Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

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English - Or. English

**ENVIRONMENT DIRECTORATE**
**JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

# DOSSIER ON GOLD NANOPARTICLES

**Series on the Safety of Manufactured Nanomaterials**
**No. 44**

*This document is only available in PDF format.*

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**OECD Environment, Health and Safety Publications**

**Series on the Safety of Manufactured Nanomaterials**

**No. 44**

# DOSSIER ON GOLD NANOPARTICLES


**Environment Directorate**
**ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT**
**Paris, 2015**

***Dossiers also published in the Series on the Safety of Manufactured Nanomaterials:***

No. 45, *Dossier on Cerium oxide (2015)*

No. 46, *Dossier on Dendrimers (2015)*

No. 47, *Dossier on Nanoclays (2015)*

No. 48, *Dossier on Fullerenes (2015)*

No. 49, *Dossier on Multiwalled Carbon Nanotubes (MWCNTs) (2015)*

No. 50, *Dossier on Single-Walled Carbon Nanotubes (SWCNTs) (2015)*

No. 51, *Dossier on Silicon dioxide (2015)*

No. 52, *Dossier on Zinc oxide (2015)*

No. 53, *Dossier on Silver nanoparticles (2015)*

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The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents;** and **Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/chemicalsafety/).

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# PREAMBLE

In November 2007, OECD's Working Party on Manufactured Nanomaterials (WPMN) launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The outcomes of the Testing Programme were intended to assess the applicability of the existing ***test guidelines***[1] to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions. Understanding the properties of NMs is crucial to choose appropriate strategies for hazard identification, risk assessment or risk management measures. The Testing Programme involved delegations from OECD member countries, some non-member economies and other stakeholders. The broad international representation, from a range of delegations enabled the programme to pool expertise and resources without which this programme would not have been possible.

Before launching the Testing Programme, the WPMN first identified a broad list of possible nanomaterials, and the list was later adjusted to a final selection of eleven MNs for testing[2]. This list comprised: i) fullerenes (C60); ii) single-walled carbon nanotubes (SWCNTs); iii) multi-walled carbon nanotubes (MWCNTs); iv) silver nanoparticles; v) titanium dioxide; vi) cerium oxide; vii) zinc oxide; viii) silicon dioxide; ix) dendrimers; x) nanoclays; and xi) gold nanoparticles. One fundamental criterion for selecting these materials was that they should be either in commercial use at the time or expected to be in the near future. At the same time, other considerations were also given attention, such as the production volume of the materials, the likely availability of such materials for testing and the existing information that would readily be available on the materials.

It was also agreed that 59 endpoints would be addressed[3] for each material corresponding to the following categories: i) nanomaterial information/ identification; ii) physical-chemical properties and material characterisation; iii) environmental fate; iv) toxicological and eco-toxicological effects; v) environmental toxicology; vi) mammalian toxicology; and vii) material safety. These endpoints were judged to be most important based largely on the general experience of testing chemicals, while taking into account the potentially different or new properties of nanomaterials. It is worth noticing that it was not expected that testing for all of the listed endpoints would be necessary for each of the selected MNs.

To assist with the Testing Programme, the WPMN developed two documents: i) a *Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials* [ENV/JM/MONO(2009)21]; and ii) *Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme* (Guidance Manual) in 2009, which was subsequently updated in 2010

[1] The OECD Test Guidelines are a collection of internationally agreed test methods used by government, industry and independent laboratories. They are used to determine the safety of chemicals.

http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

[2] Originally Iron nanoparticles, Aluminium, Carbon black, and Polystyrene were suggested but later withdrawn and replaced by gold nanoparticles.

[3] As specified in the Guidance Manual, "address" includes the term "completed" which provides that all dossiers will contain the identified endpoint information. Note that for some endpoints (for example, solubility) it is specified that the endpoint must be "completed". In such instances "completed" means that all Dossiers will be providing this endpoint information.

[ENV/JM/MONO(2009)20/REV][4]. The objective of this *Guidance Manual* was to guide sponsors[5] in the testing of the materials while ensuring that the information collected was reliable, accurate, consistent and therefore also comparable. The *Guidance Manual* addressed a whole range of issues including the organisation of the work.

The *Guidance Manual* contains detailed information on the selected endpoints for testing and recommendations on sample preparation and dosimetry.

The *Guidance Manual* also described the development of *Dossier Development Plans* (DDPs). These plans were prepared by Lead sponsors, Co-sponsors together with contributors to describe the specific plan for the testing of each nanomaterial including when and where the testing will be undertaken and by whom. The DDPs also included information on the materials to be tested as well as information on issues such as sample preparation and dosimetry. Each of the DDPs was prepared and reviewed by the WPMN before testing work began.

Based on the lessons learned during the Testing Programme, the WPMN also developed *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials* [ENV/JM/MONO(2012)40]. This latter document is an update of an earlier text first published in 2010.

The work on OECD's Testing Programme was completed by the end of 2013. In June 2014 the WPMN agreed that for each nanomaterial the dataset would be published in IUCLID printed format[6] [7]. The document will include the protocols and methods to allow their wider use (regulators and researchers).

The dataset in this document has been declassified and made publicly available and it is expected regulators and researchers will wish to use it. Due to a broad dissemination of the data and the exploratory setting in which they were developed there are a number of limitations in using the data of which potential users should be aware. The programme focused on answering scientific questions in the field of the OECD test guidelines but not to provide conclusions on the hazard or risk of the materials selected. The absence of data for some endpoints may be a gap for some endpoints but for other end points there may not if the data was not considered necessary. Although the programme ensured a broad participation of many stakeholders it was not intended to arrive at any pre-defined regulatory datasets requirements or risk assessment decisions. It was recognised from the beginning that the exploratory nature of the work would require subsequent follow-up work for example to review the specific needs that may arise when

[4] It is worth noting that while the *Guidance Manual for Sponsors* was primarily intended as a guide to WPMN's Testing Programme, it is also expected that it will be of value to anyone involved in testing NMs.

[5] The Guidance Manual noted, for example, that there could be three levels of participation to the programme. Lead sponsors, who would assume responsibility for conducting or coordinating all of the testing, determined to be appropriate for each of the endpoints for a specific nanomaterial. In some cases, "joint lead" arrangements were developed. Co-sponsors conducted some of the testing determined to be appropriate and feasible to address the endpoints for a specific listed nanomaterial. Contributors provided test data, reference or testing materials or other relevant information to the lead and co-sponsors.

[6] IUCLID is a software programme for the administration of data on chemical substances. Although it was originally developed to fulfill requirements in the EU for the evaluation and control of the risks of existing chemical substances, it is used by many others.

[7] SIAR = SIDS Initial Assessment Report (SIDS = Screening Information Data Set)

performing risk assessment of nanomaterials. In this context, the programme's ultimate goal, to add to the knowledge of the properties of nanomaterials, would form a cornerstone.

# FOREWORD

As part of its Programme on the Safety of Manufactured Nanomaterials, OECD launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The Testing Programme mainly aimed to assess the applicability of the existing test guidelines to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions.

This document presents the Dossier of the Gold Nanoparticles (NM 330) which was prepared under the leadership of South Africa. This nanomaterial has been tested for a number of endpoints for: i) Nanomaterials Information / Identification; ii) Physical-Chemical Properties; iii) Environmental Fate; iv) Environmental Toxicology; v) Mammalian Toxicology; and vi) Material Safety. They have been analysed using OECD Guidelines for the Testing of Chemicals (TG)8. The data is presented in an IUCLID9 style format and includes the protocols and methods used (see Preamble).

South Africa, including the National Centre for Occupational Health (NIOH), Mintek (South Africa), the University of Johannesburg and NIOH, led the Testing Programme on Gold Nanoparticles. This included the determination of the tests that were appropriate for Gold Nanoparticles, performing a number of tests, as well as coordinating tests performed and inputs provided by other participating countries and stakeholder, from Canada, the European Union, the Germany (Fraunhofer), the Republic of Korea (Hoseo University), the United States and the Business and Industry Advisory Committee to the OECD (BIAC).

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.

[8] http://www.oecd.org/env/testguidelines

[9] IUCLID is a software programme for the administration of data on chemical substances. It was originally developed to fulfil requirements in the EU for the evaluation and control of the risks of existing chemical substances. It is specifically relevant in the context of an international programme for the initial assessment of chemical substances.

# ACKNOWLEDGMENTS

The OECD Secretariat and the WPMN is thankful to South Africa for leading the Testing Programme on Gold Nanoparticles (NM 330). They are specifically grateful to Pr. Mary Gulumian and to Dr. Melissa Vetten from the National Centre for Occupational Health (NIOH), as well as to Mintek (South Africa) and to the University of Johannesburg for their support. In addition, they appreciate the efforts made by other participating countries / organisations: Canada, the European Union, the Germany (Fraunhofer), the Republic of Korea (Hoseo University), the United States and the Business & Industry Advisory Committee to the OECD (BIAC).

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### *Endpoint study record: 7440-57-5, Partition coefficient, Author, Year, Summary level, Study level*

**Administrative Data**

**Study result type** experimental result **Study period** No data

**Reliability** other: no data

**Rationale for reliability incl. deficiencies** No data

**Data source**

**Reference**

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| other: no data | | | | | | | | | |

**Data access**

other: no data

**Cross-reference to same study**

No cross-reference

**Materials and methods**

**Partition coefficient type**

octanol-water

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

## Type of method

other: no data

## Principles of method if other than guideline

The particles are highly hydrophilic. However, efforts to determine their LogP values were fruitless as no particles crossed into the octanol phase.

## GLP compliance

no data

## Test materials

## Details on test material

- Name of test material (as cited in study report): cAuNPs

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Any other information on results incl. tables

The particles are highly hydrophilic. However, efforts to determine their LogP values were fruitless as no particles crossed into the octanol phase.

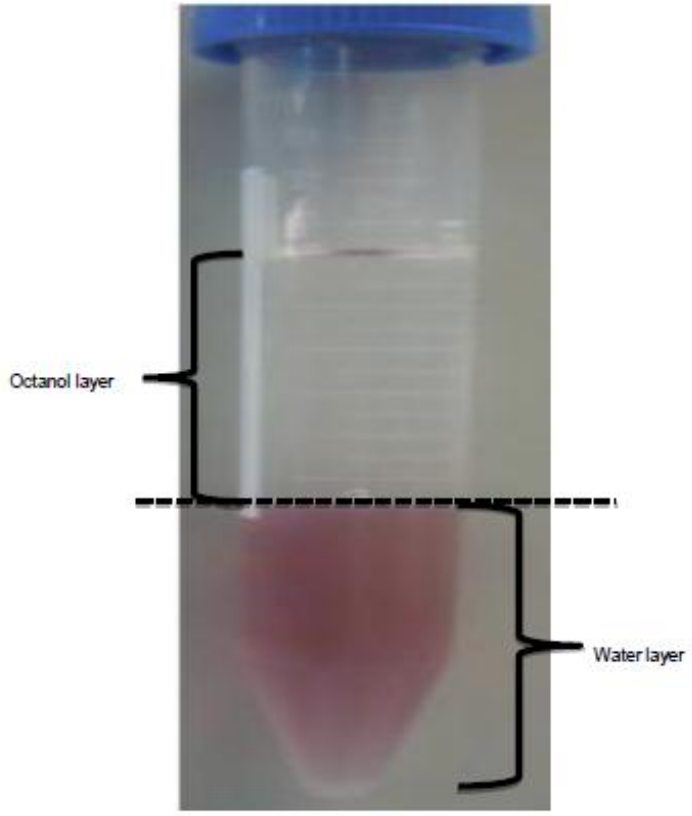
## Overall remarks, attachments

## Remarks on results including tables and figures

None

**Attached background material**

| Attached document | Remarks |
|---|---|
| Octanol-Water Partition Coefficient.pdf / 17.95 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Conclusions
No data

### Executive summary
Not available

### Cross-reference to other study
No cross-reference

# 4.8 Water solubility

## *Endpoint study record: 7440-57-5, Water solubility, Anonymous, Year, RS, K*

### Administrative Data

**Purpose flag** key study; robust study summary

**Study result type** experimental result **Study period** No data

**Reliability** other: no data

**Rationale for reliability incl. deficiencies** No data

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| other: no data | | | | | | | | | |

### Data access

other: data submitter is data owner or has Letter of Access

### Data protection claimed

yes

### Cross-reference to same study

No cross-reference

### Materials and methods

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 105 (Water Solubility) | no data |

### Type of method

flask method

### Principles of method if other than guideline

Water Solubility/Dispersibility of 14 nm cAuNPs (gold nanoparticles) was determined by using Flask method.

### GLP compliance

no data

## Test materials

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): 14 nm cAuNPs- Source: Mintek, South Africa

## Confidential details on test material
No data

***Details on methods***
Test conditions Dilution water source: Distilled and deionized (18MΩ.cm) Stock and test solutions: 14 nm cAuNPs

## Any other information on materials and methods incl. tables
None

## Results and discussions

***Details on results***
The particles are highly hydrophilic. Efforts to determine their LogP values were fruitless as no particles crossed into the octanol phase. This indicates the highly hydrophilic nature of 14 nm cAuNPs.

## Any other information on results incl. tables
None

## Overall remarks, attachments

## Remarks on results including tables and figures
None

## Applicant's summary and conclusion

## Interpretation of results
other: highly hydrophilic

## Conclusions
Under the test conditions, no gold nanoparticles crossed into the octanol phase and their LogP values were not determined, which indicates that 14 nm cAuNPs are highly hydrophilic.

**Executive summary**

A study was conducted to determine the water solubility/dispersibility of 14 nm cAuNPs using Flask method. Dilution water source was distilled and deionized (18MΩ.cm) water and stock and test solutions were 14 nm cAuNPs.

The particles are highly hydrophilic. Efforts to determine their LogP values were fruitless as no particles crossed into the octanol phase. This indicates the highly hydrophilic nature of 14 nm cAuNPs.

Under the test conditions, no gold nanoparticles crossed into the octanol phase and their LogP values were not determined, which indicates that 14 nm cAuNPs are highly hydrophilic.

**Cross-reference to other study**

No cross-reference

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### *Endpoint study record: 7440-57-5, Additional physico-chemical information - Representative Electron Microscopy (Tem) Picture(S), Author, Year, Summary level, Study level*

#### Administrative Data

**Study result type** experimental result **Study period** No data

**Reliability** other: no data

**Rationale for reliability incl. deficiencies** No data

#### Data source

#### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| other: no data | | | | | | | | | |

#### Data access

other: no data

#### Cross-reference to same study

No cross-reference

#### Materials and methods

#### Endpoint investigated

other: Representative Electron Microscopy

#### Principles of method if other than guideline

Representative Electron Microscopy

#### GLP compliance

no data

#### Test materials

#### Identity of test material same as for substance defined in section 1 (if not read-across)

yes

#### Test material form

nanomaterial

## Details on test material
- Name of test material (as cited in study report): cAuNPs (14 nm)

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Results
No data

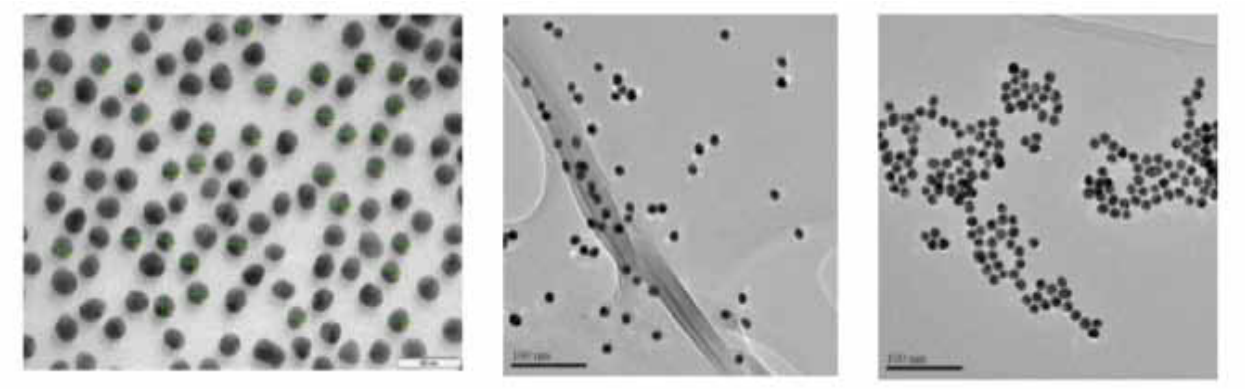
## Any other information on results incl. tables
See the attached document for Figure: Representative TEM images of 14 nm cAuNPs

## Overall remarks, attachments

## Remarks on results including tables and figures
None

## Attached background material

| Attached document | Remarks |
|---|---|
| Representative TEM images of 14 nm cAuNPs.pdf / 33.88 KB (application/octet-stream) | |



## Applicant's summary and conclusion

## Conclusions
No information

## Executive summary
No information

### Cross-reference to other study
No cross-reference

## 4.24 Agglomeration/aggregation

### *Endpoint study record: 7440-57-5, Agglomeration-aggregation, Turkevich, 1951, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2010 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Turkevich J, Stevenson PC and Hillier J. | 1951 | A Study of the Nucleation and Growth Process in the Synthesis of Colloidal Gold. | Discuss. Faraday Soc. 11: 55-75 | | | | | |

### Data access
other: data submitter is data owner or has Letter of Access

### Data protection claimed
yes

### Cross-reference to same study
No cross-reference

### Materials and methods

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | other guideline: Guidance manual for the testing of manufactured nanomaterials [OECD's sponsorship programme, ENV/JM/MONO(2009)20/REV] | no data |

## Method
other: DLS, TEM and UV-vis

## Principles of method if other than guideline
Not applicable

## Details on methods and data evaluation
Agglomeration/Aggregation State of 14 nm gold nanoparticles (cAuNPs) was tested using Zetasizer and Transmission Electron Microscopy (TEM).Test conditions:Dilution water source: Distilled and deionized (18MΩ.cm)Stock and test solutions: 14 nm cAuNPsExposure period (duration): > 6 months

## Data gathering

## Instruments
Analytical monitoring: Zetasizer (Nano ZS, Randburg, South Africa), TEM (JEM-2100, JEOL, South Africa)

## Calibration
None

## Reproducibility
None

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Reference material/nanomaterial and Sample identification number

| Identifier | Identity |
|---|---|
| reference material/nanomaterial | cAuNPs |

## Test material form
other: nanomaterial

## Confidential details on test material
No data

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Agglomerate/Aggregate size

**Percentile** other: no data
**Mean** 14 nm
**Medium** deionized water (18 MΩ·cm)
**Remarks** TEM results
**Percentile** other: no data
**Mean** 33 nm
**Medium** deionized water (18 MΩ·cm)
**Remarks** DLS results

## Agglomeration/ Aggregation Index

**Remarks** AI = DLS particle size / TEM particle size = 2.35

## Overall remarks, attachments

## Remarks on results incl. tables and figures

The UV-vis results showed the SPR band at 520 nm which is indicative of 14 nm gold nanoparticles. TEM results confirmed that the cAuNPs were indeed of an average size of 14 nm and that they are well dispersed. Interestingly larger size readings for the same cAuNPs were registered using DLS technique (Zetasizer) with the average being 33 nm. While this might indicate aggregation, it is not entirely true as DLS always appear to register higher particle sizes than those of TEM and hence is a less accurate technique in determining the actual size of the nanoparticles. This is presumably due to the fact that in solution the particles are relatively close and subject to Brownian motion thereby making the light scattering by the particles in the DLS erroneous. Nevertheless the technique can be used to determined aggregation indices (AI). In this case cAuNPs sizes were measured with both TEM and DLS and the aggregation index (AI) calculated as follows:
AI = DLS particle size / TEM particle size = 2.35
Where there is no aggregation, AI should be approximately 1. Thus, from the results of cAuNPs above, it shows that these particles aggregate over time.

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Conclusions

Under the test conditions, the 14 nm cAuNPs were monodispersed as seen from the TEM images. However measurements by DLS technique gave large size readings compared to TEM. It is not recommended to use DLS as a final technique to determine the size of the particles.

### Executive summary

A study was conducted to determine the agglomeration/aggregation state of 14 nm gold nanoparticles (cAuNPs) by using Zetasizer and Transmission Electron Microscopy (TEM).

**Test conditions**
Dilution water source: Distilled and deionized (18MΩ.cm)
Stock and test solutions: 14 nm cAuNPs
Exposure period (duration): > 6 months

The UV-vis results showed the SPR band at 520 nm which is indicative of 14 nm gold nanoparticles. TEM results confirmed that the cAuNPs were indeed of an average size of 14 nm and that they are well dispersed.The UV-vis results showed the SPR band at 520 nm which is indicative of 14 nm gold nanoparticles. TEM results confirmed that the cAuNPs were indeed of an average size of 14 nm and that they are well dispersed. Interestingly larger size readings for the same cAuNPs were registered using DLS technique (Zetasizer) with the average being 33 nm. While this might indicate aggregation, it is not entirely true as DLS always appear to register higher particle sizes than those of TEM and hence is a less accurate technique in determining the actual size of the nanoparticles. This is presumably due to the fact that in solution the particles are relatively close and subject to Brownian motion thereby making the light scattering by the particles in the DLS erroneous. Nevertheless the technique can be used to determined aggregation indices (AI). In this case cAuNPs sizes were measured with both TEM and DLS and the aggregation index (AI) calculated as follows:
AI = DLS particle size / TEM particle size = 2.35
Where there is no aggregation, AI should be approximately 1. Thus, from the results of cAuNPs above, it shows that these particles aggregate over time.
Under the test conditions, the 14 nm cAuNPs were monodispersed as seen from the TEM images. However measurements by DLS technique gave large size readings compared to TEM. It is not recommended to use DLS as a final technique to determine the size of the particles.

### Cross-reference to other study

No cross-reference

## 4.25 Crystalline phase

### *Endpoint study record: 7440-57-5, Crystalline phase - Transmission electron microscopy, Anonymous, 2012, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2010 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Turkevich J, Stevenson PC and Hillier J. | 1951 | Nucleation and Growth Process in the Synthesis of Colloidal Gold. | Discuss. Faraday Soc. 11: 55-75 | | | | | |
| study report | Anonymous | 2012 | Transmission Electron Microscopy Analysis of Nanoparticles | | | | | | |

## Data access

other: data submitter is data owner or has Letter of Access

## Data protection claimed

yes

## Cross-reference to same study

Section 4.25: 7440-57-5, Crystalline phase - x-ray diffraction, Anonymous, 2013, RS, K

## Materials and methods

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

## Method

transmission electron microscopy (TEM)

## Principles of method if other than guideline

A study was conducted to determine the crystalline phase of gold nanoparticles (14 nm cAuNPs) by Transmission electron microscopy.

## Details on methods and data evaluation

5 drops of a concentrated sample (cAuNPs) was added to the copper coated grid and allowed to dry before Transmission electron microscopy (TEM) analysis.

## Data gathering

## Instruments

No data

## Calibration
No data

## Reproducibility
No data

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Reference material/nanomaterial and Sample identification number

| Identifier | Identity |
|---|---|
| reference material/nanomaterial | cAuNPs |

## Test material form
other: nanomaterial

## Confidential details on test material
No data

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Crystallographic composition
None

## Remarks on results incl. tables
Particle size: The nanoparticle size was determined by TEM image analysis. The cAuNPs size was 14 ± 2 nm nanoparticles. The particles were well dispersed.

## Overall remarks, attachments

## Remarks on results including tables and figures
None

## Applicant's summary and conclusion

## Conclusions

Under the test conditions, cAuNPs were found to be well dispersed with a narrow size distribution.

## Executive summary

A study was conducted to determine the crystalline phase of gold nanoparticles (14 nm cAuNPs) by Transmission electron microscopy. 5 drops of a concentrated sample (cAuNPs) was added to the copper coated grid and allowed to dry before Transmission electron microscopy (TEM) analysis.
The nanoparticle size was determined by TEM image analysis. The cAuNPs size was 14 ± 2 nm nanoparticles. The particles were well dispersed.

Under the test conditions, cAuNPs were found to be well dispersed with a narrow size distribution.

## Cross-reference to other study

No cross-reference

# *Endpoint study record: 7440-57-5, Crystalline phase - x-ray diffraction, Anonymous, 2013, RS, K*

## Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2013 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Turkevich J, Stevenson PC and Hillier J. | 1951 | A Study of the Nucleation and Growth Process in the Synthesis of Colloidal Gold. | Discuss. Faraday Soc. 11: 55-75 | | | | | |
| study report | Anonymous | 2012 | Transmission Electron Microscopy Analysis of Nanoparticles | | | | | | |

## Data access

other: data submitter is data owner or has Letter of Access

## Data protection claimed
yes

## Cross-reference to same study
Section 4.25: 7440-57-5, Crystalline phase - Transmission electron microscopy, Anonymous, 2012, RS, K

## Materials and methods

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

## Method
x-ray diffraction (XRD)

## Principles of method if other than guideline
A study was conducted to determine the crystalline phase of gold nanoparticles (14 nm cAuNPs) by X-ray diffractometer.

