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

13-Jan-2016

English - Or. English

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

Cancels & replaces the same document of 16 June 2015

DOSSIER ON SILVER NANOPARTICLES
- PART 7 -

Series on the Safety of Manufactured Nanomaterials
No. 53

This document is only available in PDF format.

JT03388930

ENV/JM/MONO(2015)16/PART7
Unclassified

English - Or. English

OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 53

DOSSIER ON SILVER NANOPARTICLES
- PART 7 -

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

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

No. 44, *Dossier on Gold nanoparticles (2015)*

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. 54, *Dossier on Titanium dioxide (2015)*

ABOUT THE OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

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/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org

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 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. Finally, the reader should note

[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)

that the data contained within this dossier is raw data and has not been evaluated by either the programme sponsors or the WPMN. Any conclusions found within this dossier are the responsibility of the researchers who made them.

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 Silver Nanoparticles which was prepared under the leadership of the Republic of Korea and the United States. 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 IUCLID[9] style format and includes the protocols and methods used (see Preamble).

The Republic of Korea and the United States co-led the Testing Programme on Silver Nanoparticles. This included the determination of the tests that were appropriate for Silver Nanoparticles, performing a number of tests, as well as coordinating tests performed and inputs provided by the National Institute of Environmental Research of Korea, the United States and the Business & Industry Advisory Committee to the OECD (BIAC).

Due to the large amount of information generated through the OECD Testing Programme on Silver Nanoparticles, the Dossier has been split into seven parts.

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 Working Party on Manufactured Nanomaterials wish to thank the Republic of Korea and the United States for co-leading the Testing Programme for Silver Nanoparticles. They are specifically grateful to the Ministry of Environment, the Ministry of Trade, Industry and Energy, the Ministry of Food and Drug Safety, to Jo Eunhye, Kim Pilje, Lee Byoungcheun and Yoon Junheon from the National Institute of Environmental Research of Korea, and to Dr. Philip Sayre from the Environment Protection Agency, USA. In addition, we appreciate the efforts made by other countries / organisations that participated in the Testing Programme, in particular to Australia, Canada, the European Commission (EC), France, Germany, the Netherlands and the Nordic Council of Ministries, as well as the Business & Advisory Committee to the OECD (BIAC).

TABLE OF CONTENTS

PART 1

PART 2

PART 3

PART 4

PART 5

PART 6

PART 7

6.3.2 Toxicity to terrestrial arthropods

Endpoint study record: Toxicity to terrestrial arthropods.001

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e-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	Ministry of Environment, Korea	2010	Development of environment management system with exposure and impact analysis of the environments exposed to nanomaterials						

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test materials

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

yes

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): Silver nanoparticles powder
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance): no data
- Smiles notation (if other than submission substance): no data
- InChl (if other than submission substance): no data
- Structural formula attached as image file (if other than submission substance): no data

- Substance type: Inorganic
- Physical state: Powder
- Analytical purity: no data
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data
- Isomers composition: no data
- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data
- Radiochemical purity (if radiolabelling): no data
- Specific activity (if radiolabelling): no data
- Locations of the label (if radiolabelling): no data
- Expiration date of radiochemical substance (if radiolabelling): no data
- Stability under test conditions: no data
- Storage condition of test material: no data
- Other: Sigma Aldrich (USA)

TEST ORGANISM
- Common name: nematode
- Strain: Caenorhabdities elegans- Source: no data
- Age at study initiation (mean and range, SD): no data
- Weight at study initiation (mean and range, SD): no data
- Length at study initiation (length definition, mean, range and SD): no data
- Valve height at study initiation, for shell deposition study (mean and range, SD): no data
- Peripheral shell growth removed prior to test initiation: no data
- Method of breeding: no data
- Feeding during test: no data
- Food type: no data
- Amount: no data
- Frequency: no data

Analytical monitoring

no

Vehicle

no data

Test organisms

Test organisms (species)

other: Caenorhabdities elegans

Study design

Test duration type

short-term toxicity

Limit test

no

Total exposure duration

24 h

Test conditions

Any other information on materials and methods incl. tables

Test temperature

20 oC

Nominal and measured concentrations

Nominal Concentrations: 0.1, 0.2, 1.5, 1, 2, 4 mg/L

Details on test conditions

C. elegans: 20℃, NGM agar media as culture media, OP50 as diet
C. elegans: test period (24 hrs), measurement (Microarray)

Reference substance (positive control)

no data

Results and discussions

Any other information on results incl. tables

Short-term survival and growth experiments were compared with the sensitivity of physiological level responses of C. elegans to AgNPs (Table 4.3). Short-term testing only provided a snapshot of the physiological status, thus longer term testing was conducted to evaluate the effects on reproductive potential. Although AgNPs exposure did not affect the survival and growth of the wild type, reproduction was seriously affected, with the number of offspring per individual dramatically decreased (70% of the controls in 0.1 and 0.5 mg/L AgNPs). The mutant strains' survival and growth response were not different from the wild type, but the reproductive responses of the mtl-2 (gk125) and sod-3 (gk235) mutants were less sensitive (40-60% less at 0.1 mg/L and 10% at 0.5 mg/L) to AgNPs exposure than the wild type, while the response of the daf-12 (rh286) mutant was similar to the wild type.

Remarks on results including tables and figures

See attached file: Table 4.1

Overall remarks, attachments

Attached background material

Attached document	Remarks
Table 4.1.docx / 83.9 KB (application/octet-stream) ***Table 4.1. Ecotoxicological indicators investigated after exposure to AgNPs in wild type and mutant strains of C. elegans***	

exposure duration	parameters	strains	AgNPs (mg/L) 0.05	0.1	0.5
24 h	survival	wild type*(N2)*	1.00 ± 0.000	1.00 ± 0.000	0.98 ± 0
		mtl-2(gk125)	1.00 ± 0.000	1.00 ± 0.000	0.98 ± 0
		sod-3(gk235)	1.00 ± 0.000	1.00 ± 0.000	0.98 ± 0
		daf-12(rh286)	1.00 ± 0.000	1.00 ± 0.000	1.00 ± 0
	growth	wild type*(N2)*	0.97 ± 0.018	0.96 ± 0.022	0.94 ± 0
		mtl-2(gk125)	0.98 ± 0.007	0.97 ± 0.015	1.04 ± 0
		sod-3(gk235)	1.00 ± 0.021	1.00 ± 0.018	1.02 ± 0
		daf-12(rh286)	0.99 ± 0.008	1.00 ± 0.006	0.97 ± 0
72 h	reproduction	wild type*(N2)*	0.83 ± 0.052[a]	0.32 ± 0.023[b]	0.32 ± 0
		mtl-2(gk125)	0.79 ± 0.019[a]	0.79 ± 0.087[a]	0.41 ± 0
		sod-3(gk235)	1.06 ± 0.053	0.88 ± 0.040[a]	0.40 ± 0
		daf-12(rh286)	0.54 ± 0.075[b]	0.35 ± 0.036[b]	0.25 ± 0

Applicant's summary and conclusion

Conclusions

AgNPs exerted considerable toxicity in C. elegans, showing a dramatic decrease in reproduction potential.

Endpoint study record: Toxicity to terrestrial arthropods.002

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e-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	Chemosphere	2008	Comparative and combined toxicities of toluene and methyl tert-butyl ether to an Asian earthworm Perionyx excavatus						
study report	Nanotechnology	2009	Nano-silver-a review of available data and knowledge gaps in human and environmental risk assessment						
study report	Ecotoxicol Environ Safety	2004	A new ultrasound protocol extrusion of coelomocytes cells from the earthworm Eisenia fetida						
study report	Pedologia	2003	Annual changes in coelomocytes of four earthworm species						

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

GLP compliance

no

Test materials

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

yes

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): citrate capping silver nanoparticle
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance): no data
- Smiles notation (if other than submission substance): no data
- InChl (if other than submission substance): no data

- Structural formula attached as image file (if other than submission substance): no data
- Substance type: Inorganic
- Physical state: Powder
- Analytical purity: no data
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data
- Isomers composition: no data
- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data
- Radiochemical purity (if radiolabelling): no data
- Specific activity (if radiolabelling): no data
- Locations of the label (if radiolabelling): no data
- Expiration date of radiochemical substance (if radiolabelling): no data
- Stability under test conditions: no data
- Storage condition of test material: no data
- Other:

● Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.):
- The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20%.
- Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

● Basic morphology: Spherical
● Description of surface chemistry (e.g., coating or modification): Citrate capping
● Major commercial uses: Antibacterial coating
● Known catalytic activity
● Method of production (e.g., precipitation, gas phase)
● Producer/provider: ABC NANOTECH Co., LTD, KOREA

Analytical monitoring

no

Vehicle

no

Test organisms

Test organisms (species)

other: Eisenia andrei, Perionyx excavatus

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Limit test

no

Total exposure duration

7 d

Test conditions

Any other information on materials and methods incl. tables

Details on test conditions

Test conditions:

● Description of the test item preparation protocol
● Description of the test item
- Artificial soil test : Each test unit contained 5 g of dried NP-amended soil, and DI water was added to give an overall moisture content of about 35% of the dry weight. 15 replicates for E. andrei and 10 replicates for P. excavatus each treatment are applied during 7 days.
- Neutral red retention (NRR) assay : After artificial soil test, earthworms were separated from the cultures, rinsed thoroughly with distilled water and kept on damp filter paper at 25 oC in darkness to remove gut contents for three hours. NRR assay was conducted by a method of Hendawi et al (2004) and Kurek and Plytycz (2003) with a slight modification
- Bioaccumulation factor (BAF) : To determine NP accumulation in plant tissue after 2 d, all plants were washed thoroughly with distilled water to remove the test medium. Samples were oven dried at 70 oC for 24 h, weighed, and digested by adding 3 ml of 70% HNO3 at hot block digester. Elemental analysis in digests was performed by ICP-AES. Nanoparticle concentrations in plant tissues were determined based on the relationship between the cAgNP suspension and Ag ion. A known concentration of cAgNPs was dissolved in concentrated HNO3, and the concentration of Ag ion extracted from the cAgNP suspension was determined by ICP-AES. A direct linear relationship was obtained. Nanoparticle concentrations were then calculated based on the relationship between the cAgNP and Ag ion.
● Description of the test vehicle/media/matrix
- Preparation of NP-amended soil :
ⅰ) The test NPs were suspended for each concentration in DI water.
ⅱ) The NP suspension and DI water were added to OECD artificial soil for a final moisture content of 100 % w/w(soil: water=1:1).
ⅲ) The slurry of steps “ ⅱ ” were evenly mixed with the kneader to make the concentration grade of the NPs.
ⅳ) The slurry of steps “ⅲ” was treated in the freezing dryer for 24 hours
● Homogeneity and stability in test media and conditions
The homogeneity and stability in NP-amended soil was confirmed in follows.
1) TEM and SEM measurements
2) TEM-EDS measurements
3) Ionization measurement using ICP-AES analysis & ion toxicity test
To confirm homogeneously distributed in test soil, TEM measurements was used. The NPs in soil was identified by TEM-EDS information. And the NP-dissolution on test medium was measured using ICP-AES.

Reliability:

● The Homogeneity and stability in artificial soil was confirmed by TEM and TEM-EDS measurements.
● The NRR assay was performed according to Hendawi et al. (2004) and Kurek and Plytycz (2003).

Any other information on materials and methods incl. tables

Method

● In vitro
- Test system : Neutral Red Retention(NRR) assay, Bioaccumulation factor (BAF) analysis
● In vivo
- Species : Eisenia andrei (red tiger worm), Perionyx excavatus (blue worm)
● Sample administration : NP-amended soil
● Exposure duration : 7 days
● Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
- "methods used comparable to guidelines" : The guideline was not suggest method that the nanoparticles are homogeneously distributed in test soil. Most NPs were insoluble, so a few modification of guidelines were needed to avoid aggregation of NPs and homogeneously distribution in soil.
- The endpoint according to the TG 207 is survival of earthworm exposed toxicity materials. However, most of the survival effect level of NPs was very high concentration. So we suggest other assay as endpoint
- To confirm coelomocytes effect of earthworm exposed NPs, we used NRR(Neutral Red Retention) assay according to Hendawi et al. (2004) and Kurek and Plytycz (2003).
- To measure uptake of NPs of earthworm exposed NPs, BAFs was measured.
● Analytics (in particular, analytical verification of doses or concentrations)
● Statistics : Arithmetic means
● Any other relevant information : No

Results and discussions

Any other information on results incl. tables

● Coelomocytes viability
- 7d-NOEC for E. andrei : >2000 mg/kg
- 7d-NOEC for P. excavatus : >2000 mg/kg

● Pinocytosis
- 7d-NOEC for E. andrei : 300 mg/kg
- 7d-NOEC for P. excavatus : >2000 mg/kg
● BAF assay
- BAF for E. andrei : 0.0006 kg/kg worm (in 2000 mg/kg)
- BAF for P. excavatus : 0.0039 kg/kg worm (in 2000 mg/kg)

Overall remarks, attachments

Remarks on results including tables and figures

7d-NOEC values (pinocytosis) for E. andrei exposed to cAgNPs were 300 mg/kg dry soil as a result of NRR assay. Pinocytosis effect was reduced approximately 4% at the maximum concentration of 2000 mg/kg compare to control based on NRR assay. In the case of P. excavatus, coelomocyte density and pinocytosis effect were not changed with increasing cAgNP concentrations. The uptake of cAgNP by earthworms increased with increasing cAgNP concentrations. The bioaccumulation factors of cAgNP were calculated to be 0.001 and 0.004 for E. andrei and P. excavatus, respectively.Dissolution rate of ion

from cAgNPs was about 0.043 %. The lower dissolution could be due to sorption of ion to soil particles and the subsequent concentration of ion in pore water was reduced. The effect of silver ion was assessed. The 7d-EC50(survival) and NOEC(survival) for E. andrei were estimated to be 530(397-705) mg/kg and 300 mg/kg, respectively. As a while, there is no significant effect on the coelomocyte viability and pinosytosis activity. The loverall results show that the effect is mostly due to the nanoparticles rather than ion.In case of E. andrei, 7d-NOEC values (pinocytosis) were 300 mg/kg dry soil as a result of NRR assay and pinocytosis effect was reduced approximately 4 % at the maximum concentration of 2000 mg/kg. However, in the case of P. excavatus, coelomocyte density and pintocytosis effect were not changed with increasing cAgNP concentrations. The reasons for this involve changes in the physicochemical properties of nanoparticles in soil. Pore water harbors a range of electrolytes that significantly increase the aggregation of cAgNPs in soil. It is possible that the aggregates were larger than the pore size of the earthworm cell, and thus some micro-sized aggregates did not pass through the earthworm cells. Greater aggregation may be the principal reason for the reduced toxicity of cAgNPs in soil media. Additionally, Ag NPs can be absorbed by clay surfaces (Wijnhoven et al., 2009)

Endpoint study record: Toxicity to terrestrial arthropods.003

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e-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	ASTM	2008	Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans						
study report	Mechanism of Ageing and Development	2008	Effect of a potent antioxidant, platinum nanoparticle on the lifespan of Caenorhabditis elegans						
study report	Environmental Toxicology and Chemistry	2009	Toxicity of manufactured zinc oxide nanoparticles in the nematode Caenorhabditis elegans						
study report	Environmental Science Technology	2009	Ecotoxicity of silver nanoparticles on the soil nematode Caenorhabditis elegans						
study report	Environmental Toxicology and Pharmacology	2009	Ecotoxicological investigation of CeO2 and TiO2 nanoparticles on the soil nematode Canorhabditis elegans using gene expression, growth, fertility, and mortality as endpoints						
study report	Environmental Pollution	2009	Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode Caenorhabditis elegans						

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

GLP compliance

no

Test materials

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

yes

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): citrate capping silver nanoparticle
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance): no data
- Smiles notation (if other than submission substance): no data
- InChl (if other than submission substance): no data
- Structural formula attached as image file (if other than submission substance): no data
- Substance type: Inorganic
- Physical state: Powder
- Analytical purity: no data
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data
- Isomers composition: no data
- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data
- Radiochemical purity (if radiolabelling): no data
- Specific activity (if radiolabelling): no data
- Locations of the label (if radiolabelling): no data
- Expiration date of radiochemical substance (if radiolabelling): no data
- Stability under test conditions: no data
- Storage condition of test material: no data
- Other:

● Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.):

- The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20 %.
- Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

● Basic morphology: Spherical
● Description of surface chemistry (e.g., coating or modification): Citrate capping
● Major commercial uses: Antibacterial coating
● Known catalytic activity
● Method of production (e.g., precipitation, gas phase)
● Producer/provider: ABC NANOTECH Co., LTD, KOREA

Analytical monitoring

no

Vehicle

no

Test organisms

Test organisms (species)

other: Caenorhabditis elegans

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Limit test

no

Total exposure duration

24 h

Test conditions

Any other information on materials and methods incl. tables

Test conditions:

● Description of the test item preparation protocol
● Description of the test item
- NP NGM agar test : The one of 72hr adult or ten individuals of 24hr juveniles were transferred to NP NGM agar medium in four replicates for reproduction or survival test, respectively. Exposure period is 24 hours. To estimate survival rate and reproduction rate, they were observed under microscope.
● Description of the test vehicle/media/matrix
- Preparation of NP NGM agar medium : NGM(Nematode Growth Medium : 3 g/L NaCl, 2.5 g/L Peptone, 17 g/L Agar, 25ml 1M PPP, 1ml 1M $CaCl_2 \cdot 2H_2O$, 1ml 1M $MgSO_4 \cdot 7H_2O$, 1ml cholesterol) was prepared, autoclaved (121 oC, 15min), and cooled to 60~65 oC. NPs suspension was mixed with NGM with same rate (1:1). And about 0.2 mL of mixed solution was added to 24 well microplate (ID 17mm×height 17mm, volume 3.8 ml for each well), and immediately harden in the refrigerator to prevent nanoparticle precipitation.
● Homogeneity and stability in test media and conditions
The homogeneity and stability in NP NGM agar medium was confirmed in follows.
1) SEM measurements
2) High resolution nano scale microscopic measurement
3) Ionization measurement using ICP-AES analysis & ion toxicity test
To confirm homogeneously distributed in test medium, SEM measurements was used. And high resolution nano scale microscopic measurement was used for confirmation of distribution as a screening. And the NP-dissolution on test medium was measured using ICP-AES.

Reliability:

Caenorhabditis elegans is recommended as soil toxicity test species by ASTM

Results and discussions

Any other information on results incl. tables

Details on results

● NP NGM agar test
- 24h-LC50 for survival rate : 55.03 (28.51-105.14) mg/L
- 24h-NOEC for survival rate : <10 mg/L
- 24h-EC50 for reproduction rate : >100 mg/L
- 24h-NOEC for reproduction rate : 1 mg/L
● Ag ion toxicity test
- 24h-LC50 for survival rate : 22.31 (10.88-45.72) mg/L
- 24h-NOEC for survival rate : >0.5 mg/L
- 24h-EC50 for reproduction rate : >10 mg/L
- 24h-NOEC for reproduction rate : 1 mg/L L

Remarks on results including tables and figures

- Figure4.2. shows the adverse effects of cAgNPs on the epidermis of exposed C. elegans.
- SEM image of controls(A), clean epidermis without scarring was confirmed. However, individuals exposed to 10mg/L(B) or 100mg/L(C and D) of cAgNPs of evidenced epidermal fissuring.

Overall remarks, attachments

Attached background material

Attached document	Remarks
Figure4.2, 4.3.docx / 1.68 MB (application/octet-stream)	

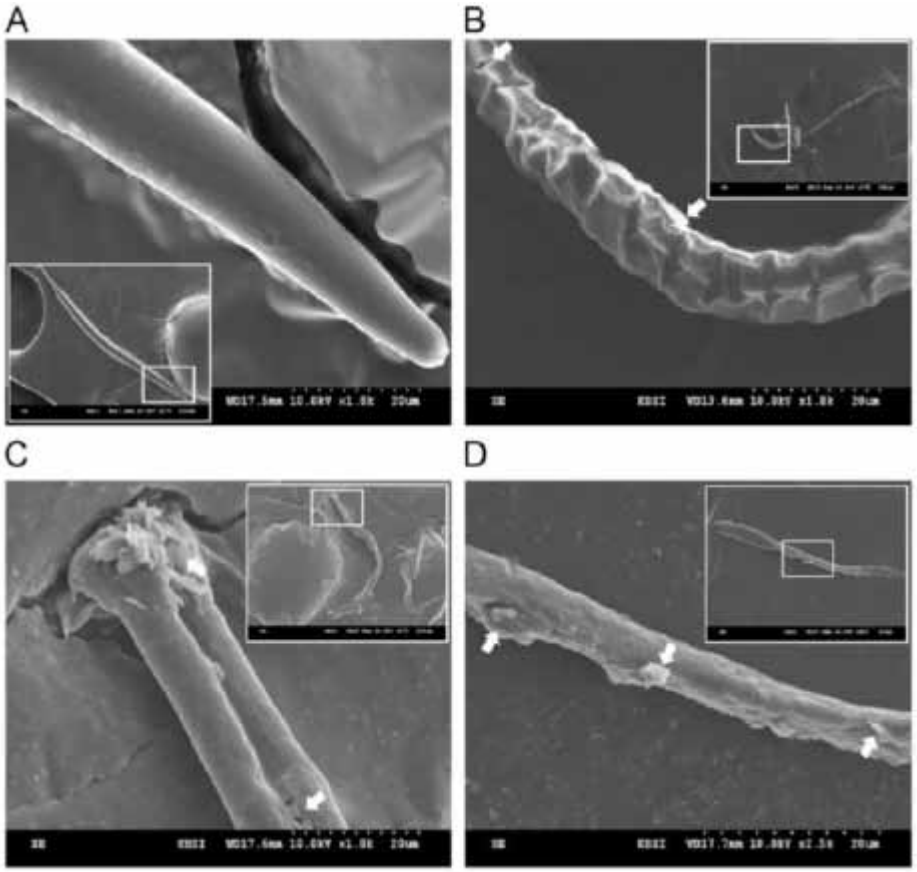


Figure. 4.2. Scanning electronmicrographof C. elegans-exposed cAgNPs: (A) control, (B) 10mg/L, and (CandD) 100mg/L. The white arrows indicate epidermal divisions and necrosis

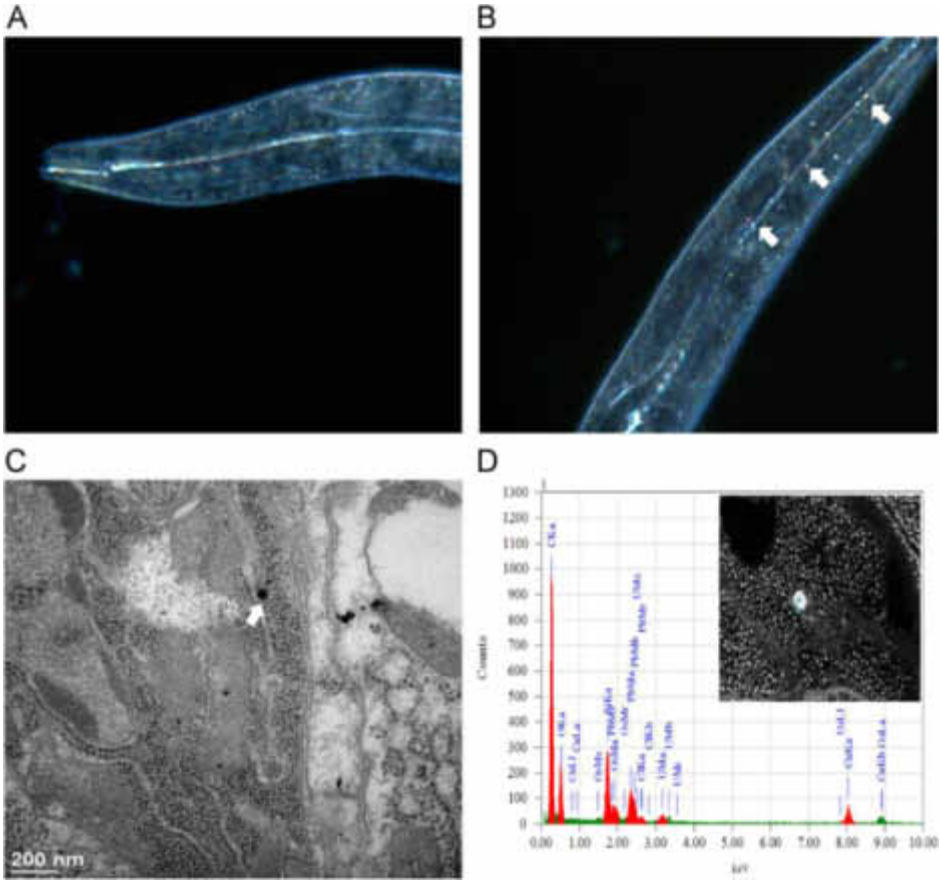


Fig. 4.3. High resolution-microscopicimageof C. elegans. (A)Untreated C. elegans (x1000). (B) Exposed to cAgNPs of 100mg/L. The white arrows indicate the particles putatively identified as cAgNPs. (C) Transmission-electron micrographs of C. elegans exposed to cAgNPs of 100mg/L.(D) Spectrum of energy-dispersive spectroscopy (EDS) of dense spot for TEM scan. The components of electron dense spot on C. elegans were identified by osmium and uranium that were originated from TEM pretreatment.

Applicant's summary and conclusion

Conclusions

The effect of NPs on survival of C. elegans showed a dose-dependent decrease up to 100 mg/L. There was no significant effect on C. elegans, at the concentrations greater than 100 mg/L of cAgNP. This phenomenon was due to the nanoparticle aggregation. Similar trend was observed in the reproduction test. The number of larva born during the exposure period has a trend of being reduced up to 10 mg/L in comparison with control. This indicated that cAgNPs adversely induced the reproductive effect on adult of C. elegans. Exposure groups were observed under the high resolution microscope and TEM. It seems that the nanoparticles were distributed along the digestion organ. But TEM

Endpoint study record: Toxicity to terrestrial arthropods.004

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e-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	Enviromental Toxicology and Chemistry	2006	Assessment of stress-related gene expression in the heavy metal-exposed nematode Caenorhabditis elegans: a potential biomarker for metal-induced toxicity monitoring and environmental risk assessment.						
study report	Toxicology	2007	Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode Caenorhabditis elegans						
study report	Environemental Toxicology and Chemistry	1990	Aquatic toxicity testing using the nematode, Caenorhabditis elegans						

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

GLP compliance

no

Test materials

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

yes

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): citrate capping silver nanoparticle
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance): no data
- Smiles notation (if other than submission substance): no data
- InChl (if other than submission substance): no data
- Structural formula attached as image file (if other than submission substance): no data
- Substance type: Inorganic
- Physical state: Powder
- Analytical purity: no data
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data
- Isomers composition: no data
- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data
- Radiochemical purity (if radiolabelling): no data
- Specific activity (if radiolabelling): no data
- Locations of the label (if radiolabelling): no data
- Expiration date of radiochemical substance (if radiolabelling): no data
- Stability under test conditions: no data
- Storage condition of test material: no data
- Other:

● Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.):
- The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20 %.
- Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

● Basic morphology: Spherical
● Description of surface chemistry (e.g., coating or modification): Citrate capping
● Major commercial uses: Antibacterial coating
● Known catalytic activity
● Method of production (e.g., precipitation, gas phase)
● Producer/provider: ABC NANOTECH Co., LTD, KOREA

Analytical monitoring

no

Vehicle

no

Test organisms

Test organisms (species)

other: Caenorhabditis elegans

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Limit test

no

Total exposure duration

24 d

Test conditions

Any other information on materials and methods incl. tables

Test conditions:
● Description of the test item preparation protocol
● Description of the test item
- Caenorhabditis elegans exposed to cAgNPs for 24 hours. The exposure concentrations of cAgNPs and Ag+ were 0.05, 0.5, 5 mg/L.
- The Ribonucleic acid (RNA) isolation of C. elegans was prepared by using the TRIzol reagent, chloroform, isoprophanol, 75 % ethanol, and RNase-free water (diethylpyrocarbonate, DEPC) according to standard protocol from Animal Genomics laboratory, Konkuk University in Seoul, Korea.
- The deoxyribonucleic acid (DNA) elimination from total RNA was conducted with 10X DNase Ⅰ buffer, DNase Ⅰ (RNase-free) following the manufacturer's standard instructions (Promega corporation, USA). The complementary- DNA (cDNA) synthesis from total RNA and Oligo dT primers was performed with reverse transcription-polymerase chain reaction (RT-PCR), using a MAXYGENE Thermal Cycler (AXYGEN Scientific, California, USA) in accordance with standard protocol of manufacturer (Bioneer, Korea).
- PCR product was prepared by using PCR PreMix solution, cDNA, primers, distilled water, and RT-PCR followed the manufacturer's standard instructions (Bioneer, Korea).
- The sequences of primers were determined from the C. elegans database (http://www.wormbase.org).
- Stress-related genes used in this study were heat shock proteins (hsp-16.2 and hsp-16.41), glutathione-S-transferase 4 (gst-4), cytochrome P450 family protein 35A2 (cyp35a2), and metallothioneins (mt-1 and mt-2). Actin mRNA was applied to express the level normalization of the used genes.
- PCR products were visualized with electrophoresis on agarose gel containing 1X Tris-borate buffer, 1%

agarose, and Ethidium bromide (EtBr). The bands induced by stress-related genes were observed with digital camera and ultraviolet transilluminator (CoreBioSystem, Korea).
● Description of the test vehicle/media/matrix- Preparation of NP NGM agar medium : NGM(Nematode Growth Medium : 3 g/L NaCl, 2.5 g/L Peptone, 17 g/L Agar, 25ml 1M PPP, 1ml 1M $CaCl_2 \cdot 2H_2O$, 1ml 1M $MgSO_4 \cdot 7H_2O$, 1ml cholesterol) was prepared, autoclaved (121 oC, 15min), and cooled to 60~65 oC. NPs suspension was mixed with NGM with same rate (1:1). And about 0.2 mL of mixed solution was added to 24-well microplate (ID 17mm×height 17mm, volume 3.8 ml for each well), and immediately harden in the refrigerator to prevent nanoparticle precipitation.
● Homogeneity and stability in test media and conditionsThe homogeneity and stability in LB medium was confirmed in follows.
1) SEM measurements
2) High resolution nano scale microscopic measurement
3) Ionization measurement using ICP-AES analysis & ion toxicity test
To confirm homogeneously distributed in test medium, SEM measurements was used. And high resolution nano scale microscopic measurement was used for confirmation of distribution as a screening. And the NP-dissolution on test medium was measured using ICP-AES.
Reliability:
- manufacturer's standard instructions

Any other information on materials and methods incl. tables

Method

● In vitro
- Test system : stress-related gene expression test
● In vivo
- Species : Caenorhabditis elegans
- Target gene : metallothionein (mt-1), metallothionein (mt-2), heat shock protein (hsp-16.2), heat shock protein (hsp-16.41), glutathione S-transferase (gst-4), cytochrome P450 family protein 35A2 (cyp-35a2)
● Sample administration : NP NGM agar medium
● Exposure route :
● Exposure duration : 24 days
● Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
- "no guidelines available" : There was no toxicity assay to evaluate effect of gene or DNA on OECD guideline.
● Analytics (in particular, analytical verification of doses or concentrations) :
● Statistics : Arithmetic means
● Any other relevant information : No

Results and discussions

Any other information on results incl. tables

In the stress-related gene expression test using C. elegans, the expression of mt-1, mt-2, hsp-162, hsp-16.41, gst-4, and cyp35a2 were not showed any significance with increasing cAgNP concentration

Overall remarks, attachments

Remarks on results including tables and figures

● SOS chromotestExpressions of mt-1, mt-2, hsp-162, hsp-16.41, gst-4, and cyp35a2 were not observed

with concentration-related significance. As mentioned in previous study (Roh et al., 2007), we confirmed that stress-related gene expression can be more sensitive endpoint than typical ecotoxicological ones (ex. Lethality, immobilization, growth, and etc.), even if it is not chemical-specific. In conclusion, there were no the lethal effects of cAgNPs at the level of the whole organism, and it showed that cAgNPs don't induce the stress of C. elegans in the genetic levels.cAgNPs don't induce the stress of C. elegans in the genetic levels.

6.3.3 Toxicity to terrestrial plants

Endpoint study record: Toxicity to terrestrial plants.001

Administrative Data

Study result type	**experimental result**
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e-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	Environmental Toxicology and Chemistry	2008	Toxicity and Bioavatilability of copper nanoparticles to the terretrial plants mung bean(Phaseolus radiatus) and Wheat(Triticum aestivum): Plant agar test for water-insoluble nanoparticles						
study report	Chemosphere	2010	Effects of rare earth oxide nanoparticles on root elongation of plants						
study report	Woo-Mi Lee et al	2012	Effect of silver nanoparticles in crop plants Phaseolus radiatus and Sorghum bicolor: Media effect on phytotoxicity	chemosphere, 86(5): 491-499					
study report	Woo-Mi Lee et al.	2008	Toxicity and Bioavatilability of copper nanoparticles to the terretrial plants mung bean(Phaseolus radiatus) and Wheat(Triticum aestivum): Plant agar	Environmental Toxicology and Chemistry, 27(9): 1915-1921					

			test for water-insoluble nanoparticles						
study report	Ma et al.	2010	Effects of rare earth oxide nanoparticles on root elongation of plants	Chemosphere, 78: 273-279					

Materials and methods

Principles of method if other than guideline

Method

● In vitro
- Test system : Bioaccumulation factor (BAF) analysis
● In vivo
- Species : Phaseolus radiatus (mung bean), Sorghum bicolor (sorghum)
● Sample administration : The dual agar medium
● Exposure route : Contacted directly
● Exposure duration : 48 hours
● Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
- "no guidelines available": The guideline was not suggest method that the nanoparticles are homogeneously distributed. Most NPs were insoluble, so new test guidelines were needed to avoid aggregation of NPs and homogeneously distribution.
- We suggest test using the dual agar medium to evaluated toxicity of the NPs. To prevented NP dissolution by transition of chemical, physical condition, we did not use the solvent to improve the solubility of NPs.
● Analytics (in particular, analytical verification of doses or concentrations)
● Statistics : Arithmetic means, TSK, dunnett's program
● Any other relevant information : No

GLP compliance

no

Test materials

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

yes

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): citrate capping silver nanoparticle
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance): no data
- Smiles notation (if other than submission substance): no data
- InChl (if other than submission substance): no data
- Structural formula attached as image file (if other than submission substance): no data

- Substance type: Inorganic
- Physical state: Powder
- Analytical purity: no data
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data
- Isomers composition: no data
- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data
- Radiochemical purity (if radiolabelling): no data
- Specific activity (if radiolabelling): no data
- Locations of the label (if radiolabelling): no data
- Expiration date of radiochemical substance (if radiolabelling): no data
- Stability under test conditions: no data
- Storage condition of test material: no data
- Other:

● Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.):

- The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20%.
- Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

● Basic morphology: Spherical
● Description of surface chemistry (e.g., coating or modification): Citrate capping
● Major commercial uses: Antibacterial coating
● Known catalytic activity
● Method of production (e.g., precipitation, gas phase)
● Producer/provider: ABC NANOTECH Co., LTD, KOREA

Analytical monitoring

no

Vehicle

no

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Limit test

no

Total exposure duration

24 h

Test conditions

Any other information on materials and methods incl. tables

Test conditions:

● Description of the test item preparation protocol
● Description of the test item
- The dual agar test (DAT) : Ten plant seedlings were placed just above the surface of the dual agar medium. The test units were placed in an incubator at a controlled temperature of 25±1 ℃ in the dark. Exposure period was 48 hours. Each agar concentration was prepared in three replicates. After an incubation period of two days, the plants were separated from the agar media and seedling growth was measured.
- Bioaccumulation factor (BAF) : To determine NP accumulation in plant tissue after 2 d, all plants were washed thoroughly with distilled water to remove the test medium. Samples were oven dried at 70℃ for 24 h, weighed, and digested by adding 3 ml of 70% HNO3 at hot block digester. Elemental analysis in digests was performed by ICP-AES. Nanoparticle concentrations in plant tissues were determined based on the relationship between the NP suspension and ion. A known concentration of cAgNPs was dissolved in concentrated HNO3, and the concentration of Ag ion extracted from the cAgNP suspension was determined by ICP-AES. A direct linear relationship was obtained. Nanoparticle concentrations were then calculated based on the relationship between the cAgNP and Ag ion.
● Description of the test vehicle/media/matrix
- Preparation of the dual agar medium : Each test unit contained 30ml of agar culture media (20ml of 2.5% agar and 10ml of 1% agar) with a specific concentration of NPs. The dual agar media of a 20 mL of 2.5% culture agar covered with a 10 mL of 1% culture agar were used in the tests. Twenty milliliters of 2.5% agar solution contained each NPs concentration, and immediately harden in a freezer to avoid the possible precipitation of nanoparticles. Ten milliliters of 1% agar solution was distributed evenly to the 2.5% agar media.
● Homogeneity and stability in test media and conditions
The homogeneity and stability in agar medium was confirmed in follows.
1) TEM and SEM measurements
2) TEM-EDS measurements
3) High resolution nano scale microscopic measurement
4) Ionization measurement using ICP-AES analysis & ion toxicity test
To confirm homogeneously distributed in test soil, TEM measurements was used. The NPs in soil was identified by TEM-EDS information. And high resolution nano scale microscopic measurement was used for confirmation of distribution as a screening. And the NP-dissolution on test medium was measured using ICP-AES.

Reliability:

● The DAT test was performed according to Lee et al., 2008

Any other information on materials and methods incl. tables

Test conditions:
● Description of the test item preparation protocol

● Description of the test item
- The dual agar test (DAT) : Ten plant seedlings were placed just above the surface of the dual agar medium. The test units were placed in an incubator at a controlled temperature of 25±1 ℃ in the dark. Exposure period was 48 hours. Each agar concentration was prepared in three replicates. After an

incubation period of two days, the plants were separated from the agar media and seedling growth was measured.
- Bioaccumulation factor (BAF) : To determine NP accumulation in plant tissue after 2 d, all plants were washed thoroughly with distilled water to remove the test medium. Samples were oven dried at 70℃ for 24 h, weighed, and digested by adding 3 ml of 70% HNO3 at hot block digester. Elemental analysis in digests was performed by ICP-AES. Nanoparticle concentrations in plant tissues were determined based on the relationship between the NP suspension and ion. A known concentration of cAgNPs was dissolved in concentrated HNO3, and the concentration of Ag ion extracted from the cAgNP suspension was determined by ICP-AES. A direct linear relationship was obtained. Nanoparticle concentrations were then calculated based on the relationship between the cAgNP and Ag ion.
● Description of the test vehicle/media/matrix
- Preparation of the dual agar medium : Each test unit contained 30ml of agar culture media (20ml of 2.5% agar and 10ml of 1% agar) with a specific concentration of NPs. The dual agar media of a 20 mL of 2.5% culture agar covered with a 10 mL of 1% culture agar were used in the tests. Twenty milliliters of 2.5% agar solution contained each NPs concentration, and immediately harden in a freezer to avoid the possible precipitation of nanoparticles. Ten milliliters of 1% agar solution was distributed evenly to the 2.5% agar media.
● Toxicity test in agar medium
- The toxicity tests were conducted in a Petri dish test unit (87 mm x 18 mm). Each test unit contained 30 mL of agar culture media (20 mL of 0.5% agar and 10 mL of 0.25% agar) along with a specific concentration of nanoparticles

- Ten plant seedlings were placed just above the surface of the agar in the test units. The test units were placed in dark incubator at a controlled temperature of 25 ± 1 ℃. After a 2-d incubation period, the plants were separated from the agar media and seedling growth was measured. The length of each seedling was measured to the nearest 10 x 3 m with a ruler. The AgNP concentrations of 0, 5, 10, 20, and 40 mg L-1 were prepared in three replicate test units per treatment. The growth responses of the test species exposed to a range of AgNPs concentrations were recorded. Agar media without nanoparticles was used as a control.

● Toxicity test in soil medium
- The AgNPs solution and soil were mixed to adjust the concentration gradient of the AgNPs within the soil to 0, 100, 300, 500, 1000, and 2000 mg kg_1 dry soil. The soil toxicity tests were conducted in a glass jar test unit (ID = 7.0 x 10-2 m, volume = 2.7 x 10-4 L) at a controlled temperature of 25 ± 1 ℃. The lamp was used to stimulate seedling growth during the 5-d incubation periods, which allows all seedling

● Silver ions released from AgNP and Ag+ toxicity

- Agar media : As for the measurement of dissolution in agar media, nanoparticle suspensions of 5 and 40 mg L-1 were prepared without agar, incubated for 2 d, and subsequently filtered through a nanomembrane filter (NE 2540-90, Woongjin Chemical, Korea) using stirred ultra filtration cells (Model 8050, Millipore, USA).

- Soil media : As for the ionization measurements in the soil, 40% moisture was introduced to the AgNPs-amended soil in the 500, 1000, and 2000 mg kg_1 dry soil samples, and maintained at the aforementioned conditions for 5 d. Samples were filtered with Whatman No. 2 filters (Whatman International, Maidstone, UK) and 0.2-lm nylon membranes (Whatman International, Maidstone, UK).

● Homogeneity and stability in test media and conditions
The homogeneity and stability in agar medium was confirmed in follows.

1) TEM and SEM measurements
2) TEM-EDS measurements
3) High resolution nano scale microscopic measurement
4) Ionization measurement using ICP-AES analysis & ion toxicity test
To confirm homogeneously distributed in test soil, TEM measurements was used. The NPs in soil was identified by TEM-EDS information. And high resolution nano scale microscopic measurement was used for confirmation of distribution as a screening. And the NP-dissolution on test medium was measured using ICP-AES.
Reliability:
● The DAT test was performed according to Lee et al., 2008

Results and discussions

Any other information on results incl. tables

In the DAT test, it was found that the growth of P. radiatus and S. bicolor was decreased with increasing cAgNP concentrations. The EC50s of the cAgNPs of P. radiatus and S. bicolor were 13 (10-17) and 26 (5-139) mg/L, respectively. The 48hr-EC50s of root and shoot of S. bicolor were <5 and >40 mg/L, respectively. The brown tip and necrosis were found in exposed roots of P. radiatus and S. bicolor. These phenomenons were also reported in the previous studies (Lee et al., 2008; Ma et al., 2010).The NP accumulated in P. radiatus and S. bicolor in DAT was increased dependently on the exposure concentration. The bioaccumulation in the DATs of P. radiatus and S. bicolor were 5.47±0.36 and 3.29±2.66 mg/kg, respectively, in the maximum exposure concentration of 40 mg/L. The bioaccumulation factors of P. radiatus and S. bicolor were calculated to be 0.14 and 0.08 L/kg, respectively. In case of the soil test, the cAgNPs accumulation in roots of P. radiatus and S. bicolor were measured to be 15.38±4.03 and 12.46±6.74 mg/kg plant respectively in the highest concentration of 2000 mg/kg dry soil. On the other hand, the cAgNPs accumulations in the shoot were measured to be 1.91±1.07 and 1.47±1.14 mg/kg plant for P. radiatus and S. bicolor, respectively. The bioaccumulation factors of root of P. radiatus and S. bicolor were calculated to be 0.008 and 0.006 kg /kg, respectively. On the other hand, the bioaccumulation factors of shoot of P. radiatus and S. bicolor were calculated to be 0.001 and 0.001 kg/kg, respectively.

Overall remarks, attachments

Remarks on results including tables and figures

● High resolution-microscopic images- Brown tips and necrosis were detected in the exposed roots of both plants. When the cross sectionally cut thin samples of P. radiatus root were examined under a high resolution- microscope, the morphology of the root tissue in the controls were created in the form of epidermis, cortex, endodermis, phloem, and xylem such that it grew normally, but the root exposed to 40 mg L-1 of AgNPs was abnormally developed compared to the controls● Transmission-electron micrographs (TEM)- TEM image of the root tissue of P. radiatus and S. bicolor exposed AgNPs in agar of 100 mg L-1 and 40 mg L-1, respectively. The EDS scan confirmed that the particles found in the TEM images were AgNPs.- This images that AgNPs were uptaken and accumulated in the cell

Attached background material

Attached document	Remarks
Fig4.1, Fig4.2.docx / 2.37 MB (application/octet-stream)	

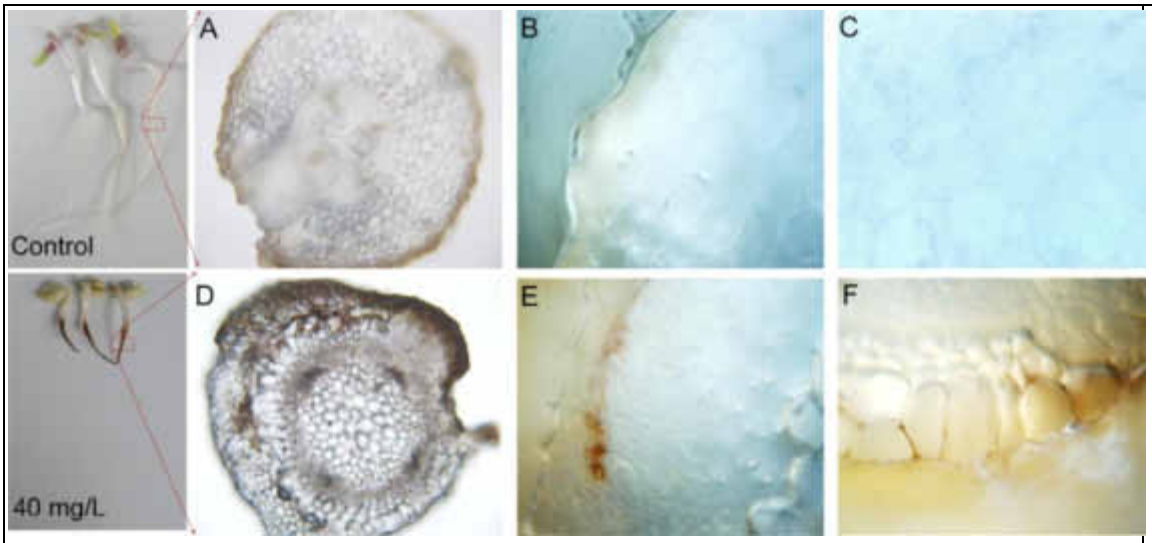


Figure 1. Fig. 4.1. High resolution-microscopic images of roots of Phaseolus radiatus exposed to AgNPs: (A) control (25_), (B) root cell epidermis, control (400_), (C) root cell cortex,control (1000_), (D) 40 mg L_1 (25_), (E) root cell epidermis, 40 mg L_1 (400_), and (F) root cell cortex, 40 mg L_1 (1000_).

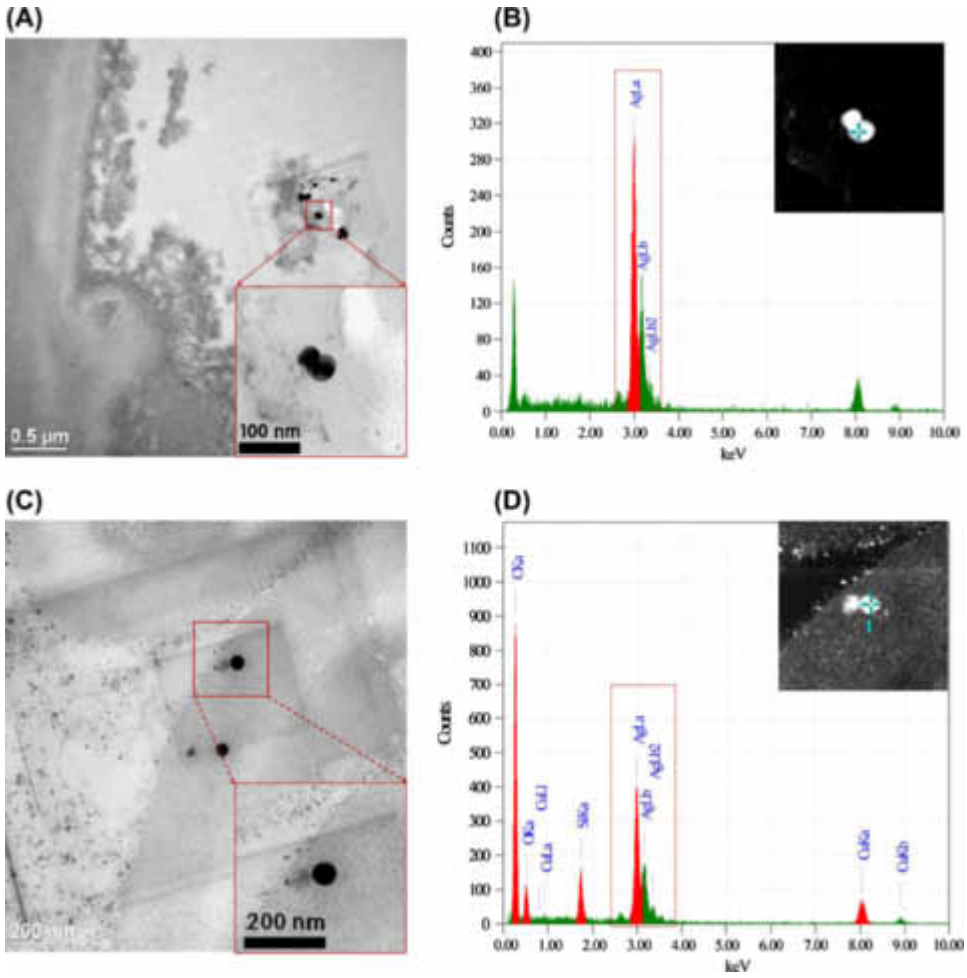


Fig. 4.2. Transmission-electron micrographs of the roots of Phaseolus radiatus and Sorghum bicolor exposed to AgNPs in agar medium of 100 mg L^{-1} and 40 mg L^{-1}, respectively: (A) Phaseolus radiatus, (B) spectrum of energy-dispersive spectroscopy (EDS) of dense spot for TEM scan in A, (C) Sorghum bicolor, and (D) spectrum of EDS of dense spot for TEM scan in C.

Applicant's summary and conclusion

Conclusions

Nanoparticles seem to be transported to plant tissue when the roots absorb moisture from the plant agar. Exposure to cAgNPs has been associated with adverse effects on the seedling growth of both plants. And P. radiatus was more sensitive than T. aestivum to Cu NPs, and even necrosis was found in exposed roots of P. radiatus. Growth of T. aestivum was measured by shoot and root length, and root growth was shown to be a more sensitive endpoint than shoot growth. These observations suggest that only a small portion of NPs could be transported within the plant from the roots to the shoots and that the site of toxic action might be the root, where uptake of NPs occurs during uptake of moisture and nutrients.

Executive summary

Plant agar media were used for easy dispersion of NPs without precipitation in the present study. The growth rates of the both plants were inhibited as a result of exposure to NPs. Seedling growth after a 2-d exposure was used as a toxicity endpoint.

Endpoint study record: Toxicity to terrestrial plants.002

Administrative Data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Uhram Song, HeejuJun, BruceWaldman, JinkyuRoh, YounghunKim, JongheopYi, EunJuLee.	2013	Functionalanalysesofnanoparticletoxicity:Acomparativestudy of theeffectsofTiO2 and Agontomatoes(Lycopersiconesculentum)	Ecotoxicology and Environmental Safety, 93, 60-67					

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Principles of method if other than guideline

Method
● In vitro
- Test system : Not applicable
● In vivo
- Species : Tomato (Lycopersicon esculentum)
● Physicochemical conditions of growth chamber: temperature: 24 ℃, humidity: 60 %, photoperiod: 18 hrs light, light intensity: 300 μE/m2/s.
● Used materials: 100 mm/15 mm petri dish, one piece of filter paper for each petri dish, 5 mL of de-ionized water (germination) or test solution (root length)
● Exposure route : Contacted directly
● Exposure duration :14 days, 6 weeks
● Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
- "no guidelines available" : The guideline was not suggest method that the nanoparticles are homogeneously distributed. Most NPs were insoluble, so new test guidelines were needed to avoid aggregation of NPs and homogeneously distribution.
- We suggest test using the dual agar medium to evaluated toxicity of the NPs. To prevented NP dissolution by transition of chemical, physical condition, we did not use the solvent to improve the

solubility of NPs.

● Analytics (in particular, analytical verification of doses or concentrations)
● Any other relevant information : No

Test materials

Details on test material

- Name of test material (as cited in study report): Silver powder (AgNPs)
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance):
- Smiles notation (if other than submission substance):
- InChl (if other than submission substance):
- Structural formula attached as image file (if other than submission substance): see Fig.
- Substance type:
- Physical state:
- Analytical purity:
- Impurities (identity and concentrations):
- Composition of test material, percentage of components:
- Isomers composition:
- Purity test date:
- Lot/batch No.:
- Expiration date of the lot/batch:
- Radiochemical purity (if radiolabelling):
- Specific activity (if radiolabelling):
- Locations of the label (if radiolabelling):
- Expiration date of radiochemical substance (if radiolabelling):
- Stability under test conditions:
- Storage condition of test material:
- Other: NAMATECH Co. Ltd (Korea)

Overall remarks, attachments

Remarks on results including tables and figures

Germination and root elongation
- Every treated plant showed almost full germination after 12 days
- Possibly AgNPs did not fully penetrate the seed coat and endosperm and thus had limited effects on the embryos.
- AgNPs, which do not ionize in water, might find the seed coat and endosperm to be effective barriers, especially when they are agglomerated.
- Those treated with AgNP showed significant decreases in root growth even at the lowest (50 mg/L) concentrations while those exposed to the highest concentration (5000 mg/L) failed to show significant increase in root growth throughout the experimental period.
- After 14 days, AgNPs showed a 2 to 3 fold increase diameter in 100mg/L treatment. AgNPs treatment was accompanied by a smaller diameter at every concentration, which allows seedlings to absorb it more easily.

Greenhouse experiment

- Low chlorophyll contents (mg/L) : 7.88±0.51 in the 0mg/kg

4.41±0.18 in the 100mg/kg
3.82±0.65 in the 1000mg/kg
- TAC values (nM/mg-protein) : 0.10±0.00, 0.12±0.00 in the 100mg/kg treatment
0.13±0.02 in the 1000mg/kg
- SOD values (U/mg protein) : 0.24 in the 0mg/kg
0.24 in the 100mg/kg
0.28 in the 1000mg/kg

Applicant's summary and conclusion

Conclusions

AgNPs results in significantly decreased root elongation at every concentration. In greenhouse experiments, mature plants showed evidence of phytotoxicity due to AgNPs by exhibiting low chlorophyll contents, higher SOD, and less fruit production.

Endpoint study record: 7440-22-4, Toxicity to terrestrial plants, Anonymous, Year, Summary level, Study level

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	No data
Rationale for reliability incl. deficiencies	The DAT test was performed according to Lee et al., 2008		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Lee WM, An YJ, Yoon H and Kweon HS.	2008	Toxicity and Bioavailability of Copper Nanoparticles to the Terrestrial Plants Mung bean (Phaseolus radiatus) and Wheat (Triticum aestivum): Plant Agar Test for Water-Insoluble Nanoparticles.	Environmental Toxicology and Chemistry, 27(9): 1915-1921					
publication	Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, Zhao Y and Chai Z.	2010	Effects of Rare Earth Oxide Nanoparticles on Root Elongation of Plants.	Chemosphere, 78: 273-279.					
publication	Lee WM, Kwak JI and An YJ.	2012	Effect of Silver Nanoparticles in Crop Plants Phaseolus radiatus and Sorghum bicolor: Media Effect on Phytotoxicity.	Chemosphere, 86(5): 491-499.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available	other guideline: The guideline was not suggest method that the nanoparticles are homogeneously distributed. Most NPs were insoluble, so new test guidelines were needed to avoid aggregation of NPs and homogeneously distribution.	no data

Principles of method if other than guideline

We suggest test using the dual agar medium to evaluated toxicity of the NPs. To prevented NP dissolution by transition of chemical, physical condition, we did not use the solvent to improve the solubility of NPs.

