

**ENVIRONMENT DIRECTORATE  
CHEMICALS COMMITTEE**

**Working Party on Manufactured Nanomaterials**

**GOLD NANOPARTICLE OCCUPATIONAL EXPOSURE ASSESSMENT IN A PILOT SCALE  
FACILITY**

**NANOMATERIALS EXPOSURE CASE STUDY**

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This document is part of ongoing efforts by the National Institute for Occupational Health (NIOH) to contribute to the responsible development of nanotechnology in the Republic of South Africa (RSA). This is in line with the Nanotechnology Health, Safety and Environment (HSE) Research Platform of the South African government Department of Science and Technology (DST).

Since its establishment at the 6<sup>th</sup> WPMN meeting [[ENV/CHEM/NANO\(2009\)24](#)], Steering Group 8 (SG8) has developed, among other things, case studies on exposure assessment for manufactured nanomaterials, focused mainly on the nanomaterials addressed by the OECD Sponsorship Programme as well as those for which information is available.

This report is on exposure assessment case study of nano-gold (AuNP), which has been conducted in a pilot-scale facility to perform an exposure assessment during the synthesis of AuNPs. The main objective of this project is to identify tasks that may result in emissions of AuNPs into the environment during their synthesis.

This report is an update of the earlier draft discussed at WPMN-13, where it was agreed that the document will be re-submitted for further comments prior to the request for declassification.

***ACTION REQUIRED:***                      ***The WPMN is invited to take note of the report and provide inputs as appropriate.***

## ABBREVIATIONS

|                  |   |
|------------------|---|
| APS              | Aerodynamic Particle Sizer                            |
| Au               | Gold  |
| AuNPs            | Gold Nanoparticles                                    |
| cm               | Centimetre  |
| CPC              | Condensation Particle Counter                         |
| DMA              | Differential Mobility Analyzer                        |
| DST              | Department of Science and Technology                  |
| EDS              | Energy Dispersive X-ray Spectrometer                  |
| HCl              | Hydrochloric acid                                     |
| HNO <sub>3</sub> | Nitric acid   |
| ICP-MS           | Inductively Coupled Plasma Mass Spectrometer          |
| IQC              | Internal Quality Control                              |
| LOD              | Limit of Detection                                    |
| MCE              | Membrane Cellulose Ester                              |
| NIOH             | National Institute for Occupational Health            |
| NIOSH            | National Institute for Occupational Health and Safety |
| nm               | Nanometre   |
| NRV              | Nano Reference Value                                  |
| OECD             | Organisation for Economic Cooperation and Development |
| OPC              | Optical Particle Counter                              |
| PNC              | Particle Number Concentration                         |
| RSA              | Republic of South Africa                              |
| SEM              | Scanning Electron Microscopy                          |
| SMPS             | Scanning Mobility Particle Sizer                      |
| TEM              | Transmission Electron Microscopy                      |
| TSI              | Trust Science Innovation                              |
| TWA              | Time Weighted Average                                 |
| UPC              | Ultrafine Particle Counter                            |

## GLOSSARY

**Nanomaterial** – material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale (International Standards Organisation 2008).

**Nanoparticle** – a nano-object with all three external dimensions in the nanoscale size range of approximately 1nm to 100nm (International Standards Organisation 2008).

**Nanoscale** – size range from approximately 1 nm to 100 nm

**National Institute for Occupational Health (NIOH)** – A division of the National Health Laboratory Service responsible for the promotion of good occupational health in South Africa

**Particle number concentration (PNC)** – concentration of all particles within a defined size range

**Peak particle exposure** – the highest particle number or mass recorded during the nanotechnology process.

**Pilot-scale facility** – Pilot plant scale-up facility for production of large volumes of AuNPs

**Process particle exposure (eight-hour TWA)** – particle number concentration or mass resulting from the nanotechnology operation multiplied by measurement time and divided by eight hours.

**Time-weighted average** - These are calculations allocating a measured exposure to the interval of time during which the exposure occurred. A worker may have an elevated exposure during one interval and a lower exposure in the next time interval.

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## Introduction

1. In the absence of conclusive proof about the toxicity of AuNPs, monitoring exposure to these particles during their synthesis is of paramount importance to their human health risk assessment. Among the conventional methods for synthesis of AuNPs the most widely used is the reduction of gold (III) derivatives (Daniel and Astruc 2004). The reduction process causes  $\text{Au}^{3+}$  to be reduced to neutral gold atoms which further become supersaturated and precipitated as more gold atoms aggregate to form sub-nanogold particles. The aim of this study was to perform an exposure assessment during the synthesis of eighty litres (80 L) of 14 nm AuNPs using the citrate reduction of chloroauric acid ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) in water. The study was conducted using a combination of particle number concentration counters and filter based air (personal and area) sampling. The main objective was to identify tasks that may result in emission of AuNPs into the environment during their synthesis.

2. The study was conducted at the pilot-scale facility an AuNP research and development laboratory. This laboratory produces a variety of nanomaterials including AuNP for use in various applications in health (diagnostics & therapeutics) and water (treatment & analysis).