## Details on methods and data evaluation
The Powder X-ray diffraction (p-XRD) analysis of drop-coated films of cAuNPs on glass was performed on the high resolution Bruker D8 Advance Powder Diffractometer, fitted with a LinxEye detector, Fe-filter and Co-Kα radiation operated at 35 kV and 40 mA with a wavelength K-alpha1 as 1.78897 Å. XRD patterns were recorded in the range of 3° and 80° [2θ] angle, and a step size of 0.02° [2θ] with a counting time of 3 seconds per step.

## Data gathering

## Instruments
The Powder X-ray diffraction (p-XRD) analysis of drop-coated films of cAuNPs on glass was performed on the high resolution Bruker D8 Advance Powder Diffractometer, fitted with a LinxEye detector, Fe-filter and Co-Kα radiation operated at 35 kV and 40 mA with a wavelength K-alpha1 as 1.78897 Å.

## Calibration
No data

## Reproducibility
No data

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Reference material/nanomaterial and Sample identification number

| Identifier | Identity |
|---|---|
| reference material/nanomaterial | cAuNPs |

## Test material form

other: nanomaterial

## Confidential details on test material

No data

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Crystalline Phase

**Common Name** Gold nanoparticles (cAuNPs)

**Crystal System** cubic

**Bravais lattice** face - centred cubic

**Crystallographic planes** Bragg reflections, i.e. (111), (200) and (220)

## Crystallographic composition

None

## Remarks on results incl. tables

XRD results obtained shows Bragg reflections, i.e. (111), (200) and (220), which typifies gold metal with a face centred cubic (fcc) structure. However, the broadness of the peaks confirms that the 14 nm cAuNPs were not crystalline enough during analysis. This is because the size of the crystalline domains within a nanoparticle is dependent on the fabrication method, which can minimize the size of crystalline domains within particles.
See the attached document for figure: X-ray diffraction (XRD) of cAuNPs

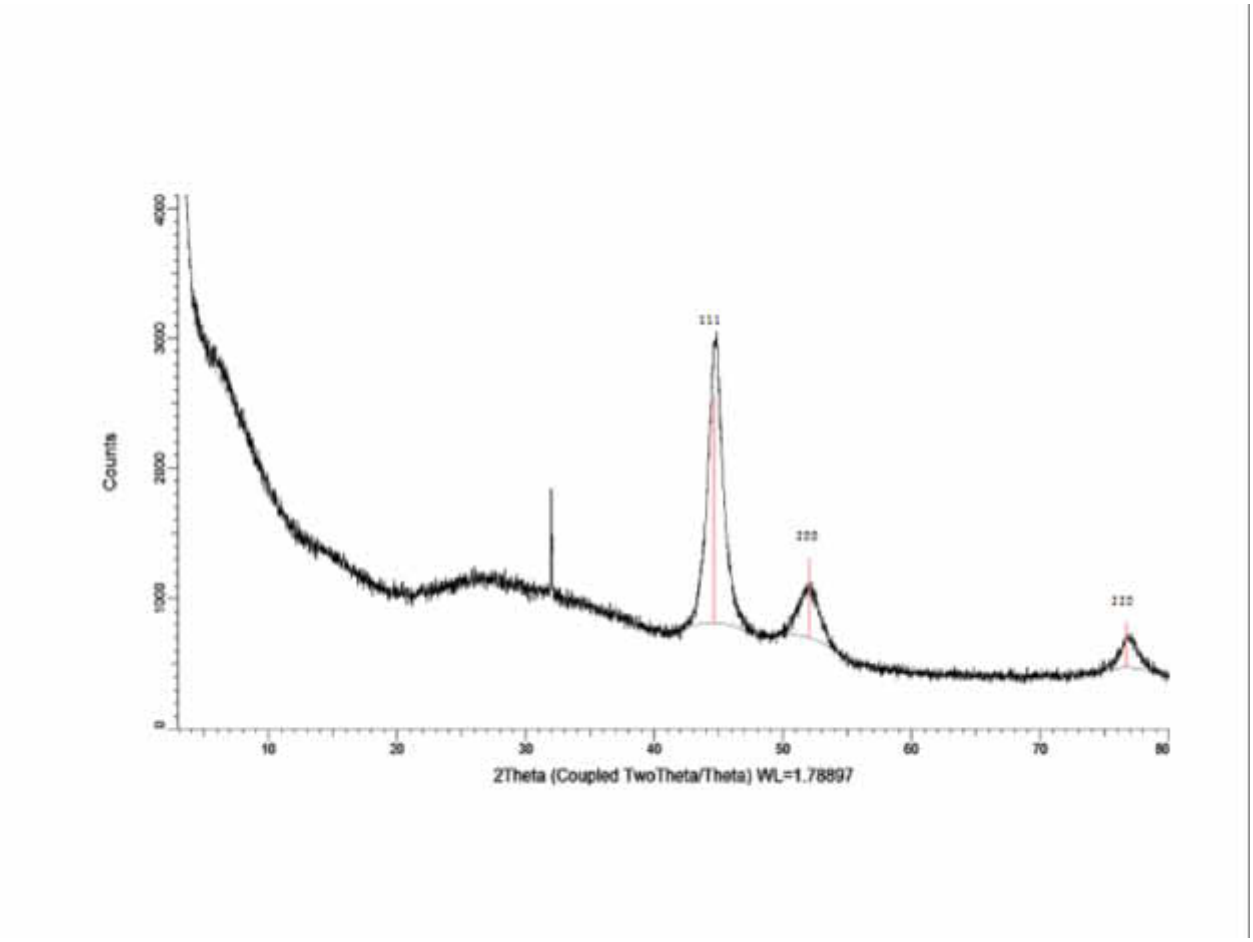
## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Attached background material

**Attached document** Crystalline Phase - X-ray diffraction (XRD) of cAuNPs.pdf / 25.46 KB (application/octet-stream)




## Applicant's summary and conclusion

## Conclusions

Under the test conditions, XRD results obtained shows Bragg reflections, i.e. (111), (200) and (220), which typifies gold metal with a face centred cubic (fcc) structure. However, the broadness of the peaks confirms that the 14 nm cAuNPs were not crystalline enough during analysis.

## Executive summary

A study was conducted to determine the crystalline phase of gold nanoparticles (14 nm cAuNPs) by X-ray diffractometer. The Powder X-ray diffraction (p-XRD) analysis of drop-coated films of cAuNPs on glass was performed on the high resolution Bruker D8 Advance Powder Diffractometer, fitted with a LinxEye detector, Fe-filter and Co-Kα radiation operated at 35 kV and 40 mA with a wavelength K-alpha1 as 1.78897 Å. XRD patterns were recorded in the range of 3° and 80° [2θ] angle, and a step size of 0.02° [2θ] with a counting time of 3 seconds per step.

The XRD results obtained shows Bragg reflections, i.e. (111), (200) and (220), which typifies gold metal with a face centred cubic (fcc) structure. However, the broadness of the peaks confirms that the 14 nm

cAuNPs were not crystalline enough during analysis. This is because the size of the crystalline domains within a nanoparticle is dependent on the fabrication method, which can minimize the size of crystalline domains within particles.

Under the test conditions, XRD results obtained shows Bragg reflections, i.e. (111), (200) and (220), which typifies gold metal with a face centred cubic (fcc) structure. However, the broadness of the peaks confirms that the 14 nm cAuNPs were not crystalline enough during analysis.

### Cross-reference to other study

No cross-reference

## 4.26 Crystallite and grain size

### *Endpoint study record: 7440-57-5, Crystallite and grain size, Author, Year, Summary level, Study level*

### Administrative Data

**Study result type** experimental result

**Reliability** other: no data

**Rationale for reliability incl. deficiencies** No data

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| other: no data | | | | | | | | | |

### Data access

other: no data

### Cross-reference to same study

No cross-reference

### Materials and methods

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

### Method

x-ray diffraction (XRD)

### Principles of method if other than guideline
No data

### Details on methods and data evaluation
No data

### Data gathering

### Instruments
No data

### Calibration
No data

### Reproducibility
No data

### GLP compliance
no data

### Test materials

### Identity of test material same as for substance defined in section 1 (if not read-across)
CAS number

### Test material form
nanomaterial

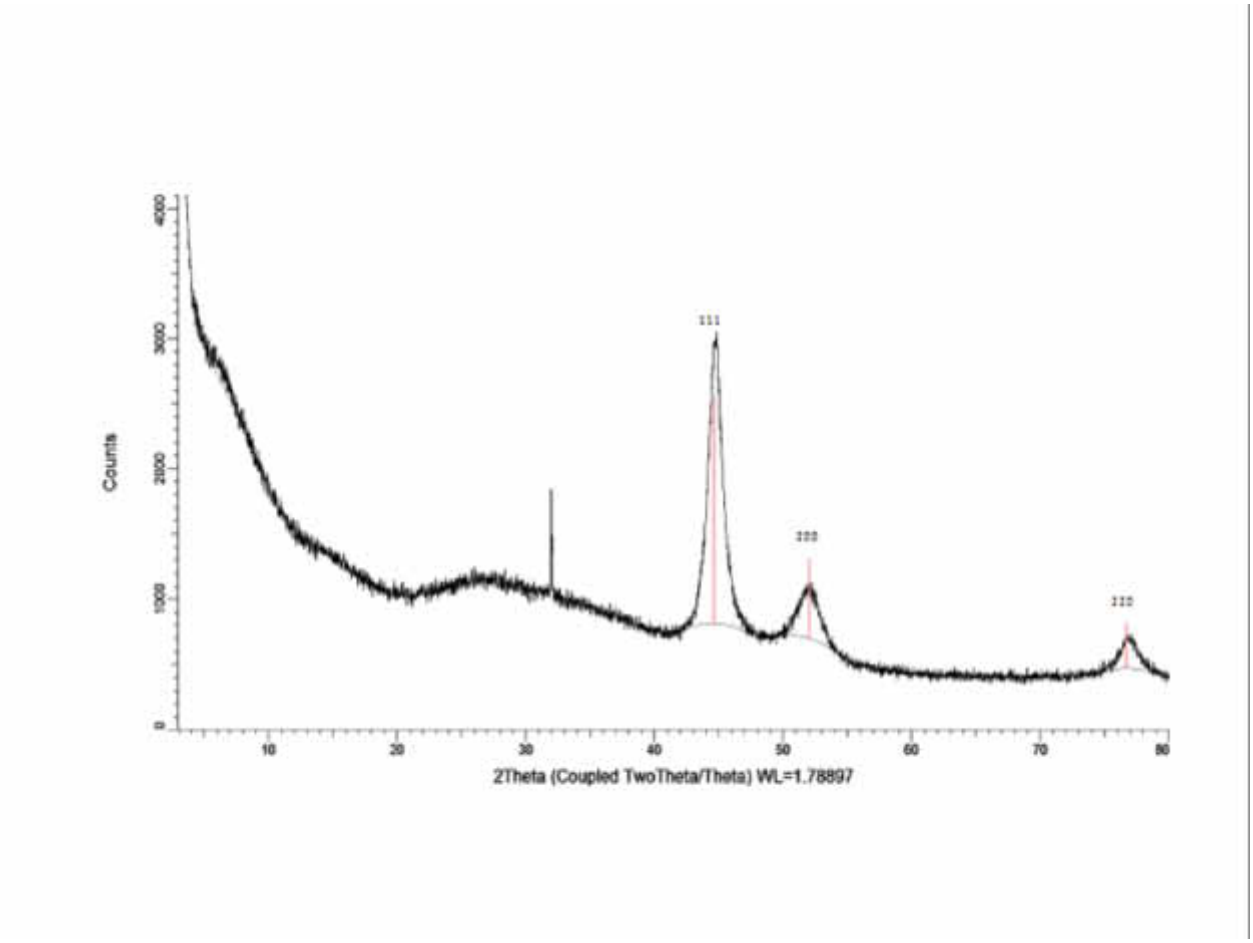
### Any other information on materials and methods incl. tables
None

### Results and discussions

### Remarks on results including tables and figures
As indicated earlier (vide supra), the XRD results (see the attached document - figure) suggest not only that the crystallite are smaller, but presents amorphous characteristics. Thus, size was not calculated.

## Attached background material

**Attached document** X-ray diffraction (XRD) of cAuNPs.doc / 63 KB (application/octet-stream)




## Remarks on results incl. tables

None

## Applicant's summary and conclusion

## Conclusions

Not available

## Executive summary

Not available

## Cross-reference to other study

No cross-reference

## 4.27 Aspect ratio/shape

## 4.28 Specific surface area

### *Endpoint study record: 7440-57-5, Specific surface area, Author, Year, Summary level, Study level*

#### Administrative Data

**Study result type** experimental result **Study period** No data

**Reliability** other: no data

**Rationale for reliability incl. deficiencies** No data

#### Data source

#### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| other: no data | | | | | | | | | |

#### Data access

other: no data

#### Cross-reference to same study

No cross-reference

#### Materials and methods

#### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

#### Methods

BET

#### Principles of method if other than guideline

No data

#### Details on methods and data evaluation

Owing to the amount of sample required in addition to the cumbersome nature of BET technique for minute samples, surface area determination by BET technique was aborted.

**Data gathering**

**Instruments**
Not applicable

**Calibration**
Not applicable

**Reproducibility**
Not applicable

**GLP compliance**
no data

**Test materials**

**Test material form**
other: nanomaterial

**Any other information on materials and methods incl. tables**
None

**Overall remarks, attachments**

**Remarks on results including tables and figures**
None

**Results and discussions**

**Remarks on results incl. tables**
None

**Applicant's summary and conclusion**

**Conclusions**
No data

**Executive summary**
No data available

**Cross-reference to other study**
No cross-reference

# 4.29 Zeta potential

## *Endpoint study record: 7440-57-5, Zeta potential, Anonymous, 2010, RS, K*

### Administrative Data

**Purpose flag** key study; robust study summary

**Study result type** experimental result **Study period** 2010

**Reliability** other: no data

**Rationale for reliability incl. deficiencies** No data

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | no data | 2010 | no data | | | | | | |

### Data access

other: data submitter is data owner or has Letter of Access

### Data protection claimed

yes

### Cross-reference to same study

No cross-reference

### Materials and methods

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | other guideline: Guidance manual for the testing of manufactured nanomaterials [OECD's sponsorship programme, ENV/JM/MONO(2009)20/REV] | no data |

### Methods

other: Laser Doppler Micro-electrophoresis

### Principles of method if other than guideline

Not applicable

### Details on methods and data evaluation

The surface charge of the 14 nm cAuNPs was established by measuring the zeta potential using a Malvern

zetasizer machine.Dilution water source: deionised water (18 MΩ.cm)Stock and test solutions preparation: cAuNPs suspension was diluted with deionised water.Stability of the test chemical solutions: Stable at low temperature, 4°C for 1 yearExposure vessel type: 50 mL polypropylene conical tubeTest temperature range: 25 °C

## Data gathering

## Instruments

Malvern zetasizer machine was used to measuring the zeta potential.

## Calibration

None

## Reproducibility

None

## GLP compliance

no

## Test materials

## Test material form

other: nanomaterial

## Confidential details on test material

Stock and test solutions preparation: cAuNPs suspension was diluted with deionised water.Stability of the test chemical solutions: Stable at low temperature, 4°C for 1 year

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Zeta Potential

**Zeta Potential** -32.2 mV

**in medium** deionised water (18 MΩ.cm)

**Remarks** high surface charge confers stability to the particles as they repel each other in solution.

## Remarks on results incl. tables

None

### Overall remarks, attachments

### Remarks on results including tables and figures
None

### Applicant's summary and conclusion

### Conclusions
Under the test conditions, the zeta potential for 14 nm cAuNPs is -32.2 mV, thus the particles have a high surface charge that confers stability over a long period of time, i.e. ≥ 1 year.

### Executive summary
A study was conducted to determine the surface charge of gold nanoparticles (14 nm cAuNPs) by measuring the zeta potential using a Malvern zetasizer machine. Test conditions were as follows:
Dilution water source: deionised water (18 MΩ.cm)
Stock and test solutions preparation: cAuNPs suspension was diluted with deionised water.
Stability of the test chemical solutions: Stable at low temperature, 4° C for 1 year
Exposure vessel type: 50 mL polypropylene conical tube
Test temperature range: 25 °C
The zeta potential recorded was -32.2 mV. This shows that the particles are negatively charged. This is in agreement with the fact that the surface coating is citrate molecules. Such high surface charge confers stability to the particles as they repel each other in solution.
Under the test conditions, the zeta potential for 14 nm cAuNPs is -32.2 mV, thus the particles have a high surface charge that confers stability over a long period of time, i.e. ≥ 1 year.

### Cross-reference to other study
No cross-reference

## 4.30 Surface chemistry

## 4.31 Dustiness

## 4.32 Porosity

## 4.33 Pour density

## 4.34 Photocatalytic activity

## 4.35 Radical formation potential

## 4.36 Catalytic activity

# 5. ENVIRONMENTAL FATE AND PATHWAYS

# 6. ECOTOXICOLOGICAL INFORMATION

## 6.1 Aquatic toxicity

### 6.1.1 Short-term toxicity to fish

### *Endpoint study record: 7440-57-5, Short-term toxicity to fish, Anonymous, 2013, RS, K*

#### Administrative Data

**Purpose flag** key study; robust study summary

**Study result type** experimental result **Study period** 2012-2013

**Reliability** 2 (reliable with restrictions)

**Rationale for reliability incl. deficiencies** Non-GLP study conducted according to OECD Guideline 203 with deviations: details on test item, pH of water, no. of fishes per concentration, test procedure and statistical procedures not reported

#### Data source

#### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | Anonymous | 2013 | No data | | | | | | |

#### Data access

other: data submitter is data owner or has letter of access

#### Data protection claimed

yes, but willing to share

#### Cross-reference to same study

No cross-reference

#### Materials and methods

#### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 203 (Fish, Acute Toxicity Test) | yes (details on test item, pH of water, no. of fishes per concentration, test procedure and statistical procedures not reported) |

## Principles of method if other than guideline
Not applicable

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS- Source: Mintek, South Africa

## Confidential details on test material
No data

***Details on properties of test surrogate or analogue material***
No data

## Analytical monitoring
no

***Details on sampling***
Not applicable

***Details on analytical methods***
Not applicable

## Vehicle
no data

## Test organisms

## Test organisms (species)
other: Danio rerio, and Poecilia reticulata and indigenous southern African species Pseudocrenilabrus philander, Tilapia sparrmanii and Labeobarbus aeneus

***Details on test organisms***

TEST ORGANISM
- Common name: Fish
- Source: Test organisms were cultured at Centre for Aquatic Research laboratories using protocols developed in-house over the past 15 years.
- Parental fish were held in 150 L aquaria.

ACCLIMATION
- Acclimation period: Fry were collected from laboratory 7-12 days prior to start of test to allow for acclimation to test conditions.
- Acclimation conditions (same as test or not): Yes
- Type and frequency of food: Fish were fed daily with NutriMin® flake food and supplemented with frozen Chironomus sp. (bloodworms) every other day.
- Health during acclimation (any mortality observed): The brood stock was visually checked every working day for mortality, illness or abnormal behaviour. No prophylactic treatment of fish took place. Only healthy fish without diseases and abnormalities were used as parental fish for the production of fertilised eggs.

## Study design

## Test type

no data

## Water media type

freshwater

## Limit test

no

## Total exposure duration

96 h

***Post exposure observation period***

None

## Test conditions

***Hardness***

Water Media Type: ISO moderately hard freshwater (1/5 strength) was used as test water and to prepare the test suspension (58.8 mg CaCl2.2H2O; 24.7 mg MgSO4.7H2O; 13.0 mg NaHCO3; 1.15 mg KCl) corresponding to 76 - 100 % Dissolved Oxygen; 271 - 328 µS/cm (conductivity)

***Test temperature***

Holding temperature: Between 22-26 °C ± 2 °C according to test species.

***pH***

No data

***Dissolved oxygen***
76 - 100 % Dissolved Oxygen

***Salinity***
No data

***Nominal and measured concentrations***
- Nominal concentration: 100 mg/L as stock solution and 0.16 to 45 mg/L

***Details on test conditions***
TEST MEDIUM / WATER PARAMETERS
- Holding water: A mixture of RO (reverse osmosis) water and tap water (10%) was used as holding water.
- The following water chemistry data are recorded regularly in the testing facility: pH, conductivity, dissolved oxygen content, DOC content, temperature and TDS. During preparation and performance of the test, all values were within the admissible ranges.
- Conductivity: 271 - 328 µS/cm
- Intervals of water quality measurement: No renewal was undertaken since no visible deposition of nanogold appeared during the exposure period and water quality conditions remained within acceptable ranges.

OTHER TEST CONDITIONS
- Photoperiod: Light/dark cycle was 12 h/12 h
- Particle size distribution and zeta potential of gold in water was determined at the onset of the test and upon completion of the 96 h exposure period.

OTHERS:
- The test beakers were filled with moderately hard water and allowed to stabilise for 24 h prior to the onset of the exposures.
- The dilution series for the exposures were prepared on the day of commencing exposures using a stock solution consisting of 100 mg/l cAuNPs, which were added to the test vessels to achieve the nominal concentrations in each test vessel.
- The test organisms were introduced into the test vessels on the same day and exposed to the different concentrations for a period of 96 h.
- As there was no visible agglomeration or sedimentation of gold nanoparticles during the exposure period it was assumed that there was a homogenous distribution of particles and no agitation was applied.
- Concomitant exposures of cAuNPs DIS were undertaken where the same volume of dispersant without any nanomaterials was added to the exposure beakers.

***Reference substance (positive control)***
no

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Effect concentrations

| Duration | Endpoint | Effect conc. | Nominal/Measured | Conc. based on | Basis for effect | Remarks (e.g. 95% CL) |
|---|---|---|---|---|---|---|
| 96 h | LC50 | > 45 mg/L | nominal | test mat. | mortality | |

***Details on results***

- Mortality of control: Mortalities in the control groups ranged between 0 and 4 % and were within acceptable levels.
- Mortality in test item group: Mortalities are not over 20 % for any of the exposure concentrations (range between 0.16 to 45 mg/L). Highest percentage mortalities (20-30 %) for cAuNPs were recorded between 10-20 mg/L for the five species tested.
- Toxicity generally decreased with an increase in exposure concentrations above 15 mg/L.
- Characterisation indicated that mean particle size increased at concentrations above 10 mg/L due to agglomeration.
- Toxicity of dispersant without the nanomaterials was generally more toxic than exposures including the nanomaterials, indicating an antagonisticeffect of the nanogold on the dispersant toxicity. However toxicities never increased above 50 %.

***Results with reference substance (positive control)***

Not applicable

***Reported statistics and error estimates***

No data

## Any other information on results incl. tables

None

## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Validity criteria fulfilled

yes

## Conclusions

Under the test conditions, the 96 h LC50 is greater than 45 mg/L in five species. The dispersant cAuNPs DIS was more toxic than the nanogold (cAuNPs). Highest percentage mortalities (20-30%) for cAuNPs were recorded between 10-20 mg/l for the five species tested.

## Executive summary

In an acute aquatic toxicity study, performed in accordance with OECD Guideline 203, 5 species of fishes (Danio rerio, and Poecilia reticulata and indigenous southern African species Pseudocrenilabrus philander,

Tilapia sparrmanii and Labeobarbus aeneus) were exposed to Dispersant cAuNPs DIS or Gold nanoparticles (cAuNPs) at the concentrations ranging from 0.16 to 45 mg/L for 96 h. Control group was also studied simultaneously. Mortality was recorded.
Mortalities were not over 20 % for any of the exposure concentrations (range between 0.16 to 45 mg/L). Highest percentage mortalities (20-30 %) for cAuNPs were recorded between 10-20 mg/L for the five species tested. Toxicity generally decreased with an increase in exposure concentrations above 15 mg/L. Mortalities in the control groups ranged between 0 and 4 % and were within acceptable levels. Toxicity of dispersant without the nanomaterials was generally more toxic than exposures including the nanomaterials, indicating an antagonistic effect of the nanogold on the dispersant toxicity. However toxicities never increased above 50 %.
Under the test conditions, the 96 h LC50 is greater than 45 mg/L in five species. The dispersant cAuNPs DIS was more toxic than the nanogold (cAuNPs). Highest percentage mortalities (20-30%) for cAuNPs were recorded between 10-20 mg/l for the five species tested.

