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GOLD NANOPARTICLE OCCUPATIONAL EXPOSURE ASSESSMENT IN A PILOT SCALE FACILITY

Nanomaterials Exposure Case Study

Series on the Safety of Manufactured Nanomaterials
No. 77

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

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NANOMATERIALS EXPOSURE CASE STUDY

IOMC INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2016

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FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the "state of the art" for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee in September 2006. This programme concentrates on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. This programme promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

The document presents a case study on exposure assessment 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 document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, pesticides and Biotechnology of the OECD.

ABBREVIATIONS

ACF	Autocorrelation function
APS	Aerodynamic Particle Sizer
AR	autoregressive
ARIMA	Autoregresion Integrated Moving Average
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
ENPs	Engineered nanoparticles
FE-SEM	Field-Emission Scanning Electron Microscopy
FE-TEM	Field-Emission Transmission Electron Microscopy
HCl	Hydrochloric acid
HNO_3	Nitric acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
IQC	Internal Quality Control
LBPC	Local Background particle concentration
LOD	Limit of Detection
LPRV	Local Particle Reference Value
ma	Moving average
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
OEL	Occupational Exposure Limit
OPC	Optical Particle Counter
PACF	Partial autocorrelation
PNC	Particle Number Concentration
PPE	Personal protective equipment
RSA	Republic of South Africa
SEM	Scanning Electron Microscopy
SER	Economic Council of the Netherlands
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 gold nanoparticles (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 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 assess exposure to nanoparticles during the synthesis of eighty litres (80 L) of 14 nm AuNPs 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 at 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

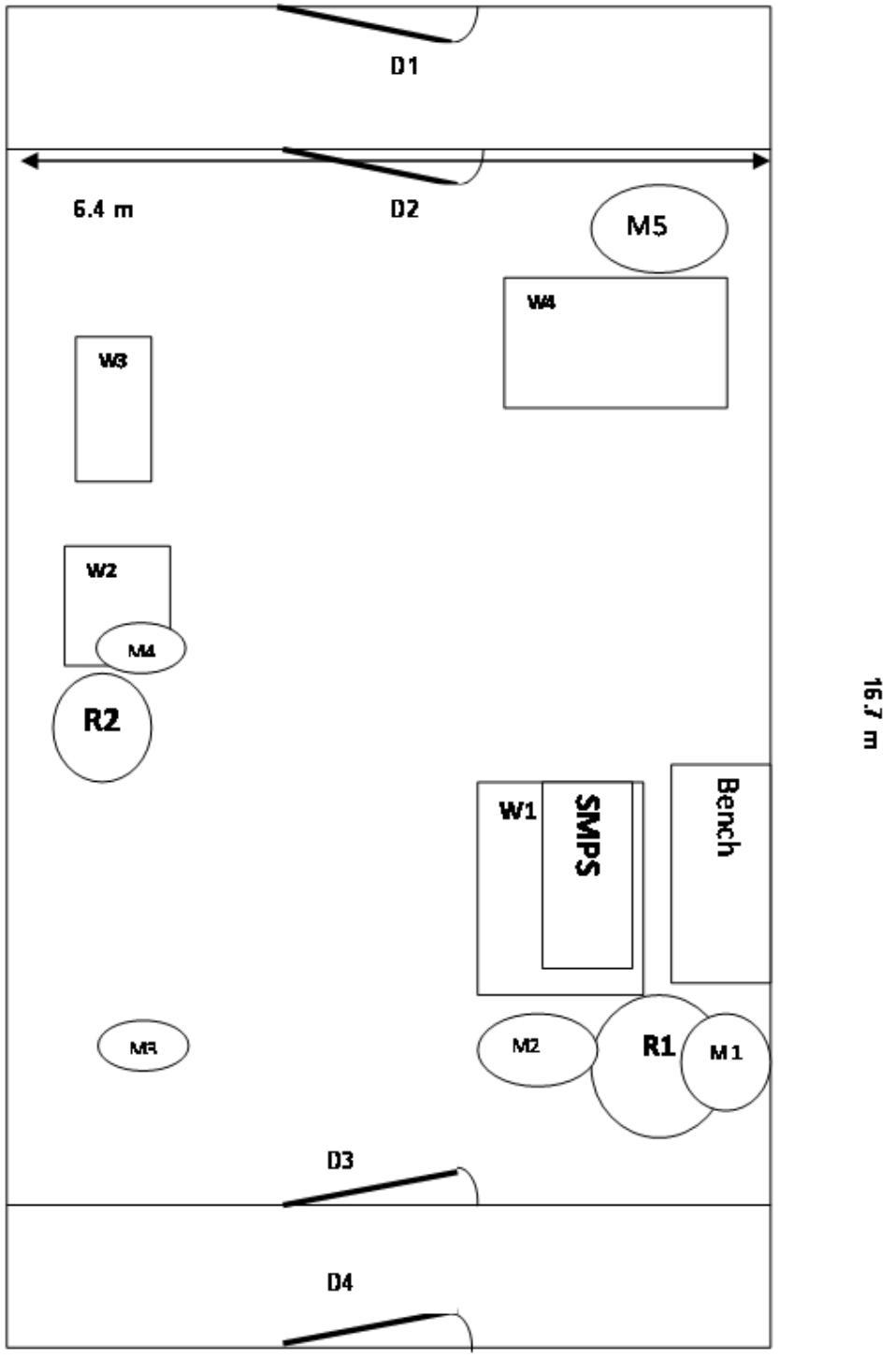
3. The exposure assessment utilized in this survey followed a tiered approach as described by Methner et al (2010). The approach included:

