlation was found between the other elements assessed here and the different potential sources of exposure to nanoparticles (Supplementary information).

In summary, the main finding of this paper is that the prevalence of patients with a concentration higher than the LoB is generally higher in the nano/ion fraction than in the micro/submicro fraction. In the nano/ion fraction, the essential trace elements Cu, Fe and Zn exhibited a high prevalence while that of other elements was relatively low (under 20%).

In conclusion, many *in vivo* animal studies reported in the literature have shown the fetotoxicity of nanoparticles after maternal pulmonary exposure, especially in rodents [11,23,33,34]. Nanoparticles can cross the placental barrier and this translocation can be associated with adverse effects such as an impairment of normal growth and development of the fetuses [34]. This raises concerns for public health and care is recommended regarding potential exposure to nanoparticles for pregnant women. In this context, our results showing low concentrations and low prevalence of most of the assessed elements in the nano/ion fraction of amniotic fluids are reassuring. Further research is needed to draw firm conclusions on the developmental toxicity of engineered nanoparticles in humans but the present paper can provide a useful basis for further evaluation of the fetal toxicity of nanoparticles.

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## **Declarations of interest**

None.

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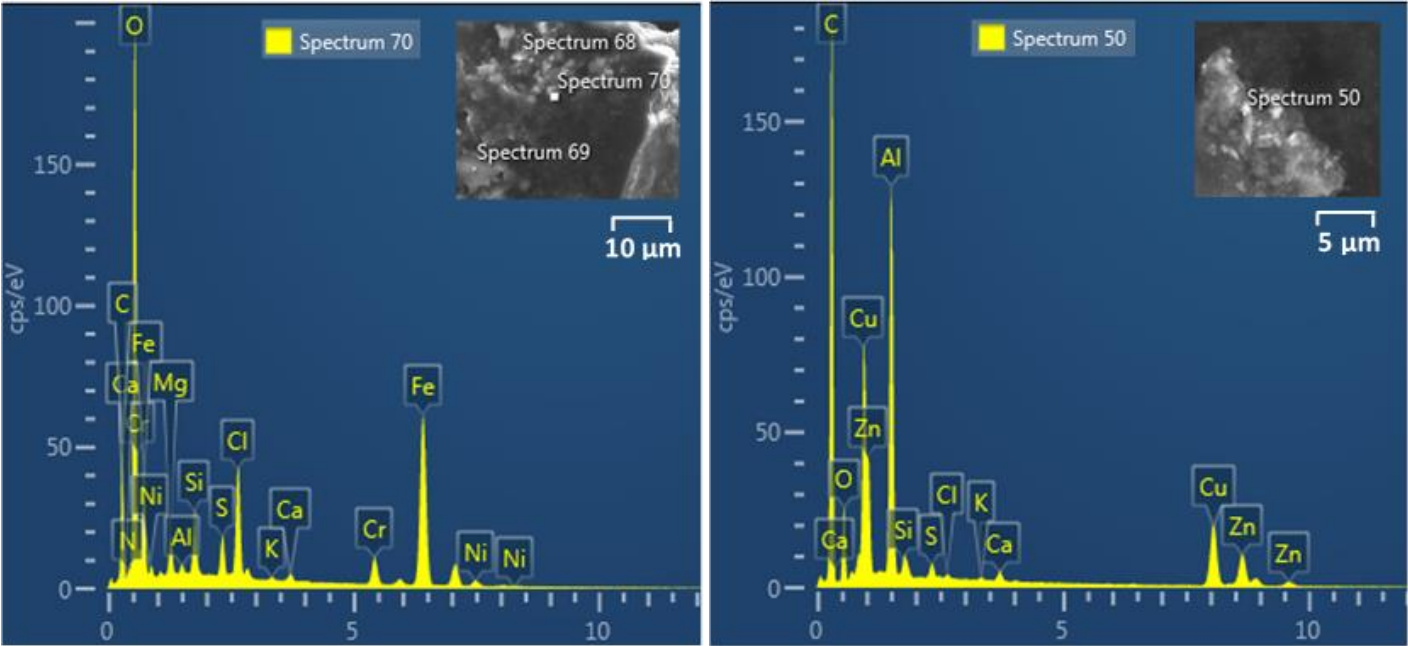
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## **Figure captions**

**Figure 1** – Observation of nanoparticles in the nano/ion fraction of amniotic fluids by scanning electron microscopy and associated EDX spectra.