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): Citrate capping silver nanoparticle
- Source: ABC NANOTECH Co., LTD, KOREA
- Structural formula/molecular structure: Ag
- Composition of test material, including degree of purity in %: The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20 %. Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

Other:
- Basic morphology: Spherical
- Description of surface chemistry (e.g., coating or modification): Citrate capping
- Major commercial uses: Antibacterial coating

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

Test substance characterisation:
To confirm the charaterisation, homogeneity and (short term) stability of prepared test items, the follows were used.
- DLS size measurement
- Zeta potential measurement
- SEM measurementsFrom the measurement of DLS, SEM, and Zeta potential, the size and aggregation level of NP suspension in test media was estimated.
- Microscope (equipped with a high-resolution adaptor): The homogeneity of the nanoparticles in the agar media was measured with a microscope.
- pH meter: It was verified, using a digital pH meter, that as the result of measurement for the exposure concentration of each of the AgNPs

Analytical monitoring

no

Details on sampling

Not applicable

Details on analytical methods

Not applicable

Vehicle

no data

Details on test substrate

No data

Test organisms

Test organisms

Species	other: Phaseolus radiatus, Sorghum bicolor
Plant group	other: no data
Details on test organisms	- Common name: Phaseolus radiatus (mung bean), Sorghum bicolor (sorghum)

Study design

Study type

other: no data

Test duration type

short-term toxicity

Test type

other: seedling growth

Substrate type

other: agar medium and soil medium

Limit test

no

Total exposure duration

48 h

Post exposure observation period

None

Test conditions

Test temperature

See the "Details on test conditions"

pH

No data

Moisture

No data

Nominal and measured concentrations

Toxicity test in agar medium: AgNP concentrations of 0, 5, 10, 20 and 40 mg/LToxicity test in soil medium: 0, 100, 300, 500, 1000 and 2000 mg/kg dry soil

Details on test conditions

TEST SYSTEM: Bioaccumulation factor (BAF) analysis - Dual agar test (DAT): Ten plant seedlings were placed just above the surface of the dual agar medium. The test units were placed in an incubator at a controlled temperature of 25±1 °C in the dark. Exposure period was 48 hours. Each agar concentration was prepared in three replicates. After an incubation period of two days, the plants were separated from the agar media and seedling growth was measured.
- Bioaccumulation factor (BAF): To determine NP accumulation in plant tissue after 2 d, all plants were washed thoroughly with distilled water to remove the test medium. Samples were oven dried at 70 °C for 24 h, weighed, and digested by adding 3 ml of 70% HNO3 at hot block digester. Elemental analysis in digests was performed by ICP-AES. Nanoparticle concentrations in plant tissues were determined based on the relationship between the NP suspension and ion. A known concentration of cAgNPs was dissolved in concentrated HNO3, and the concentration of Ag ion extracted from the cAgNP suspension was determined by ICP-AES. A direct linear relationship was obtained. Nanoparticle concentrations were then calculated based on the relationship between the cAgNP and Ag ion.Description of the test vehicle/media/matrix- Preparation of the dual agar medium: Each test unit contained 30 mL of agar culture media (20 mL of 2.5% agar and 10 mL of 1% agar) with a specific concentration of NPs. The dual agar media of a 20 mL of 2.5% culture agar covered with a 10 mL of 1% culture agar were used in the tests. Twenty milliliters of 2.5% agar solution contained each NPs concentration, and immediately harden in a freezer to avoid the possible precipitation of nanoparticles. Ten milliliters of 1% agar solution was distributed evenly to the 2.5% agar media.Toxicity test in agar medium
- The toxicity tests were conducted in a Petri dish test unit (87 mm x 18 mm). Each test unit contained 30 mL of agar culture media (20 mL of 0.5% agar and 10 mL of 0.25% agar) along with a specific concentration of nanoparticles.
- Ten plant seedlings were placed just above the surface of the agar in the test units. The test units were placed in dark incubator at a controlled temperature of 25 ± 1 °C. After a 2-d incubation period, the plants were separated from the agar media and seedling growth was measured. The length of each seedling was measured to the nearest 10 x 3 m with a ruler. The AgNP concentrations of 0, 5, 10, 20, and 40 mg/L were prepared in three replicate test units per treatment. The growth responses of the test species exposed to a range of AgNPs concentrations were recorded. Agar media without nanoparticles was used as a control.Toxicity test in soil medium
- The AgNPs solution and soil were mixed to adjust the concentration gradient of the AgNPs within the soil to 0, 100, 300, 500, 1000, and 2000 mg/kg dry soil. The soil toxicity tests were conducted in a glass jar test unit (ID = 7.0 x 10^-2 m, volume = 2.7 x 10^-4 L) at a controlled temperature of 25 ± 1 °C. The lamp was used to stimulate seedling growth during the 5-d incubation periods, which allows all seedling.Silver ions released from AgNP and Ag+ toxicity
- Agar media : As for the measurement of dissolution in agar media, nanoparticle suspensions of 5 and 40

mg/L were prepared without agar, incubated for 2 d, and subsequently filtered through a nanomembrane filter (NE 2540-90, Woongjin Chemical, Korea) using stirred ultra-filtration cells (Model 8050, Millipore, USA).
- Soil media : As for the ionization measurements in the soil, 40% moisture was introduced to the AgNPs-amended soil in the 500, 1000, and 2000 mg/kg dry soil samples, and maintained at the aforementioned conditions for 5 d. Samples were filtered with Whatman No. 2 filters (Whatman International, Maidstone, UK) and 0.2-lm nylon membranes (Whatman International, Maidstone, UK).Homogeneity and stability in test media and conditionsThe homogeneity and stability in agar medium was confirmed in follows.
- TEM and SEM measurements
- TEM-EDS measurements- High resolution nano scale microscopic measurement- Ionization measurement using ICP-AES analysis & ion toxicity testTo confirm homogeneously distributed in test soil, TEM measurements was used. The NPs in soil was identified by TEM-EDS information. And high resolution nano scale microscopic measurement was used for confirmation of distribution as a screening. And the NP-dissolution on test medium was measured using ICP-AES.

Reference substance (positive control)

no

Any other information on materials and methods incl. tables

None

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
other: Phaseolus radiatus and Sorghum bicolor	48 h	EC50	EC50s of the cAgNPs of P. radiatus and S. bicolor were 13 (10-17) and 26 (5-139) mg/L, respectively	meas. (arithm. mean)	test mat.	seedling emergence	
other: Phaseolus radiatus and Sorghum bicolor	48 h	EC50	EC50s of root and shoot of S. bicolor were <5 and >40 mg/L, respectively.	meas. (arithm. mean)	test mat.	seedling emergence	

Details on results

- In the DAT test, it was found that the growth of P. radiatus and S. bicolor was decreased with increasing cAgNP concentrations. The EC50s of the cAgNPs of P. radiatus and S. bicolor were 13 (10-17) and 26 (5-139) mg/L, respectively. The 48 h-EC50s of root and shoot of S. bicolor were <5 and >40 mg/L, respectively. The brown tip and necrosis were found in exposed roots of P. radiatus and S. bicolor. These phenomenons were also reported in the previous studies (Lee et al., 2008; Ma et al., 2010).
- The NP accumulated in P. radiatus and S. bicolor in DAT was increased dependently on the exposure concentration. The bioaccumulation in the DATs of P. radiatus and S. bicolor were 5.47 ± 0.36 and 3.29 ± 2.66 mg/kg, respectively, in the maximum exposure concentration of 40 mg/L. The bioaccumulation factors of P. radiatus and S. bicolor were calculated to be 0.14 and 0.08 L/kg, respectively. In case of the soil test, the cAgNPs accumulation in roots of P. radiatus and S. bicolor were measured to be 15.38 ± 4.03 and 12.46 ± 6.74 mg/kg plant respectively in the highest concentration of 2000 mg/kg dry soil. On the other hand, the cAgNPs accumulations in the shoot were measured to be 1.91 ± 1.07 and 1.47 ± 1.14

mg/kg plant for P. radiatus and S. bicolor, respectively. The bioaccumulation factors of root of P. radiatus and S. bicolor were calculated to be 0.008 and 0.006 kg /kg, respectively. On the other hand, the bioaccumulation factors of shoot of P. radiatus and S. bicolor were calculated to be 0.001 and 0.001 kg/kg, respectively.

Results with reference substance (positive control)

Not applicable

Reported statistics and error estimates

Arithmetic means, TSK, dunnett's program

Any other information on results incl. tables

High resolution-microscopic images
- Brown tips and necrosis were detected in the exposed roots of both plants. When the cross sectionally cut thin samples of P. radiatus root were examined under a high resolution
- microscope, the morphology of the root tissue in the controls were created in the form of epidermis, cortex, endodermis, phloem, and xylem such that it grew normally, but the root exposed to 40 mg/Lof AgNPs was abnormally developed compared to the controls.

Transmission-electron micrographs (TEM)
- TEM image of the root tissue of P. radiatus and S. bicolor exposed AgNPs in agar of 100 mg/L and 40 mg/L, respectively. The EDS scan confirmed that the particles found in the TEM images were AgNPs.
- This images that AgNPs were uptaken and accumulated in the cell.

See the attached document for figures - High resolution-microscopic and TEM image

Overall remarks, attachments

Remarks on results including tables and figures

Nanoparticles seem to be transported to plant tissue when the roots absorb moisture from the plant agar. Exposure to cAgNPs has been associated with adverse effects on the seedling growth of both plants. And P. radiatus was more sensitive than T. aestivum to Cu NPs, and even necrosis was found in exposed roots of P. radiatus. Growth of T. aestivum was measured by shoot and root length, and root growth was shown to be a more sensitive endpoint than shoot growth. These observations suggest that only a small portion of NPs could be transported within the plant from the roots to the shoots and that the site of toxic action might be the root, where uptake of NPs occurs during uptake of moisture and nutrients.

Attached background material

Attached document	Remarks
Toxicity to terrestrial plants.pdf / 161.73 KB (application/octet-stream)	
Test substance identification: ● Nanomaterial name (chemical name (e.g. IUPAC) and, if different, name from list): citrate capping silver nanoparticle ● CAS Number : 7440-22-4 ● Structural formula/molecular structure : Ag ● Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.): - The stock of silver nanoparticles was black colloidal suspension, and included degree	

of purity in 20%.

- Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

- Basic morphology: Spherical
- Description of surface chemistry (e.g., coating or modification): Citrate capping
- Major commercial uses: Antibacterial coating
- Known catalytic activity
- Method of production (e.g., precipitation, gas phase)
- Producer/provider: ABC NANOTECH Co., LTD, KOREA
- *Batch no, and any other information* useful to univocally identify the material used :
- Other relevant information :

Test substance characterisation:

To confirm the charaterisation, homogeneity and (short term) stability of prepared test items, the follows were used.

1) DLS size measurement

2) Zeta potential measurement

3) SEM measurements

From the measurement of DLS, SEM, and Zeta potential, we estimated the size and aggregation level of NP suspension in test media.

Research Field

- Characterisation
- Physico-chemical properties
- Environmental Fate
- Ecotoxicology
- Toxicology
- Exposure Assessment Environment (including environmental safety)
- Exposure Assessment Human (including workplace safety)

Study result type:

- Experimental result
- Estimated by calculation
- Read-across
- QSAR
- Other

Method

- *In vitro*
  - *Test system* : Bioaccumulation factor (BAF) analysis
- *In vivo*
  - *Species* : *Phaseolus radiatus* (mung bean), *Sorghum bicolor* (sorghum)
- Sample administration : The dual agar medium
- Exposure route : Contacted directly
- Exposure duration : 48 hours
- Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
  - *"no guidelines available"* : The guideline was not suggest method that the nanoparticles are homogeneously distributed. Most NPs were insoluble, so new test guidelines were needed to avoid aggregation of NPs and homogeneously distribution.
  - We suggest test using the dual agar medium to evaluated toxicity of the NPs. To prevented NP dissolution by transition of chemical, physical condition, we did not use the solvent to improve the solubility of NPs.
- Analytics (in particular, analytical verification of doses or concentrations)
- Statistics : Arithmetic means, TSK, dunnett's program
- Any other relevant information : No

Test conditions:

- Description of the test item preparation protocol
- Description of the test item
  - *The dual agar test (DAT)* : Ten plant seedlings were placed just above the surface of the dual agar medium. The test units were placed in an incubator at a controlled temperature of 25±1 ℃ in the dark. Exposure period was 48 hours. Each agar concentration was prepared in three replicates. After an incubation period of two days, the plants were separated from the agar media and seedling growth was measured.
  - *Bioaccumulation factor (BAF)* : To determine NP accumulation in plant tissue after 2 d, all plants were washed thoroughly with distilled water to remove the test medium. Samples were oven dried at 70℃ for 24 h, weighed, and digested by adding 3 ml of 70% HNO_3 at hot block digester. Elemental analysis in digests was performed by ICP-AES. Nanoparticle concentrations in plant tissues were determined based on the relationship between the NP suspension and ion. A known concentration of cAgNPs was dissolved in concentrated HNO_3, and the concentration of Ag ion extracted from the cAgNP suspension was determined by ICP-AES. A direct linear relationship was obtained. Nanoparticle concentrations were then calculated based on the relationship between the cAgNP and Ag ion.
- Description of the test vehicle/media/matrix

- *Preparation of the dual agar medium* : Each test unit contained 30ml of agar culture media (20ml of 2.5% agar and 10ml of 1% agar) with a specific concentration of NPs. The dual agar media of a 20 mL of 2.5% culture agar covered with a 10 mL of 1% culture agar were used in the tests. Twenty milliliters of 2.5% agar solution contained each NPs concentration, and immediately harden in a freezer to avoid the possible precipitation of nanoparticles. Ten milliliters of 1% agar solution was distributed evenly to the 2.5% agar media.

● Homogeneity and stability in test media and conditions

The homogeneity and stability in agar medium was confirmed in follows.

1) TEM and SEM measurements

2) TEM-EDS measurements

3) High resolution nano scale microscopic measurement

4) Ionization measurement using ICP-AES analysis & ion toxicity test

To confirm homogeneously distributed in test soil, TEM measurements was used. The NPs in soil was identified by TEM-EDS information. And high resolution nano scale microscopic measurement was used for confirmation of distribution as a screening. And the NP-dissolution on test medium was measured using ICP-AES.

Reliability:

● The DAT test was performed according to Lee et al., 2008

Results:

In the DAT test, it was found that the growth of *P. radiatus* and *S. bicolor* was decreased with increasing cAgNP concentrations. The EC50s of the cAgNPs of *P. radiatus* and *S. bicolor* were 13 (10-17) and 26 (5-139) mg/L, respectively. The 48hr-EC50s of root and shoot of *S. bicolor* were <5 and >40 mg/L, respectively. The brown tip and necrosis were found in exposed roots of *P. radiatus* and *S. bicolor*. These phenomenons were also reported in the previous studies (Lee et al., 2008; Ma et al., 2010).

The NP accumulated in *P. radiatus* and *S. bicolor* in DAT was increased dependently on the exposure concentration. The bioaccumulation in the DATs of *P. radiatus* and *S. bicolor* were 5.47±0.36 and 3.29±2.66 mg/kg, respectively, in the maximum exposure concentration of 40 mg/L. The bioaccumulation factors of *P. radiatus* and *S. bicolor* were calculated to be 0.14 and 0.08 L/kg, respectively. In case of the soil test, the cAgNPs accumulation in roots of *P. radiatus* and *S. bicolor* were measured to be 15.38±4.03 and 12.46±6.74 mg/kg plant respectively in the highest concentration of 2000 mg/kg dry soil. On the other hand, the cAgNPs accumulations in the shoot were measured to be 1.91±1.07 and 1.47±1.14 mg/kg plant for *P. radiatus* and *S. bicolor*, respectively. The bioaccumulation factors of root of *P. radiatus* and *S. bicolor* were calculated to be 0.008 and 0.006 kg /kg, respectively. On the other hand, the bioaccumulation factors of shoot of *P. radiatus* and *S. bicolor* were calculated to be 0.001 and 0.001 kg/kg, respectively.

Discussion/Remarks:

Nanoparticles seem to be transported to plant tissue when the roots absorb moisture from the plant agar. Exposure to cAgNPs has been associated with adverse effects on the seedling growth of both plants. And *P. radiatus* was more sensitive than *T. aestivum* to Cu NPs, and even necrosis was found in exposed roots of *P. radiatus*. Growth of *T. aestivum* was measured by shoot and root length, and root growth was shown to be a more sensitive endpoint than shoot growth. These observations suggest that only a small portion of NPs could be transported within the plant from the roots to the shoots and that the site of toxic action might be the root,

where uptake of NPs occurs during uptake of moisture and nutrients.

Conclusion:

Plant agar media were used for easy dispersion of NPs without precipitation in the present study. The growth rates of the both plants were inhibited as a result of exposure to NPs. Seedling growth after a 2-d exposure was used as a toxicity endpoint.

Reference:

Woo-Mi Lee et al., 2008, Toxicity and Bioavatilability of copper nanoparticles to the terretrial plants mung bean(*Phaseolus radiatus*) and Wheat(*Triticum aestivum*): Plant agar test for water-insoluble nanoparticles, Environmental Toxicology and Chemistry, 27(9): 1915-1921

Ma et al., 2010, Effects of rare earth oxide nanoparticles on root elongation of plants, Chemosphere, 78: 273-279

Applicant's summary and conclusion

Validity criteria fulfilled

no data

Conclusions

Plant agar media were used for easy dispersion of NPs without precipitation in the present study. The growth rates of the both plants were inhibited as a result of exposure to NPs. Seedling growth after a 2-d exposure was used as a toxicity endpoint.

Executive summary

No summary was available

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Toxicity to terrestrial plants, Song, 2013, Summary level, Study level

Administrative Data

Study result type	experimental result	Study period	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
publication	Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J and Lee EJ.	2013	Functional Analyses of Nanoparticle Toxicity: A Comparative Study of the Effects of TiO2 and Ag on Tomatoes (Lycopersicon esculentum).	Ecotoxicology and Environmental Safety, 93: 60-67.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available	other guideline: The guideline was not suggest method that the nanoparticles are homogeneously distributed. Most NPs were insoluble, so new test guidelines were needed to avoid aggregation of NPs and homogeneously distribution.	no data

Principles of method if other than guideline

We suggest test using the dual agar medium to evaluated toxicity of the NPs. To prevented NP dissolution by transition of chemical, physical condition, we did not use the solvent to improve the solubility of NPs.

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): Citrate capping silver nanoparticle
- Source: ABC NANOTECH Co., LTD, KOREA
- Structural formula/molecular structure: Ag
- Composition of test material, including degree of purity in %: The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20 %. Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

Other:
- Basic morphology: Spherical
- Description of surface chemistry (e.g., coating or modification): Citrate capping

- Major commercial uses: Antibacterial coating

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

Test substance characterisation:
To confirm the charaterisation, homogeneity and (short term) stability of prepared test items, the follows were used.
- ELS (ELS-Z2, Otsuka Electronics, Japan) : Hydrodynamic diameter, zeta potential
- Microscope (AURIGA, Carl Zeiss, Germany) : Particle size diameter
- ICP emission spectrometer (ICPS-1000V,shimadzu, Japan)

Analytical monitoring

no

Details on sampling

Not applicable

Details on analytical methods

Not applicable

Vehicle

no data

Details on test substrate

No data

Test organisms

Test organisms

Species	Lycopersicon esculentum
Plant group	other: no data
Details on test organisms	- Common name: Tomato

Study design

Study type

other: no data

Test duration type

long-term toxicity

Test type

seed germination/root elongation toxicity test

Substrate type

filter paper

Limit test

no

Total exposure duration

Remarks 14 days and 6 weeks

Post exposure observation period

None

Test conditions

Test temperature

24 °C

pH

No data

Moisture

Humidity: 60 %

Nominal and measured concentrations

Exposure concentrations: 50 to 5000 mg/L

Details on test conditions

TEST SYSTEM
- Test container: 100 mm/15 mm petri dish, one piece of filter paper for each petri dish, 5 mL of de-ionized water (germination) or test solution (root length)
Exposure route: Contacted directly

GROWTH CONDITIONS
- Photoperiod: 18 h light- Light intensity: 300 μE/m2/s.
- Temperature: 24 °C
- Humidity: 60 %

TEST CONCENTRATIONS
- Test concentrations: 50 to 5000 mg/L

Reference substance (positive control)

no

Any other information on materials and methods incl. tables

None

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
Lycopersicon esculentum		other: no data					

Details on results

Germination and root elongation:
- Every treated plant showed almost full germination after 12 days
- Possibly AgNPs did not fully penetrate the seed coat and endosperm and thus had limited effects on the embryos.
- AgNPs, which do not ionize in water, might find the seed coat and endosperm to be effective barriers, especially when they are agglomerated.
- Those treated with AgNP showed significant decreases in root growth even at the lowest (50 mg/L) concentrations while those exposed to the highest concentration (5000 mg/L) failed to show significant increase in root growth throughout the experimental period.
- After 14 days, AgNPs showed a 2 to 3 fold increase diameter in 100 mg/L treatment. AgNPs treatment was accompanied by a smaller diameter at every concentration, which allows seedlings to absorb it more easily.

Results with reference substance (positive control)

Not applicable

Reported statistics and error estimates

No data

Any other information on results incl. tables

Greenhouse experiment
- Low chlorophyll contents (mg/L): 7.88 ± 0.51 in the 0 mg/kg; 4.41 ± 0.18 in the 100 mg/kg; 3.82 ± 0.65 in the 1000 mg/kg
- TAC values (nM/mg-protein): 0.10 ± 0.00, 0.12 ± 0.00 in the 100 mg/kg treatment; 0.13 ± 0.02 in the 1000 mg/kg
- SOD values (U/mg protein): 0.24 in the 0 mg/kg; 0.24 in the 100 mg/kg; 0.28 in the 1000 mg/kg

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Validity criteria fulfilled

no data

Conclusions

AgNPs results in significantly decreased root elongation at every concentration. In greenhouse experiments, mature plants showed evidence of phytotoxicity due to AgNPs by exhibiting low chlorophyll contents, higher SOD, and less fruit production.

Executive summary

No summary was available

Cross-reference to other study

No cross-reference

Endpoint study record: Toxicity to terrestrial plants-Phaseolus.001

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Schlich		Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (OECD 208)		Fraunhofer Institute for Molecular Biology and Applied Ecology	UMSICHT			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 208 (Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test)	no

GLP compliance

no

Test materials

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

yes

Test material identity

Identifier	Identity
CAS number	7440-22-4
EC number	231-131-3
IUPAC name	Silver (1+)

Details on test material

NM-300KPrimary particle size - 15nm2 mL of NM-300K contained 200 mg of silver nanoparticles

Analytical monitoring

no

Study design

Study type

laboratory study

Test duration type

short-term toxicity

Test type

seedling emergence toxicity test

Substrate type

natural soil

Limit test

no

Total exposure duration

14 d

Test conditions

Test temperature

22°C ± 10°C

pH

pH: 4.85 (CaCl2); 6.05 (H2O)

Moisture

humidity: 70% ± 25%

Nominal and measured concentrations

Nominal: 1.5 / 4.5 / 13.5 / 40.5 / 121.5 mg/kg dry soil

Details on test conditions

photoperiod: minimum 16 hour light;light intensity: 350 ± 50 µE/m2/s. Additional lighting may be necessary if intensity decreases below 200 µE/m2/s, wavelength 400 - 700 nm except for certain species whose light requirements are less.

Any other information on materials and methods incl. tables

a) 200 mg silver nanoparticle in 2 mL stock dispersion (NM-300K)b) 2 mL NM-300K containing 200 mg Ag + 8 mL deionised waterc) 1 min shaking followed by 15 min sonication (ultrasonic bath; 35 kHz)Silver nanoparticles were applied by mixing the test material and air-dried carrier soil with the same physicochemical propertiesas the test soil. Enough Ag-NPs were added to the carrier to achieve the final test concentration when 5% carrier soil and95% test soil were mixed to homogeneity. The soil was mixed with a spoon instead of a pestle to avoid modifying the Ag-NPs.Uncontaminated soil (at 20–30% WHCmax) was spread on a plate, and the spiked carrier soil was evenly distributed over thetest soil before mixing. The mixed soil was adjusted to 55% WHCmax using deionized water. This procedure was preformedfor every test concentration.

Results and discussions

Effect concentrations

Species	Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
Phaseolus aureus	14 d	EC25	33.5 mg/kg soil dw	nominal	test mat.		

Applicant's summary and conclusion

Validity criteria fulfilled

yes

6.3.4 Toxicity to soil microorganisms

Endpoint study record: Toxicity to soil microorganisms-N-Trans.001

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Schlich	2012	N-Transformation (OECD 216)		Fraunhofer Institute for Molecular Biology and Applied Ecology	UMSICHT			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 216 (Soil Microorganisms: Nitrogen Transformation Test)	no

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
CAS number	7440-22-4
EC number	231-131-3
IUPAC name	Silver (1+)
other: Reference Material/Nanomaterial	NM-300K

Details on test material

NM-300KPrimary particle size - 15nm2 mL of NM-300K contained 200 mg of silver nanoparticles

Analytical monitoring

no

Details on sampling

100 g dry soil were sampled after 3 hours, 7 days, 21 days and 28 days.
Incubation: According to guideline

Details on analytical methods

No analytical monitoring.

Vehicle

no

Details on preparation and application of test substrate

a) 200 mg silver nanoparticle in 2 mL stock dispersion (NM-300K) b) 2 mL NM-300K containing 200 mg Ag + 8 mL deionised waterc) 1 min shaking followed by 15 min sonication (ultrasonic bath; 35 kHz)
Silver nanoparticles were applied by mixing the test material and air-dried carrier soil with the same physicochemical propertiesas the test soil. Enough Ag-NPs were added to the carrier to achieve the final test concentration when 5% carrier soil and 95% test soil were mixed to homogeneity. The soil was mixed with a spoon instead of a pestle to avoid modifying the Ag-NPs.
Uncontaminated soil (at 20–30% WHCmax) was spread on a plate, and the spiked carrier soil was evenly distributed over thetest soil before mixing. The mixed soil was adjusted to 55% WHCmax using deionized water. This procedure was preformedfor every test concentration.

Test organisms

Test organisms (inoculum)

soil

Study design

Total exposure duration

28 d

Test conditions

Test temperature

Test temperature: 20±2 °C

Moisture content

Water content of the soil: 55% of maximum water holding capacity

Organic carbon content (% dry weigth)

Corg [%] 1.02

Nitrogen content (% dry weigth)

Ntotal [mg/kg] 0.78

Nominal and measured concentrations

Nominal concentrations:1) 15 mg/kg dry soil2) 45 mg/kg dry soil3) 100 mg/kg dry soil4) 200 mg/kg dry soil

Details on test conditions

Amount of soil: 2000 g dry weight of soilTest container: During the test period, the test soil was incubated in 5 L plastic buckets.
Geographical reference of sampling site (latitude, longitude): See www.refesol.de
Parameter RefeSol 01A 1 - 02 Soil type Dystric Cambisol
Properties loamy sand, medium acid, very light humic
Sand [%] 70.1Silt [%] 24.2Clay [%] 5.7pH (CaCl2) 4.85 (CaCl2); 6.05 (H2O)
Corg [%] 1.02Ntotal [mg/kg] 0.78CECeff [mmolc/kg] 19.0
Feox [mmolc/kg] 1.57Alox [mmolc/kg] 0.95
RefeSol soils were selected as reference soils on behalf of the German Federal Environment Agency (Umweltbundesamt UBA) and they are known to be suitable for testing the influence of substances on the habitat function of soils (bioavailability, effects on organisms).
RefeSol 01A matches the properties stated in various OECD terrestrial ecotoxicological guidelines (e.g. tests with plants and soil microflora). The soils were sampled in the field and stored in high-grade stainless steel basins with drainage and ground contact in the grounds of the Fraunhofer IME in Schmallenberg. During all our experiments, red clover was sown on the stored soils and no pesticides were used. Appropriate amounts of soil were sampled 1–4 weeks before the test. If the soil was too wet for sieving, it was dried at room temperature to 20–30% of the maximum water holding capacity (WHCmax) with periodic turning to avoid surface drying. If the tests did not start immediately after sieving, the soil was stored in the dark at 4°C under aerobic conditions

Results and discussions

Details on results

Control: soil + lupine meal according to guideline
Control2: soil + lupine meal + dispersant (amount of dispersant as used to reach 45 mg/kg)
Control3: soil + lupine meal + dispersant (amount of dispersant as used to reach 200 mg/kg)
The nitrogen transformation was strongly stimulated by the dispersant NM-300K DIS by both tested concentrations. A comparison of the dispersant control 3 with the corresponding test concentration shows a significant reduction of the nitrogen transformation by 81.4%.

Reported statistics and error estimates

The variation between replicate control samples should be less than ± 15%.Day 0: CV% = 6.8%Day 7: CV% = 8.5%Day 28: CV% = 2.9%

Applicant's summary and conclusion

Validity criteria fulfilled

yes

Conclusions

NM-300K seems to inhibit the nitrogen transformation of the soil micro-organism. However, the Dispersant (NM-300K DIS) hampers the investigation.
Depending on the test concentration, instead of the N-Transformation test the potential ammonium oxidation should be observed.

Endpoint study record: Toxicity to soil microorganisms-C-Trans.001

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Schlich	2012	C-Transformation Test (OECD 217)		Fraunhofer Institute for Molecular Biology and Applied Ecology	UMSICHT			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 217 (Soil Microorganisms: Carbon Transformation Test)	no

GLP compliance

yes

Test materials

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

yes

Test material identity

Identifier	Identity
CAS number	7440-22-4
EC number	231-131-3
IUPAC name	Silver (1+)
other: Reference Material/Nanomaterial	NM-300K

Details on test material

NM-300KPrimary particle size - 15nm2 mL of NM-300K contained 200 mg of silver nanoparticles

Confidential details on test material

-

Details on properties of test surrogate or analogue material

-

Analytical monitoring

no

Details on sampling

100 g dry soil were sampled after 3 hours, 7 days, 21 days and 28 days.Incubation: According to guideline

Details on analytical methods

No analytical monitoring was performed.

Details on preparation and application of test substrate

a) 200 mg silver nanoparticle in 2 mL stock dispersion (NM-300K)b) 2 mL NM-300K containing 200 mg Ag + 8 mL deionised waterc) 1 min shaking followed by 15 min sonication (ultrasonic bath; 35 kHz)
Silver nanoparticles were applied by mixing the test material and air-dried carrier soil with the same physicochemical propertiesas the test soil. Enough Ag-NPs were added to the carrier to achieve the final test concentration when 5% carrier soil and95% test soil were mixed to homogeneity. The soil was mixed with a spoon instead of a pestle to avoid modifying the Ag-NPs.
Uncontaminated soil (at 20–30% WHCmax) was spread on a plate, and the spiked carrier soil was evenly distributed over thetest soil before mixing. The mixed soil was adjusted to 55% WHCmax using deionized water. This procedure was preformedfor every test concentration.

Test organisms

Test organisms (inoculum)

soil

Study design

Total exposure duration

28 d

Test conditions

Test temperature

Test temperature: 20±2 °C

Moisture content

Water content of the soil: 55% of maximum water holding capacity

Organic carbon content (% dry weigth)

Corg [%] 1.02Ntotal [mg/kg] 0.78

Nitrogen content (% dry weigth)

Ntotal [mg/kg] 0.78

Nominal and measured concentrations

1) 1.67 mg/kg dry soil2) 5.0 mg/kg dry soil3) 15.0 mg/kg dry soil4) 45.0 mg/kg dry soil5) 100.0 mg/kg dry soil

Details on test conditions

Amount of soil: 2000 g dry weight of soil
Test container: During the test period, the test soil was incubated in 5 L plastic buckets.Geographical reference of sampling site (latitude, longitude): See www.refesol.de
RefeSol soils were selected as reference soils on behalf of the German Federal Environment Agency (Umweltbundesamt UBA) and they are known to be suitable for testing the influence of substances on the habitat function of soils (bioavailability, effects on organisms). RefeSol 01A matches the properties stated in various OECD terrestrial ecotoxicological guidelines (e.g. tests with plants and soil microflora). The soils were sampled in the field and stored in high-grade stainless steel basins with drainage and ground contact in the grounds of the Fraunhofer IME in Schmallenberg. During all our experiments, red clover was sown on the stored soils and no pesticides were used. Appropriate amounts of soil were sampled 1–4 weeks before the test. If the soil was too wet for sieving, it was dried at room temperature to 20–30% of the maximum water holding capacity (WHCmax) with periodic turning to avoid surface drying. If the tests did not start immediately after sieving, the soil was stored in the dark at 4°C under aerobic conditions
Soil:Parameter RefeSol 01A 1 – 02 Soil type Dystric Cambisol
Properties loamy sand, medium acid, very light humicSand [%] 70.1Silt [%] 24.2Clay [%] 5.7pH (CaCl2) 4.85 (CaCl2); 6.05 (H2O)Corg [%] 1.02
Ntotal [mg/kg] 0.78CECeff [mmolc/kg] 19.0Feox [mmolc/kg] 1.57Alox [mmolc/kg] 0.95

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)
28 d	EC10	0.9				
28 d	EC50	13				

Details on results

EC10: 0.9 mg/kg dry soil; EC50: 13 mg/kg dry soil

Time Replicates Treatment
0,0 d Control Control2 1.67 mg/kg 5 mg/kg 15 mg/kg 45 mg/kg 100 mg/kg
1 2.4 4.8 3.2 4 4.8 4 2.4
2 2.5 4.8 3.2 4 4 3.2 3.2
3 2.4 4.8 4 4 4 4 3.2
4 2.4 5.6 4.8 4 3.2
#Replicates 4 4 3 3 4 4 4
Mean 2.43 5.00 3.47 4.00 4.40 3.80 3.00
Std.Dev 0.05 0.40 0.46 0.00 0.46 0.40 0.40
CV% 2.1 8.0 13.3 0.0 10.5 10.5 13.3
7,0 d Replicates

1 2.4 3.2 2.4 2.4 1.6 1.6 1.6
2 2.4 3.2 2.4 1.6 1.6 0.8 1.6
3 2.4 2.4 2.4 1.6 1.6 0.8 0.8
4 3.3 2.4 2.4 1.6
#Replicates 3 3 3 4 4 4 4
Mean 2.40 2.93 2.40 2.23 1.80 1.40 1.40
Std.Dev 0.00 0.46 0.00 0.81 0.40 0.77 0.40
CV% 0.0 15.7 0.0 36.4 22.2 54.7 28.6
21,0 d Replicates
1 2.4 2.4 0.8 1.6 0.8 0.8 1.6
2 2.4 2.4 1.6 0.8 0.8 0.8 0.8
3 2.4 1.6 1.6 0.8 0.8 0.8 1.6
4 0.8 0.8 2.4 1.6
#Replicates 3 3 4 3 4 4 4
Mean 2.40 2.13 1.20 1.07 0.80 1.20 1.40
Std.Dev 0.00 0.46 0.46 0.46 0.00 0.80 0.40
CV% 0.0 21.7 38.5 43.3 0.0 66.7 28.6
28,0 d
1 1.6 1.6 1.6 0.8 0.8 0.8 0.8
2 1.6 2.4 1.6 0.8 0.8 0.8 0.8
3 1.6 1.6 1.6 0.8 0.8 0.8 0.8
4 1.6 0.8 1.6 0.8 0.8 0.8
#Replicates 4 3 4 4 4 4 4
Mean 1.60 1.87 1.40 1.00 0.80 0.80 0.80
Std.Dev 0.00 0.46 0.40 0.40 0.00 0.00 0.00
CV% 0.0 24.7 28.6 40.0 0.0 0.0 0.0

Results with reference substance

Control 2: Soil with the highest concentration of dispersant used in the test.

Reported statistics and error estimates

The variation between replicate control samples should be less than ± 15%.
Day 0: CV% = 2.1 % ;
Day 7: CV% = 0.0 %
Day 21: CV% = 0.0 %;
Day 28: CV% = 0.0 %

Applicant's summary and conclusion

Validity criteria fulfilled

yes (The variation between replicate control samples should be less than ± 15%.)

Endpoint study record: Toxicity to soil microorganisms.001

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e-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	Chemosphere	2009	Effect of antimony on the microbial growth and the activities of soil enzymes						
study report	Soil Biology and Biochemistry	1998	Soil oxygen status and dehydrogenase activity						
study report	Biology and Fertility of Soils	1989	Short-term assay of soil urease activity using colorimetric determination of ammonium						
study report	Soil Biology and Biochemistry	1997	Dehydrogenase activity in soil: a comparison between the TTC and INT assay under their optimum conditions						
study report	Soil Biology and Biochemistry	2001	Complementarity of bioassays and microbial activity measurements for the evaluation of hydrocarboncontaminated soils quality						
study report	Methods of soil analysis Part 2 - Microbiological and Biochemical Properties-SSSA Book Series	1994	Soil enzymes						
study report	Soil Biology and Biochemistry	2001	Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils						

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

GLP compliance

no

Test materials

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

yes

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): citrate capping silver nanoparticle
- Molecular formula (if other than submission substance): Ag
- Molecular weight (if other than submission substance): no data
- Smiles notation (if other than submission substance): no data
- InChl (if other than submission substance): no data
- Structural formula attached as image file (if other than submission substance): no data
- Substance type: Inorganic
- Physical state: Powder
- Analytical purity: no data
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data
- Isomers composition: no data
- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data
- Radiochemical purity (if radiolabelling): no data
- Specific activity (if radiolabelling): no data
- Locations of the label (if radiolabelling): no data
- Expiration date of radiochemical substance (if radiolabelling): no data
- Stability under test conditions: no data
- Storage condition of test material: no data
- Other:

● Composition of nanomaterial being tested, including degree of purity in %, nature of known impurities including isomers and by-products (in % or ppm) or additive(s) (in ppm or %) (e.g. stabilising agents, surfactants or inhibitors. As appropriate.):

- The stock of silver nanoparticles was black colloical suspension, and included degree of purity in 20 %.
- Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.

● Basic morphology: Spherical
● Description of surface chemistry (e.g., coating or modification): Citrate capping
● Major commercial uses: Antibacterial coating
● Known catalytic activity
● Method of production (e.g., precipitation, gas phase)
● Producer/provider: ABC NANOTECH Co., LTD, KOREA

Analytical monitoring

no

Vehicle

no

Study design

Total exposure duration

7 d

Test conditions

Moisture content

Test conditions:
● Description of the test item preparation protocol
● Description of the test item
- Urease : Urease activity was measured using colorimetric determination of ammonium according to Kandeler and Gerber (1988)
- Dehydrogenase (DHA) : Dehydrogenase activity was determined following the procedures adapted from Gong (1997) and Brohon et al. (2001)
- Acid phosphatase (APA), Arylsulfatase (ASA), and ß-glucosidase (BGA) : Activities of acid phosphatase, arylsulfatase, ß-glucosidase were determined following the procedures adapted from Tabatabai (1994).
- Hydrolysis of fluorescein diacetate (FDA) : The activity of FDA hydrolase was determined following the procedures adapted from Adam and Duncan (2001).
● Description of the test vehicle/media/matrix
- Preparation of nature soil polluted to NPs : Soil was surface sample (0-5 cm) collected from the campus of Konkuk University (Seoul, Republic of Korea). Samples were air dried for 3 days, sieved(<2 mm), and stored in plastic bags at 4 oC until test. Unamended soil was used as a control. Two kinds of method blank were prepared to correct background absorbance. Soil treated with 3.5 mL of formaldehyde to inhibit microbial activity was used as a method blank to correct the absorbance of soil extract. Amended soil with cAgNPs in the absence of substrate was used as the second method blank to correct the absorbance due to cAgNPs themselves. Second method blanks were prepared for each concentration of cAgNPs used in the present study. The moisture content was adjusted to about 60 % of the water holding capacity and then the soils were incubated at 25 oC for in the dark
● Homogeneity and stability in test media and conditions
The NP-dissolution on test medium was measured using ICP-AES.

Reliability:

Kandeler and Gerber (1988); Gong (1997) and Brohon et al. (2001); Tabatabai (1994); Adam and Duncan (2001)

Any other information on materials and methods incl. tables
Method

● In vitro
- Test system : Not applicable
● In vivo

- Species : urease, dehydrogenase, acid phosphatase, arylsulfatase, ß-glucosidase, and hydrolysis of fluorescein diacetate (FDA)
● Sample administration : Natural soil, Konkuk Univ. (Seoul, Korea)
● Exposure route :
● Exposure duration : 7 days
● Description of the method and give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy, or other.
- "no guidelines available" : There was no toxicity assay using soil enzyme activity on OECD guideline. Soil enzyme activities indicate the potential of the soil to support biochemical processes (Brzezinska et al., 1998)
- Enzyme activity assay was used as a sensitive indicator of the adverse effect of toxicity materials.
- We suggest test using soil enzyme activity to evaluated toxicity of the NPs. Nature soil was polluted by NPs to detect the activity of 6 species enzyme.
● Analytics (in particular, analytical verification of doses or concentrations) :
● Statistics : Arithmetic means
● Any other relevant information : No

Results and discussions

Any other information on results incl. tables

attached file: Results

Overall remarks, attachments

Attached background material

Attached document	Remarks
Results.docx / 15.23 KB (application/octet-stream) Results ● Enzyme activity effects	

	EC50/NOEC (mg/kg dry soil)		
	0day	1day	7day
DHA	EC50 : 217.19 (63.55-742.26) NOEC : 1	NOEC : 10	EC50 : 107.98 (62.82-185.61) NOEC : 10
FDA	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC: 1	EC50 : >1000 NOEC : 100
Urease	EC50 : 34.55 (19.20-62.17) NOEC : 10	EC50 : 24.29 (13.34-44.23) NOEC : <1	EC50 : 14.20 (8.78-22.97) NOEC : <1
APA	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : >1000

ASA	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : 10
BGA	EC50: >1000 NOEC: >1000	EC50 : >1000 NOEC : 1	EC50 : >1000 NOEC : 100

● Ion toxicity test : There was no efffect of enzyme activity within maximum of NP ionization concentration.

Applicant's summary and conclusion

Conclusions

Soil enzymes are difficult to extract from soils, they are characterized by measuring their activity under a strict set of conditions such as a substrate, pH butter and temperature. Change in activity in each cAgNPs treatment was expressed as a percentage of the mean of control treatment. The results showed that there is an adverse effect of cAgNPs on the activities of five representative enzymes and the hydrolysis of FDA in soil environment. Activity of dehydrogenase, ASA, urease immediately declined after cAgNP amendment with increasing concentration. The urease and dehydrogenase exhibits more sensitive to cAgNPs than other four enzymes. No significant effect of silver ions, which can be dissolved from cAgNPs under experimental condition, was observed on the activities of soil enzymes.

Executive summary

Using Soil enzyme activities, we can assess of adverse effect on biochemical processes that can change chemicals introduced in soil to bioavailable substances. In this study, there are significant effect on 6 enzyme activities by cAgNPs and it indicates that cAgNPs could affect to diverse terrestrial species directly and indirectly.

Endpoint study record: Toxicity to soil microorganisms.002

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	2e - Study well.....

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

GLP compliance

no

Test organisms

Test organisms (inoculum)

other:

Study design

Total exposure duration

48 h

Test conditions

Moisture content

no data reported

6.3.6 Toxicity to other above-ground organisms

Endpoint study record: 7440-22-4, Toxicity to other above-ground organisms, Anonymous, Year, Summary level, Study level

Administrative Data

Study result type	experimental result	Study period	No data
Rationale for reliability incl. deficiencies	Kandeler and Gerber (1988); Gong (1997) and Brohon et al. (2001); Tabatabai (1994); Adam and Duncan (2001)		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Kandeler E and Gerber H.	1988	Short-Term Assay of Soil Urease Activity Using Colorimetric Determination of Ammonium.	Biology and Fertility of Soils, 6:68-72.					
review article or handbook	Tabatabai MA.	1994	Soil enzymes.	Methods of soil analysis Part 2 - Microbiological and Biochemical Properties-SSSA Book Series, No. 5, 775-833.					
publication	Gong P.	1997	Dehydrogenase Activity in Soil: A Comparison between the TTC and INT Assay under Their Optimum conditions.	Soil Biology and Biochemistry, 29:211-214.					
publication	Brzezinska	1998	Soil Oxygen Status	Soil Biology and					

	M, Stepniewska Z and Stepniewski W.		and Dehydrogenase Activity.	Biochemistry, 30:1783-1790.					
publication	Adam G and Duncan H.	2001	Development of a Sensitive and Rapid Method for the Measurement of Total Microbial Activity Using Fluorescein Diacetate (FDA) in a Range of Soils.	Soil Biology and Biochemistry, 33:943-951.					
publication	Brohon B, Delolme C and Gourdon R.	2001	Complementarity of Bioassays and Microbial Activity Measurements for the Evaluation of Hydrocarbon Contaminated Soils Quality.	Soil Biology and Biochemistry, 33:883-891.					
publication	An YJ and Kim MJ.	2009	Effect of Antimony on the Microbial Growth and the Activities of Soil Enzymes.	Chemosphere, 74:654-659.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
no guideline available		

Principles of method if other than guideline

- "no guidelines available" : There was no toxicity assay using soil enzyme activity on OECD guideline. Soil enzyme activities indicate the potential of the soil to support biochemical processes (Brzezinska et al., 1998).- Enzyme activity assay was used as a sensitive indicator of the adverse effect of toxicity materials.- We suggest test using soil enzyme activity to evaluated toxicity of the NPs. Nature soil was polluted by NPs to detect the activity of 6 species enzyme.

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): Citrate capping silver nanoparticle
- Source: ABC NANOTECH Co., LTD, KOREA
- Structural formula/molecular structure: Ag
- Composition of test material, including degree of purity in %: The stock of silver nanoparticles was black colloidal suspension, and included degree of purity in 20 %. Citrate (0.5-1.5 %) was used as a stabilizer of the colloidal particles.
Other:- Basic morphology: Spherical- Description of surface chemistry (e.g., coating or modification): Citrate capping
- Major commercial uses: Antibacterial coating

Confidential details on test material

No data

Details on properties of test surrogate or analogue material

Test substance characterisation:To confirm the charaterisation, homogeneity and (short term) stability of prepared test items, the follows were used.- DLS size measurement- Zeta potential measurement- SEM measurementsFrom the measurement of DLS, SEM, and Zeta potential, the size and aggregation level of NP suspension in test media was estimated.

Analytical monitoring

no

Details on sampling

Not applicable

Details on analytical methods

Not applicable

Vehicle

no data

Details on test medium and application

No data

Test organisms

Test organisms (species)

other: urease, dehydrogenase, acid phosphatase, arylsulfatase, ß-glucosidase, and hydrolysis of fluorescein diacetate (FDA)

Details on test organisms

Not applicable

Study design

Study type

other: no data

Limit test

no

Total exposure duration

7 d

Post exposure observation period

None

Test conditions

Test temperature

No data

Humidity

No data

Photoperiod and lighting

No data

Nominal and measured concentrations

No data

Details on test conditions

- Urease: Urease activity was measured using colorimetric determination of ammonium according to Kandeler and Gerber (1988)
- Dehydrogenase (DHA): Dehydrogenase activity was determined following the procedures adapted from Gong (1997) and Brohon et al. (2001)
- Acid phosphatase (APA), Arylsulfatase (ASA), and ß-glucosidase (BGA): Activities of acid phosphatase, arylsulfatase, ß-glucosidase were determined following the procedures adapted from Tabatabai (1994).
- Hydrolysis of fluorescein diacetate (FDA): The activity of FDA hydrolase was determined following the procedures adapted from Adam and Duncan (2001).
- Preparation of nature soil polluted to NPs: Soil was surface sample (0-5 cm) collected from the campus of Konkuk University (Seoul, Republic of Korea). Samples were air dried for 3 days, sieved (<2 mm), and stored in plastic bags at 4 °C until test. Unamended soil was used as a control. Two kinds of method blank were prepared to correct background absorbance. Soil treated with 3.5 mL of formaldehyde to inhibit microbial activity was used as a method blank to correct the absorbance of soil extract. Amended soil with cAgNPs in the absence of substrate was used as the second method blank to correct the absorbance due to cAgNPs themselves. Second method blanks were prepared for each concentration of cAgNPs used in the present study. The moisture content was adjusted to about 60 % of the water holding capacity and then the soils were incubated at 25 °C in the dark.
- Homogeneity and stability in test media and conditionsThe NP-dissolution on test medium was measured using ICP-AES.

Reference substance (positive control)

no

Any other information on materials and methods incl. tables

None

Results and discussions

Details on results

- Ion toxicity test: There was no effect of enzyme activity within maximum of NP ionization concentration.
- See table 6.3.6/1 for enzyme activity effects

Results with reference substance (positive control)

Not applicable

Reported statistics and error estimates

Arithmetic means

Any other information on results incl. tables

Table 6.3.6/1: Enzyme activity effects

Enzyme activity	**EC50/NOEC (mg/kg dry soil)**		
	0 day	1 day	7 day
DHA	EC50 : 217.19 (63.55-742.26) NOEC : 1	NOEC : 10	EC50 : 107.98 (62.82-185.61) NOEC : 10
FDA	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC: 1	EC50 : >1000 NOEC : 100
Urease	EC50 : 34.55 (19.20-62.17) NOEC : 10	EC50 : 24.29 (13.34-44.23) NOEC : <1	EC50 : 14.20 (8.78-22.97) NOEC : <1
APA	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : >1000
ASA	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : 10	EC50 : >1000 NOEC : 10
BGA	EC50: >1000 NOEC: >1000	EC50 : >1000 NOEC : 1	EC50 : >1000 NOEC : 100

Overall remarks, attachments

Remarks on results including tables and figures

Soil enzymes are difficult to extract from soils, they are characterized by measuring their activity under a strict set of conditions such as a substrate, pH butter and temperature. Change in activity in each cAgNPs treatment was expressed as a percentage of the mean of control treatment. The results showed that there is an adverse effect of cAgNPs on the activities of five representative enzymes and the hydrolysis of FDA in soil environment. Activity of dehydrogenase, ASA, urease immediately declined after cAgNP amendment with increasing concentration. The urease and dehydrogenase exhibits more sensitive to cAgNPs than other four enzymes. No significant effect of silver ions, which can be dissolved from cAgNPs under experimental condition, was observed on the activities of soil enzymes.

Applicant's summary and conclusion

Validity criteria fulfilled

no data

Conclusions

Using Soil enzyme activities, we can assess of adverse effect on biochemical processes that can change chemicals introduced in soil to bioavailable substances. In this study, there is significant effect on 6 enzyme activities by cAgNPs and it indicates that cAgNPs could affect to diverse terrestrial species directly and indirectly.

Executive summary

No summary was available

Cross-reference to other study

No cross-reference

6.4 Biological effects monitoring

6.5 Biotransformation and kinetics

6.6 Additional ecotoxicological information

Endpoint study record: Toxicity to sludge-Respiration inhibition.001

Administrative Data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Schlich	2012	Respiration inhibition test (OECD 209)		Fraunhofer Institute for Molecular Biology and Applied Ecology	UMSICHT			

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD 209	

Test materials

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

yes

Test material identity

Identifier	Identity
CAS number	7440-22-4
EC number	231-131-3

IUPAC name	silver (1+)
other: Reference Material/Nanomaterial	NM-300K

Details on test material

NM-300KPrimary particle size - 15nm2 mL of NM-300K contained 200 mg of silver nanoparticles

Confidential details on test material

Any other information on materials and methods incl. tables

Room temperature was kept at 20 ± 2°CDry content of sludge: 4 g/L ± 10%Resulting dry content of sludge in the test vessel: 1.6 g/LTest was performed in 1 L glass flasks, washed with dishwashing detergent.Sewage sludge from the municipal sewage treatment plant in Schmallenberg, Germany.
Synthetic sewage feed composed according to the guideline was used:
The medium should be prepared to contain the following constituents at the stated amounts:
- peptone 16 g
- meat extract (or a comparable vegetable extract) 11 g
- urea 3 g
- sodium chloride (NaCl) 0.7 g
- calcium chloride dihydrate (CaCl2 * 2H2O) 0.4 g
- magnesium sulphate heptahydrate (MgSO4 * 7H2O) 0.2 g
- anhydrous potassium monohdydrogen phosphate (K2HPO4) 2.8gdistilled or deionized water to 1 litre.

Preparation of test material: 8 mL of deionized water were added into the stock dispersion of NM-300K. 1 min shaking followed by 15 min sonication (ultrasonic bath; 35 kHz).
Test concentration:1) 1 mg/L2) 3 mg/L3) 9 mg/L4) 27 mg/L5) 50 mg/L6) 81 mg/L7) 100 mg/L

Results and discussions

Any other information on results incl. tables

Validity of the test: 3,5-dichlorphenole was used as reference. The EC50 has to be between 5 and 30 mg/L. The variation between replicate control samples should be less than ± 15%.For NM-300K an EC10 of 27.9 mg/L and an EC50 of 43.0 mg/L was determined.

Overall remarks, attachments

Remarks on results including tables and figures

-

Applicant's summary and conclusion

Conclusions

-

Executive summary

-

Endpoint study record: Surface Charge-Dependent Toxicity of Silver Nanoparticles (Badawy US EPA)

Administrative Data

Study result type	experimental result	Study period	Approximately 2009 to 2011
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Testing was completed under a U.S. EPA, Office of Research and Development research plan inlcuding a quality assurance project plan and in acordance with EPA QA/QC guidance. Standard USEPA or OECD harmonized guidelines (with modifications) were used where available.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	El Badawy, A.; Silva, R. G.; Morris, B.; Schekel, K. G.; Suidan, M., T.; Tolaymat, T. M.	2011	Surface Charge-Dependent Toxicity of Silver Nanoparticles	Environmental Science and Technology					

Materials and methods

Type of information

Rapid screening tests were employed. With additional confimatory testing, the standard biological oxygen demand (BOD5) test method might be considered for OECD standardized testing as a rapid screening assay for evaluating the toxicty of nanomaterials.The toxicity of AgNPs to gram positive bacillus species was investigated. The standard biological oxygen demand (BOD5) test was used to determine the oxygen consumption after 5 days of exposure of the microorganisms to Citrate-AgNPs. A Live/Dead ® BacLightTM kit with two color fluorescence assay coupled with fluorescence spectroscopy was employed to perform bacterial viability tests. The standard biological oxygen demand (BOD5) test was used to determine the oxygen consumption after 5 days of exposure of the microorganisms to the AgNPs. The tested organisms were PolySeed (Gram-positive bacillus species) obtained from, Interlab® Supply USA.

GLP compliance

no

Test materials

Details on test material

The H2-AgNPs (13 nm, -22 mV) have uncoated surfaces. The Citrate-AgNPs (10 nm, - 38 mV) are electrostatically stabilized through the ionization of the carboxyl groups of the citrate molecule adsorbed on the surface of the AgNPs.The PVP-AgNPs (12 nm, -10 mV) are stabilized through the steric repulsion caused by the adsorption of PVP on the surface of the AgNP. The BPEI-AgNPs (10 nm, + 40 mV) are electrosterically stabilized due to the adsorption of the BPEI molecules on the nanoparticle surface. The four investigated silver nanoparticles were purified to remove residual impurities form the synthesized silver nanoparticles suspenions using 10 KD polyethersulfone ultrafiltration membranes.

Results and discussions

Any other information on results incl. tables

The results clearly demonstrated that AgNPs exhibited surface charge-dependent toxicity to the investigated bacillus species. The toxicty followed the order of BPEI-AgNPs > PVP-AgNPs > H2-AgNPs > Citrate-AgNPs. The more negative Citrate-AgNPs were the least toxic, whereas the positively charged BPEI-AgNPs were the most toxic NPs. The ionization of the citrate molecules, coating the Citrate-AgNPs, has resulted in a zeta potential of -38 mV which is similar to that of the tested bacillus species (-37mV under test conditions). The carboxyl,phosphate and amino groups on the cellular membrane of the Gram-positive bacteria provide the organisms with a negative charge. Thus, there is a high degree of repulsion, between the negatively charged Citrate- AgNPs and the bacillus cells, which forms an electrostatic barrier that limits the cell-particle interactions thus reducing the toxicity. As the magnitude of the negative zeta potential gradually decrease, H2-AgNPs (-22 mV) and PVP-AgNPs (-10 mV), the electrostatic barrier is reduced which increases the chances of cell-particle interactions and results in higher toxicity. The repulsion turned to attraction when the bacteria were exposedto the positively charged BPEI-AgNPs (+40 mV). This allows for a higher degree of interactions which causes a greater toxicity.The BPEI-AgNPs, PVP-AgNPs, H2-AgNPs, and Citrate-AgNPs completely inhibited the bacterial activity at conenctrations gretaer than 3 ppb, 10 ppb, 100 ppb and 800 ppb, respectively.

Overall remarks, attachments

Remarks on results including tables and figures

The results demonstrate the importance of the physical interactions, between the AgNP and the bacterium, on the toxicity of AgNPs. These physical interactions are highly governed by the surface charge of not only the AgNPs but also of the cellular membranes of the bacteria examined. The results suggest that unless the electrostatic barrier between the AgNPs and the bacteria is overcome, other toxicity factors (i.e., shape and size) may have minimal impact on the toxicity of these nanoparticles. As a result, surface charge is one of the most important factors that has to be taken into consideration when evaluating the toxicity of AgNPs in the environment. In addition, it is expected that surface charge-dependent toxicity behavior might eventually be a good tool for the prediction of the toxicological behavior of various types of AgNPs.