- An information gathering on the synthesis of AuNPs in the facility (tier 1);
- An assessment of nanoparticle emission during AuNP synthesis using hand held particle counters (tier 2);
- An assessment of nanoparticle emission during AuNP synthesis using a combination of mobility particle sizers focusing on particle number concentration and size, and personal and area air sampling for particle characterisation (tier 3).

MONITORING LOCATION

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



4. 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 and the one used during this exercise); 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. It is important to indicate that only R1 glass reactor was used in the measurement.

5. The temperature in the facility varied from 22 to 25^{o}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 Au nanoparticles on the day of this study. Other possible sources of nanoparticle in the laboratory could be combustion nanoparticles from the reactor or those originating from outside.

6. The AuNPs were synthesized in a 100 litre glass chemical reactor (R1 in Figure 1) that had previously been cleaned with a hydrochloric and nitric acid (HCl/HNO_3) 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 $HAuCl_4.3H_2O$ 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) (Table1). 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.

Table 1. AuNPs synthesis process

Activities	Duration in minutes	Number of workers	Location
Preparation	14	3	R1
Synthesis	21	3	R1
Post synthesis	90	3	R1
Harvesting	44	1	R1

PARTICLE NUMBER CONCENTRATION SAMPLING

7. In this tier 1, 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 2 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 particles/cm^3. 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^3) of sampled air, and has an upper limit of detection of 100,000 particles/cm^3.

8. The desk top particle counters used were the Scanning Mobility Particle Counter (SMPS) and Aerodynamic Particle Sizer (APS), 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 10^7 particles/cm^3.The size range determination depends on the flow rate of the SMPS. The APS provides for both PNC and PND in 52 channels in the size range 300nm to 20um and has an upper limit of detection of 10000 particles/cm^3.

Table 2: 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 ParticleCounter (TSI UPC, Model 8525),	Total number	10 – 1000nm
HACH Met One Handheld Particle Counter (HHPC-6)	Number, Size distribution	300nm - 5µm

9. 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

10. 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.

11. Estimation of ultrafine particle (< 300nm) number concentration from the Condensation Particle Counter (CPC) was done following Peters et al., (2009) using the formula below including the Optical Particle Counter (OPC):

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

12. The AuNP synthesis via the citrate-reduction method occurs at temperatures above 100 ^{o}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

13. 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.