Figure 1



**Table 1** - Population characteristics in terms of demographic data, medical history and data about the amniocentesis. Please note that although the initial number of patients in our cohort is 100, some data were missing or unanswered in the questionnaire, thus, for each characteristic the number of patients with solid data is indicated (n).

| <b>Demographic data</b>  |                 |               |
|--|-----------------|---------------|
| Age (years): mean $\pm$ SD; [min – max]; n=100                         | 33.4 $\pm$ 6.1  | [18 - 44]     |
| Body mass index (kg/m <sup>2</sup> ): mean $\pm$ SD; [min – max]; n=93 | 25.5 $\pm$ 5.9  | [17.2 - 49.5] |
| Gravidity: mean $\pm$ SD; [min – max]; n=94                            | 2.4 $\pm$ 1.4   | [1 - 7]       |
| 1: number; %   | 29              | 30.9          |
| 2  | 30              | 31.9          |
| 3  | 16              | 17            |
| > 4  | 19              | 20.2          |
| Parity: mean $\pm$ SD; [min – max]; n=94                               | 1.02 $\pm$ 1.05 | [0 - 4]       |
| 0: number; %   | 37              | 39.4          |
| 1  | 29              | 30.9          |
| 2  | 19              | 20.2          |
| > 3  | 9               | 9.5           |
| Smoker: number; %; n=95  | 14              | 14.7          |
| Alcohol consumption: number; %; n=95                                   | 0               | 0             |
| Medication intake: number ; %; n=100                                   | 34              | 34            |
| <b>Medical history</b>   |                 |               |
| Miscarriage before 14 weeks: number; %; n=93                           | 19              | 20.4          |
| Fetal death: number; %; n=94   | 2               | 2.1           |
| Fetal malformation: number; %; n=94                                    | 6               | 6.4           |
| Premature delivery: number; %; n=94                                    | 7               | 7.4           |
| Miscarriage after 14 weeks: number; %; n=94                            | 5               | 5.3           |
| Therapeutic abortion: number; %; n=94                                  | 5               | 5.3           |
| <b>Amniocentesis</b>   |                 |               |
| Gestational age (weeks): mean $\pm$ SD; [min – max]; n=98              | 18.8 $\pm$ 4.8  | [15-35]       |
| 15-17: number; %   | 66              | 67.4          |
| 18-22  | 13              | 13.3          |
| 23-32  | 17              | 17.3          |
| > 32   | 2               | 2             |
| Indication: number; %; n=98  |                 |               |
| Abnormal maternal serum markers  | 51              | 52            |
| Increased nuchal translucency  | 9               | 9.2           |
| Ultrasonographic signs   | 22              | 22.5          |
| Genetic abnormality history  | 12              | 12.2          |
| Amniodrainage  | 3               | 3.1           |
| Seroconversion   | 1               | 1             |

**Table 2** - Data relative to the potential exposure to nanoparticles. Please note that some patients had several leisure and heating source, thus total % is higher than 100%. Please also note that as for Table 1, because of missing data, the number of patients with full data (n) is indicated for each topic.

| <b>Environment</b>                          |      |      |
|---|------|------|
| Living area: number; %; n=99                |      |      |
| Urban                                       | 44   | 44.4 |
| Suburban                                    | 20   | 20.2 |
| Rural                                       | 35   | 35.4 |
| Leisure: number; %; n=100                   |      |      |
| None  | 63   | 63   |
| Gardening                                   | 20   | 20   |
| Handiwork                                   | 13   | 13   |
| Indoor sport                                | 3    | 3    |
| Outdoor sport                               | 18   | 18   |
| Transport: number; %; n=100                 |      |      |
| No car                                      | 10   | 10   |
| Diesel                                      | 62   | 62   |
| Petrol                                      | 21   | 21   |
| Both  | 5    | 5    |
| Other                                       | 2    | 2    |
| Heating: number; %; n=97                    |      |      |
| Electric                                    | 32   | 33   |
| Gas   | 34   | 35.1 |
| Wood  | 29   | 29.9 |
| Collective                                  | 14   | 14.4 |
| Oil-fired                                   | 10   | 10.3 |
| <b>Hygiene and cosmetic products; n=100</b> |      |      |
| Soap/shower gel (mean number/week)          | 7.1  |      |
| Shampoo (mean number/week)                  | 3.2  |      |
| Deodorant (mean number/week)                | 6    |      |
| Toothpaste (mean number/week)               | 12.6 |      |
| Make-up (mean number/week)                  | 4.3  |      |
| Cream (mean number/week)                    | 5.3  |      |
| Lotion (mean number/week)                   | 2.2  |      |
| Serum (mean number/week)                    | 0.6  |      |
| Hair care (mean number/month)               | 2.6  |      |
| Hair coloration (mean number/year)          | 3.6  |      |
| Fragrance (mean number/month)               | 11.3 |      |
| Sunscreen (mean number/month)               | 1.6  |      |
| <b>Occupational activity; n=93</b>          |      |      |
| Office work, secretariat: number; %         | 26   | 28   |
| Health: number; %                           | 18   | 19.3 |
| In contact with children: number; %         | 13   | 14   |