### Cross-reference to other study

No cross-reference

## 6.1.2 Long-term toxicity to fish

## *Endpoint study record: 7440-57-5, Long-term toxicity to fish, Anonymous, 2013, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2012-2013 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | Anonymous | 2013 | No data | | | | | | |

### Data access

other: data submitter is data owner or has letter of access

### Data protection claimed

yes, but willing to share

## Cross-reference to same study
No cross-reference

## Materials and methods

## Life stage / endpoint studied
other: Fish, test with fish embryos)

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | other guideline: OECD Draft guideline (Fish, test with fish embryos) | no data |

## Principles of method if other than guideline
Not applicable

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS
- Source: Mintek, South Africa

## Confidential details on test material
No data

***Details on properties of test surrogate or analogue material***
No data

## Analytical monitoring
no

***Details on sampling***
Not applicable

***Details on analytical methods***
Not applicable

## Vehicle
no data

## Test organisms

## Test organisms (species)
Danio rerio

***Details on test organisms***
TEST ORGANISM
- Source: Danio rerio, was cultured at Fraunhofer IME under inbreeding conditions since more than 20 years.
- Parental fish were held in 150 L aquaria. At time of egg collection, parental fish were about 18 months old (maximum age for parental fish is 2 years).
- Stock density was approximately 80 fish per vessel.

ACCLIMATION
- Type and frequency of food: Fish were fed daily ad libitum with TetraMinR Hauptfutter (Tetra Werke, Melle, Germany) and brine shrimp nauplii (Artemia salina).
- Health during acclimation: The broodstock were visually checked every working day for mortality, illness, parasites or abnormal behaviour. No prophylactic treatment of fish took place. Only healthy fish without diseases and abnormalities were used as parental fish for the production of fertilised eggs.- Fertilisation rate was checked to fulfil the quality criterion of at least 50 % for accepting the batch as parental fish for the production of fertilised eggs for a study.

METHOD FOR PREPARATION AND COLLECTION OF FERTILIZED EGGS
- Method of collection of fertilised eggs: Eggs were collected with spawning-trays (made of glass) that were placed at the bottom of the holding vessels. The trays were covered with a lattice (stainless steel), to prevent the adults from predating on the eggs, and artificial plant substrate to stimulate spawning into the tray). Lighting (one neon lamp per vessel, light intensity approximately 1000 lux, measured 5 cm above the water surface in the middle of the test vessel) induced mating of fish and spawning. The collected eggs were transferred from the spawning-tray onto a sieve, rinsed with clean water in order to remove faeces and food waste, put into glass dishes and incubated at 26 °C.

## Study design

## Test type
static

## Water media type
freshwater

## Limit test
no

## Total exposure duration

96 h

***Post exposure observation period***

None

## Test conditions

***Hardness***

Water Media Type: Water Media Type: ISO moderately hard freshwater (1/5 strength) was used as test water and to prepare the test suspension (58.8 mg CaCl2.2H2O; 24.7 mg MgSO4.7H2O; 13.0 mg NaHCO3; 1.15 mg KCl) corresponding to 76 - 100 % Dissolved Oxygen; 271 - 328 µS/cm (conductivity)

***Test temperature***

26.0 ± 1 °C

***pH***

No data

***Dissolved oxygen***

76 - 100 % Dissolved Oxygen

***Salinity***

No data

***Nominal and measured concentrations***

Dispersant cAuNPs DIS - 10 and 50 % cAuNPs (gold in dispersant) - 0.004 to 22 mg/L

***Details on test conditions***

TEST SYSTEM
- Test vessel: Polystyrene multi-well dishes (24 wells; NUNC, Denmark) with a total volume of 5 mL per well and flat bottom were used as test vessels.
- Type: Closed- After 24 h and 48 h coagulated eggs and abnormalities in genesis were recorded. After 72 and 96 h hatching behaviour was documented. After collecting the eggs, a pool of 50 – 100 undifferentiated eggs was transferred with a widened and deburred pipette tip into each of the beakers prepared with test dispersion and control water to guarantee an exposure to the test substance in the early genesis state. Time from spawning until transfer into the test solutions did not exceed one hour.
- No. of fertilized eggs per well: One fertilised egg/well
- No. of wells per concentration (replicates): Other 20 wells were filled with the test dispersions (2 mL per well).
- No. of wells per control (replicates): 4 wells for the control, which was filled with 2 mL ISO water (1/5 strength) per well.
- Renewal rate of test solution (frequency/flow rate): The flow through rate was adjusted to achieve a 2-fold exchange of water per day.

OTHER TEST CONDITIONS
- Photoperiod: Light/dark cycle of 12/12 h

EFFECT PARAMETERS MEASURED:
- All eggs (20 in the control and 20 in every test concentration) were observed and evaluated every 24 h, using an inverse microscope.
- Embryos were checked for the following abnormalities after 24 h (coagulation, absence of somites, tails not separated from yolk sacs, no eye development, no spontaneous movement) and 96 h (coagulation, no heartbeat, no circulation, no pigmentation, oedema).

***Reference substance (positive control)***

no

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Effect concentrations

| Duration | Endpoint | Effect conc. | Nominal/Measured | Conc. based on | Basis for effect | Remarks (e.g. 95% CL) |
|---|---|---|---|---|---|---|
| 96 h | NOEC | > 22 mg/L | nominal | test mat. | other: embryo abnormalities | |

***Details on results***

- Tests with the dispersant cAuNPs DIS showed a concentration-effect relationship for abnormities of the embryos. At 50 % dispersant all embryos died. In the presence of 10 % dispersant the larvae hatched after a reduced embryo development period. Some of them showed a lower heartbeat or missing blood circulation.
- In contrast, cAuNPs (gold in dispersant) caused no abnormities after 24 and 48 h. Heartbeat and hatching behaviour were comparable to the control.

***Results with reference substance (positive control)***

Not applicable

***Reported statistics and error estimates***

No data

## Any other information on results incl. tables

None

## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Validity criteria fulfilled

yes

### Conclusions

The dispersant on its own (cAuNPs DIS) showed a high toxicity. This toxicity is reduced in the presence of gold nanoparticles with no toxicity being observed at concentrations between of 0.004 and 22 mg/L.

### Executive summary

In a chronic toxicity to fish, fertilised eggs of*Danio rerio*was exposed to Dispersant cAuNPs DIS (10 and 50 %) or Gold nanoparticles (cAuNPs at 0.004 to 22 mg/L) for 96 h under static conditions. Control group was also studied simultaneously. All eggs (20 in the control and 20 in every test concentration) were observed and evaluated every 24 h, using an inverse microscope. Embryos were checked for the following abnormalities after 24 h (coagulation, absence of somites, tails not separated from yolk sacs, no eye development, no spontaneous movement) and 96 h (coagulation, no heartbeat, no circulation, no pigmentation, oedema).

Tests with the dispersant cAuNPs DIS showed a concentration-effect relationship for abnormities of the embryos. At 50% dispersant all embryos died. In the presence of 10 % dispersant the larvae hatched after a reduced embryo development period. Some of them showed a lower heartbeat or missing blood circulation. In contrast, cAuNPs (gold in dispersant) caused no abnormities after 24 and 48 h. Heartbeat and hatching behaviour were comparable to the control.

The dispersant on its own (cAuNPs DIS) showed a high toxicity. This toxicity is reduced in the presence of gold nanoparticles with no toxicity being observed at concentrations between of 0.004 and 22 mg/L.

### Cross-reference to other study

No cross-reference

## 6.1.3 Short-term toxicity to aquatic invertebrates

## *Endpoint study record: 7440-57-5, Short-term toxicity to aquatic invertebrates (Daphnia), Anonymous, 2013, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2012-2013 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Non-GLP study conducted according to OECD Guideline 202 with deviations: details on test item, pH of water, no. of daphnids per concentration and summary tables of results not reported; light/dark cycle of 12/12 h was maintained instead of recommended 16 h light and 8 h dark cycle | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | Anonymous | 2013 | No data | | Frauenhofer Institute | | | | |

## Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) | yes (details on test item, pH of water, no. of daphnids per concentration and summary tables of results not reported; light/dark cycle of 12/12 h was maintained instead of recommended 16 h light and 8 h dark cycle ) |

## Principles of method if other than guideline

Not applicable

## GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS
- Source: Mintek, South Africa

## Confidential details on test material
No data

***Details on properties of test surrogate or analogue material***
No data

## Analytical monitoring
no

***Details on sampling***
Not applicable

***Details on analytical methods***
Not applicable

## Vehicle
no data

## Test organisms

## Test organisms (species)
other: Daphnia magna and Daphnia pulex

***Details on test organisms***
TEST ORGANISM
- Source: Daphnia spp. were cultured at Fraunhofer IME and at the Centre for Aquatic Research laboratories using protocols developed in-house over the past number of years.
- Age at study initiation: Young female Daphnia (parent animals) aged less than 24 h

ACCLIMATION
- Acclimation period: One week; Batches of 30 to 50 animals of at least 3 weeks old adult Daphnia were held at room temperature in approx. 1.8 L dilution water.
- Type and frequency of food: Daphnids were fed daily with an algal suspension (Desmodesmus subspicatus) and LiquizellR (HOBBY). At the Centre for Aquatic Research Daphnia were fed with a suspension of 6.3 g trout pellets, 2.6 g brewers yeast and 0.5 g alfalfa. 30 mL of this suspension were given to 1 L Daphnia medium.

## Study design

## Test type
static

## Water media type
freshwater

## Limit test

no

## Total exposure duration

48 h

**Remarks** The daphnids were exposed without aeration.

***Post exposure observation period***

None

## Test conditions

***Hardness***

Water Media Type: ISO moderately hard freshwater (1/5 strength) was used as test water and to prepare the test suspension (58.8 mg CaCl2.2H2O; 24.7 mg MgSO4.7H2O; 13.0 mg NaHCO3; 1.15 mg KCl) corresponding to 76 - 100 % Dissolved Oxygen; 271 - 328 µS/cm (conductivity)

***Test temperature***

20–21 °C ± 1.5°C

***pH***

No data

***Dissolved oxygen***

76 - 100 % Dissolved Oxygen

***Salinity***

No data

***Nominal and measured concentrations***

cAuNPs: Daphnia pulex were exposed to a nominal concentration range of 0.5 to 20 mg/L.cAuNPs DIS: up to 100 % at the highest dispersant concentration

***Details on test conditions***

TEST SYSTEM
- Test vessel: Plastic apparatus
- Aeration: ISO water was used as holding- and dilution water and was aerated for 24 h.
- Renewal rate of test solution: Water was changed once per week.

TEST MEDIUM / WATER PARAMETERS
- Source/preparation of dilution water: ISO water was used as holding- and dilution water
- Conductivity: 271 - 328 µS/cm
- The following water chemistry data were regularly recorded in the testing facility, and were: pH, conductivity, dissolved oxygen.

OTHER TEST CONDITIONS
- Photoperiod: Light/dark cycle of 12 h/12 h

- Light intensity: Not more than 1000 lux

EFFECT PARAMETERS MEASURED:
- Immobilisation was recorded after 24 and 48 h of exposure.

OTHERS:
Immobilisation in the treatments and in the control were analysed for statistically significant differences using appropriate statistical methods.

***Reference substance (positive control)***
no

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Effect concentrations

| Duration | Endpoint | Effect conc. | Nominal/Measured | Conc. based on | Basis for effect | Remarks (e.g. 95% CL) |
|---|---|---|---|---|---|---|
| 48 h | LC50 | > 20 mg/L | nominal | test mat. | mobility | |

***Details on results***
Immobility:
- In the control and in the test vessels containing the gold dispersion, no immobilisation was detected after an incubation period of 24 h; 5 % immobilisation occurred after an incubation period of 48 h.
- Immobility was only recorded at 10 mg/L with maximum immobility recorded of 33 % at 20 mg/L.
- The dispersant (cAuNPs DIS) displayed a concentration-effect relationship and had greater toxicity than the nanogold and dispersant mixture.
- In the presence of the dispersant, the toxicity of cAuNPs (gold nanoparticles) decreased, whereas the toxicity of the dispersant alone was much more toxic (i.e. up to 100 % at the highest dispersant concentration).

***Results with reference substance***
Not applicable

***Reported statistics and error estimates***
No data

## Any other information on results incl. tables
None

## Overall remarks, attachments

## Remarks on results including tables and figures
None

### Applicant's summary and conclusion

### Validity criteria fulfilled
yes

### Conclusions
In the presence of the dispersant, the toxicity of cAuNPs (gold nanoparticles) decreased, whereas the toxicity of the dispersant alone was much more toxic (i.e. up to 100 % at the highest dispersant concentration). The maximum effect of 33 % immobility was recorded at 20 mg/L cAuNPs.

### Executive summary
In an acute aquatic toxicity study, performed in accordance with OECD Guideline 202, Daphnia spp. Were exposed to cAuNPs at 0.5 to 20 mg/L and cAuNPs DIS at up to 100 % dispersant concentration for 48 h under static conditions. Control group was also studied simultaneously. Immobilisation was recorded after 24 and 48 h.
In the control and in the test vessels containing the gold dispersion, no immobilisation was detected after an incubation period of 24 h; 5 % immobilisation occurred after an incubation period of 48 h. Immobility was only recorded at 10 mg/L with maximum immobility recorded of 33 % at 20 mg/L. The dispersant (cAuNPs DIS) displayed a concentration-effect relationship and similarly to the fish toxicity tests had greater toxicity than the nanogold and dispersant mixture.
In the presence of the dispersant, the toxicity of cAuNPs (gold nanoparticles) decreased, whereas the toxicity of the dispersant alone was much more toxic (i.e. up to 100 % at the highest dispersant concentration). The maximum effect of 33 % immobility was recorded at 20 mg/L cAuNPs.

### Cross-reference to other study
No cross-reference

## 6.1.4 Long-term toxicity to aquatic invertebrates

## 6.1.5 Toxicity to aquatic algae and cyanobacteria

## *Endpoint study record: 7440-57-5, Toxicity to aquatic algae, Anonymous, 2013, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2012-2013 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Non-GLP study conducted according to OECD Guideline 201 with deviations: details on test item, concentrations of test substance, replicates, incubation temperature, results of biomass concentration not reported | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | Anonymous | 2013 | No data | | Fraunhofer Institute and University of Johannesburg | | | | |

## Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 201 (Alga, Growth Inhibition Test) | yes (details on test item, concentrations of test substance, replicates, incubation temperature, results of biomass concentration not reported ) |

## Principles of method if other than guideline

Not applicable

## GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS
- Source: Mintek, South Africa

## Confidential details on test material
No data

***Details on properties of test surrogate or analogue material***
No data

## Analytical monitoring
no

***Details on sampling***
Not applicable

***Details on analytical methods***
Not applicable

## Vehicle
no data

## Test organisms

## Test organisms (species)
Pseudokirchnerella subcapitata

***Details on test organisms***
TEST ORGANISM
- Source (laboratory, culture collection): Pseudokirchnerella subcapitata were cultured at Fraunhofer IME and at the Centre for Aquatic Research laboratories using protocols developed in-house over the past number of years.
- Method of cultivation: Three days prior to testing, a pre-culture of the test alga Pseudokirchnerella subcapitata was established in sterile growth medium, according to test OECD guideline no. 201, to obtain exponentially growing algae. All stock solutions for the OECD medium were prepared with purified water processed using an ELGA "PURELAB Ultra". Cell concentrations were calculated using an electronic particle counter (CASY 1 Model TT, Schärfe System, Reutlingen, Germany). The cultures were kept in suspension by rotary shaking at 100 rpm on a Multitron Incubation Shaker (INFORS, Switzerland).

## Study design

## Test type
no data

## Limit test
no

## Total exposure duration
72 h

***Post exposure observation period***
None

## Test conditions

***Hardness***
No data

***Test temperature***
No data

***pH***
No data

***Dissolved oxygen***
No data

***Salinity***
No data

***Nominal and measured concentrations***
No data

***Details on test conditions***
TEST SYSTEM
- Test vessel: 24 and 96 well microplate method

GROWTH MEDIUM
- Standard medium used: Yes; OECD growth medium

WATER MEDIA TYPE: OECD algal medium (TG201)

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) :
- Determination of cell concentrations: Cell concentrations were calculated using an electronic particle counter (CASY 1 Model TT, Schärfe System, Reutlingen, Germany).
- Chlorophyll measurement: Yes
- In the test, algal biomass was determined after 0, 24, 48 and 72 h by recording the fluorescence intensity using a Tecan Spectrafluorplus microtiter plate reader. The fluorescence signal was converted into cell numbers using a calibration curve. For comparative purposes the Algal toxkit F was used by the laboratories at the University of Johannesburg.

***Reference substance (positive control)***
no

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Effect concentrations

| Duration | Endpoint | Effect conc. | Nominal/Measured | Conc. based on | Basis for effect | Remarks (e.g. 95% CL) |
|---|---|---|---|---|---|---|
| 72 h | other: no data | | | | | |

***Details on results***

- Dispersant cAuNPs DIS displaying higher toxicity and this toxicity were reduced in the presence of gold nanoparticles.
- In the presence of the two highest concentrations of cAuNPs (gold nanoparticles in dispersant) and ultrapure water as diluent, the fluorescence at day 0 fell below the background value. Subtraction of the background values resulted in negative values. After 24 h fluorescence values above the background values were determined. It was assumed that the low fluorescence values at test start were not an indicator for toxicity but were due to shading effect of the nanomaterials influencing the fluorescence and absorbance readings.
- EC50 values that were obtained were not deemed reliable and these tests were therefore being repeated and using chlorophyll-a production as an indicator of growth rather than the standard fluorescence.

***Results with reference substance (positive control)***

Not applicable

***Reported statistics and error estimates***

No data

## Any other information on results incl. tables

None

## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Validity criteria fulfilled

no data

## Conclusions

In the presence of the dispersant (citrate buffer) algal growth was stimulated in a dose dependent factor. The interference of the nanogold particles with the fluorescence assay makes TG201 unsuitable to determine the effect of NM-330 on algal growth. It is recommended that an alternative measure of growth, i.e. chlorophyll a concentration be used.

## Executive summary

In an algal toxicity study performed in accordance with OECD guideline 201, Pseudokirchnerella subcapitata was exposed toCitrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS

at two unknown concentrations for 72 h. Cell concentrations were calculated using an electronic particle counter. Algal biomass was determined after 0, 24, 48 and 72 h by recording the fluorescence intensity using a Tecan Spectrafluorplus microtiter plate reader.
Dispersant cAuNPs DIS showed higher toxicity and this toxicity was reduced in the presence of gold nanoparticles. In the presence of the two highest concentrations of cAuNPs (gold nanoparticles in dispersant) and ultrapure water as diluent, the fluorescence at day 0 fell below the background value. Subtraction of the background values resulted in negative values. After 24 h fluorescence values above the background values were determined. It was assumed that the low fluorescence values at test start were not an indicator for toxicity but were due to shading effect of the nanomaterials influencing the fluorescence and absorbance readings. The EC50 values that were obtained were not deemed reliable and these tests are therefore being repeated and using chlorophyll-a production as an indicator of growth rather than the standard fluorescence.
In the presence of the dispersant (citrate buffer) algal growth was stimulated in a dose dependent factor. The interference of the nanogold particles with the fluorescence assay makes TG201 unsuitable to determine the effect of NM-330 on algal growth. It is recommended that an alternative measure of growth, i.e. chlorophyll a concentration be used.

### Cross-reference to other study

No cross-reference

## 6.1.6 Toxicity to aquatic plants other than algae

## 6.1.7 Toxicity to microorganisms

### *Endpoint study record: 7440-57-5, Toxicity to microorganisms, Anonymous, 2013, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | 2012-2013 |
| **Reliability** | 4 (not assignable) | | |
| **Rationale for reliability incl. deficiencies** | This is not a suitable test to determine the effects of nanogold on micro-organisms. | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | Anonymous | 2013 | No data | | | | | | |

### Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | other guideline: ISO 11348-2:2007: Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test). | no data |

## Principles of method if other than guideline

The purpose of the test was to determine the effects of a nanogold suspension on the growth of Vibrio fischeri. Exponentially growing test organisms were exposed to the test substance in batch cultures over a period of thirty minutes.

## GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report):Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS- Source: Mintek, South Africa

## Confidential details on test material

No data

***Details on properties of test surrogate or analogue material***

No data

## Analytical monitoring

no

***Details on sampling***
Not applicable

***Details on analytical methods***
Not applicable

## Vehicle
no data

## Test organisms

## Test organisms (species)
Vibrio fisheri

***Details on inoculum***
No data

## Study design

## Test type
no data

## Limit test
no

## Total exposure duration
30 min

***Post exposure observation period***
None

## Test conditions

***Hardness***
Not applicable

***Test temperature***
22 ± 1 °C

***pH***
No data

***Dissolved oxygen***
Not applicable

***Salinity***
Not applicable

***Nominal and measured concentrations***
Nominal concentrations: 0.10, 0.21, 0.42, 0.83 and 1.67 mg/L

***Details on test conditions***
TEST SYSTEM
- No. of replicates per concentration: 3
- No. of replicates per control: 6

WATER MEDIA TYPE: ISO
Culture medium containing nutrient stocks made up using distilled water.

EFFECT PARAMETERS MEASURED (with observation intervals if applicable):
- Bioluminescence was determined using a Luminometer.

***Reference substance (positive control)***
no

## Results and discussions

## Effect concentrations

| Duration | Endpoint | Effect conc. | Nominal/Measured | Conc. based on | Basis for effect | Remarks (e.g. 95% CL) |
|---|---|---|---|---|---|---|
| 30 min | other: no data | | | | | |

***Details on results***
- At all concentrations, an increase in bioluminescence were recorded indicating that the presence of particles in the exposure media has a direct influence on the method. This is therefore not regarded as a suitable method to determine the toxicity to bacteria.

***Results with reference substance (positive control)***
Not applicable

***Reported statistics and error estimates***
No data

## Any other information on results incl. tables
None

## Overall remarks, attachments

## Remarks on results including tables and figures
None

### Applicant's summary and conclusion

### Validity criteria fulfilled
not applicable

### Conclusions
The dispersant cAuNPs DIS was able to demonstrate effects on bioluminescence but due to the interference of the cAuNPs (gold nanoparticles in dispersant) particles with the bioluminescence it was concluded that this is not a suitable test to determine the effects of nanogold on micro-organisms

### Executive summary
A study was conducted to assess the effect of gold nanoparticles (Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS) on micro-organisms. In this study, *Vibrio fischeri* was exposed to the test substance at the nominal concentrations of 0.10, 0.21, 0.42, 0.83 and 1.67 mg/L and were incubated at 22 ± 1°C over a period of thirty minutes. Twenty cm cuvettes were used with 3 replicates per concentration and 6 replicates for the control. Bioluminescence was determined using a Luminometer.

At all concentrations, an increase in bioluminescence was recorded indicating that the presence of particles in the exposure media has a direct influence on the method. This is therefore not regarded as a suitable method to determine the toxicity to bacteria.

The dispersant cAuNPs DIS was able to demonstrate effects on bioluminescence but due to the interference of the cAuNPs (gold nanoparticles in dispersant) particles with the bioluminescence it was concluded that this is not a suitable test to determine the effects of nanogold on micro-organisms.

### Cross-reference to other study
No cross-reference

## 6.1.8 Toxicity to other aquatic organisms

# 6.2 Sediment toxicity

## *Endpoint study record: 7440-57-5, Sediment toxicity, Anonymous, 2013, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2012-2013 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Non-GLP study conducted according to OECD Guideline 219 with deviations: details on test item, source of organisms and breeding conditions, age of test animals, feeding larvae, number of replicates in treatment and details on results not reported | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | Anonymous | 2013 | No data | | | | | | |

## Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 219 (Sediment-Water Chironomid Toxicity Test Using Spiked Water) | yes (details on test item, source of organisms and breeding conditions, age of test animals, feeding larvae, number of replicates in treatment and details on results not reported) |

## Principles of method if other than guideline

Not applicable

## GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Citrate coated 14 nm cAuNPs and citrate buffer used as dispersant cAuNPs DIS
- Source: Mintek, South Africa

## Confidential details on test material
No data

***Details on properties of test surrogate or analogue material***
No data

## Analytical monitoring
no

***Details on sampling***
Not applicable

***Details on analytical methods***
Not applicable

## Vehicle
no data

***Details on sediment and application***
PREPARATION OF SPIKED SEDIMENT
- Pooling or mixing of different substrates: Artificial sediment consisting of Sphagnum peat, air-dried, finely ground (5 %), Kaolinite, air-dried (20 %), industrial quartz sand, air-dried (75 %).
- Method of mixing: The test substrate was wetted with deionised water to reach a water content of 25 % - 30 %.
- According to the guideline a water content between 30 % and 50 % is recommended. The laboratory experience showed that lower water content results in a more homogenous distribution of the sediment in the individual vessels.
- Pulverised calcium carbonate of chemically pure quality (CaCO3) was added to adjust the pH of the final mixture of the sediment to 7.0 ± 0.5. Organic carbon content of the final mixture was 1.8 % which was within the demanded range of 2 % ± 0.5 %.