14. 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.

15. 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 and only used natural ventilation.

AIR SAMPLING

- **Personal sampling**

16. 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 synthesized AuNPs were harvested the following day by employee D for 44 minutes and both area and personal sampling was done using MCE filters. For area sampling a high volume pump run at 7L/min, and for personal sampling a standard volume pump run at 4L/min was used for the duration of the harvesting period.

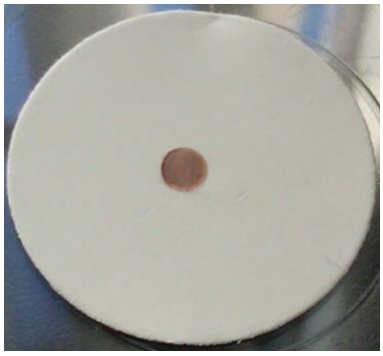
Figure 3: A worker with personal sampling train attached

Area sampling

17. Area samples were collected by placing open faced MCE filters (25mm diameter and 0.8µm pore size) before sampling and as close as possible to the source and in other areas within the facility and drawing air using pumps at a flow rate of 7L/min.

18. Area and personal sampling was done in duplicate, one sample for metal analysis by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and another for electron microscopy analysis (morphology and elemental analysis). A carbon coated Transmission Electron Microscopy (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

19. 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)

20. 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)

21. The concentration of the gold particles in the 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_3 (Trace Select Ultra 65%) and further digestion at 90˚C for 30mins. After cooling 100 µl of 1ppm ^{193}Ir internal standard was added and the samples brought up 10ml final volume with 18.2MΩ/cm water.

22. 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.

23. Two aqueous Internal Quality Controls (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'.

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.

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

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

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

Exposure assessment

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

Particle-number concentration

26. The emission of nanoparticles was measured before (background levels) and during the preparation, synthesis, and post-synthesis phase using a combination of real-time particle counters. Table 3, below summarises real-time particle number concentration results.

Table 3: Nanoparticle emission during AuNP synthesis

Activities profile	**7 – 290nm (SMPS)**		**10 – < 300nm (P-Trak)***	
	Average ± SD (#/cm^3)	**Range** (#/cm^3)	**Average ± SD** (#/cm^3)	**Range** (#/cm^3)
Background	7901**±297**	7450 – 8330	**7171 ± 3**	7167 - 7177
Preparation phase	7361**±210**	6860 - 7610	**6593 ± 144**	6369 - 6857
Synthesis phase	10295**± 216**	7700 - 13600	**8449± 2419**	6524 – 13690-
Post-synthesis phase	9535 **± 1870**	7070 - 13900	**7671 ±1326**	5904 - 10358

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

27. The particle number concentration measured with the SMPS (i.e. particles from 7.5nm to 283.9nm) ranged from a background level of 7901 particles/cm^3 to a peak of 10295particles/cm^3 during the 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 7171particles/cm^3 to a peak of 8449particles/cm^3 during the synthesis phase. Particles concentrations varied significantly from background to synthesis (P 0.000) as shown in figures 5 and 6.