|  |    |      |
|--|----|------|
| In contact with chemicals: number; %     | 6  | 6.5  |
| In contact with food products: number; % | 7  | 7.5  |
| In contact with animals: number; %       | 1  | 1.1  |
| Other: number; %                         | 11 | 11.8 |
| Unemployed: number; %                    | 11 | 11.8 |

**Table 3** - Concentration of 12 elements in the nano/ion and micro/submicro fractions of amniotic fluids from 100 patients. LoB: limit of blank, SD: standard deviation.

|   | Ag        |                 | Al        |                 | Be        |                 | Co        |                 | Cr        |                 | Cu        |                 |
|---|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|
|   | Nano /ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion | Micro/ submicro |
| Number of patients with a concentration > LoB | 3         | 1               | 15        | 1               | 8         | 1               | 1         | 1               | 13        | 0               | 100       | 2               |
| Mean concentration (ppb) ± SD                 | 5 ± 2     | 16940           | 48 ± 17   | 141             | 2 ± 1     | 1               | 19        | 318             | 174 ± 6   | -               | 63 ± 34   | 48 ± 27         |
| Median  | 4         | 16940           | 40        | 141             | 2         | 1               | 19        | 318             | 173       | -               | 55        | 48              |
| Min   | 4         | 16940           | 36        | 141             | 2         | 1               | 19        | 318             | 164       | -               | 32        | 29              |
| Max   | 7         | 16940           | 92        | 141             | 3         | 1               | 19        | 318             | 182       | -               | 328       | 68              |

|   | Fe        |                 | Ni        |                 | Si        |                 | Ti        |                 | W           |                 | Zn        |                 |
|---|-----------|-----------------|-----------|-----------------|-----------|-----------------|-----------|-----------------|-------------|-----------------|-----------|-----------------|
|   | Nano /ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion | Micro/ submicro | Nano/ ion   | Micro/ submicro | Nano/ ion | Micro/ submicro |
| Number of patients with a concentration > LoB | 100       | 8               | 11        | 12              | 1         | 0               | 3         | 1               | 4           | 0               | 32        | 1               |
| Mean concentration (ppb) ± SD                 | 247 ± 509 | 434 ± 541       | 23 ± 9    | 9 ± 6           | 4590      | -               | 12 ± 2    | 15              | 3848 ± 4521 | -               | 69 ± 144  | 308             |
| Median  | 132       | 240             | 20        | 7               | 4590      | -               | 11        | 15              | 1783        | -               | 19        | 308             |
| Min   | 30        | 185             | 17        | 3               | 4590      | -               | 10        | 15              | 1226        | -               | 1         | 308             |
| Max   | 4580      | 1768            | 49        | 21              | 4590      | -               | 15        | 15              | 10600       | -               | 754       | 308             |



### Supplementary information

Investigation of potential correlations between the nanoparticle burden in amniotic fluids and the potential sources of exposure to nanoparticles (obtained through a questionnaire completed by the patients).