## Test organisms

## Test organisms (species)
other: First instar larvae from the dipteran Chironomus riparius

***Details on test organisms***
TEST ORGANISM
- Sediment-dwelling larvae (first instar) of the fresh water dipteran Chironomus riparius were placed in a sediment-water test system with defined artificial sediment.

## Study design

## Study type
other: no data

## Test duration type
long-term toxicity

## Test type
no data

## Water media type
no data

## Type of sediment
artificial sediment

## Limit test
no

## Total exposure duration
28 d

***Post exposure observation period***
None

## Test conditions

***Hardness***
No data

***Test temperature***
20 ± 2 °C

***pH***
pH of the final mixture of the sediment: 7.0 ± 0.5

***Dissolved oxygen***
No data

***Salinity***
No data

***Ammonia***
No data

***Nominal and measured concentrations***
cAuNPs: 0.44 – 22 mg/LcAuNPs DIS: up to 50 % (highest test concentration)

***Details on test conditions***

TEST SYSTEM

- Test container (material, size): Glass vessels (600 mL) were used as test vessels. The vessels were filled up to a height of 1.5 cm with 128.2 g wet artificial sediment (corresponding to 95 g dry mass). The containers were covered with glass plates.
- Overlying water volume: The overlaying water was 6 cm high ca. 1:4 sediment:water ratio)
- Depth of sediment and overlying water: 1:4- After 10 days, emergence traps were placed on the test vessels, the glass plates remained on the emergence traps to avoid evaporation.
- Aeration: Yes
- Aeration frequency and intensity: Aeration of overlaying water was provided through a glass pipette fixed 2-3 cm above the sediment layer (at least 1 bubble /second).
- Sediment was put into the test vessels. Tap water (125 mL) was added and the sediment-water system was left under gentle aeration for several days prior to adding the test organisms.

EXPOSURE REGIME

-No. of organisms per container (treatment): Batches of 20 larvae were placed into each vessel.
-After an incubation period of 24 h, 135 mL of the freshly prepared stock dispersion of the nanoparticles was added. A further 10 mL of tap water were used to rinse the vessels containing the stock dispersions. To avoid separation of sediment ingredients during addition of test water and stock dispersion, the surface of the water column was covered with a stainless steel disc while water was poured onto it. The disc was removed immediately afterwards. Due to the large amount of stock dispersion the dispersion admixed while being added to the water column. No further mixing was applied in order to avoid disturbance of the sediment.

OTHER TEST CONDITIONS

- Photoperiod: 16 h photoperiod
- Light intensity: 500 –1000 lux

EFFECT PARAMETERS MEASURED:

- Chironomid emergence was measured as the endpoint at the end of the test, i.e. after 28 days of incubation.
- Development time and total number of fully emerged male and female midges were determined. Test vessels were observed daily for visual assessment of abnormal behaviour. Emergence was counted daily. After identification the midges were removed from the test vessel. At test end, the test vessels were observed for visible pupae that had failed to emerge.
- Emergence rate, development time and rate, and sensitivity of the sexes in the treatment test systems and in the control were analysed for statistically significant differences using appropriate statistical methods.

***Reference substance (positive control)***

no

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Effect concentrations

| Duration | Endpoint | Effect conc. | Nominal/Measured | Conc. based on | Basis for effect | Remarks (e.g. 95% CL) |
|---|---|---|---|---|---|---|
| 28 d | NOEC | 50 % | nominal | test mat. | other: Emergence rate, development time and rate | |

***Details on results***

- Toxicity was observed for the dispersant (cAuNPs DIS) at the highest test concentration. The larvae were fully grown even though there was a delay in the development. However, no larvae emerged as the organisms died before hatching. In the presence of gold this effect did not occur.
- For the concentrations resulting in emergence [cAuNPs: all test concentrations (0.44 – 22 mg/L); cAuNPs DIS all test concentrations except the highest test concentration] no statistical difference between the treated vessels and the control was observed for the development time and the emergence rate. For the development rate a statistical difference was calculated. However, no concentration-effect relationship and no difference between thevessels with cAuNPs (gold in dispersant) and cAuNPs DIS (dispersant) was obvious. Therefore, it was concluded that the statistical difference in the development rate is not substance related effect but probably indicates biological variability.

***Results with reference substance (positive control)***

Not applicable

***Reported statistics and error estimates***

See the "Details on results"

## Any other information on results incl. tables

None

## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Validity criteria fulfilled

yes

## Conclusions

The dispersant cAuNPs DIS in the highest test concentration showed an obvious effect. No emergence was observed. In contrast, cAuNPs (gold nanoparticles in dispersant) showed no effect, even at the highest test concentration of 50 %. Gold nanoparticles compensated the effect of the dispersant.

**Executive summary**

In a sediment toxicity study,performed in accordance with OECD Guideline 219, sediment-dwelling larvae (first instar) of the fresh water dipteran*Chironomus riparius*were placed in a sediment-water test system with defined artificial sediment and the overlaying water was spiked with the test item at a defined range of concentrations (cAuNPs: 0.44 – 22 mg/L; cAuNPs DIS: up to 50 % (highest test concentration)) for 28 days. The test item was applied once. Chironomid emergence was measured as the endpoint at the end of the test, i.e. after 28 days of incubation. Emergence rate, development time and rate, and sensitivity of the sexes in the treatment test systems and in the control were analysed for statistically significant differences using appropriate statistical methods.

Toxicity was observed for the dispersant (cAuNPs DIS) at the highest test concentration. The larvae were fully grown even though there was a delay in the development. However, no larvae emerged as the organisms died before hatching. In the presence of gold this effect did not occur. For the concentrations resulting in emergence [cAuNPs: all test concentrations (0.44 – 22 mg/L); cAuNPs DIS all test concentrations except the highest test concentration] no statistical difference between the treated vessels and the control was observed for the development time and the emergence rate. For the development rate a statistical difference was calculated. However, no concentration-effect relationship and no difference between the vessels with cAuNPs (gold in dispersant) and cAuNPs DIS (dispersant) was obvious. Therefore, it was concluded that the statistical difference in the development rate is not substance related effect but probably indicates biological variability.

The dispersant cAuNPs DIS in the highest test concentration showed an obvious effect. No emergence was observed. In contrast, cAuNPs (gold nanoparticles in dispersant) showed no effect, even at the highest test concentration of 50 %. Gold nanoparticles compensated the effect of the dispersant.

**Cross-reference to other study**

No cross-reference

## 6.3 Terrestrial toxicity

## 6.4 Biological effects monitoring

## 6.5 Biotransformation and kinetics

## 6.6 Additional ecotoxicological information

# 7. TOXICOLOGICAL INFORMATION

## 7.1 Toxicokinetics, metabolism and distribution

### 7.1.1 Basic toxicokinetics

## *Endpoint study record: 7440-57-5, Basic toxicokinetics, Lu, Year, RS, K*

### Administrative Data

**Purpose flag** key study; robust study summary

**Study result type** experimental result **Study period** 2011-2012

**Reliability** 2 (reliable with restrictions)

**Rationale for reliability incl. deficiencies** Study was extensively repeated with appropriate statistics.

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Lu Z, Ma G, Veinot JGC and Wong CS | | Influence of Nanoparticles on Phase I Biotransformation of Persistent Organic Pollutants by Cytochrome P-45. | Environ Sci Technol, submitted. | | | | | |

### Data access

data published

### Cross-reference to same study

No cross-reference

### Materials and methods

### Type of method

in vitro

### Objective of study

metabolism

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

### Principles of method if other than guideline

In Phase I biotransformation test, rat cytochrome P-450 2B1 (CYP 2B1) was used to biotransform 2,2’,3,5’,6-pentachlorobiphenyl (PCB 95, CAS#38379-99-6) in vitro, and citrate-capped Au nanoparticles were added to determine effect of nanomaterials on biotransformation.

### GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Radiolabelling
no

## Details on test material
- Name of test material (as cited in study report): cAuNPs- Test Substance Remarks: Created by Jon Veinot's group following a procedure similar to that of Ji et al., 2007, J. Am. Chem. Soc. 129, 13939-13948

## Confidential details on test material
No data

## Test animals

## Species
other: in vitro

***Details on test animals and environmental conditions***
Not applicable

## Administration / exposure

## Route of administration
other: in vitro

## Vehicle
acetone (60 µL/mL)

***Details on exposure***
100 ng/mL PCB 95 substrate incubated with 5 pmol CYP 2B1, 50 µL solution A, 10 µL solution B, and either 0, 10, or 200 µM AuNPs in 110 mM potassium phosphate buffer (pH 7.4). Samples collected from 3-60 min at 10 min intervals (or less depending on need) to establish PCB 95 biotransformation kinetics.

## Duration and frequency of treatment / exposure
Not applicable

## Doses / concentrations
0, 10 and 200 µM AuNPs- Actual Au NP dose not measured. PCB 95 dose measured by GC/MS at starting levels, decreasing with incubation time.

## No. of animals per sex per dose
Not applicable

## Control animals
other: not applicable

***Positive control***
Not applicable

***Statistics***
Student's t-test and ANOVA

## Any other information on materials and methods incl. tables
Cell Type: Purified rat CYP 2B1 + cytochrome P-450 reductase + cytochrome b5 and NADPH regeneration system (solution A: 31 mM NADP+, 66 mM gluclose-6-phosphate and 66 mM MgCl2 in water; solution B: 40 U/ml glucose-6-phosphate dehydrogenase in 5 mM sodium citrate), all from BD Biosciences.

## Results and discussions

## Preliminary studies
Not applicable

## Main ADME results

| Type | |
|---|---|
| metabolism | No metabolites collected. |

## Pharmacokinetic studies

## Details on absorption
Not applicable

## Details on distribution in tissues
Not applicable

## Details on excretion
Not applicable

## Metabolite characterisation studies

## Metabolites identified
no

## Details on metabolites
AuNPs inhibited PCB 95 biotransformation when present at 20 µM, but could enhance biotransformation at 1 µM. It was found that AuNPs reduced ionic strength of local microenvironment through cation sorption or sequestration within Au NP double layer, through various indirect experiments. In vitro enzyme system was more efficient at producing and regenerating NADPH at lower ionic strengths, thereby increasing rate of PCB 95 biotransformation.

## Bioaccessibility

## Any other information on results incl. tables
None

## Overall remarks, attachments

## Remarks on results including tables and figures
None

## Applicant's summary and conclusion

## Interpretation of results
no data

## Conclusions
Any charged nanoparticle that sequesters ions and influences ionic strength of local microenvironment will affect any enzymatic process that depends on ionic strength for function e.g., co-factor production. This is an indirect toxicity effect of such nanoparticles.

## Executive summary
In Phase I biotransformation test, rat cytochrome P-450 2B1 (CYP 2B1) was used to biotransform 2,2',3,5',6-pentachlorobiphenyl (PCB 95, CAS#38379-99-6) in vitro, and citrate-capped Au nanoparticles were added to determine effect of nanomaterials on biotransformation. 100 ng/mL PCB 95 substrate incubated with 5 pmol CYP 2B1, 50 µL solution A, 10 µL solution B, and either 0, 10, or 200 µM AuNPs in 110 mM potassium phosphate buffer (pH 7.4). Samples collected from 3-60 min at 10 min intervals (or less depending on need) to establish PCB 95 biotransformation kinetics. Actual Au NP dose not measured. PCB 95 dose measured by GC/MS at starting levels, decreasing with incubation time.
AuNPs inhibited PCB 95 biotransformation when present at 20 µM, but could enhance biotransformation at 1 µM. It was found that AuNPs reduced ionic strength of local microenvironment through cation sorption or sequestration within Au NP double layer, through various indirect experiments. In vitro enzyme system was more efficient at producing and regenerating NADPH at lower ionic strengths, thereby increasing rate of PCB 95 biotransformation.

Any charged nanoparticle that sequesters ions and influences ionic strength of local microenvironment will affect any enzymatic process that depends on ionic strength for function e.g., co-factor production. This is an indirect toxicity effect of such nanoparticles.

### Cross-reference to other study

No cross-reference

## 7.2 Acute Toxicity

## 7.3 Irritation / corrosion

## 7.4 Sensitisation

## 7.5 Repeated dose toxicity

### 7.5.1 Repeated dose toxicity: oral

### 7.5.2 Repeated dose toxicity: inhalation

### *Endpoint study record: 7440-57-5, Repeated dose toxicity-inhalation, Anonymous, 2012, RS, K*

#### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2012 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Non-GLP study conducted similarly to OECD 412 Guideline with deviations: details on test item, details of inhalation chamber, body weight and environmental conditions of experimental room not reported; haematology and clinical chemistry not reported; individual and summary tables of results not reported | | |

#### Data source

#### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | 2012 | In Vivo Toxicity Assessment using Standard Nanoparticles (Gold Nanoparticle): Size Dependent Lung Deposition and Clearance of Gold Nanoparticles. | | KRISS, Korea | | | | |

## Data access
other: data submitter is data owner or has Letter of Access

## Data protection claimed
yes, but willing to share

## Cross-reference to same study
No Cross-reference

## Materials and methods

## Test type
subacute

## Limit test
no

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| equivalent or similar to | OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day) | yes (details on test item, details of inhalation chamber, body weight and environmental conditions of experimental room not reported; haematology and clinical chemistry not reported; individual and summary tables of results not reported) |

## Principles of method if other than guideline
Not applicable

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles
- Size: 14 and 95 nm- Analytical purity: 99.99 %

## Confidential details on test material
No data

## Test animals

## Species
rat

## Strain
Sprague-Dawley

## Sex
male

***Details on test animals and environmental conditions***
TEST ANIMALS
- Age at study initiation: 8 weeks

## Administration / exposure

## Route of administration
other: Inhalation-particulate

## Type of inhalation exposure
no data

## Vehicle
other: HEPA filtered clean air was supplied to negative control group

***Details on inhalation exposure***
No data

## Analytical verification of doses or concentrations
no

***Details on analytical verification of doses or concentrations***
No data

## Duration of treatment / exposure
Duration of test: 28 DaysExposure period: 5 Days, 3 & 23 days of recovery

## Frequency of treatment
continuous exposure, 6 h/day, 5 days/week

## Doses/concentrations

Gold nanoparticles 14 nm at concentration of 12.58 µg/m^3 and Gold nanoparticles 95 nm at concentration of 13.7 µg/m3

**Basis** no data

## MMAD / GSD

Gold nanoparticles 14 nm (CMD 12.8 nm GSD 1.14) at concentration of 12.58 µg/m^3 Gold nanoparticles 95 nm (CMD 105.4 nm GSD 1.29) at concentration of 13.7 µg/m^3

## No. of animals per sex per dose

24 males per group

## Control animals

yes

***Details on study design***

- Post-exposure recovery period in satellite groups: 3 and 23 days

***Positive control***

Not applicable

## Examinations

***Observations and examinations performed and frequency***

CLINICAL OBSERVATIONS: Yes
- Time schedule: General clinical observations were performed daily

BODY WEIGHT: Yes
- Time schedule for examinations: Once in a week

FOOD AND WATER CONSUMPTION: Yes
- Time schedule for examinations: Once in a week

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: Ophthalmologic examination was made prior to the grouping of animals and at 1 week prior to termination of the study.

HAEMATOLOGY AND CLINICAL CHEMISTRY: No

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

***Sacrifice and pathology***

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
- Histopathology, BAL fluid analysis, and organ weight measurement were performed at the end of the

treatment.

***Other examinations***
None

***Statistics***
One way analysis of variance (ANOVA) test followed by Dunnett's test

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Effect levels

| Endpoint | Effect level | Based on | Sex | Basis for effect level / Remarks |
|---|---|---|---|---|
| NOAEL | not determined | test mat. | male | no toxicity effects were observed |

## Results of examinations

***Clinical signs and mortality***
no effects

***Body weight and weight gain***
no effects

***Food consumption***
no effects

***Water consumption***
no data

***Ophthalmoscopic examination***
no data

***Haematology***
not examined

***Clinical chemistry***
not examined

***Urinalysis***
not examined

***Neurobehaviour***
not examined

***Organ weights***
no effects

***Gross pathology***
no effects

***Histopathology: non-neoplastic***
no effects

***Details on results***
CLINICAL SIGNS AND MORTALITY:
No clinical signs or mortality was observed.

BODY WEIGHT AND WEIGHT GAIN:
No significant difference among dose group was observed.

FOOD CONSUMPTION:
No difference among dose group was observed.

GROSS PATHOLOGY:
No significant gross effects were observed during the 5-day exposure period and 23 days of recovery period

ORGAN WEIGHTS:
No significant difference among dose group was observed.

HISTOPATHOLOGY:
No significant findings in the lungs were observed.

OTHER FINDINGS
Tissue distribution: Distribution in lung, small amount of gold nanoparticles translocated from lungs to liver and spleen.

## Any other information on results incl. tables

Toxicokinetics:
**Table 7.5.2/1: Toxicokinetics - results**

| Particle size | t1/2 (day) | Cmax (ng/mL) | $AUC_{all}$(ng.day/mL) | $AUC_{inf}$(ng.day/mL) | $MRT_{all}$(day) | $MRT_{inf}$(day) |
|---|---|---|---|---|---|---|
| 14 nm | 44.5 | 830.1 | 19416.5 | 55539.4 | 11.9 | 64.2 |
| 95 nm | 179.5 | 430.4 | 10523.1 | 105678.5 | 13.7 | 259.7 |

## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Conclusions

- STIS gold nanoparticle inhalation study using 14 and 95 nm gold nanoparticle showed size dependent clearance from the lung, showing faster clearance of 14 nm than 95 nm.
- Gold nanoparticles showed biopersistence in the lung tissue.
- Gold nanoparticles did not translocate extrapulmonary to other tissues such as kidney, brain and testis. Small number of gold nanoparticle translocated from lung to liver and spleen.

## Executive summary

In a repeated dose toxicity study conducted similarly to the OECD 412 Guideline, male Sprague-Dawley rats (24 animals per group) were administered with Gold nanoparticles 14 nm (CMD 12.8 nm GSD 1.14) at concentration of 12.58 μg/$m^3$and 95 nm (CMD 105.4 nm GSD 1.29) at concentration of 13.7 μg/$m^3$by inhalation-particulate through continuous exposure, 6 h/day, 5 days/week, for 5 days with 23 days of recovery period. HEPA filtered clean air was supplied to negative control group. Examinations during the study included: mortality, clinical observation of animals, body weight change, monitoring of food and water consumption, ophthalmological examination, gross pathology, measurement of organ weights and histopathology.

No mortality or clinical signs were observed. No significant difference in body weight and food consumption was observed in any of the dose groups. No significant gross effects were observed in any of the dose groups. No significant difference was observed in organ weight in any of the dose groups. No significant histopathological findings were observed in lungs. Toxicokinetics revealed that 14 and 95 nm gold nanoparticle showed size dependent clearance from the lung, showing faster clearance of 14 nm than 95 nm. Gold nanoparticles showed biopersistence in the lung tissue. Gold nanoparticles did not translocate extrapulmonary to other tissues such as kidney, brain and testis. Small number of gold nanoparticle translocated from lung to liver and spleen.

## Cross-reference to other study

No Cross-reference

# *Endpoint study record: 7440-57-5, Repeated dose toxicity-inhalation, Sung, 2011, RS, K*

## Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2007 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | GLP Study conducted according to OECD 413 Guideline with deviations: details on test item, details on the inhalation chamber, actual concentrations in the inhalation chamber, body weight and environmental conditions and individual and summary tables of results not reported; NOAEL interpretation was not discussed | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | 2009 | Subchronic (90-Day) Inhalation Toxicity Study of Gold Nanoparticles in Rats | | KCL (Korea conformity Laboratory) | | | | |
| publication | Sung JH, Ji JH, Park JD, Song MY, Song KS, Ryu HR, Yoon JU, Jeon KS, Jeong J, Han BS, Chung YH, Chang HK, Lee JH, Kim DW, Kelman BJ and Yu IJ. | 2011 | Subchronic Inhalation Toxicity of Gold Nanoparticles | Particle and Fibre Toxicology, 8: 16 | KCL (Korea conformity Laboratory) | | | | |

## Data access

other: data submitter is data owner or has Letter of Access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No Cross-reference

## Materials and methods

## Test type

subchronic

**Limit test** no

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day) | yes (details on test item, details on the inhalation chamber, actual concentrations in the inhalation chamber, body weight and environmental conditions and individual and summary tables of results not reported; NOAEL interpretation was not discussed) |

## Principles of method if other than guideline

Not applicable

## GLP compliance

yes

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles - Average diameter was 4-5 nm- Analytical purity: 99.99 %

## Confidential details on test material
No data

## Test animals

## Species
rat

## Strain
Sprague-Dawley

## Sex
male/female

***Details on test animals and environmental conditions***
TEST ANIMALS
- Age at study initiation: 7 weeks

## Administration / exposure

## Route of administration
other: Inhalation-particulate

## Type of inhalation exposure
no data

## Vehicle
other: HEPA filtered clean air was supplied to negative control group

***Details on inhalation exposure***
Preparation of test material:
- Gold nanoparticles were generated from solid gold wire by nanoparticle generator (ISO/10801).

Concentration was adjusted by flow rate with mass flow controller. Average diameter was 4-5 nm.

## Analytical verification of doses or concentrations

no

***Details on analytical verification of doses or concentrations***

No data

## Duration of treatment / exposure

90 days

## Frequency of treatment

Continuous exposure, 6 h/day, 5 days/week, for 90 days

## Doses/concentrations

1.85 × 10^6 particles/cm3 (20.02 μg/m3); 2.36 × 10^5 particles/cm3 (0.38 μg/m3), 2.36 × 10^4 particles/cm3 (0.04 μg/m3)

**Basis** no data

## MMAD / GSD

- High-concentration chamber: Geometric mean diameter (GMD), concentration, and surface area of the gold nanoparticles measured by the DMAS were 5.06 nm, 1.85 × 10^6 particles/cm3, 20.02 μg/m3, and 3.64 × 10^8 nm2/cm3, respectively
- Middle-concentration chamber: Geometric mean diameter (GMD), concentration, and surface area of the gold nanoparticles measured by the DMAS were 4.12 nm, 2.36 × 10^5 particles/cm3, 0.38 μg/m3 and 1.68 × 10^7 nm2/cm3, respectively,
- Low-concentration chamber: Geometric mean diameter (GMD), concentration, and surface area of the gold nanoparticles measured by the DMAS were 4.3 nm, 2.36 × 10^4 particles/cm3, 0.04 μg/m3, and 1.9 × 10^6 nm2/cm3, respectively.
- The diameters were log normally distributed between 1 and 6 nm, and the CMD (count median diameter) and GSD were 2.47 nm and 1.42, respectively.

## No. of animals per sex per dose

10 animals per sex per dose

## Control animals

yes

***Details on study design***

No data

***Positive control***

Not applicable

## Examinations

***Observations and examinations performed and frequency***

CLINICAL OBSERVATIONS: Yes
- Time schedule: General clinical observations were performed daily

BODY WEIGHT: Yes
- Time schedule for examinations: Once in a week

FOOD AND WATER CONSUMPTION: Yes
- Time schedule for examinations: Once in a week

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: Ophthalmologic examination was made prior to the grouping of animals and at 1 week prior to termination of the study.

HAEMATOLOGY AND CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: During necropsy

URINALYSIS: Yes
- Time schedule for collection of urine: Urinalysis was performed on the last week of treatment with 5 animals per sex per dose.

NEUROBEHAVIOURAL EXAMINATION: No

***Sacrifice and pathology***

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
- Histopathology and organ weight measurement were performed at the end of the treatment.

***Other examinations***

BAL fluid analysis, lung and kidney function test were performed at the end of the treatment.

***Statistics***

One way analysis of variance (ANOVA) test followed by Dunnett's test

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Effect levels

| Endpoint | Effect level | Based on | Sex | Basis for effect level / Remarks |
|---|---|---|---|---|
| NOAEL | 0.38 µg/m3 | test mat. | male/female | |
| LOAEL | 20.02 µg/m3 | test mat. | male/female | |

## Results of examinations

***Clinical signs and mortality***
no effects

***Body weight and weight gain***
yes

***Food consumption***
no effects

***Water consumption***
no data

***Ophthalmoscopic examination***
no effects

***Haematology***
no effects

***Clinical chemistry***
no effects

***Urinalysis***
no effects

***Neurobehaviour***
not examined

***Organ weights***
no effects

***Gross pathology***
no effects

***Histopathology: non-neoplastic***
yes

***Details on results***
CLINICAL SIGNS AND MORTALITY:
No clinical signs or mortality was observed.

BODY WEIGHT AND WEIGHT GAIN:
A significant body weight gain was noted in the low-dose female group ($p<0.05$) when compared to the control, middle-dose, and high-dose groups after 4 weeks of exposure.