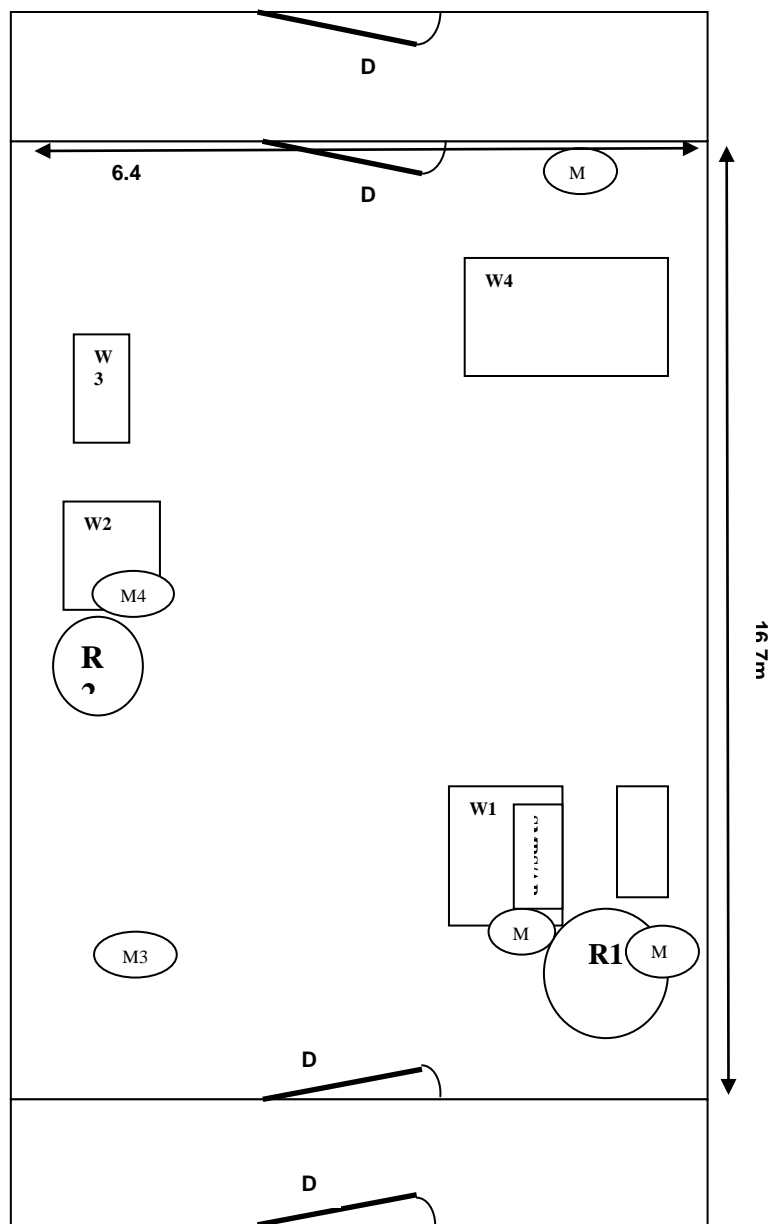
## Materials and Methods

3. The exposure assessment utilized in this survey followed a tiered approach as described by Methner et al (2010). The approach includes;

- An information gathering on the synthesis of AuNPs in the facility (tier 1);
- An assessment of AuNPs using scanning mobility sizer focusing on particle number concentration and size and personal and area air sampling (tiers 2 and 3)

## Monitoring location

4. The schematic layout of the pilot-scale facility for the synthesis of AuNPs is shown in Figure 1 below.



**Figure 1: Layout of pilot-scale facility for AuNP synthesis.**

5. W1 to W4 stand for the different work areas in the facility; R1 and R2 denote the two glass reactors in the facility (with R1 at 100 litres being the biggest); M1 to M5 represent the area monitoring positions (M1 – 30cm from R1, M2 – 40cm from R1, M3 – 4m from R1, M4 – 7.2m from R1 and M5 – 10m from R1), and D1 to D4 stand for the doors in the facility.

6. The temperature in the facility varied from 22 to 25°C and the relative humidity was around 50%. The doors to the synthesis facility, especially doors 2 and 3, were kept closed at all times. There were no other activities taking place inside the laboratory that could generate nanoparticles on the day of this study.

7. The AuNPs were synthesized in a 100 litre glass chemical reactor (R1 in Figure 1) that had previously been cleaned with an HCl/HNO<sub>3</sub> solution. The synthesis was carried out in a three phase process that included preparation, the actual synthesis and post-synthesis. The preparation phase included boiling of 72 litres of highly pure water (approximately 14 minutes). The synthesis phase consisted of three steps that involved addition of previously heated HAuCl<sub>4</sub>·3H<sub>2</sub>O to the boiling water, and bringing the whole solution to the boil (12 minutes), followed by addition of previously heated citrate solution, with the latter step done as fast as possible (1 minute). The colour progression went from an initial yellow to colourless to greyish to bluish and a final red colour (8 minutes). The post-synthesis phase which is the final phase consisted of continuous stirring of the solution for one and a half hours after switching off the heat once the desired colour in the previous phase has been obtained.