**Table S1** – Pearson correlation coefficients calculated between the concentration for each element assessed in the nano/ion fraction of amniotic fluid and the potential sources of exposure to nanoparticles.

|           | <b>Living area</b> | <b>Leisure</b> | <b>Transport</b> | <b>Heating</b> | <b>Soap</b> | <b>Shower gel</b> | <b>Shampoo</b> | <b>Deodorant</b> | <b>Toothpaste</b> |
|-----------|--------------------|----------------|------------------|----------------|-------------|-------------------|----------------|------------------|-------------------|
| <b>Ag</b> | -0.175             | -0.042         | -0.035           | -0.112         | 0.053       | 0.060             | 0.073          | 0.118            | 0.196             |
| <b>Al</b> | 0.037              | -0.099         | -0.184           | -0.040         | -0.040      | 0.012             | -0.119         | -0.119           | -0.201            |
| <b>Be</b> | -0.195             | -0.067         | 0.117            | -0.085         | 0.174       | -0.026            | -0.043         | -0.090           | 0.203             |
| <b>Co</b> | 0.010              | 0.064          | 0.149            | 0.156          | -0.061      | 0.170             | -0.011         | 0.030            | 0.092             |
| <b>Cr</b> | 0.040              | -0.104         | 0.025            | -0.108         | -0.056      | 0.065             | 0.010          | 0.057            | 0.004             |
| <b>Cu</b> | -0.059             | -0.086         | -0.069           | -0.154         | 0.011       | 0.044             | -0.058         | -0.016           | -0.061            |
| <b>Fe</b> | -0.042             | -0.092         | -0.031           | -0.137         | -0.035      | -0.034            | -0.084         | -0.009           | -0.083            |
| <b>Ni</b> | -0.107             | -0.078         | 0.018            | 0.033          | 0.120       | 0.152             | 0.006          | -0.018           | 0.211             |
| <b>Si</b> | -0.103             | -0.064         | -0.201           | 0.045          | -0.003      | -0.117            | -0.117         | -0.175           | -0.214            |
| <b>Ti</b> | -0.018             | 0.175          | -0.005           | 0.033          | 0.077       | 0.005             | 0.058          | -0.011           | -0.101            |
| <b>W</b>  | -0.123             | 0.001          | -0.131           | -0.086         | 0.198       | 0.067             | 0.206          | 0.060            | 0.292             |
| <b>Zn</b> | -0.066             | -0.105         | -0.009           | -0.143         | 0.036       | 0.034             | -0.016         | 0.064            | 0.001             |

|           | <b>Make-up</b> | <b>Cream</b> | <b>Lotion</b> | <b>Serum</b> | <b>Hair care</b> | <b>Hair coloration</b> | <b>Fragrance</b> | <b>Sunscreen</b> | <b>Occupational activity</b> |
|-----------|----------------|--------------|---------------|--------------|------------------|------------------------|------------------|------------------|------------------------------|
| <b>Ag</b> | 0.158          | -0.009       | 0.050         | -0.042       | -0.008           | -0.040                 | -0.003           | -0.058           | 0.101                        |
| <b>Al</b> | -0.036         | -0.015       | -0.015        | -0.003       | 0.031            | 0.047                  | -0.154           | -0.030           | 0.183                        |
| <b>Be</b> | -0.036         | 0.066        | -0.005        | -0.071       | 0.066            | -0.063                 | -0.116           | 0.056            | 0.007                        |
| <b>Co</b> | -0.079         | -0.143       | -0.063        | 0.278        | 0.034            | -0.074                 | 0.134            | -0.042           | -0.066                       |
| <b>Cr</b> | 0.056          | 0.020        | 0.018         | -0.004       | 0.023            | 0.052                  | -0.039           | -0.088           | 0.239                        |
| <b>Cu</b> | 0.038          | 0.056        | -0.022        | -0.042       | -0.161           | -0.011                 | -0.104           | -0.023           | 0.018                        |
| <b>Fe</b> | 0.105          | -0.016       | -0.079        | -0.053       | -0.083           | 0.073                  | -0.042           | -0.123           | 0.010                        |
| <b>Ni</b> | -0.005         | -0.029       | 0.044         | -0.082       | -0.081           | -0.084                 | -0.080           | -0.016           | -0.050                       |
| <b>Si</b> | -0.113         | 0.046        | -0.063        | -0.025       | 0.034            | -0.074                 | -0.081           | -0.042           | 0.126                        |
| <b>Ti</b> | -0.009         | -0.054       | 0.242         | 0.105        | 0.224            | 0.087                  | 0.010            | 0.062            | -0.152                       |
| <b>W</b>  | 0.033          | 0.041        | 0.108         | -0.035       | -0.002           | -0.045                 | 0.049            | -0.059           | 0.055                        |
| <b>Zn</b> | 0.123          | 0.094        | 0.033         | -0.054       | -0.060           | -0.037                 | -0.065           | -0.099           | 0.015                        |