FOOD CONSUMPTION:
No difference among dose group was observed.

OPHTHALMOSCOPIC EXAMINATION:
No symptoms detected.

HAEMATOLOGY:
No significant dose-related differences were observed for the hematology values.

CLINICAL CHEMISTRY:
No significant dose-related differences were observed for the blood biochemical values.URINALYSIS: No difference among dose group was observed.

GROSS PATHOLOGY:
No significant gross effects were observed during the 90-day exposure period.

ORGAN WEIGHTS:
No significant difference among dose group was observed.

HISTOPATHOLOGY:
Minor focal inflammation with an inflammatory infiltrate of mixed cell type (lymphocyte/neutrophil/macrophage) was noted in both treated male and female rats. The increases were dose-dependent in female rats.

OTHER FINDINGS:
- Lung function: Among the pulmonary function test parameters, there were significant changes in tidal volume and minute volume during the 90 days of gold nanoparticle exposure ($p < 0.01–0.05$). Dose-dependent tidal volume decreases in male rats led to minute volume decreases in the high-dose animals. A tendency towards a dose-dependent decrease in the tidal volume appeared to be present in female rats, but was not statistically significant.
- Blood clotting time: No significant differences were found between the control and any treated animals in APPT or PT.
- Tissue distribution: Distribution in lung, kidney, liver, blood, brain and olfactory nerve was prominent and concentration dependent.

## Any other information on results incl. tables

None

## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Applicant's summary and conclusion

## Conclusions

Under the test conditions, the No Observed Adverse Effect Level (NOAEL) of Gold nanoparticles was considered to be 2.36 × 10^5 particles/cm3 (0.38 μg/m3) in rats.

### Executive summary

In a repeated dose toxicity study conducted according to the OECD Guideline 413, Gold nanoparticles was administered by inhalation-particulate to groups of Sprague-Dawley rats (10 animals/sex/dose) at the concentrations of $1.85 \times 10^6$particles/$cm^3$(20.02 μg/$m^3$); $2.36 \times 10^5$particles/$cm^3$(0.38 μg/$m^3$), $2.36 \times 10^4$particles/$cm^3$(0.04 μg/$m^3$) by continuous exposure, 6 h/day, 5 days/week, for 90 days. HEPA filtered clean air was supplied to negative control group. Examinations during the study included: mortality, clinical observation of animals, body weight change, monitoring of food and water consumption, laboratory investigations: haematology, blood clinical chemistry, ophthalmological examination, urinalysis, gross pathology, measurement of organ weights and histopathology.

No mortality or clinical signs were observed. There was a significant body weight gain in the low-dose female group ($p<0.05$) when compared to the control, middle-dose, and high-dose groups after 4 weeks of exposure. No significant difference in food consumption was observed in any of the dose groups. No significant difference was observed in hematology and clinical chemistry in any of the dose groups. No abnormalities were observed in ophthalmoscopic examination. No significant gross effects were observed during the 90-day exposure period. No significant difference was observed in organ weight in any of the dose groups. The only significant changes in histopathology occurred in, where there was minor focal inflammation with an inflammatory infiltrate of mixed cell type (lymphocyte/neutrophil/macrophage) were noted in both treated male and female rats. The increases were dose-dependent in female rats. Among the pulmonary function test parameters, there were significant changes in tidal volume and minute volume during the 90 days of gold nanoparticle exposure ($p <0.01–0.05$). Dose-dependent tidal volume decreases in male rats led to minute volume decreases in the high-dose animals. A tendency towards a dose-dependent decrease in the tidal volume appeared to be present in female rats, but was not statistically significant.

Under the test conditions, the No Observed Adverse Effect Level (NOAEL) of Gold nanoparticles was considered to be $2.36 \times 10^5$particles/$cm^3$(0.38 μg/$m^3$) in rats.

### Cross-reference to other study

No Cross-reference

## 7.5.3 Repeated dose toxicity: dermal

## 7.5.4 Repeated dose toxicity: other routes

## *Endpoint study record: 7440-57-5, Repeated dose toxicity - intravenous, Anonymous, 2011, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2011 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Non-GLP study conducted according to OECD 407 Guideline with deviations: test item administered by intravenous route, details on test item, body weight, environmental conditions of experimental room not reported; individual and summary tables of results not reported | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | 2011 | In Vivo Toxicity Assessment using Standard Nanoparticles (Gold Nanoparticle) | | KRISS | | | | |

## Data access

other: data submitter is data owner or has Letter of Access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No Cross-reference

## Materials and methods

## Test type

subacute

## Limit test

no

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | other guideline: OECD Test Guideline 407 (Repeated dose 28 days oral toxicity study in rodents) | yes (test item administered by intravenous route, details on test item, body weight, environmental conditions of experimental room not reported; individual and summary tables of results not reported) |

## Principles of method if other than guideline

Not applicable

## GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles
- Size: 14 nm
- Source: Mintek, South Africa
- Analytical purity: 99.99 %

## Confidential details on test material
No data

## Test animals

## Species
rat

## Strain
Sprague-Dawley

## Sex
male

***Details on test animals and environmental conditions***
TEST ANIMALS
- Age at study initiation: 7 weeks

## Administration / exposure

## Route of administration
intravenous

## Vehicle
other: citrate

***Details on exposure***
No data

## Analytical verification of doses or concentrations
no

## Duration of treatment / exposure
28 days

## Frequency of treatment
once daily, 7 injections/week for 4 weeks

## Doses / concentrations
10 and 100 µg/kg bw/day

## No. of animals per sex per dose
8 male animals per dose (5 for 28 days, and 3 for 28 day recovery)

## Control animals
yes, concurrent vehicle

***Details on study design***
- Post-exposure recovery period in satellite groups: 28 days

## Examinations

***Observations and examinations performed and frequency***
CLINICAL OBSERVATIONS: Yes
- Time schedule: General clinical observations were performed daily.

BODY WEIGHT: Yes
- Time schedule for examinations: Once in a week

FOOD AND WATER CONSUMPTION: Yes
- Time schedule for examinations: Once in a week

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: Ophthalmologic examination was made prior to the grouping of animals and at 1 week prior to termination of the study.

HAEMATOLOGY AND CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: During necropsy
- Anaesthetic used for blood collection: No data
- Animals fasted: No data

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

***Sacrifice and pathology***
GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes; Histopathology and organ weight measurement were performed at the end of the treatment.

***Other examinations***
None

***Statistics***
One way analysis of variance (ANOVA) test followed by Dunnett's test.

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Effect levels

| Endpoint | Effect level | Based on | Sex | Basis for effect level / Remarks |
|---|---|---|---|---|
| NOAEL | 100 µg/kg bw/day | test mat. | male | no toxicity effects were observed |

## Results of examinations

***Clinical signs and mortality***
no effects

***Body weight and weight gain***
no effects

***Food consumption***
no effects

***Water consumption***
no data

***Ophthalmoscopic examination***
no data

***Haematology***
no effects

***Clinical chemistry***
no effects

***Urinalysis***
not examined

***Neurobehaviour***
not examined

***Organ weights***
no effects

***Gross pathology***
no effects

***Histopathology: non-neoplastic***
no effects

***Details on results***
CLINICAL SIGNS AND MORTALITY:
No clinical signs or mortality was observed.

BODY WEIGHT AND WEIGHT GAIN:
No significant difference among dose group was observed.

FOOD CONSUMPTION:
No difference among dose group was observed.

HAEMATOLOGY:
No significant dose-related differences were observed for the hematology values.

CLINICAL CHEMISTRY:
No significant dose-related differences were observed for the blood biochemical values.

GROSS PATHOLOGY:
No significant gross effects were observed.

ORGAN WEIGHTS:
No significant difference among dose group was observed.

HISTOPATHOLOGY:
No significant difference among dose group was observed.

OTHER FINDINGS
Tissue distribution: Dose dependent distribution in lung, kidney, liver, and kidneys. No significant distribution in blood, brain and testis.
Blood clotting time: No significant differences were found between the control and any treated animals in APPT or PT.

## Any other information on results incl. tables
None

## Overall remarks, attachments

## Remarks on results including tables and figures
None

### Applicant's summary and conclusion

### Conclusions

Under the test conditions, the No Observed Adverse Effect Level (NOAEL) of Gold nanoparticles 14 nm was considered to be 100 µg/kg bw/day in male rats.

### Executive summary

In a repeated dose toxicity study conducted according to the OECD Guideline 407, Gold nanoparticles (size: 14 nm) was administered daily by intravenous route to groups of male Sprague-Dawley rats (5 animals/dose) (3 animals/dose used for recovery groups), at the dose levels of 0 (control), 10 and 100 µg/kg bw/day for 28 days and recovery groups then maintained without treatment for a further 28 days. Control animals were treated with vehicle (citrate) alone. Examinations during the study included: mortality, clinical observation of animals, body weight change, monitoring of food and water consumption, laboratory investigations: haematology, blood clinical chemistry, gross pathology, measurement of organ weights and histopathology.
No mortality or clinical signs were observed. No significant difference in body weight, food and water consumption was observed in any of the dose groups. No significant difference was observed in hematology and clinical chemistry in any of the dose groups. No significant gross effects were observed in any of the dose groups. No significant difference was observed in organ weight in any of the dose groups. No significant difference among dose group was observed for histopathology.
Under the test conditions, the No Observed Adverse Effect Level (NOAEL) of Gold nanoparticles 14 nm was considered to be 100 µg/kg bw/day in male rats.

### Cross-reference to other study

No Cross-reference

## 7.6 Genetic toxicity

## 7.6.1 Genetic toxicity in vitro

## *Endpoint study record: 7440-57-5, Genetic toxicity in vitro - chromosome aberration test, Anonymous, 2013, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | 2012-2013 |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Non-GLP study conducted according to OECD 473 Guideline with deviations: test performed without metabolic activation only; details of test item, details of cell culture, incubation time and temperature and test conditions, metabolic activation and historical negative/positive controls not reported | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | 2013 | No information | No information | No information | | | | |

## Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Type of genotoxicity

chromosome aberration

## Type of study

in vitro mammalian chromosome aberration test

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test) | yes (details of test item, details of cell culture, incubation time and temperature and test conditions, metabolic activation and historical negative/positive controls not reported) |

## Principles of method if other than guideline

Not applicable

## GLP compliance

no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Confidential details on test material
No data

## Method

## Target gene
None

## Species/strain

| | |
|---|---|
| **Species/strain** | Chinese hamster Ovary (CHO) |
| **Details on mammalian cell lines (if applicable)** | No data available |
| **Metabolic activation** | without |

## Test concentrations
0.375, 0.75, 1.5 and 3 nM

***Vehicle***
- Vehicle(s)/solvent(s) used: Culture media

## Controls

| | |
|---|---|
| **Negative controls** | no |
| **Solvent / vehicle controls** | yes (culture media) |
| **True negative controls** | no |
| **Positive controls** | yes |
| **Positive control substance** | other: For the positive control, 5 mM Ethylmethanesulfonate (EMS, CAS No. 62-50-0) was used. |

***Details on test system and conditions***
METHOD OF APPLICATION:
In culture medium

FREQUENCY OF DOSING:
single treatment

DURATION
- Exposure duration: No data available
- Fixation time (start of exposure up to fixation or harvest of cells): No data available

SPINDLE INHIBITOR (cytogenetic assays):
No data available

STAIN (for cytogenetic assays):
No data available

NUMBER OF REPLICATIONS:
3 replicates per concentration

NUMBER OF CELLS EVALUATED:
No data available

NUMBER OF METAPHASES ANALYZED:
Minimum 95

***Evaluation criteria***

A concentration-related increase or a reproducible increase in the number of cells with chromosome aberrations was considered to be positive.

***Statistics***

Not applied

## Any other information on materials and methods incl. tables

None

## Results and discussions

## Test results

| | |
|---|---|
| **Species/strain** | Chinese hamster Ovary (CHO) |
| **Metabolic activation** | without |
| **Test system** | all strains/cell types tested |
| **Genotoxicity** | ambiguous (without metabolic activation) |
| **Cytotoxicity** | no |
| **Vehicle controls valid** | yes |
| **Negative controls valid** | not applicable |
| **Positive controls valid** | yes |

***Additional information on results***

TEST-SPECIFIC CONFOUNDING FACTORS:
No data available

CYTOTOXICITY:
Cytotoxicity not observed

COMPARISON WITH HISTORICAL CONTROL DATA:
No data available

## Any other information on results incl. tables

**Table 7.6.1/1: Results of chromosomal aberration**

| Sample | No. of metaphases | No. of aberrations per metaphase cells | % aberration |
|---|---|---|---|
| Negative control (CHO cells) | 100 | 10 | 10 |
| Positive control (EMS, 5 mM) | 95 | 72 | 76 |
| cAuNP 0.375 nM | 120 | 41 | 34 |
| cAuNP 0.75 nM | 128 | 37 | 29 |
| cAuNP 1.5 nM | 130 | 30 | 23 |
| cAuNP 3 nM | 100 | 37 | 37 |

**As can be seen in the table, treatment with cAuNPs induced chromosomal aberrations at a frequency of between 23 – 37 % as compared to 10% in the negative control and 76% in the positive control. However, no concentration dependent increase in aberrations was observed.**

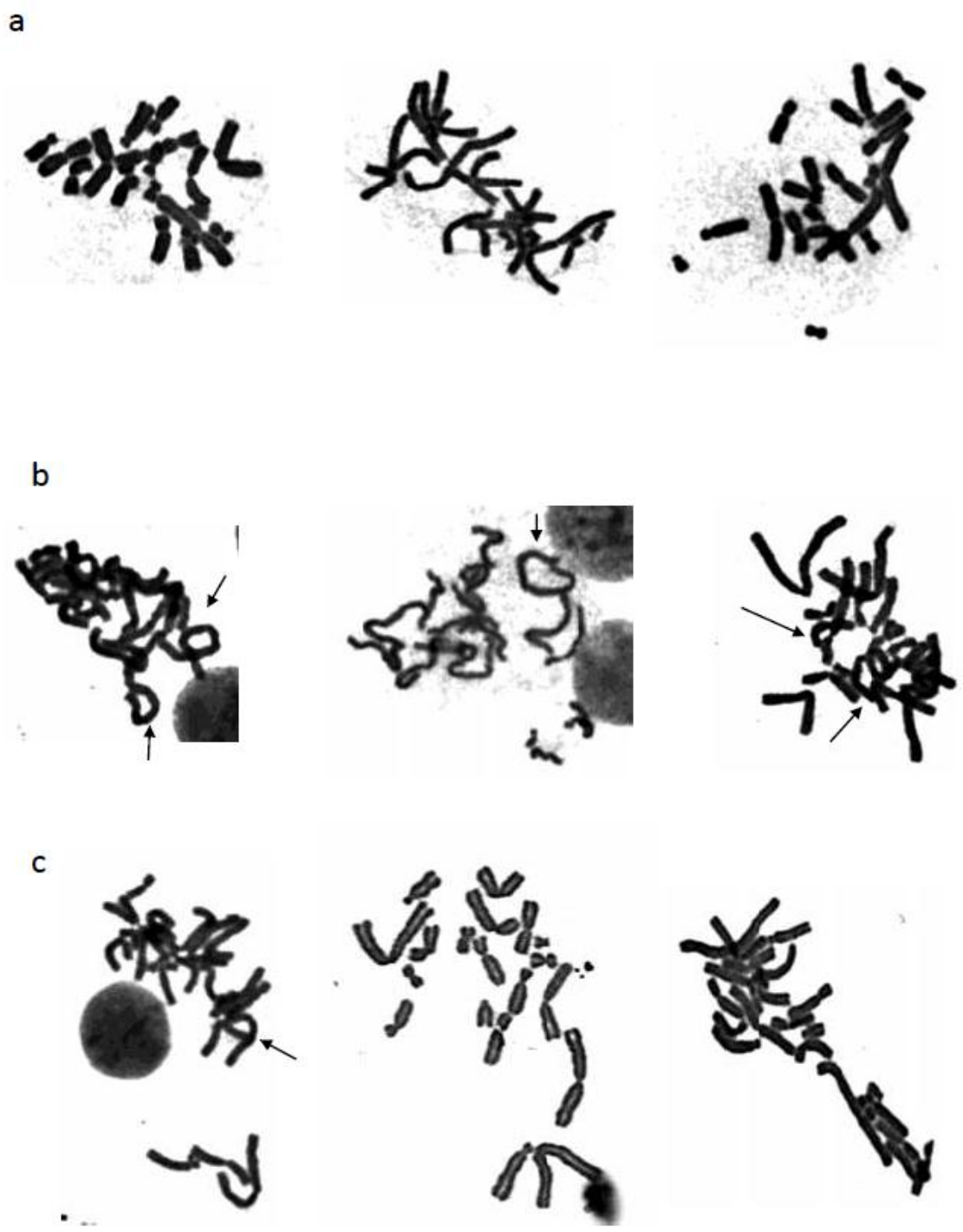
## Overall remarks, attachments

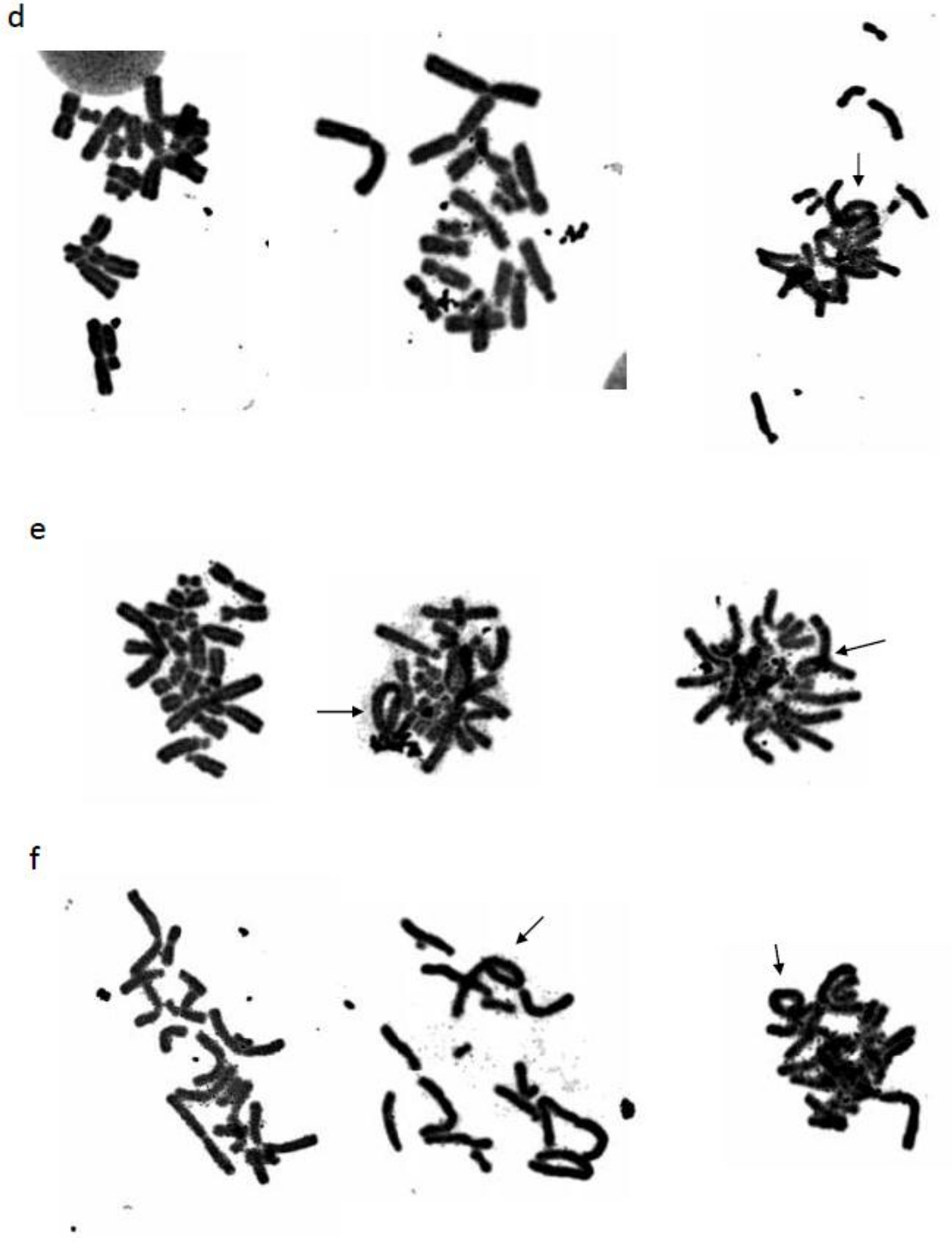
## Remarks on results including tables and figures

None

## Attached background material

| Attached document | Remarks |
|---|---|
| Gold nanoparticles-chromosomal aberrations figures.pdf / 166.45 KB (application/octet-stream) | |


a
b
c


d
e
f

## Applicant's summary and conclusion

## Interpretation of results
ambiguous without metabolic activation

## Conclusions
From the results obtained, one can conclude that gold nanoparticles do induce chromosomal aberrations but there is no concentration related increase in the observed aberrations. The experiment needs to be repeated to determine the reproducibility of aberrations.

## Executive summary
In an in vitro chromosome aberration test performed according to OECD Guideline 473, Chinese Hamster Ovary cellswere exposed to Gold nanoparticles at the concentrations of 0.375, 0.75, 1.5 and 3 nM, without metabolic activation. Negative control (CHO cells) and positive controls (ethylmethanesulphonate at 5 mM) were used.
No cytotoxicity was observed. Treatment with cAuNPs induced chromosomal aberrations at a frequency of between 23 – 37 % as compared to 10 % in the negative control and 76 % in the positive control. However, no concentration dependent increase in aberrations was observed.
Under the test conditions, Gold nanoparticles induced chromosomal aberrations in CHO cells, without metabolic activation, but there is no concentration related increase in the observed aberrations. The experiment needs to be repeated to determine the reproducibility of aberrations.

## Cross-reference to other study
No cross-reference

# *Endpoint study record: 7440-57-5, Genetic toxicity in vitro-Ames test, Anonymous, 2013, RS, K*

## Administrative Data

**Purpose flag** key study; robust study summary

**Study result type** experimental result **Study period** 2012-2013

**Reliability** 2 (reliable with restrictions)

**Rationale for reliability incl. deficiencies** Non-GLP study conducted according to OECD 471 Guideline with deviations: test performed without metabolic activation only; details of test item, no. of cells per culture, incubation time and temperature, metabolic activation, no. of revertant colonies and historical negative/positive controls not reported

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Maron DM and Ames BN. | 1983 | Revised Methods for the Salmonella Mutagenicity Test. | Mutation research, 113:173-215. | | | | | |

## Data access
other: data submitter is data owner or has letter of access

## Data protection claimed
yes, but willing to share

## Cross-reference to same study
No cross-reference

## Materials and methods

## Type of genotoxicity
gene mutation

## Type of study
bacterial reverse mutation assay (e.g. Ames test)

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| according to | OECD Guideline 471 (Bacterial Reverse Mutation Assay) | yes (test performed without metabolic activation only; details of test item, no. of cells per culture, incubation time and temperature, metabolic activation, no. of revertant colonies and historical negative/positive controls not reported) |

## Principles of method if other than guideline
Not applicable

## GLP compliance
no

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Confidential details on test material
No data

## Method

## Target gene
None

## Species/strain

| | |
|---|---|
| **Species/strain** | S. typhimurium, other: TA97a, TA98, TA100 and TA102 |
| **Details on mammalian cell lines (if applicable)** | Not applicable |
| **Metabolic activation** | without |

## Test concentrations
A range of 10 to 162 μg/plate

### *Vehicle*
- Vehicle(s)/solvent(s) used: Distilled water

## Controls

| | |
|---|---|
| **Negative controls** | no |
| **Solvent / vehicle controls** | yes (distilled water) |
| **Positive controls** | yes |
| **Positive control substance** | other: sodium azide (SA; 5 μg/plate) for TA100 strain; mitomycin-C (MMC; 0.5 μg/plate) for TA102; 2-nitrofluorene for TA98 (2-NF; 2.5 μg/plate); and 9-aminoacridine (9AA; 50 μg/plate) for TA97a strain. |
| **Remarks** | Sodium azide was dissolved in distilled water; whilst other positive controls were dissolved in dimethylsulfoxide |

### *Details on test system and conditions*
METHOD OF APPLICATION:
No data available

FREQUENCY OF DOSING:
Single Treatment

DURATION
- Exposure duration: No data available

NUMBER OF REPLICATIONS:
Triplicate

DETERMINATION OF CYTOTOXICITY
- Method: Cytotoxicity was determined by examining the bacterial background lawn.