Applicant's summary and conclusion

Cross-reference to other study

Legal entity: NAPIRAhub OECD-WPMN Silver UUID NAPI-ab57fd40-aacc-4f1c-8d8b-6559efd7c0cb Dossier UUID 0 Author admin / (No legal entity) Date 2009-07-13 14:39:34 CEST Remarks General information Legal entity name NAPIRAhub OECD-WPMN Silver Legal entity type other: research centre Remarks Legal entity for the OECD-WPMN Silver NAPIRAhub Contact information Contact address Address EC/JRC/IHCP Via E. Fermi Postal code I-21020 Town Ispra Region / State VA Country Italy E-mail jrc-info-napirahub@ec.europa.eu

7. TOXICOLOGICAL INFORMATION

7.1 Toxicokinetics, metabolism and distribution

7.1.1 Basic toxicokinetics

Endpoint study record: 7440-22-4, Basic toxicokinetics, Lee, 2013, 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	GLP study conducted according to OECD 417 Guideline with deviations: details on test item, acclimation, housing and feeding conditions 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	Toxicokinetics Study of Silver Nanoparticles in Rabbit.		ChemOn		National Institute of Environmental Research (NIER), Korea		
publication	Lee Y, Kim P, Yoon J, Lee B, Choi K, Kil KH and Park K.	2013	Serum Kinetics, Distribution and Excretion of Silver in Rabbits following 28 Days after a Single Intravenous Injection of Silver Nanoparticles.	Nanotoxicology. 7(6): 1120-30.					

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 method

in vivo

Objective of study

toxicokinetics

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 417 (Toxicokinetics)	yes (details on test item, acclimation, housing and feeding conditions not reported)

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

Radiolabelling

no

Details on test material

- Name of test material (as cited in study report): Citrate capped silver nanoparticle (cAgNPs)- Source: ABC Nanotech Co., Ltd., Korea

Confidential details on test material

No data

Test animals

Species

rabbit

Strain

New Zealand White

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Weight at study initiation: 2.4 kg

Administration / exposure

Route of administration

intravenous

Vehicle

other: 5 % glucose

Details on exposure

No data

Duration and frequency of treatment / exposure

Single dose

Doses / concentrations

0.5 and 5 mg/kg bw

No. of animals per sex per dose

4 males per dose

Control animals

yes

Positive control

Not applicable

Details on study design

No data

Details on dosing and sampling

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)
- Tissues and body fluids sampled: Blood, urine, faeces and tissues (liver, kidney, spleen, lung, brain, testis, and thymus)
Time and frequency of sampling:
- Blood sampling was performed from ear vein of treated animals at 0 min (before treatment), 5 min, 10 min, 30 min, 1, 2, 6 and 12 h, 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days, respectively. Blood samples were also collected from non-treated control group at 1, 7 and 28 days
- Faeces and urine were collected for 24 h at 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days after treatment. Faeces were homogenized with 10 volume of deionized water, and a part was taken and frozen at -80 °C for subsequent analysis. Collected urine was also homogenized, and a part was taken and frozen for subsequent analysis.
Tissues Sampled: Tissues (liver, kidney, spleen, lung, brain, testis, and thymus) from 4 rabbits of a treated group were obtained for AgNPs distribution analysis at 1, 7 and 28 days after treatment.
- Pharmacokinetic analysis: Bioavailability, tissue distribution and excretion ratios were calculated using the KFDA model (BA-Calc 2007 1.0.0, KFDA, Seoul, Korea).
- Chemical analysis: Samples (about 200 mg) were digested in a mixed solution of 7 mL of 70 % HNO3 and 1 mL of 30 % H2O2 using a microwave digestion system, and subjected to inductively coupled plasma-mass spectrometry (ICP-MS) for analysis.
- Metabolites: Not applicable

Statistics

One way analysis of variance (ANOVA) test, Duncan's or Dunnett's multiple range test

Any other information on materials and methods incl. tables

General examination: Any immediate signs of toxicity and body weight changes, Urinalysis, Hematology analysis, Absolute and relative (organ-to-BW ratios) weights of the organs, Histopathological examination.
Urinalysis Sampled: At 1, 7, and 28 days after treatment, urinalysis was conducted to determine glucose, bilirubin, ketone body, specific gravity, pH, protein, urobilinogen, nitrite, occult blood and leukocyte levels in the urine.

Results and discussions

Preliminary studies

Not applicable

Pharmacokinetic studies

Details on distribution in tissues

AgNPs were easily accumulated in the body and retained for a long time after a single intravenous injection in rabbits, although a decline in blood concentration was observed. The half-time of release of silver from organs into blood could not be calculated because more than 50 % of the AgNPs was still retained in all the tested organs until the final day of 28 days.

Details on excretion

Excretion through feces after injection supports the idea of biliary excretion of AgNPs, which needs further investigation on the detailed mechanisms of AgNPs excretion.

Toxicokinetic parameters

Test #1 No. other: AUC(last) - 0.90 ± 0.16 and 3.65 ± 0.68 μg•day/mL in 0.5 and 5 mg/kg bw-treated groups, respectively

Test #1 No. Half-life 1st: t1/2 was 16.3 ± 2.9 days and 11.7 ± 1.3 days in 0.5 and 5 mg/kg bw-treated groups, respectively.

Metabolite characterisation studies

Details on metabolites

Not applicable

Bioaccessibility

Bioaccessibility testing results

Not applicable

Any other information on results incl. tables

Serum Kinetics:The AUC(last) was 0.90 ± 0.16 and 3.65 ± 0.68 μg•day/mL in 0.5 and 5 mg/kg bw-treated groups, respectively. The t1/2 was 16.3 ± 2.9 days and 11.7 ± 1.3 days in 0.5 and 5 mg/kg bw-treated groups, respectively.
Mortality: None.
Body weight: The average weight-gain was 431 g in the control group, 416 g in low-dose treated group,

and 599 g in high-dose treated group (N=4)
See the attached document for information on tables/figures of results

Overall remarks, attachments

Remarks on results including tables and figures

None

Attached background material

Attached document	Remarks
Toxicokinetics Study of Silver Nanoparticles in Rabbit.pdf / 650.3 KB (application/octet-stream) **Test Substances** Citrate capped silver nanoparticles (cAgNPs), CAS No. 7440-22-4 (silver) Remarks: Manufactured by ABC Nanotech Co., Ltd. (Korea) **Methods** Methods/guideline followed: OECD Test Guideline 417, "Toxicokinetics" Type: *In vivo* GLP: Yes Year (study performed): 2011 **Test Conditions** Species: Rabbit Strain: New Zealand White Sex: Male Cell Type: Not applicable Body weight: 2.4 kg Number of Animals: 4 rabbits per dose Route: Intravenous injection (i.v.) Vehicle: 5% glucose Dose(s) used: 0.5 or 5 mg/kg body weight, single dose Statistical Methods: One way analysis of variance (ANOVA) test, Duncan's or Dunnett's multiple range test Actual Dose(s): Not applicable	

Excretion Routes: Urine and feces General examination: Any immediate signs of toxicity and body weight changes, Urinalysis, Hematology analysis, Absolute and relative (organ-to-BW ratios) weights of the organs, Histopathological examination. Blood sampling was performed from ear vein of treated animals at 0 min (before treatment), 5 min, 10 min, 30 min, 1h, 2h, 6h, 12h, 1d, 2d, 3d, 4d, 5d, 6d, 7d, 14d, 21d, and 28d, respectively. Blood samples were also collected from non-treated control group at 1d, 7d, and 28d, Feces and urine were collected for 24 hoursat 1d, 2d, 3d, 4d, 5d, 6d, 7d, 14d, 21d, and 28d after treatment. Feces were homogenized with 10 volume of deionized water, and a part was taken and frozen at -80°C for subsequentanalysis. Collected urine was also homogenized, and a part was taken and frozen for subsequentanalysis. Urinalysis Sampled: . At 1d, 7d, and 28d after treatment, urinalysis was conducted to determine glucose, bilirubin, ketone body, specific gravity, pH, protein, urobilinogen, nitrite, occult blood and leukocyte levels in the urine. Tissues Sampled: Tissues (liver, kidney, spleen, lung, brain, testis, and thymus) from 4 rabbits of a treated group were obtained for AgNPs distribution analysis at 1d, 7d, and 28d after treatment. Metabolites: Not applicable Metabolites CAS: Not applicable Chemical analysis: Samples (about 200 mg)were digested in a mixed solution of 7 mL of 70% HNO_3 and 1 mL of 30% H_2O_2 using a microwave digestion system, and subjected to inductively coupled plasma-mass spectrometry (ICP-MS) for analysis. Pharmacokinetic analysis: Bioavailability, tissue distribution and excretion ratios were calculated using the KFDA model (BA-Calc 2007 1.0.0, KFDA, Seoul, Korea). **Results** Mortality: None. Body weight: The average weight-gain was 431g in the control group, 416g in low-dose treated group, and 599g in high-dose treated group (N=4) Serum Kinetics The AUC(last) was 3.65±0.68 μg•day/ml in 5 mg/kg-treated group and 0.90±0.16 μg•day/ml in 0.5 mg/kg-treated group, respectively. The T1/2 was 11.7±1.3 day in high-dose treated group and 16.3±2.9 day in low-dose treated group, respectively. Blood:	

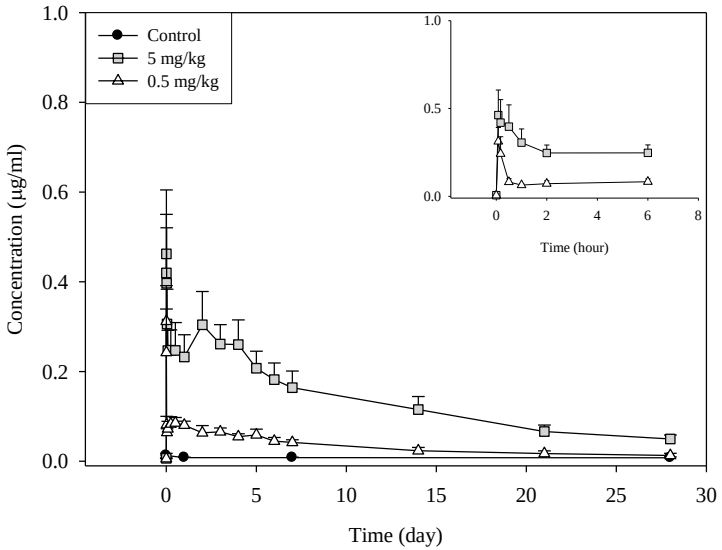
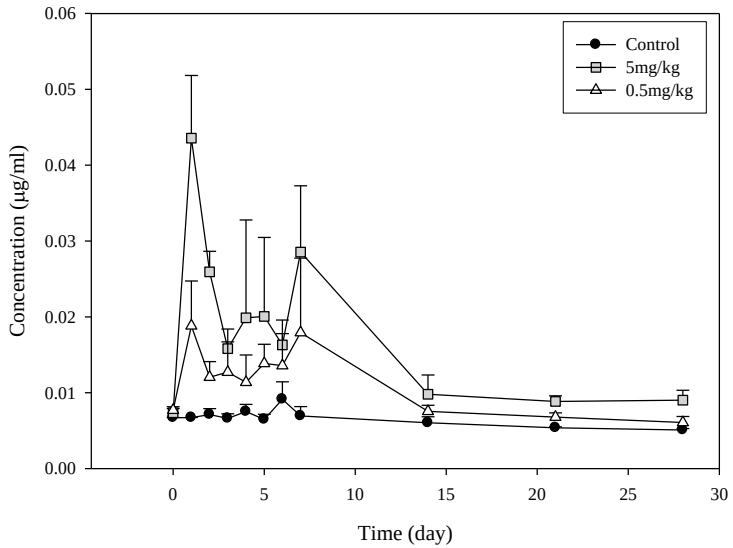


Fig.2 Concentration-time curves of silver in serum of New Zealand White rabbits for 28 days after a single intravenous of AgNPs injection

Urine:

Fig. 3 Concentration-time curves of silver in urine of New Zealand White rabbits for 28 days after a single intravenous of AgNPs



Feces:

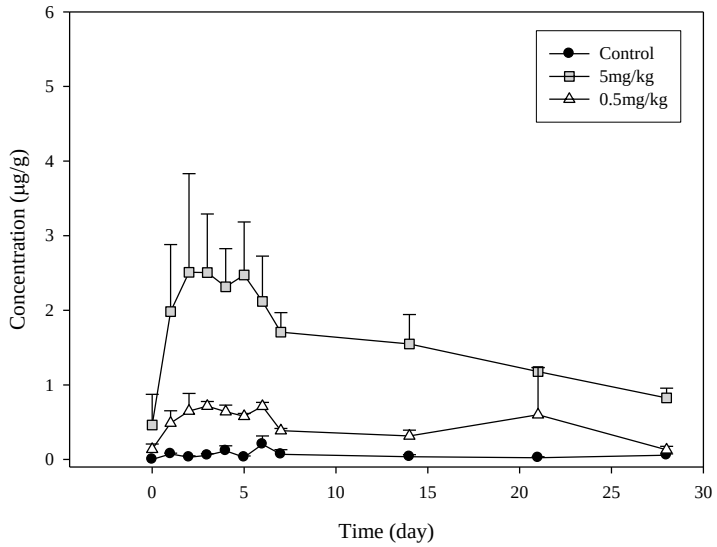


Fig. 4 Concentration-time curves of silver in feces of New Zealand White rabbits for 28 days after a single intravenous injection of AgNPs

Tissue:

Table 1. Silver concentration in organs of rabbits sacrificed at 1d, 7d, and 28d after a single intravenous injection of AgNPs

Dose	Days	Liver	Kidney	Spleen	Lung	Brain	Testis	Thymus
Low (0.5 mg/kg)	1day	0.771±0.250	0.382±0.134	0.387±0.191	0.173±0.152	0.070±0.022	0.147±0.036	0.044±0.011
	7day	1.102±0.214	0.031±0.004	0.534±0.181	0.040±0.005	0.075±0.055	0.127±0.024	0.027±0.012
	28day	0.532±0.061	0.018±0.005	0.305±0.187	0.018±0.003	0.040±0.002	0.035±0.011	0.012±0.001
High (5 mg/kg)	1day	1.017±0.170	0.555±0.221	0.631±0.026	0.333±0.157	0.130±0.040	0.579±0.201	0.228±0.089
	7day	1.241±0.680	0.662±0.250	0.937±0.239	0.351±0.149	0.405±0.292	0.452±0.104	0.146±0.064
	28day	1.120±0.120	0.622±0.113	0.670±0.086	0.398±0.190	0.222±0.055	0.385±0.156	0.406±0.179

Hematological data:

Table 3. Hematological data at 1 day after a single intravenous injection of AgNPs

Tests	Units	Groups		
		Control (0 mg/kg)	High (5 mg/kg)	Low (0.5 mg/kg)
RBC	(10^6/μL)	6.5±0.5[a)]	4.9±0.4**	4.7±0.4**
HCT	(%)	41.4±3.7	32.6±2.7**	30.8±2.7**
HGB	(g/dL)	12.8±0.8	10.2±0.9**	9.6±0.8**
MCV	(fL)	63.4±2.5	66.4±1.5	65.8±1.3
MCH	(pg)	19.7±0.6	20.7±0.8	20.6±0.6
MCHC	(g/dL)	31.0±0.9	31.1±0.7	31.3±0.5

RDW	(%)	13.6±0.4	13.4±1.5	14.1±0.4
HDW	(g/dL)	2.1±0.1	2.3±0.2	2.3±0.2
PLT	(10^3/μL)	485.5±102.4	365.3±25.9	393.0±74.5
MPV	(fL)	6.4±0.2	7.1±0.3**	7.0±0.2**
WBC	(10^3/μL)	7.5±2.8	8.6±1.2	6.9±1.3
NEU	(%)	27.9±9.5	37.4±4.4	29.1±14.3
LYM	(%)	62.2±10.5	53.6±5.4	63.2±13.6
MONO	(%)	5.0±1.7	2.8±1.8	4.0±2.2
EOS	(%)	1.7±1.4	2.9±0.4	1.0±0.5
BASO	(%)	2.5±0.4	2.9±0.6	2.4±0.3
LUC	(%)	0.7±0.4	0.5±0.2	0.4±0.1
N		4[b)]	4	4

**: Represents a significant difference at $P<0.01$ level compared with the Vehicle control.

a) Data are expressed as Mean ± S.D.

b) Number of animals.

Serum biochemistry:

Table 4. Clinical biochemistry data at 1 day after a single intravenous injection of AgNPs

Tests	Units	Groups		
		Control (0 mg/kg)	High (5 mg/kg)	Low (0.5 mg/kg)
AST	(IU/L)	47.4±22.5[a)]	34.5±16.4	27.3±7.1
ALT	(IU/L)	74.2±30.2	49.3±18.7	49.3±11.1
ALP	(IU/L)	158.5±26.5	139.5±56.9	148.8±29.7

CPK	(IU/L)	1289.8±963.2	2215.5±1882.6	5155.0±3628.1
TBIL	(mg/dL)	0.2±0.0	0.2±0.0	0.1±0.1
GLU	(mg/dL)	135.9±4.5	130.8±3.1	140.3±1.7
TCHO	(mg/dL)	57.0±12.5	60.0±9.6	40.0±0.0*
TG	(mg/dL)	101.3±36.1	96.0±55.3	157.5±54.4
TP	(g/dL)	6.1±0.6	5.2±0.2*	5.2±0.4*
ALB	(g/dL)	4.0±0.3	3.4±0.0	2.9±0.3
BUN	(mg/dL)	19.4±1.3	15.4±1.8	16.3±3.0
CRE	(mg/dL)	1.0±0.2	0.9±0.2	0.8±0.1
IP	(mg/dL)	6.7±0.2	6.0±0.6	6.2±0.8
Ca^{2+}	(mg/dL)	13.4±0.3	11.7±0.2*	11.9±0.2*
Na^{+}	(mmol/L)	143.1±3.5	141.7±2.1	140.5±1.2
K^{+}	(mmol/L)	5.3±1.0	4.7±0.4	5.6±0.3
Cl^{-}	(mmol/L)	102.3±2.6	106.9±2.0*	103.6±1.9
N		4[b)]	4	4

***/** Represents a significant difference at *P*<0.05 / *P*<0.01 levels compared with the vehicle control.**
a) Data are expressed as Mean ± S.D.
b) Number of animals

Histopathological analysis:

Table 5. Individual histopathological findings at 1d, 7d, and 28 d after a single intravenous injection of AgNPs (5 mg/kg)

Group	Control (0 mg/kg)	High (5 mg/kg)											
	28 day	1day				7day				28day			
Testis													
Degeneration, germ cell	2	1	1	1	1	1	1	1	1	2	1	1	1
Kidney	N			N					N	N			N
Inflammatory cell infiltration	-	1	1	-	1	1	1	1	-	-	1	1	-
Liver	N												
Necrosis, multifocal	-	1	-	-	-	-	-	-	-	-	-	-	-
Infiltration, mixed cell	-	1	1	1	1	1	2	1	1	2	1	1	1
Pigmentation	-	1	-	-	-	1	1	1	1	1	1	1	1
Spleen	N		N		N	N		N	N	N	N	N	
Agonal congestion/hemorrhage	-	-	-	P	-	-	P	-	-	-	-	-	-
Hematopoiesis extramedullary, - erythropoiesis	-	1	-	-	-	-	-	-	-	-	-	-	1
Thymus	N	N	N	N	N	N	N	N	N	N	N	N	N
Lung						N			N	N	N		N
Accumulation, foamy macrophages	-	1	1	-	-	-	-	1	-	-	-	1	-
Infiltration, mononuclear cell	-	1	-	1	1	-	1	1	-	-	-	1	-
Inflammatory cell infiltration	1	-	1	-	-	-	1	-	-	-	-	-	-
Hyperplasia, alveolar wall	-	-	-	-	-	-	-	1	-	-	-	-	-
Brain	N	N	N	N	N	N	N	N	N	N	N	N	N
Cerebrum	-	-	-	-	-	-	-	-	-	-	-	-	-
Diencephalon	-	-	-	-	-	-	-	-	-	-	-	-	-
Cerebellum	-	-	-	-	-	-	-	-	-	-	-	-	-
Medulla oblongata	-	-	-	-	-	-	-	-	-	-	-	-	-

"1"=Minimal; "2"=Slight; "3"=Moderate; "N"=Normal; "-"=No finding; "P"=Present

Conclusions

AgNPs were easily accumulated in the body and retained for a long time after a single intravenous injection in rabbits, although a decline in blood concentration was observed. The half-time of serum kinetic were 11.7±1.3 days in 5 mg/kg-treated group, and 16.3±2.9 days in 0.5 mg/kg-treated group,

respectively. The half-time of release of silver from organs into blood could not be calculated because more than 50% of the AgNPs was still retained in all the tested organs until the final day of 28 days. Excretion through feces after injection supports the idea of biliary excretion of AgNPs, which needs further investigation on the detailed mechanisms of AgNPs excretion. **Reference** National Institute of Environmental Research (NIER), Korea, 2011. Toxicokinetics study of silver nanoparticles in rabbit, tested by ChemOn	

Applicant's summary and conclusion

Interpretation of results

other: bioaccumulation potential based on study results

Conclusions

AgNPs were easily accumulated in the body and retained for a long time after a single intravenous injection in rabbits, although a decline in blood concentration was observed. The half-time of serum kinetic were 11.7 ± 1.3 days in 5 mg/kg bw-treated group, and 16.3 ± 2.9 days in 0.5 mg/kg bw-treated group, respectively. The half-time of release of silver from organs into blood could not be calculated because more than 50% of the AgNPs was still retained in all the tested organs until the final day of 28 days. Excretion through feces after injection supports the idea of biliary excretion of AgNPs, which needs further investigation on the detailed mechanisms of AgNPs excretion.

Executive summary

In a toxicokinetics study, conducted according to OECD Guideline 417 and in compliance with GLP, Citrate capped silver nanoparticle (cAgNPs) was administered to New Zealand White rabbits (4 males/dose) at 0.5 and 5 mg/kg bw as single dose via intravenous injection. Blood sampling was performed from ear vein of treated animals at 0 min (before treatment), 5 min, 10 min, 30 min, 1, 2, 6 and 12 h, 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days, respectively. Blood samples were also collected from non-treated control group at 1, 7 and 28 days. Faeces and urine were collected for 24 h at 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days after treatment. Faeces were homogenized with 10 volume of deionized water, and a part was taken and frozen at -80 °C for subsequent analysis. Collected urine was also homogenized, and a part was taken and frozen for subsequent analysis. Tissues (liver, kidney, spleen, lung, brain, testis, and thymus) from 4 rabbits of a treated group were obtained for AgNPs distribution analysis at 1, 7 and 28 days after treatment. Bioavailability, tissue distribution and excretion ratios were calculated.
No mortality was observed. AgNPs were easily accumulated in the body and retained for a long time after a single intravenous injection in rabbits, although a decline in blood concentration was observed. The AUC(last) was 0.90 ± 0.16 and 3.65 ± 0.68 µg•day/mL in 0.5 and 5 mg/kg bw-treated group, respectively. The t1/2 was 16.3 ± 2.9 days and 11.7 ± 1.3 days in 0.5 and 5 mg/kg bw-treated group, respectively. The half-time of release of silver from organs into blood could not be calculated because more than 50 % of the AgNPs was still retained in all the tested organs until the final day of 28 days. Excretion through feces after injection supports the idea of biliary excretion of AgNPs, which needs further investigation on the detailed mechanisms of AgNPs excretion.
AgNPs were easily accumulated in the body and retained for a long time after a single intravenous injection in rabbits, although a decline in blood concentration was observed.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Basic toxicokinetics, Park, 2011, RS, K

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	2009
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	GLP study conducted according to OECD 417 Guideline with deviations: details on test item, acclimation, housing and feeding conditions 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	2009	The Hazardous Profile of Silver Nanoparticles.				National Institute of Environmental Research (NIER), Korea		
publication	Park K, Park EJ, Chun IK, Choi K, Lee SH, Yoon J and Lee BC.	2011	Bioavailability and Toxicokinetics of Citrate-Coated Silver Nanoparticles in Rats.	Arch Pharm Res. 34(1): 153-158.					

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 method

in vivo

Objective of study

toxicokinetics

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 417 (Toxicokinetics)	yes (details on test item, acclimation, housing and feeding conditions not reported)

Principles of method if other than guideline

Not applicable

GLP compliance

yes (A part of experiments such as animal treatment and bio-sampling were performed under GLP.)

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): Citrate capped silver nanoparticle (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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: 6 weeks
- Weight at study initiation: Approximately 250 g

Administration / exposure

Route of administration

other: oral gavage (p.o.) or intravenous injection (i.v.)

Vehicle

other: deionised water

Details on exposure

No data

Duration and frequency of treatment / exposure

Single dose

Doses / concentrations

1 and 10 mg/kg bw

No. of animals per sex per dose

Four rats per dose. In total, thirty two rats were used for whole experiments.

Control animals

no data

Positive control

Not applicable

Details on study design

No data

Details on dosing and sampling

PHARMACOKINETIC STUDY (Absorption, distribution, excretion)
- Tissues and body fluids sampled: Blood and tissues (liver, lung, and kidney)
- Time and frequency of sampling:Blood was collected at 10 min, 1, 2, 4, 8, 24, 48 and 96 h after a single treatment.Liver, lung, and kidney were obtained at 24 and 96 h after treatment.
- Excretion Routes: Urine and feces
- Pharmacokinetic analysis: Bioavailability, tissue distribution and excretion ratios were calculated using BA Calc 2006 computer software (Ver. 1.0.0.; Korea Food and Drug Administration).
- Chemical analysis: Samples (about 200 mg)were digested in a mixed solution of 7 mL of 70 % HNO3 and 1 mL of 30 % H2O2 using a microwave digestion system, and subjected to inductively coupled plasma-mass spectrometry (ICP-MS) for analysis.

Statistics

One way analysis of variance (ANOVA) test

Any other information on materials and methods incl. tables

None

Results and discussions

Preliminary studies

Not applicable

Main ADME results

Type	
absorption	absorption rates of cAgNPs through gastrointestinal tract were very low bioavailability is 1.2 % (1 mg/kg bw) and 4.2 % (10 mg/kg bw). Most of orally administered cAgNPs were found in feces, suggesting a poor absorption rate.

Metabolite characterisation studies

Details on metabolites

Not applicable

Bioaccessibility

Bioaccessibility testing results

Not applicable

Any other information on results incl. tables

- Oral bioavailability: 1.2 % (1 mg/kg bw) or 4.2 % (10 mg/kg bw).
- AUC(last), AUC(inf), Cmax, Tmax, and T1/2: Please refer to figure and tables in the attached document.
- Liver was found to be the main organ where absorbed silver nanoparticles were accumulated.
- Most of orally administered silver nanoparticles were excreted via feces.

Overall remarks, attachments

Remarks on results including tables and figures

None

Attached background material

Attached document	Remarks
Toxicokinetic parameters.pdf / 91.55 KB (application/octet-stream) **Test Substances** Citrate capped silver nanoparticles (cAgNPs), CAS No. 7440-22-4 (silver) Remarks: Manufactured by ABC Nanotech Co., Ltd. (Korea) **Methods** Methods/guideline followed: OECD Test Guideline 417, "Toxicokinetics" Type: *In vivo* GLP: A part of experiments such as animal treatment and bio-sampling were performed under GLP. Year (study performed): 2009 **Test Conditions** Species: Rat Strain: Sprague-Dawley Sex: Male Cell Type: Not applicable Age: Six weeks old Body weight: Approximately 250 g (standard deviation was not presented) Number of Animals: Four rats per dose. In total, thirty two rats were used for whole experiments. Route: Oral gavage (p.o.) or intravenous injection (i.v.) Vehicle: Deionised water Dose(s) used: 1 or 10 mg/kg body weight, single dose Statistical Methods: One way analysis of variance (ANOVA) test Actual Dose(s): Not applicable Excretion Routes: Urine and feces	

Body Fluids Sampled: Blood was collected at 10 min, 1, 2, 4, 8, 24, 48 and 96 hrs after a single treatment.
Tissues Sampled: Liver, lung, and kidney were obtained at 24 and 96 hrs after treatment.
Metabolites: Not applicable
Metabolites CAS: Not applicable
Chemical analysis: Samples (about 200 mg)were digested in a mixed solution of 7 mL of 70% HNO_3 and 1 mL of 30% H_2O_2 using a microwave digestion system, and subjected to inductively coupled plasma-mass spectrometry (ICP-MS) for analysis.
Pharmacokinetic analysis: Bioavailability, tissue distribution and excretion ratios were calculated using BA Calc 2006 computer software (Ver. 1.0.0.; Korea Food and Drug Administration).

Results

Oral bioavailability: 1.2 % (1 mg/kg) or 4.2% (10 mg/kg).
$AUC_{(last)}$, $AUC_{(inf)}$, C_{max}, T_{max}, and $T_{1/2}$: Please refer to Fig. 5.1 and Table 5.1.
Liver was found to be the main organ where absorbed silver nanoparticles were accumulated.
Most of orally administered silver nanoparticles were excreted via feces (Table 5.3.).

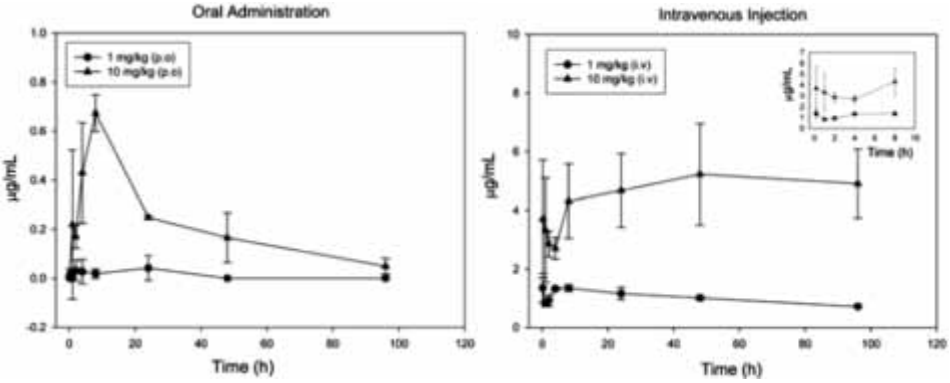


Fig.5.1. Blood concentrations of cAgNPs in rats after a single oral (left) or intravenous (right) administration (AgNPs were measured as total silver concentration by ICP-MS. Rats were treated with AgNPs (1 mg/kg and 10 mg/kg) by a single oral administration (left) or intravenous injection (right). Rats were sacrificed at the designated time (10 min, 1, 2, 4, 8, 24, 48, and 96 h, n = 4). Control animals were sacrificed after vehicle treatment (designated as time 0 h*).*

Table 5.1. Toxicokinetic parameters of cAgNPs in rats

group	$AUC_{(last)}$ (min · μg/mL)	$AUC_{(inf)}$ (min · μg/mL)	C_{max} (μg/mL)	T_{max} (min)	$T_{1/2}$ (min)
1 mg/kg (i.v)	5791.6	11944.3	1.351	10	5948
10 mg/kg (i.v)	27569.7	-	5.223	2880	-
1 mg/kg (p.o)	69.7	-	0.083	1440	-
10 mg/kg (p.o)	1166.0	1294.2	0.673	480	1813

Table 5.2. Excretion of cAgNPs through urine and feces

group	treated group (24 h)	
	urine (μg/mL)	feces (μg/g)
1 mg/kg (i.v)	0.007 ± 0.013	1.5 ± 2.3
10 mg/kg (i.v)	0.018 ± 0.311	85.2 ± 55.1**
1 mg/kg (p.o)	0.003 ± 0.005	377.6 ± 173.8**
10 mg/kg (p.o)	0.042 ± 0.031	1663.1 ± 522.2**

*$p < 0.05$, **$p < 0.01$.

Conclusions

The absorption rates of cAgNPs through gastrointestinal tract were very low– bioavailability is 1.2% (1 mg/kg) and 4.2% (10 mg/kg). Most of orally administered cAgNPs were found in feces, suggesting a poor absorption rate.

Reference

National Institute of Environmental Research (NIER), Korea, 2009. The Hazardous profile of silver nanoparticles

Applicant's summary and conclusion

Interpretation of results

other: poor oral absorption

Conclusions

The absorption rates of cAgNPs through gastrointestinal tract were very low- bioavailability was 1.2 % (1 mg/kg bw) and 4.2 % (10 mg/kg bw). Most of orally administered cAgNPs were found in feces, suggesting a poor absorption rate.

Executive summary

In a toxicokinetics study, conducted according to OECD Guideline 417 and in compliance with GLP, Citrate capped silver nanoparticle (cAgNPs) was administered to Sprague-Dawley rats (4 males/dose) at 1 and 10 mg/kg bw as single dose via oral gavage (p.o) or intravenous injection (i.v). Blood was collected at 10 min, 1, 2, 4, 8, 24, 48 and 96 h after a single treatment. Liver, lung, and kidney were obtained at 24 and 96 h after treatment.Excretion ofcAgNPsvia feces and urine was determined at 24 h after treatment.Control animals were sacrificed after vehicle treatment (at time 0 h). Bioavailability, tissue distribution and excretion ratios were calculated.

Oral bioavailability: 1.2 % (1 mg/kg bw) or 4.2 % (10 mg/kg bw)
AUC(last), AUC(inf), Cmax, Tmax, and T1/2:
At 1 mg/kg bw (i.v): 5791.6 min. μg/mL, 11944.3 min. μg/mL, 1.351 μg/mL, 10 min and 5948 min, respectively
At 10 mg/kg bw (p.o): 1166.0 min. μg/mL, 1294.2 min. μg/mL, 0.673 μg/mL, 480 min and 1813 min, respectively

AUC(last), Cmax and Tmax:
At 1 mg/kg bw (p.o): 69.7 min. µg/mL, 0.083 µg/mL and 1440 min, respectively
At 10 mg/kg bw (i.v): 27569.7 min. µg/mL, 5.223 µg/mL and 2880 min, respectively
Liver was found to be the main organ where absorbed silver nanoparticles were accumulated. Most of orally administered silver nanoparticles were excreted via feces.
The absorption rates of cAgNPs through gastrointestinal tract were very low- bioavailability was 1.2 % (1 mg/kg bw) and 4.2 % (10 mg/kg bw). Most of orally administered cAgNPs were found in feces, suggesting a poor absorption rate.

Cross-reference to other study

No cross-reference

7.2 Acute Toxicity

7.2.1 Acute toxicity: oral

Endpoint study record: 7440-22-4, Acute toxicity-oral (non-GLP), Anonymous, 2010, 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	Non-GLP study conducted according to OECD 423 Guideline with deviations: details on test item, body weight, housing and feeding conditions, details of clinical observations, necropsy findings not reported and 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	2010	Studies on Hazardous Properties of Manufactured Silver Nanoparticles			NIER No. 2010-49-1224	National Institute of Environmental Research, 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

acute toxic class method

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	yes (details on test item, body weight, housing and feeding conditions, details of clinical observations, necropsy findings not reported and 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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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

oral: gavage

Vehicle

other: Deionised water

Details on oral exposure

VEHICLE
- Concentration in vehicle: 30 and 200 mg/mL
- Amount of vehicle (if gavage): 10 mL/kg bw
MAXIMUM DOSE VOLUME APPLIED: 10 mL/kg bw
CLASS METHOD (if applicable)
- Rationale for the dose selection: A starting dose was 300 mg/kg bw, resulting in no observable symptom. Therefore, the dose was increased up to 2000 mg/kg bw.

Doses

300 and 2000 mg/kg bw

No. of animals per sex per dose

6 males/dose
- Experiments were performed twice and three animals were used for each experiment

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Necropsy of survivors performed: No
- Other examinations performed: No

Statistics

No data

Any other information on materials and methods incl. tables

None

Results and discussions

Preliminary study (if fixed dose study)

Not applicable

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male	LD50	> 2000 mg/kg bw	test mat.		Mortality or clinical sign was not observed

Mortality

No mortality was observed.

Clinical signs

No clinical signs were observed.

Body weight

No data

Gross pathology

Not determined

Other findings

None

Any other information on results incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

cAgNPs tested can be classified as GHS category 5 which are of relatively low acute toxicity.

Applicant's summary and conclusion

Interpretation of results

other: no data

Criteria used for interpretation of results

not specified

Conclusions

Single oral administration of cAgNPs did not induce any signs of mortality up to 2000 mg/kg bw.

Executive summary

In an acute oral toxicity study performed according to OECD Guideline 423, groups (6 male rats/dose) of Sprague Dawley rats were given a single oral (gavage) dose of Citrate capped silver nanoparticles (cAgNPs) at 300 and 2000 mg/kg bw. Animals were then observed for mortality and clinical signs for 14 days.
No mortality or clinical signs were observed. In this study, the oral LD50 of test item was considered to be higher than 2000 mg/kg bw in male rats.

Under the test conditions, the oral LD50 for cAgNPs is higher than 2000 mg/kg bw in male rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Acute toxicity-oral, Anonymous, 2010, 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	GLP study conducted according to OECD 423 Guideline with deviations: details on test item, body weight, housing and feeding conditions, details of clinical observations, necropsy findings not reported and 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	2010	Acute Oral Toxicity Study of Silver Nanoparticles in Rats		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy(MKE), 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

acute toxic class method

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 423 (Acute Oral toxicity - Acute Toxic Class Method)	yes (details on test item, body weight, housing and feeding conditions, details of clinical observations, necropsy findings not reported and individual and summary tables of results not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

Confidential details on test material

No data

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: 8-9 weeks

Administration / exposure

Route of administration

oral: gavage

Vehicle

other: 1.0 % citrate solution

Details on oral exposure

VEHICLE
- Concentration in vehicle: 30 and 200 mg/mL
- Amount of vehicle (if gavage): 10 mL/kg bw
MAXIMUM DOSE VOLUME APPLIED: 10 mL/kg bw
DOSAGE PREPARATION:
Vehicle was added and mixed with 1.46 or 9.77 mL of the test substances (20.48 %) to make 10 mL for the treatment of 300 and 2000 mg/kg bw-treated group, respectively.
CLASS METHOD (if applicable)
- Rationale for the dose selection: A starting dose was 300 mg/kg bw, resulting in no observable symptom. Therefore, the dose was increased up to 2000 mg/kg bw.

Doses

300 and 2000 mg/kg bw

No. of animals per sex per dose

6 females/dose

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Necropsy of survivors performed: No
- Other examinations performed: No

Statistics

No data

Any other information on materials and methods incl. tables

Table 7.2.1/1: Degree of macro dispersion of silver nanomaterial in 1 % citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824± 0.014	< 70.592± 0.362
5	> 4	3.021± 0.002	< 75.533± 0.038
2.5	> 4	2.137± 0.004	< 53.467± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Preliminary study (if fixed dose study)

Not applicable

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD50	> 2000 mg/kg bw	test mat.		Mortality or clinical sign was not observed

Mortality

No mortality was observed.

Clinical signs

No clinical signs were observed.

Body weight

No data

Gross pathology

Not determined

Other findings

None

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: no data

Criteria used for interpretation of results

not specified

Conclusions

Single oral administration of cAgNPs did not induce any signs of toxicity up to 2000 mg/kg bw.

Executive summary

In an acute oral toxicity study performed according to OECD Guideline 423 and in compliance with GLP, groups (6 female rats/dose) of Sprague Dawley rats were given a single oral (gavage) dose of Citrate capped silver nanoparticles (cAgNPs) at 300 and 2000 mg/kg bw. Animals were then observed for mortality and clinical signs for 14 days.
No mortality or clinical signs were observed. In this study, the oral LD50 of test item was considered to be higher than 2000 mg/kg bw in female rats.

Under the test conditions, the oral LD50 for cAgNPs is higher than 2000 mg/kg bw in female rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

7.2.2 Acute toxicity: inhalation

7.2.3 Acute toxicity: dermal

Endpoint study record: 7440-22-4, Acute toxicity-dermal, Kim, 2013, 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	GLP study conducted according to OECD 402 Guideline with deviations: details on test item, body weight, housing and feeding conditions, necropsy findings not reported and 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	2010	Acute Dermal Toxicity Study of Silver Nanoparticles in Rats		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE)		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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

standard acute method

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 402 (Acute Dermal Toxicity)	yes (details on test item, body weight, housing and feeding conditions, necropsy findings not reported and individual and summary tables of results not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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: 8 weeks for male and 11 weeks for female

Administration / exposure

Type of coverage

no data

Vehicle

other: 1.0 % citrate solution

Details on dermal exposure

No data

Duration of exposure

24 h

Doses

2000 mg/kg bw

No. of animals per sex per dose

5

Control animals

yes

Details on study design

- Duration of observation period following administration: 14 days

Statistics

None

Any other information on materials and methods incl. tables

For treatment, vehicle was added and mixed with 29.30 mL of test Substances to make 30 mL. Volume administered or concentration: 10 mL/kg bw

Table 7.2.3/1: Degree of macro dispersion of silver nanomaterial in 1 % citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A**: Absorbance units of dispersed Silver nanoparticle**
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Preliminary study (if fixed dose study)

Not applicable

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD50	> 2000 mg/kg bw	test mat.		Mortality or clinical sign was not observed.

Mortality

No mortality was observed.

Clinical signs

No clinical signs were observed.

Body weight

No data

Gross pathology

No data

Other findings

None

Any other information on results incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

Although the dose tested was specified as 2000 mg/kg bw, 2400 mg/kg bw was administered. It is because the specific gravity of 1.2 was not considered in preparation for the test material.

Applicant's summary and conclusion

Interpretation of results

other: no data

Criteria used for interpretation of results

not specified

Conclusions

The acute dermal exposure to cAgNPs did not induce any sign of toxicity up to 2000 mg/kg bw.

Executive summary

In an acute dermal toxicity study (limit test) performed according to OECD Guideline 402 and in compliance with GLP, a group of Sprague Dawley rats (5/sex/dose) were given a single dermal application of Citrate capped silver nanoparticles (cAgNPs) at 2000 mg/kg bw for 24 h.
Animals were then observed for mortality and clinical signs for 14 days.
No mortality or clinical signs were observed. In this study, the combined dermal LD50 of cAgNPs was considered to be higher than 2000 mg/kg bw in rats.

Under the test conditions, the acute dermal LD50 of cAgNPs is higher than 2000 mg/kg bw in rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Acute toxicity-dermal, Kim, 2013, 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	GLP study conducted according to OECD 402 Guideline with deviations: details on test item, body weight, housing and feeding conditions, necropsy findings not reported and 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	2010	Acute Dermal Toxicity Study of Silver Nanoparticles in Rats		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE)		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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

standard acute method

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 402 (Acute Dermal Toxicity)	yes (details on test item, body weight, housing and feeding conditions, necropsy findings not reported and individual and summary tables of results not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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: 8 weeks for male and 11 weeks for female

Administration / exposure

Type of coverage

no data

Vehicle

other: 1.0 % citrate solution

Details on dermal exposure

No data

Duration of exposure

24 h

Doses

2000 mg/kg bw

No. of animals per sex per dose

5

Control animals

yes

Details on study design

- Duration of observation period following administration: 14 days

Statistics

None

Any other information on materials and methods incl. tables

For treatment, vehicle was added and mixed with 29.30 mL of test Substances to make 30 mL. Volume administered or concentration: 10 mL/kg bw

Table 7.2.3/1: Degree of macro dispersion of silver nanomaterial in 1 % citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Preliminary study (if fixed dose study)

Not applicable

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD50	> 2000 mg/kg bw	test mat.		Mortality or clinical sign was not observed.

Mortality

No mortality was observed.

Clinical signs

No clinical signs were observed.

Body weight

No data

Gross pathology

No data

Other findings

None

Any other information on results incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

Although the dose tested was specified as 2000 mg/kg bw, 2400 mg/kg bw was administered. It is because the specific gravity of 1.2 was not considered in preparation for the test material.

Applicant's summary and conclusion

Interpretation of results

other: no data

Criteria used for interpretation of results

not specified

Conclusions

The acute dermal exposure to cAgNPs did not induce any sign of toxicity up to 2000 mg/kg bw.

Executive summary

In an acute dermal toxicity study (limit test) performed according to OECD Guideline 402 and in compliance with GLP, a group of Sprague Dawley rats (5/sex/dose) were given a single dermal application of Citrate capped silver nanoparticles (cAgNPs) at 2000 mg/kg bw for 24 h. Animals were then observed for mortality and clinical signs for 14 days.
No mortality or clinical signs were observed. In this study, the combined dermal LD50 of cAgNPs was considered to be higher than 2000 mg/kg bw in rats.

Under the test conditions, the acute dermal LD50 of cAgNPs is higher than 2000 mg/kg bw in rats therefore it is not classified according to the Annex VI to the Directive 67/548/EEC and the CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

7.2.4 Acute toxicity: other routes

7.3 Irritation / corrosion

7.3.1 Skin irritation / corrosion

Endpoint study record: 7440-22-4, Skin irritation-corrosion, 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	GLP study conducted according to OECD 404 Guideline with deviations: details on test item, age at start of study, body weight, housing and feeding conditions, tabulation of irritation response 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	Skin Irritation/Corrosion Study of Silver Nanoparticles in Rabbits		KTR(Korea Testing & Research Institute)		National Institute of Environmental Research (NIER), 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

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	yes (details on test item, age at start of study, body weight, housing and feeding conditions, tabulation of irritation response not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea
- pH: Not determined

Confidential details on test material

No data

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

No data

Test system

Type of coverage

no data

Preparation of test site

shaved

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.5 mL - Concentration (if solution): Undiluted

Duration of treatment / exposure

4 h

Observation period

Erythema, eschar formation and oedema formation were assessed 1, 24, 48, 72 h and 14 days after

application.

Number of animals

3 females

Control animals

yes

Details on study design

TEST SITE
- Area of exposure: 0.5 mL/6 cm2 skin - A small area, approximately 6 cm2, on the back was shaved 24 h before testing. The Test substance was applied to a gauze patch, and, then to the skin.
REMOVAL OF TEST SUBSTANCE
- Washing (if done): No data
SCORING SYSTEM: According to OECD Test Guideline 404

Any other information on materials and methods incl. tables

None

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
primary dermal irritation index (PDII)	mean	24, 48 and 72 h	0		other: not applicable	

Irritant/corrosive response data

Erythema, eschar formation or oedema formation was not observed in all the treated animals.

Other effects

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

Interpretation of results

not irritating

Criteria used for interpretation of results

not specified

Conclusions

cAgNPs were not an irritant to skin of New Zealand white rabbit.

Executive summary

In a primary dermal irritation study performed according to OECD Guideline 404 and in compliance with GLP, three female New Zealand White rabbits were dermally exposed to 0 or 0.5 mL/6 cm^2skin of Citrate capped silver nanoparticles (cAgNPs) for 4 h. Erythema and eschar formation and oedema formation were assessed 1, 24, 48 and 72 h after application.
Erythema, eschar formation or oedema formation was not observed in all the treated animals. The primary irritation index was 0.0. In this study, cAgNPs is not a skin irritant on female rabbits.
Under the test conditions, cAgNPs is not classified as irritating to skin of rabbits according to the criteria of Annex VI to the Directive 67/548/EEC and CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Skin irritation-corrosion, Kim, 2013, 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	GLP study conducted according to OECD 404 Guideline with deviations: details on test item, age at start of study, body weight, housing and feeding conditions, tabulation of irritation response 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	2010	Acute Skin Irritation/Corrosion Study of Silver Nanoparticles in Rabbits		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE), Korea		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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 method

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	yes (details on test item, age at start of study, body weight, housing and feeding conditions, tabulation of irritation response not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea
- pH: Not determined

Confidential details on test material

No data

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

No data

Test system

Type of coverage

no data

Preparation of test site

shaved

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.5 mL
- Concentration (if solution): Undiluted

Duration of treatment / exposure

4 h

Observation period

Erythema, eschar formation and oedema formation were assessed 1, 24, 48 and 72 h after application.

Number of animals

3 males

Control animals

other: 1.0 % citrate solution was used for negative control

Details on study design

TEST SITE
- Area of exposure: 0.5 mL/6 cm2 skin (=approximately, 0.1 g/6 cm2 skin)
- A small area, approximately 6 cm2, on the back was shaved 24 h before testing. The Test substance was applied to a gauze patch, and, then to the skin. For control group, vehicle, 1.0 % citrate solution was applied to the skin instead of the test material.

REMOVAL OF TEST SUBSTANCE
- Washing (if done): No data

SCORING SYSTEM: According to OECD Test Guideline 404

Any other information on materials and methods incl. tables

Table: 7.3.1/1: Degree of macro dispersion of silver nanomaterial in 1% citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
primary dermal irritation index (PDII)	mean	24, 48 and 72 h	0		other: not applicable	

Irritant/corrosive response data

Erythema, eschar formation or oedema formation was not observed in all the treated animals.

Other effects

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

Interpretation of results

not irritating

Criteria used for interpretation of results

not specified

Conclusions

cAgNPs were not an irritant to skin of New Zealand white rabbit.

Executive summary

In a primary dermal irritation study performed according to OECD Guideline 404 and in compliance with GLP, three male New Zealand White rabbits were dermally exposed to 0.5 mL/6 cm^2skin of Citrate capped silver nanoparticles (cAgNPs) for 4 h. 1.0 % citrate solution was used for negative control. Erythema and eschar formation and oedema formation were assessed 1, 24, 48 and 72 h after application.
Erythema, eschar formation or oedema formation was not observed in all the treated animals. The primary irritation index was 0.0. In this study, cAgNPs is not a skin irritant on male rabbits.
Under the test conditions, cAgNPs is not classified as irritating to skin of rabbits according to the criteria of Annex VI to the Directive 67/548/EEC and CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

7.3.2 Eye irritation

Endpoint study record: 7440-22-4, Eye irritation, Kim, 2013, 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	GLP study conducted according to OECD 405 Guideline with deviations: details on test item, age at start of study, body weight, housing and feeding conditions, tabulation of irritation response 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	2010	Corrosive and Irritations Study on Silver Nanoparticles using Rabbits		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE), Korea		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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 405 (Acute Eye Irritation / Corrosion)	yes (details on test item, age at start of study, body weight, housing and feeding conditions, tabulation of irritation response not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

Confidential details on test material

No data

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

No data

Test system

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.1 mL/eye (=approximately, 0.02 g/eye)

Duration of treatment / exposure

No data

Observation period

1, 24, 48 and 72 h after application

Number of animals

3 males

Control animals

no

Details on study design

SCORING SYSTEM: Ocular reactions of conjunctivae, cornea and iris were scored according to the "GRADING OF OCULAR LESIONS" in OECD TG No. 405.
TOOL USED TO ASSESS SCORE: Hand-slit lamp

Any other information on materials and methods incl. tables

Table: 7.3.2/1: Degree of macro dispersion of silver nanomaterial in 1% citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Overall irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
overall irritation score	mean	24, 48 and 72 h	0		other: not applicable	

Irritant/corrosive response data

Any ocular reaction was not observed in all the animals. Therefore, the index of acute ocular irritation was 0.0.

Other effects

None

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

not irritating

Criteria used for interpretation of results

not specified

Conclusions

cAgNPs were non-irritant to eye of New Zealand white rabbit.

Executive summary

In an eye irritation study conducted according to the OECD Guideline 405 and in compliance with GLP, 3 male rabbits of the New Zealand White strain were exposed to0.1 mL/eye (=approximately, 0.02 g/eye) of Citrate capped silver nanoparticles (cAgNPs)in one eye while the another eye remained untreated and served as control. The eyes were examined and the changes were observed at 1, 24, 48 and 72 h after application andocular reactions of conjunctivae, cornea and iris were scored according to the "grading of ocular lesions" in OECD Guideline 405.
Any ocular reaction was not observed in all the animals. Irritation scores for cornea, iris, conjunctivae and chemosis were 0, 0, 0 and 0, respectively. Therefore, the index of acute ocular irritation was 0.0.In this study, cAgNPs is not an eye irritant on male rabbits.
Under the test conditions, cAgNPs is not classified as irritating to eyes of rabbits according to the criteria of Annex VI to the Directive 67/548/EEC and CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

7.4 Sensitisation

7.4.1 Skin sensitisation

Endpoint study record: 7440-22-4, Skin sensitisation, 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	GLP study conducted according to OECD Guideline 406 with deviations: details on test item, acclimation period, individual weights of animals at the conclusion of the test, housing and feeding conditions 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	Skin Sensitization Study of Silver Nanoparticles in Guinea Pig.		KTR (Korea Testing & Research Institute)		National Institute of Environmental Research (NIER), 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

Type of method

in vivo

Type of study

Guinea pig maximisation test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 406 (Skin Sensitisation)	yes (details on test item, acclimation period, individual weights of animals at the conclusion of the test, housing and feeding conditions not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd

Confidential details on test material

No data

Test animals

Species

guinea pig

Strain

other: CrlOri:HA

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: 5 weeks
- Weight at study initiation: 282.34 - 346.39 g

Test system

Traditional sensitisation test

Route of induction exposure

other: dermal

Route of challenge exposure

other: dermal

Vehicle

other: Induction vehicle: distilled water; Challenge vehicle: 0.1 % 1,chloro-2,4-dinitrobenzene (DNCB)

Concentration

Induction concentration: 20.48 %Challenge concentration: 20.48 %

No. of animals per dose

Ten males for positive control group, ten males for negative control group and twenty males for treatment group.

Details on study design (Traditional tests)

MAIN STUDYA. INDUCTION EXPOSURE (dermal Induction)
- No. of exposures: Three - Exposure period: 6 h
- Test groups: 0.4 mL of test substance used for a 2.5x2.5 cm2 occlusive patch.
- Control group: For negative control, distilled water was applied instead of test material.
- Site: No data
- Frequency of applications: Induction process was repeated a total of two times on the 7th and 14th day after the initial induction.
- Duration: Days 1-7
- Concentrations: 20.48 %B. CHALLENGE EXPOSURE (dermal)
- No. of exposures: One
- Day(s) of challenge: Day 27
- Exposure period: 24 h
- Test groups: 0.4 mL of test substance used for a 2.5x2.5 cm2 occlusive patch.
- Control group: For negative control, 0.1 % (w/v) DNCB was applied instead of test material.
- Site: No data
- Concentrations: 20.48 %
- Evaluation (hr after challenge): 24 and 48 h
- Skin reaction was graded according to "Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions" in OECD TG No. 406.

Challenge controls

0.1 % (w/v) DNCB

Positive control substance(s)

yes (1 % (w/v) DNCB)

LLNA

Any other information on materials and methods incl. tables

None

Results and discussion

Positive control results

1 % (w/v) DNCB induced skin sensitization (100 %).

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
1st reading	24	test group	20.48 %	0	20	No skin reaction was observed in any of the treated groups at 24 h after the challenge
1st reading	48	test group	20.48 %	0	20	No skin reaction was observed in any of the treated groups at 48 h after the challenge
1st reading	24	negative control	0 %	0	10	No skin reaction was observed in control group
1st reading	48	negative control	0 %	0	10	No skin reaction was observed in control group
1st reading	24	positive control	1 % (w/v) DNCB	10	10	Skin reaction was observed in all treated animals at 24 h after the challenge
1st reading	48	positive control	1 % (w/v) DNCB	10	10	Skin reaction was observed in all treated animals at 48 h after the challenge

LLNA

Any other information on results incl. tables

Table 7.4.1/1: Evaluation of skin response

Group	Number of animal	Observation time (h)	Score of skin response				Mean score	Sensitization rate (%)
			0	1	2	3		
G1	20	24	20	0	0	0	0.0	0.0
		48	20	0	0	0	0.0	0.0
G2	10	24	10	0	0	0	0.0	0.0
		48	10	0	0	0	0.0	0.0
G3	10	24	0	3	2	5	2.2	100
		48	0	4	3	3	1.9	100

Overall remarks, attachments

Remarks on results including tables and figures

Grades: 1, weak class
There was no observed any skin reaction in all treated groups at 24 and 48 h after the challenge

Applicant's summary and conclusion

Interpretation of results

other: weak sensitising

Criteria used for interpretation of results

not specified

Conclusions

cAgNPs were a weak sensitizer in Guinea pig.