### Particle number concentration sampling

8. In this tier a number of complimentary portable and desktop particle counters were used for the real-time monitoring of temporal changes in particle number concentration (PNC) and size distribution (PND) of particles between 5nm and 20µm (see Table 1 and Figure 3, below). The portable particle counters used were the Hand-held Particle Counter (HHPC-6) from HACH Instruments and the Ultrafine Particle Counter (UPC or P-Trak) from TSI Inc., Shoreview, MN. The HHPC-6 measures PNC expressed as the total number of particles per litre of air (particles/L), in six channels: 300, 500, 1,000, 3,000, 5,000, and 10,000 nm, and has an upper limit of detection of 70,000 particles/L. The UPC measures particle number concentration in the size range 10 –1,000 nm, expressed as the total number of particles per cubic centimetre (particles/cm<sup>3</sup>) of sampled air, and has an upper limit of detection of 100,000 particles/cm<sup>3</sup>.

9. The desk top particle counters used were the Scanning Mobility Particle Counter and Aerodynamic Particle Sizer, both from TSI Inc., Shoreview, MN. The SMPS determines both PNC and PND in 167 channels in the size range 2.5 nm to 1,000 nm, has an upper limit of detection of 10<sup>7</sup> particles/cm<sup>3</sup>. The APS provides for both PNC and PND in 52 channels in the size range 300nm to 20µm and has an upper limit of detection of 10000 particles/cm<sup>3</sup>.

**Table 1: Real time measurement instruments used in the study**

| Name   | Metric                    | Range        |
|--|---------------------------|--------------|
| Scanning Mobility Particle Counter (TSI SMPS Model 3080) equipped with a long Differential Mobility Analyzer (TSI DMA Model 3081). | Number, Size distribution | 5 -1000nm    |
| Aerodynamic Particle Sizer (TSI APS Model 3321)  | Number, Size distribution | 500nm - 20µm |
| P-Trak Ultrafine Particle Counter (TSI UPC, Model 8525),   | Total number              | 10 – 1000nm  |
| HACH Met One Handheld Particle Counter (HHPC-6)  | Number, Size distribution | 300nm - 5µm  |



10. The desktop particle counters, SMPS and APS, were placed as close as possible to the reactor (about 30 to 40cm away from the main reactor opening), see Figure 2, below.



**Figure 2: Chemical reactor with real –time measurement instruments, SMPS and APS, with occupational hygiene pumps on top.**

11. The SMPS was run at an aerosol flow rate of 1L/min and sheath flow rate of 10L/min, scanning a size range between 7.5nm to 283.9nm, with a 2 minute time resolution.

12. Estimation of ultrafine particle (< 300nm) number concentration from the CPC was done following Peters et al., (2009) using the formula below;

$$N = N_{CPC} - [N_{OPC}(300nm) + N_{OPC}(500nm) + N_{OPC}(700nm) + N_{OPC}(1000nm)]$$

### **Air sampling**

- **Personal sampling**

13. Sampling trains (see Figure 3 below), consisting of 25mm Membrane Cellulose Ester (MCE) filters (pore size 0.8µm) and standard volume pumps operated at 4L/min, were attached at breathing zones of three laboratory workers directly involved in the synthesis process (employee A, B and C), and one indirectly involved (employee D). Air monitoring was done for the duration of their tasks which lasted for 139 minutes. The harvesting of the AuNPs was done on the following day by employee D and both area and personal sampling was done using MCE filters. For area sampling a high volume pump run at 7L/min for 44 minutes was used, and for personal sampling a standard volume pump run at 4L/min for 44 minutes was used.



**Figure 3: A worker with personal sampling train attached.**

- **Area sampling**

14. Area samples were collected by placing open faced MCE filters (25mm diameter and 0.8 $\mu$ m pore size) as close as possible to the source and in other areas within the facility and drawing air using pumps at a flow rate of 4L/min.

15. Area and personal sampling was done in duplicate, one sample for metal analysis by ICP-MS and another for electron microscopy analysis (morphology and elemental analysis). A carbon coated TEM grid was attached to the centre of the filter for electron microscopy to collect particles directly onto the grid, as shown in Figure 4 below.



**Figure 4: Carbon coated TEM copper grid on a MCE filter**

## Nanoparticles characterisation

16. Characterisation of the particles collected on filters from personal and area sampling (including the cascade impactor) was done using Field-Emission Transmission Electron Microscopy (FE-TEM) and Field-Emission Scanning Electron Microscopy (FE-SEM).

- **Field-Emission Transmission Electron Microscopy (FE-TEM)**

17. Particles collected directly onto TEM grids were analysed using a JEOL JEM-2100F Field Emission Transmission Electron Microscope equipped with an Oxford INCA Energy TEM 250 Energy Dispersive X-ray Spectrometer (EDS) system.