### *Evaluation criteria*
A positive result was obtained for the following criteria; a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per

plate in at least one strain with or without metabolic activation system.

***Statistics***
None

## Any other information on materials and methods incl. tables
None

## Results and discussions

## Test results

| | |
|---|---|
| **Species/strain** | S. typhimurium, other: TA97a, TA98, TA100 and TA102 |
| **Metabolic activation** | without |
| **Test system** | all strains/cell types tested |
| **Genotoxicity** | negative (without metabolic activation) |
| **Cytotoxicity** | no |
| **Vehicle controls valid** | yes |
| **Negative controls valid** | not applicable |
| **Positive controls valid** | yes |

***Additional information on results***
TEST-SPECIFIC CONFOUNDING FACTORS
- Precipitation: None

CYTOTOXICITY:
- Bacterial lawn was present at all tested concentrations, indicating no cytotoxicity.

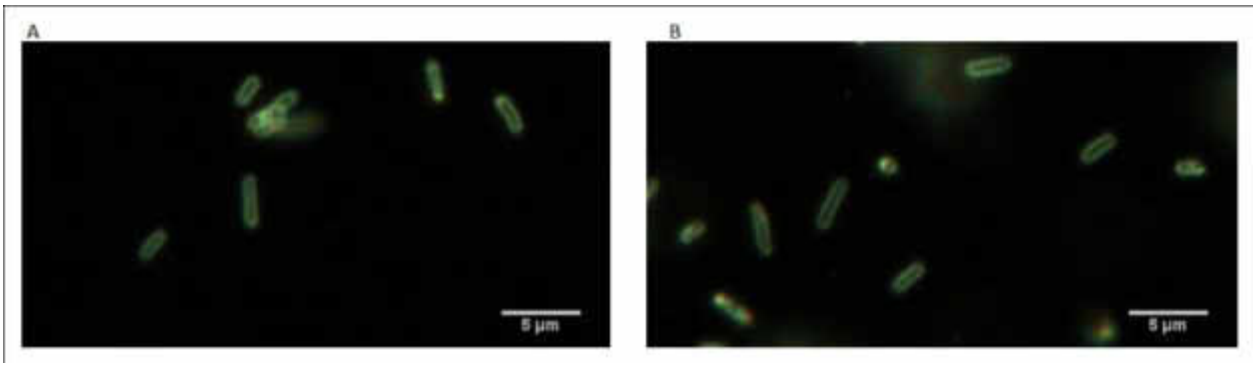
## Any other information on results incl. tables
Refer the attached document for Dark field image of bacterial strain TA98, (A) untreated and (B) treated with 162 µg/plate cAuNPs.

## Overall remarks, attachments

## Remarks on results including tables and figures
Following incubation of the TA98 strain with the gold nanoparticles, the bacteria were observed using dark-field microscopy. Based on these observations, gold nanoparticles do not enter the bacteria. Figure shows images captured at 100x magnification using the dark-field CytoViva system. Hyperspectral Imaging confirmed that no cAuNPs were present in the treated bacteria.

## Attached background material

| Attached document | Remarks |
|---|---|
| Dark field image of bacterial strain TA98.pdf / 113.15 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Interpretation of results
negative without metabolic activation

### Conclusions
No dose related increase in revertants was observed following treatment with cAuNPs, and thus are regarded as not genotoxic under the test conditions; however this may be due to the observation that the nanoparticles did not enter the bacteria.

### Executive summary
In a reverse gene mutation assay in bacteria, performed according to the OECD Guideline 471, strains of *Salmonella typhimurium* (TA97a, TA98, TA100 and TA102) and were exposed to Gold nanoparticles (cAuNPs) at the concentrations of 10 -162 µg/plate without metabolic activation. Vehicle and positive control groups were also included in mutagenicity tests.
Bacterial lawn was present at all tested concentrations, indicating no cytotoxicity. Following incubation of the TA98 strain with the gold nanoparticles, the bacteria were observed using dark-field microscopy. Based on these observations, gold nanoparticles do not enter the bacteria. Hyperspectral imaging confirmed that no cAuNPs were present in the treated bacteria. No dose related increase in revertants was observed following treatment with cAuNPs, and thus are regarded as not genotoxic under the test conditions; however this may be due to the observation that the nanoparticles did not enter the bacteria.
Under the test conditions, Gold nanoparticles are not considered as mutagenic in this bacterial system.

### Cross-reference to other study
No cross-reference

### 7.6.2 Genetic toxicity in vivo

## 7.7 Carcinogenicity

## 7.8 Toxicity to reproduction

## 7.9 Specific investigations

### 7.9.1 Neurotoxicity

### 7.9.2 Immunotoxicity

### 7.9.3 Specific investigations: other studies

### *Endpoint study record: 7440-57-5, Specific investigations - cell impedance, Anonymous, Year, SS, S*

#### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

#### Data source

#### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

#### Data access

other: data submitter is data owner or has letter of access

## Data protection claimed
yes, but willing to share

## Cross-reference to same study
No cross-reference

## Materials and methods

## Type of effects studied
cytotoxicity

## Type of method
in vitro

## Endpoint addressed
other: cytotoxicity

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline
The Roche xCELLigence RTCA single plate (SP) instrument was used to measure electrode impedance across the base of the wells. This impedance is influenced by the presence of cells cultured on plates on top of these electrodes, and therefore the CI values are used to monitor cell viability. cAuNPs were added to wells with culture media but in the absence of cells. BEAS-2B cells were seeded alongside to give a comparison of CI that would be obtained during an experiment. A scan was acquired every 15 mins.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

### Confidential details on test material
No data

### Test animals

### Species
other: BEAS-2B cells

### Administration / exposure

### Duration of treatment / exposure
Not applicable

### Frequency of treatment
Not applicable

### Post exposure period
Not applicable

### No. of animals per sex per dose
Not applicable

### Examinations

### Any other information on materials and methods incl. tables
The Roche xCELLigence RTCA single plate (SP) instrument was used to measure electrode impedance across the base of the wells. cAuNPs were added to wells with culture media but in the absence of cells. BEAS-2B cells were seeded alongside to give a comparison of CI that would be obtained during an experiment. A scan was acquired every 15 mins.

### Results and discussions

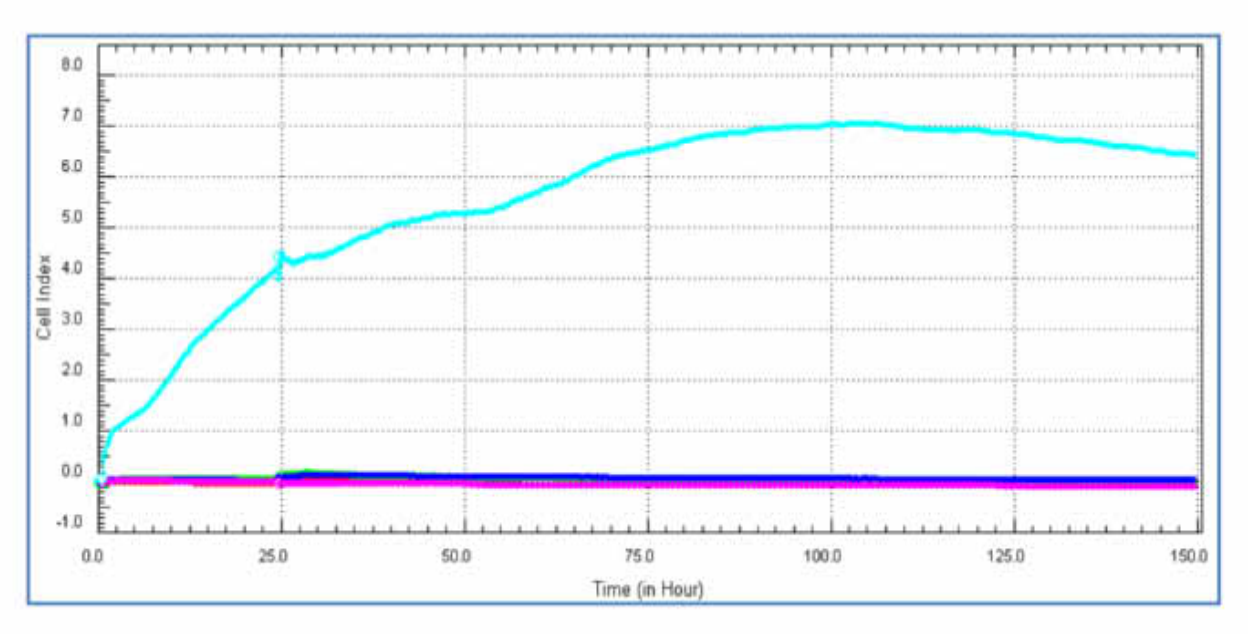
### Details on results
cAuNPs showed no increase in CI as compared to the media-only wells. The CI from both the media-only and the media with particles wells is negligible compared to the CI reading obtained from well with proliferating BEAS-2B cells.

### Any other information on results incl. tables
See the attached document for figure: Cell Index obtained from untreated BEAS-2B cells, media only wells, and media and cAuNP wells

### Overall remarks, attachments
### Remarks on results including tables and figures
None

## Attached background material

| Attached document | Remarks |
|---|---|
| Cell impedance - xCELLigence system.pdf / 15.24 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Conclusions

Under the test conditions, cAuNPs do not interfere with the measurement of CI values using the xCELLigence system. cAuNPs are capable of interfering with in vitro toxicity assays, either through direct optical interference or through interference of the reaction of the assay. Interference is assay specific and therefore each assay needs to be validated individually.

### Executive summary

The Roche xCELLigence RTCA single plate (SP) instrument was used to measure electrode impedance across the base of the wells. cAuNPs were added to wells with culture media but in the absence of cells. BEAS-2B cells were seeded alongside to give a comparison of CI that would be obtained during an experiment. A scan was acquired every 15 mins.
cAuNPs showed no increase in CI as compared to the media-only wells. The CI from both the media-only and the media with particles wells was negligible compared to the CI reading obtained from well with proliferating BEAS-2B cells.
Under the test conditions, cAuNPs do not interfere with the measurement of CI values using the xCELLigence system.

### Cross-reference to other study

No cross-reference

## *Endpoint study record: 7440-57-5, Specific investigations - cellular uptake, Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | | No information | | | | | | |

### Data access

other: data submitter is data owner or has letter of access

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

No cross-reference

### Materials and methods

### Type of effects studied

other: cellular uptake

### Type of method

in vitro

### Endpoint addressed

other: cellular uptake

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline

The Cytoviva Hyperspectral Imaging (HSI) consists of patented illumination technology that integrates onto a standard optical microscope to create high signal-to-noise, darkfield images. Integrated with this is a Visible and Near-Infrared HSI which enables spectral quantification of the sample being analysed. The system captures the unique reflectance spectra of objects in the microscope field of view and this spectral signature can be collected to form a library. Images of scanned samples can be mapped against the library.

## GLP compliance

no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Confidential details on test material

No data

## Test animals

## Species

other: in vitro - HEK 293, BEAS 2B and CHO cells

## Administration / exposure

## Duration of treatment / exposure

1, 4 and 6 h

## Frequency of treatment

Not applicable

## Post exposure period

Not applicable

## No. of animals per sex per dose

Not applicable

## Examinations

## Any other information on materials and methods incl. tables

BEAS 2B, HEK 293, and CHO cells were treated with cAuNPs for 1, 4 and 6 h. Following treatment, cells were washed, fixed and immobilized. Dark-field images were captured at 60x magnification using the CytoViva HSI system. HSI scans were acquired of the cAuNPs alone and of the cells incubated with particles. Spectral profiles were collected from the HSI scans.

## Results and discussions

## Details on results

- Uptake was observed in all cell lines to various extends. - BEAS-2B cells: BEAS-2B cells showed high levels of uptake, even after an hour of incubation. The particles seemed to accumulate and form aggregates within the cells. - HEK 293 cells: The cells treated with 14 nm AuNPs had cellular uptake after an hour although this uptake was not as high as in BEAS-2B cells. - CHO cells: Minimal AuNPs uptake was observed in the CHO cells after an hour, however an increase and high accumulation of the nanoparticles was observed after 4 and 6 h.

## Any other information on results incl. tables

See the attached document for figure: Cytoviva Hyperspectral Imaging

## Overall remarks, attachments

## Remarks on results including tables and figures

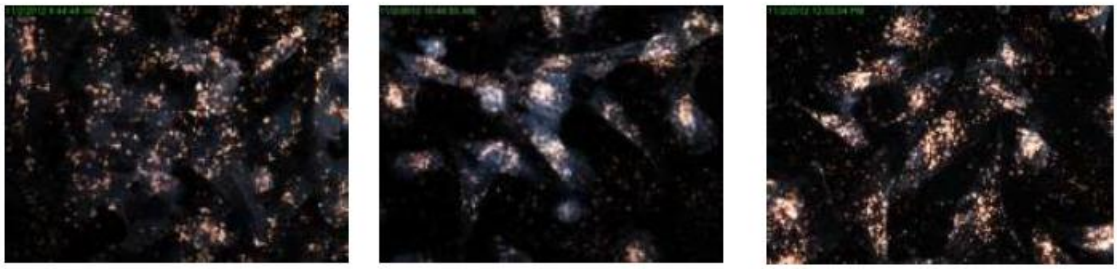
None

## Attached background material

| Attached document | Remarks |
|---|---|
| Cytoviva Hyperspectral Imaging - cellular uptake.pdf / 128.77 KB (application/octet-stream) | |

BEAS-2B

1hr 4hr 6hr

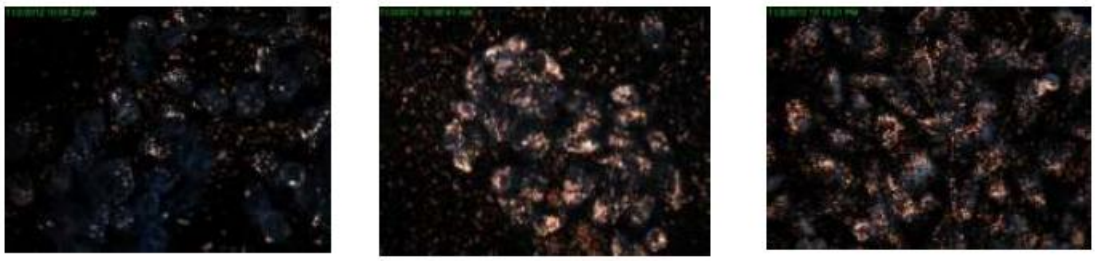


HEK 293

1hr 4hr 6hr

CHO

1hr 4hr 6hr



## Applicant's summary and conclusion

## Conclusions

Not available

## Executive summary

A study was conducted to determine the cellular uptake of cAuNPs in BEAS 2B, HEK 293 and CHO cells using the CytoViva HSI system. The cells were treated with cAuNPs for 1, 4 and 6 h and then cells were washed, fixed and immobilized. Dark-field images were captured at 60x magnification using the CytoViva HSI system. HSI scans were acquired of the cAuNPs alone and of the cells incubated with particles. Spectral profiles were collected from the HSI scans.
Uptake was observed in all cell lines to various extends. BEAS-2B cells showed high levels of uptake, even after an hour of incubation. The particles seemed to accumulate and form aggregates within the cells. HEK 293 cells treated with 14 nm AuNPs had cellular uptake after an hour although this uptake was not as high as in BEAS-2B cells. Minimal AuNPs uptake was observed in the CHO cells after an hour, however an increase and high accumulation of the nanoparticles was observed after 4 and 6 h.
Under the test conditions, the cellular uptake of cAuNPs was observed in BEAS 2B, HEK 293 and CHO cells.

## Cross-reference to other study

No cross-reference

# *Endpoint study record: 7440-57-5, Specific investigations - cytotoxicity (ATP-based assay), Anonymous, Year, SS, S*

## Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

## Data access
other: data submitter is data owner or has letter of access

## Data protection claimed
yes, but willing to share

## Cross-reference to same study
No cross-reference

## Materials and methods

## Type of effects studied
cytotoxicity

## Type of method
in vitro

## Endpoint addressed
other: cytotoxicity

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline
Traditional assay was used to assess the cytoxicity of cAuNPs : the ATP CellTiter Glo assay (Promega) which measures the levels of ATP in metabolically active cells. In a cytotoxicity study (ATP-based assay), BEAS-2B cells were treated with 1 and 5 nM cAuNPs for one hour. The CellTiter-Glo® Reagent was added directly to cells in multiwell plates. Luciferin is catalyzed by luciferase in the presence of Mg2+, ATP and molecular oxygen to generate a stable luminescent signal.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

**Details on test material**
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

**Confidential details on test material**
No data

**Test animals**

**Species**
other: in vitro - BEAS 2B cells

**Administration / exposure**

**Duration of treatment / exposure**
One hour

**Frequency of treatment**
Not applicable

**Post exposure period**
Not applicable

**Doses / concentrations**
1 and 5 nM
Basis no data

**No. of animals per sex per dose**
Not applicable

**Examinations**

**Any other information on materials and methods incl. tables**
BEAS-2B cells were allowed to proliferate for 24 h before AuNP treatment. Untreated control wells contained media. Cells were treated with 1 or 5 nM cAuNPs for one hour. The CellTiter-Glo® Reagent was added directly to cells in multiwell plates. Luciferin is catalyzed by luciferase in the presence of $Mg^{2+}$, ATP and molecular oxygen to generate a stable luminescent signal.

**Results and discussions**

**Details on results**
cAuNPs were toxic in a concentration dependent manner.

## Any other information on results incl. tables

See the attached document for figure: Toxicity of cAuNPs observed from the ATP-based assay

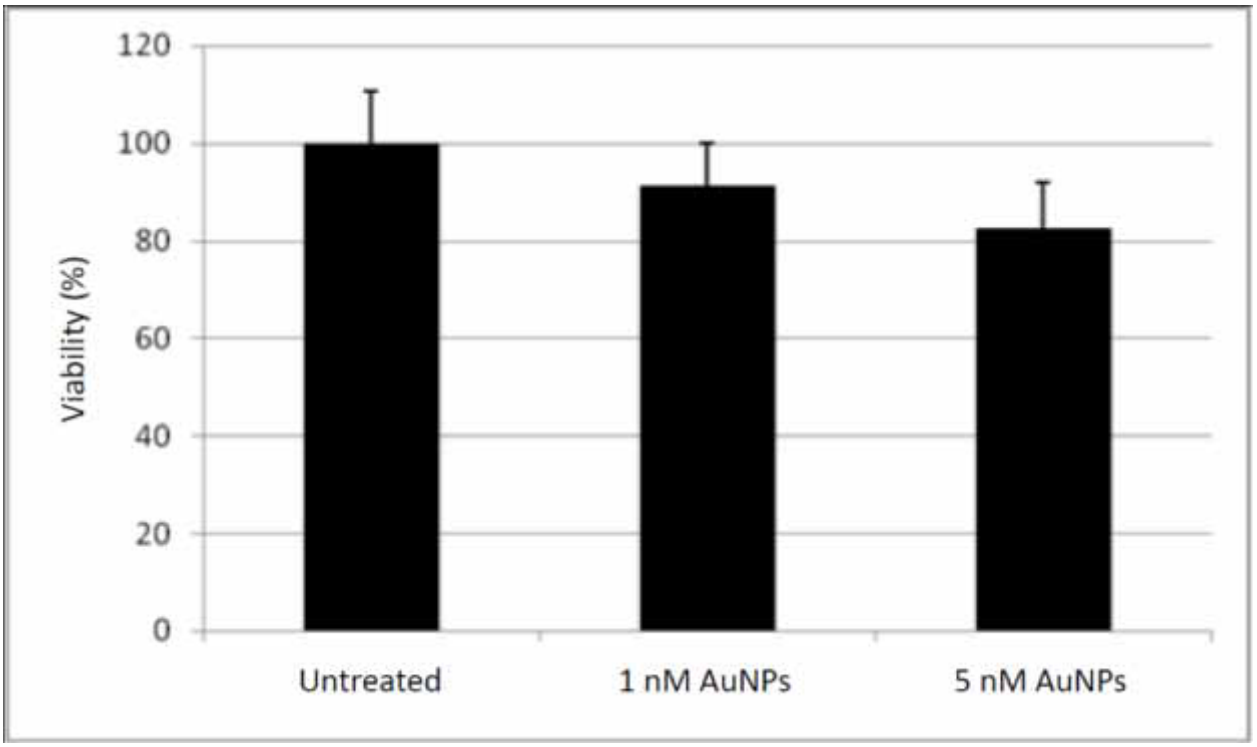
## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Attached background material

| Attached document | Remarks |
|---|---|
| Toxicity of cAuNPs observed from the ATP-based assay.pdf / 88.62 KB (application/octet-stream) | |




## Applicant's summary and conclusion

## Conclusions

Contradictory results between the XTT-, LDH-, and ATP-based assays could be caused by interference of the nanoparticles with the assay. This potential interference needs to be investigated and carefully controlled.

## Executive summary

In a cytotoxicity study (ATP-based assay), BEAS-2B cells were treated with 1 and 5 nM cAuNPs for one hour. The CellTiter-Glo® Reagent was added directly to cells in multiwell plates. Luciferin is catalyzed by luciferase in the presence of Mg2+, ATP and molecular oxygen to generate a stable luminescent signal. Untreated control wells contained media alone.

Results showed that cAuNPs were toxic in a concentration dependent manner.
Under the test conditions, cAuNPs were toxic toBEAS-2B cellsin a concentration dependent manner.

### Cross-reference to other study
No cross-reference

## *Endpoint study record: 7440-57-5, Specific investigations - cytotoxicity (LDH release assay), Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

### Data access
other: data submitter is data owner or has letter of access

### Data protection claimed
yes, but willing to share

### Cross-reference to same study
No cross-reference

### Materials and methods
### Type of effects studied
cytotoxicity

## Type of method
in vitro

## Endpoint addressed
other: cytotoxicity

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline
Traditional assay was used to assess the cytoxicity of cAuNPs: CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega) which measures the release of lactate dehydrogenase (LDH). In a LDH release assay (cytotoxicity study), BEAS-2B cells were treated with 1 and 5 nM cAuNPs for one hour. This assay measures the release of LDH through the measurement of the fluorescence of the final assay product, resorufin.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Confidential details on test material
No data

## Test animals

## Species
other: in vitro - BEAS 2B cells

## Administration / exposure

## Duration of treatment / exposure
One hour

### Frequency of treatment
Not applicable

### Post exposure period
Not applicable

### Doses / concentrations
1 and 5 nM

**Basis** no data

### No. of animals per sex per dose
Not applicable

### Examinations

### Any other information on materials and methods incl. tables
BEAS-2B cells were allowed to proliferate for 24 h before AuNP treatment. Untreated control wells contained media. Cells were treated with 1 or 5 nM cAuNPs for one hour. Lysis solution provided with the kit was used to generate maximum LDH Release. LDH released into the culture medium was measured via the conversion of resazurin into resorufin. Fluorescence was measured at 560 nm excitation, 590 nm emission.

### Results and discussions

### Details on results
cAuNPs was nontoxic to BEAS-2B cells in LDH release assay.

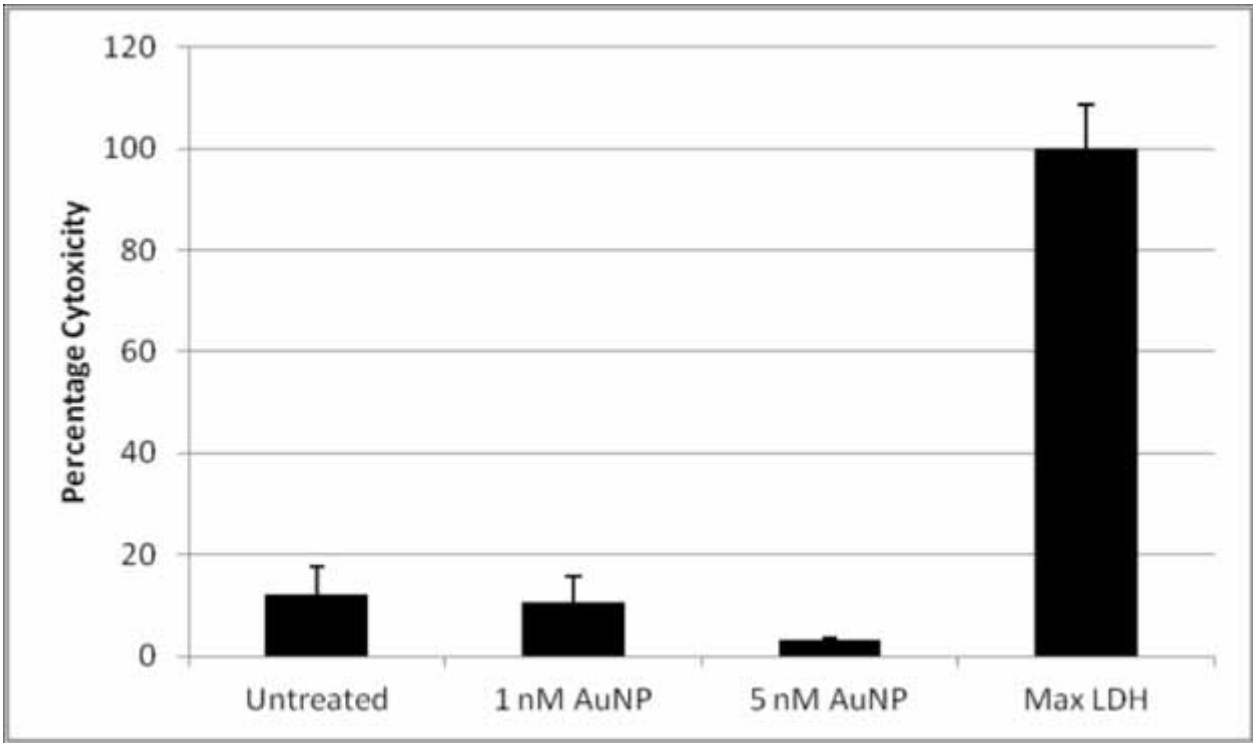
### Any other information on results incl. tables
See the attached document for figure: Toxicity of cAuNPs observed from the LDH-based assay

### Overall remarks, attachments

### Remarks on results including tables and figures
None

## Attached background material

| Attached document | Remarks |
|---|---|
| Toxicity of cAuNPs observed from the LDH-based assay.pdf / 87.63 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Conclusions

Contradictory results between the XTT-, LDH-, and ATP-based assays could be caused by interference of the nanoparticles with the assay. This potential interference needs to be investigated and carefully controlled.