Executive summary

In a Magnusson & Kligman maximisation study (GPMT) performed according to OECD Guideline 406 and in compliance with GLP, groups of CrlOri:HA guinea pigs (20 males) were induced dermally with 0.4 mL of Citrate capped silver nanoparticles (cAgNPs) in a 2.5x2.5 cm^2occlusive patch. This induction process was repeated a total of two times on the 7th and 14th day after the initial induction. A challenge was performed on the 27th day with a 0.1 % (w/v) DNCB and the test substance. For negative control, however, distilled water was applied while for the positive control, 1 % (w/v) DNCB was applied instead of the test substance. Ten males for positive control group and ten males for negative control group were used. Skin reaction was graded according to "Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions" in OECD TG No. 406.
No skin reaction was observed in any of the treated groups at 24 and 48 h after the challenge. 1 % (w/v) DNCB induced skin sensitization (100 %).
cAgNPs were a weak sensitizer in Guinea pig.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Skin sensitisation, Kim, 2013, 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	GLP study conducted according to OECD Guideline 406 with deviations: details on test item, acclimation period, individual weights of animals at the conclusion of the test, housing and feeding conditions and 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	2010	Skin Sensitization Study of Silver Nanoparticles in Guinea Pig.		KCL(Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE), KOREA		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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 method

in vivo

Type of study

Guinea pig maximisation test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 406 (Skin Sensitisation)	yes (details on test item, acclimation period, individual weights of animals at the conclusion of the test, housing and feeding conditions and positive controls not reported)

Principles of method if other than guideline

Not applicable

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd

Confidential details on test material

No data

Test animals

Species

guinea pig

Strain

Hartley

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: 4.5-5 weeks
- Weight at study initiation: 340.89-365.24 g

Test system

Traditional sensitisation test

Route of induction exposure

other: intradermal (1st Induction), dermal (2nd Induction)

Route of challenge exposure

other: dermal

Vehicle

other: 1.0 % citrate solution

Concentration

Induction concentration: 20.48 %Challenge concentration: 20.48 %

No. of animals per dose

Ten males for control group, and twenty males for treatment group.

Details on study design (Traditional tests)

MAIN STUDYA. INDUCTION EXPOSURE (intradermal (1st Induction))
- No. of exposures: One
- Exposure period: 1 week
- Test groups: Three pairs of intradermal injection of 0.1 mL volume (20.48 %) were given in the shoulder region at 1st induction.
- Control group: For negative control, 1.0 % citrate solution was applied instead of test material.
- Site: Shoulder region
- Frequency of applications: Single application
- Duration: Days 1-7
- Concentrations: 20.48 %

B. INDUCTION EXPOSURE (dermal (2nd Induction))
- No. of exposures: One
- Exposure period: 48 h
- Test groups: The volume of the test substance used for a 2x4 cm2 occlusive patch was 0.5 mL (20.48 %) at 2nd induction phase.
- Control group: For negative control, 1.0 % citrate solution was applied instead of test material.
- Site: Shoulder region
- Frequency of applications: Single application
- Duration: 2 weeks
- Concentrations: 20.48 %

C. CHALLENGE EXPOSURE (dermal)
- No. of exposures: One
- Day(s) of challenge: Week 3

- Exposure period: 24 h
- Test groups: The volume of the test substance used for a 2x4 cm2 occlusive patch was 0.5 mL at challenge phase.
- Control group: For negative control, 1.0 % citrate solution was applied instead of test material.
- Site: Shoulder region
- Concentrations: 20.48 %
- Evaluation (hr after challenge): 24 and 48 h
- Skin reaction was graded according to "Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions" in OECD TG No. 406.

Challenge controls

1.0 % citrate solution

Positive control substance(s)

no data

LLNA

Any other information on materials and methods incl. tables

Table: 7.4.1/1: Degree of macro dispersion of silver nanomaterial in 1% citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussion

Positive control results

No data

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
1st reading	24	test group	20.48 %	1	20	In treated group, 1 animal showed grade 1 erythema at 24 h after the challenge. But, no other skin reaction was observed in the other animals.
1st reading	48	test group	20.48 %	1	20	In treated group, 1 animal showed grade 1 erythema at 48 h after the challenge. But, no other skin reaction was observed in the other animals.
1st reading	24	negative control	0 %	0	10	No skin reaction was observed in control group
1st reading	48	negative control	0 %	0	10	No skin reaction was observed in control group

LLNA

Any other information on results incl. tables

Table 7.4.1/1: Skin sensitization results

Group	**Number of animal**	**Observation time (h)**	**Score of skin response**				**Sensitization rate (%)**
			0	1	2	3	
G1	20	24	19	1	0	0	5.0
		48	19	1	0	0	5.0
G2	10	24	10	0	0	0	0.0
		48	10	0	0	0	0.0

Overall remarks, attachments

Remarks on results including tables and figures

Grades: 1, weak class
Results Remarks: In treated group, 1 animal showed grade 1 erythema at 24 or 48 h after the challenge. But, no other skin reaction was observed in the other animals. One out of twenty tested animals (5%) exhibited grade 1 erythema at 24 or 48 h after challenge, but no other skin reaction was observed in the other animals.

Applicant's summary and conclusion

Interpretation of results

other: weak sensitising

Criteria used for interpretation of results

not specified

Conclusions

cAgNPs were a weak sensitizer in Guinea pig.

Executive summary

In a Magnusson & Kligman maximisation study (GPMT) performed according to OECD Guideline 406 and in compliance with GLP, groups of Hartley guinea pigs (20 males) were induced with three pairs of intradermal injection ofCitrate capped silver nanoparticles (cAgNPs) of0.1 mL volume (20.48 %) were given in the shoulder region at 1st induction.The volume of the test substance used for a 2x4 cm^2occlusive patch was 0.5 mL at 2nd induction and challenge phase. The patch was lasted for 48 h at 2nd induction phase and lasted for 24 h at challenge phase. For negative control (10 males), 1.0 % citrate solution was applied instead of test material. Interval between the first and second induction was 1 week and, after 2 weeks later, challenge was performed. Skin reaction was graded according to "Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions" in OECD TG No. 406.
One out of twenty tested animals (5 %) exhibited grade 1 erythema at 24 or 48 h after challenge, but no other skin reaction was observed in the other animals. In this study, cAgNPs were a weak sensitizer in Guinea pig.
Under these test conditions, cAgNPs are classified as R43 "May cause sensitisation by skin contact" according to the Annex VI to the Directive 67/548/EEC and "Category 1" according to the CLP Regulation (EC) N° (1272-2008).

Cross-reference to other study

No cross-reference

7.5 Repeated dose toxicity

7.5.1 Repeated dose toxicity: oral

Endpoint study record: 7440-22-4, Repeated dose toxicity-oral (28 days), Anonymous, 2010, 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	Non-GLP study conducted according to OECD 407 Guideline with deviations: details on test item, acclimatisation, body weight, housing and feeding conditions, food and water consumption, haematology, histopathology, statistical analysis, individual and summary tables 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	2010	Studies on Hazardous Properties of Manufactured Silver Nanoparticles.		National Institute of Environmental Research (NIER), Korea	NIER No. 2010-49-1224			

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	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	yes (details on test item, acclimatisation, body weight, housing and feeding conditions, food and water consumption, haematology, histopathology, statistical analysis, individual and summary tables 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 capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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

oral: drinking water

Vehicle

other: deionised water

Details on oral exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

28 days

Frequency of treatment

Continuous treatment by drinking water

Doses/concentrations

0, 25, 100 and 400 mg/kg bw/day

Basis nominal in water

No. of animals per sex per dose

5

Control animals

other: Control was provided with normal drinking water without cAgNPs

Details on study design

No data

Positive control

Not applicable

Examinations

Observations and examinations performed and frequency

CLINICAL OBSERVATIONS: No
MORTALITY OBSERVATIONS: Yes
BODY WEIGHT: Yes
FOOD CONSUMPTION AND WATER CONSUMPTION: No
OPHTHALMOSCOPIC EXAMINATION: No
HAEMATOLOGY: No
CLINICAL CHEMISTRY: Yes
- Parameters checked: Serum glutamate pyruvate transaminase (GPT) and blood urea nitrogen (BUN)
URINALYSIS: No
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes, Liver was examined macroscopically
HISTOPATHOLOGY: Not tested

Other examinations

None

Statistics

Statistical analysis was not performed

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/female	no significant toxicity was observed.

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

not examined

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

not examined

Haematology

not examined

Clinical chemistry

yes

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

not examined

Gross pathology

yes

Histopathology: non-neoplastic

not examined

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY
- No significant toxicity was observed.
- No mortality was observed.
BODY WEIGHT AND WEIGHT GAIN
- No significant difference among treated groups was observed.
CLINICAL CHEMISTRY
- Serum GPT was increased while blood urea nitrogen (BUN) was decreased in female rats, which showed dose-dependency.

Any other information on results incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

According to the description, serum glutamate pyruvate transaminase (GPT) was increased while blood urea nitrogen (BUN) was decreased in female rats, which exhibited dose-dependent tendency. However, only average values were presented and no information was obtained regarding deviations of each value. Additionally, statistical analysis was not performed. Therefore, conclusions made in this report are hardly supported by the data presented.

Applicant's summary and conclusion

Conclusions

cAgNPs tested did not induce any toxicity in the repeated dose 28-day oral toxicity study in rodents.

Executive summary

In a repeated dose oral toxicity study conducted according to the OECD Guideline 407, Citrate capped silver nanoparticles (cAgNPs) was administered by oral (via drinking water) to groups of Sprague-Dawley rats (5/sex/dose) at the dose-levels of 0, 25, 100 and 400 mg/kg bw/day for 28 days. Examinations during the study included: mortality, toxic effects, body weight, clinical biochemistry and macroscopic examination.

No significant toxicity or mortality was observed. No significant difference in body weight was observed in any of the dose groups. Serum GPT was increased while blood urea nitrogen (BUN) was decreased in female rats, which showed dose-dependency.

cAgNPs tested did not induce any toxicity in the repeated dose 28-day oral toxicity study in rodents.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Repeated dose toxicity-oral (90 days), Anonymous, 2010, 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	Non-GLP study conducted according to OECD 408 Guideline with deviations: details on test item, acclimatisation, body weight, housing and feeding conditions, food and water consumption, haematology, histopathology, statistical analysis, individual and summary tables 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	2010	Studies on Hazardous Properties of Manufactured Silver Nanoparticles.		National Institute of Environmental Research (NIER), Korea	NIER No. 2010-49-1224			

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 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	yes (details on test item, acclimatisation, body weight, housing and feeding conditions, food and water consumption, haematology, histopathology, statistical analysis, individual and summary tables 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 capped silver nanoparticles (cAgNPs)- Source: ABC Nanotech Co., Ltd., Korea

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

oral: drinking water

Vehicle

other: deionised water

Details on oral exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

90 days

Frequency of treatment

Continuous treatment by drinking water

Doses/concentrations

0, 25, 100 and 400 mg/kg bw/day

Basis nominal in water

No. of animals per sex per dose

10

Control animals

other: Control was provided with normal drinking water without cAgNPs

Details on study design

No data

Positive control

Not applicable

Examinations

Observations and examinations performed and frequency

CLINICAL OBSERVATIONS: No

MORTALITY OBSERVATIONS: Yes
BODY WEIGHT: Yes
FOOD CONSUMPTION AND WATER CONSUMPTION: No
OPHTHALMOSCOPIC EXAMINATION: No
HAEMATOLOGY: No
CLINICAL CHEMISTRY: Yes
- Parameters checked: Serum triglyceride and total bilirubin
URINALYSIS: No
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: No

Other examinations

None

Statistics

Statistical analysis was not performed

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/female	no significant toxicity was observed.

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

not examined

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

not examined

Haematology

not examined

Clinical chemistry

yes

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

not examined

Gross pathology

yes

Histopathology: non-neoplastic

not examined

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY
- No significant toxicity was observed.
- No mortality was observed.
BODY WEIGHT AND WEIGHT GAIN
- No significant difference among treated groups was observed.
CLINICAL CHEMISTRY
- Serum triglyceride (TG) was decreased while total bilirubin levels were increased in treated groups. However, there was no dose-dependent tendency and statistical significance was not tested.

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

cAgNPs tested did not exhibit any toxicity in the repeated dose for a 90-day oral toxicity study in rodents.

Executive summary

In a repeated dose oral toxicity study conducted according to the OECD Guideline 408, Citrate capped silver nanoparticles (cAgNPs) was administered by oral (via drinking water) to groups of Sprague-Dawley rats (10/sex/dose) at the dose-levels of 0, 25, 100 and 400 mg/kg bw/day for 90 days. Examinations during the study included: mortality, toxic effects, body weight, clinical biochemistry and macroscopic examination.

No significant toxicity or mortality was observed. No significant difference in body weight was observed in any of the dose groups. Serum triglyceride (TG) was decreased while total bilirubin levels were increased in treated groups. However, there was no dose-dependent tendency and statistical significance was not tested.

cAgNPs tested did not exhibit any toxicity in the repeated dose for a 90-day oral toxicity study in rodents.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Repeated dose toxicity-oral, Hong, 2014, 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	GLP study conducted according to OECD 422 Guideline with deviations: details on test item, acclimatisation, body weight, housing and feeding conditions 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	No information				National Institute of Environmental Research (NIER), Korea,		
publication	Hong JS, Kim S, Lee SH, Jo E, Lee B, Yoon J, Eom IC, Kim HM, Kim P, Choi K, Lee MY, Seo YR, Kim Y, Lee Y, Choi J and Park K.	2014	Combined Repeated-Dose Toxicity Study of Silver Nanoparticles with the Reproduction/Developmental Toxicity Screening Test.	Nanotoxicology, 8: 349-362.					

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

combined repeated dose and reproduction / developmental screening

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	yes (details on test item, acclimatisation, body weight, housing and feeding conditions not reported)

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): Citrate capped silver nanoparticles (AgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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

oral: unspecified

Vehicle

water

Details on oral exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

42 days

Frequency of treatment

Exposure period: Once a day
Male: 14 days before mating, 14 days during the mating, and 14 days of post-mating
Female: 14 days before mating, during the mating and gestation, and 4 days of lactation

Doses/concentrations

62.5, 125 and 250 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

50

Control animals

other: Control was provided with sterilizer water without AgNPs

Details on study design

No data

Positive control

Not applicable

Examinations

Observations and examinations performed and frequency

CLINICAL OBSERVATIONS: No
MORTALITY OBSERVATIONS: Yes

BODY WEIGHT: Yes
FOOD CONSUMPTION AND WATER CONSUMPTION: Yes
OPHTHALMOSCOPIC EXAMINATION: No
HAEMATOLOGY: Yes
CLINICAL CHEMISTRY: Yes
URINALYSIS: Yes
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes

Other examinations

None

Statistics

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SPSS Statistical Analysis Systems (SPSS 10.1 Base, SPSS Korea Data Solution. Co.Ltd.). The data were presented as mean ± SD. Variance in the numerical data was checked using Levene's test. If the variance was homogeneous, the one-way ANOVA test was conducted to determine which pairs of group comparison were significantly different. If this test showed significance between the groups, the data were analyzed by the multiple comparison procedure of the Dunnet's post-hoc 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	> 250 mg/kg bw/day (nominal)	test mat.	male/female	no significant toxicity was observed.

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

no effects

Water consumption and compound intake (if drinking water study)

no effects

Ophthalmoscopic examination

not examined

Haematology

no effects

Clinical chemistry

yes

Urinalysis

no effects

Neurobehaviour

not examined

Organ weights

yes

Gross pathology

no effects

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY: No mortality was observed. Alopecia was observed in the vehicle control and treatment groups of both sexes. Salivation was observed in 1 female of the 250 mg/kg bw/day group on the Day 1 of gestation.
BODY WEIGHT AND WEIGHT GAIN: No significant difference among treated groups was observed.
FOOD AND WATER CONSUMPTION: Ad libitum
HAEMATOLOGY: No statistically significant changes in hematological analysis were observed in any of the treatment groups, including recovery group.
CLINICAL CHEMISTRY AND URINALYSIS:
- In the serum biochemical analysis and urinalysis, no treatment-related changes were observed. In both sexes, decreases in inorganic phosphorus (IP) and aspartate aminotransferase (AST), and an increase in chloride (Cl) were also considered to be spontaneous because these were neither dose-related nor within the normal ranges, or no AgNPs caused histopathological findings in related organs.- A statistically significant decrease in Aspartate aminotransferase (AST) was observed in females of the 250 mg/kg bw/day group. In recovery group, a statistically significant decrease and increase in Inorganic phosphorus and Chloride were observed in males.
ORGAN WEIGHTS- In recovery groups, a statistically significant increase in absolute and relative weights of liver was observed in males, and an increase in absolute weights of kidneys and adrenal glands were observed in females.

Any other information on results incl. tables

See the attached document for information on tables of results

Overall remarks, attachments

Remarks on results including tables and figures

According to the description, serum glutamate pyruvate transaminase (GPT) was increased while blood

urea nitrogen (BUN) was decreased in female rats, which exhibited dose-dependent tendency. However, only average values were presented and no information was obtained regarding deviations of each value. Additionally, statistical analysis was not performed. Therefore, conclusions made in this report are hardly supported by the data presented.

Attached background material

Attached document	Remarks
OECD 422 tables - results.pdf / 820.88 KB (application/octet-stream)	

Applicant's summary and conclusion

Conclusions

The AgNPs didn't cause any statistically significant changes in the endpoints of repeated dose toxicity including body weight gain, water and food consumption, hematology, serum biochemistry, histopathology, urinalysis, and necropsy. Alopecia seemed to be the only minor symptom of AgNPs treatment.

Executive summary

In a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test conducted according to OECD Guideline 422 and in compliance with GLP, Silver nanoparticles (AgNPs) was administered by oral route to groups of Sprague-Dawley rats (50/sex/dose) at the dose-levels of 62.5, 125 and 250 mg/kg bw/day for 42 days (Male : 14 days before mating, 14 days during the mating, and 14 days of post-mating , Female :14 days before mating, during the mating and gestation, and 4 days of lactation). Examinations during the study included: clinical observations, mortality, bodyweight, food and water consumption, haematology, blood chemistry, organ weight, gross and histopathological examinations.
No significant toxicity or mortality was observed. Alopecia was observed in the vehicle control and treatment groups of both sexes. Salivation was observed in 1 female of the 250 mg/kg bw/day group on the Day 1 of gestation. No significant difference in body weight, food and water consumption were observed in any of the dose groups. No statistically significant changes in hematological analysis were observed in any of the treatment groups, including recovery group. In the serum biochemical analysis and urinalysis, no treatment-related changes were observed. In both sexes, decreases in inorganic phosphorus (IP) and aspartate aminotransferase (AST), and an increase in chloride (Cl) were also considered to be spontaneous because these were neither dose-related nor within the normal ranges. In recovery groups, a statistically significant increase in absolute and relative weights of liver was observed in males, and an increase in absolute weights of kidneys and adrenal glands were observed in females. No gross or histopathological findings were observed at necropsy.
Under the test conditions, the No Observed Adverse Effect Level (NOAEL) of AgNPs was considered to be higher than 250 mg/kg bw/day in Sprague-Dawley rats.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Repeated dose toxicity-oral, Jeong, 2010, RS, K

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	2009
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	GLP study conducted according to OECD 407 Guideline with deviations: details on acclimatisation, body weight, housing and feeding conditions, clinical observations, food and water consumption, haematology, clinical chemistry, statistical analysis, individual and summary tables not reported		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Jeong GN, Jo UB, Ryu HY, Kim YS, Song KS and Yu IJ.	2010	Histochemical Study of Intestinal Mucins after Administration of Silver Nanoparticles in Sprague-Dawley Rats.	Arch Toxicol. 84:63–69					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	yes (details on acclimatisation, body weight, housing and feeding conditions, clinical observations, food and water consumption, haematology, clinical chemistry, statistical analysis, individual and summary tables not reported)

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): Silver nanoparticle (cAgNPs)
- Source: Silver nanoparticles (52.7–70.9 nm, average; 60 nm) were purchased from Namatech Co., Ltd., Korea.
- Analytical purity: At least 99.98 % pure

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: 6 weeks

Administration / exposure

Route of administration

oral: unspecified

Vehicle

CMC (carboxymethyl cellulose) (0.5 % in water)

Details on oral exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

28 days

Frequency of treatment

Once daily for 28 days

Doses/concentrations

0, 30, 300 and 1000 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

10

Control animals

yes, concurrent vehicle

Details on study design

No data

Positive control

Not applicable

Examinations

Observations and examinations performed and frequency

CLINICAL OBSERVATIONS: Yes; Following
OECD TG 407BODY WEIGHT: Yes
FOOD CONSUMPTION AND WATER CONSUMPTION: No
OPHTHALMOSCOPIC EXAMINATION: No
HAEMATOLOGY: No
CLINICAL CHEMISTRY: No
URINALYSIS: No
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
- After collecting their blood, the rats were sacrificed by cervical dislocation. The ileum, colon, and rectum were all carefully removed, then weighed and fixed in a 10 %-formalin solution containing neutral phosphate buffered saline.
HISTOPATHOLOGY: Yes, organs were embedded in paraffin, stained with hematoxylin and eosin (H–E) and a Periodic acid Schiff (PAS) reaction, and examined under a light microscope.

Other examinations

None

Statistics

Not described

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
no NOAEL identified				

Results of examinations

Clinical signs and mortality

not examined

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

not examined

Food efficiency

not examined

Water consumption and compound intake (if drinking water study)

not examined

Ophthalmoscopic examination

not examined

Haematology

not examined

Clinical chemistry

not examined

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

not examined

Gross pathology

no data

Histopathology: non-neoplastic

not examined

Histopathology: neoplastic

yes

Details on results

BODY WEIGHT AND WEIGHT GAIN
- No significant difference among treated groups was observed.
HISTOPATHOLOGY
- The experimental samples at the high and middle dose revealed luminal and surface particles, plus silver nanoparticles were found within the tissue. Higher numbers of silver nanoparticles were also found in the lamina propria (connective tissue under the epithelia) in both the small and large intestine, and in the tip of the upper villi in the ileum and protruding surface of the fold in the colon.
- In the control rat ileum, the goblet cells in the villi and crypt epithelium were revealed as densely stained mucus granules that filled the apical cytoplasm. Meanwhile, in the silver nanoparticle-administered rats, the ileal mucosa exhibited two different regions, where the first showed no change in the architecture of the epithelia compared to the control rats, while the other showed disordered goblet cells. In the silver nanoparticle-treated rats, the numbers of goblet cells that had released their mucus granules were higher than those in the controls, there were also large amounts of mucus material in the crypt lumen and ileal lumen, and cell shedding at the tip of the villi was frequent. - In rats, the proximal colon differs from the distal colon with respect to the surface structure, the form of the crypt, and the depth of the crypt. However, this study was limited to the proximal region of the colon, and no significant changes in the goblet cells from the proximal colon were observed for the silver nanoparticles-treated rats. In contrast, the goblet cells in the rectum of the silver nanoparticle-treated rats were found to have released their mucus granules and were occasionally destroyed.

Any other information on results incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

The treated samples showed luminal and surface particles and the tissue also contained silver nanoparticles. A dose-dependent increased accumulation of silver nanoparticles was observed in the lamina propria in both the small and large intestine, and also in the tip of the upper villi in the ileum and protruding surface of the fold in the colon. The silver nanoparticle-treated rats exhibited higher numbers of goblet cells that had released their mucus granules than the controls, resulting in more mucus materials in the crypt lumen and ileal lumen. Moreover, cell shedding at the tip of the villi was frequent. Lower amounts of neutral and acidic mucins were found in the goblet cells in the silver nanoparticle-treated rats, plus the amount of sialomucins was increased, while the amount of sulfomucins was decreased. In particular, in the colon of the silver nanoparticle-treated rats, sialyated mucins were detected in the lamina propria, the connective tissue under the epithelia.

Attached background material

Attached document	Remarks
28 days toxicity-histopathology figures.pdf / 211.65 KB (application/octet-stream) **Test Substances** Citrate capped silver nanoparticle (cAgNPs), CAS No. 7440-22-4 (silver) Remarks: Manufactured by ABC Nanotech Co., Ltd. (Korea) **Methods** Methods/guideline followed: OECD Test Guideline 407, “Repeated dose 28-day oral toxicity study in rodents” Test type: *In vivo*	

GLP: No Year (study performed): 2010 Species: Rat Strain: Sprague-Dawley Route of administration: Oral administration via drinking water Duration of test: 28 Days Doses/concentration levels: 0, 25, 100 and 400 mg/kg body weight Sex: Both male and female Exposure period: 28 Days Frequency of treatment: Continuous treatment by drinking water Control group and treatment: Control was provided with normal drinking water without cAgNPs Post exposure observation period: Not applicable Statistical Methods: Statistical analysis was not performed **Test Conditions** Age at study initiation: Seven weeks old No. of animals per sex per dose: Five Vehicle: Deionised water Satellite groups and reasons they were added: Not applicable Clinical observations performed and frequency: Not performed Organs examined at necropsy (macroscopic and microscopic): Liver was examined macroscopically and clinical biochemistry was performed. **Results** NOAEL (NOEL): Not determined LOAEL (LOEL): Not determined Actual dose received by dose level by sex (if known): Not applicable Toxic response/effects by dose level: No significant toxicity was observed Statistical results (as appropriate): Not described Remarks: According to the description, serum glutamate pyruvate transaminase (GPT) was increased while blood urea nitrogen (BUN) was decreased in female rats, which exhibited dose-dependent tendency. However, only average values were presented and no information was obtained regarding deviations of each value. Additionally, statistical analysis was not performed. Therefore, conclusions made in this report are hardly supported by the data presented. Body weight: No significant difference among groups Food/water consumption: Not described Description, severity, time of onset and duration of clinical signs: Not applicable Ophthalmologic findings incidence and severity: Not tested Haematological findings incidence and severity: Not tested Clinical biochemistry findings incidence and severity: Serum GPT was increased while blood urea nitrogen (BUN) was decreased in female rats, which showed dose-dependency. Mortality and time to death: None	

Gross pathology incidence and severity: Not tested Organ weight changes: Not tested Histopathology incidence and severity: Not tested **Conclusions** cAgNPs tested did not induce any toxicity in the repeated dose 28-day oral toxicity study in rodents. Remarks: None **References** National Institute of Environmental Research (NIER), Korea, 2010. Studies on hazardous properties of manufactured silver nanoparticles, (NIER No. 2010-49-1224) **Test Substances** Silver nanoparticle (cAgNPs), CAS No. 7440-22-4 (silver), at least 99.98% pure Remarks: The silver nanoparticles (52.7–70.9 nm, average; 60 nm) were purchased from Namatech Co., Ltd. (Korea) **Methods** Methods/guideline followed: OECD Test Guideline 407, "Repeated dose 28-day oral toxicity study in rodents" Test type: *In vivo* GLP: Yes Year (study performed): 2009 Species: Rat Strain: Sprague-Dawley Route of administration: Oral administration((vehicle : 0.5% carboxy methyl cellulose) Duration of test: 28 Days Doses/concentration levels: 0, 30, 300 and 1000 mg/kg body weight Sex: Both male and female Exposure period: 28 Days Frequency of treatment: once per day for 28 days Control group and treatment: Control was provided with 0.5% carboxy methyl cellulose in water Post exposure observation period: Not applicable Statistical Methods: Not described **Test Conditions** Age at study initiation: Six weeks old No. of animals per sex per dose: ten Vehicle: 0.5% carboxy methyl cellulose in water Satellite groups and reasons they were added: Not applicable Clinical observations performed and frequency: Following OECD TG 407	

Organs examined at necropsy (macroscopic and microscopic): After collecting their blood, the rats were sacrificed by cervical dislocation. The ileum, colon, and rectum were all carefully removed, then weighed and fixed in a 10%-formalin solution containing neutral phosphate buffered saline. Thereafter, the organs were embedded in paraffin, stained with hematoxylin and eosin (H–E) and a Periodic acid Schiff (PAS) reaction, and examined under a light microscope. **Results** NOAEL (NOEL): Not determined LOAEL (LOEL): Not determined Actual dose received by dose level by sex (if known): Not applicable Toxic response/effects by dose level: A dose-dependent increased accumulation of silver nanoparticles was observed in the lamina propria in both the small and large intestine, and also in the tip of the upper villi in the ileum and protruding surface of the fold in the colon. Statistical results (as appropriate): Not described Remarks: The treated samples showed luminal and surface particles and the tissue also contained silver nanoparticles. A dose-dependent increased accumulation of silver nanoparticles was observed in the lamina propria in both the small and large intestine, and also in the tip of the upper villi in the ileum and protruding surface of the fold in the colon. The silver nanoparticle-treated rats exhibited higher numbers of goblet cells that had released their mucus granules than the controls, resulting in more mucus materials in the crypt lumen and ileal lumen. Moreover, cell shedding at the tip of the villi was frequent. Lower amounts of neutral and acidic mucins were found in the goblet cells in the silver nanoparticle-treated rats, plus the amount of sialomucins was increased, while the amount of sulfomucins was decreased. In particular, in the colon of the silver nanoparticle-treated rats, sialyated mucins were detected in the lamina propria, the connective tissue under the epithelia. Body weight: No significant difference among groups Food/water consumption: Not described Description, severity, time of onset and duration of clinical signs: Not described Ophthalmologic findings incidence and severity: Not described Haematological findings incidence and severity: Not described Clinical biochemistry findings incidence and severity: Not described Mortality and time to death: Not described Gross pathology incidence and severity: Not described Organ weight changes: Not described Histopathology incidence and severity: The experimental samples at the high and middle dose revealed luminal and surface particles, plus silver nanoparticles were found within the tissue. Higher numbers of silver nanoparticles were also found in the lamina propria (connective tissue under the epithelia) in both the small and large intestine, and in the tip of the upper villi in the ileum and protruding surface of the fold in the colon (figures not shown). In the control rat ileum, the goblet cells in the villi and crypt epithelium were revealed as densely stained mucus granules that filled the apical cytoplasm (Fig. 1a). Meanwhile, in the silver nanoparticle-administered rats, the ileal mucosa exhibited two different regions, where the first showed no change in the architecture of the epithelia compared to the control rats, while the other	

showed disordered goblet cells. In the silver nanoparticle-treated rats, the numbers of goblet cells that had released their mucus granules were higher than those in the controls, there were also large amounts of mucus material in the crypt lumen and ileal lumen (Fig. 1b), and cell shedding at the tip of the villi was frequent. In rats, the proximal colon differs from the distal colon with respect to the surface structure, the form of the crypt, and the depth of the crypt. However, this study was limited
to the proximal region of the colon, and no significant changes in the goblet cells from the proximal colon were observed for the silver nanoparticles-treated rats. In contrast, the goblet cells in the rectum of the silver nanoparticle-treated rats were found to have released their mucus granules and were occasionally destroyed (Fig. 2b).

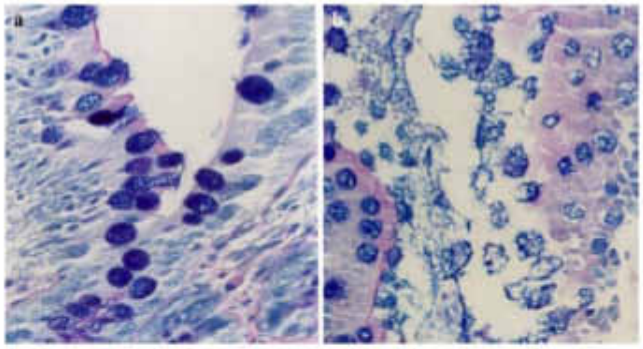


Fig. 1 AB pH 2.5-PAS staining of ileum mucosa from control and silver nanoparticle-administered rats. In the silver nanoparticle-administered rats (b), higher numbers of goblet cells were found to have released their mucus granules, resulting in more mucus materials in the crypt lumen and ileal lumen, plus the staining intensity of the goblet cells was lower when compared with that for the control rats (a). Magnification ×400

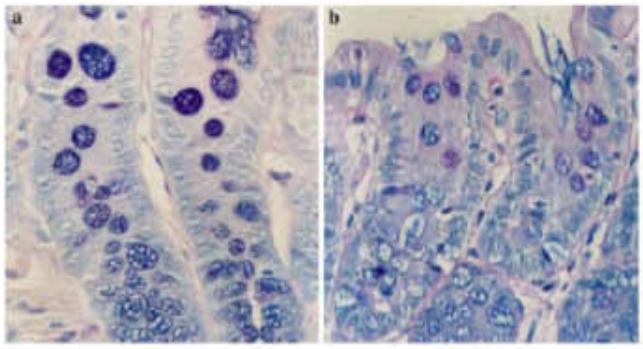


Fig. 2 AB pH 2.5-PAS staining of rectum mucosa from control and silver nanoparticle-administered rats. In the control rectum mucosa (a), goblet cells with densely stained granules were observed along the length of the crypt. Meanwhile, in the silver nanoparticle-treated rectum mucosa (b), the number of stained mucus cells was decreased, the crypt lumen was expanded, and mucus was released from the goblet cells. Magnification ×400

Conclusions

Silver nanoparticles are a powerful intestinal secretagogue and induce an abnormal mucin composition in the intestinal mucosa.

References

Histochemical study of intestinal mucins after administration of silver nanoparticles in Sprague–Dawley rats. Arch Toxicol (2010) 84:63–69

Applicant's summary and conclusion

Conclusions

Under the test conditions, Silver nanoparticles are a powerful intestinal secretagogue and induce an abnormal mucin composition in the intestinal mucosa.

Executive summary

In a repeated dose oral toxicity study conducted according to the OECD Guideline 407 and in compliance with GLP, Silver nanoparticles (cAgNPs) was administered by oral route to groups of Sprague-Dawley rats (10/sex/dose) at the dose-levels of 0, 30, 100 and 1000 mg/kg bw/day, once daily for 28 days. Examinations during the study included: mortality, toxic effects, body weight, macroscopic and microscopic examination.

No significant difference in body weight was observed in any of the dose groups. The treated samples showed luminal and surface particles and the tissue also contained silver nanoparticles. A dose-dependent increased accumulation of silver nanoparticles was observed in the lamina propria in both the small and large intestine, and also in the tip of the upper villi in the ileum and protruding surface of the fold in the colon. The silver nanoparticle-treated rats exhibited higher numbers of goblet cells that had released their mucus granules than the controls, resulting in more mucus materials in the crypt lumen and ileal lumen. Moreover, cell shedding at the tip of the villi was frequent. Lower amounts of neutral and acidic mucins were found in the goblet cells in the silver nanoparticle-treated rats, plus the amount of sialomucins was increased, while the amount of sulfomucins was decreased. In particular, in the colon of the silver nanoparticle-treated rats, sialyated mucins were detected in the lamina propria, the connective tissue under the epithelia.

Under the test conditions, Silver nanoparticles are a powerful intestinal secretagogue and induce an abnormal mucin composition in the intestinal mucosa.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Repeated dose toxicity-oral, Kim, 2010, 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 408 Guideline with deviations: details of test substance formulation, details on acclimatisation, individual weights of animals at the start of the test, housing and feeding conditions, 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	2008	Subchronic (90-Day) Oral Toxicity Study of Silver Nanoparticles in Rats		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE), Korea		
publication	Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chung YH, Chang HK, Lee JH, Oh KH, Kelman BJ, Hwang IK and Yu IJ.	2010	Subchronic Oral Toxicity of Silver Nanoparticles.	Part Fibre Toxicol. 7:20.					

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 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	yes (details of test substance formulation, details on acclimatisation, individual weights of animals at the start of the test, housing and feeding conditions, individual and summary tables of results not reported)

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): Silver powder
- Source: NAMATECH Co., Ltd., Korea
- Analytical purity: 99.98 %

Confidential details on test material

No data

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: 5 weeks

Administration / exposure

Route of administration

oral: gavage

Vehicle

CMC (carboxymethyl cellulose) (0.5 %)

Details on oral exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

90 days

Frequency of treatment

Once daily for 90 days

Doses/concentrations

0, 30, 125 and 500 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

10

Control animals

yes, concurrent vehicle

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. On the first day treatment, observations were made every hour.
BODY WEIGHT: Yes
- Time schedule for examinations: Once weekly
FOOD AND WATER CONSUMPTION: Yes
- Time schedule for examinations: Once weekly
OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: Ophthalmological examination was made prior to the grouping of animals and at the termination of the study, using an ophthalmoscope.
HAEMATOLOGY AND CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: At necropsy
- Anaesthetic used for blood collection: No data- Animals fasted: No data
URINALYSIS: Yes
- Time schedule for collection of urine: Urinalysis was performed at the last week of treatment
- Metabolism cages used for collection of urine: No data- Animals fasted: No data
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
- Organs examined at necropsy (macroscopic and microscopic): Liver, kidneys, adrenals, testes, epididymis, uterus, ovaries, thymus, spleen, brain, heart, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, pancreas, trachea, lungs, aorta, gonads, sciatic nerve, prostate, urinary bladder, lymph nodes, bone marrow, mammary gland, tongue, skin and eyes

Other examinations

None

Statistics

Chi-Square test or one way analysis of variance (ANOVA) test followed by Duncan test or Dunnett's T 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	< 30 mg/kg bw/day (actual dose received)	test mat.	male/female	
LOAEL	125 mg/kg bw/day (actual dose received)	test mat.	male/female	

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

yes

Food consumption and compound intake (if feeding study)

no effects

Water consumption and compound intake (if drinking water study)

no effects

Ophthalmoscopic examination

no effects

Haematology

yes

Clinical chemistry

yes

Urinalysis

no data

Neurobehaviour

not examined

Organ weights

yes

Gross pathology

no effects

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

not examined

Details on results

CLINICAL SIGNS AND MORTALITY
- No remarkable clinical signs were observed.
- No death was observed in all treated groups.

BODY WEIGHT AND WEIGHT GAIN
- Decrease in body weight gain was observed in male rats treated with 500 mg/kg bw/day after 4, 5 week and 7~13 weeks.

FOOD AND WATER CONSUMPTION
- No significant differences in food and water consumption between treated and control groups.

HAEMATOLOGY
- Increase of monocyte number in female rats treated with 500 mg/kg bw/day, and decrease of reticulocytes in female rats treated with 30 mg/kg bw/day. No difference in prothrombin time and activated partial thromboplastin time among groups.

CLINICAL CHEMISTRY
- Total cholesterol and total bilirubin were elevated in male rats treated with 125 mg/kg bw/day of silver powder, and total cholesterol was increased in male rats treated with 500 mg/kg bw/day. Decreases in magnesium, total protein, and inorganic phosphorus were detected in female rats treated with 125 mg/kg bw/day. The increases in total cholesterol and alkaline phosphatase and the decreases in magnesium, total protein, and inorganic phosphate were observed in female rats treated with 500 mg/kg bw/day of silver powder.

ORGAN WEIGHTS
- No significant organ-weight changes were observed in either the male and female rats except for an increase in the weight of the left testis for the 500 mg/kg bw/day male rats, and for decrease in the weight of right kidney for the 30 and 125 mg/kg bw/day female rats.

GROSS PATHOLOGY
- No significant differences between treated and control groups.

HISTOPATHOLOGY: NON-NEOPLASTIC
- Bile duct hyperplasia and focal inflammation in liver were prominent, although dose-response relationship was not detected.

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

NOAEL was less than 30 mg/kg bw/day and potential target organ is liver.

Executive summary

In a repeated dose oral toxicity study conducted according to the OECD Guideline 408 and in compliance with GLP, Silver powder was administered by oral gavage to groups of Fischer 344 rats (10/sex/dose) at the dose-levels of 0, 30, 125 and 500 mg/kg bw/day for 90 days. Examinations during the study included: mortality, clinical signs, body weight, food and water consumption, haematology, clinical chemistry, ophthalmoscope,organ weights,gross and histopathologicalexamination.

No mortality or clinical signs were observed. Decrease in body weight gain was observed in male rats treated with 500 mg/kg bw/day after 4, 5 week and 7~13 weeks. No significant differences in food and water consumption between treated and control groups. Increase of monocyte number in female rats treated with 500 mg/kg bw/day, and decrease of reticulocytes in female rats treated with 30 mg/kg bw/day. No difference in prothrombin time and activated partial thromboplastin time among groups. Total cholesterol and total bilirubin were elevated in male rats treated with 125 mg/kg bw/day of silver powder, and total cholesterol was increased in male rats treated with 500 mg/kg bw/day. Decreases in magnesium, total protein, and inorganic phosphorus were detected in female rats treated with 125 mg/kg bw/day. The increases in total cholesterol and alkaline phosphatase and the decreases in magnesium, total protein, and inorganic phosphate were observed in female rats treated with 500 mg/kg bw/day of silver powder. No significant organ-weight changes were observed in either the male and female rats except for an increase in the weight of the left testis for the 500 mg/kg bw/day male rats, and for decrease in the weight of right kidney for the 30 and 125 mg/kg bw/day female rats. No significant difference in gross pathology was observed between treated and control groups. Bile duct hyperplasia and focal inflammation in liver were prominent, although dose-response relationship was not detected.
Under the test conditions, the LOAEL and NOAEL of Silver powder was 125 mg/kg bw/day and < 30 mg/kg bw/day, respectively in Fischer 344 rats.

Cross-reference to other study

No cross-reference

Endpoint study record: Repeated dose toxicity: oral.004-NAMATECH

Administrative Data

Study result type	experimental result	Study period	2009
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	1a - GLP guideline study(OECD)		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report		2010	Histochemical study of intestinal mucins after administration of silver nanoparticles in Sprague–Dawley rats						

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

other:

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	

GLP compliance

yes (incl. certificate)

Test materials

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): Silver nanoparticle
- Molecular formula (if other than submission substance): Ag
- Substance type: Inorganic
- Physical state: Liquid
- Analytical purity: 99.98%
- Impurities (identity and concentrations): No data
- Composition of test material, percentage of components: No data
- Isomers composition: No data- Purity test date: No data
- Lot/batch No.: No data
- Expiration date of the lot/batch: No data
- Stability under test conditions: No data
- Storage condition of test material: dark condition(4oC)
- Other: Composition of the nanomaterials(including purity, impurities and additives): Black colloidal suspension obtained from ABC Nanotech Co. Ltd. (Korea). Citrate (0.5-1.5%) was used as a stabilizer for

the colloidal particles. The sizes of cAgNPs, specified by the manufacturer, range from 5 to 25 nm (average 10 nm). Characteristics/identifiers specific to nanomaterials: To confirm the characteristics, homogeneity and stability of test nanoparticles, the following were used:
1) dynamic light scattering (DLS) and transmission electron microscopy (TEM): size/ size distribution and agglomeration/aggregation state,
2) electrophoretic light scattering (ELS): zeta potential/surface charge,
3) X-ray diffraction (XRD): crystalline phase/crystallite size.

Confidential details on test material
- Analytical purity: 99.98%
- Impurities (identity and concentrations): no data
- Composition of test material, percentage of components: no data- Purity test date: no data
- Lot/batch No.: no data
- Expiration date of the lot/batch: no data- Isomers composition: no data
- Other: Particle size : 52.7-70.9 nm, average; 60 nm

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Administration / exposure

Route of administration

oral: drinking water

Vehicle

CMC (carboxymethyl cellulose)

Duration of treatment / exposure

Details on oral exposure Control group and treatment: Control was provided with 0.5% carboxy methyl cellulose in water Duration of treatment / exposure 28 Days

Frequency of treatment

Once per day for 28 days

Doses/concentrations

0, 30, 300 and 1,000mg/kg bw

Basis no data

No. of animals per sex per dose

10

Control animals

yes

Examinations

Any other information on materials and methods incl. tables

Observations and examinations performed and frequency

Clinical observations performed and frequency: Following OECD TG 407

Sacrifice and pathology

GROSS PATHOLOGY: No data
HISTOPATHOLOGY: Yes (see table)

Other examinations

Organs examined at necropsy (macroscopic and microscopic): After collecting their blood, the rats were sacrificed by cervical dislocation. The ileum, colon, and rectum were all carefully removed, then weighed and fixed in a 10%-formalin solution containing neutral phosphate buffered saline.Thereafter, the organs were embedded in paraffin, stained with hematoxylin and eosin (H–E) and a Periodic acid Schiff (PAS) reaction, and examined under a light microscope.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL				Basis for N

Results of examinations

Any other information on results incl. tables

Observations

Clinical signs and mortality

no data

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

no data

Food efficiency

no data

Water consumption and compound intake (if drinking water study)

no data

Ophthalmoscopic examination

no data

Haematology

no data

Clinical chemistry

no data

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

no data

Gross pathology

no data

Histopathology: non-neoplastic

no data

Histopathology: neoplastic

yes

Details on results

Histopathology incidence and severity: The experimental samples at the high and middle dose revealed luminal and surface particles, plus silver nanoparticles were found within the tissue. Higher numbers of silver nanoparticles were also found in the lamina propria (connective tissue under the epithelia) in both the small and large intestine, and in the tip of the upper villi in the ileum and protruding surface of the fold in the colon (figures not shown).In the control rat ileum, the goblet cells in the villi and crypt epithelium were revealed as densely stained mucus granules that filled the apical cytoplasm (Fig. 1a). Meanwhile, in the silver nanoparticle-administered rats, the ileal mucosa exhibited two different regions, where the first showed no change in the architecture of the epithelia compared to the control rats, while the other showed disordered goblet cells. In the silver nanoparticle-treated rats, the numbers of goblet cells that had released their mucus granules were higher than those in the controls, there were also large amounts of mucus material in the crypt lumen and ileal lumen (Fig. 1b), and cell shedding at the tip of the villi was frequent. In rats, the proximal colon differs from the distal colon with respect to the surface structure, the form of the crypt, and the depth of the crypt. However, this study was limitedto the proximal region of the colon, and no significant changes in the goblet cells from the proximal colon were observed for the silver nanoparticles-treated rats. In contrast, the goblet cells in the rectum of the silver nanoparticle-treated rats were found to have released their mucus granules and were occasionnally destroyed (Fig. 2b).

Overall remarks, attachments

Attached background material

Attached document	Remarks
silver_0.jpg / 300.51 KB (image/pjpeg)	

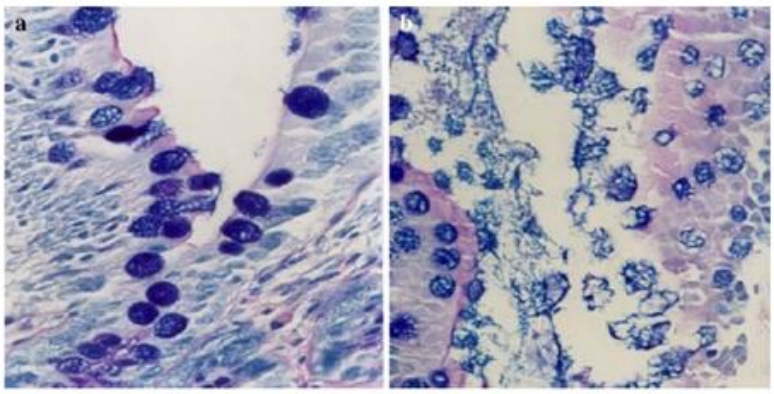


Fig. 1 AB pH 2.5-PAS staining of ileum mucosa from control and silver nanoparticle-administered rats. In the silver nanoparticle-administered rats (**b**), higher numbers of goblet cells were found to have released their mucus granules, resulting in more mucus materials in the crypt lumen and ileal lumen, plus the staining intensity of the goblet cells was lower when compared with that for the control rats (**a**). Magnification ×400

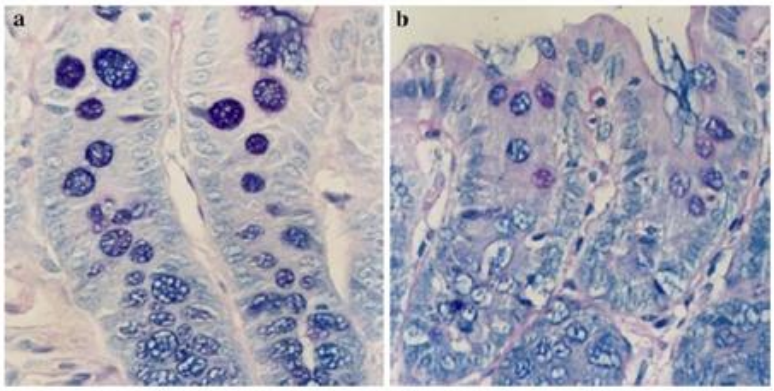


Fig. 2 AB pH 2.5-PAS staining of rectum mucosa from control and silver nanoparticle-administered rats. In the control rectum mucosa (**a**), goblet cells with densely stained granules were observed along the length of the crypt. Meanwhile, in the silver nanoparticle-treated rectum mucosa (**b**), the number of stained mucus cells was decreased, the crypt lumen was expanded, and mucus was released from the goblet cells. Magnification ×400

Applicant's summary and conclusion

Conclusions

Silver nanoparticles are a powerful intestinal secretagogue and induce an abnormal mucin composition in the intestinal mucosa.

Endpoint study record: Repeated dose toxicity: oral.005

Administrative Data

Study result type	experimental result
Study period	2011
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	1a - GLP guideline study(OECD)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	National Institute of Environmental Research(NIER)	2011	Combined repeated dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test		Korea Testing & Research Institute (KTR)	TBH-555			
study report	Jeong-Sup Hong., Suhyon Kim., Sang Hee Lee., Eunhye Jo., Byungcheun Lee., Junheon Yoon., Ig-Chun Eom., Hyun-Mi Kim., Pilje Kim., Kyunghee Choi., Moo Yeol Lee., Yeong-Rok Seo., Younghun Kim.,Yeonjin Lee., Jonghye Choi., Kwangsik Park.	2013	Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test.	Nanotoxicology, 2013; Early Online, 1–14					

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

combined repeated dose and reproduction / developmental screening

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	yes

GLP compliance

yes

Test materials

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): Citrate capped silver nanoparticles
- Molecular formula (if other than submission substance): Ag
- Substance type: Inorganic- Physical state: Liquid
- Analytical purity: 20.29%
- Impurities (identity and concentrations): No data
- Composition of test material, percentage of components: No data
- Isomers composition: No data
- Purity test date: No data
- Lot/batch No.: No data
- Expiration date of the lot/batch: No data
- Stability under test conditions: No data
- Storage condition of test material: dark condition(4℃)
- Other: Composition of the nanomaterials(including purity, impurities and additives): Black colloidal suspension obtained from ABC Nanotech Co. Ltd. (Korea). Citrate (0.5-1.5%) was used as a stabilizer for the colloidal particles.

The sizes of AgNPs, specified by the manufacturer, range from 5 to 25 nm (average 10 nm).
Characteristics/identifiers specific to nanomaterials: To confirm the characteristics, homogeneity and stability of test nanoparticles, the following were used:
1) dynamic light scattering (DLS) and transmission electron microscopy (TEM): size/ size distribution and agglomeration/aggregation state,
2) electrophoretic light scattering (ELS): zeta potential/surface charge,
3) X-ray diffraction (XRD): crystalline phase/crystallite size.

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Administration / exposure

Route of administration

oral: gavage

Vehicle

water

Duration of treatment / exposure

Male : 14 days before mating, 14 days during the mating, and 14 days of post-mating
Female :14 days before mating, during the mating and gestation, and 4 days of lactation Details on test animals and environmental conditions
TEST ANIMALS
- Source: ORIENTBIO
- Age at study initiation: 8~9 week
- Weight at study initiation: no data
- Fasting period before study: no data
- Housing: no data
- Diet (e.g. ad libitum): ad libritum
- Water (e.g. ad libitum): ad libritum
- Acclimation period: 7 days
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20.5 - 23.5 ℃
- Humidity (%): 47.7 - 62.0 %
- Air changes (per hr): 10 - 15 h,
- Photoperiod (hrs dark / hrs light): 12h /12h
IN-LIFE DATES:
From: To:
Details on oral exposure no data

Frequency of treatment

once a day

Doses/concentrations

62.5, 125, 250 mg/kg

Basis nominal in water

No. of animals per sex per dose

50 / male, female / 62.5, 125, 250 mg/kg

Control animals

yes

Examinations

Any other information on materials and methods incl. tables

Statistics

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SPSS Statistical Analysis Systems (SPSS 10.1 Base, SPSS Korea Data Solution. Co.Ltd.). The data were presented as mean ± SD. Variance in the numerical data was checked using Levene's test. If the variance was homogeneous, the one-way
ANOVA test was conducted to determine which pairs of group comparison were significantly different. If this test showed significance between the groups, the data were analyzed by the multiple comparison procedure of the Dunnet’s post-hoc test.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	> 250 mg/kg bw/day (nominal)		male/female	Basis for effect level / Remarks overall effectsclinical signs; mortality; body weight; food consumption; food efficiency; water consumption and compound intake; ophthalmoscopic examination; haematology; clinical chemistry; urinalysis; gross pathology; organ weights; histopathology; other:; no effec

Results of examinations

Any other information on results incl. tables

Observations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption and compound intake (if feeding study)

no effects

Food efficiency

no effects

Water consumption and compound intake (if drinking water study)

no effects

Ophthalmoscopic examination

no effects

Haematology

no effects

Clinical chemistry

no effects

Urinalysis

no effects

Neurobehaviour

no effects

Organ weights

yes

Gross pathology

no effects

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

no effects

Details on results

Organ weight changes: In recovery groups, a statistically significant increase in absolute and relative weights of liver was observed in males, and an increase in absolute weights of kidneys and adrenal gland were observed in females.Serum biochemistry findings incidence and severity: A statistically significant decrease in Aspartate aminotransferase(AST) was observed in females of the 250 mg/kg bw/day group. In recovery group, a statistically significant decrease and increase in Inorganic phosphorus and Chloried were observed in males

Overall remarks, attachments

Remarks on results including tables and figures

Remarks: According to the description, serum glutamate pyruvate transaminase (GPT) was increased while blood urea nitrogen (BUN) was decreased in female rats, which exhibited dose-dependent tendency. However, only average values were presented and no information was obtained regarding deviations of each value. Additionally, statistical analysis was not performed. Therefore, conclusions made in this report are hardly supported by the data presented.

Attached background material

Attached document

Table 1. Hematological.jpg / 101.5 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of male rats		5	5	5	5	5	5
RBC	(M/μl)	8.29 ± 0.50	8.45 ± 0.67	8.27 ± 0.26	8.13 ± 0.41	8.33 ± 0.31	8.23 ± 0.15
Hb	(g/dl)	14.9 ± 0.5	15.2 ± 1.0	15.1 ± 0.2	14.8 ± 0.8	14.9 ± 0.4	14.9 ± 0.5
HCT	(%)	44.4 ± 1.6	44.5 ± 2.7	44.0 ± 0.7	44.1 ± 2.3	43.5 ± 1.2	43.6 ± 1.3
MCV	(fl)	53.7 ± 2.7	52.8 ± 1.8	53.2 ± 1.6	54.3 ± 2.0	52.3 ± 2.0	52.9 ± 1.5
MCH	(pg)	18.0 ± 0.9	18.0 ± 0.9	18.3 ± 0.5	18.2 ± 0.7	17.9 ± 0.8	18.2 ± 0.7
MCHC	(g/dl)	33.4 ± 0.1	34.1 ± 0.7	34.5 ± 1.0	33.5 ± 0.5	34.2 ± 0.3	34.4 ± 0.5
RDW	(%)	12.0 ± 0.4	12.0 ± 0.3	12.5 ± 0.7	12.5 ± 0.9	13.1 ± 0.6	12.6 ± 0.8
HDW	(g/dl)	3.03 ± 0.10	3.11 ± 0.14	3.23 ± 0.28	3.11 ± 0.12	2.82 ± 0.13	2.85 ± 0.11
Reti	(G/l)	153.8 ± 27.2	147.0 ± 27.4	175.2 ± 57.7	172.0 ± 27.6	189.8 ± 28.4	160.3 ± 26.5
Reti	(%)	1.86 ± 0.37	1.74 ± 0.27	2.11 ± 0.67	2.11 ± 0.29	2.29 ± 0.42	1.95 ± 0.33
PLT	(K/μl)	1246.4 ± 64.9	1233.4 ± 34.6	1255.6 ± 113.2	1060.0 ± 382.4	1129.4 ± 79.6	1153.2 ± 101.9
MPV	(fl)	11.3 ± 0.7	11.0 ± 0.6	11.4 ± 0.3	13.0 ± 3.8	9.5 ± 0.4	9.9 ± 0.3
PT	(sec)	20.0 ± 2.3	23.4 ± 9.3	18.8 ± 5.9	20.7 ± 4.2	16.7 ± 1.2	16.1 ± 2.2
APTT	(sec)	22.3 ± 2.7	23.5 ± 4.1	20.9 ± 2.5	23.1 ± 3.2	18.9 ± 1.3	18.0 ± 3.1
WBC	(K/μl)	8.81 ± 2.16	7.89 ± 1.36	8.28 ± 1.31	7.07 ± 2.29	6.02 ± 2.81	4.82 ± 2.58
WBC differential counting							
NE	(K/μl)	1.70 ± 0.71	1.31 ± 0.18	1.40 ± 0.36	0.99 ± 0.43	0.83 ± 0.40	0.83 ± 0.64
	(%)	19.2 ± 6.5	16.7 ± 1.6	17.0 ± 3.6	13.5 ± 2.8	15.0 ± 5.4	16.1 ± 3.7
LY	(K/μl)	6.63 ± 1.71	6.09 ± 1.07	6.43 ± 1.14	5.63 ± 1.75	4.97 ± 2.51	3.75 ± 1.89
	(%)	75.3 ± 5.8	77.2 ± 2.0	77.5 ± 3.8	80.1 ± 3.4	81.2 ± 6.4	78.6 ± 4.3
MO	(K/μl)	0.25 ± 0.10	0.27 ± 0.05	0.27 ± 0.07	0.23 ± 0.11	0.13 ± 0.06	0.15 ± 0.07
	(%)	2.7 ± 0.5	3.4 ± 0.3	3.3 ± 0.8	3.3 ± 1.1	2.2 ± 0.7	3.3 ± 1.7
EO	(K/μl)	0.11 ± 0.03	0.10 ± 0.05	0.11 ± 0.06	0.10 ± 0.05	0.05 ± 0.01	0.06 ± 0.03
	(%)	1.3 ± 0.5	1.2 ± 0.4	1.3 ± 0.6	1.3 ± 0.4	0.9 ± 0.4	1.2 ± 0.6
BA	(K/μl)	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
	(%)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
LUC	(K/μl)	0.09 ± 0.06	0.11 ± 0.07	0.05 ± 0.02	0.10 ± 0.05	0.03 ± 0.01	0.02 ± 0.01
	(%)	1.1 ± 0.9	1.3 ± 0.7	0.7 ± 0.3	1.5 ± 0.7	0.5 ± 0.3	0.5 ± 0.2

RBC, Red blood cell; Hb, Haemoglobin; HCT, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; RDW, Red cell distribution width; HDW, Haemoglobin distribution width; Reti, Reticulocytes; PLT, Platelet count; MPV, Mean platelet volume; WBC, White blood cell; NE, Neutrophils; LY, Lymphocytes; MO, Monocytes; EO, Eosinophils; BA, Basophils; LUC, Large unstained cell.