- **Metal analysis by Inductive Coupled Plasma-Mass Spectroscopy (ICP-MS)**

18. The concentration of the gold particles in filters was analysed following the NIOSH 7303 method. Briefly, MCE filters were transferred to NUNC 15ml digestion tubes, 1.25ml HCl (Trace Select 37%) added, the tubes covered with caps and digested at 90°C for 30 minutes. After digestion, the samples were allowed to cool for 5 minutes, followed by the addition of 1.25ml HNO<sub>3</sub> (Trace Select Ultra 65%) and further digestion at 90°C for 30mins. After cooling 100 µl of 1ppm <sup>193</sup>Ir internal standard was added and the samples brought up 10ml final volume with 18.2MΩ/cm water.

19. The digested filter samples were analysed using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer with an Octopole Reaction System. The instrument was calibrated with aqueous calibration standards prepared with 18.2MΩ/cm water. Aliquots of each sample were analysed in triplicate. The detection limits (LOD) were 0.025 µg/L / 0.00025 µg/filter.

20. Two aqueous IQCs' and 2 spiked filter IQCs' were analysed for quality assurance and control at the beginning of the analysis, after every 10 samples and at the end of the analysis. The calibration and internal standards and the IQCs' were prepared from certified Au and Ir stock standard solutions. The results obtained were deemed accurate and precise based on the calibration, %RSD and from the results obtained for the aqueous IQCs' and spiked filter IQCs'.

21. The filters were weighed before and after sampling. The difference between the pre and post average weight of blank filters was used to correct for the total weight of the filter samples.

22. Estimation of gold concentration in the air was done by using the formula:

$$C \text{ (mg/m}^3\text{)} = \frac{C_1V_1 - C_2V_2}{V}$$

Where:

$C_1$ =Concentration of Gold per 1L sample

$V_1$ =Solution Volume of the sample

$C_2$ =Concentration of Gold in a laboratory blank

$V_2$ =Solution Volume of the blank

$V$ =Volume of air sampled

### **Review of gold nanoparticles synthesis process**

23. AuNPs are synthesized by the reduction of chloroauric acid ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) in aqueous media, using citrate as the used reducing agent. The reduction process causes  $\text{Au}^{3+}$  to be reduced to neutral gold atoms. The atoms become supersaturated and are precipitated as more gold atoms aggregate to form sub-nanogold particles. This process occurs at temperatures above 100 °C, and may result in the emission of nanoparticles as a result of evaporation. It was therefore assumed that exposure may occur if no control measures are in place to contain evaporation. As a result, a walk-through survey was carried out to investigate this possibility.

### **Walk-through survey**

24. A walk-through survey was done at the gold nanoparticle synthesis facility to; (i) determine the presence of emission control measures, (ii) assess nanoparticle emission (using hand held particle counters), and (iii) identify potentially exposed personnel.

25. Walk-through surveys were done at a research and development (R & D) laboratory and a pilot-scale facility. At the R & D laboratory, synthesis of 200 ml of AuNPs was carried in an extractor hood. Personal protective equipment (PPE) such as laboratory coats and hand gloves were used during the synthesis of AuNPs. For the assessment of nanoparticle emission; background particle number concentration was measured before the synthesis process and at the perimeters of process enclosures during operation of the process. The addition of citrate to the heated chloroauric acid solution was observed to result in the emission of nanoparticles compared to the other tasks.

26. Because the synthesis of large volumes of AuNP is carried out at a pilot scale-up facility, a walk through survey was also conducted in this facility. AuNP synthesis in this facility is carried in large glass reactors with volume capacities ranging from 50L to 100L. The facility has no temperature control or ventilation system.

### **Exposure assessment**

27. The exposure assessment was conducted in the pilot scale facility during the production of 80 litres AuNPs.

28. Mass concentration (gravimetric analysis) is not sufficient to measure exposure to nanomaterials as these materials have insignificant mass; consequently, particle number concentration and size distribution measurements are a recommended metric Methner et al (2010).

### **Particle-number concentration**

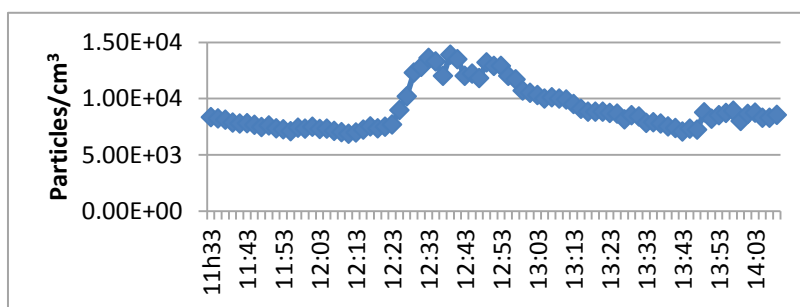
29. The emission of nanoparticles was measured before (background levels) and during the synthesis of AuNPs using a combination of real-time particle counters. Table 2, below summarises real-time particle number concentration results.