### Executive summary

In a cytotoxicity study (LDH release assay), BEAS-2B cells were treated with 1 and 5 nM cAuNPs for one hour. This assay measures the release of LDH through the measurement of the fluorescence of the final assay product, resorufin. Untreated control wells contained media alone. Fluorescence was measured at 560 nm excitation, 590 nm emission.
The measurement of LDH release assay shows the cAuNPs to be nontoxic at both concentrations.
Under the test conditions, cAuNPs was nontoxic to BEAS-2B cells.

### Cross-reference to other study

No cross-reference

## *Endpoint study record: 7440-57-5, Specific investigations - cytotoxicity (XTT assay), Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

### Data access
other: data submitter is data owner or has letter of access

### Data protection claimed
yes, but willing to share

### Cross-reference to same study
No cross-reference

### Materials and methods

### Type of effects studied
cytotoxicity

### Type of method
in vitro

### Endpoint addressed
other: cytotoxicity

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline

Traditional assay was used to assess the cytoxicity of cAuNPs: “the In vitro Toxicology Assay Kit, XTT based (Sigma-Aldrich) whereby XTT tetrazolium salt is reduced by the mitochondria to form a colorimetric product". In a cytotoxicity study, BEAS-2B cells were treated with 1 and 5 nM cAuNPs for one hour and XTT reagent was added, incubated and read at 450 nm.

## GLP compliance

no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Confidential details on test material

No data

## Test animals

## Species

other: in vitro - BEAS 2B cells

## Administration / exposure

## Duration of treatment / exposure

One hour

## Frequency of treatment

Not applicable

## Post exposure period

Not applicable

## Doses / concentrations

1 and 5 nM

Basis no data

## No. of animals per sex per dose

Not applicable

## Examinations

## Any other information on materials and methods incl. tables

BEAS-2B cells were allowed to proliferate for 24 h before AuNP treatment. Untreated control wells contained media. Cells were treated with 1 or 5 nM cAuNPs for one hour. Positive control wells received 500 μM hydrogen peroxide. XTT reagent was added, incubated and read at 450 nm.

## Results and discussions

## Details on results

XTT assay results showed that a decrease in absorbance, indicative of a decrease in cell viability, as evident from the hydrogen peroxide positive control. Based on the XTT assay, it can be deduced that at 1 nM the AuNPs exhibit only mild toxicity, however at 5 nM there is an increase in viability as compared to the untreated control cells.

## Any other information on results incl. tables

See the attached document for figure: Toxicity of cAuNPs observed from the XTT-based assay

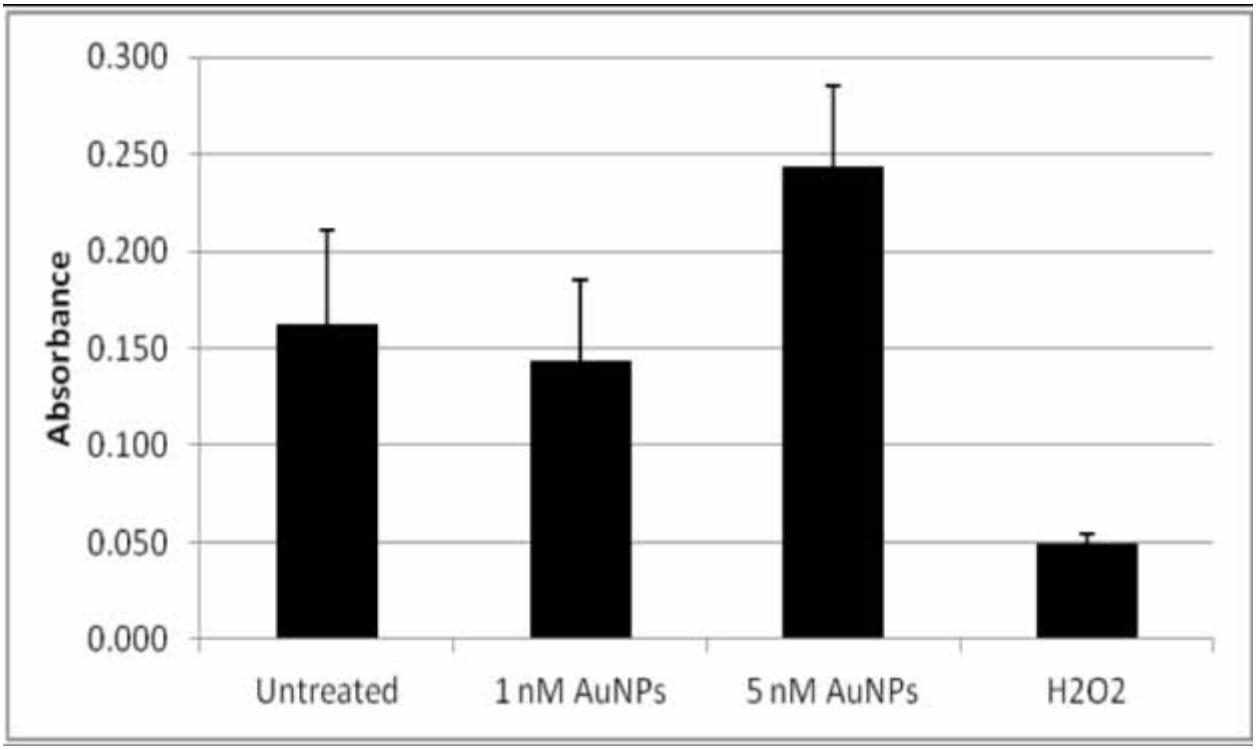
## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Attached background material

| Attached document | Remarks |
|---|---|
| Toxicity of cAuNPs observed from the XTT-based assay.pdf / 88.85 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Conclusions

Contradictory results between the XTT-, LDH-, and ATP-based assays could be caused by interference of the nanoparticles with the assay. This potential interference needs to be investigated and carefully controlled.

### Executive summary

In a cytotoxicity study, BEAS-2B cells were treated with cAuNPs at 1 and 5 nM for one hour and XTT reagent was added, incubated and read at 450 nm. Positive control wells received 500 µM hydrogen peroxide.

Results showed that a decrease in absorbance, indicative of a decrease in cell viability, as evident from the hydrogen peroxide positive control. Based on the XTT assay, it can be deduced that at 1 nM the AuNPs exhibit only mild toxicity, however at 5 nM there is an increase in viability as compared to the untreated control cells.

Under the test conditions, the AuNPs exhibit only mild toxicity at 1 nM, however at 5 nM there is an increase in viability as compared to the untreated control cells.

### Cross-reference to other study

No cross-reference

## *Endpoint study record: 7440-57-5, Specific investigations - cytotoxicity using cell impedance, Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | | No information | | | | | | |

### Data access

other: data submitter is data owner or has letter of access

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

No cross-reference

### Materials and methods

### Type of effects studied

cytotoxicity

### Type of method

in vitro

### Endpoint addressed

other: cytotoxicity

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline

The Roche xCELLigence RTCA single plate (SP) instrument was used to measure Cell Index (CI) which is a measure of the electrode impedance which reflects the state of the ionic environment at the electrode/solution interface. This impedance is influenced by the presence of cells cultured on plates on top of these electrodes and therefore used to monitor cell viability. In cytotoxicity study, HEK 293, BEAS 2B and CHO cells were treated with the cAuNPs in Roche xCELLigence RTCA single plate (SP) instrument and then scans were acquired. The experiments with the BEAS-2B cells was run for 6 days, the HEK 293 cells for 36 h, and the CHO cells for 80 h.

## GLP compliance

no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Confidential details on test material

No data

## Test animals

## Species

other: in vitro - HEK 293, BEAS 2B and CHO cells

## Administration / exposure

## Duration of treatment / exposure

Not applicable

## Frequency of treatment

Not applicable

## Post exposure period

Not applicable

## Doses / concentrations

0.5, 1, 2 and 5 nM

**Basis** no data

## No. of animals per sex per dose

Not applicable

## Examinations

## Any other information on materials and methods incl. tables

Cytotoxicity studies were conducted using the Roche xCELLigence RTCA single plate (SP) instrument. HEK 293, BEAS 2B and CHO cells were seeded and placed in the RTCA station and allowed to proliferate for 24 h prior to treatment, during which time a scan was acquired every 15 minutes. The cells were then treated with the cAuNPs and scans were acquired.

## Results and discussions

## Details on results

Each cell line exhibited its own distinct growth curve and experiments were stopped once the cell viability of the untreated cells began to decrease. The experiments with the BEAS-2B cells was run for 6 days, the HEK 293 cells for 36 h, and the CHO cells for 80 h. The curves of the treated cells show the same trends as the untreated cells and therefore concluded that the cAuNPs were nontoxic.

## Any other information on results incl. tables

See the attached document for figure: Cytotoxicity using cell impedance

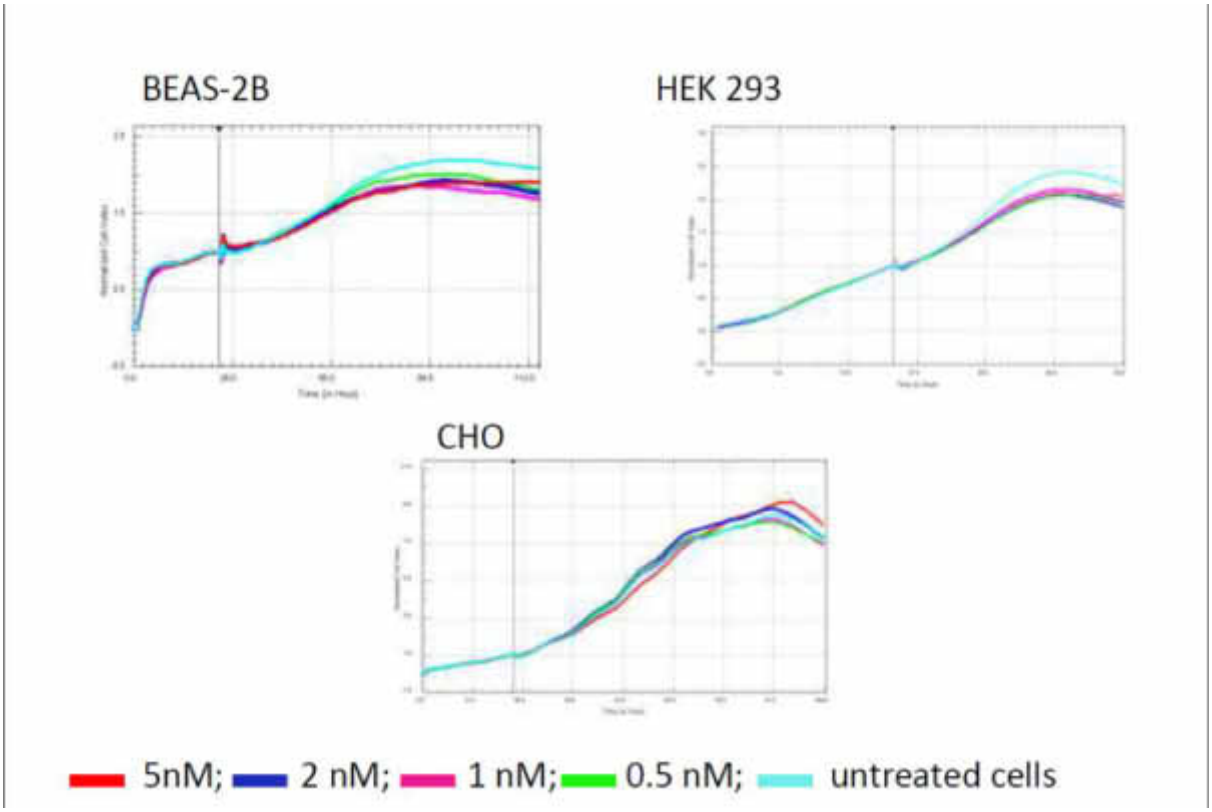
## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Attached background material

| Attached document | Remarks |
|---|---|
| Cytotoxicity using cell impedance.pdf / 55.86 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Conclusions

The treated cells followed the trend of the untreated control cells and therefore it was concluded that the cAuNPs were nontoxic.

### Executive summary

In cytotoxicity study, HEK 293, BEAS 2B and CHO cells were treated with the cAuNPs at 0.5, 1, 2 and 5 nM in Roche xCELLigence RTCA single plate (SP) instrument and then scans were acquired. The experiments with the BEAS-2B cells was run for 6 days, the HEK 293 cells for 36 h, and the CHO cells for 80 h.

Each cell line exhibited its own distinct growth curve and experiments were stopped once the cell viability of the untreated cells began to decrease. The results of the treated cells show the same trends as the untreated cells and therefore concluded that the cAuNPs were nontoxic.

Under the test conditions, cAuNPs were nontoxic to BEAS 2B, HEK 293 and CHO cells.

### Cross-reference to other study

No cross-reference

# 7.10 Exposure related observations in humans

## 7.10.1 Health surveillance data

## 7.10.2 Epidemiological data

## 7.10.3 Direct observations: clinical cases, poisoning incidents and other

## 7.10.4 Sensitisation data (humans)

## 7.10.5 Exposure related observations in humans: other data

### *Endpoint study record: 7440-57-5, Exposure related observations in humans-other data, Anonymous, Year, RS, K*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | key study; robust study summary | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| study report | No information | | No information | | | | | | |

### Data access

other: data submitter is data owner or has letter of access

### Data protection claimed

yes, but willing to share

### Cross-reference to same study

No cross-reference

## Materials and methods

## Type of information

Background: The synthesis and subsequent use of nanoparticles in a wide variety of applications is one of the rapidly expanding research areas in South Africa in the last decade. This in turn has led to an increasing likelihood of occupational exposure to these nanoparticles. The aim of this survey was to perform an exposure assessment during the synthesis of AuNPs using the citrate reduction method.The exposure assessment of AuNPs was conducted at an AuNP research and development laboratory. This laboratory produces a variety of nanomaterials including AuNP for research in various applications in health (diagnostics & therapeutics) and water (treatment & analysis).

## Endpoint addressed

other: occupational exposure

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline available | | |

## Principles of method if other than guideline

The exposure assessment survey was conducted using a combination of particle number concentration measurement and filter based (personal and area) sampling. The main objective was to identify processes and tasks that may result in emission of AuNPs into the environment during their synthesis.

## GLP compliance

no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)

yes

## Test material form

nanomaterial

## Details on test material

- Name of test material (as cited in study report): Gold nanoparticles (AuNPs)

## Method

## Details on study design

The exposure assessment survey was conducted using a combination of particle number concentration measurement and filter based (personal and area) sampling. The main objective was to identify processes and tasks that may result in emission of AuNPs into the environment during their synthesis.

## Exposure assessment

measured

## Any other information on materials and methods incl. tables

Real time measurement instruments used in the study.

***Table 7.10.5/1: Exposure assessment***

| Name | Metric | Range | Portability |
|---|---|---|---|
| Scanning Mobility Particle Counter (TSI SMPS Model 3080) equipped with a long Differential Mobility Analyzer (TSI DMA Model 3081). | Number, Size distribution | 5 -1000 nm | Desk top |
| Aerodynamic Particle Sizer (TSI APS Model 3321) | Number, Size distribution | 500 nm-20 μm | Desk top |
| P-Trak Ultrafine Particle Counter (TSI UPC, Model 8525) | Total number | 10-1000 nm | Hand held |
| HACH Met One Handheld Particle Counter (HHPC-6) | Number, Size distribution | 300 nm-5 μm | Hand held |
| Naneum Nano-ID | Size distribution by mass | 2 nm-20 μm | Portable |

At the time of this exposure assessment a volume of 80L of AuNP was synthesized using a citrate reduction method of chloroauric acid.

For the purpose of this report only the real-time particle measurement results are presented as the results from characterisation of particles captured on the filters were not yet available at the time of writing this report.

Real-time local background particle exposure was measured in terms of number concentration when the process of interest in not in operation was determined to be 7965 particles per cubic centimetre of air.

Real-time particle number and mass concentration data at emission points was measured at the perimeters of process enclosures and extraction ventilation, during operation of the process.

## Results and discussions

## Results

- The measurement of nanomaterials in the laboratory did not increase appreciably during the tasks monitored. Only after 30 minutes after the addition of the citrate solution was there an increase in the number of nanomaterials measured by the SMPS. The observed increase was almost twice the calculated local particle reference value.
- Similarly to the SMPS the TSI P-Trak result also show an increase in the number of total particles (10 – 1000 nm) at about the same time. At this stage it was not clear whether the observed increase was from the process or from another as yet unidentified source within the laboratory. It is hoped that the area sampling filter will shed a light on the nature and possibly the source of the measured nanoparticles.

## Any other information on results incl. tables

See the attached document for figure: Particle number concentration measured

## Overall remarks, attachments

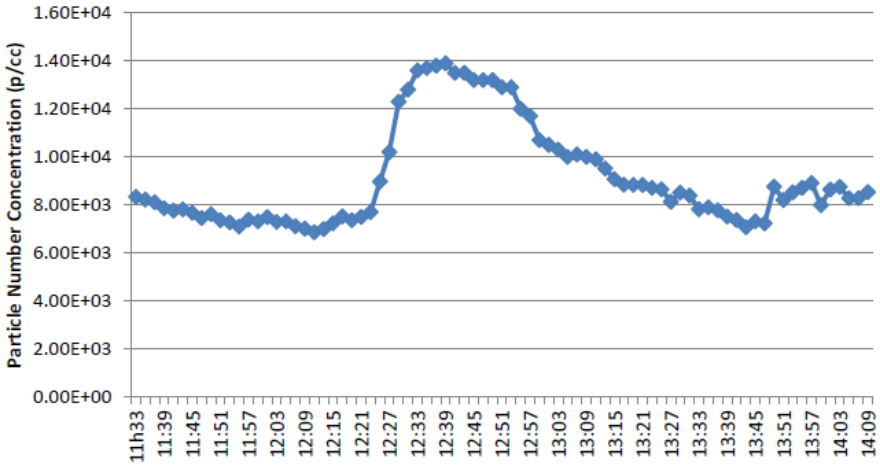
## Remarks on results including tables and figures

None

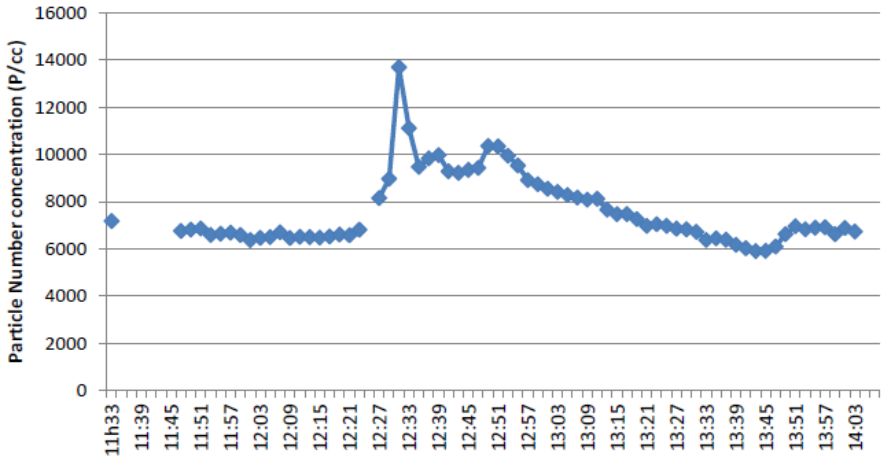
## Attached background material

| Attached document | Remarks |
|---|---|
| Human Exposure.pdf / 91.08 KB (application/octet-stream) | |

Particle number concentration measured by SMPS during AuNP synthesis




Particle number concentration measured by TSI- P-Trak during AuNP synthesis



### Applicant's summary and conclusion

### Conclusions

From the results obtained so far it will seem that further assessments may not need to be carried out, as the OECD general excursion guidance criteria has not been exceeded. According to the OECD general excursion guidance criteria (OECD 2012), a nanotechnology process could be considered to require further assessment if; (a) short term exposures/emissions exceed three times the local particle reference value for more than a total of 30 minutes during a work day; and/or, (b) a single short term value exceeds five times the local particle reference value.

### Executive summary

The exposure assessment survey was conducted using a combination of particle number concentration measurement and filter based (personal and area) sampling. The main objective was to identify processes and tasks that may result in emission of AuNPs into the environment during their synthesis. Real time measurement instruments used in the study.

Real-time local background particle exposure was measured in terms of number concentration when the process of interest in not in operation was determined to be 7965 particles per cubic centimetre of air. Real-time particle number and mass concentration data at emission points was measured at the perimeters of process enclosures and extraction ventilation, during operation of the process. The measurement of nanomaterials in the laboratory did not increase appreciably during the tasks monitored. Only after 30 minutes after the addition of the citrate solution was there an increase in the number of nanomaterials measured by the SMPS. The observed increase was almost twice the calculated local particle reference value. Similarly to the SMPS the TSI P-Trak result also show an increase in the number of total particles (10 – 1000nm) at about the same time. At this stage it was not clear whether the observed increase was from the process or from another as yet unidentified source within the laboratory. It is hoped that the area sampling filter will shed a light on the nature and possibly the source of the measured nanoparticles.

From the results obtained so far it will seem that further assessments may not need to be carried out, as the OECD general excursion guidance criteria has not been exceeded.

### Cross-reference to other study

No cross-reference

## 7.11 Toxic effects on livestock and pets

## 7.12 Additional toxicological information

### *Endpoint study record: 7440-57-5, Additional toxicological information - interference (ATP assay), Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

## Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Type of information

Background: Kroll et al (2009) summarized nanoparticle characteristics such as high adsorption capacity, surface charge, and catalytic activity as potential causes of the interference of nanoparticles with toxicity assays. In another publication the same group suggested an approach to prevent interference of nanoparticles with in vitro toxicity assays by altering assay protocols or lowering particle concentrations. However the authors did observe that interference was assay- and particle-specific, and recommended that each in vitro methodology to be evaluated for each individual nanoparticle (Kroll et al., 2012). Therefore the cAuNPs were tested for interference with some commonly used in vitro tests.

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline

Luminescent signal of the cAuNPs was measured. To test for further interference of the cAuNPs with the conversion of substrate to product, cAuNPs(1 and 5 nM) were incubated with the CellTiter-Glo® Reagent (CellTiter-Glo® Luminescent Cell Viability Assay, Promega) and 1.5 µM ATP.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Method

## Any other information on materials and methods incl. tables
Luminescent signal of the cAuNPs was measured. To test for further interference of the cAuNPs with the conversion of substrate to product, cAuNPs(1 and 5 nM) were incubated with the CellTiter-Glo® Reagent (CellTiter-Glo® Luminescent Cell Viability Assay, Promega) and 1.5 μM ATP.