Table 2. Hematological.jpg / 103.04 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of female rats		5	5	5	5	4	5
RBC	(M/μl)	6.98 ± 0.36	6.63 ± 0.25	6.61 ± 0.45	6.86 ± 0.41	7.28 ± 0.75	7.36 ± 0.32
Hb	(g/dl)	12.9 ± 0.4	12.5 ± 0.5	12.6 ± 1.4	12.8 ± 0.4	15.0 ± 0.1	14.8 ± 0.4
HCT	(%)	38.6 ± 1.6	37.5 ± 1.8	37.9 ± 4.2	38.0 ± 1.3	43.6 ± 1.1	42.5 ± 0.9
MCV	(fl)	55.4 ± 1.7	56.6 ± 3.3	57.3 ± 3.4	55.5 ± 2.0	60.5 ± 8.2	57.8 ± 1.9
MCH	(pg)	18.5 ± 0.6	18.7 ± 0.9	19.0 ± 1.3	18.7 ± 0.6	20.9 ± 2.4	20.1 ± 0.6
MCHC	(g/dl)	33.5 ± 0.3	33.1 ± 0.5	33.1 ± 0.5	33.6 ± 0.4	34.6 ± 0.8	34.9 ± 0.4
RDW	(%)	15.4 ± 1.6	16.5 ± 1.2	15.6 ± 0.9	16.2 ± 2.8	14.6 ± 0.5	14.2 ± 0.7
HDW	(g/dl)	2.92 ± 0.22	2.99 ± 0.11	2.98 ± 0.31	2.91 ± 0.24	2.25 ± 0.15	2.29 ± 0.03
Reti	(G/l)	426.9 ± 84.2	550.9 ± 159.2	411.0 ± 36.7	462.0 ± 165.2	214.8 ± 106.2	156.1 ± 24.1
Reti	(%)	6.14 ± 1.36	8.35 ± 2.59	6.26 ± 0.82	6.85 ± 2.83	3.10 ± 1.96	2.12 ± 0.33
PLT	(K/μl)	2021.4 ± 220.2	1958.8 ± 174.4	2070.8 ± 263.7	1965.0 ± 123.3	1421.5 ± 187.5	1403.0 ± 128.6
MPV	(fl)	9.8 ± 2.1	10.1 ± 1.4	10.1 ± 1.3	9.5 ± 1.5	11.0 ± 0.9	10.9 ± 0.4
PT	(sec)	13.7 ± 0.4	14.2 ± 0.6	14.5 ± 0.5	14.5 ± 0.6	14.3 ± 0.9	14.2 ± 0.6
APTT	(sec)	14.1 ± 2.1	13.8 ± 1.1	14.0 ± 2.7	15.1 ± 1.6	14.4 ± 0.7	14.3 ± 0.9
WBC	(K/μl)	5.07 ± 1.09	5.93 ± 2.31	7.02 ± 3.80	3.65 ± 1.88	5.83 ± 2.27	5.19 ± 0.99
WBC differential counting							
NE	(K/μl)	1.10 ± 0.15	1.36 ± 0.86	1.90 ± 2.36	0.93 ± 0.47	3.03 ± 1.80	1.70 ± 1.15
	(%)	22.3 ± 4.0	21.3 ± 5.5	22.8 ± 19.2	25.8 ± 7.9	48.7 ± 15.3	30.8 ± 15.8
LY	(K/μl)	3.76 ± 0.99	4.33 ± 1.38	4.76 ± 2.76	2.58 ± 1.39	2.53 ± 0.89	3.26 ± 0.66
	(%)	73.7 ± 4.2	74.5 ± 5.1	72.7 ± 21.8	70.2 ± 8.2	46.6 ± 14.6	64.9 ± 16.6
MO	(K/μl)	0.11 ± 0.02	0.13 ± 0.05	0.20 ± 0.26	0.09 ± 0.04	0.20 ± 0.06	0.15 ± 0.07
	(%)	2.3 ± 0.4	2.4 ± 0.6	2.4 ± 2.1	2.4 ± 0.8	3.5 ± 0.8	2.9 ± 0.9
EO	(K/μl)	0.04 ± 0.02	0.06 ± 0.03	0.05 ± 0.03	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
	(%)	0.8 ± 0.2	1.1 ± 0.5	0.8 ± 0.4	0.8 ± 0.2	0.6 ± 0.3	0.7 ± 0.3
BA	(K/μl)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
	(%)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
LUC	(K/μl)	0.04 ± 0.02	0.04 ± 0.02	0.09 ± 0.11	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
	(%)	0.7 ± 0.3	0.6 ± 0.3	1.0 ± 0.9	0.6 ± 0.1	0.6 ± 0.3	0.6 ± 0.3

RBC, Red blood cell; Hb, Haemoglobin; HCT, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; RDW, Red cell distribution width; HDW, Haemoglobin distribution width; Reti, Reticulocytes; PLT, Platelet count; MPV, Mean platelet volume; WBC, White blood cell; NE, Neutrophils; LY, Lymphocytes; MO, Monocytes; EO, Eosinophils; BA, Basophils; LUC, Large unstained cell.

Table 3. Blood biochemical .jpg / 79.62 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of male rats		5	5	5	5	5	5
TP	(g/dl)	5.7 ± 0.1	5.7 ± 0.2	5.8 ± 0.1	5.7 ± 0.3	5.5 ± 0.2	5.6 ± 0.1
ALB	(g/dl)	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
A/G		0.6 ± 0.1	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1
T-BIL	(mg/dl)	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01
ALP	(U/l)	382.6 ± 54.7	454.4 ± 58.5	369.2 ± 51.4	458.8 ± 85.4	376.4 ± 76.7	390.2 ± 45.3
AST	(U/l)	102.4 ± 27.7	118.4 ± 10.5	109.0 ± 26.4	112.0 ± 45.4	115.2 ± 7.6	125.4 ± 13.6
ALT	(U/l)	34.4 ± 11.0	37.6 ± 5.1	35.8 ± 6.7	34.6 ± 7.3	37.2 ± 4.1	41.8 ± 4.1
CREA	(mg/dl)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
BUN	(mg/dl)	13.4 ± 1.0	14.6 ± 1.4	12.8 ± 1.3	13.4 ± 0.6	14.4 ± 2.4	14.2 ± 1.4
T-CHO	(mg/dl)	61.8 ± 11.8	54.6 ± 11.0	66.4 ± 9.1	53.6 ± 14.8	63.2 ± 19.2	50.2 ± 10.7
TG	(mg/dl)	47.2 ± 22.4	39.6 ± 14.9	56.2 ± 23.5	50.8 ± 36.8	46.2 ± 13.7	56.6 ± 14.9
GLU	(mg/dl)	160.2 ± 17.1	143.0 ± 16.4	147.8 ± 9.8	158.6 ± 10.5	176.4 ± 12.8	163.2 ± 6.2
CA	(mg/dl)	10.1 ± 0.3	10.0 ± 0.3	10.1 ± 0.1	10.1 ± 0.2	9.7 ± 0.2	9.9 ± 0.1
IP	(mg/dl)	7.7 ± 0.5	7.6 ± 0.4	7.8 ± 0.6	7.7 ± 0.4	7.5 ± 0.2	6.8 ± 0.3**
GGT	(U/l)	0.5 ± 0.8	0.0 ± 0.4	0.1 ± 0.3	0.3 ± 0.4	0.2 ± 0.5	0.3 ± 0.5
CK	(IU/l)	280.8 ± 144.8	263.0 ± 136.6	333.8 ± 210.6	336.2 ± 412.4	463.2 ± 75.2	572.4 ± 239.3
Na	(mmol/l)	143.3 ± 1.0	142.8 ± 0.7	142.3 ± 0.2	143.0 ± 1.1	141.0 ± 0.9	142.2 ± 1.0
K	(mmol/l)	4.28 ± 0.15	4.07 ± 0.42	4.22 ± 0.27	4.12 ± 0.17	4.21 ± 0.22	4.31 ± 0.13
Cl	(mmol/l)	109.6 ± 1.0	109.1 ± 1.0	107.8 ± 0.7	109.1 ± 1.6	106.6 ± 0.7	108.5 ± 1.2*

*Significantly different from values of control group at $p < 0.05$; **At $p < 0.01$; TP, Total protein; ALB, Albumin; A/G, Albumin/Globulin ratio; T-BIL, Total bilirubin; ALP, Alkaline phosphate; ALT, Alanine aminotransferase; CREA, Creatinine; BUN, Blood urea nitrogen; T-CHO, Total cholesterol; TG, Triglyceride; GLU, Glucose; CA, Calcium; IP, Inorganic phosphorus; GGT, Gamma glutamyltranspeptidase; CK, Creatine kinase; Na, Sodium, K, Potassium; Cl, Chloride.

Table 4. Blood biochemical.png / 66.5 KB (image/png)

		Main group				Recovery group	
Dose (mg/kg bw/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of female rats		5	5	5	5	4	5
TP	(g/dl)	6.1 ± 0.1	5.8 ± 0.3	5.7 ± 0.5	5.9 ± 0.2	5.7 ± 0.2	5.6 ± 0.3
ALB	(g/dl)	2.4 ± 0.1	2.3 ± 0.2	2.2 ± 0.5	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
A/G		0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
T-BIL	(mg/dl)	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.05 ± 0.02
ALP	(U/l)	209.0 ± 64.9	328.8 ± 92.4	281.0 ± 90.6	265.4 ± 45.5	349.8 ± 70.6	306.8 ± 85.6
AST	(U/l)	123.2 ± 15.2	121.2 ± 14.9	96.6 ± 15.3	85.6 ± 14.0*	139.3 ± 30.1	95.6 ± 9.2
ALT	(U/l)	45.8 ± 5.4	50.6 ± 8.1	44.8 ± 7.5	44.4 ± 7.4	103.3 ± 13.9	88.0 ± 9.1
CREA	(mg/dl)	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
BUN	(mg/dl)	16.2 ± 3.3	15.9 ± 3.0	17.7 ± 2.7	15.6 ± 1.8	21.0 ± 5.5	21.4 ± 1.1
T-CHO	(mg/dl)	91.6 ± 23.0	77.6 ± 21.8	79.4 ± 22.9	84.0 ± 20.9	127.5 ± 15.7	122.0 ± 17.4
TG	(mg/dl)	157.6 ± 51.9	95.8 ± 30.1	72.0 ± 13.5	98.2 ± 42.7	245.3 ± 70.6	165.8 ± 97.0
GLU	(mg/dl)	95.2 ± 11.8	110.0 ± 16.5	105.6 ± 17.2	104.8 ± 18.1	112.5 ± 4.9	124.4 ± 8.3
CA	(mg/dl)	9.8 ± 0.4	10.3 ± 0.5	10.4 ± 0.5	10.0 ± 0.3	8.3 ± 0.4	9.3 ± 0.6
IP	(mg/dl)	8.0 ± 0.9	9.1 ± 1.1	9.5 ± 1.3	7.4 ± 1.5	10.7 ± 1.7	9.8 ± 1.9
GGT	(U/l)	0.3 ± 0.6	0.1 ± 0.4	0.3 ± 0.9	0.1 ± 0.5	0.3 ± 0.3	0.0 ± 0.3
CK	(IU/l)	734.4 ± 298.7	610.6 ± 133.1	455.0 ± 270.0	262.6 ± 103.1	700.0 ± 220.6	267.0 ± 77.8
Na	(mmol/l)	134.0 ± 3.3	139.5 ± 4.8	137.5 ± 5.5	134.8 ± 6.2	135.1 ± 1.0	134.8 ± 1.6
K	(mmol/l)	4.68 ± 0.48	4.45 ± 0.22	4.78 ± 0.38	4.00 ± 0.28	4.50 ± 0.15	4.39 ± 0.22
Cl	(mmol/l)	98.3 ± 3.6	102.5 ± 2.8	101.9 ± 5.5	99.7 ± 4.9	97.6 ± 2.1	97.9 ± 2.2

*Significantly different from values of control group at $p < 0.05$; TP; Total protein, ALB; Albumin; A/G, Albumin/Globulin ratio; T-BIL, Total bilirubin; T-BIL, Total bilirubin; ALP, Alkaline phosphate; ALT, Alanine aminotransferase; bw, Body weight; CREA, Creatinine; BUN, Blood urea nitrogen; T-CHO, Total cholesterol; TG, Triglyceride; GLU, Glucose; CA, Calcium; IP, Inorganic phosphorus; GGT, Gamma glutamyltranspeptidase; CK, Creatine kinase; Na, Sodium; K, Potassium; Cl, Chloride.

Table 5. Organ weight of male rats given AgNPs.jpg / 131.71 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg bw/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of male rats		10	10	10	10	5	5
Body weight (g)		501.0 ± 48.4	485.0 ± 35.2	484.9 ± 43.1	497.3 ± 41.1	493.9 ± 28.9	501.7 ± 22.9
Liver	(g)	12.47 ± 1.46	12.49 ± 1.29	12.66 ± 1.92	12.64 ± 1.47	12.03 ± 0.53	13.39 ± 0.74*
	(g%)	2.49 ± 0.12	2.58 ± 0.19	2.60 ± 0.19	2.54 ± 0.12	2.44 ± 0.11	2.67 ± 0.10*
Kidney-Left	(g)	1.72 ± 0.16	1.63 ± 0.12	1.65 ± 0.16	1.70 ± 0.18	1.58 ± 0.14	1.65 ± 0.11
	(g%)	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.32 ± 0.02	0.33 ± 0.03
Kidney-Right	(g)	1.72 ± 0.17	1.61 ± 0.14	1.64 ± 0.18	1.71 ± 0.12	1.62 ± 0.22	1.62 ± 0.12
	(g%)	0.34 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.35 ± 0.02	0.33 ± 0.03	0.32 ± 0.03
Spleen	(g)	0.87 ± 0.10	0.83 ± 0.10	0.89 ± 0.16	0.88 ± 0.16	0.85 ± 0.15	0.85 ± 0.14
	(g%)	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.03	0.18 ± 0.03	0.17 ± 0.02	0.17 ± 0.02
Adrenal gland-Left	(mg)	41.4 ± 9.32	39.9 ± 7.81	41.7 ± 7.85	44.8 ± 12.56	36.0 ± 7.58	33.4 ± 7.50
	(mg%)	8.3 ± 1.77	8.1 ± 1.52	8.7 ± 1.77	9.1 ± 2.13	7.4 ± 2.07	6.6 ± 1.14
Adrenal gland-Right	(mg)	39.6 ± 6.82	36.8 ± 5.79	41.4 ± 5.87	41.2 ± 9.19	40.4 ± 4.88	33.8 ± 6.61
	(mg%)	8.0 ± 1.25	7.7 ± 1.34	7.8 ± 1.06	8.1 ± 1.60	8.0 ± 1.00	6.6 ± 1.14
Testis-Left	(g)	1.80 ± 0.18	1.75 ± 0.11	1.80 ± 0.21	1.78 ± 0.13	1.86 ± 0.22	1.75 ± 0.12
	(g%)	0.36 ± 0.06	0.36 ± 0.03	0.37 ± 0.04	0.36 ± 0.04	0.38 ± 0.06	0.35 ± 0.03
Testis-Right	(g)	1.79 ± 0.16	1.78 ± 0.10	1.78 ± 0.21	1.77 ± 0.15	1.90 ± 0.17	1.78 ± 0.14
	(g%)	0.36 ± 0.06	0.37 ± 0.03	0.37 ± 0.04	0.36 ± 0.04	0.39 ± 0.05	0.36 ± 0.03
Brain	(g)	1.95 ± 0.66	2.14 ± 0.10	2.17 ± 0.11	2.16 ± 0.12	2.13 ± 0.09	2.08 ± 0.15
	(g%)	0.39 ± 0.13	0.44 ± 0.04	0.45 ± 0.04	0.44 ± 0.03	0.43 ± 0.04	0.42 ± 0.03
Pituitary gland	(mg)	17.5 ± 3.60	16.4 ± 4.81	15.5 ± 5.97	14.5 ± 4.70	12.8 ± 0.84	10.0 ± 4.06
	(mg%)	3.5 ± 0.71	3.5 ± 0.97	3.2 ± 1.23	2.9 ± 0.74	2.6 ± 0.55	2.0 ± 1.22
Lung	(g)	1.75 ± 0.14	1.64 ± 0.16	1.73 ± 0.20	1.83 ± 0.16	1.72 ± 0.15	1.74 ± 0.16
	(g%)	0.35 ± 0.03	0.34 ± 0.02	0.36 ± 0.02	0.37 ± 0.05	0.35 ± 0.03	0.35 ± 0.02
Heart	(g)	1.73 ± 0.31	1.65 ± 0.19	1.73 ± 0.21	1.66 ± 0.42	1.65 ± 0.05	1.82 ± 0.22
	(g%)	0.35 ± 0.06	0.34 ± 0.02	0.36 ± 0.05	0.34 ± 0.08	0.33 ± 0.02	0.36 ± 0.04
Thymus	(g)	0.49 ± 0.18	0.46 ± 0.04	0.46 ± 0.12	0.47 ± 0.11	0.41 ± 0.11	0.36 ± 0.07
	(g%)	0.10 ± 0.03	0.10 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.07 ± 0.01
Thyroid gland	(mg)	34.5 ± 9.22	26.8 ± 7.85	31.2 ± 10.51	34.3 ± 14.57	24.4 ± 3.61	24.4 ± 7.34
	(mg%)	9.0 ± 3.16	5.6 ± 1.96	6.4 ± 2.01	6.7 ± 2.50	4.0 ± 5.48	4.0 ± 5.48
Salivary gland	(g)	0.79 ± 0.11	0.78 ± 0.09	0.87 ± 0.10	0.90 ± 0.09	0.83 ± 0.09	0.88 ± 0.14
	(g%)	0.16 ± 0.02	0.16 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.03
Seminal vesicles	(g)	3.01 ± 0.61	2.62 ± 0.69	2.24 ± 0.63	2.49 ± 0.49	2.99 ± 0.43	3.17 ± 0.58
	(g%)	0.61 ± 0.15	0.54 ± 0.14	0.46 ± 0.11	0.50 ± 0.10	0.60 ± 0.07	0.63 ± 0.13
Epididymis-Left	(g)	0.78 ± 0.06	0.76 ± 0.07	0.77 ± 0.08	0.78 ± 0.06	0.81 ± 0.10	0.84 ± 0.03
	(g%)	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.01
Epididymis-Right	(g)	0.78 ± 0.07	0.79 ± 0.10	0.79 ± 0.08	0.79 ± 0.07	0.85 ± 0.07	0.85 ± 0.08
	(g%)	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.02	0.17 ± 0.02

*Significantly different from values of control group at $p < 0.05$; bw, Body weight; g%, relative weight of organ (g) to body weight (g); mg%, relative weight of organ (mg) to body weight (g).

Table 6. Organ weight.jpg / 120.22 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg bw/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of female rats		9	10	10	10	4	5
Body weight (g)		299.8 ± 17.7	322.1 ± 40.0	322.9 ± 23.3	318.5 ± 16.8	308.7 ± 12.1	330.0 ± 15.5
Liver	(g)	8.96 ± 0.76	10.05 ± 2.25	10.05 ± 1.53	9.53 ± 0.56	11.31 ± 0.62	11.89 ± 0.54
	(g%)	2.99 ± 0.15	3.10 ± 0.43	3.11 ± 0.35	2.99 ± 0.15	3.67 ± 0.23	3.60 ± 0.06
Kidney-Left	(g)	0.97 ± 0.05	1.03 ± 0.12	1.00 ± 0.09	1.00 ± 0.09	1.04 ± 0.09	1.17 ± 0.07*
	(g%)	0.32 ± 0.03	0.32 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	0.36 ± 0.02
Kidney-Right	(g)	0.99 ± 0.05	1.05 ± 0.14	1.04 ± 0.08	1.02 ± 0.08	1.09 ± 0.05	1.17 ± 0.04*
	(g%)	0.33 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.32 ± 0.02	0.35 ± 0.01	0.35 ± 0.01
Spleen	(g)	0.62 ± 0.09	0.68 ± 0.15	0.72 ± 0.21	0.67 ± 0.15	0.59 ± 0.08	0.65 ± 0.07
	(g%)	0.21 ± 0.02	0.21 ± 0.04	0.22 ± 0.07	0.21 ± 0.04	0.19 ± 0.03	0.20 ± 0.02
Adrenal gland-Left	(mg)	45.0 ± 5.36	40.2 ± 5.69	46.6 ± 9.57	46.3 ± 7.82	35.8 ± 1028	53.0 ± 6.32*
	(mg%)	15.2 ± 2.05	12.7 ± 2.45	14.4 ± 3.44	14.6 ± 2.59	11.75 ± 3.50	15.8 ± 1.30
Adrenal gland-Right	(mg)	43.0 ± 7.63	41.8 ± 5.35	41.3 ± 12.25	43.4 ± 5.91	42.8 ± 4.79	45.8 ± 4.82
	(mg%)	14.3 ± 2.45	13.0 ± 2.16	12.8 ± 3.97	13.7 ± 2.36	13.8 ± 2.06	14.0 ± 1.22
Ovary-Left	(g)	0.056 ± 0.015	0.059 ± 0.010	0.064 ± 0.016	0.060 ± 0.009	0.052 ± 0.005	0.054 ± 0.006
	(g%)	0.019 ± 0.005	0.019 ± 0.005	0.020 ± 0.006	0.019 ± 0.003	0.017 ± 0.002	0.016 ± 0.002
Ovary-Right	(g)	0.061 ± 0.011	0.061 ± 0.015	0.067 ± 0.009	0.062 ± 0.015	0.041 ± 0.009	0.047 ± 0.009
	(g%)	0.020 ± 0.003	0.019 ± 0.004	0.021 ± 0.003	0.019 ± 0.005	0.013 ± 0.003	0.014 ± 0.003
Brain	(g)	1.96 ± 0.10	1.97 ± 0.10	1.99 ± 0.06	2.02 ± 0.05	1.93 ± 0.08	2.01 ± 0.03
	(g%)	0.66 ± 0.04	0.62 ± 0.07	0.62 ± 0.04	0.64 ± 0.03	0.62 ± 0.03	0.61 ± 0.03
Pituitary gland	(mg)	14.6 ± 6.17	15.1 ± 5.51	13.7 ± 5.27	20.8 ± 5.01	15.5 ± 1.91	13.8 ± 2.39
	(mg%)	4.8 ± 1.72	4.6 ± 1.65	4.7 ± 0.95	6.5 ± 1.35	5.0 ± 0.82	4.2 ± 0.84
Lung	(g)	1.34 ± 0.11	1.40 ± 0.28	1.43 ± 0.16	1.46 ± 0.19	1.35 ± 0.09	1.45 ± 0.08
	(g%)	0.45 ± 0.03	0.43 ± 0.05	0.44 ± 0.04	0.46 ± 0.06	0.44 ± 0.05	0.44 ± 0.04
Heart	(g)	1.12 ± 0.08	1.25 ± 0.14	1.19 ± 0.09	1.21 ± 0.14	1.22 ± 0.03	1.33 ± 0.13
	(g%)	0.37 ± 0.03	0.39 ± 0.04	0.37 ± 0.03	0.38 ± 0.03	0.40 ± 0.02	0.40 ± 0.03
Thymus	(g)	0.34 ± 0.07	0.36 ± 0.11	0.36 ± 0.07	0.40 ± 0.13	0.25 ± 0.06	0.26 ± 0.06
	(g%)	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.04	0.08 ± 0.02	0.08 ± 0.01
Thyroid gland	(mg)	21.8 ± 5.78	14.7 ± 4.88	18.6 ± 6.45	21.5 ± 9.76	18.5 ± 3.11	23.8 ± 8.58
	(mg%)	7.1 ± 2.20	4.6 ± 1.43	5.7 ± 2.16	6.6 ± 2.84	7.5 ± 5.0	8.0 ± 4.47
Salivary gland	(g)	0.66 ± 0.15	0.63 ± 0.12	0.62 ± 0.11	0.64 ± 0.10	0.65 ± 0.07	0.62 ± 0.09
	(g%)	0.22 ± 0.04	0.19 ± 0.03	0.19 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.19 ± 0.03
Uterus	(g)	1.16 ± 0.31	0.97 ± 0.13	1.06 ± 0.21	1.12 ± 0.41	0.54 ± 0.13	0.55 ± 0.11
	(g%)	0.39 ± 0.10	0.30 ± 0.04	0.33 ± 0.06	0.35 ± 0.13	0.18 ± 0.04	0.16 ± 0.03

*Significantly different from values of control group at $p < 0.05$; bw, Body weight; g%, relative weight of organ (g) to body weight (g); mg%, relative weight of organ (mg) to body weight (g).

Table 7. Histopathological.jpg / 103.77 KB (image/pjpeg)

Group		Male				Female			
		Treated group		Recovery group		Treated group		Recovery group	
Dose (mg/kg bw/day)		Control	250	Control	250	Control	250	Control	250
No. of animals examined		10	10	5	5	9	10	4	5
Organs	Histopathological findings								
Liver	No abnormalities detected	6	5	3	3	4	3	2	4
	Focal necrosis/minimal	1	0	0	0	0	0	0	0
	Parenchymal necrosis/minimal	0	0	0	0	0	1	0	0
	Inflammatory cells/minimal	4	5	2	2	2	0	2	1
	Extramedullary haematopoiesis/minimal	0	0	0	0	5	5	0	0
	Hepatocyte vacuolation/minimal	1	0	0	0	1	3	0	0
	Clear cell focus/minimal	0	0	0	0	0	1	0	0
Kidney	No abnormalities detected	7	6	3	3	2	5	1	1
	Interstitial inflammatory cells/minimal	3	1	0	1	0	3	0	0
	Cortical tubular basophilia/minimal	2	1	2	1	0	1	0	0
	Tubular basophilia/ minimal/slight	0	1/1	0	1	0	0	0	0
	Medullary tubular dilatation/minimal	0	0	0	0	0	0	0	1
	Cortical scar/minimal	0	1	0	0	0	0	1	0
	Cystic tubules	0	1	0	0	0	0	0	0
	Pelvic mineralisation/minimal	0	0	0	0	1	0	2	1
	Cortico-medullary mineralisation/minimal	0	0	0	0	7	5	1	4
Adrenal gland	No abnormalities detected	8	7	5	2	9	10	3	5
	Cortical vacuolation/minimal	2	3	0	3	0	0	0	0
	Cortical hypertrophy/minimal	0	0	0	0	0	0	1	0
Heart	No abnormalities detected	7	7	4	4	9	10	3	4
	Myocardial inflammatory cells/minimal/slight	2/1	3	1	1	0	0	1	1
Lung	No abnormalities detected	7	4	3	3	6	5	3	4
	Alveolar macrophages/minimal	1	1	0	0	0	2	1	0
	Perivascular inflammatory/minimal	2	3	1	0	2	2	0	0
	Subpleural inflammatory cells/minimal	0	1	0	0	0	0	0	0
	Cholesterol granuloma/minimal	0	2	1	2	1	1	0	0
	Granulomas	0	0	0	0	0	2	0	1
	Alveolitis	0	0	0	0	0	1	0	0
Ovary	No abnormalities detected					9	10	4	5
Spleen	No abnormalities detected	9	7	3	2	1	1	3	5
	Extramedullary haematopoiesis/minimal/slight/moderate	1	3	1/1	3	2/3/1	2/4/3	1	0
Prostate gland/vagina	No abnormalities detected	7	5	1	3	9	10	4	5
	Concretion	0	0	1	0	0	0	0	0
	Interstitial inflammatory cells/minimal/slight	3	4/1	2/2	2	0	0	0	0
Testis	No abnormalities detected	10	10	5	5				
Thymus	No abnormalities detected	10	9	5	5	9	10	4	5
	Cortical apoptosis/minimal	0	1	0	0	0	0	0	0
Thyroid gland	No abnormalities detected	5	8	4	5	7	7	1	4
	Ultimobranchial cysts	4	2	1	0	1	2	2	1
	Lymphoid cell hyperplasia/minimal	1	0	0	0	0	0	1	0
Stomach	No abnormalities detected	9	10	5	5	9	10	3	5
	Submucosal oedema and inflammatory cells/minimal	1	0	0	0	0	0	1	0
Urinary bladder	No abnormalities detected	8	8	5	5	9	9	4	5
	Submucosalinflammatory cells/minimal	1	0	0	0	0	1	0	0
Pancreas	No abnormalities detected	9	9	3	4	9	10	4	4
	Fat infiltration/minimal	1	0	1	0	0	0	0	0
	Granulomas/slight	0	0	0	1	0	0	0	0
	Acinar cell apoptosis/minimal	0	0	0	0	0	0	0	1
	Inflammatory cells/minimal	0	1	1	0	0	0	0	0

bw, Body weight.

Group		Male				Female			
		Treated group		Recovery group		Treated group		Recovery group	
Dose (mg/kg bw/day)		Control	250	Control	250	Control	250	Control	250
No. of animals examined		10	10	5	5	9	10	4	5
Organs	Histopathological findings								
Liver	No abnormalities detected	6	5	3	3	4	3	2	4
	Focal necrosis/minimal	1	0	0	0	0	0	0	0
	Parenchymal necrosis/minimal	0	0	0	0	0	1	0	0
	Inflammatory cells/minimal	4	5	2	2	2	0	2	1
	Extramedullary haematopoiesis/minimal	0	0	0	0	5	5	0	0
	Hepatocyte vacuolation/minimal	1	0	0	0	1	3	0	0
	Clear cell focus/minimal	0	0	0	0	0	1	0	0
Kidney	No abnormalities detected	7	6	3	3	2	5	1	1
	Interstitial inflammatory cells/minimal	3	1	0	1	0	3	0	0
	Cortical tubular basophilia/minimal	2	1	2	1	0	1	0	0
	Tubular basophilia/ minimal/slight	0	1/1	0	1	0	0	0	0
	Medullary tubular dilatation/minimal	0	0	0	0	0	0	0	1
	Cortical scar/minimal	0	1	0	0	0	0	1	0
	Cystic tubules	0	1	0	0	0	0	0	0
	Pelvic mineralisation/minimal	0	0	0	0	1	0	2	1
	Cortico-medullary mineralisation/minimal	0	0	0	0	7	5	1	4
Adrenal gland	No abnormalities detected	8	7	5	2	9	10	3	5
	Cortical vacuolation/minimal	2	3	0	3	0	0	0	0
	Cortical hypertrophy/minimal	0	0	0	0	0	0	1	0
Heart	No abnormalities detected	7	7	4	4	9	10	3	4
	Myocardial inflammatory cells/minimal/slight	2/1	3	1	1	0	0	1	1
Lung	No abnormalities detected	7	4	3	3	6	5	3	4
	Alveolar macrophages/minimal	1	1	0	0	0	2	1	0
	Perivascular inflammatory/minimal	2	3	1	0	2	2	0	0
	Subpleural inflammatory cells/minimal	0	1	0	0	0	0	0	0
	Cholesterol granuloma/minimal	0	2	1	2	1	1	0	0
	Granulomas	0	0	0	0	0	2	0	1
	Alveolitis	0	0	0	0	0	1	0	0
Ovary	No abnormalities detected					9	10	4	5
Spleen	No abnormalities detected	9	7	3	2	1	1	3	5
	Extramedullary haematopoiesis/minimal/slight/moderate	1	3	1/1	3	2/3/1	2/4/3	1	0
Prostate gland/vagina	No abnormalities detected	7	5	1	3	9	10	4	5
	Concretion	0	0	1	0	0	0	0	0
	Interstitial inflammatory cells/minimal/slight	3	4/1	2/2	2	0	0	0	0
Testis	No abnormalities detected	10	10	5	5				
Thymus	No abnormalities detected	10	9	5	5	9	10	4	5
	Cortical apoptosis/minimal	0	1	0	0	0	0	0	0
Thyroid gland	No abnormalities detected	5	8	4	5	7	7	1	4
	Ultimobranchial cysts	4	2	1	0	1	2	2	1
	Lymphoid cell hyperplasia/minimal	1	0	0	0	0	0	1	0
Stomach	No abnormalities detected	9	10	5	5	9	10	3	5
	Submucosal oedema and inflammatory cells/minimal	1	0	0	0	0	0	1	0
Urinary bladder	No abnormalities detected	8	8	5	5	9	9	4	5
	Submucosalinflammatory cells/minimal	1	0	0	0	0	1	0	0
Pancreas	No abnormalities detected	9	9	3	4	9	10	4	4
	Fat infiltration/minimal	1	0	1	0	0	0	0	0
	Granulomas/slight	0	0	0	1	0	0	0	0
	Acinar cell apoptosis/minimal	0	0	0	0	0	0	0	1
	Inflammatory cells/minimal	0	1	1	0	0	0	0	0

bw, Body weight.

Applicant's summary and conclusion

Conclusions

The AgNPs didn't cause any statistically significant changes in the endpoints of repeated dose toxicity including body weight gain, water and food consumption, hematology, serum biochemistry, histopathology, urinalysis, and necropsy.Alopecia seemed to be the only minor symptom of AgNPs treatment.There was no statistically significant differences among the groups in the endpoints of reproduction/developmental toxicity tests including mating, fertility and pregnancy rate, gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post natal day 0 and day 4. Based on the findings, the NOAEL of AgNPs for repeated dose toxicity with reproductive/developmental screening test was considered to be 250 mg/kg

7.5.2 Repeated dose toxicity: inhalation

Endpoint study record: 7440-22-4, Repeated dose toxicity-inhalation, Ji, 2007, RS, K

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	2006
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	GLP study conducted according to OECD 412 Guideline with deviations: details of inhalation chamber, body weight, housing and feeding conditions, 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
publication	Ji JH, Jung JH, Kim SS, Yoon JU, Park JD, Choi BS, Chung YH, Kwon IH, Jeong J, Han BS, Shin JH, Sung JH, Song KS and Yu IJ.	2007	Twenty-Eight- Day Inhalation Toxicity Study of Silver Nanoparticles in Sprague-Dawley Rats.	Inhal Toxicol. 19(10): 857-71.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	yes (details of inhalation chamber, body weight, housing and feeding conditions, individual and summary tables of results not reported)

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): Silver powder - Source: Daedeok Science, Korea- Analytical purity: 99.98 %

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: 8 weeks

Administration / exposure

Route of administration

inhalation

Type of inhalation exposure

no data

Vehicle

other: fresh air

Details on inhalation exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

4 weeks

Frequency of treatment

Once a day, 6 h/day, 5 days/week, for 4 weeks

Doses/concentrations

low-dose group (target dose, 1.2×10^4 particles/cm3, 1.2×10^6 nm2/cm2), middle-dose group (target dose, 1.2 × 10^5 particles/cm3, 8.5 × 10^7 nm2/cm2), and high-dose group (target dose, 1.2 × 10^6 particles/ cm3, 1.8 × 10^9 nm2/cm2).

Basis nominal conc.

MMAD / GSD

Actual dose received by dose level by sex: the geometric mean diameter (GMD), geometric standard deviation (GSD), and total number concentration of silver nanoparticles were as follows- High-concentration chamber: 15.38 nm, 1.58, and 1.63 × 10^6 particles/ cm3, respectively- Medium-concentration chamber: 12.60 nm, 1.53, and 1.60 × 10^5 particles/ cm3, respectively- Low concentration chamber: 12.61 nm, 1.52, and 1.66 ×10^4 particles/ cm3, respectively.

No. of animals per sex per dose

10

Control animals

other: fresh-air control

Details on study design

No data

Positive control

Examinations

Observations and examinations performed and frequency

Not applicable
CLINICAL OBSERVATIONS: Yes
- Time schedule: Animals were examined daily on weekdays for any evidence of exposure related effects, including respiratory, dermal, behavioral, nasal, or genitourinary changes suggestive of irritancy.
BODY WEIGHT: Yes
- Time schedule for examinations: Body weights were evaluated at the time of purchase, at the time of grouping, once a week during the inhalation exposure, and before necropsy.
FOOD AND WATER CONSUMPTION: Yes
OPHTHALMOSCOPIC EXAMINATION: No
HAEMATOLOGY AND CLINICAL CHEMISTRY: Yes
URINALYSIS: No
NEUROBEHAVIOURAL EXAMINATION: No
GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
- Organs examined at necropsy (macroscopic and microscopic): The adrenal glands, bladder, testes, ovaries, uterus, epididymis, seminal vesicle, heart, thymus, thyroid gland, trachea, esophagus, tongue, prostate, lungs, nasal cavity, kidneys, spleen, liver, pancreas, and brain were all removed carefully. These organs were then weighed and fixed in a 10 % formalin solution containing neutral phosphate-buffered saline. Thereafter, the organs were embedded in paraffin, stained with hematoxylin and eosin, and examined under light microscopy.

Sacrifice and pathology

Other examinations

None

Statistics

A multiple variance analysis and Duncan’s multiple range tests

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
LOAEL	1.2×10^4 particles/cm3 (=1.2×10^6 nm2/cm2)	test mat.	male/female	

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no effects

Food consumption

no effects

Water consumption

no effects

Ophthalmoscopic examination

not examined

Haematology

yes

Clinical chemistry

yes

Urinalysis

not examined

Neurobehaviour

not examined

Organ weights

no effects

Gross pathology

no effects

Histopathology: non-neoplastic

yes

Details on results

CLINICAL SIGNS AND MORTALITY
- No remarkable clinical signs were observed.
- No death was observed in all treated groups.

BODY WEIGHT AND WEIGHT GAIN
- No significant change in body weight was observed.

FOOD AND WATER CONSUMPTION
- No significant change in food and water consumption was observed.

HAEMATOLOGY
- There was no significant dose-related changes in the hematology values for the male rats. The percentage of neutrophils and eosinophils increased significantly ($p < 0.05$) in female rats in the low-dose group when compared with the control. The MCH in the female rats in the middle-dose group increased significantly ($p < 0.05$) when compared with the female rats in the high-dose group.

CLINICAL CHEMISTRY
- The high-dose group revealed significantly increased ($p < 0.05$) calcium in both the male and female rats when compared with the control, and increased total protein ($p < 0.05$) in the male rats when compared with the control. Meanwhile, the low-dose group of male rats showed increased gamma-GT ($p < 0.05$)

when compared with the control group. The exact meaning of these differences was impossible to clarify.

ORGAN WEIGHTS
- No significant organ weight changes were observed.

GROSS PATHOLOGY
- No significant difference among dose group was observed.

HISTOPATHOLOGY: NON-NEOPLASTIC
- Histopathological examination of the male rat livers revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle- and high-dose groups, respectively. For the female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high-dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high-dose group and one case among the female rats in the high-dose group. The other organs, including the kidneys, spleen, lungs, adrenals, heart, reproductive organs, brain, and nasal cavity, were also examined histopathologically, with no distinct findings.
- The silver concentration in the lung tissue from the groups exposed to silver nanoparticles for 28 days revealed a statistically significant ($p < 0.01$) dose-dependent increase. Although no clear blood silver concentrations were detected for any of the dose groups, a clear increase ($p < 0.05$) was observed in the liver silver concentration for the high-dose group, along with a statistically significant ($p < 0.01$) increase in the brain silver concentration. The olfactory bulb, which showed higher silver-concentration levels than the brain, also revealed a dose-dependent increase ($p < 0.01$) in both the male and female rats.

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

The current 28-day study of silver nanoparticle inhalation in Sprague-Dawley rats indicated that the silver nanoparticle doses used did not cause any significant health effects. But, the lung silver concentration exhibited a dose dependent increase following silver nanoparticle inhalation exposure.

Executive summary

In a repeated dose toxicity study conducted according to the OECD 412 Guideline and in compliance with GLP,Silver nanoparticles was administered by inhalation to groups of Sprague-Dawley rats (10 animals/sex/dose) at the concentrations oflow-dose group (target dose, 1.2×104particles/cm3, 1.2×106nm2/cm2), middle-dose group (target dose, 1.2 × 105particles/cm3, 8.5 × 107nm2/cm2), and high-dose group (target dose, 1.2 × 106particles/ cm3, 1.8 × 109nm2/cm2) for6 h/day, 5 days/week, for 90 days. Fresh-air used as control.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 and food/water

consumption was observed in any of the dose groups. There were no significant dose-related changes in the hematology values for the male rats. The percentage of neutrophils and eosinophils increased significantly ($p < 0.05$) in female rats in the low-dose group when compared with the control. The MCH in the female rats in the middle-dose group increased significantly ($p < 0.05$) when compared with the female rats in the high-dose group. The high-dose group revealed significantly increased ($p < 0.05$) calcium in both the male and female rats when compared with the control, and increased total protein ($p < 0.05$) in the male rats when compared with the control. Meanwhile, the low-dose group of male rats showed increased gamma-GT ($p < 0.05$) when compared with the control group. The exact meaning of these differences was impossible to clarify. No significant gross pathological or organ weight changes were observed. Histopathological examination of the male rat livers revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle- and high-dose groups, respectively. For the female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high-dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high-dose group and one case among the female rats in the high-dose group. The other organs, including the kidneys, spleen, lungs, adrenals, heart, reproductive organs, brain, and nasal cavity, were also examined histopathologically, with no distinct findings. The silver concentration in the lung tissue from the groups exposed to silver nanoparticles for 28 days revealed a statistically significant ($p < 0.01$) dose-dependent increase. Although no clear blood silver concentrations were detected for any of the dose groups, a clear increase ($p < 0.05$) was observed in the liver silver concentration for the high-dose group, along with a statistically significant ($p < 0.01$) increase in the brain silver concentration. The olfactory bulb, which showed higher silver-concentration levels than the brain, also revealed a dose-dependent increase ($p < 0.01$) in both the male and female rats.

Under the test conditions, the Lowest Observed Adverse Effect Level (LOAEL) of Silver nanoparticles was considered to be1.2×104particles/cm3(=1.2×106nm2/cm2) in Sprague-Dawley rats.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Repeated dose toxicity-inhalation, Kim, 2009, RS, K

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	2009
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	GLP study conducted according to OECD 413 Guideline with deviations: details on the inhalation chamber, housing and feeding conditions, clinical observations, body weight, food/water consumption, clinical pathology, ophthalmoscopy and 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
publication	Kim WY, Kim J, Park JD, Ryu HY and Yu IJ.	2009	Histological Study of Gender Differences in Accumulation of Silver Nanoparticles in Kidneys of Fischer 344 Rats.	J Toxicol Environ Health A. 72: 1279-1284.					

Data access

data published

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 the inhalation chamber, housing and feeding conditions, clinical observations, body weight, food/water consumption, clinical pathology, ophthalmoscopy and individual and summary tables of results not reported)

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): Silver powder
- Source: NAMATECH Co., Ltd., Korea
- Size: 52.7–70.9 nm, average 60 nm
- Analytical purity: 99.98 %

Confidential details on test material

No data

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: 6 weeks

Administration / exposure

Route of administration

inhalation

Type of inhalation exposure

no data

Vehicle

other: 0.5 % carboxymethyl cellulose

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

90 days

Frequency of treatment

Once daily for 90 days

Doses/concentrations

low (30 mg CMC/kg/d), intermediate (125 mg CMC/kg/d), and high (500 mg CMC/kg/d)

Basis no data

MMAD / GSD

No data

No. of animals per sex per dose

4 groups (10 rats in each group)

Control animals

other: 0.5 % carboxymethyl cellulose

Details on study design

No data

Positive control

Not applicable

Examinations

Observations and examinations performed and frequency

No data was available

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
- Organs examined at necropsy (macroscopic and microscopic): Lungs and nasal cavity were removed and fixed in a 10 % formalin solution containing neutral phosphate-buffered saline. The specimens were dehydrated in a graded ethanol series and embedded in paraffin. Sections were cut at a thickness of 3 to 4 μm, deparaffinized, and stained with hematoxylin and eosin (H&E) to study the histological structure. The paraffin-embedded tissue sections were deparaffinised with xylene and hydrated with grade alcohol. The tissues were then treated with 0.5 % Triton X-100 in phosphate-buffered saline (PBS) and washed with PBS and deionized water several times. HQ SILVER (Nanoprobes, Yaphank, NY) using equal amounts of the three components (initiator, moderator, and activator) was prepared in the dark, mixed thoroughly, and the tissue sections were incubated with it for 15 min. After several washings with deionized water, the slides were stained with hematoxylin, dehydrated with grade alcohol, and mounted using Canada balsam.

Other examinations

None

Statistics

Not applicable

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
no NOAEL identified				

Results of examinations

Clinical signs and mortality

no effects

Body weight and weight gain

no data

Food consumption

no data

Food efficiency

no data

Water consumption

no data

Ophthalmoscopic examination

no data

Haematology

no data

Clinical chemistry

no data

Urinalysis

no data

Neurobehaviour

no data

Organ weights

no data

Gross pathology

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

no data

Details on results

MORTALITY
- No death was observed in all treated groups.
HISTOPATHOLOGY:
- Gender-differentiated Ag nanoparticle accumulation in the kidneys: Female rats showed a higher accumulation of Ag nanoparticles in all regions of the kidney, including the cortex, outer medulla, and inner medulla. When compared with the male specimens, the female glomerulus in the cortex displayed a higher accumulation, corresponding to concentration differences previously described by Kim et al. (2008). The outer stripe of the outer medulla (OSOM) also demonstrated a different Ag nanoparticle accumulation, along with the inner medulla (IM), including the middle portion of the inner medulla (IMm) and terminal portion of the inner medulla (IMt). The difference was even more evident at higher magnification of kidneys. The basement membrane of the glomerulus in the cortex and renal tubules in the outer medulla in

the females showed a higher accumulation of Ag nanoparticles. The nucleus of the interstitial cells in the inner medulla also displayed a strong positive response. In addition, the basement membrane of the inner medullary collecting ducts showed a higher accumulation of Ag nanoparticles in females when compared with those of males.
- Most of the Ag nanoparticles appeared to be located in the basement membrane. The urinary bladder also showed a preferential deposition of Ag nanoparticles in the basement membrane of the transitional epithelium. The capsule and medulla demonstrated a strong positive response to Ag staining in the adrenal gland. Further, the zona glomerulosa (ZG) and zona reticularis (ZR) in the cortex showed strong positive reactions, with Ag nanoparticles likely distributed in the capillary basement membrane in females. The cytoplasm and nuclei in some of the medulla cells also displayed positive reactions to Ag nanoparticles.

Any other information on results incl. tables

None

Overall remarks, attachments

Remarks on results including tables and figures

None

Attached background material

Attached document	Remarks
Inhalation toxicity - histopathology figures.pdf / 606.43 KB (application/octet-stream) **Test Substances** Identity (purity): Silver, CAS No. 7440-22-4 (silver), Purity: 99.99% Test substance was silver nanoparticles generated from solid silver wire by nanoparticle generator (ISO/10801). Concentration was adjusted by flow rate with mass flow controller. Cumulative median diameter (CMD) was 18.48(1.45) (GM (GSD)) nm. For the high concentration chamber, the geometric mean diameter, total number concentration, and surface area of silver nanoparticles measured by the DMAS were 18.93 (geometric standard deviation [GSD], 1.59) nm, 2.85×10^{6} particles/cm^3, and $6.61/cm^9$ nm^2/cm^3, respectively, whereas the measurements for the middle concentration chamber were 18.33 nm, 1.43tio^6 particles/cm^3, and $2.37/cm^9$ nm^2/cm^3, respectively, and those for the low concentration chamber were 18.12 nm, 6.64×10^{5} particles/cm^3, and $1.08/cm^9$ nm^2/cm^3, respectively. The diameters were log normally distributed between 6 and 55 nm. **Methods** Methods/guideline followed: OECD Test Guideline 413, “Subchronic inhalation toxicity: 90-day study” Test type: *In vivo* GLP: Yes Year (study performed):2007 Species: Rat Strain: Sprague-Dawley Route of administration: Inhalation-particulate Duration of test: 90 Days Doses/concentration levels: 0, 0.6×10^{6} particles/cm^3 (=49 μg/m^3), 1.4×10^{6} particles/cm^3 (=133 μg/m^3), 3.0×10^{6} particles/cm^3 (=515 μg/m^3) Sex: Both male and female Exposure period: 90 Days Frequency of treatment: continuous exposure, 6 hrs/day, 5 days/week, for 90 days	

Control group and treatment: filtered clean air Post exposure observation period: Not applicable Statistical Methods: one way analysis of variance (ANOVA) test followed by Dunnett's test **Test Conditions** Age at study initiation: Eight weeks old No. of animals per sex per dose: Ten animals per sex per dose Vehicle: Not applicable. HEPA filtered clean air was supplied to negative control group. Satellite groups and reasons they were added: Not applicable Clinical observations performed and frequency: General clinical observations were performed daily. Ophthalmological examination was made prior to the grouping of animals and at 1 week prior to termination of the study. Body weights and food/water consumptions were measured once in a week. Urinalysis was performed on the last week of treatment with 5 animals per sex per dose. Hematology and clinical biochemistry were performed with the blood collected at the necropsy. Histopathology, BAL fluid analysis, lung and kidney function test, micronuclei formation test and organ weight measurement were performed at the end of the treatment. **Results** NOAEL (NOEL): 1.0×10^6 particles/cm^3 (=100 μg/m^3) LOAEL (LOEL): Not determined Actual dose received by dose level by sex: Not applicable Toxic response/effects by dose level: Not observed Statistical results (as appropriate): Not applicable General clinical observation: No symptom was found. Body weight: Decreased in female exposed to 1.4×10^6 particles/cm^3 from 3 week exposure. Food consumption: No difference among groups Urinalysis: No significant difference among groups Ophthalmological examination: No symptom was detected. Organ weight: No significant difference was found among groups.	

Necropsy: In male, cervical lymph node congestive spot, spleen hypertrophy, bladder congestive spot, intestinal nodule, and brain retraction were detected. In female, cyst in ovary, spleen hypertrophy, black spot in liver and adrenal gland, and intestinal nodule were found.
Haematologic analysis: No significant difference found among groups.
Clinical biochemistry: Increases of creatinine and total protein and decreases of chloride were detected in male exposed to 0.6×10^6 particles/cm^3. However, all the values were within normal range and dose-response was not detected.
Histopathology: Liver and lung were appeared to be the target organs of inhaled silver nanoparticles.
BAL analysis: Concentration dependent increase in albumin, lactate dehydrogenase and total protein were found in female rat.
Lung function: Decreases in tidal volume were observed in the male rats, which was concentration-dependent. In case of female, such decrease was detected in the group exposed to 1.4×10^6 particles/cm^3.
Micronuclei test: No significant difference was found among groups.
Blood clotting time: No change was detected in prothrombin time and activated partial **t**hromboplastin time. Significant increase in erythrocyte clotting was found in female rats exposed to 3.0×10^6 particles/cm^3.
Tissue distribution: Distribution in lung, kidney, liver, blood, brain and olfactory nerve was prominent and concentration dependent.

Conclusions
Remarks: NOAEL was 1.0×10^6 particles/cm^3 or 100 μg/m^3.

References
KFDA, Korea, 2008. Subchronic (90-day) inhalation toxicity study of silver nanoparticles in rats, tested by KCL (Korea conformity Laboratory)
Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, Song MY, Jeong J, Han BS, Han JH, Chung YH, Chang HK, Lee JH, Cho MH, Kelman BJ, Yu IJ. 2009. Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci. 108 (2) : 452-61

Test Substances
Identity(Purity): Silver powder, CAS No. 7440-22-4(silver) Purity : 99.98 %
Remarks: Manufactured by Daedeok Science (Korea)

Methods Methods/guideline followed: OECD test guideline 412 Type: *In vivo* GLP: Yes Year (study performed): 2006 Species: Rat Strain: Sprague-Dawley Sex: Both male and female Route of administration: Inhalation Doses/concentration levels: the fresh-air control, low-dose group (target dose, 1.2×10^4 particles/cm^3, 1.2×10^6 nm^2/cm^2), middle-dose group (target dose, 1.2×10^5 particles/cm^3, 8.5×10^7 nm^2/cm^2), and high-dose group (target dose, 1.2×10^6 particles/cm^3, 1.8×10^9 nm^2/cm^2). Duration of test: Silver nanoparticles for 6 h/day, 5 days/wk, for 4 wk. Exposure period: 4 weeks Frequency of treatment: Once a day Post exposure observation period: Not applicable Statistical Methods: A multiple variance analysis and Duncan's multiple range tests **Test Conditions** Age at study initiation: 8-week old male and female No. of animals per sex per dose: 4 groups (10 rats in each group): the fresh-air control, low-dose group (target dose, 1.2×10^4 particles/cm^3, 1.2×10^6 nm^2/cm^2), middle-dose group (target dose, 1.2×10^5 particles/cm^3, 8.5×10^7 nm^2/cm^2), and high-dose group (target dose, 1.2×10^6 particles/cm^3, 1.8×10^9 nm^2/cm^2). Satellite groups and reasons they were added: Not applicable Clinical observations performed and frequency: The animals were examined daily on weekdays for any evidence of exposurerelated effects, including respiratory, dermal, behavioral, nasal, or genitourinary changes suggestive of irritancy. The body weights were evaluated at the time of purchase, at the time of grouping, once a week during the inhalation exposure, and before necropsy. Organs examined at necropsy (macroscopic and microscopic): The adrenal glands, bladder, testes, ovaries, uterus, epididymis, seminal vesicle, heart, thymus, thyroid gland, trachea, esophagus, tongue, prostate, lungs,	

nasal cavity, kidneys, spleen, liver, pancreas, and brain were all removed carefully. These organs were then weighed and fixed in a 10% formalin solution containing neutral phosphate-buffered saline. Thereafter, the organs were embedded in paraffin, stained with hematoxylin and eosin, and examined under light microscopy. **Results** NOAEL (NOEL): Not determined LOAEL (LOEL): Not determined Actual dose received by dose level by sex: In the high-concentration chamber, the geometric mean diameter (GMD), geometric standard deviation (GSD), and total number concentration of silver nanoparticles were 15.38 nm, 1.58, and 1.63×10^6 particles/cm^3, respectively; in the medium-concentration chamber, they were 12.60 nm, 1.53, and 1.60×10^5 particles/cm^3, respectively; and in the low concentration chamber, they were 12.61 nm, 1.52, and 1.66×10^4 particles/cm^3, respectively. Toxic response/effects by dose level: LOAEL : low-dose group (target dose, 1.2×10^4 particles/cm^3, 1.2×10^6 nm^2/cm^2) Body weight: No significant change Food/water consumption: No significant change Description, severity, time of onset and duration of clinical signs: No remarkable clinical sign Haematological findings incidence and severity: The high-dose group revealed significantly increased ($p < .05$) calcium in both the male and female rats when compared with the control, and increased total protein ($p < .05$) in the male rats when compared with the control. Meanwhile, the low-dose group of male rats showed increased gamma-GT ($p < .05$) when compared with the control group. The exact meaning of these differences was impossible to clarify. Clinical biochemistry findings incidence and severity: There were no significant dose-related changes in the hematology values for the male rats. The percentage of neutrophils and eosinopils increased significantly ($p < .05$) in female rats in the low-dose group when compared with the control. The MCH in the female rats in the middle-dose group increased significantly ($p < .05$) when compared with the female rats in the high-dose group. Mortality and time to death: No death was observed in all treated groups. Gross pathology incidence and severity: No significant differences between treated male and female rats and control group. Organ weight changes: No significant organ-weight changes Histopathology incidence and severity: Histopathological examination of the male rat livers revealed one case of cytoplasmic vacuolization in the	

control, four cases in the low-dose group, and one case each in the middle- and high-dose groups, respectively. For the female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high-dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high-dose group and one case among the female rats in the high-dose group. The other organs, including the kidneys, spleen, lungs, adrenals, heart, reproductive
organs, brain, and nasal cavity, were also examined histopathologically, with no distinct findings.
The silver concentration in the lung tissue from the groups exposed to silver nanoparticles for 28 days revealed a statistically significant ($p < .01$) dose-dependent increase. Although no clear blood silver concentrations were detected for any of the dose groups, a clear increase ($p < .05$) was observed in the liver silver concentration for the high-dose group, along with a statistically significant ($p < .01$) increase in the brain silver concentration. The olfactory bulb, which showed higher silver-concentration levels than the brain, also revealed a dose-dependent increase ($p < .01$) in both the male and female rats.