**Table 2: Nanoparticle emission during AuNP synthesis**

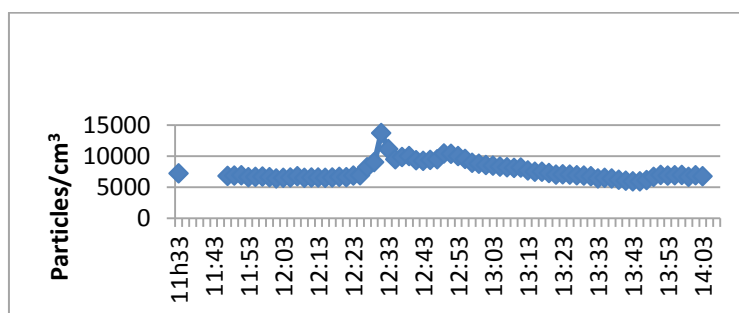
|                      | 7 – 290nm (SMPS) | 10 – < 300nm (P-Trak)* |
|----------------------|------------------|------------------------|
| Background           | 7966             | 6926                   |
| Preparation phase    | 7965             | 6982                   |
| Synthesis phase      | 7358             | 6460                   |
| Post-synthesis phase | 9469             | 7593                   |

\*  $N = N_{CPC} - [N_{OPC}(300nm) + N_{OPC}(500nm) + N_{OPC}(700nm) + N_{OPC}(1000nm)]$  Equation 1

30. The particle number concentration measured with the SMPS (i.e. particles from 7.5nm to 283.9nm) ranged from a background level of 7966 particles/cm<sup>3</sup> to a peak of 9469 particles/cm<sup>3</sup> during the post-synthesis phase. Similarly, the TSI P-Trak indicated an increase in the number of total particles, ranging from 10nm to 300nm (see Equation 1, above), from a background level of 6926 particles/cm<sup>3</sup> to a peak of 7593 particles/cm<sup>3</sup> during the post synthesis phase.



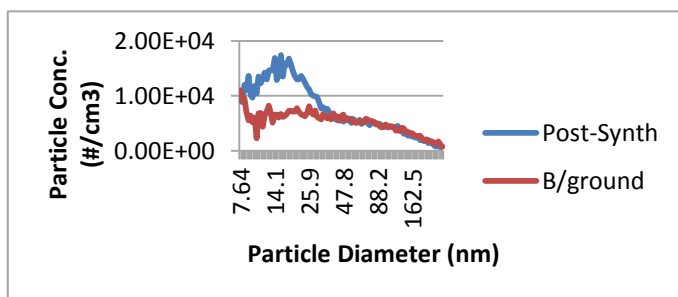
**Figure 5: Real-time particle number concentration measured by TSI-SMPS during AuNP synthesis**



**Figure 6: Real-time particle number concentration measured with TSI-UPC**

### Particle size distribution

31. The SMPS, operating in the range from 7.5nm to 283.9nm, showed an increase in the concentration of particles starting from 7.5nm to 35nm (average ~16nm) during the post-synthesis phase (at particle emission peak) compared to the background phase. This confirms that the peak concentration observed consisted of nano-sized particles, most probably emitted from the synthesis process.



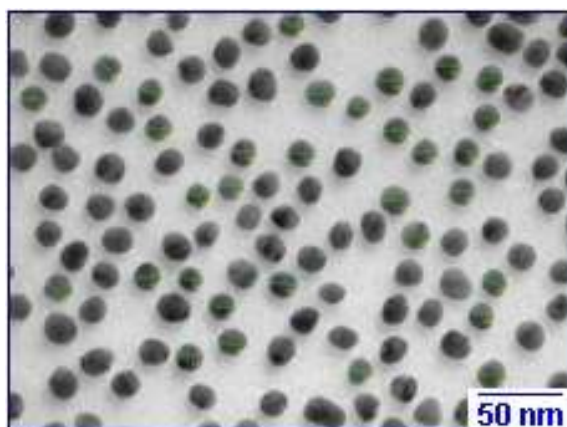
**Figure 7: Particle size distribution**

### Particle characterisation

32. One of the limitations of real-time particle counters is that they cannot distinguish between background (e.g. incidental) and engineered nanoparticles. As a result off-line methods, e.g. TEM, SEM and ICP were used to analyse particles collected on filters.