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

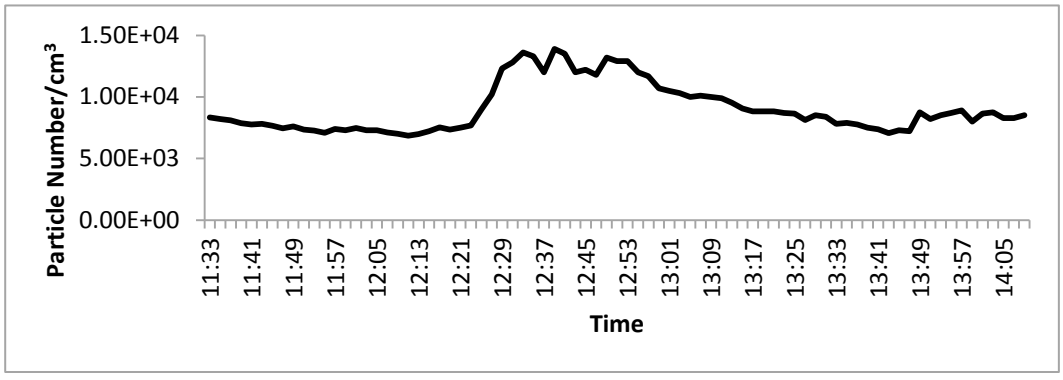
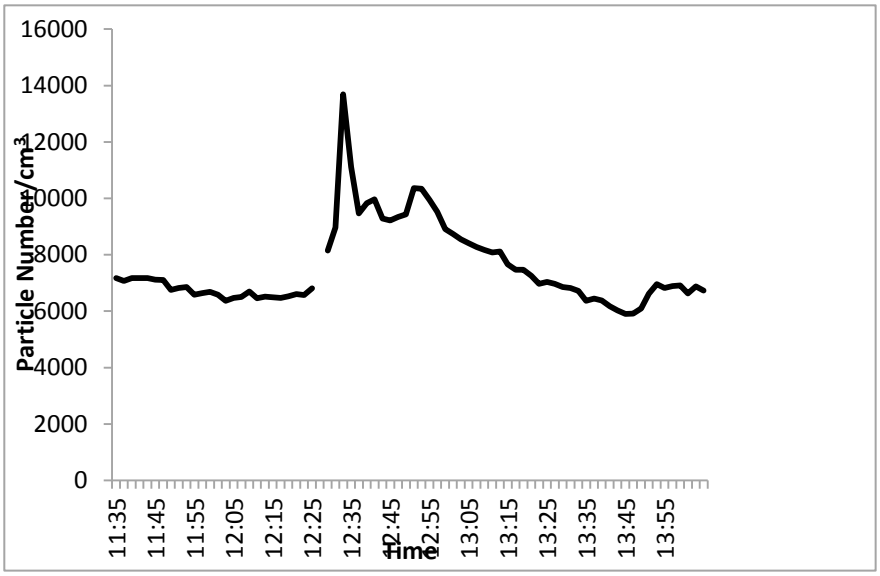


Figure 6: Real-time particle number concentration measured with TSI-UPC



PARTICLE SIZE DISTRIBUTION

28. The emission of nanoparticles was measured by establishing the Local background particle concentration and concentration of particles generated by the synthesis of AuNPs using a combination of real-time particle counters. Statistical analysis was done in using Autoregresion Integrated Moving Average (ARIMA) for time series data as suggested in the scientific literature (Klein et al., 2011; 2015). Table 4, below summarises real-time particle number concentration results. The geometrical mean was 9031 (± 1974) of the nanoparticles activity versus the background nanoparticles concentrations (7966 ± 973). Number concentration of nanoparticles has shown a significant increase (P: 0.0010) between the local background particle concentration.

Table 4. Background and nanoactivities particles concentration

Parameters	Values
Local Background particle concentration (LBPC) (number = #)	7966 # cm^3
Activity particle number concentration (#)	9031 # cm^3
P - value	0.0010
Sigma2	231 # cm^3
ar1	0.812
ar2	0.151
ma1	0.999
R^2	0.057
variance	2720814

29. The Autocorrelation function (ACF) and partial autocorrelation (PACF) used for the correction of the correlation have shown respectively significance in background and gold nanoparticle concentration from the synthesis at 2 lags and one lag within the ARIMA model (2,0,1). The autoregressive (AR) (2) sum was close to 1 which means that the series investigated returns to the mean slowly and the moving average (ma) close to 1 which meant that the fraction of the last period shock in the `series was still felt in the current period. The SMPS, operating in the range from7.5nm to 283.9nm, showed an increase in the concentration of particles starting from 7.5nm to 35nm (average ~16nm) during the 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