Conclusions
The current 28-day study of silver nanoparticle inhalation in Sprague-Dawley rats indicated that the silver nanoparticle doses used did not cause any significant health effects. But, the lung silver concentration exhibited a dose dependent increase following silver nanoparticle inhalation exposure.

References
OECD Guidelines for the Testing of Chemicals No. 412 'Repeated dose inhalation toxicity: 28-day or 14-day study'(Adopted May, 1981)
Ji JH et. al., Twenty-Eight-Day Inhalation Toxicity Study of Silver Nanoparticles in Sprague-Dawley Rats. Inhalation Toxicology, 19:857–871, 2007

Test Substances
Identity(Purity): Silver powder, CAS No. 7440-22-4(silver) Purity : 99.98 % (52.7–70.9 nm, average 60 nm)
Remarks: Manufactured by NAMATECH Co., Ltd (Korea)

Methods
Methods/guideline followed: OECD test guideline 413

Type: *In vivo*
GLP: Yes
Year (study performed):2009

Species: Rat Strain: Fischer 344 (F344) Sex: Both male and female Route of administration: Inhalation Doses/concentration levels: vehicle control (0.5% carboxymethyl cellulose), low (30 mg CMC/kg/d), intermediate (125 mg CMC/kg/d), and high (500 mg CMC/kg/d) Exposure period: 90 days Frequency of treatment: Once a day Post exposure observation period: Not applicable Statistical Methods: Not applicable **Test Conditions** Age at study initiation: 6-week old male and female No. of animals per sex per dose: 4 groups (10 rats in each group): vehicle control (0.5% carboxymethyl cellulose), low (30 mg CMC/kg/d), intermediate (125 mg CMC/kg/d), and high (500 mg CMC/kg/d) Satellite groups and reasons they were added: Not applicable Clinical observations performed and frequency: Not applicable Organs examined at necropsy (macroscopic and microscopic): Lungs and nasal cavity were removed and fixed in a 10% formalin solution containing neutral phosphate-buffered saline. The specimens were dehydrated in a graded ethanol series and embedded in paraffin. Sections were cut at a thickness of 3 to 4 μm, deparaffinized, and stained with hematoxylin and eosin (H&E) to study the histological structure. The paraffin-embedded tissue sections were deparaffinised with xylene and hydrated with grade alcohol. The tissues were then treated with 0.5% Triton X-100 in phosphate-buffered saline (PBS) and washed with PBS and deionized water several times. HQ SILVER (Nanoprobes, Yaphank, NY) using equal amounts of the three components (initiator, moderator, and activator) was prepared in the dark, mixed thoroughly, and the tissue sections were incubated with it for 15 min. After several washings with deionized water, the slides were stained with hematoxylin, dehydrated with grade alcohol, and mounted using Canada balsam.	

Results NOAEL (NOEL): Not determined LOAEL (LOEL): Not determined Mortality and time to death: No death was observed in all treated groups. Histopathology incidence and severity: Figure 1 shows the gender-differentiated Ag nanoparticle accumulation in the kidneys. Female rats showed a higher accumulation of Ag nanoparticles in all regions of the kidney, including the cortex, outer medulla, and inner medulla (Figure 1). When compared with the male specimens, the female glomerulus in the cortex displayed a higher accumulation (Figure 1, D and E vs. K and L), corresponding to concentration differences previously described by Kim et al. (2008). The outer stripe of the outer medulla (OSOM) also demonstrated a different Ag nanoparticle accumulation (Figure 1, F and G vs. M and N), along with the inner medulla (IM), including the middle portion of the inner medulla (IMm) and terminal portion of the inner medulla (IMt) (Figure 1, I and J vs. P and Q). The difference was even more evident at higher magnification of kidneys (Figure 2). The basement membrane of the glomerulus in the cortex (Figure 2, A and B vs. H and I) and renal tubules in the outer medulla in the females (Figure 2, C and D vs. J and K) showed a higher accumulation of Ag nanoparticles. The nucleus of the interstitial cells in the inner medulla also displayed a strong positive response (Figure 2F and G). In addition, the basement membrane of the inner medullary collecting ducts showed a higher accumulation of Ag nanoparticles in females when compared with those of males (Figure 2, F and G vs. M and N). Most of the Ag nanoparticles appeared to be located in the basement membrane. The urinary bladder also showed a preferential deposition of Ag nanoparticles in the basement membrane of the transitional epithelium (Figure 3). The capsule and medulla demonstrated a strong positive response to Ag staining in the adrenal gland (Figure 4, C and D vs. E and F). Further, the zona glomerulosa (ZG) (Figure 5, E vs. H) and zona reticularis (ZR) (Figure 5, E vs. I) in the cortex showed strong positive reactions, with Ag nanoparticles likely distributed in the capillary basement membrane in females. The cytoplasm and nuclei in some of the medulla cells also displayed positive reactions to Ag nanoparticles (Figure 5, F and J).	

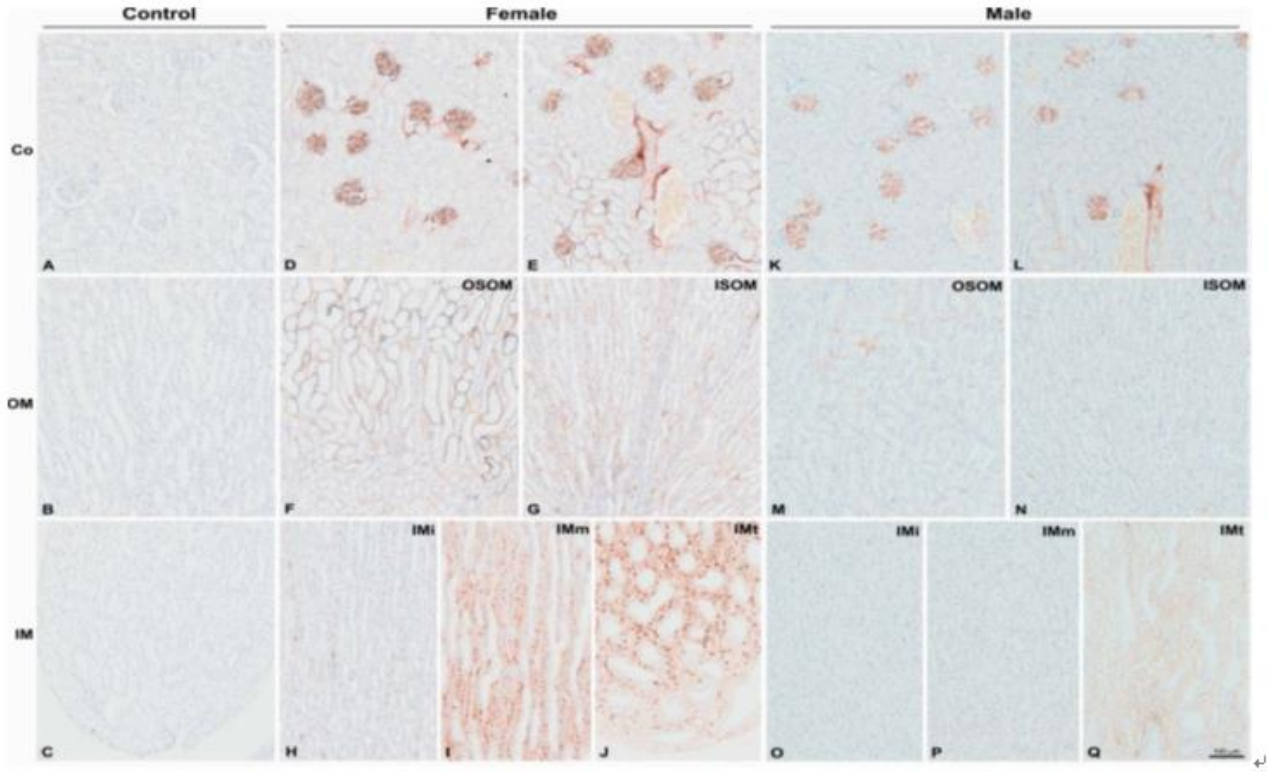


FIG. 1. Low magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 100 μm.

FIG. 1. Low magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 100 μm.

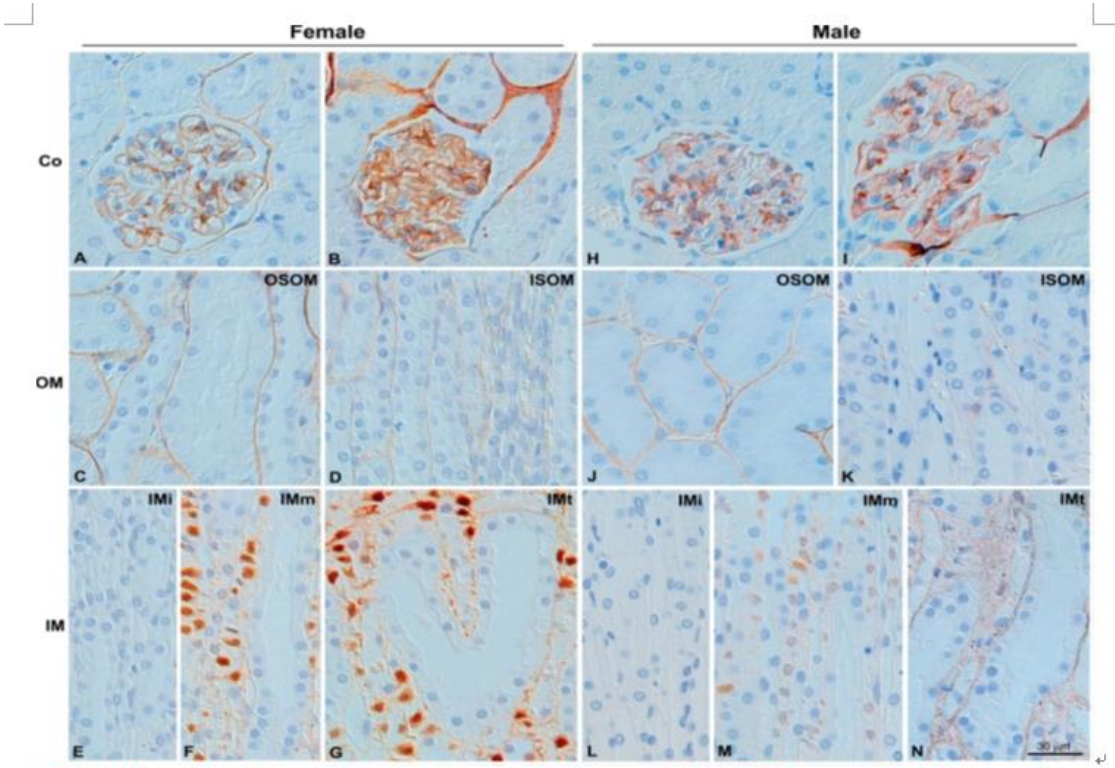


FIG. 2. High magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 30 μm.

FIG. 2. High magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 30 μm.

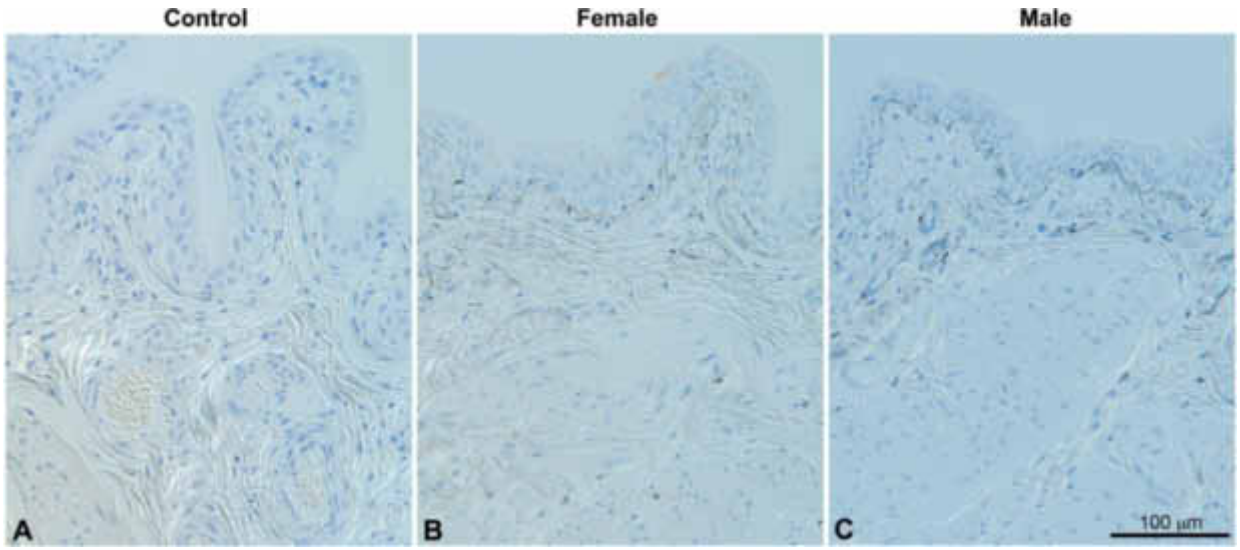


FIG. 3. Urinary bladder. The bar indicates 100 µm.

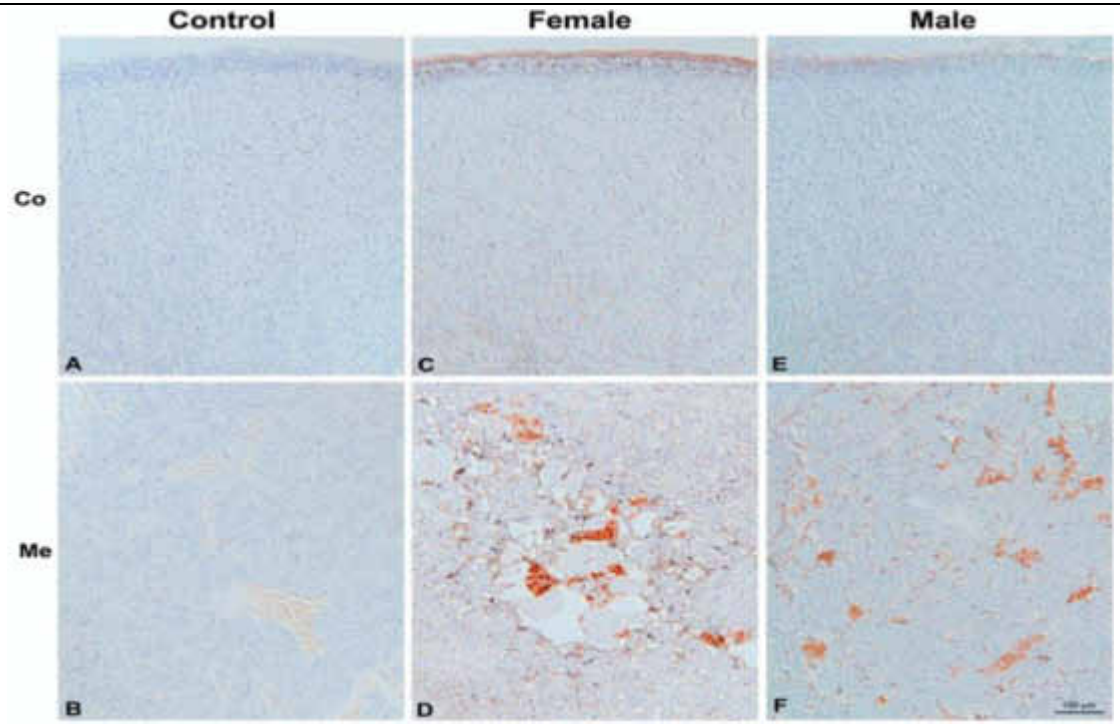


FIG. 4. Adrenal gland. The bar indicates 100 µm

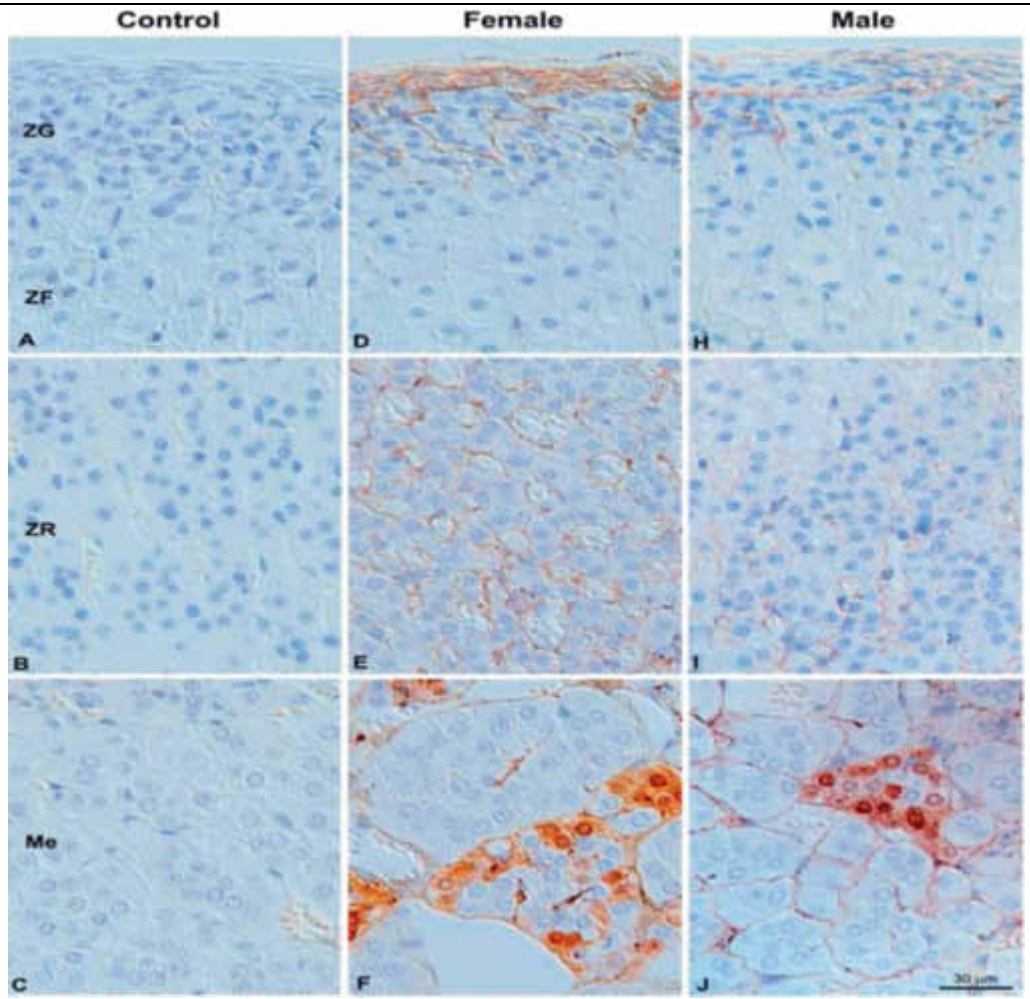


FIG. 5. Adrenal gland with high magnification. ZG, zona glomerulosa; ZF, zona fasciculate; ZR, zona reticularis; Me, medulla. The bar indicates 30 µm.

Conclusions Female rats showed a higher accumulation of Ag nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males. **References** Kim WY et. al., Histological Study of Gender Differences in Accumulation of Silver Nanoparticles in Kidneys of Fischer 344 Rats. Journal of Toxicology and Environmental Health, Part A, 72: 1279–1284, 2009	

Applicant's summary and conclusion

Conclusions

Under the test conditions, female rats showed a higher accumulation of Silver nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males.

Executive summary

In a repeated dose toxicity study conducted according to the OECD Guideline 413 and in compliance with GLP, Silver nanoparticles was administered by inhalation to four groups ofFischer 344rats (10 rats in each group; males and females) at the concentrations of 0 (vehicle control - 0.5 % carboxymethyl cellulose), 30, 125 and 500 mg/kg bw/day, once daily for 90 days. Mortality and histopathological examination was performed.
No mortality was observed. Female rats showed a higher accumulation of Ag nanoparticles in all regions of the kidney, including the cortex, outer medulla, and inner medulla. when compared with the male specimens, the female glomerulus in the cortex displayed a higher accumulation, corresponding to concentration differences previously described by Kim et al. (2008). The outer stripe of the outer medulla (OSOM) also demonstrated a different Ag nanoparticle accumulation, along with the inner medulla (IM), including the middle portion of the inner medulla (IMm) and terminal portion of the inner medulla (IMt). The difference was even more evident at higher magnification of kidneys. The basement membrane of the glomerulus in the cortex and renal tubules in the outer medulla in the females showed a higher accumulation of Ag nanoparticles. The nucleus of the interstitial cells in the inner medulla also displayed a strong positive response. In addition, the basement membrane of the inner medullary collecting ducts showed a higher accumulation of Ag nanoparticles in females when compared with those of males. Most of the Ag nanoparticles appeared to be located in the basement membrane. The urinary bladder also showed a preferential deposition of Ag nanoparticles in the basement membrane of the transitional epithelium. The capsule and medulla demonstrated a strong positive response to Ag staining in the adrenal gland. Further, the zona glomerulosa (ZG) and zona reticularis (ZR) in the cortex showed strong positive reactions, with Ag nanoparticles likely distributed in the capillary basement membrane in females. The cytoplasm and nuclei in some of the medulla cells also displayed positive reactions to Ag nanoparticles.
Under the test conditions, female rats showed a higher accumulation of Silver nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Repeated dose toxicity-inhalation, Sung, 2009, 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 the inhalation chamber, body weight and housing and feeding conditions and 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	2008	Subchronic (90-Day) Inhalation Toxicity Study of Silver Nanoparticles in Rats.		KCL (Korea conformity Laboratory)		KFDA, Korea		
publication	Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, Song MY, Jeong J, Han BS, Han JH, Chung YH, Chang HK, Lee JH, Cho MH, Kelman BJ and Yu IJ.	2009	Subchronic Inhalation Toxicity of Silver Nanoparticles.	Toxicol Sci. 108(2): 452-61.					

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 the inhalation chamber, body weight and housing and feeding conditions and individual and summary tables of results not reported)

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): Silver nanoparticles
- Analytical purity: 99.99 %
- Silver nanoparticles generated from solid silver wire by nanoparticle generator (ISO/10801).
- The diameters were log normally distributed between 6 and 55 nm.

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: 8 weeks

Administration / exposure

Route of administration

other: inhalation-particulate

Type of inhalation exposure

whole body

Vehicle

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

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE
- Concentration was adjusted by flow rate with mass flow controller.
Geometric mean diameter, total number concentration, and surface area of silver nanoparticles measured by the DMAS were as follows:
- High concentration chamber: 18.93 (geometric standard deviation [GSD], 1.59) nm, 2.85×10^6 particles/ cm3, and 6.61/ cm9 nm2/cm3, respectively.
- Middle concentration chamber: 18.33 nm, 1.43 x10^6 particles/cm3, and 2.37/cm9 nm2/cm3,

respectively.
- Low concentration chamber: 18.12 nm, 6.64×10^5 particles/cm3, and 1.08/ cm9 nm2/cm3, respectively. The diameters were log normally distributed between 6 and 55 nm.

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

Not applicable

Duration of treatment / exposure

90 days

Frequency of treatment

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

Doses/concentrations

0, 0.6x10^6 particles/cm3 (=49 µg/m3), 1.4 x10^6 particles/ cm3 (=133 µg/m3) and 3.0 x10^6 particles/ cm3 (=515 µg/m3)

Basis nominal conc.

MMAD / GSD

Cumulative median diameter (CMD) was 18.48(1.45) (GM (GSD)) nm.

No. of animals per sex per dose

10

Control animals

other: filtered clean air

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 weekly
FOOD AND WATER CONSUMPTION: Yes
- Time schedule for examinations: Once weekly
OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: Ophthalmological 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: At necropsy
- Anaesthetic used for blood collection: No data

- Animals fasted: No data
URINALYSIS: Yes
- Time schedule for collection of urine: Urinalysis was performed on the last week of treatment with 5 animals per sex per dose.
- Metabolism cages used for collection of urine: No data
- Animals fasted: No data
NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes
HISTOPATHOLOGY: Yes
- Histopathology, BAL fluid analysis, lung and kidney function test, micronuclei formation test 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/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

yes

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

no data

Details on results

CLINICAL SIGNS AND MORTALITY
- No clinical signs were observed.
BODY WEIGHT AND WEIGHT GAIN
- Decrease in body weight in females exposed to 1.4x10^6 particles/cm3 from 3 week exposure.
FOOD CONSUMPTION
- No significant difference in food consumption was observed in any of the dose groups.
OPHTHALMOSCOPIC EXAMINATION
- No symptom was detected.
HAEMATOLOGY
- No significant difference in haematology was observed between treated and control groups.
CLINICAL CHEMISTRY
- Increases of creatinine and total protein and decreases of chloride were detected in male exposed to 0.6x10^6 particles/cm3. However, all the values were within normal range and dose-response was not detected.
URINALYSIS
- No significant difference in urinalysis was observed between treated and control groups.
ORGAN WEIGHTS
- No significant difference was found among groups.
GROSS PATHOLOGY
- In male, cervical lymph node congestive spot, spleen hypertrophy, bladder congestive spot, intestinal nodule, and brain retraction were detected. In female, cyst in ovary, spleen hypertrophy, black spot in liver and adrenal gland, and intestinal nodule were observed.
HISTOPATHOLOGY: NON-NEOPLASTIC
- Liver and lung were appeared to be the target organs of inhaled silver nanoparticles.
OTHER FINDINGS
- BAL analysis: Concentration dependent increase in albumin, lactate dehydrogenase and total protein were found in female rat.
- Lung function: Decreases in tidal volume were observed in the male rats, which was concentration-dependent. In case of female, such decrease was detected in the group exposed to 1.4x10^6 particles/cm3.
- Micronuclei test: No significant difference was found among groups.
- Blood clotting time: No change was detected in prothrombin time and activated partial thromboplastin time. Significant increase in erythrocyte clotting was found in female rats exposed to 3.0x10^6

particles/cm3.
- 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 NOAEL of Silver nanoparticles was 1.0x10^6 particles/cm3 or 100 µg/m3 in Sprague-Dawley rats.

Executive summary

In a repeated dose toxicity study conducted according to the OECD Guideline 413 and in compliance with GLP, Silver nanoparticles was administered by inhalation-particulate to groups of Sprague-Dawley rats (10 animals/sex/dose) at the concentrations of0, $0.6x10^6$particles/cm^3(=49 µg/m^3), 1.4 $x10^6$particles/ cm^3(=133 µg/m^3) and 3.0 $x10^6$particles/ cm^3(=515 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. Decrease in body weight in females exposed to $1.4x10^6$particles/$cm^3$from 3 week exposure. No significant difference in food consumption was observed in any of the dose groups. No significant difference in haematology and urinalysis were observed between treated and control groups. Increases of creatinine and total protein and decreases of chloride were detected in male exposed to $0.6x10^6$particles/cm^3. However, all the values were within normal range and dose-response was not detected. No abnormalities were observed in ophthalmoscopic examination. In male, cervical lymph node congestive spot, spleen hypertrophy, bladder congestive spot, intestinal nodule, and brain retraction were detected. In female, cyst in ovary, spleen hypertrophy, black spot in liver and adrenal gland, and intestinal nodule were observed. No significant difference was observed in organ weight s in any of the dose groups. Liver and lung were appeared to be the target organs of inhaled silver nanoparticles. BAL analysis revealed concentration dependent increase in albumin, lactate dehydrogenase and total protein were found in female rat. Decreases in tidal volume were observed in the male rats, which was concentration-dependent. In case of female, such decrease was detected in the group exposed to $1.4x10^6$particles/cm^3. No change was detected in prothrombin time and activated partial thromboplastin time. Significant increase in erythrocyte clotting was found in female rats exposed to $3.0x10^6$particles/cm^3. Distribution in lung, kidney, liver, blood, brain and olfactory nerve was prominent and concentration dependent.

Under the test conditions, the NOAEL of Silver nanoparticles was $1.0x10^6$particles/cm^3or 100 µg/m^3in Sprague-Dawley rats.

Cross-reference to other study

No cross-reference

7.5.3 Repeated dose toxicity: dermal

Endpoint study record: Repeated dose toxicity: inhalation.002

Administrative Data

Study result type experimental result Study period 2006

Reliability 1 (reliable without restriction)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Ji JH et al.	2007	Twenty-Eight-Day Inhalation Toxicity Study of Silver Nanoparticles in Sprague-Dawley Rats.	Inhalation Toxicology, 19:857–871					
publication	OECD	1981	OECD Guidelines for the Testing of Chemicals No. 412 'Repeated dose inhalation toxicity: 28-day or 14-day study'	Adopted May, 1981					

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

other:

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	no

GLP compliance

yes

Test materials

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

Details on test animals and environmental conditions Age at study initiation: 8-week old male and female Administration / exposure Route of administration inhalation Type of inhalation exposure no data Details on inhalation exposure Duration of test: Silver nanoparticles for 6 h/day, 5 days/wk, for 4 wk. Analytical verification of doses or concentrations yes Details on analytical verification of doses or concentrations the fresh-air control, low-dose group (target dose, 1.2×10^4 particles/cm^3, 1.2×10^6 nm^2/cm^2), middle-dose group (target dose, 1.2 × 105 particles/cm3, 8.5 × 10^7 nm^2/cm^2), and high-dose group (target dose, 1.2 × 10^6 particles/cm^3, 1.8 × 10^9 nm2/cm^2). MMAD / GSD In the high-concentration chamber, the geometric mean diameter (GMD), geometric standard deviation (GSD), and total number concentration of silver nanoparticles were 15.38 nm, 1.58, and 1.63 × 10^6 particles/cm^3, respectively; in the medium-concentration chamber, they were 12.60 nm, 1.53, and 1.60 × 105 particles/cm^3, respectively; and in the low concentration chamber, they were 12.61 nm, 1.52, and 1.66 ×10^4 particles/cm3, respectively.Details on study design no data

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

Age at study initiation: 8-week old male and female

Administration / exposure

Type of coverage

no data

Vehicle

no data

Details on exposure

Duration of test: Silver nanoparticles for 6 h/day, 5 days/wk, for 4 wk. Route of administration inhalation Type of inhalation exposure no data

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

the fresh-air control, low-dose group (target dose, 1.2×10^4 particles/cm^3, 1.2×10^6 nm^2/cm^2), middle-dose group (target dose, 1.2 × 105 particles/cm3, 8.5 × 10^7 nm^2/cm^2), and high-dose group (target dose, 1.2 × 10^6 particles/cm^3, 1.8 × 10^9 nm2/cm^2).

Duration of treatment / exposure

Silver nanoparticles for 6 h/day, 5 days/wk, for 4 wk.

Frequency of treatment

Once a day.

Doses/concentrations

the fresh-air control, low-dose group (target dose, 1.2×104 particles/cm3, 1.2×106 nm2/cm2), middle-dose group (target dose, 1.2 × 105 particles/cm3, 8.5 × 107 nm2/cm2), and high-dose group (target dose, 1.2 × 106 particles/cm3, 1.8 × 109 nm2/cm2).

Basis other: Analytical conc.

No. of animals per sex per dose

4 groups (10 rats in each group)

Control animals

yes

Details on study design

MMAD / GSD In the high-concentration chamber, the geometric mean diameter (GMD), geometric standard deviation (GSD), and total number concentration of silver nanoparticles were 15.38 nm, 1.58, and 1.63 × 10^6 particles/cm^3, respectively; in the medium-concentration chamber, they were 12.60 nm, 1.53, and 1.60 × 105 particles/cm^3, respectively; and in the low concentration chamber, they were 12.61 nm, 1.52, and 1.66 ×10^4 particles/cm3, respectively.Details on study design no data

Examinations

Observations and examinations performed and frequency

Frequency of treatment: Once a day
Post exposure observation period: Not applicable
Clinical observations performed and frequency: The animals were examined daily on weekdays for any evidence of exposurerelated effects, including respiratory, dermal, behavioral, nasal, or genitourinary changes suggestive of irritancy.
Body weights : at the time of purchase, at the time of grouping, once a week during the inhalation exposure, and before necropsy.

Sacrifice and pathology

Organs examined at necropsy (macroscopic and microscopic): The adrenal glands, bladder, testes, ovaries, uterus, epididymis, seminal vesicle, heart, thymus, thyroid gland, trachea, esophagus, tongue, prostate, lungs, nasal cavity, kidneys, spleen, liver, pancreas, and brain were all removed carefully.

Statistics

A multiple variance analysis and Duncan’s multiple range tests

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
LOAEL		other: not determined	male/female	
NOAEL		other: Not determined	male/female	

Results of examinations

Clinical signs and mortality

no effects

Dermal irritation

no effects

Body weight and weight gain

no effects

Food consumption

no effects

Food efficiency

no effects

Water consumption

no effects

Ophthalmoscopic examination

no data

Haematology

yes

Clinical chemistry

no effects

Urinalysis

no data

Neurobehaviour

no data

Organ weights

no effects

Gross pathology

no effects

Histopathology: non-neoplastic

yes

Histopathology: neoplastic

no effects

Details on results

-Body weight: No significant change
-Food/water consumption: No significant change
-Description, severity, time of onset and duration of clinical signs: No remarkable clinical sign
-Haematological findings incidence and severity: The high-dose group revealed significantly increased (p

<.05) calcium in both the male and female rats when compared with the control, and increased total protein ($p < .05$) in the male rats when compared with the control. Meanwhile, the low-dose group of male rats showed increased gamma-GT ($p < .05$) when compared with the control group. The exact meaning of these differences was impossible to clarify. Clinical biochemistry findings incidence and severity: There were no significant dose-related changes in the hematology values for the male rats. The percentage of neutrophils and eosinopils increased significantly ($p < .05$) in female rats in the low-dose group when compared with the control. The MCH in the female rats in the middle-dose group increased significantly ($p < .05$) when compared with the female rats in the high-dose group.
-Mortality and time to death: No death was observed in all treated groups.
-Gross pathology incidence and severity: No significant differences between treated male and female rats and control group.
-Organ weight changes: No significant organ-weight changes
-Histopathology incidence and severity: Histopathological examination of the male rat livers revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle- and high-dose groups, respectively. For the female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high-dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high-dose group and one case among the female rats in the high-dose group. The other organs, including the kidneys, spleen, lungs, adrenals, heart, reproductive organs, brain, and nasal cavity, were also examined histopathologically, with no distinct findings.The silver concentration in the lung tissue from the groups exposed to silver nanoparticles for 28 days revealed a statistically significant ($p < .01$) dose-dependent increase. Although no clear blood silver concentrations were detected for any of the dose groups, a clear increase ($p < .05$) was observed in the liver silver concentration for the high-dose group, along with a statistically significant ($p < .01$) increase in the brain silver concentration. The olfactory bulb, which showed higher silver-concentration levels than the brain, also revealed a dose-dependent increase ($p < .01$) in both the male and female rats.

Overall remarks, attachments

Remarks on results including tables and figures

Actual dose received by dose level by sex: In the high-concentration chamber, the geometric meandiameter (GMD), geometric standard deviation (GSD), and total number concentration of silver nanoparticles were 15.38 nm, 1.58, and 1.63 × 106 particles/cm3, respectively; in the medium-concentration chamber, they were 12.60 nm, 1.53, and 1.60 × 105 particles/cm3, respectively; and in the low concentration chamber, they were 12.61 nm, 1.52, and 1.66 ×104 particles/cm3, respectively.
Toxic response/effects by dose level:
LOAEL : low-dose group (target dose, 1.2×104 particles/cm3, 1.2×106 nm2/cm2)Body weight: No significant changeFood/water consumption: No significant changeDescription, severity, time of onset and duration of clinical signs: No remarkable clinical sign
Haematological findings incidence and severity: The high-dose group revealed significantly increased($p <.05$) calcium in both the male and female rats when compared with the control, and increased total protein ($p < .05$) in the male rats when compared with the control. Meanwhile, the low-dose group of male rats showed increased gamma-GT ($p < .05$) when compared with the control group. The exact meaning of these differences was impossible to clarify.
Clinical biochemistry findings incidence and severity: There were no significant dose-related changes in the hematology values for the male rats. The percentage of neutrophils and eosinopils increased significantly ($p < .05$) in female rats in the low-dose group when compared with the control. The MCH in the female rats in the middle-dose group increased significantly ($p < .05$) when compared with the female rats in the high-dose group.
Mortality and time to death: No death was observed in all treated groups.

Gross pathology incidence and severity: No significant differences between treated male and female rats and control group.
Organ weight changes: No significant organ-weight changes
Histopathology incidence and severity: Histopathological examination of the male rat livers revealed one case of cytoplasmic vacuolization in the control, four cases in the low-dose group, and one case each in the middle- and high-dose groups, respectively. For the female rats, two cases each of cytoplasmic vacuolization were detected in the control and low-dose group, respectively, six cases in the middle dose group, and seven cases in the high-dose group. Two cases of hepatic focal necrosis were detected among the male rats in the high-dose group and one case among the female rats in the high-dose group. The other organs, including the kidneys, spleen, lungs, adrenals, heart, reproductiveorgans, brain, and nasal cavity, were also examined histopathologically, with no distinct findings.The silver concentration in the lung tissue from the groups exposed to silver nanoparticles for 28 days revealed a statistically significant ($p < .01$) dose-dependent increase. Although no clear blood silver concentrations were detected for any of the dose groups, a clear increase ($p < .05$) was observed in the liver silver concentration for the high-dose group, along with a statistically significant ($p < .01$) increase in the brain silver concentration. The olfactory bulb, which showed higher silver-concentration levels than the brain, also revealed a dose-dependent increase ($p < .01$) in both the male and female rats.

Applicant's summary and conclusion

Conclusions

The current 28-day study of silver nanoparticle inhalation in Sprague-Dawley rats indicated that the silver nanoparticle doses used did not cause any significant health effects. But, the lung silver concentration exhibited a dose dependent increase following silver nanoparticle inhalation exposure.

Endpoint study record: Repeated dose toxicity: inhalation.003

Administrative Data

Study result type experimental result Study period 2009

Reliability 1 (reliable without restriction)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Kim WY et. al.		Histological Study of Gender Differences in Accumulation of Silver Nanoparticles in Kidneys of Fischer 344 Rats	Journal of Toxicology and Environmental Health, Part A, 72: 1279–1284					

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

subchronic

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Test Guideline(Repeated Dose 90-Day Oral Toxicity Study in Rodents)	no data

GLP compliance

yes (incl. certificate)

Test materials

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

Purity : 99.98 % Particle size : 52.7–70.9 nm, average 60 nm

Test animals

Species

rat

Strain

Fischer 344

Sex

male/female

Details on test animals and environmental conditions

Age at study initiation: 6-week old male and female

Administration / exposure

Vehicle

CMC (carboxymethyl cellulose)

Details on exposure

Route of administration inhalation Type of inhalation exposure no data Details on inhalation exposure no data

Duration of treatment / exposure

90 days.

Frequency of treatment

Once a day.

Doses/concentrations

vehicle control (0.5% carboxymethyl cellulose), low (30 mg CMC/kg/d), intermediate (125 mg CMC/kg/d), and high (500 mg CMC/kg/d) Basis no data

No. of animals per sex per dose

4 groups (10 rats in each group)

Details on study design

yes

Examinations

Observations and examinations performed and frequency

No data

Sacrifice and pathology

Organs examined at necropsy (macroscopic and microscopic): Yes

Statistics

Not applicable

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
LOAEL ((LOEL))			male/female	Not determined

Results of examinations

Clinical signs and mortality

no effects

Dermal irritation

no data

Body weight and weight gain

no data

Food consumption

no data

Food efficiency

no data

Water consumption

no data

Ophthalmoscopic examination

no data

Haematology

no data

Clinical chemistry

no data

Urinalysis

no data

Neurobehaviour

no data

Gross pathology

no data

Histopathology: non-neoplastic

no effects

Histopathology: neoplastic

no effects

Any other information on results incl. tables

Histopathology incidence and severity: Figure 1 shows the gender-differentiated Ag nanoparticle accumulation in the kidneys. Female rats showed a higher accumulation of Ag nanoparticles in all regions of the kidney, including the cortex, outer medulla, and inner medulla (Figure 1). When compared with the male specimens, the female glomerulus in the cortex displayed a higher accumulation (Figure 1, D and E vs. K and L), corresponding to concentration differences previously described by Kim et al. (2008). The outer stripe of the outer medulla (OSOM) also demonstrated a different Ag nanoparticle accumulation (Figure 1, F and G vs. M and N), along with the inner medulla (IM), including the middle portion of the inner medulla (IMm) and terminal portion of the inner medulla (IMt) (Figure 1, I and J vs. P and Q). The difference was even more evident at higher magnification of kidneys (Figure 2). The basement membrane of the glomerulus in the cortex (Figure 2, A and B vs. H and I) and renal tubules in the outer medulla in the females (Figure 2, C and D vs. J and K) showed a higher accumulation of Ag nanoparticles. The nucleus of the interstitial cells in the inner medulla also displayed a strong positive response (Figure 2F and G). In addition, the basement membrane of the inner medullary collecting ducts showed a higher accumulation of Ag nanoparticles in females when compared with those of males (Figure 2, F and G vs. M and N).Most of the Ag nanoparticles appeared to be located in the basement membrane. The urinary bladder also showed a preferential deposition of Ag nanoparticles in the basement membrane of the transitional epithelium (Figure 3). The capsule and medulla demonstrated a strong positive response to Ag staining in the adrenal gland (Figure 4, C and D vs. E and F). Further, the zona glomerulosa (ZG) (Figure 5, E vs. H) and zona reticularis (ZR) (Figure 5, E vs. I) in the cortex showed strong positive reactions, with Ag nanoparticles likely distributed in the capillary basement membrane in females. The cytoplasm and nuclei in some of the medulla cells also displayed positive reactions to Ag nanoparticles (Figure 5, F and J).

Overall remarks, attachments
Attached background material
Attached document

fig1.jpg / 264.41 KB (image/pjpeg)

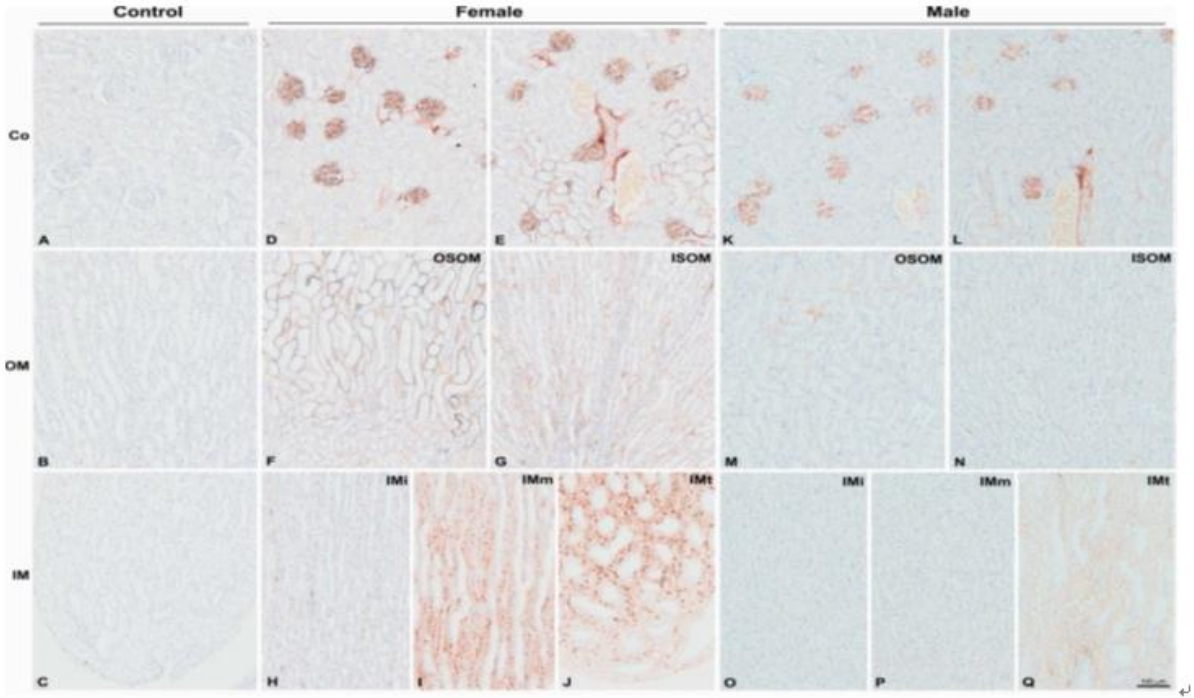


FIG. 1. Low magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 100 μm.

fig 2.jpg / 356.07 KB (image/pjpeg)

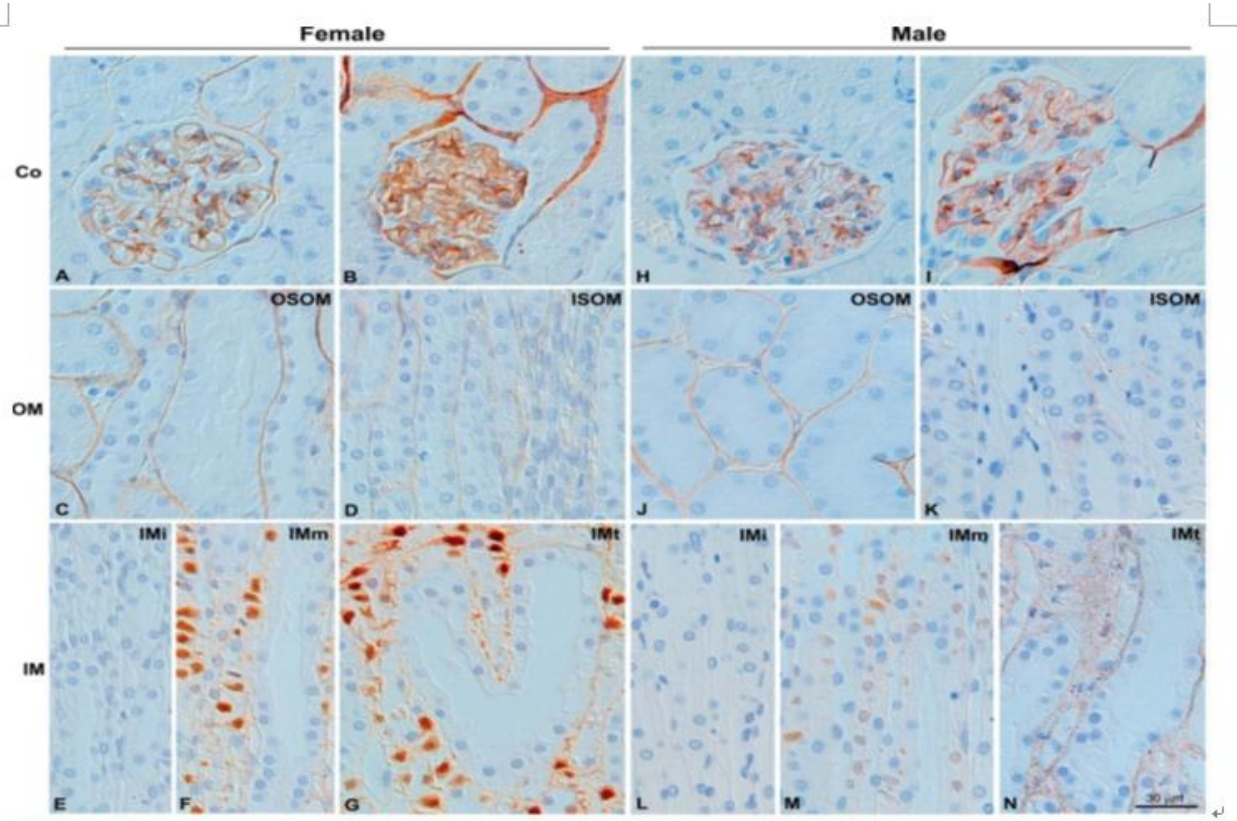


FIG. 2. High magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 30 μm.

fig 3.jpg / 159.48 KB (image/pjpeg)

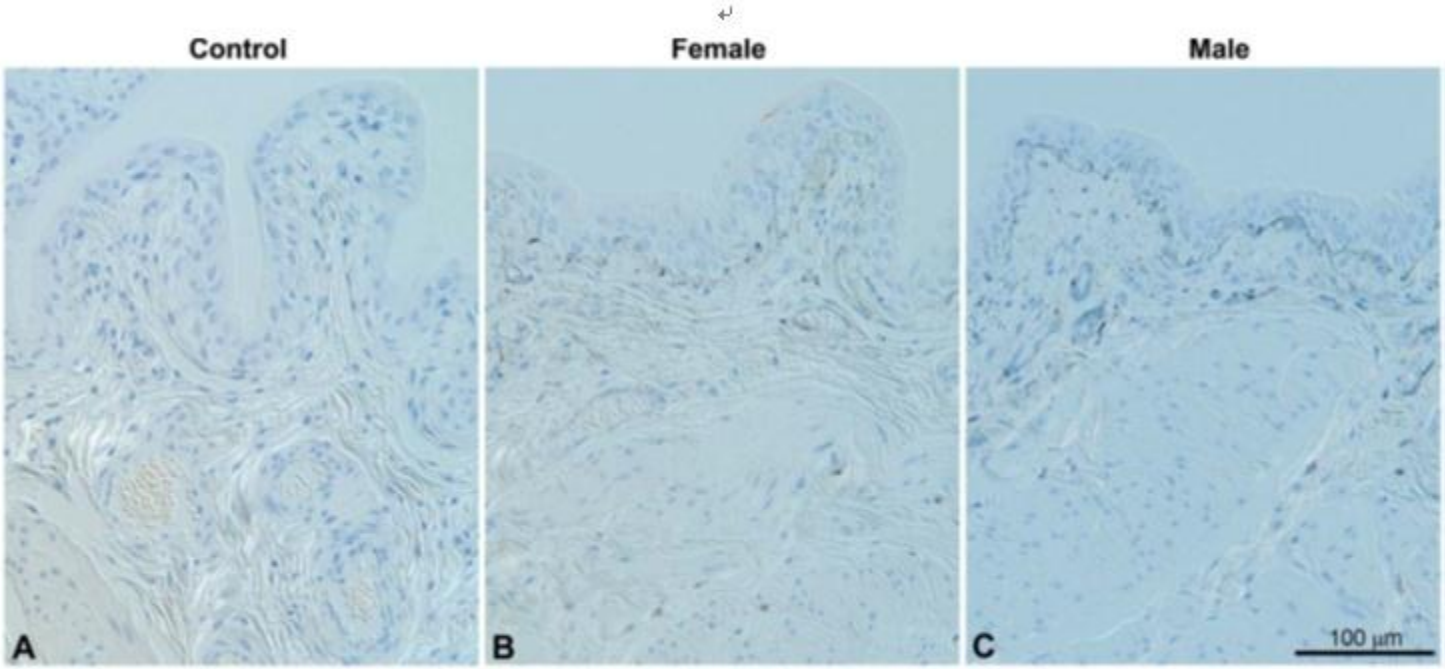


FIG. 3. Urinary bladder. The bar indicates 100 µm.

fig 4.jpg / 229.3 KB (image/pjpeg)

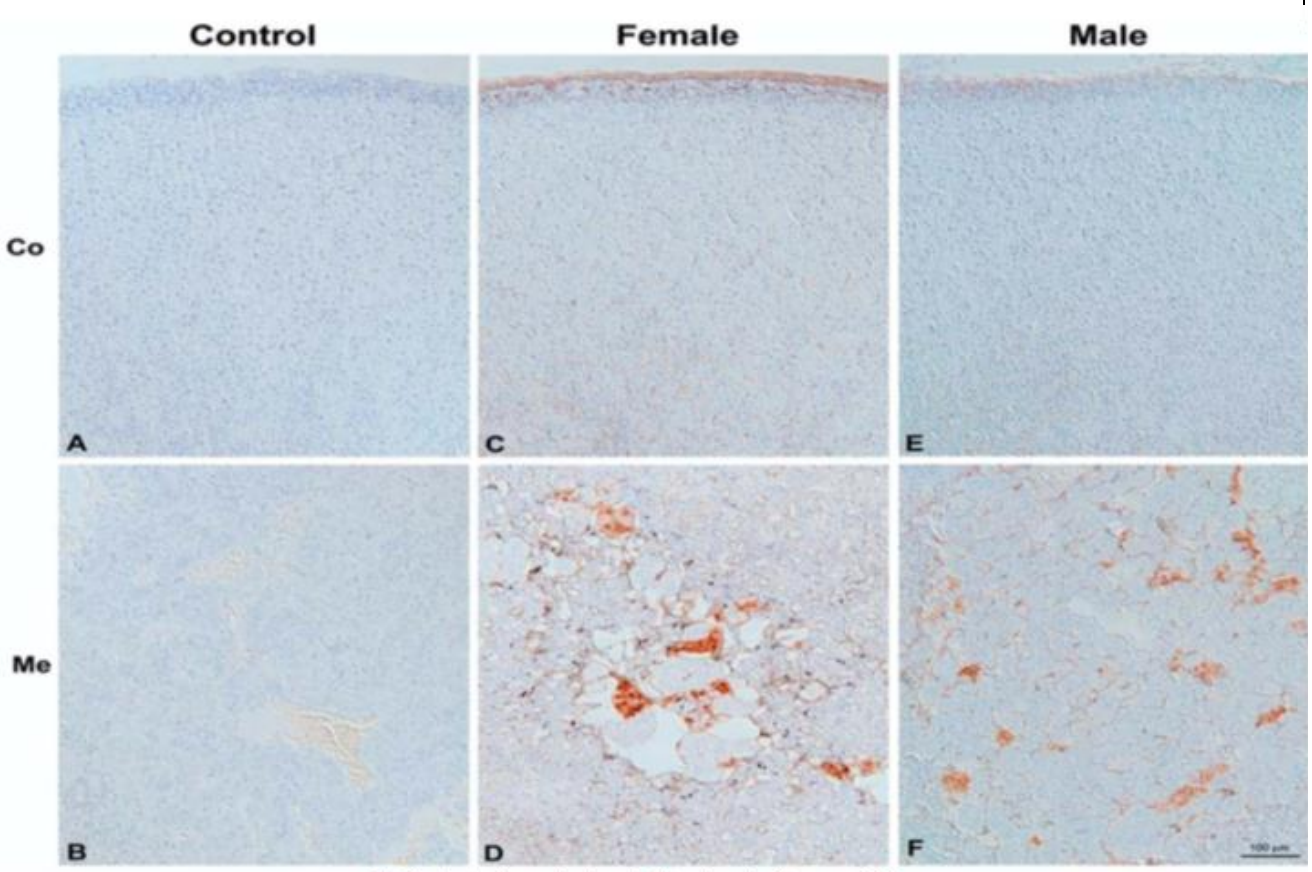


FIG. 4. Adrenal gland. The bar indicates 100 μm

fig 5.jpg / 391.54 KB (image/pjpeg)

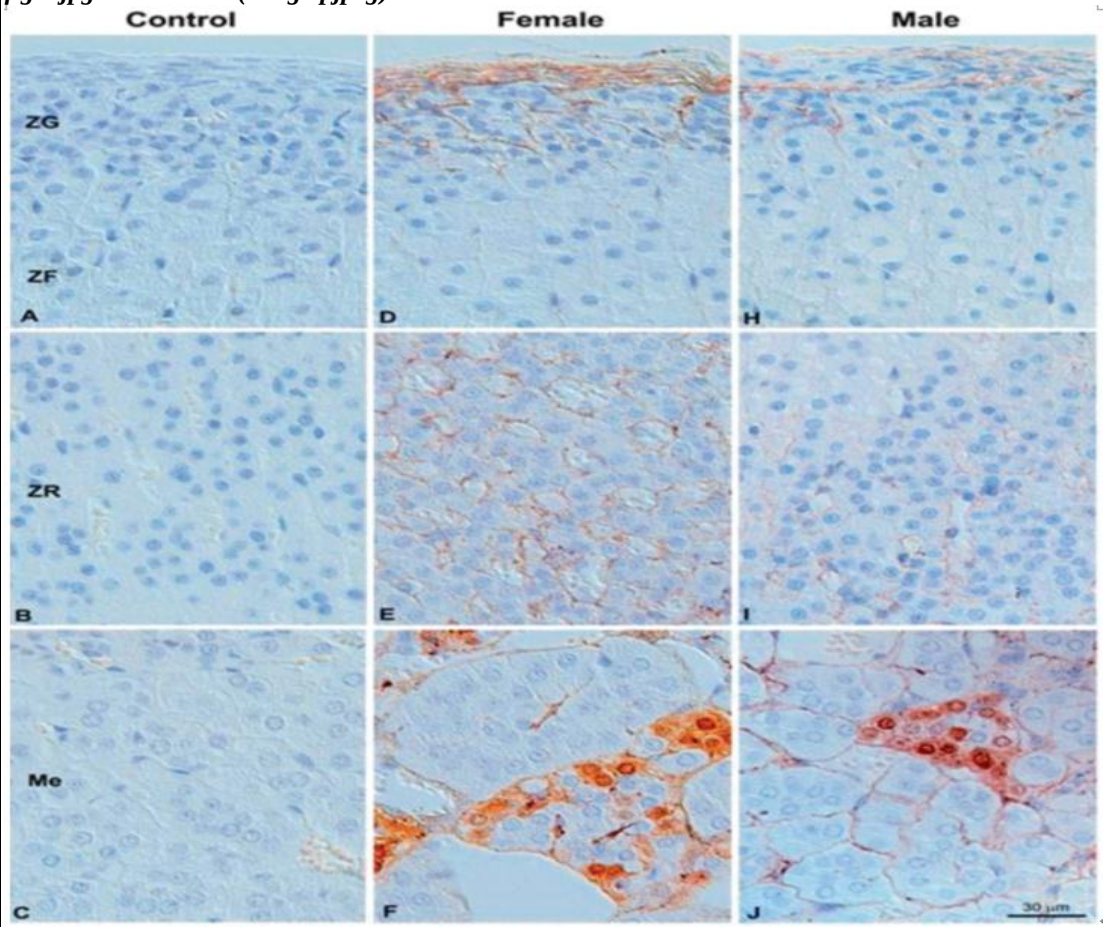


FIG. 5. Adrenal gland with high magnification. ZG, zona glomerulosa; ZF, zona fasciculate; ZR, zona reticularis; Me, medulla. The bar indicates 30 µm.