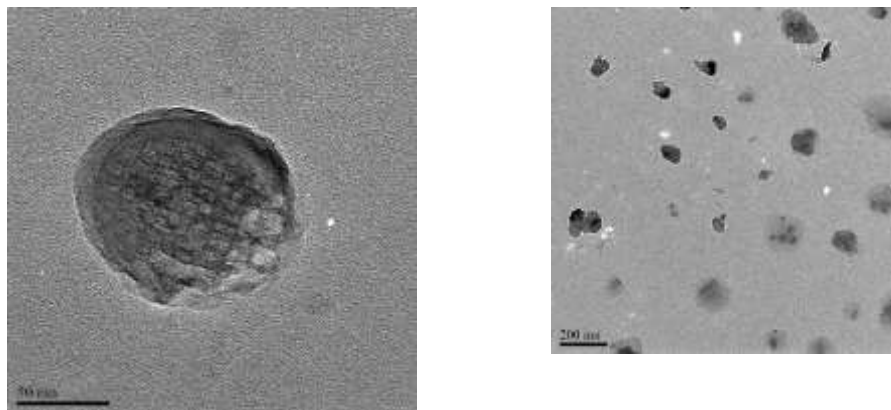
- **FE-TEM**

33. Figure below, shows TEM images of individual, spherical engineered gold nanoparticles.

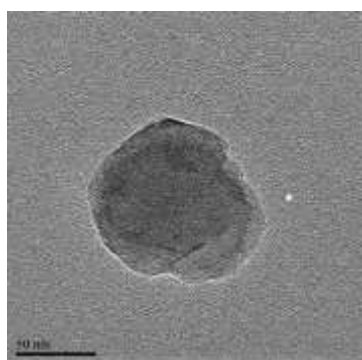


**Figure 8: TEM image of 14 nm gold nanoparticles**

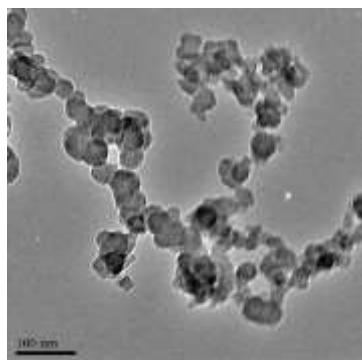
34. FE-TEM showed the presence of nanoparticles in the area samples collected near and up to about 7m from the synthesis reactor. The nanoparticles ranged from approximately 20nm to at least 500nm; with aggregated/agglomerated/coagulated particles ranging from 50nm to about 500nm (see Figures 9 to 12).



**Figure 9: TEM images of particles collected 30 cm from the source.**



**Figure 10: TEM images of particles collected 40 cm from the source.**



**Figure 11: TEM images of particles collected 4 m from the source.**

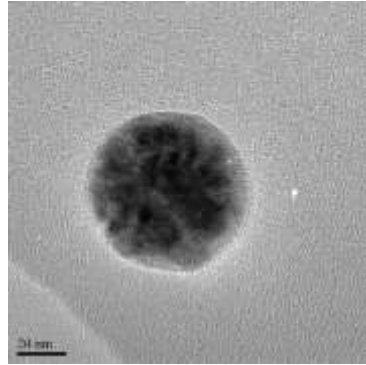


Figure 12: TEM images of particles collected 7.2 m from the source.

35. Elemental analysis by TEM-EDX showed that some of the nanoparticles were of gold origin.

- **FE-SEM**

36. FE-SEM analysis of filter-based samples showed a wide range of particles and spot analysis indicated the presence of gold particles as well as silicates. These results also indicate the presence of agglomerates/aggregates/coagulated particles.

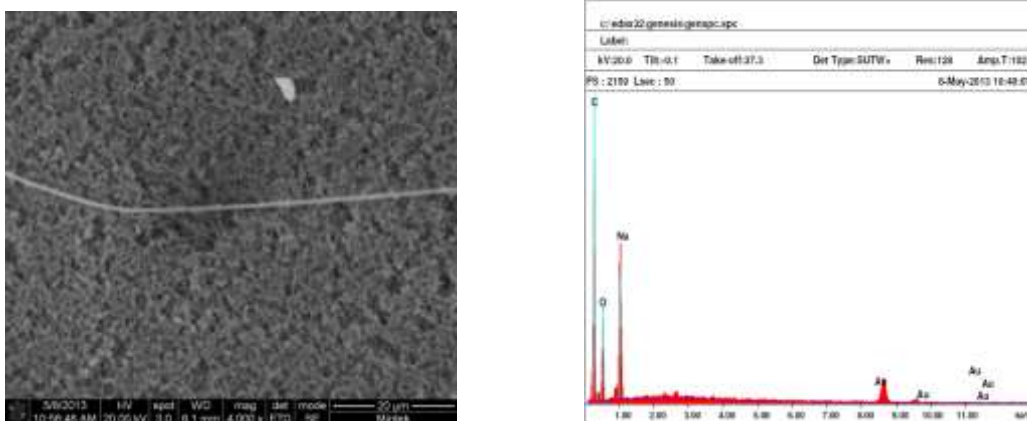


Figure 13: FE-SEM particle image from a personal filter sample (employee A)