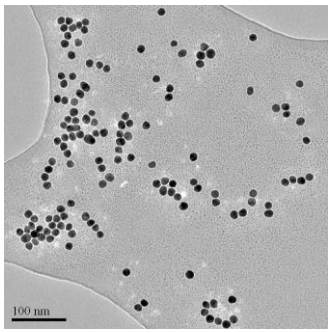
PARTICLE CHARACTERIZATION

30. 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

30. Figure 7 shows TEM images of individual, spherical engineered gold nanoparticles.

Figure 7: A representative TEM micrograph of 14 nm gold nanoparticles synthesized bycitrate reduction method (adapted from Sosibo et al., 2015)



31. FE-TEM showed the presence of nanoparticles in the area samples collected near and up to about 7m from the synthesis reactor as described in Figure 3 above. The particle size ranged from approximately 20nm to at least 500nm; with aggregated/agglomerated/coagulated particles ranging from 50nm to about 500nm (see Figures 8 to 11).

Figure 8: TEM images of particles collected 30 cm from the source

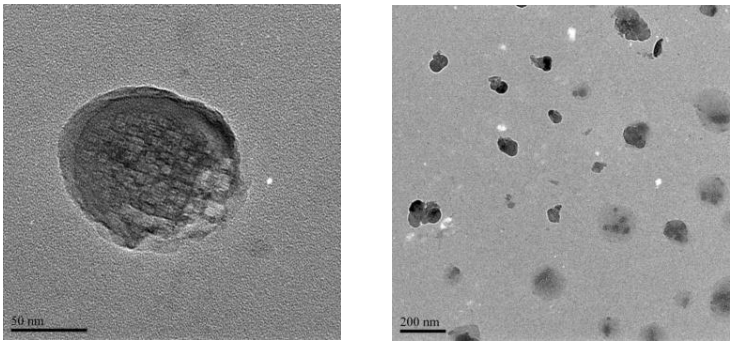


Figure 9: TEM images of particles collected 40 cm from the source

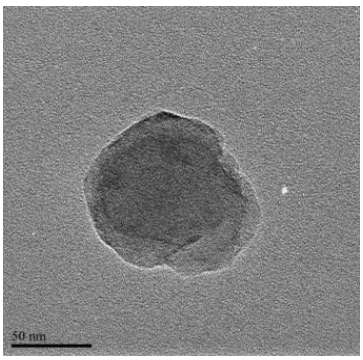


Figure 10: TEM images of particles collected 4 m from the source

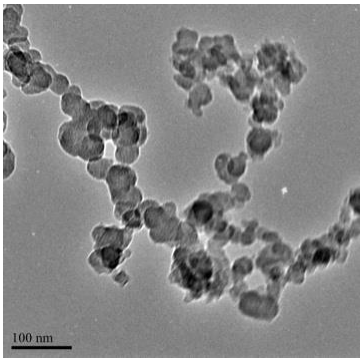
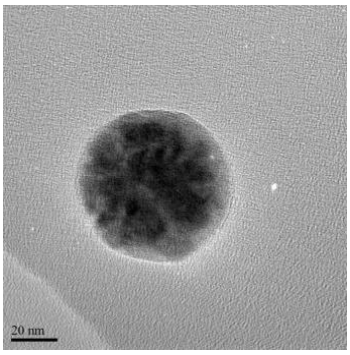


Figure 11: TEM images of particles collected 7.2 m from the source



FE-SEM

32. FE-SEM analysis of filter-based samples showed a wide range of particles including agglomerates/aggregates/coagulated particles and spot analysis indicated the presence of gold particles as well as silicates.

33. The filter sample for employee A had the following elemental composition C (72.6% wt), O (23.4% wt), Au (2.2% wt) and Si (1.8% wt) with the filter sample 30 cm from the source having the following elements C (71,7% wt), O (24.3% wt) and Au (3.98% wt).

Metal Analysis by ICP-MS

34. 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 µg/cm^3, with the highest concentration measured 30 cm from the reactor opening.