Applicant's summary and conclusion

Conclusions

Female rats showed a higher accumulation of Ag nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males.

7.5.4 Repeated dose toxicity: other routes

7.6 Genetic toxicity

7.6.1 Genetic toxicity in vitro

Endpoint study record: 7440-22-4, Genetic toxicity in vitro-Ames test, Kim, 2013, 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	GLP study conducted according to OECD 471 Guideline with deviations: details on test item, strain characteristics, media used, no. of cells/culture, treatment procedure, incubation time and temperature, individual and summary tables of results and historical negative (solvent/vehicle) and positive control data 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	Bacterial Mutation Test on Silver Nanoparticles.		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE), Korea		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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 (details on test item, strain characteristics, media used, no. of cells/culture, treatment procedure, incubation time and temperature, individual and summary tables of results and historical negative (solvent/vehicle) and positive control data not reported)

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): Citrate capped silver nanoparticles (cAgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

Confidential details on test material

No data

Method

Target gene

None

Species/strain

Species/strain S. typhimurium TA 1535, TA 1537, TA 98 and TA 100

Details on Not applicable

mammalian cell lines (if applicable)	
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	S9 mix 0.1 mL (=0.01 mL S9)/plate; S9 fraction prepared from liver homogenates of Sprague-Dawley male rat induced with Aroclor 1254
Species/strain	**E. coli WP2 uvr A**
Details on mammalian cell lines (if applicable)	Not applicable
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	S9 mix 0.1 mL (=0.01 mL S9)/plate; S9 fraction prepared from liver homogenates of Sprague-Dawley male rat induced with Aroclor 1254

Test concentrations

20.48 % of cAgNP solution was employed for this test.
Preliminary range-finding test:0.31, 0.63, 1.25, 2.5 and 5.0 µL/plate (approximately 62, 126, 250, 500 and 1000 µg/plate)
Main test:
Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2uvr
A: - Without S9 mix: 0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16 and 0.31 µL/plate which are approximately 0, 1, 2, 4, 8, 16, 32 and 62 µg/plate relatively. Salmonella typhimurium TA98, TA1535, TA1537 and Escherichia coli WP2uvr
A: - With S9 mix: 0, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25 and 2.5 µL/plate which are approximately 0, 8, 16, 32, 62, 126, 250 and 500 µg/plate relatively.
Salmonella typhimurium TA100:- With S9 mix: 0, 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, and 1.25 µL/plate which are approximately 0, 4, 8, 16, 32, 62, 126 and 250 µg/plate.

Vehicle

- Vehicle(s)/solvent(s) used: 1.0 % citrate solution

Controls

Negative controls	no
Solvent / vehicle controls	yes (1.0 % citrate solution)
True negative controls	no

Positive controls	yes
Positive control substance	other: without S9 mix: Furylfuramide for TA98, TA100 and WP2uvrA, Sodium azide for TA1535, 9-aminoacridine hydrochloride hydrate for TA1537; With S9 mix: 2-aminoanthracene for all tested bacterial species
Remarks	All the chemicals were dissolved in dimethylsulfoxide and treated to bacteria along with the test substance

Details on test system and conditions

METHOD OF APPLICATION: No data
FREQUENCY OF DOSING: Single treatment
DURATION- Exposure duration: No data
NUMBER OF REPLICATES: 3

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

Not applied

Any other information on materials and methods incl. tables

Table: 7.6.1/1: Degree of macro dispersion of silver nanomaterial in 1% citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Test results

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative

Cytotoxicity	yes
Vehicle controls valid	no data
Negative controls valid	not applicable
Positive controls valid	no data
Species/strain	E. coli WP2 uvr A
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	yes
Vehicle controls valid	no data
Negative controls valid	not applicable
Positive controls valid	no data

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
- Precipitation: Not observed
RANGE-FINDING/SCREENING STUDIES:Cytotoxic concentration
- With metabolic activation: >0.63 µL/plate (TA98 and TA1537); 1.25 µL/plate (TA100, TA1535 and WP2uvrA)
- Without metabolic activation: >0.16 µL/plate (TA100); 0.31 µL/plate (TA98, TA1535, TA1537 and WP2uvrA)

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

negative without metabolic activation

negative with metabolic activation

Conclusions

cAgNPs did not induce an increase in bacterial colony formation, and thus are not genotoxic under the test conditions.

Executive summary

In a bacterial reverse mutation assay, performed according to the OECD Guideline 471 and in compliance with GLP, strains of*Salmonella typhimurium*(TA 1535, TA 1537, TA 98 and TA 100) and*Escherichia coli (WP2uvrA)*were exposed to Citrate capped silver nanoparticles (20.48 % cAgNPs) at the following concentrations:

Preliminary range-finding test:
0.31, 0.63, 1.25, 2.5 and 5.0 µL/plate (62, 126, 250, 500 and 1000 µg/plate)
Main test:
Salmonella typhimurium TA98, TA1535, TA1537 and Escherichia coli WP2uvrA:
- Without S9 mix: 0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16 and 0.31 µL/plate which are approximately 0, 1, 2, 4, 8, 16, 32 and 62 µg/plate relatively.
- With S9 mix: 0, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25 and 2.5 µL/plate which are approximately 0, 8, 16,32, 62, 126, 250 and 500 µg/plate relatively.
Salmonella typhimurium TA100: 0, 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, and 1.25 µL/plate which are approximately 0, 4, 8, 16, 32, 62, 126 and 250 µg/plate.
Metabolic activation system used in this test was S9 mix 0.1 mL (=0.01 mL S9)/plate; S9 fraction prepared from liver homogenates of Sprague-Dawley male rat induced with Aroclor 1254. Vehicle and positive control groups were also included in mutagenicity tests.

In range finding test, cytotoxic concentrations were as follows: with metabolic activation: >0.63 µL/plate (TA98 and TA1537); 1.25 µL/plate (TA100, TA1535 and WP2uvrA); without metabolic activation: >0.16 µL/plate (TA100); 0.31 µL/plate (TA98, TA1535, TA1537 and WP2uvrA). No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to test item at any concentrations in either the presence or absence of S9 mix. The positive and vehicle controls induced the appropriate responses in the corresponding strains indicating the validity of the study.
Under the test conditions, cAgNPs are not considered as mutagenic in S. typhimurium (TA 1535, TA 1537, TA 98 and TA 100) and E. coli (WP2uvrA) strains.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Genetic toxicity in vitro-chromosomal aberration, Kim, 2013, 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	GLP study conducted according to OECD 473 Guideline with deviations: details on media, cell and culture conditions, incubation time and temperature, chromosome preparation, no. of metaphases analyzed, individual and summary tables and historical negative and positive control data 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 Vitro Mammalian Chromosome Aberration Test of Silver Nanoparticles using Cultured Chinese Hamster Ovary (CHO-K1) Cells.		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE), Korea		
publication	Kim JS, Song KS, Sung JH, Ryu HR, Choi BG, Cho HS, Lee JK and Yu IJ.	2013	Genotoxicity, Acute Oral and Dermal Toxicity, Eye and Dermal Irritation and Corrosion and Skin Sensitisation Evaluation of Silver Nanoparticles.	Nanotoxicology, 7(5): 953-960					

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 on media, cell and culture conditions, incubation time and temperature, chromosome preparation, no. of metaphases analyzed, individual and summary tables and historical negative and positive control data not reported)

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): Citrate capped silver nanoparticles (cAgNPs)- Source: ABC Nanotech Co., Ltd., Korea- Analytical purity: 20.48 % (determined by KSD2717)

Confidential details on test material

No data

Method

Target gene

None

Species/strain

Species/strain	other: Chinese hamster ovary fibroblast, CHO-K1
Details on mammalian cell lines (if applicable)	No data
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	S9 mix 0.5 mL (=0.15 mL S9)/60 mm plate; S9 fraction prepared from liver homogenates of Sprague-Dawley male rat induced with Aroclor 1254

Test concentrations

20.48 % of cAgNP solution was employed for this test.Preliminary range-finding test:To determine chromosome aberration test dose level, Relative Cell Count (RCC) was calculated for all cultures treated with the Test Substances and control substance at the 8 dose levels - 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5 and 5 µL/mLMain test:Tests without S9 mix: 0, 0.001, 0.003 and 0.005 µL/mL for 24 h treatment, and 0, 0.005, 0.010 and 0.019 µL/mL for 6 h treatment and 18 h recovery period.Tests with S9 mix: 0, 0.039, 0.078 and 0.156 µL/mL

Vehicle

- Vehicle(s)/solvent(s) used: Sterilized distilled water

Controls

Negative controls	yes (1.0 % citrate solution)
Solvent / no vehicle	

controls

True negative controls	no
Positive controls	yes
Positive control substance	other: 0.04 µg/µL of mitomycin C (in the absence of S9 mix) and 10 µg/µL of cyclophosphamide (in the presence of S9 mix)

Details on test system and conditions

FREQUENCY OF DOSING: Single treatment
DURATION
- Exposure duration: No data
- Fixation time (start of exposure up to fixation or harvest of cells): No data
SPINDLE INHIBITOR (cytogenetic assays): No data
STAIN (for cytogenetic assays): No data
NUMBER OF REPLICATIONS: Two replicate cultures were used for each concentration level
NUMBER OF CELLS EVALUATED: No data

Evaluation criteria

Statistical analysis was performed to determine the increase in frequency of aberrant metaphase and polyploidy. A concentration-related increase or a reproducible increase in the number of cells with chromosome aberrations was considered to be positive.

Statistics

Chi-Square test for comparison with control and linear logistic regression test for dose-response relationship.

Any other information on materials and methods incl. tables

Follow up study was performed because metaphasic cells were not observed in the first test.

Results and discussions

Test results

Species/strain	Chinese hamster Ovary (CHO)
Metabolic activation	with and without
Test system	strain/cell type: Chinese hamster ovary fibroblast, CHO-K1
Genotoxicity	negative
Cytotoxicity	yes
Vehicle controls valid	not applicable
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
- Precipitation: Not observed
RANGE-FINDING/SCREENING STUDIES:
Cytotoxic concentrationWith metabolic activation: 0.156 µL/mL
Without metabolic activation: 0.078 µL/mL (6 h treatment) and 0.078 µL/mL (24 h treatment)

Mitotic index: Not determined
MAIN STUDY:
Frequency of reversions/mutations/aberrations, polyploidy as appropriate:
- Test without S9 mix: frequencies of aberrant metaphase in 24 h treated cells were 0.5, 0.5, 0.0, 0.5 for 0, 0.001, 0.003, 0.005 µL/mL treated cells, respectively. Cells treated for 6 h and recovered for 18 h exhibited 0.5, 2.0, 0.5 and 1.5 of frequency in 0, 0.005, 0.010 and 0.019 µL/mL treated groups, respectively.
- Test with S9 mix: frequency of aberrant metaphase was 0.5 in all groups.

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

negative without metabolic activation

negative with metabolic activation

Conclusions

cAgNPs did not induce an increase in chromosomal aberration. Therefore, it is not genotoxic under the test conditions.

Executive summary

In an in vitro chromosome aberration test performed according to OECD Guideline 473 and in compliance with GLP, Chinese hamster ovary fibroblast, CHO-K1 cells were exposed to Citrate capped silver nanoparticles (20.48 % cAgNPs) at the following concentrations:
Preliminary range-finding test:
To determine chromosome aberration test dose level, Relative Cell Count (RCC) was calculated for all cultures treated with the Test Substances and control substance at the 8 dose levels - 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5 and 5 µL/mL
Main test:
Tests without S9 mix: 0, 0.001, 0.003 and 0.005 µL/mL for 24 h treatment, and 0, 0.005, 0.010 and 0.019 µL/mL for 6 h treatment and 18 h recovery period.
Tests with S9 mix: 0, 0.039, 0.078 and 0.156 µL/mL
Metabolic activation system used in this test was S9 mix 0.5 mL (=0.15 mL S9)/60 mm plate; S9 fraction prepared from liver homogenates of Sprague-Dawley male rat induced with Aroclor 1254. Negative and positive control groups were also included in this test.

In the range finding test, cytotoxic concentration were as follows: with metabolic activation: 0.156 µL/mL; without metabolic activation: 0.078 µL/mL (6 h treatment) and 0.078 µL/mL (24 h treatment).In test without S9 mix, frequencies of aberrant metaphase in 24 h treated cells were 0.5, 0.5, 0.0, 0.5 for 0, 0.001, 0.003, 0.005 µL/mL treated cells, respectively. Cells treated for 6 h and recovered for 18 h exhibited 0.5, 2.0, 0.5 and 1.5 of frequency in 0, 0.005, 0.010 and 0.019 µL/mL treated groups, respectively. In test with S9 mix, frequency of aberrant metaphase was 0.5 in all groups. No significant increases in chromosomal aberrations were observed at any concentrations in either the presence or absence of S9 mix.

Under the test conditions, cAgNPs are not considered as clastogenic in Chinese hamster ovary fibroblast, CHO-K1 cells with and without metabolic activation.

Cross-reference to other study

No cross-reference

7.6.2 Genetic toxicity in vivo

Endpoint study record: 7440-22-4, Genetic toxicity in vivo - Micronucleus test, Kim, 2008, 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 474 Guideline with deviations: details on acclimation, body weight, housing and feeding conditions, positive controls, bone marrow cells preparation, individual and summary tables of results and historical control data 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	2007	Mammalian Erythrocyte Micronucleus Test of Silver Nanoparticles in Rat.		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE) Korea		
publication	Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD, Choi BS, Lim R, Chang HK, Chung YH, Kwon IH, Jeong J, Han BS and Yu IJ.	2008	Twenty-Eight-Day Oral Toxicity, Genotoxicity, and Gender-Related Tissue Distribution of Silver Nanoparticles in Sprague-Dawley Rats.	Inhal Toxicol. 20(6): 575-583.					

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

micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	yes (details on acclimation, body weight, housing and feeding conditions, positive controls, bone marrow cells preparation, individual and summary tables of results and historical control data not reported)

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): Silver nanoparticles
- Source: NAMATECH Co., Ltd., Korea
- Purity: 99.98 %

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: 5 weeks

Administration / exposure

Route of administration

oral: gavage

Vehicle(s)

- Vehicle(s)/solvent(s) used: 0.5% carboxy methyl cellulose (CMC)

Details on exposure

No data

Duration of treatment / exposure

28 days

Frequency of treatment

Once daily for 28 days

Post exposure period

Not applicable

Doses / concentrations

0, 30, 300 and 1000 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

10

Control animals

yes, concurrent vehicle

Positive control(s)

None

Examinations

Tissues and cell types examined

No data

Details of tissue and slide preparation

Sampling times and number of samples: Once at the end of test; three slides per animal

Evaluation criteria

Test was considered to be valid if the ratio of polychromatic erythrocytes (PCE)/[PCE+normochromatic erythrocytes (NCE)] is >0.1. A dose-related increase in the incidence of micronucleated immature erythrocytes (MNPCE) from 2000 immature erythrocytes and/or a clear increase in the number of micronucleated cells in a single dose group at a single sampling time were considered to be positive.

Statistics

One way analysis of variance (ANOVA) test

Any other information on materials and methods incl. tables

Clinical observations performed: General clinical observations were performed every day
Organs examined at necropsy (macroscopic and microscopic): Liver, kidneys, adrenals, testes, epididymis, uterus, ovaries, thymus, spleen, brain, heart, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, pancreas, trachea, lungs, aorta, gonads, sciatic nerve, prostate, urinary bladder, lymph nodes, bone marrow, mammary gland, tongue, skin and eyes

Table: 7.6.2/1: Degree of macro dispersion of silver nanomaterial in 1% citric acid solution

Concentration (%)	Silver nanoparticle		
	A (A.U.)	B (A.U.)	DOM (%)
20	> 4	> 4	< 100
10	> 4	2.824 ± 0.014	< 70.592 ± 0.362
5	> 4	3.021 ± 0.002	< 75.533 ± 0.038
2.5	> 4	2.137 ± 0.004	< 53.467 ± 0.101

A: Absorbance units of dispersed Silver nanoparticle
B: Absorbance units of centrifuged supernatant
A.U.: Absorbance units
DOM: Degree of macro dispersion (B/AX100)

Results and discussions

Test results

Sex male/female

Genotoxicity negative

Toxicity no effects

Vehicle controls valid no data

Negative controls valid not applicable

Positive controls valid not applicable

Additional information on results

RESULTS OF DEFINITIVE STUDY
- Treatment of test substance did not show any cytotoxic effects to bone marrow cells in all treated groups.
- Induction of micronuclei (for Micronucleus assay): Treatment of test substance did not induce significant increase in micronuclei formation in all treated groups.
- Ratio of PCE/NCE (for Micronucleus assay): No effect
- Statistical evaluation: Not significant

Any other information on results incl. tables

No clinical signs or mortality were observed.

No significant changes in body weight, food/water consumption were observed.
NOAEL: 30 mg/kg bw/day; LOAEL: 300 mg/kg bw/day

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

Oral administration of silver nano did not increase the incidence of micronuclei formation, suggesting it is not genotoxic under the test conditions.

Executive summary

In an in vivo bone marrow micronucleus assay performed according to OECD Guideline 474 and in compliance with GLP, groups of Sprague-Dawley rats (10/sex/dose) were orally (gavage) administered with Silver nanoparticles at the dose levels of 0, 30, 300 and 1000 mg/kg bw/day for 28 days. Polychromatic (PCE) and normochromatic (NCE) erythrocyte ratio was established to determine the toxicity and incidence of micronucleated immature erythrocytes (MNPCE) from 2000 immature erythrocytes was scored.
Treatment of test substance did not show any cytotoxic effects to bone marrow cells and did not induce significant increase in micronuclei formation in all treated groups. No clinical signs or mortality were observed. No significant changes in body weight, food/water consumption were observed. The LOAEL and NOAEL was 300 and 30 mg/kg bw/day, respectively.

Oral administration of silver nano did not increase the incidence of micronuclei formation, suggesting it is not genotoxic under the test conditions.

Cross-reference to other study

No cross-reference

Endpoint study record: 7440-22-4, Genetic toxicity in vivo - Micronucleus test, Kim, 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	GLP study conducted according to OECD 474 Guideline with deviations: details on acclimation, body weight, positive controls, bone marrow cells preparation and historical control data 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	Mammalian Erythrocyte Micronucleus Test of Silver Nanoparticles in Rat.		KCL (Korea conformity Laboratory)		Ministry of Knowledge and Economy (MKE) Korea		
publication	Kim JS, Sung JH, Ji JH, Song KS, Lee JH, Kang CS and Yu IJ.	2011	In vivo Genotoxicity of Silver Nanoparticles after 90-day Silver Nanoparticle Inhalation Exposure.	Safety and Health at Work, 2 (1): 65-69.					

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

micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	yes (details on acclimation, body weight, positive controls, bone marrow cells preparation and historical control data not reported)

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): Silver nanoparticles
- Source: ABC Nanotech Co., Ltd., Korea
- Purity: 99.98 %

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: 6 weeks
- Housing: Rats were housed in polycarbonate cages (5 rats per cage)
ENVIRONMENTAL CONDITIONS
- Temperature (°C): 23 ± 2 °C
- Humidity (%): 55 ± 7 %
- Photoperiod (hrs dark / hrs light): 12-h light/dark cycle

Administration / exposure

Route of administration

inhalation

Vehicle(s)

No data

Details on exposure

Sample preparation: The nanoparticle generator was operated at 30 L/min and the resulting nanoparticles were mixed with a 200 L/min main flow through a high-concentration chamber. Using the MFC for the first particle sampler, a portion of the high nanoparticle concentration was diverted to a middle-concentration chamber and diluted to the MFC flow rate. In the same way, a portion of the middle nanoparticle concentration was also diverted to the low-concentration chamber and diluted to the MFC flow rate. The flow rates for the high, middle and low doses were 47.02 ± 0.14 lpm, 6.76 ±0.16 lpm and 5.42 ± 0.18 lpm (mean ± S.E.), respectively.

Duration of treatment / exposure

90 days

Frequency of treatment

6 h/day, 5 days/week, for 13 weeks

Post exposure period

Not applicable

Doses / concentrations

0.7 × 10^6 particles/cm3 (low dose), 1.4 × 10^6 particles/cm3 (middle dose), and 2.9 × 10^6 particles/cm3 (high dose)

Basis nominal conc.

No. of animals per sex per dose

4 groups (10 rats in each group)

Control animals

other: fresh-air

Positive control(s)

None

Examinations

Tissues and cell types examined

No data

Details of tissue and slide preparation

Sampling times and number of samples: Once at the end of test; three slides per animal

Evaluation criteria

To evaluate the bone marrow toxicity, the PCE / PCE + NCE ratio was calculated based on a total of 200 erythrocytes using these slides.

Statistics

One way analysis of variance (ANOVA) test

Any other information on materials and methods incl. tables

Dose groups: low-dose group (target dose, 0.6 × 106particles/cm3, 1.0 × 109nm2/cm2), middle-dose group (target dose, 1.4 × 106 particles/cm3, 2.5 × 109nm2/cm2), and high-dose group (target dose, 3.0 × 106particles/cm3, 5.0× 109nm2/cm2)Organs examined at necropsy (macroscopic and microscopic): Fluorescent microscope (Leica, Germany)

Results and discussions

Test results

Sex	male/female
Genotoxicity	negative

Toxicity	no effects
Vehicle controls valid	no data
Negative controls valid	not applicable
Positive controls valid	not applicable

Additional information on results

RESULTS OF DEFINITIVE STUDY
- Induction of micronuclei (for Micronucleus assay): There were no statistically significant differences in the micronucleated polychromatic erythrocytes or in the ratio of polychromatic erythrocytes among the total erythrocytes after silver nanoparticle exposure when compared with the control.
- Ratio of PCE/(PCE + NCE) (for Micronucleus assay): No effect
- Statistical results: No effect

Any other information on results incl. tables

Table 7.6.2/1: Frequency of MN PCEs and PCE / (PCE + NCE) ratio in bone marrow of male rats

Dose (mg/kg bw/day)	No. of rats (Male)	Frequency of MN PCEs in every 2000 PCEs (Mean ± SE, %)	PCE / (PCE + NCE) (Mean ± SE, %)
0	10	0.14 ± 0.10	0.36 ± 0.10
Low	10	0.13 ± 0.09	0.39 ± 0.07
Middle	10	0.21 ± 0.09	0.31 ± 0.05
High	10	0.18 ± 0.13	0.30 ± 0.08

Table 7.6.2/2: Frequency of MN PCEs and PCE / (PCE + NCE) ratio in bone marrow of female rats

Dose (mg/kg bw/day)	No. of rats (Female)	Frequency of MN PCEs in every 2000 PCEs (Mean ± SE, %)	PCE / (PCE + NCE) (Mean ± SE, %)
0	10	0.14 ± 0.08	0.29 ± 0.08
Low	10	0.09 ± 0.06	0.30 ± 0.09
Middle	10	0.08 ± 0.06	0.35 ± 0.08
High	10	0.13 ± 0.10	0.31 ± 0.08

MN PCE: micronucleated polychromatic erythrocytes, PCE: polychromatic erythrocytes, NCE: normochromatic erythrocytes.
No clinical signs or mortality were observed.
No significant changes in body weight, food/water consumption were observed.
NOAEL - 30 mg/kg bw / LOAEL - 125 mg/kg bw

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

The present results suggested that a 90 day exposure to silver nanoparticles by inhalation did not induce genetic toxicity in male or female rat bone marrow in vivo.

Executive summary

In an in vivo bone marrow micronucleus assay performed according to OECD Guideline 474 and in compliance with GLP, four groups of Sprague-Dawley rats (10 rats in each group) were administered via inhalation with Silver nanoparticles at the dose levels of fresh-air control, 0.7×10^6particles/cm^3(low dose), 1.4×10^6particles/cm^3(middle dose), and 2.9×10^6particles/cm^3(high dose) for 6 h/day, 5 days/week, for 13 weeks. Polychromatic (PCE) and PCE/normochromatic (NCE) erythrocyte ratio was established to determine the toxicity and incidence of micronucleated immature erythrocytes (MNPCE) from immature erythrocytes was scored.
There were no statistically significant differences in the micronucleated polychromatic erythrocytes or in the ratio of polychromatic erythrocytes among the total erythrocytes after silver nanoparticle exposure when compared with the control. No clinical signs or mortality were observed. No significant changes in body weight, food/water consumption were observed. The LOAEL and NOAEL was 125 and 30 mg/kg bw/day, respectively.
The present results suggested that a 90 day exposure to silver nanoparticles by inhalation did not induce genetic toxicity in male or female rat bone marrow in vivo.

Cross-reference to other study

No cross-reference

7.7 Carcinogenicity

7.8 Toxicity to reproduction

7.8.1 Toxicity to reproduction

Endpoint study record: 7440-22-4, Toxicity to reproduction, Hong, 2014, 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	GLP study conducted according to OECD 422 Guideline with deviations: details on test item, acclimation, housing and feeding conditions, mating procedure, body weight, food/water consumption, haematology, clinical biochemistry and histopathology 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	No information				National Institute of Environmental Research (NIER), Korea,		
publication	Hong JS, Kim S, Lee SH, Jo E, Lee B, Yoon J, Eom IC, Kim HM, Kim P, Choi K, Lee MY, Seo YR, Kim Y, Lee Y, Choi J and Park K.	2014	Combined Repeated-Dose Toxicity Study of Silver Nanoparticles with the Reproduction/Developmental Toxicity Screening Test.	Nanotoxicology. 8: 349-62.					

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

fertility

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	yes (details on test item, acclimation, housing and feeding conditions, mating procedure, body weight, food/water consumption, haematology, clinical biochemistry and histopathology not reported)

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): Citrate capped silver nanoparticle (AgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

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

oral: unspecified

Vehicle

other: sterilized water

Details on exposure

No data

Details on mating procedure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

No data

Duration of treatment / exposure

42 Days

Frequency of treatment

Male: 14 days before mating, 14 days during the mating, and 14 days of post-mating
Female :14 days before mating, during the mating and gestation, and 4 days of lactation
Exposure period: Once a day

Details on study schedule

No data

Doses / concentrations

62.5, 125 and 250 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

50

Control animals

other: control was provided with normal drinking water without AgNPs

Further details on study design

No data

Positive control

Not applicable

Examinations

Parental animals: Observations and examinations

CLINICAL OBSERVATIONS: Yes
- Time schedule: All the animals were monitored once a day during the experimental period.
BODY WEIGHT: No
FOOD AND WATER CONSUMPTION: No

Estrous cyclicity (Parental animals)

Examination of estrus cycle was performed.

Sperm parameters (Parental animals)

No data

Litter observations

No data

Postmortem examinations (Parental animals)

No data

Postmortem examinations (Offspring)

No data

Statistics

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SPSS Statistical Analysis Systems (SPSS 10.1 Base, SPSS Korea Data Solution. Co.Ltd.). The data were presented as mean ± SD. Variance in the numerical data was checked using Levene's test. If the variance was homogeneous, the one-way ANOVA test was conducted to determine which pairs of group comparison were significantly different. If this test showed significance between the groups, the data were analyzed by the multiple comparison procedure of the Dunnet's post-hoc test.

Reproductive indices

No data

Offspring viability indices

No data

Any other information on materials and methods incl. tables

None

Results and discussions

Effect levels

Endpoint	Generation	Sex	Effect level	Based on	Basis for effect level / Remarks
NOAEL	P	male/female	> 250 mg/kg bw/day (actual dose received)	test mat.	

Results of examinations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

no data

Reproductive function: estrous cycle (parental animals)

no effects

Reproductive function: sperm measures (parental animals)

not examined

Reproductive performance (parental animals)

no effects

Organ weights (parental animals)

no data

Gross pathology (parental animals)

no data

Histopathology (parental animals)

no data

Details on results (parental animals)

- No treatment-related changes in detailed functional observation were observed in any of the treatment groups.
- No statistically significant differences were observed in the following parameters examined: gestation period, the number of corpora lutea and implantation, delivery rate.
- Examination of Estrus Cycle : No significant toxicity was observed
- Mating evaluation : No significant toxicity was observed

Results of examinations: offspring

Viability (offspring)

no effects

Clinical signs (offspring)

no effects

Body weight (offspring)

no effects

Sexual maturation (offspring)

no effects

Organ weights (offspring)

no data

Gross pathology (offspring)

no effects

Histopathology (offspring)

no data

Details on results (offspring)

No statistically significant differences were observed in the following parameters examined: the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4.

Any other information on results incl. tables

See the attached document for information on tables of results

Overall remarks, attachments

Remarks on results including tables and figures

None

Attached background material

Attached document	Remarks
Reproductive and littering findings of the female rats treated with AgNPs.pdf / 76.01 KB (application/octet-stream) Test Substances Citrate capped silver nanoparticle (AgNPs), CAS No. 7440-22-4 (silver) Remarks: Manufactured by ABC Nanotech Co., Ltd. (Korea) Methods Methods/guideline followed: OECD Guideline 422 "Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test" Test type: *In vivo* GLP: Yes Year (study performed): 2011 Species: Rat Strain: Sprague-Dawley Route of administration: Oral administration Duration of test: 42 Days Doses/concentration levels: 62.5, 125, 250 mg/kg Sex: Both male and female Exposure period: once a day Frequency of treatment: Male : 14 days before mating, 14 days during the mating, and 14 days of post-mating , Female :14 days before mating, during the mating and gestation, and 4 days of lactation Control group and treatment: Control was provided with normal drinking water without AgNPs Post exposure observation period: Not applicable Statistical Methods: Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SPSS Statistical Analysis Systems (SPSS 10.1 Base, SPSS Korea Data Solution. Co.Ltd.). The data were presented as mean ± SD. Variance in the numerical data was checked using Levene's test. If the variance was homogeneous, the one-way ANOVA test was conducted to determine which pairs of group comparison were significantly different. If this test showed significance between the groups, the data were analyzed by the multiple comparison procedure of the Dunnet's post-hoc test. Test Conditions Age at study initiation: Seven weeks old No. of animals per sex per dose: 50 / male, female / 62.5, 125, 250 mg/kg Vehicle: Sterilized water Satellite groups and reasons they were added: Not applicable Clinical observations performed and frequency: All the animals were monitored once a day during the experimental period. Results Ophthalmologic findings incidence and severity: Not tested Examination of Estrus Cycle : No significant toxicity was observed Mating evaluation : No significant toxicity was observed Funtional observation : No significant toxicity was observed Conclusions No treatment-related changes in detailed functional observation were observed in any of the	

treatment groups. No statistically significant differences were observed in the following parameters examined: gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4. NOAEL of the test article are considered to be more than 250mg/kg bw/day for general toxicity in parent animals.

Table 8. Reproductive and littering findings of the female rats treated with AgNPs

Dose (mg/kg bw/day)		0 (Control)	62.5	125	250
No. of females		15	10	10	15
Precoital interval (day)		4.0 ± 0.3	4.2 ± 0.2	4.1 ± 0.4	4.2 ± 0.5
Mating rate (%)		100	100	100	100
Fertility rate (%)		100	100	100	100
Pregnancy rate (%)		86.7	100	100	100
Functional observation of P					
Male					
No. of male rats		5	5	5	5
Auditory response		+	+	+	+
Pupillary reflex		+	+	+	+
Nociceptive reflex		4.8 ± 0.9	5.0 ± 1.3	4.0 ± 1.6	4.2 ± 1.1
Rotarod	Time (sec)	7.7 ± 4.0	22.2 ± 15.5	10.4 ± 11.5	8.9 ± 6.3
	Distance (m)	0.28 ± 0.14	0.86 ± 0.50	0.38 ± 0.42	0.33 ± 0.23
Passive avoidance	1st	41.2 ± 17.4	25.6 ± 18.5	20.3 ± 11.7	27.0 ± 11.2
	2nd	38.1 ± 28.9	83.3 ± 54.6	51.2 ± 46.7	19.1 ± 12.2
Female					
No. of female rats		5	5	5	5
Auditory response		+	+	+	+
Pupillary reflex		+	+	+	+
Nociceptive reflex		5.1 ± 1.2	4.2 ± 2.4	4.6 ± 1.8	4.0 ± 0.9
Rotarod	Time (sec)	14.0 ± 4.8	13.2 ± 9.6	13.9 ± 10.5	14.2 ± 4.3
	Distance (m)	0.51 ± 0.17	0.48 ± 035	0.51 ± 0.38	0.53 ± 0.16
Passive avoidance	1st	46.6 ± 19.0	23.6 ± 15.3	13.5 ± 5.1	14.0 ± 9.5
	2nd	29.0 ± 35.4	24.1 ± 18.7	11.9 ± 6.8	16.9 ± 13.1
F1 rats					
Duration of pregnancy (days)		21.5 ± 0.5	21.6 ± 0.5	21.5 ± 0.5	22 ± 0.5
No. of corpora lutea		27.3 ± 7.5	28.2 ± 4.5	30.2 ± 3.1	26.1 ± 4.3
No. of implantation		14.6 ± 1.8	15.9 ± 1.5	15.1 ± 1.9	15.5 ± 1.51
Pre-implantation loss (%)		43.2	43.7	49.6	39.5
Post-implantation loss (%)		0.0	1.8	0.0	0.7
Delivery rate (%)		86.7	100	100	100
Sex rate (male/female)		0.96	0.99	0.88	1.03
No. of survival/0 days		14.6 ± 1.8	15.6 ± 1.4	15.1 ± 1.9	15.4 ± 1.4
No. of survival/4 days		14.3 ± 1.8	15.5 ± 1.4	15.0 ± 1.8	15.3 ± 1.4
Survival rate/4 days (%)		97.9 ± 3.2	99.4 ± 1.9	99.4 ± 2.0	99.1 ± 2.3
Body weight of offspring (g)					
Male	Day 0	6.9 ± 0.5	6.8 ± 0.7	6.9 ± 0.7	7.0 ± 0.7
	Day 4	10.0 ± 1.0	9.6 ± 1.1	9.5 ± 1.6	10.0 ± 1.0
Female	Day 0	6.6 ± 0.4	6.3 ± 0.6	6.3 ± 0.7	6.6 ± 0.6
	Day 4	10.0 ± 1.0	9.0 ± 1.1	8.9 ± 1.6	9.4 ± 0.9

bw, Body weight.

Applicant's summary and conclusion

Conclusions

No treatment-related changes in detailed functional observation were observed in any of the treatment groups. No statistically significant differences were observed in the following parameters examined: gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4. NOAEL of the test article are considered to be more than 250 mg/kg bw/day for general toxicity in parent animals.

Executive summary

In a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test conducted according to OECD Guideline 422 and in compliance with GLP, Citrate capped silver nanoparticle (AgNPs) was orally administered to groups of Sprague-Dawley rats at 0, 62.5, 125 and 250 mg/kg bw/day for 42 days (Male: 14 days before mating, 14 days during the mating, and 14 days of post-mating; Female: 14 days before mating, during the mating and gestation, and 4 days of lactation). During the study, data was recorded on mortality, clinical signs, estrus cycle, mating evaluation, functional observation, gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies and body weights of pups.
No treatment-related changes in detailed functional observation were observed in any of the treatment groups. No statistically significant differences were observed in the following parameters examined: gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4. NOAEL of the test article are considered to be more than 250 mg/kg bw/day for general toxicity in parent animals.
Under the test condition, the No Observed Adverse Effect Level (NOAEL) of AgNPs is considered to be more than 250mg/kg bw/day for general toxicity in parent animals..

Cross-reference to other study

No cross-reference

Endpoint study record: Toxicity to reproduction.001

Administrative Data

Study result type	experimental result	Study period	2011
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	1a - GLP guideline study(OECD)		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	National Institute of Environmental Research(NIER)	2011	Combined repeated dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test		Korea Testing & Research Institute (KTR)	TBH-555			
study report	Jeong-Sup Hong., Suhyon Kim., Sang Hee Lee., Eunhye Jo., Byungcheun Lee., Junheon Yoon., Ig-Chun Eom., Hyun-Mi Kim., Pilje Kim., Kyunghee Choi., Moo Yeol Lee., Yeong-Rok Seo., Younghun Kim.,Yeonjin Lee., Jonghye Choi., Kwangsik Park.,	2013	Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test.	Nanotoxicology,1–14					

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

screening

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)	yes

Test materials

Test material identity

Identifier	Identity
CAS name	Silver
CAS number	7440-22-4

Details on test material

Reference Material/Nanomaterial and Sample identification number Identifier Reference Material/Nanomaterial
- Name of test material (as cited in study report): Citrate capped silver nanoparticles
- Molecular formula (if other than submission substance): Ag
- Substance type: Inorganic
- Physical state: Liquid
- Analytical purity: 20.29%
- Impurities (identity and concentrations): No data
- Composition of test material, percentage of components: No data
- Isomers composition: No data
- Purity test date: No data
- Lot/batch No.: No data
- Expiration date of the lot/batch: No data
- Stability under test conditions: No data
- Storage condition of test material: dark condition(4℃)
- Other: Composition of the nanomaterials(including purity, impurities and additives): Black colloidal suspension obtained from ABC Nanotech Co. Ltd. (Korea). Citrate (0.5-1.5%) was used as a stabilizer for the colloidal particles. The sizes of AgNPs, specified by the manufacturer, range from 5 to 25 nm (average 10 nm).

Characteristics/identifiers specific to nanomaterials: To confirm the characteristics, homogeneity and stability of test nanoparticles, the following were used:
1) dynamic light scattering (DLS) and transmission electron microscopy (TEM): size/ size distribution and agglomeration/aggregation state,
2) electrophoretic light scattering (ELS): zeta potential/surface charge,
3) X-ray diffraction (XRD): crystalline phase/crystallite size.

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
- Source: no data
- Age at study initiation: 8~9 week
- Weight at study initiation: no data
- Fasting period before study: no data
- Housing: no data
- Diet (e.g. ad libitum): ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 7 day

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20.5 - 23.5 ℃
- Humidity (%): 47.7 - 62.0 %
- Air changes (per hr): 10 - 15 h,
- Photoperiod (hrs dark / hrs light): 12h /12h

Administration / exposure

Route of administration

oral: gavage

Type of inhalation exposure (if applicable)

no data

Vehicle

water

Details on exposure

No data

Details on mating procedure

- M/F ratio per cage:1
- Length of cohabitation:no data
- Proof of pregnancy:vaginal plug and sperm in vaginal smear day 0 of pregnancy
- After ... days of unsuccessful pairing replacement of first male by another male with proven fertility.
- Further matings after two unsuccessful attempts: no data
- After successful mating each pregnant female was caged (how): 1 pregnant female /cage
- Any other deviations from standard protocol: no data

Duration of treatment / exposure

Male : 14 days before mating, 14 days during the mating, and 14 days of post-mating
Female :14 days before mating, during the mating and gestation, and 4 days of lactation

Frequency of treatment

Once a day

Details on study schedule

no data

Doses / concentrations

62.5, 125, 250 mg/kg

Basis nominal in water

No. of animals per sex per dose

50 / male, female / 62.5, 125, 250 mg/kg

Control animals

yes

Further details on study design

No data

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: once a day
- Cage side observations checked in table [No.?] were included.

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: once a dayBODY WEIGHT: Yes
- Time schedule for examinations: once a week during pre-mating, mating and recovery periods. During pregnancy and lactation periods, body weights were measured as follows; day 0 (the day of pregnancy), 3, 6, 9, 12, 15, 18 and 20 of gestation of pregnant rats, and day 0 (the day of delivery) and 4 post-partum of offspring

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): no data
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No data

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): no data
- Time schedule for examinations: no data

OTHER: no data

Estrous cyclicity (Parental animals)

Vaginal smear was taken daily from each female during the premating period. Regularity and length of the cycle was examined.

Sperm parameters (Parental animals)

no data

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: Yes
- If yes, maximum of 5/sex/litter pups/litter ; excess pups were killed and discarded.

PARAMETERS EXAMINEDThe following parameters were examined in [F1] offspring: Yes[number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, weight gain, physical or behavioural abnormalities, other:]

GROSS EXAMINATION OF DEAD PUPS: Yes[no / yes, for external and internal abnormalities; possible cause of death was/was not determined for pups born or found dead.]

Postmortem examinations (Parental animals)

SACRIFICE
- Male animals: All surviving animals were sacrificed after 28 days of treatment
- Maternal animals: All surviving animals were sacrificed after 4 days post-partum pups
GROSS NECROPSY
- Gross necropsy consisted of external surface & all orifices, cranial cavity, external surface of the brain & spinal cord, nasal cavity & paranasal sinus, thoracic, abdominal and pelvic cavities & their viscera and tissues & organs
HISTOPATHOLOGY / ORGAN WEIGHTS
The tissues indicated in below were prepared for microscopic examination and weighed, respectively.liver, spleen, heart, lung, brain, thymus, kidneys, adrenal glands, thyroid, pituitary gland, salivary gland, ovaries, testis, epididymis, seminal vesicles and uterus

Postmortem examinations (Offspring)

SACRIFICE
- The F1 offsprings
- These animals were subjected to postmortem examinations (macroscopic and/or microscopic examination) as follows: Yes

GROSS NECROPSY
- Gross necropsy consisted of [external and internal examinations including liver, kidney, lung and brain]
HISTOPATHOLOGY / ORGAN WEIGTHS
no data

Results and discussions

Effect levels

Endpoint	Generation	Sex	Effect level	Based on	Basis for effect level / Remarks
NOAEL		male/female	> 250 mg/kg bw/day (nominal)		Overall effects:clinical signs; mortality; body weight; food consumption and compound intake; food efficiency; water consumption and compound intake; gross pathology; organ weights; histopathology;mating index; fertility index; % females mating > 1st estrous; number of implantation sites; duration of pregnancy; birth index; live birth index; pregnancy index; litter size; litter weight; pup weight; sex ratio; survival index; viability index; lactation index; sperm characterization; other:

Results of examinations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

no effects

Test substance intake (parental animals)

no effects

Reproductive function: estrous cycle (parental animals)

no effects

Reproductive function: sperm measures (parental animals)

no effects

Reproductive performance (parental animals)

no effects

Organ weights (parental animals)

no effects

Gross pathology (parental animals)

no effects

Histopathology (parental animals)

no effects

Details on results (parental animals)

no effects

Results of examinations: offspring

Viability (offspring)

no effects

Clinical signs (offspring)

no effects

Body weight (offspring)

no effects

Sexual maturation (offspring)

no effects

Organ weights (offspring)

no effects

Gross pathology (offspring)

no effects

Histopathology (offspring)

no effects

Details on results (offspring)

no effects

Overall remarks, attachments

Remarks on results including tables and figures

Ophthalmologic findings incidence and severity: Not testedExamination of Estrus Cycle : No significant toxicity was observedMating evaluation : No significant toxicity was observedFuntional observation : No significant toxicity was observed

Attached background material

Attached document

Table 1. Hematological.jpg / 101.5 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of male rats		5	5	5	5	5	5
RBC	(M/μl)	8.29 ± 0.50	8.45 ± 0.67	8.27 ± 0.26	8.13 ± 0.41	8.33 ± 0.31	8.23 ± 0.15
Hb	(g/dl)	14.9 ± 0.5	15.2 ± 1.0	15.1 ± 0.2	14.8 ± 0.8	14.9 ± 0.4	14.9 ± 0.5
HCT	(%)	44.4 ± 1.6	44.5 ± 2.7	44.0 ± 0.7	44.1 ± 2.3	43.5 ± 1.2	43.6 ± 1.3
MCV	(fl)	53.7 ± 2.7	52.8 ± 1.8	53.2 ± 1.6	54.3 ± 2.0	52.3 ± 2.0	52.9 ± 1.5
MCH	(pg)	18.0 ± 0.9	18.0 ± 0.9	18.3 ± 0.5	18.2 ± 0.7	17.9 ± 0.8	18.2 ± 0.7
MCHC	(g/dl)	33.4 ± 0.1	34.1 ± 0.7	34.5 ± 1.0	33.5 ± 0.5	34.2 ± 0.3	34.4 ± 0.5
RDW	(%)	12.0 ± 0.4	12.0 ± 0.3	12.5 ± 0.7	12.5 ± 0.9	13.1 ± 0.6	12.6 ± 0.8
HDW	(g/dl)	3.03 ± 0.10	3.11 ± 0.14	3.23 ± 0.28	3.11 ± 0.12	2.82 ± 0.13	2.85 ± 0.11
Reti	(G/l)	153.8 ± 27.2	147.0 ± 27.4	175.2 ± 57.7	172.0 ± 27.6	189.8 ± 28.4	160.3 ± 26.5
Reti	(%)	1.86 ± 0.37	1.74 ± 0.27	2.11 ± 0.67	2.11 ± 0.29	2.29 ± 0.42	1.95 ± 0.33
PLT	(K/μl)	1246.4 ± 64.9	1233.4 ± 34.6	1255.6 ± 113.2	1060.0 ± 382.4	1129.4 ± 79.6	1153.2 ± 101.9
MPV	(fl)	11.3 ± 0.7	11.0 ± 0.6	11.4 ± 0.3	13.0 ± 3.8	9.5 ± 0.4	9.9 ± 0.3
PT	(sec)	20.0 ± 2.3	23.4 ± 9.3	18.8 ± 5.9	20.7 ± 4.2	16.7 ± 1.2	16.1 ± 2.2
APTT	(sec)	22.3 ± 2.7	23.5 ± 4.1	20.9 ± 2.5	23.1 ± 3.2	18.9 ± 1.3	18.0 ± 3.1
WBC	(K/μl)	8.81 ± 2.16	7.89 ± 1.36	8.28 ± 1.31	7.07 ± 2.29	6.02 ± 2.81	4.82 ± 2.58
WBC differential counting							
NE	(K/μl)	1.70 ± 0.71	1.31 ± 0.18	1.40 ± 0.36	0.99 ± 0.43	0.83 ± 0.40	0.83 ± 0.64
	(%)	19.2 ± 6.5	16.7 ± 1.6	17.0 ± 3.6	13.5 ± 2.8	15.0 ± 5.4	16.1 ± 3.7
LY	(K/μl)	6.63 ± 1.71	6.09 ± 1.07	6.43 ± 1.14	5.63 ± 1.75	4.97 ± 2.51	3.75 ± 1.89
	(%)	75.3 ± 5.8	77.2 ± 2.0	77.5 ± 3.8	80.1 ± 3.4	81.2 ± 6.4	78.6 ± 4.3
MO	(K/μl)	0.25 ± 0.10	0.27 ± 0.05	0.27 ± 0.07	0.23 ± 0.11	0.13 ± 0.06	0.15 ± 0.07
	(%)	2.7 ± 0.5	3.4 ± 0.3	3.3 ± 0.8	3.3 ± 1.1	2.2 ± 0.7	3.3 ± 1.7
EO	(K/μl)	0.11 ± 0.03	0.10 ± 0.05	0.11 ± 0.06	0.10 ± 0.05	0.05 ± 0.01	0.06 ± 0.03
	(%)	1.3 ± 0.5	1.2 ± 0.4	1.3 ± 0.6	1.3 ± 0.4	0.9 ± 0.4	1.2 ± 0.6
BA	(K/μl)	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
	(%)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
LUC	(K/μl)	0.09 ± 0.06	0.11 ± 0.07	0.05 ± 0.02	0.10 ± 0.05	0.03 ± 0.01	0.02 ± 0.01
	(%)	1.1 ± 0.9	1.3 ± 0.7	0.7 ± 0.3	1.5 ± 0.7	0.5 ± 0.3	0.5 ± 0.2

RBC, Red blood œll; Hb, Haemoglobin; HCT, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; RDW, Red cell distribution width; HDW, Haemoglobin distribution width; Reti, Reticulocytes; PLT, Platelet count; MPV, Mean platelet volume; WBC, White blood cell; NE, Neutrophils; LY, Lymphocytes; MO, Monocytes; EO, Eosinophils; BA, Basophils; LUC, Large unstained cell.

Table 2. Hematological.jpg / 103.04 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of female rats		5	5	5	5	4	5
RBC	(M/µl)	6.98 ± 0.36	6.63 ± 0.25	6.61 ± 0.45	6.86 ± 0.41	7.28 ± 0.75	7.36 ± 0.32
Hb	(g/dl)	12.9 ± 0.4	12.5 ± 0.5	12.6 ± 1.4	12.8 ± 0.4	15.0 ± 0.1	14.8 ± 0.4
HCT	(%)	38.6 ± 1.6	37.5 ± 1.8	37.9 ± 4.2	38.0 ± 1.3	43.6 ± 1.1	42.5 ± 0.9
MCV	(fl)	55.4 ± 1.7	56.6 ± 3.3	57.3 ± 3.4	55.5 ± 2.0	60.5 ± 8.2	57.8 ± 1.9
MCH	(pg)	18.5 ± 0.6	18.7 ± 0.9	19.0 ± 1.3	18.7 ± 0.6	20.9 ± 2.4	20.1 ± 0.6
MCHC	(g/dl)	33.5 ± 0.3	33.1 ± 0.5	33.1 ± 0.5	33.6 ± 0.4	34.6 ± 0.8	34.9 ± 0.4
RDW	(%)	15.4 ± 1.6	16.5 ± 1.2	15.6 ± 0.9	16.2 ± 2.8	14.6 ± 0.5	14.2 ± 0.7
HDW	(g/dl)	2.92 ± 0.22	2.99 ± 0.11	2.98 ± 0.31	2.91 ± 0.24	2.25 ± 0.15	2.29 ± 0.03
Reti	(G/l)	426.9 ± 84.2	550.9 ± 159.2	411.0 ± 36.7	462.0 ± 165.2	214.8 ± 106.2	156.1 ± 24.1
Reti	(%)	6.14 ± 1.36	8.35 ± 2.59	6.26 ± 0.82	6.85 ± 2.83	3.10 ± 1.96	2.12 ± 0.33
PLT	(K/µl)	2021.4 ± 220.2	1958.8 ± 174.4	2070.8 ± 263.7	1965.0 ± 123.3	1421.5 ± 187.5	1403.0 ± 128.6
MPV	(fl)	9.8 ± 2.1	10.1 ± 1.4	10.1 ± 1.3	9.5 ± 1.5	11.0 ± 0.9	10.9 ± 0.4
PT	(sec)	13.7 ± 0.4	14.2 ± 0.6	14.5 ± 0.5	14.5 ± 0.6	14.3 ± 0.9	14.2 ± 0.6
APTT	(sec)	14.1 ± 2.1	13.8 ± 1.1	14.0 ± 2.7	15.1 ± 1.6	14.4 ± 0.7	14.3 ± 0.9
WBC	(K/µl)	5.07 ± 1.09	5.93 ± 2.31	7.02 ± 3.80	3.65 ± 1.88	5.83 ± 2.27	5.19 ± 0.99
WBC differential counting							
NE	(K/µl)	1.10 ± 0.15	1.36 ± 0.86	1.90 ± 2.36	0.93 ± 0.47	3.03 ± 1.80	1.70 ± 1.15
	(%)	22.3 ± 4.0	21.3 ± 5.5	22.8 ± 19.2	25.8 ± 7.9	48.7 ± 15.3	30.8 ± 15.8
LY	(K/µl)	3.76 ± 0.99	4.33 ± 1.38	4.76 ± 2.76	2.58 ± 1.39	2.53 ± 0.89	3.26 ± 0.66
	(%)	73.7 ± 4.2	74.5 ± 5.1	72.7 ± 21.8	70.2 ± 8.2	46.6 ± 14.6	64.9 ± 16.6
MO	(K/µl)	0.11 ± 0.02	0.13 ± 0.05	0.20 ± 0.26	0.09 ± 0.04	0.20 ± 0.06	0.15 ± 0.07
	(%)	2.3 ± 0.4	2.4 ± 0.6	2.4 ± 2.1	2.4 ± 0.8	3.5 ± 0.8	2.9 ± 0.9
EO	(K/µl)	0.04 ± 0.02	0.06 ± 0.03	0.05 ± 0.03	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
	(%)	0.8 ± 0.2	1.1 ± 0.5	0.8 ± 0.4	0.8 ± 0.2	0.6 ± 0.3	0.7 ± 0.3
BA	(K/µl)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
	(%)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
LUC	(K/µl)	0.04 ± 0.02	0.04 ± 0.02	0.09 ± 0.11	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
	(%)	0.7 ± 0.3	0.6 ± 0.3	1.0 ± 0.9	0.6 ± 0.1	0.6 ± 0.3	0.6 ± 0.3

RBC, Red blood cell; Hb, Haemoglobin; HCT, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; RDW, Red cell distribution width; HDW, Haemoglobin distribution width; Reti, Reticulocytes; PLT, Platelet count; MPV, Mean platelet volume; WBC, White blood cell; NE, Neutrophils; LY, Lymphocytes; MO, Monocytes; EO, Eosinophils; BA, Basophils; LUC, Large unstained cell.