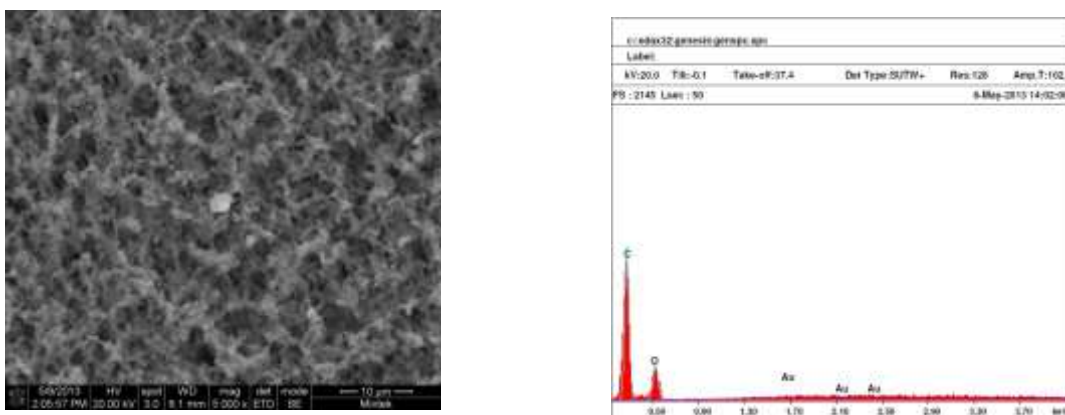


Figure 14: FE-SEM images of particles collected 30 cm from the source.



- **Metal analysis by ICP-MS**

37. The filter sampling, with the exception of the one done during a decanting process, was done throughout the whole shift and not task specific. Metal analysis by ICP-MS showed that all the filters analysed (personal or area) had gold indicating presence of gold in the sampled laboratory air. For the area samples the gold concentration ranged 0.015 to 0.15  $\mu\text{g}/\text{cm}^3$ , with the highest concentration measured 30 cm from the reactor opening.

38. Results for the four workers sampled also indicated exposure to gold nanoparticles, with measured gold concentrations ranging from 0.00028 to 0.015  $\mu\text{g}/\text{cm}^3$ , with the workers responsible for addition of citrate and supervising the synthesis process being the highly exposed at 0.1  $\mu\text{g}/\text{cm}^3$  (TWA – 0.012  $\mu\text{g}/\text{cm}^3$ ) and 0.12  $\mu\text{g}/\text{cm}^3$  (0.015  $\mu\text{g}/\text{cm}^3$ , respectively). The workers responsible for the addition of the chloroauric acid had the least exposure at 0.002  $\mu\text{g}/\text{cm}^3$  (TWA – 0.0002  $\mu\text{g}/\text{cm}^3$ ). The task of decanting the AuNP did not result in much exposure, 0.00007  $\text{mg}/\text{cm}^3$  (TWA – 0.000002  $\text{mg}/\text{cm}^3$ ).

**Table 3:  $\text{Au}^{3+}$  mass concentration of personal samples**

|            | Air concentration<br>( $\mu\text{g}/\text{m}^3$ ) | TWA<br>( $\mu\text{g}/\text{m}^3$ ) | Job description  |
|------------|---|-------------------------------------|--|
| Employee A | 0.00225   | 0.000281                            | Addition of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ |
| Employee B | 0.102468  | 0.0128                              | Addition of citrate and monitoring of colour changes   |
| Employee C | 0.127133  | 0.0158                              | Supervising synthesis process                          |
| Employee D | 0.0706  | 0.00235                             | Decanting of AuNP                                      |

39. Filter concentration of the field blanks were below the detection limit of the ICP, which 0.00025  $\mu\text{g}/\text{ml}$

**Table 4:  $\text{Au}^{3+}$  mass concentration of area samples during synthesis of AuNPs**

| Location              | Air concentration ( $\mu\text{g}/\text{m}^3$ ) |
|-----------------------|--|
| Pre-background        | 0.0609   |
| 30cm from the source  | 0.153  |
| 40cm from the source  | 0.150  |
| 4m from the source    | 0.0259   |
| 7.2cm from the source | 0.015  |
| 10m from the source   | 0.0911   |

## Comments and Conclusions

- **Real-time particle monitoring**

40. In this study a combination of real-time particle counting instruments were used, including Condensation Particle Counters (TSI-SMPS and TSI-Ultrafine Particle Counter) and Optical Particle Counters (HHPC-6 and TSI-APS). A combination of the TSI-UPC and HHPC-6 has previously been used and recommended to assess emission of nanoparticles from synthesis processes. As the former instrument measures total number concentration of particles in the range 10 to 1000nm and the latter measures particle number distribution of particles from 300nm to 20um, a high particle count in the TSI-UPC and a high particle count in the small size range (300nm – 500nm) in the HHPC-6 would indicate the presence of nano-ranged particles Methner et al (2010). In addition, HHPC-6 results in the size range 300nm to 1000nm were subtracted from the TSI-UPC results to give an indication of particles less than 300nm (Peters et al 2009). Using this approach, together with the SMPS, our study has confirmed the emission of nanoparticles during the post-synthesis phase in the manufacturing of AuNPs. The peak particle exposure concentration measured was (9469 particles/cm<sup>3</sup>) consisted mainly of nanoparticles in the size range 7.5nm to 35nm.