35. Results for the four workers sampled also indicated exposure to gold nanoparticles, with measured gold concentrations ranging from 0.00028 to 0.015 µg/cm^3, with the workers responsible for addition of citrate and supervising the synthesis process being the highly exposed at 0.1 µg/cm^3 (Time Weighted Average (TWA) – 0.012 µg/cm^3) and 0.12 µg/cm^3 (TWA 0.015 µg/cm^3) respectively (Tables 5 and 6). The workers responsible for the addition of the chloroauric acid had the lowest exposure at 0.002 µg/cm^3 (TWA – 0.0002 µg/cm^3). The task of decanting the AuNP resulted in an exposure of 0.00007 mg/cm^3 (TWA – 0.000002 mg/cm^3). Filter concentration of the field blanks were below the ICP detection limit of 0.00025 µg/ml

Table 5: Au^{3+} mass concentration of personal samples as measured by ICP-MS

	Air concentration ($\mu g/m^3$)	**TWA ($\mu g/cm^3$)**	**Job description**
Employee A	0.00225	0.000281	Addition of $HAuCl_4.3H_2O$
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

Table 6: Au^{3+} mass concentration of area samples during synthesis of AuNPs

Location	**Air concentration ($\mu g/ cm^3$)**
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

36. 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).

COMMENTS AND CONCLUSIONS

Real-time particle monitoring

37. Emission of nanoparticles from the synthesis of 100 ml AuNPs by citrate-reduction at temperatures above 100^{o}C was detected using hand held particle counters at a research and development laboratory, with the emission observed to happen during the addition of citrate to the heated chloroauric acid solution. During the up scaled production of 40L AuNPs a significant increase ($p < 0.0000$) in nanoparticle number concentration (10295 particles/cm^3) was observed during the synthesis stage compared to background (7901 particles/cm^3).

38. 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 7 (van Broekhuizen et al. 2012). The OECD excursion guidance criteria are used in decision making process based on the particle control values.

39. 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 significant exposure.

40. Our results indicate that there may be no significant exposure to Au nanoparticles 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 Nano Reference Value (NRV), i.e. 20 000 particles/cm^3. However, these results do not suggest that the risk of exposure to Au nanoparticles is low; as a result it is recommended that the precautionary principle is applied to safeguard the health of researchers as engineered particles occurring from synthesis of AuNPs at workplace can present potential hazard if considering inhalation as major path of exposure (Jiménez et al.,2014; Bekker et al., 2015, Debia et al., 2016; Fonseca et al. 2016).

Table 7: Provisional nano reference values for the four classes of engineered nanoparticles (ENPs) **(**Adapted from van Broekhuizen et al., 2012)

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^3	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^3	20,000 particles/cm^3	Ag, Au, CeO_2, CoO, Fe, Fe_xO_y, La, Pb, Sb_2O_5, SnO_2,
2b	Persistent granular nanomaterials in the range of 1–100nm and fibre form nanomaterials.	<6,000 kg/m^3	40,000 particles/cm^3	- Al_2O_3, SiO_2, TiN, TiO_2, ZnO, nanoclay Carbon Black, C_{60}, dendrimers, polystyrene, - Nanofibres with excluded asbestos-like effects
3	Non-persistent granular nanomaterial.	-	Applicable Occupational Exposure Limit (OEL)	e.g. NaCl-, lipid- ,flour-, sucrose- particles.
NB: For short term peak concentrations: $NRV_{15min\text{-}TWA} = 2 \times NRV_{8hr\text{-}TWA}$				

Particle characterisation

41. 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.

42. FE-TEM and FE-SEM 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 is recommended in resource challenged settings as it has the advantage of avoiding further sample preparation procedures and cost effective.

43. The highest air concentration of emitted AuNPs in the facility was closer to the reactor at 42ng/cm^3 and the lowest was 3.91ng/cm^3 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^3, 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).

44. 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.

- TWA levels were very low and particle emission was less than the proposed nano reference values (NRV) and below the OECD excursion guidance criteria, however further research need to be undertaken to assess the adverse health effects of exposure of AuNP

- 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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