## Results and discussions

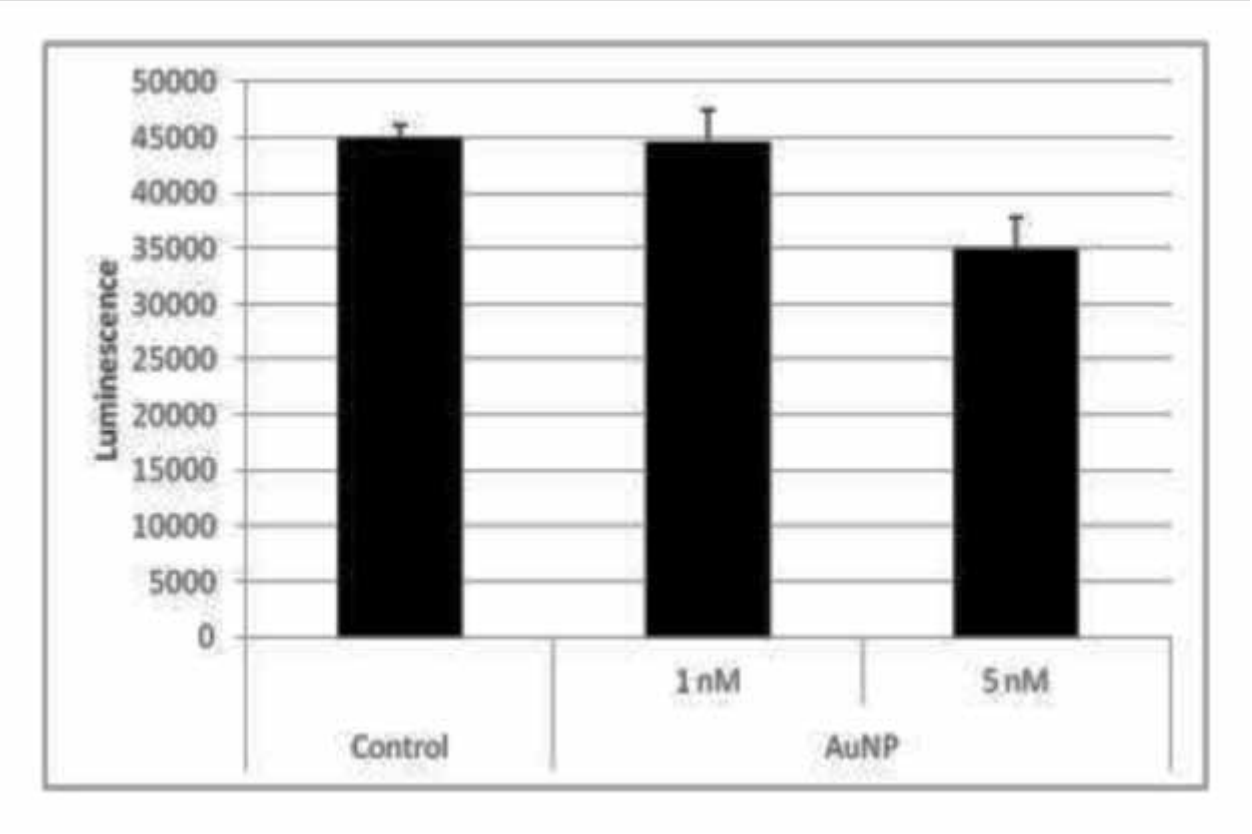
## Any other information on results incl. tables
No meaningful luminescent signal was detected with the particles alone. However, when a further experiment was conducted to investigate the effects of the AuNPs on the reaction that occurs during the ATP-based assay, namely the conversion of luciferin substrate to luminescent oxyluciferin in the presence of ATP, it can be seen that with an increase in AuNP concentration, a decrease in luminescent signal was observed, suggesting that the AuNPs were interfering with the conversion of luciferin to oxyluciferin at high concentrations.
See the attached document for figure: Luminescent signal obtained from the incubation of luciferin substrate and ATP in the presence of AuNPs

## Overall remarks, attachments

## Remarks on results including tables and figures
None

## Attached background material

| Attached document | Remarks |
|---|---|
| Luminescent signal obtained from the incubation of luciferin substrate and ATP in the presence of AuNPs.pdf / 87.51 KB (application/octet-stream) | |




## Applicant's summary and conclusion

### Conclusions

Although cAuNPs did not interfere with the detection of the luminescent signal, they were shown to interfere with the reaction on which this assay is based. cAuNPs are capable of interfering with in vitro toxicity assays, either through direct optical interference or through interference of the reaction of the assay. Interference is assay specific and therefore each assay needs to be validated individually.

### Executive summary

In an ATP based assay, luminescent signal of the cAuNPs was measured. To test for further interference of the cAuNPs with the conversion of substrate to product, cAuNPs (1 and 5 nM) were incubated with the CellTiter-Glo® Reagent and 1.5 μM ATP.

No meaningful luminescent signal was detected with the particles alone. However, when a further experiment was conducted to investigate the effects of the AuNPs on the reaction that occurs during the ATP-based assay, namely the conversion of luciferin substrate to luminescent oxyluciferin in the presence of ATP, it can be seen that with an increase in AuNP concentration, a decrease in luminescent signal was observed, suggesting that the AuNPs were interfering with the conversion of luciferin to oxyluciferin at high concentrations.

Although cAuNPs did not interfere with the detection of the luminescent signal, they were shown to

interfere with the reaction on which this assay is based.

### Cross-reference to other study
No cross-reference

## *Endpoint study record: 7440-57-5, Additional toxicological information - interference (DCFH-DA assay), Anonymous, Year, SS, S*

### Administrative Data

**Purpose flag** supporting study

**Study result type** experimental result **Study period** No data

**Reliability** 2 (reliable with restrictions)

**Rationale for reliability incl. deficiencies** Study well documented, meets generally accepted scientific principles, acceptable for assessment

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

### Data access
other: data submitter is data owner or has letter of access

### Data protection claimed
yes, but willing to share

### Cross-reference to same study
No cross-reference

## Materials and methods

## Type of information
cAuNPs were incubated with DCF and measured at excitation 480 nm, emission 530 nm.

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline
Formation of Reactive Oxygen species (ROS) in cells indicates an increase in the cells' oxidative stress. DCFH-DA penetrates cell membrane; it is hydrolysed by cellular esterases to DCFH and converted via ROS to fluorescent DCF. The fluorescence intensity is proportional to the ROS levels within the cell. cAuNPs were incubated with DCF and measured at excitation 480 nm, emission 530 nm.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Method

## Any other information on materials and methods incl. tables
cAuNPs (0, 1 and 2 nM concentrations) were incubated with DCF (0, 1000, 2500, 5000 and 10000 nM) and measured at excitation 480 nm, emission 530 nm.

## Results and discussions

## Any other information on results incl. tables
In the absence of DCF, the cAuNPs did not produce a detectable fluorescent signal. Increasing concentrations of cAuNPs resulted in a decrease in the fluorescent intensity of the DCF. See the attached document for figure: Fluorescent signal obtained from the addition of cAuNPs to DCF at various concentrations

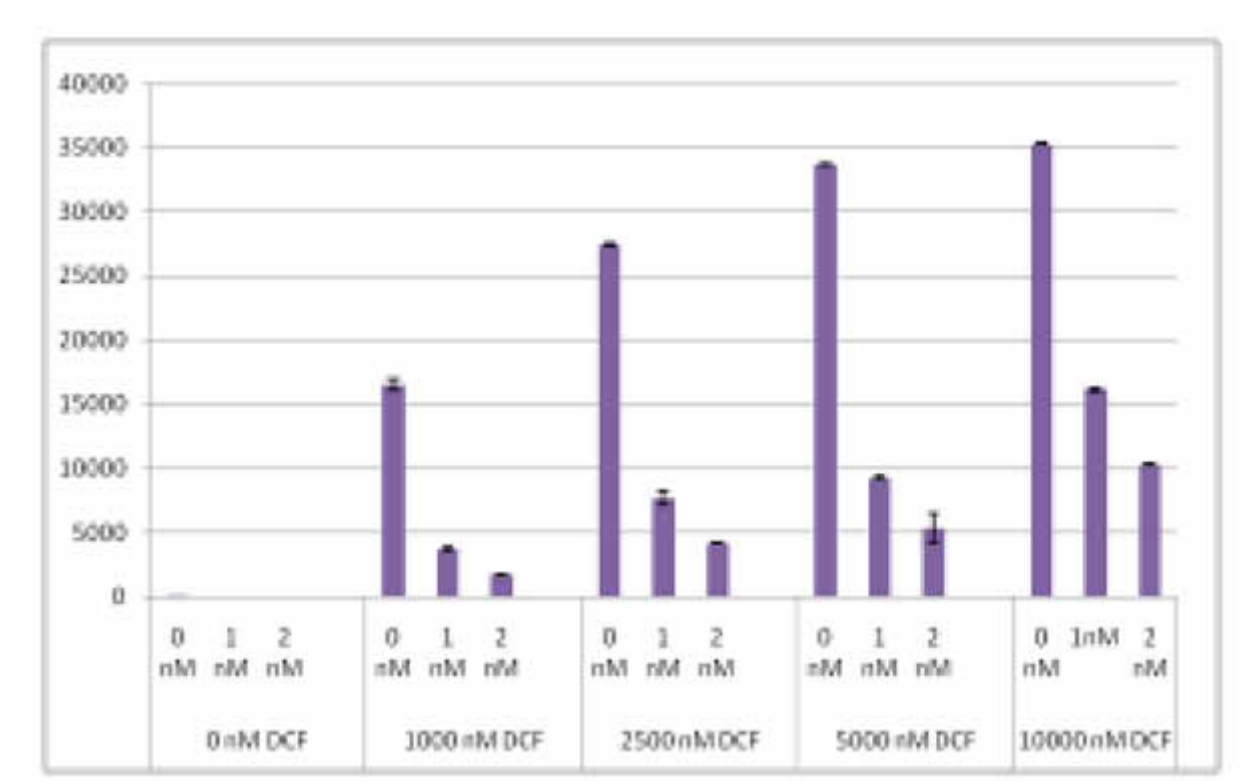
## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Attached background material

| Attached document | Remarks |
|---|---|
| Fluorescent signal obtained from the addition of cAuNPs to DCF at various concentrations.pdf / 15.96 KB (application/octet-stream) | |




## Applicant's summary and conclusion

## Conclusions

Under the test conditions, cAuNPs did not produce fluorescent signal themselves, however they appear to quench the fluorescent signal from the DCF; therefore this assay cannot be used to detect Reactive Oxygen species (ROS). cAuNPs are capable of interfering with in vitro toxicity assays, either through direct optical interference or through interference of the reaction of the assay. Interference is assay specific and therefore each assay needs to be validated individually.

## Executive summary

A study was conducted to determine the cells' oxidative stress by cAuNPs using the fluorescence intensity. cAuNPs (0, 1 and 2 nM concentrations) were incubated with DCF (0, 1000, 2500, 5000 and 10000 nM) and fluorescence was measured at excitation 480 nm, emission 530 nm.

In the absence of DCF, the cAuNPs did not produce a detectable fluorescent signal. Increasing

concentrations of cAuNPs resulted in a decrease in the fluorescent intensity of the DCF.
Under the test conditions, cAuNPs did not produce fluorescent signal themselves, however they appear to quench the fluorescent signal from the DCF; therefore this assay cannot be used to detect Reactive Oxygen species (ROS).

### Cross-reference to other study
No cross-reference

## *Endpoint study record: 7440-57-5, Additional toxicological information - interference (LDH assay), Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

### Data access
other: data submitter is data owner or has letter of access

### Data protection claimed
yes, but willing to share

### Cross-reference to same study
No cross-reference

## Materials and methods

### Type of information

Background: Kroll et al (2009) summarized nanoparticle characteristics such as high adsorption capacity, surface charge, and catalytic activity as potential causes of the interference of nanoparticles with toxicity assays. In another publication the same group suggested an approach to prevent interference of nanoparticles with in vitro toxicity assays by altering assay protocols or lowering particle concentrations. However the authors did observe that interference was assay- and particle-specific, and recommended that each in vitro methodology to be evaluated for each individual nanoparticle (Kroll et al., 2012). Therefore the cAuNPs were tested for interference with some commonly used in vitro tests.

### Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

### Principles of method if other than guideline

Luminescence of the cAuNPs was measured at 560 nm excitation, 590 nm emission. To test for further interference, cAuNPs were incubated with resazurin and NADH in order to assess the ability of the particles to interfere with the conversion of substrate to product.

### GLP compliance

no data

### Test materials

### Identity of test material same as for substance defined in section 1 (if not read-across)

yes

### Test material form

nanomaterial

### Details on test material

- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

### Method

### Any other information on materials and methods incl. tables

Luminescence of the cAuNPs was measured at 560 nm excitation, 590 nm emission. To test for further interference, cAuNPs were incubated with resazurin and NADH in order to assess the ability of the particles to interfere with the conversion of substrate to product.

## Results and discussions

### Any other information on results incl. tables

No meaningful fluorescence signal was observed from the particles alone. Also, the particles had no effect

on the conversion of substrate to product.

### Overall remarks, attachments

### Remarks on results including tables and figures
None

### Applicant's summary and conclusion

### Conclusions
Based on the results, cAuNPs do not interfere with this LDH assay. cAuNPs are capable of interfering with in vitro toxicity assays, either through direct optical interference or through interference of the reaction of the assay. Interference is assay specific and therefore each assay needs to be validated individually.

### Executive summary
In a LDH assay, luminescence of the cAuNPs was measured at 560 nm excitation, 590 nm emission. To test for further interference, cAuNPs were incubated with resazurin and NADH in order to assess the ability of the particles to interfere with the conversion of substrate to product.
No meaningful fluorescence signal was observed from the particles alone. Also, the particles had no effect on the conversion of substrate to product.
Based on the results, cAuNPs do not interfere with the LDH assay.

### Cross-reference to other study
No cross-reference

## *Endpoint study record: 7440-57-5, Additional toxicological information - interference (Limulus Amoebocyte Lysate (LAL) test) , Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

## Data source

## Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

## Data access

other: data submitter is data owner or has letter of access

## Data protection claimed

yes, but willing to share

## Cross-reference to same study

No cross-reference

## Materials and methods

## Type of information

To test for interference, cAuNPs were incubated with the colourless substrate Ac-Ile-Glu-Ala-Arg-pNA to observe activity of the particles on the substrate. In addition, cAuNPs were incubated with the reaction end-product p-nitroaniline (pNA) to observe any interference with the optical detection.

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline

The Limulus Amoebocyte Lysate (LAL) test is used to detect endotoxins and is based on the catalysis of a proenzyme to an enzyme by gram-negative bacterial endotoxin. This enzyme then catalyses the removal of pNA from a colourless substrate Ac-Ile-Glu-Ala-Arg-pNA. After the reaction is stopped, pNA is measured spectrophotometrically at 405 nm. The concentration of endotoxin is calculated from the absorbance values of standards containing known amounts of endotoxin. To test for interference, cAuNPs were incubated with the colourless substrate Ac-Ile-Glu-Ala-Arg-pNA to observe activity of the particles on the substrate. In addition, cAuNPs were incubated with the reaction end-product p-nitroaniline (pNA) to observe any interference with the optical detection.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Method

## Any other information on materials and methods incl. tables
The Limulus Amoebocyte Lysate (LAL) test is used to detect endotoxins and is based on the catalysis of a proenzyme to an enzyme by gram-negative bacterial endotoxin. This enzyme then catalyses the removal of pNA from a colourless substrate Ac-Ile-Glu-Ala-Arg-pNA. After the reaction is stopped, pNA is measured spectrophotometrically at 405 nm. The concentration of endotoxin is calculated from the absorbance values of standards containing known amounts of endotoxin. To test for interference, cAuNPs were incubated with the colourless substrate Ac-Ile-Glu-Ala-Arg-pNA to observe activity of the particles on the substrate. In addition, cAuNPs were incubated with the reaction end-product p-nitroaniline (pNA) to observe any interference with the optical detection.

## Results and discussions

## Any other information on results incl. tables
- Incubation of the substrate with cAuNPs resulted in no detectable levels of end-product, suggesting that cAuNPs do not have any catalytic activity in this reaction. - Incubation of the cAuNPs with the end-product, pNA, showed no change in absorbance reading.

## Overall remarks, attachments

## Remarks on results including tables and figures
None

## Applicant's summary and conclusion

## Conclusions
Under the test conditions, cAuNPs did not interfere with the LAL assay either by incubation with substrate or reaction end-product (p-nitroaniline). cAuNPs are capable of interfering with in vitro toxicity assays, either through direct optical interference or through interference of the reaction of the assay. Interference is assay specific and therefore each assay needs to be validated individually.

### Executive summary

In the Limulus Amoebocyte Lysate (LAL) test to detect endotoxins, cAuNPs were incubated with the colourless substrate Ac-Ile-Glu-Ala-Arg-pNA to observe activity of the particles on the substrate. In addition, cAuNPs were incubated with the reaction end-product p-nitroaniline (pNA) to observe any interference with the optical detection.
Incubation of the substrate with cAuNPs resulted in no detectable levels of end-product, suggesting that cAuNPs do not have any catalytic activity in this reaction. Incubation of the cAuNPs with the end-product, pNA, showed no change in absorbance reading.
Under the test conditions, cAuNPs did not interfere with the LAL assay either by incubation with substrate or reaction end-product (p-nitroaniline).

### Cross-reference to other study

No cross-reference

## *Endpoint study record: 7440-57-5, Additional toxicological information - interference (XTT assay), Anonymous, Year, SS, S*

### Administrative Data

| | | | |
|---|---|---|---|
| **Purpose flag** | supporting study | | |
| **Study result type** | experimental result | **Study period** | No data |
| **Reliability** | 2 (reliable with restrictions) | | |
| **Rationale for reliability incl. deficiencies** | Study well documented, meets generally accepted scientific principles, acceptable for assessment | | |

### Data source

### Reference

| Reference type | Author | Year | Title | Bibliographic source | Testing laboratory | Report no. | Owner company | Company study no. | Report date |
|---|---|---|---|---|---|---|---|---|---|
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J | 2009 | Current In Vitro Methods in Nanoparticle Risk Assessment: Limitations and Challenges. | Eur J Pharm Biopharm. 72: 370-377. | | | | | |
| publication | Kroll A, Pillukat MH, Hahn D and Schnekenburger J. | 2012 | Interference of Engineered Nanoparticles with In Vitro Toxicity Assays. | Arch Toxicol. 86(7): 1123-36. | | | | | |

### Data access

other: data submitter is data owner or has letter of access

## Data protection claimed
yes, but willing to share

## Cross-reference to same study
No cross-reference

## Materials and methods

## Type of information
Background: Kroll et al (2009) summarized nanoparticle characteristics such as high adsorption capacity, surface charge, and catalytic activity as potential causes of the interference of nanoparticles with toxicity assays. In another publication the same group suggested an approach to prevent interference of nanoparticles with in vitro toxicity assays by altering assay protocols or lowering particle concentrations. However the authors did observe that interference was assay- and particle-specific, and recommended that each in vitro methodology to be evaluated for each individual nanoparticle (Kroll et al., 2012). Therefore the cAuNPs were tested for interference with some commonly used in vitro tests.

## Test guideline

| Qualifier | Guideline | Deviations |
|---|---|---|
| no guideline followed | | |

## Principles of method if other than guideline
Absorbance by cAuNPs at 1 and 5 nM at a wavelength of 450 nm in the absence of cells but in the presence of unreduced XTT was measured.

## GLP compliance
no data

## Test materials

## Identity of test material same as for substance defined in section 1 (if not read-across)
yes

## Test material form
nanomaterial

## Details on test material
- Name of test material (as cited in study report): Gold nanoparticles (cAuNPs)

## Method

## Any other information on materials and methods incl. tables
XTT was prepared as per manufacturer's instructions (In vitro Toxicology Assay Kit, Sigma Aldrich). Absorbance by cAuNPs at 1nM and at 5 nM at a wavelength of 450 nm in the absence of cells but in the

presence of unreduced XTT was measured.

## Results and discussions

## Any other information on results incl. tables

A dose-dependent increase in absorbance was observed; therefore the cAuNPs are interfering with the absorbance reading in this assay. See the attached document for figure: The absorbance of 1 nM and 5 nM cAuNPs in culture media with XTT at 450 nm

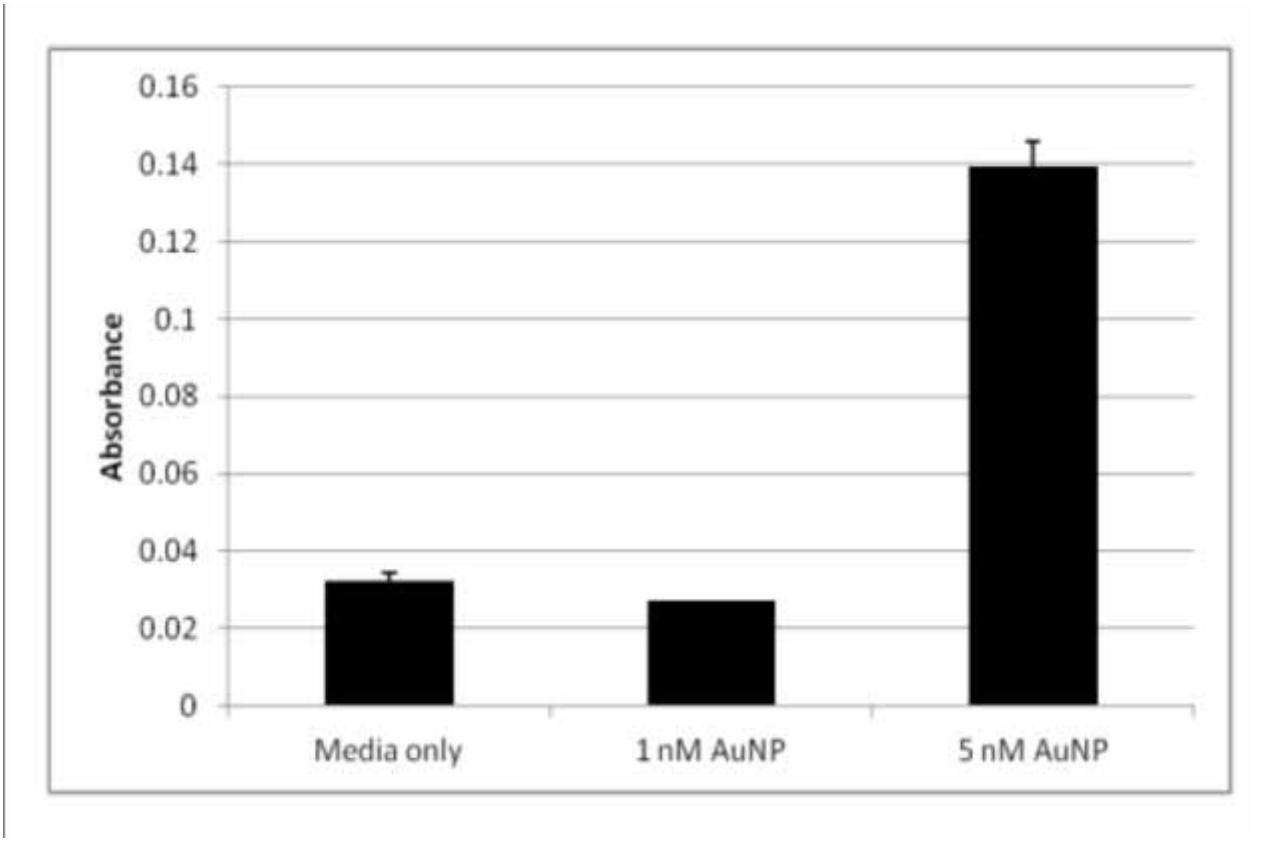
## Overall remarks, attachments

## Remarks on results including tables and figures

None

## Attached background material

| Attached document | Remarks |
|---|---|
| The absorbance of 1 nM and 5 nM cAuNPs in culture media with XTT at 450 nm.pdf / 88.77 KB (application/octet-stream) | |



### Applicant's summary and conclusion

### Conclusions

Under the test conditions, a dose-dependent increase in absorbance was observed; therefore the cAuNPs are interfering with the absorbance reading in this assay. cAuNPs are capable of interfering with in vitro toxicity assays, either through direct optical interference or through interference of the reaction of the assay. Interference is assay specific and therefore each assay needs to be validated individually.

### Executive summary

In a XTT assay, absorbance by cAuNPs at 1 and 5 nM at a wavelength of 450 nm in the absence of cells but in the presence of unreduced XTT was measured.
A dose-dependent increase in absorbance was observed; therefore the cAuNPs are interfering with the absorbance reading in this assay.

### Cross-reference to other study

No cross-reference

## 8. ANALYTICAL METHODS

## 9. RESIDUES IN FOOD AND FEEDING STUFFS

## 10. EFFECTIVENESS AGAINST TARGET ORGANISMS

## 11. GUIDANCE ON SAFE USE

## 12. LITERATURE SEARCH

## 13. ASSESSMENT REPORTS

## 14. INFORMATION REQUIREMENTS