41. As currently there are no occupational exposure guidelines for AuNPs the measured nanoparticle emission during AuNP synthesis was evaluated using an Organization for Economic Cooperation and Development (OECD) excursion guidance criteria (OECD, 2012) and Nano Reference Values recommended by the Working Conditions Committee of the Social and Economic Council of the Netherlands (SER), see Table 5 (van Broekhuizen et al. 2012). The OECD excursion guidance criteria are used in decision making process based on the particle control values.

42. Following the OECD guidance criteria a Local Particle Reference Value (LPRV) was established from the real time particle instrument background readings. According to the general *excursion guidance criteria* - a nanotechnology process could be considered to require modified controls or further assessment if emission or exposure levels exceed certain levels for defined time periods. In this study, the assumptions were that if short term emissions or exposures exceeded three times the particle control value for time periods that add up to more than a total of 30 minutes per eight-hour working day there is exposure. Our results indicate that no further assessment is needed as the measured particle concentration during synthesis was not three times above the determined reference value. Similarly, the measured nanoparticle emission was below the recommended NRV, i.e. 20 000 particles/cm<sup>3</sup>, and as a result was deemed as not requiring further action.

**Table 5: Provisional nano reference values for the four classes of ENPs**

| Class | Description  | Density                  | NRV (8-hr TWA)                   | Examples   |
|-------|--|--------------------------|----------------------------------|--|
| 1     | Rigid, biopersistent nanofibres for which effects similar to those of asbestos are not excluded. | -                        | 0.01 fibres/cm <sup>3</sup>      | SWCNT or MWCNT or metal oxide fibres for which asbestos-like effects are not excluded  |
| 2a    | Persistent granular nanomaterials in the range of 1–100nm.                                       | >6,000 kg/m <sup>3</sup> | 20,000 particles/cm <sup>3</sup> | Ag, Au, CeO <sub>2</sub> , CoO, Fe, Fe <sub>x</sub> O <sub>y</sub> , La, Pb, Sb <sub>2</sub> O <sub>5</sub> , SnO <sub>2</sub> , |

|  |   |                          |                                  |  |
|--|---|--------------------------|----------------------------------|--|
| 2b   | Persistent granular nanomaterials in the range of 1–100nm and fibre form nanomaterials. | <6,000 kg/m <sup>3</sup> | 40,000 particles/cm <sup>3</sup> | - Al <sub>2</sub> O <sub>3</sub> , SiO <sub>2</sub> , TiN, TiO <sub>2</sub> , ZnO, nanoclay<br>Carbon Black, C <sub>60</sub> , dendrimers, polystyrene, - Nanofibres with excluded asbestos-like effects |
| 3  | Non-persistent granular nanomaterial.   | -                        | Applicable OEL                   | e.g. NaCl-, lipid-, flour-, sucrose-particles.   |
| NB: For short term peak concentrations: $NRV_{15min-TWA} = 2 \times NRV_{8hr-TWA}$ |   |                          |                                  |  |

43. Adapted from van Broekhuizen et al 2012).

- **Particle characterisation**

44. Due to the fact that the current particle counting instruments cannot differentiate between engineered and incidental nanoparticles, it is recommended that filter-based samples be collected for further analysis by off-line instruments like TEM or ICP-MS to further characterize emitted particles.

45. FETEM and FESEM were used to determine the morphology (size, shape and degree of agglomeration) and chemical composition of the particles collected on membrane filters. In both instruments single and large agglomerated particles were identified, with the smallest particles observed to be approximately 20nm. The particles observed were largely spherical and confirmed to be of gold origin by EDS analysis. The presence of gold particles on the filters (area and personal) indicates that AuNPs were emitted from the synthesis process and resulted in exposure to the workers synthesizing the particles. The direct collection of particles onto TEM grids attached to membrane filters seems to be effective and needs to be further explored.

46. The highest air concentration of emitted AuNPs in the facility was closer to the reactor at 42ng/cm<sup>3</sup> and the lowest was 3.91ng/cm<sup>3</sup> at a distance of 7 m away from the reactor. Occupational exposure to AuNPs has been confirmed and quantified by ICP-MS and does not seem to be very high with the total concentration and TWA levels personal exposure levels ranging from 0.2 to 12 ng/cm<sup>3</sup>, lower than the measured area air concentration. These results confirm conventional wisdom which suggests that nanomaterials in liquid suspension generally pose lower inhalation risk to workers (Johnson et al 2010).

47. In conclusion:

- Synthesis of AuNPs in liquid suspension results in emission of /and exposure to AuNPs. Exposure during synthesis was not limited to the pristine AuNPs (i.e. primary particles) but their aggregates /agglomerates as well.
- However the TWA levels were very low and particle emission was less than the proposed nano reference values (NRV) and below the OECD excursion guidance criteria and therefore the AuNP synthesis under the conditions described in this report does not require further assessment.
- To our knowledge this is the first study to suggest that engineered gold nanoparticles are emitted during their synthesis using the citrate reduction method. This study has laid the basis for future exposure assessment of other nanoparticles/nanomaterials of interest in the country.

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