Table 3. Blood biochemical .jpg / 79.62 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of male rats		5	5	5	5	5	5
TP	(g/dl)	5.7 ± 0.1	5.7 ± 0.2	5.8 ± 0.1	5.7 ± 0.3	5.5 ± 0.2	5.6 ± 0.1
ALB	(g/dl)	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
A/G		0.6 ± 0.1	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1
T-BIL	(mg/dl)	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01
ALP	(U/l)	382.6 ± 54.7	454.4 ± 58.5	369.2 ± 51.4	458.8 ± 85.4	376.4 ± 76.7	390.2 ± 45.3
AST	(U/l)	102.4 ± 27.7	118.4 ± 10.5	109.0 ± 26.4	112.0 ± 45.4	115.2 ± 7.6	125.4 ± 13.6
ALT	(U/l)	34.4 ± 11.0	37.6 ± 5.1	35.8 ± 6.7	34.6 ± 7.3	37.2 ± 4.1	41.8 ± 4.1
CREA	(mg/dl)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.0
BUN	(mg/dl)	13.4 ± 1.0	14.6 ± 1.4	12.8 ± 1.3	13.4 ± 0.6	14.4 ± 2.4	14.2 ± 1.4
T-CHO	(mg/dl)	61.8 ± 11.8	54.6 ± 11.0	66.4 ± 9.1	53.6 ± 14.8	63.2 ± 19.2	50.2 ± 10.7
TG	(mg/dl)	47.2 ± 22.4	39.6 ± 14.9	56.2 ± 23.5	50.8 ± 36.8	46.2 ± 13.7	56.6 ± 14.9
GLU	(mg/dl)	160.2 ± 17.1	143.0 ± 16.4	147.8 ± 9.8	158.6 ± 10.5	176.4 ± 12.8	163.2 ± 6.2
CA	(mg/dl)	10.1 ± 0.3	10.0 ± 0.3	10.1 ± 0.1	10.1 ± 0.2	9.7 ± 0.2	9.9 ± 0.1
IP	(mg/dl)	7.7 ± 0.5	7.6 ± 0.4	7.8 ± 0.6	7.7 ± 0.4	7.5 ± 0.2	6.8 ± 0.3**
GGT	(U/l)	0.5 ± 0.8	0.0 ± 0.4	0.1 ± 0.3	0.3 ± 0.4	0.2 ± 0.5	0.3 ± 0.5
CK	(IU/l)	280.8 ± 144.8	263.0 ± 136.6	333.8 ± 210.6	336.2 ± 412.4	463.2 ± 75.2	572.4 ± 239.3
Na	(mmol/l)	143.3 ± 1.0	142.8 ± 0.7	142.3 ± 0.2	143.0 ± 1.1	141.0 ± 0.9	142.2 ± 1.0
K	(mmol/l)	4.28 ± 0.15	4.07 ± 0.42	4.22 ± 0.27	4.12 ± 0.17	4.21 ± 0.22	4.31 ± 0.13
Cl	(mmol/l)	109.6 ± 1.0	109.1 ± 1.0	107.8 ± 0.7	109.1 ± 1.6	106.6 ± 0.7	108.5 ± 1.2*

*Significantly different from values of control group at $p < 0.05$; **At $p < 0.01$; TP, Total protein; ALB, Albumin; A/G, Albumin/Globulin ratio; T-BIL, Total bilirubin; ALP, Alkaline phosphate; ALT, Alanine aminotransferase; CREA, Creatinine; BUN, Blood urea nitrogen; T-CHO, Total cholesterol; TG, Triglyceride; GLU, Glucose; CA, Calcium; IP, Inorganic phosphorus; GGT, Gamma glutamyltranspeptidase; CK, Creatine kinase; Na, Sodium, K, Potassium; Cl, Chloride.

Table 4. Blood biochemical.png / 66.5 KB (image/png)

		Main group				Recovery group	
Dose (mg/kg bw/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of female rats		5	5	5	5	4	5
TP	(g/dl)	6.1 ± 0.1	5.8 ± 0.3	5.7 ± 0.5	5.9 ± 0.2	5.7 ± 0.2	5.6 ± 0.3
ALB	(g/dl)	2.4 ± 0.1	2.3 ± 0.2	2.2 ± 0.5	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
A/G		0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
T-BIL	(mg/dl)	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.05 ± 0.02
ALP	(U/l)	209.0 ± 64.9	328.8 ± 92.4	281.0 ± 90.6	265.4 ± 45.5	349.8 ± 70.6	306.8 ± 85.6
AST	(U/l)	123.2 ± 15.2	121.2 ± 14.9	96.6 ± 15.3	85.6 ± 14.0*	139.3 ± 30.1	95.6 ± 9.2
ALT	(U/l)	45.8 ± 5.4	50.6 ± 8.1	44.8 ± 7.5	44.4 ± 7.4	103.3 ± 13.9	88.0 ± 9.1
CREA	(mg/dl)	0.7 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
BUN	(mg/dl)	16.2 ± 3.3	15.9 ± 3.0	17.7 ± 2.7	15.6 ± 1.8	21.0 ± 5.5	21.4 ± 1.1
T-CHO	(mg/dl)	91.6 ± 23.0	77.6 ± 21.8	79.4 ± 22.9	84.0 ± 20.9	127.5 ± 15.7	122.0 ± 17.4
TG	(mg/dl)	157.6 ± 51.9	95.8 ± 30.1	72.0 ± 13.5	98.2 ± 42.7	245.3 ± 70.6	165.8 ± 97.0
GLU	(mg/dl)	95.2 ± 11.8	110.0 ± 16.5	105.6 ± 17.2	104.8 ± 18.1	112.5 ± 4.9	124.4 ± 8.3
CA	(mg/dl)	9.8 ± 0.4	10.3 ± 0.5	10.4 ± 0.5	10.0 ± 0.3	8.3 ± 0.4	9.3 ± 0.6
IP	(mg/dl)	8.0 ± 0.9	9.1 ± 1.1	9.5 ± 1.3	7.4 ± 1.5	10.7 ± 1.7	9.8 ± 1.9
GGT	(U/I)	0.3 ± 0.6	0.1 ± 0.4	0.3 ± 0.9	0.1 ± 0.5	0.3 ± 0.3	0.0 ± 0.3
CK	(IU/l)	734.4 ± 298.7	610.6 ± 133.1	455.0 ± 270.0	262.6 ± 103.1	700.0 ± 220.6	267.0 ± 77.8
Na	(mmol/l)	134.0 ± 3.3	139.5 ± 4.8	137.5 ± 5.5	134.8 ± 6.2	135.1 ± 1.0	134.8 ± 1.6
K	(mmol/l)	4.68 ± 0.48	4.45 ± 0.22	4.78 ± 0.38	4.00 ± 0.28	4.50 ± 0.15	4.39 ± 0.22
Cl	(mmol/l)	98.3 ± 3.6	102.5 ± 2.8	101.9 ± 5.5	99.7 ± 4.9	97.6 ± 2.1	97.9 ± 2.2

*Significantly different from values of control group at $p < 0.05$; TP; Total protein, ALB; Albumin; A/G, Albumin/Globulin ratio; T-BIL, Total bilirubin; T-BIL, Total bilirubin; ALP, Alkaline phosphate; ALT, Alanine aminotransferase; bw, Body weight; CREA, Creatinine; BUN, Blood urea nitrogen; T-CHO, Total cholesterol; TG, Triglyceride; GLU, Glucose; CA, Calcium; IP, Inorganic phosphorus; GGT, Gamma glutamyltranspeptidase; CK, Creatine kinase; Na, Sodium; K, Potassium; Cl, Chloride.

Table 5. Organ weight of male rats given AgNPs.jpg / 131.71 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg bw/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of male rats		10	10	10	10	5	5
Body weight (g)		501.0 ± 48.4	485.0 ± 35.2	484.9 ± 43.1	497.3 ± 41.1	493.9 ± 28.9	501.7 ± 22.9
Liver	(g)	12.47 ± 1.46	12.49 ± 1.29	12.66 ± 1.92	12.64 ± 1.47	12.03 ± 0.53	13.39 ± 0.74*
	(g%)	2.49 ± 0.12	2.58 ± 0.19	2.60 ± 0.19	2.54 ± 0.12	2.44 ± 0.11	2.67 ± 0.10*
Kidney-Left	(g)	1.72 ± 0.16	1.63 ± 0.12	1.65 ± 0.16	1.70 ± 0.18	1.58 ± 0.14	1.65 ± 0.11
	(g%)	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.32 ± 0.02	0.33 ± 0.03
Kidney-Right	(g)	1.72 ± 0.17	1.61 ± 0.14	1.64 ± 0.18	1.71 ± 0.12	1.62 ± 0.22	1.62 ± 0.12
	(g%)	0.34 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.35 ± 0.02	0.33 ± 0.03	0.32 ± 0.03
Spleen	(g)	0.87 ± 0.10	0.83 ± 0.10	0.89 ± 0.16	0.88 ± 0.16	0.85 ± 0.15	0.85 ± 0.14
	(g%)	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.03	0.18 ± 0.03	0.17 ± 0.02	0.17 ± 0.02
Adrenal gland-Left	(mg)	41.4 ± 9.32	39.9 ± 7.81	41.7 ± 7.85	44.8 ± 12.56	36.0 ± 7.58	33.4 ± 7.50
	(mg%)	8.3 ± 1.77	8.1 ± 1.52	8.7 ± 1.77	9.1 ± 2.13	7.4 ± 2.07	6.6 ± 1.14
Adrenal gland-Right	(mg)	39.6 ± 6.82	36.8 ± 5.79	41.4 ± 5.87	41.2 ± 9.19	40.4 ± 4.88	33.8 ± 6.61
	(mg%)	8.0 ± 1.25	7.7 ± 1.34	7.8 ± 1.06	8.1 ± 1.60	8.0 ± 1.00	6.6 ± 1.14
Testis-Left	(g)	1.80 ± 0.18	1.75 ± 0.11	1.80 ± 0.21	1.78 ± 0.13	1.86 ± 0.22	1.75 ± 0.12
	(g%)	0.36 ± 0.06	0.36 ± 0.03	0.37 ± 0.04	0.36 ± 0.04	0.38 ± 0.06	0.35 ± 0.03
Testis-Right	(g)	1.79 ± 0.16	1.78 ± 0.10	1.78 ± 0.21	1.77 ± 0.15	1.90 ± 0.17	1.78 ± 0.14
	(g%)	0.36 ± 0.06	0.37 ± 0.03	0.37 ± 0.04	0.36 ± 0.04	0.39 ± 0.05	0.36 ± 0.03
Brain	(g)	1.95 ± 0.66	2.14 ± 0.10	2.17 ± 0.11	2.16 ± 0.12	2.13 ± 0.09	2.08 ± 0.15
	(g%)	0.39 ± 0.13	0.44 ± 0.04	0.45 ± 0.04	0.44 ± 0.03	0.43 ± 0.04	0.42 ± 0.03
Pituitary gland	(mg)	17.5 ± 3.60	16.4 ± 4.81	15.5 ± 5.97	14.5 ± 4.70	12.8 ± 0.84	10.0 ± 4.06
	(mg%)	3.5 ± 0.71	3.5 ± 0.97	3.2 ± 1.23	2.9 ± 0.74	2.6 ± 0.55	2.0 ± 1.22
Lung	(g)	1.75 ± 0.14	1.64 ± 0.16	1.73 ± 0.20	1.83 ± 0.16	1.72 ± 0.15	1.74 ± 0.16
	(g%)	0.35 ± 0.03	0.34 ± 0.02	0.36 ± 0.02	0.37 ± 0.05	0.35 ± 0.03	0.35 ± 0.02
Heart	(g)	1.73 ± 0.31	1.65 ± 0.19	1.73 ± 0.21	1.66 ± 0.42	1.65 ± 0.05	1.82 ± 0.22
	(g%)	0.35 ± 0.06	0.34 ± 0.02	0.36 ± 0.05	0.34 ± 0.08	0.33 ± 0.02	0.36 ± 0.04
Thymus	(g)	0.49 ± 0.18	0.46 ± 0.04	0.46 ± 0.12	0.47 ± 0.11	0.41 ± 0.11	0.36 ± 0.07
	(g%)	0.10 ± 0.03	0.10 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.07 ± 0.01
Thyroid gland	(mg)	34.5 ± 9.22	26.8 ± 7.85	31.2 ± 10.51	34.3 ± 14.57	24.4 ± 3.61	24.4 ± 7.34
	(mg%)	9.0 ± 3.16	5.6 ± 1.96	6.4 ± 2.01	6.7 ± 2.50	4.0 ± 5.48	4.0 ± 5.48
Salivary gland	(g)	0.79 ± 0.11	0.78 ± 0.09	0.87 ± 0.10	0.90 ± 0.09	0.83 ± 0.09	0.88 ± 0.14
	(g%)	0.16 ± 0.02	0.16 ± 0.02	0.18 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.03
Seminal vesicles	(g)	3.01 ± 0.61	2.62 ± 0.69	2.24 ± 0.63	2.49 ± 0.49	2.99 ± 0.43	3.17 ± 0.58
	(g%)	0.61 ± 0.15	0.54 ± 0.14	0.46 ± 0.11	0.50 ± 0.10	0.60 ± 0.07	0.63 ± 0.13
Epididymis-Left	(g)	0.78 ± 0.06	0.76 ± 0.07	0.77 ± 0.08	0.78 ± 0.06	0.81 ± 0.10	0.84 ± 0.03
	(g%)	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.01
Epididymis-Right	(g)	0.78 ± 0.07	0.79 ± 0.10	0.79 ± 0.08	0.79 ± 0.07	0.85 ± 0.07	0.85 ± 0.08
	(g%)	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.02	0.17 ± 0.02

*Significantly different from values of control group at $p < 0.05$; bw, Body weight; g%, relative weight of organ (g) to body weight (g); mg%, relative weight of organ (mg) to body weight (g).

Table 6. Organ weight.jpg / 120.22 KB (image/pjpeg)

		Main group				Recovery group	
Dose (mg/kg bw/day)	Unit	0 (Control)	62.5	125	250	0 (Control)	250
No. of female rats		9	10	10	10	4	5
Body weight (g)		299.8 ± 17.7	322.1 ± 40.0	322.9 ± 23.3	318.5 ± 16.8	308.7 ± 12.1	330.0 ± 15.5
Liver	(g)	8.96 ± 0.76	10.05 ± 2.25	10.05 ± 1.53	9.53 ± 0.56	11.31 ± 0.62	11.89 ± 0.54
	(g%)	2.99 ± 0.15	3.10 ± 0.43	3.11 ± 0.35	2.99 ± 0.15	3.67 ± 0.23	3.60 ± 0.06
Kidney-Left	(g)	0.97 ± 0.05	1.03 ± 0.12	1.00 ± 0.09	1.00 ± 0.09	1.04 ± 0.09	1.17 ± 0.07*
	(g%)	0.32 ± 0.03	0.32 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	0.36 ± 0.02
Kidney-Right	(g)	0.99 ± 0.05	1.05 ± 0.14	1.04 ± 0.08	1.02 ± 0.08	1.09 ± 0.05	1.17 ± 0.04*
	(g%)	0.33 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.32 ± 0.02	0.35 ± 0.01	0.35 ± 0.01
Spleen	(g)	0.62 ± 0.09	0.68 ± 0.15	0.72 ± 0.21	0.67 ± 0.15	0.59 ± 0.08	0.65 ± 0.07
	(g%)	0.21 ± 0.02	0.21 ± 0.04	0.22 ± 0.07	0.21 ± 0.04	0.19 ± 0.03	0.20 ± 0.02
Adrenal gland-Left	(mg)	45.0 ± 5.36	40.2 ± 5.69	46.6 ± 9.57	46.3 ± 7.82	35.8 ± 1028	53.0 ± 6.32*
	(mg%)	15.2 ± 2.05	12.7 ± 2.45	14.4 ± 3.44	14.6 ± 2.59	11.75 ± 3.50	15.8 ± 1.30
Adrenal gland-Right	(mg)	43.0 ± 7.63	41.8 ± 5.35	41.3 ± 12.25	43.4 ± 5.91	42.8 ± 4.79	45.8 ± 4.82
	(mg%)	14.3 ± 2.45	13.0 ± 2.16	12.8 ± 3.97	13.7 ± 2.36	13.8 ± 2.06	14.0 ± 1.22
Ovary-Left	(g)	0.056 ± 0.015	0.059 ± 0.010	0.064 ± 0.016	0.060 ± 0.009	0.052 ± 0.005	0.054 ± 0.006
	(g%)	0.019 ± 0.005	0.019 ± 0.005	0.020 ± 0.006	0.019 ± 0.003	0.017 ± 0.002	0.016 ± 0.002
Ovary-Right	(g)	0.061 ± 0.011	0.061 ± 0.015	0.067 ± 0.009	0.062 ± 0.015	0.041 ± 0.009	0.047 ± 0.009
	(g%)	0.020 ± 0.003	0.019 ± 0.004	0.021 ± 0.003	0.019 ± 0.005	0.013 ± 0.003	0.014 ± 0.003
Brain	(g)	1.96 ± 0.10	1.97 ± 0.10	1.99 ± 0.06	2.02 ± 0.05	1.93 ± 0.08	2.01 ± 0.03
	(g%)	0.66 ± 0.04	0.62 ± 0.07	0.62 ± 0.04	0.64 ± 0.03	0.62 ± 0.03	0.61 ± 0.03
Pituitary gland	(mg)	14.6 ± 6.17	15.1 ± 5.51	13.7 ± 5.27	20.8 ± 5.01	15.5 ± 1.91	13.8 ± 2.39
	(mg%)	4.8 ± 1.72	4.6 ± 1.65	4.7 ± 0.95	6.5 ± 1.35	5.0 ± 0.82	4.2 ± 0.84
Lung	(g)	1.34 ± 0.11	1.40 ± 0.28	1.43 ± 0.16	1.46 ± 0.19	1.35 ± 0.09	1.45 ± 0.08
	(g%)	0.45 ± 0.03	0.43 ± 0.05	0.44 ± 0.04	0.46 ± 0.06	0.44 ± 0.05	0.44 ± 0.04
Heart	(g)	1.12 ± 0.08	1.25 ± 0.14	1.19 ± 0.09	1.21 ± 0.14	1.22 ± 0.03	1.33 ± 0.13
	(g%)	0.37 ± 0.03	0.39 ± 0.04	0.37 ± 0.03	0.38 ± 0.03	0.40 ± 0.02	0.40 ± 0.03
Thymus	(g)	0.34 ± 0.07	0.36 ± 0.11	0.36 ± 0.07	0.40 ± 0.13	0.25 ± 0.06	0.26 ± 0.06
	(g%)	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.04	0.08 ± 0.02	0.08 ± 0.01
Thyroid gland	(mg)	21.8 ± 5.78	14.7 ± 4.88	18.6 ± 6.45	21.5 ± 9.76	18.5 ± 3.11	23.8 ± 8.58
	(mg%)	7.1 ± 2.20	4.6 ± 1.43	5.7 ± 2.16	6.6 ± 2.84	7.5 ± 5.0	8.0 ± 4.47
Salivary gland	(g)	0.66 ± 0.15	0.63 ± 0.12	0.62 ± 0.11	0.64 ± 0.10	0.65 ± 0.07	0.62 ± 0.09
	(g%)	0.22 ± 0.04	0.19 ± 0.03	0.19 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.19 ± 0.03
Uterus	(g)	1.16 ± 0.31	0.97 ± 0.13	1.06 ± 0.21	1.12 ± 0.41	0.54 ± 0.13	0.55 ± 0.11
	(g%)	0.39 ± 0.10	0.30 ± 0.04	0.33 ± 0.06	0.35 ± 0.13	0.18 ± 0.04	0.16 ± 0.03

*Significantly different from values of control group at $p < 0.05$; bw, Body weight; g%, relative weight of organ (g) to body weight (g); mg%, relative weight of organ (mg) to body weight (g).

Table 7. Histopathological.jpg / 103.77 KB (image/pjpeg)

Group		Male				Female			
		Treated group		Recovery group		Treated group		Recovery group	
Dose (mg/kg bw/day)		Control	250	Control	250	Control	250	Control	250
No. of animals examined		10	10	5	5	9	10	4	5
Organs	Histopathological findings								
Liver	No abnormalities detected	6	5	3	3	4	3	2	4
	Focal necrosis/minimal	1	0	0	0	0	0	0	0
	Parenchymal necrosis/minimal	0	0	0	0	0	1	0	0
	Inflammatory cells/minimal	4	5	2	2	2	0	2	1
	Extramedullary haematopoiesis/minimal	0	0	0	0	5	5	0	0
	Hepatocyte vacuolation/minimal	1	0	0	0	1	3	0	0
	Clear cell focus/minimal	0	0	0	0	0	1	0	0
Kidney	No abnormalities detected	7	6	3	3	2	5	1	1
	Interstitial inflammatory cells/minimal	3	1	0	1	0	3	0	0
	Cortical tubular basophilia/minimal	2	1	2	1	0	1	0	0
	Tubular basophilia/ minimal/slight	0	1/1	0	1	0	0	0	0
	Medullary tubular dilatation/minimal	0	0	0	0	0	0	0	1
	Cortical scar/minimal	0	1	0	0	0	0	1	0
	Cystic tubules	0	1	0	0	0	0	0	0
	Pelvic mineralisation/minimal	0	0	0	0	1	0	2	1
	Cortico-medullary mineralisation/minimal	0	0	0	0	7	5	1	4
Adrenal gland	No abnormalities detected	8	7	5	2	9	10	3	5
	Cortical vacuolation/minimal	2	3	0	3	0	0	0	0
	Cortical hypertrophy/minimal	0	0	0	0	0	0	1	0
Heart	No abnormalities detected	7	7	4	4	9	10	3	4
	Myocardial inflammatory cells/minimal/slight	2/1	3	1	1	0	0	1	1
Lung	No abnormalities detected	7	4	3	3	6	5	3	4
	Alveolar macrophages/minimal	1	1	0	0	0	2	1	0
	Perivascular inflammatory/minimal	2	3	1	0	2	2	0	0
	Subpleural inflammatory cells/minimal	0	1	0	0	0	0	0	0
	Cholesterol granuloma/minimal	0	2	1	2	1	1	0	0
	Granulomas	0	0	0	0	0	2	0	1
	Alveolitis	0	0	0	0	0	1	0	0
Ovary	No abnormalities detected					9	10	4	5
Spleen	No abnormalities detected	9	7	3	2	1	1	3	5
	Extramedullary haematopoiesis/minimal/slight/moderate	1	3	1/1	3	2/3/1	2/4/3	1	0
Prostate gland/vagina	No abnormalities detected	7	5	1	3	9	10	4	5
	Concretion	0	0	1	0	0	0	0	0
	Interstitial inflammatory cells/minimal/slight	3	4/1	2/2	2	0	0	0	0
Testis	No abnormalities detected	10	10	5	5				
Thymus	No abnormalities detected	10	9	5	5	9	10	4	5
	Cortical apoptosis/minimal	0	1	0	0	0	0	0	0
Thyroid gland	No abnormalities detected	5	8	4	5	7	7	1	4
	Ultimobranchial cysts	4	2	1	0	1	2	2	1
	Lymphoid cell hyperplasia/minimal	1	0	0	0	0	0	1	0
Stomach	No abnormalities detected	9	10	5	5	9	10	3	5
	Submucosal oedema and inflammatory cells/minimal	1	0	0	0	0	0	1	0
Urinary bladder	No abnormalities detected	8	8	5	5	9	9	4	5
	Submucosalinflammatory cells/minimal	1	0	0	0	0	1	0	0
Pancreas	No abnormalities detected	9	9	3	4	9	10	4	4
	Fat infiltration/minimal	1	0	1	0	0	0	0	0
	Granulomas/slight	0	0	0	1	0	0	0	0
	Acinar cell apoptosis/minimal	0	0	0	0	0	0	0	1
	Inflammatory cells/minimal	0	1	1	0	0	0	0	0

bw, Body weight.

Table 8. Reproductive.jpg / 66.11 KB (image/pjpeg)

Dose (mg/kg bw/day)		0 (Control)	62.5	125	250
No. of females		15	10	10	15
Precoital interval (day)		4.0 ± 0.3	4.2 ± 0.2	4.1 ± 0.4	4.2 ± 0.5
Mating rate (%)		100	100	100	100
Fertility rate (%)		100	100	100	100
Pregnancy rate (%)		86.7	100	100	100
Functional observation of P					
Male					
No. of male rats		5	5	5	5
Auditory response		+	+	+	+
Pupillary reflex		+	+	+	+
Nociceptive reflex		4.8 ± 0.9	5.0 ± 1.3	4.0 ± 1.6	4.2 ± 1.1
Rotarod	Time (sec)	7.7 ± 4.0	22.2 ± 15.5	10.4 ± 11.5	8.9 ± 6.3
	Distance (m)	0.28 ± 0.14	0.86 ± 0.50	0.38 ± 0.42	0.33 ± 0.23
Passive avoidance	1st	41.2 ± 17.4	25.6 ± 18.5	20.3 ± 11.7	27.0 ± 11.2
	2nd	38.1 ± 28.9	83.3 ± 54.6	51.2 ± 46.7	19.1 ± 12.2
Female					
No. of female rats		5	5	5	5
Auditory response		+	+	+	+
Pupillary reflex		+	+	+	+
Nociceptive reflex		5.1 ± 1.2	4.2 ± 2.4	4.6 ± 1.8	4.0 ± 0.9
Rotarod	Time (sec)	14.0 ± 4.8	13.2 ± 9.6	13.9 ± 10.5	14.2 ± 4.3
	Distance (m)	0.51 ± 0.17	0.48 ± 035	0.51 ± 0.38	0.53 ± 0.16
Passive avoidance	1st	46.6 ± 19.0	23.6 ± 15.3	13.5 ± 5.1	14.0 ± 9.5
	2nd	29.0 ± 35.4	24.1 ± 18.7	11.9 ± 6.8	16.9 ± 13.1
F1 rats					
Duration of pregnancy (days)		21.5 ± 0.5	21.6 ± 0.5	21.5 ± 0.5	22 ± 0.5
No. of corpora lutea		27.3 ± 7.5	28.2 ± 4.5	30.2 ± 3.1	26.1 ± 4.3
No. of implantation		14.6 ± 1.8	15.9 ± 1.5	15.1 ± 1.9	15.5 ± 1.51
Pre-implantation loss (%)		43.2	43.7	49.6	39.5
Post-implantation loss (%)		0.0	1.8	0.0	0.7
Delivery rate (%)		86.7	100	100	100
Sex rate (male/female)		0.96	0.99	0.88	1.03
No. of survival/0 days		14.6 ± 1.8	15.6 ± 1.4	15.1 ± 1.9	15.4 ± 1.4
No. of survival/4 days		14.3 ± 1.8	15.5 ± 1.4	15.0 ± 1.8	15.3 ± 1.4
Survival rate/4 days (%)		97.9 ± 3.2	99.4 ± 1.9	99.4 ± 2.0	99.1 ± 2.3
Body weight of offspring (g)					
Male	Day 0	6.9 ± 0.5	6.8 ± 0.7	6.9 ± 0.7	7.0 ± 0.7
	Day 4	10.0 ± 1.0	9.6 ± 1.1	9.5 ± 1.6	10.0 ± 1.0
Female	Day 0	6.6 ± 0.4	6.3 ± 0.6	6.3 ± 0.7	6.6 ± 0.6
	Day 4	10.0 ± 1.0	9.0 ± 1.1	8.9 ± 1.6	9.4 ± 0.9

bw, Body weight.

Applicant's summary and conclusion

Conclusions

No treatment-related changes in detailed functional observation were observed in any of the treatment groups. No statistically significant differences wereobserved in the following parameters examined: gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4.

7.8.2 Developmental toxicity / teratogenicity

Endpoint study record: 7440-22-4, Developmental toxicity-teratogenicity, Hong, 2014, 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	GLP study conducted according to OECD 422 Guideline with deviations: details on test item, acclimation, housing and feeding conditions, mating procedure, body weight, food/water consumption, haematology, clinical biochemistry and histopathology 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	No information				National Institute of Environmental Research (NIER), Korea,		
publication	Hong JS, Kim S, Lee SH, Jo E, Lee B, Yoon J, Eom IC, Kim HM, Kim P, Choi K, Lee MY, Seo YR, Kim Y, Lee Y, Choi J and Park K.	2014	Combined Repeated-Dose Toxicity Study of Silver Nanoparticles with the Reproduction/Developmental Toxicity Screening Test.	Nanotoxicology. 8: 349-62.					

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

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Guideline 422 “Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test”	yes (details on test item, acclimation, housing and feeding conditions, mating procedure, body weight, food/water consumption, haematology, clinical biochemistry and histopathology not reported)

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): Citrate capped silver nanoparticle (AgNPs)
- Source: ABC Nanotech Co., Ltd., Korea

Confidential details on test material

No data

Test animals

Species

rat

Strain

Sprague-Dawley

Details on test animals and environmental conditions

TEST ANIMALS
- Age at study initiation: 7 weeks

Administration / exposure

Route of administration

oral: unspecified

Vehicle

other: sterilized water

Details on exposure

No data

Analytical verification of doses or concentrations

no

Details on analytical verification of doses or concentrations

No data

Details on mating procedure

No data

Duration of treatment / exposure

42 Days

Frequency of treatment

Male: 14 days before mating, 14 days during the mating, and 14 days of post-mating Female :14 days

before mating, during the mating and gestation, and 4 days of lactationExposure period: Once a day

Duration of test

42 Days

Doses / concentrations

62.5, 125, 250 mg/kg bw/day

Basis actual ingested

No. of animals per sex per dose

50

Control animals

other: control was provided with normal drinking water without AgNPs

Further details on study design

No data

Examinations

Maternal examinations

CLINICAL OBSERVATIONS: Yes
- Time schedule: All the animals were monitored once a day during the experimental period.

Ovaries and uterine content

The ovaries and uterine content was examined after termination: Yes Examinations included:
- Number of corpora lutea: Yes
- Number of implantations: Yes
- Pre- implantations loss: Yes
- Post- implantations loss: Yes

Fetal examinations

Number of live and dead pups, percentage of live and dead pups to implantations, sex ratio, survival rate, number of neonates with external anomalies and body weights of pups on post-natal day 0 and 4

Statistics

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SPSS Statistical Analysis Systems (SPSS 10.1 Base, SPSS Korea Data Solution. Co.Ltd.). The data were presented as mean ± SD. Variance in the numerical data was checked using Levene's test. If the variance was homogeneous, the one-way ANOVA test was conducted to determine which pairs of group comparison were significantly different. If this test showed significance between the groups, the data were analyzed by the multiple comparison procedure of the Dunnet’s post-hoc test.

Indices

No data

Historical control data

None

Any other information on materials and methods incl. tables

None

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Based on	Basis for effect level / Remarks
NOAEL	developmental toxicity	> 250 mg/kg bw/day (actual dose received)	test mat.	

Maternal toxic effects

no effects

Details on maternal toxic effects

No statistically significant differences were seen in the following parameters examined- Gestation period: Gestation and up to day 4 post-partum- The number of corpora lutea and implantation: No statistically significant differences were seen- Delivery rate: No statistically significant differences were seen

Embryotoxic / teratogenic effects

no effects

Details on embryotoxic / teratogenic effects

- Number of live and dead pups: No statistically significant differences were seen
- Percentage of live and dead pups to implantations: No statistically significant differences were seen
- Pre- implantations loss: No statistically significant differences were seen
- Post- implantations loss: No statistically significant differences were seen
- Sex ratio: No statistically significant differences were seen
- Survival rate: No statistically significant differences were seen
- Number of neonates with external anomalies: No statistically significant differences were seen
- Body weights of pups on post-natal day 0 and 4: No significant toxicity was observed

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

No statistically significant differences were observed in the following parameters examined: the number of live and dead pups, the percentage of live and dead pups to implantations, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4. NOAEL of the test article are considered to be more than 250 mg/kg bw/day for general toxicity in F1 pups.

Executive summary

In a Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening

Test conducted according to OECD Guideline 422 and in compliance with GLP, Citrate capped silver nanoparticle (AgNPs) was orally administered to groups of Sprague-Dawley rats at 0, 62.5, 125 and 250 mg/kg bw/day for 42 days (Male: 14 days before mating, 14 days during the mating, and 14 days of post-mating; Female: 14 days before mating, during the mating and gestation, and 4 days of lactation). During the study, data was recorded on clinical observations, gestation period, the number of corpora lutea and implantation, delivery rate, the number of live and dead pups, the percentage of live and dead pups to implantations, pre-implantation loss, post-implantation loss, sex ratio, survival rate, number of neonates with external anomalies and body weights of pups.
No statistically significant differences were observed in the following parameters examined: the number of live and dead pups, the percentage of live and dead pups to implantations, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4. NOAEL of the test article are considered to be more than 250 mg/kg bw/day for general toxicity in F1 pups.
Under the test condition, the No Observed Adverse Effect Level (NOAEL) of AgNPs is considered to be more than 250 mg/kg bw/day for general toxicity in F1 pups.

Cross-reference to other study

No cross-reference

Endpoint study record: Developmental toxicity / teratogenicity.001

Administrative Data

Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	1a - GLP guideline study(OECD)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	National Institute of Environmental Research(NIER)	2011	Combined repeated dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test		Korea Testing & Research Institute (KTR)	TBH-555			

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: OECD Test Guideline(Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test)	

Test materials

Test material identity

Identifier	Identity
other: Identifier Reference Material/Nanomaterial	
CAS name	Silver
CAS number	7440-22-4

Details on test material

- Name of test material (as cited in study report): Citrate capped silver nanoparticles
- Molecular formula (if other than submission substance): Ag
- Substance type: Inorganic
- Physical state: Liquid
- Analytical purity: 20.29%
- Impurities (identity and concentrations): No data
- Composition of test material, percentage of components: No data
- Isomers composition: No data- Purity test date: No data
- Lot/batch No.: No data- Expiration date of the lot/batch: No data
- Stability under test conditions: No data
- Storage condition of test material: dark condition(4℃)
- Other: Composition of the nanomaterials(including purity, impurities and additives):

Black colloidal suspension obtained from ABC Nanotech Co. Ltd. (Korea).
Citrate (0.5-1.5%) was used as a stabilizer for the colloidal particles.
The sizes of AgNPs, specified by the manufacturer, range from 5 to 25 nm (average 10 nm).
Characteristics/identifiers specific to nanomaterials: To confirm the characteristics, homogeneity and stability of test nanoparticles, the following were used:
1) dynamic light scattering (DLS) and transmission electron microscopy (TEM): size/ size distribution and agglomeration/aggregation state,
2) electrophoretic light scattering (ELS): zeta potential/surface charge,
3) X-ray diffraction (XRD): crystalline phase/crystallite size.

Confidential details on test material

no data

Test animals

Species

rat

Strain

Sprague-Dawley

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Orientbio
- Age at study initiation: 8-9 week
- Weight at study initiation: no data
- Fasting period before study: no data
- Housing: no data
- Diet : ad libitum
- Water : ad libitum
- Acclimation period: 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (°C): 20.5 - 23.5 ℃
- Humidity (%): 47.7 - 62.0 %
- Air changes (per hr): 10 - 15 h,
- Photoperiod (hrs dark / hrs light): 12h /12h
IN-LIFE DATES: From: To:

Administration / exposure

Route of administration

oral: gavage

Type of inhalation exposure (if applicable)

no data

Vehicle

water

Details on exposure

no data

Details on mating procedure

- Impregnation procedure: cohoused
- If cohoused: Each morning, the females were examined for the presence of sperm or a vaginal plug. Day 0 of pregnancy was defined as the day when a vaginal plug or sperm was found- M/F ratio per cage:
1- Length of cohabitation: no data
- After ... days of unsuccessful pairing replacement of first male by another male with proven fertility. no data
- Further matings after two unsuccessful attempts: [no / yes (explain)] no data
- Verification of same strain and source of both sexes: [yes / no (explain)] no data
- Proof of pregnancy: vaginal plug and sperm in vaginal smear referred to as day 0 of pregnancy
- Any other deviations from standard protocol: no data

Duration of treatment / exposure

42 days

Frequency of treatment

once a day

Duration of test

Male : 14 days before mating, 14 days during the mating, and 14 days of post-mating Female :14 days before mating, during the mating and gestation, and 4 days of lactation

Doses / concentrations

62.5, 125, 250 mg/kg

Basis nominal in water

No. of animals per sex per dose

50 / male, female / 62.5, 125, 250 mg/kg

Control animals

yes

Further details on study design

no data

Examinations

Maternal examinations

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: once a day
- Cage side observations checked in table [No.?] were included.
DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: once a day
BODY WEIGHT: Yes
- Time schedule for examinations: once a week during pre-mating, mating and recovery periods. During pregnancy and lactation periods, body weights were measured as follows; day 0 (the day of pregnancy), 3, 6, 9, 12, 15, 18 and 20 of gestation of pregnant rats, and day 0 (the day of delivery) and 4 post-partum of offspring
FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): no data- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: No data
WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): no dat
a- Time schedule for examinations: no dataOTHER: no data

Ovaries and uterine content

The ovaries and uterine content was examined after termination: No dataExaminations included:- Gravid uterus weight: No data- Number of corpora lutea: No data- Number of implantations: No data- Number of early resorptions: No data- Number of late resorptions: No data- Other: No data

Fetal examinations

- External examinations: No data- Soft tissue examinations: No data- Skeletal examinations: No data- Head examinations: No data

Results and discussions

Effect levels

Endpoint	Effect type	Effect level	Based on	Basis for effect level / Remarks
NOAEL	developmental toxicity	> 250		overall effectsnumber pregnant; number aborting; number of implantations; no. of total litter losses by resorption; duration of pregnancy; mortality; clinical signs; body weight; ophthalmoscopic examination; clinical observations; blood analysis; haematology; clinical chemistry; urinalysis; gross pathology; organ weights; histopathology; other:

Maternal toxic effects

no effects

Embryotoxic / teratogenic effects

no effects

Overall remarks, attachments

Remarks on results including tables and figures

Ophthalmologic findings incidence and severity: Not testedObservation on parturition date and lactation periodNo statistically significant differences were seen in the following parameters examinedGestation period : gestation and up to day 4 post-partumThe number of corpora lutea and implantation : No statically significant differences were seenDelivery rate : No statically significant differences were seenThe number of live and dead pups : No statically significant differences were seenThe percentage of live and dead pups to implantations : No statically significant differences were seenPre- implantations loss : No statically significant differences were seenPost- implantations loss : No statically significant differences were seenSex ratio : No statically significant differences were seensurvival rate : No statically significant differences were seennumber of neonates with external anomalies : No statically significant differences were seenBody weights of pups on post-natal day 0 and 4 : No significant toxicity was observed

Applicant's summary and conclusion

Conclusions

No statistically significant differences were observed in the following parameters examined: the number of live and dead pups, the percentage of live and dead pups to implantations, sex ratio, survival rate, number of neonates with external anomalies, and body weights of pups on post-natal day 0 and day 4.

Cross-reference to other study

Legal entity: NAPIRAhub OECD-WPMN Silver UUID NAPI-ab57fd40-aacc-4f1c-8d8b-6559efd7c0cb Dossier UUID 0 Author admin / (No legal entity) Date 2009-07-13 14:39:34 CEST Remarks General information Legal entity name NAPIRAhub OECD-WPMN Silver Legal entity type other: research centre Remarks Legal entity for the OECD-WPMN Silver NAPIRAhub Contact information Contact address Address EC/JRC/IHCP Via E. Fermi Postal code I-21020 Town Ispra Region / State VA Country Italy E-mail jrc-info-napirahub@ec.europa.eu

7.9 Specific investigations

7.9.1 Neurotoxicity

7.9.2 Immunotoxicity

7.9.3 Specific investigations: other studies

7.10 Exposure related observations in humans

7.10.1 Health surveillance data

Endpoint study record: 7440-22-4, Health surveillance data, Lee, 2012, 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	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	Lee JH, Mun J, Park JD and Yu IJ.	2012	A Health Surveillance Case Study on Workers Who Manufacture Silver Nanomaterials.	Nanotoxicology. 6 (6): 667-669.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Study type

other: voluntary biomonitoring program

Endpoint addressed

other: occupational exposure effects

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

A health surveillance study was conducted in a workplace which manufactures silver nanomaterials, including the assessment of personal exposure levels to silver nanoparticles, a walk-through evaluation of the manufacturing process and the collection and analysis of blood and urine samples from the exposed workers.

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): Silver nanoparticles

Confidential details on test material

No data

Method

Type of population

occupational

Ethical approval

no data

Details on study design

- Sampling site: The workplace manufacturing silver nanoparticles was previously described by Lee et al. (2011). Essentially, a large-scale pilot reactor was used to manufacture silver nanoparticles <100 nm in size at a rate of 5 kg/day. Various types of precursor (wire type, powder type, liquid type) were fed through an inductively coupled plasma (ICP) torch, where the precursors were vaporised in the argon plasma. This vaporised precursor gas was then moved through a second zone of plasma produced from a passive ICP RF-antenna. In this zone, the atoms were condensed to nanoscale particles using a specific temperature gradient and cooling process. From the precursor feeding to the nanopowder collection, all the processes were conducted in a vacuum state. The connection from the reactor to the collector was a complete closed system, and thus very few silver nanoparticles were released from the collector into the workplace air. Silver nanoparticles ranging from 20 to 30 nm and manufactured as silver powders were introduced to the reactor using a torch and reacted with acetylene and oxygen gases. The manufactured silver nanoparticles were then amassed in a collector. A total of 5 workers were normally involved in the silver nanomaterial manufacturing (Lee et al. 2011).
- Personal sampling: The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 mm nominal pore-size, and 2 in. cowl) obtained from Pall Corp. (P/N 64678; Ann Arbor, MI). The filter samples for personal sampling were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5–2.0 L/min and SKC (Leland Legacy pump) operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short.

The sampling holders were also changed during the sampling period to avoid overload. The sampling with personal samplers was performed during the normal work period from 09:30 to 16:00 and typically lasted 159–350 min. The personal samplers were attached to the workers involved in the nanomaterial manufacturing. The total suspended particulate concentration was determined gravimetrically based on the NIOSH manual of analytical methods (NMAM) 0500 (1994).

Any other information on materials and methods incl. tables

Silver analysis: Silver concentrations on the filter were analysed using an ICP (Perkin Elmer optima 5300DV) based on the NMAM 7300-ICP method (NIOSH, 2003) after wet digestion using the ICP-OES Plasma Spectrometer method. The filters were digested in a microwave (MARS Xpress, CEM) for 15 min at 150 C in the presence of nitric acid. After digestion, the samples were allowed to cool and analysed by ICP.
Collection and analysis of biological samples: A trained occupational nurse collected urine and drew blood samples from 2 workers after their shift. The analysis of the blood samples included the blood biochemistry and a haematological examination. The data were reported to an occupational physician for an evaluation of their health status.

Determination of tissue silver: Blood and urine were digested with concentrated nitric acid by using the microwave digestion system (MARS 230/60,CEM). The concentration of silver in digested fluid was analysed with a flameless method using an atomic absorption spectrophotometer equipped with a Zeeman graphite furnace (Perkin Elmer 5100ZL, Zeeman Furnace Module, USA) based on the NIOSH 7300 method. The concentration of silver in the tissue was expressed as mg/dl wet weight.

Results and discussions

Results

Only 2 workers participated in the voluntary biomonitoring program. The workers were 37 and 42 years of age, male, and had worked for 7 years in the nanosilver manufacturing industry. Despite being exposed to 0.15777 and 0.10869 mg/m3 of total suspended particulate, the silver concentrations were 0.35 and 1.35 mg/m3, which are lower than the current occupational exposure limit for both silver dust and soluble silver compounds. The levels of silver in their blood and urine were also low. When the blood chemistry and haematology data were evaluated by an occupational physician, the blood data were determined to be within a normal range.

Any other information on results incl. tables

Table 7.10.1/1: Demographic data and silver concentrations related to personal exposure, and blood and urine samples

Worker	Gender/age	Occupational history (years)	TSP (mg/m3)	Air Ag (mg/m3)	Blood (mg/dl)	Urine (mg/dl)
1	Male/37	7	0.15755	0.00035	0.034	0.043
2	Male/42	7	0.10869	0.00135	0.030	ND

Overall remarks, attachments

Remarks on results including tables and figures

None

Applicant's summary and conclusion

Conclusions

The health surveillance data indicated that the nanomaterial manufacturing workers were exposed to much less than the concentrations of silver dust (100 mg/m3) or soluble silver (10 mg/m3) threshold limit values for the workplace air or silver nanoparticle 90-day inhalation toxicity NOAEL of 100 mg/m3 (Sung et al. 2009). Furthermore, their blood and urine concentrations of silver were within the normal reported range, and their health status estimated by their blood biochemistry and haematology showed no significant findings. Thus, engineering control such as a closed system from the generation of silver nanoparticles to collection can reduce exposure to silver nanoparticles and contribute to the health status of workers who manufacture silver nanomaterials.

Executive summary

A health surveillance study was conducted in a workplace manufacturing silver nanoparticles. A total of 5 workers were normally involved in the silver nanomaterial manufacturing (Lee et al. 2011), only 2 workers participated in the voluntary biomonitoring program. The workers were 37 and 42 years of age, male, and had worked for 7 years in the nanosilver manufacturing industry. The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 mm nominal pore-size, and 2 in. cowl) obtained from Pall Corp. (P/N 64678; Ann Arbor, MI). The filter samples for personal sampling were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5–2.0 L/min and SKC (Leland Legacy pump) operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short. The sampling with personal samplers was performed during the normal work period from 09:30 to 16:00 and typically lasted 159–350 min. The total suspended particulate concentration was determined gravimetrically based on the NIOSH manual of analytical methods (NMAM) 0500 (1994). Silver concentrations on the filter were analysed using an ICP (Perkin Elmer optima 5300DV) based on the NMAM 7300-ICP method (NIOSH, 2003) after wet digestion using the ICP-OES Plasma Spectrometer method. The concentration of silver in blood and urine was analysed with a flameless method using an atomic absorption spectrophotometer equipped with a Zeeman graphite furnace (Perkin Elmer 5100ZL, Zeeman Furnace Module, USA) based on the NIOSH 7300 method.
Two male workers who had worked for 7 years in the business of manufacturing silver nanomaterialwere being exposed to 0.15777 and 0.10869 mg/m^{3}of total suspended particulate, however the silver concentrations were 0.35 and 1.35 mg/m^3, which are lower than the current occupational exposure limit for both silver dust and soluble silver compounds. The health surveillance data indicated that the nanomaterial manufacturing workers were exposed to much less than the concentrations of silver dust (100 mg/m^3) or soluble silver (10 mg/m^3) threshold limit values for the workplace air or silver nanoparticle 90-day inhalation toxicity NOAEL of 100 mg/m^3(Sung*et al*. 2009). Furthermore, their blood and urine concentrations of silver were within the normal reported range, and their health status estimated by their blood biochemistry and haematology showed no significant findings.Taken together, the health surveillance indicated that the nanomaterial manufacturing workers were exposed to a much lower concentration of silver dust or soluble silver threshold limit values and showed no significant findings on their health status. Thus, engineering control such as a closed system from the generation of silver nanoparticles to collection can reduce exposure to silver nanoparticles and contribute to the health status of workers who manufacture silver nanomaterials.

Cross-reference to other study

No cross-reference

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: 7440-22-4, Exposure related observations in humans-other data, Lee, 2011, RS, K

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	Study period	2009
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	Lee JH, Kwon M, Ji JH, Kang CS, Ahn KH, Han JH and Yu IJ.	2011	Exposure Assessment of Workplaces Manufacturing Nanosized TiO2 and Silver.	Inhalation Toxicology, 23(4):226-236.					

Data access

data published

Cross-reference to same study

No cross-reference

Materials and methods

Type of information

To estimate the potential exposure of workers, personal sampling, area monitoring, and real-time monitoring using a scanning mobility particle sizer (SMPS) and dust monitor were conducted at workplaces where the workers handle nanomaterials.

Endpoint addressed

other: occupational exposure effects

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

A study was conducted at workplaces where the workers handle nanomaterials, personal sampling, area monitoring, and real-time monitoring using a scanning mobility particle sizer (SMPS) and dust monitor were performed to estimate the potential exposure of workers.

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): Silver nanoparticles

Confidential details on test material

No data

Method

Ethical approval

no data

Details on study design

See the section "Any other information on materials and methods incl. tables"

Exposure assessment

measured

Details on exposure

TYPE OF EXPOSURE: Occupational exposure
TYPE OF EXPOSURE MEASUREMENT: Personal and area sampling, Real-time aerosol monitoring, Metal analysis and Transmission electron microscopy

Any other information on materials and methods incl. tables

Table 7.10.5/1: Information on workplaces

Plant	Region (handling workers)	Manufactured materials	Process	Engineering controls	PPE use
C (Industry)	Daejeon (5)	Ag manufacturing	Induced coupled plasma with electric atomizer (ICPA type) pilot test	Natural ventilation	Working clothes
D (Industry)	Daejeon (4)	Ag application	Sodium citrate + silver nitrate	Natural ventilation, fume hood	Working clothes

Personal and area sampling: The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 μm nominal pore-size, and 2 in. cowl, open-face) obtained from Pall Corp. (P/N 64678; Ann Arbor, MI). The filter samples for personal sampling were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5–2.0 L/min and SKC (Leland Legacy pump)-operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short. The sampling holders were also changed during the sampling period to avoid overload. The sampling with personal samplers was performed during the normal work period from 09:30 to 16:00 and typically lasted from 159 to 350 min. The personal samplers were attached to the workers involved in the manufacturing nanomaterials. Some samples for area samplings were also collected on the filters by placing the samplers 1–4 m away from the manufacturing devices, the suspected emission sources for nanoparticles and several places in the workplace.
Real-time aerosol monitoring: An scanning mobility particle sizer (SMPS) combining a differential mobility analyzer (DMA, 4220, HCT Co., Ltd., Korea) and condensation particle counter (CPC, 4312, HCT Co., Ltd., 0–108 particles/cm3 detection range) was used to monitor the particle size distribution with the electrical mobility diameter ranging from 15 to 710.5 nm. Meanwhile, a dust monitor (Model 1.108, Grimm) was used to observe the particle size distribution with the diameter ranging from 0.3 to 20 μm. The workplace air was sampled at a low rate of 0.3 and 1.2 L/min for the SMPS and dust monitor, respectively. The SMPS scanned the particle sizes at a time resolution of 2.5 min (120 sec for up-scan and 30 sec for retrace), although the average times for the dust monitor were 6 sec to 1 min.
Metal analysis: After wet digestion using the inductive coupled plasma (ICP)-OES Plasma Spectrometer method, silver concentrations on the filter were analyzed using an ICP (Perkin Elmer optima 5300DV) based on the NIOSH Manual 7300-ICP method (NIOSH, 2003a). The filters were digested in a microwave (CEM MARS Xpress) for 15 min at 150 °C in the presence of nitric acid. After digestion, the samples were allowed to cool and analyzed by ICP.
Transmission electron microscopy: The air samples were analyzed according to National Institute of Occupational Safety and Health (NIOSH) analytical method 7402 (1994) and Han*et al*. (2008). The filter was coated with carbon and mounted onto carbon-coated copper grids (Veco, Eerbeek, Holland) using acetone vapor. Meanwhile, the Ag and TiO2 nanoparticles were morphologically identified using a scanning transmission electron microscope (STEM, Hitachi 7100, Tokyo) and determined by comparing the elemental composition of the Ag and TiO2 nanoparticles using an energy dispersive X-ray analyzer (EDX, KEVEX 7000Q, Foster City, CA) (Han*et al*., 2008; Lee*et al*., 2010).

Results and discussions

Results

Occupational exposure:
- Workplace C manufactured silver nanoparticles <100 nm in size using a large-scale pilot reactor. The daily production amount was 5 kg/day. Various types of precursor (the wire type, the powder type, the liquid type) are fed in the ICP torch. The precursor is vaporized in the argon plasma. This vaporized precursor gas moves through the second zone of plasma, which is produced from the passive ICP RF-antenna. In this zone, atoms condense to the nanoscale particle with some temperature gradient and the cooling process. From the precursor feeding to the nanopowder collection, all processes are fulfilled in the vacuum state. Silver nanoparticles ranging from 20 to 30 nm and manufactured as silver powders were

introduced to the reactor using a torch and reacted with acetylene and oxygen gases. The manufactured silver nanoparticles were collected in a collector. The actual silver metal concentrations ranged from 0.00002 to 0.00102 mg/m3, making them less than the silver dust 0.1 mg/m3 and silver soluble compound 0.01 mg/m3 occupational exposure limits. Transmission electron micrograph of silver nanoparticles sampled in front of source material feeding entrance showed agglomerated/aggregated silver nanoparticles.
- Workplace D manufactured silver nanoparticles by mixing sodium citrate with silver nitrate or an attrition milling operation. The daily production amount was 1 kg/day. The workplace was equipped with a fume hood and well-ventilated. The samples were supplied to the attrition miller from 10:30 into the afternoon, and a large amount of sodium citrate was weighed, added to the tank, and stirred to make it soluble. A sodium citrate solution and silver nitrate solution were then pumped into the reactor and mixed together to form the silver nanoparticles. The silver metal concentrations ranged from 0.00003 to 0.00043 mg/m3 (0.00118 mg/m3, 9.6 min), making them less than the current exposure limit of 0.1 mg/3. The number concentrations of ultrafine particles in the range of 15–710.5 nm were very low, showing 393.9- 3,525.8 particles/c m3. However, with an increase in the number of ultrafine particles, the number of fine particles larger than 0.3 μm also increased simultaneously. When the sodium citrate was added and mixed, the number of particles <50 nm increased (10:54), and the number of particles larger than 100 nm increased during the cleaning.

Any other information on results incl. tables

See the attached document for information on results

Overall remarks, attachments

Remarks on results including tables and figures

None

Attached background material

Attached document	Remarks
Human exposure.pdf / 355.87 KB (application/octet-stream)	

Test Substances

Identity (purity): Silver, CAS No. 7440-22-4 (silver)

Methods

Sampling site: The current study measured the nanoparticle concentrations inside four plants manufacturing Ag in 2009. The information related to each plant is shown in Table 5.9.

Table 5.9. Information on workplaces

Plant	Region (handling workers)	Manufactured materials	Process	Engineering controls	PPE use
C (Industry)	Daejeon (5)	Ag manufacturing	Induced coupled plasma with electric atomizer (ICPA type) pilot test	Natural ventilation	Working clothes
D (Industry)	Daejeon (4)	Ag application	Sodium citrate + silver nitrate	Natural ventilation, fume hood	Working clothes

Personal and area sampling: The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 μm nominal pore-size, and 2 in. cowl, open-face) obtained from Pall Corp. (P/N 64678; Ann Arbor, MI). The filter samples for personal sampling were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5–2.0 L/min and SKC (Leland Legacy pump)-operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short. The sampling holders were also changed during the sampling period to avoid overload. The sampling with personal samplers was performed during the normal work period from 09:30 to 16:00 and typically lasted from 159 to 350 min. The personal samplers were attached to the workers involved in the manufacturing nanomaterials. Some samples for area samplings were also collected on the filters by placing the samplers 1–4 m away from the manufacturing devices, the suspected emission sources for nanoparticles and several places in the workplace. Real-time aerosol monitoring: An scanning mobility particle sizer (SMPS) combining a differential mobility analyzer (DMA, 4220, HCT Co., Ltd., Korea) and condensation particle counter (CPC, 4312, HCT Co., Ltd., 0–108 particles/cm3 detection range) was used to monitor the particle size distribution with the electrical mobility diameter ranging from 15 to 710.5 nm. Meanwhile, a dust monitor (Model 1.108, Grimm) was used to observe the particle size distribution with the diameter ranging from 0.3 to 20 μm. The workplace air was sampled at a low rate of 0.3 and 1.2 L/min for the SMPS and dust monitor, respectively. The SMPS scanned the particle sizes at a time resolution of 2.5 min (120 sec for up-scan and 30 sec for retrace), although the average times for the dust monitor were 6 sec to 1 min. Metal analysis: After wet digestion using the inductive coupled plasma (ICP)-OES Plasma Spectrometer method, silver concentrations on the filter were analyzed using an ICP (Perkin Elmer optima 5300DV) based on the NIOSH Manual 7300-ICP method (NIOSH, 2003a). The filters were digested in a microwave (CEM MARS Xpress) for 15 min at 150°C in the presence of nitric acid. After digestion, the samples were allowed to cool and analyzed by ICP Transmission electron microscopy: The air samples were analyzed according to National Institute of Occupational Safety and Health (NIOSH) analytical method 7402 (1994) and Han *et al*. (2008). The filter was coated with carbon and mounted onto carbon-coated copper grids (Veco, Eerbeek, Holland) using acetone vapor. Meanwhile, the Ag and TiO2 nanoparticles were morphologically identified using a scanning transmission electron microscope (STEM, Hitachi 7100, Tokyo) and determined by comparing the elemental composition of the Ag and TiO2 nanoparticles using an energy dispersive X-ray analyzer (EDX, KEVEX 7000Q, Foster City, CA) (Han et al., 2008; Lee *et al*., 2010). **Results** Occupational exposure: Workplace C manufactured silver nanoparticles <100 nm in size using a large-scale pilot reactor. The daily production amount was 5 kg/day. Various types of precursor (the wire type, the powder type, the liquid type) are fed in the ICP torch. The precursor is vaporized in the argon plasma. This vaporized precursor gas moves through the second zone of plasma, which is produced from the passive ICP RF-antenna. In this zone, atoms condense to the nanoscale particle with some temperature gradient and the cooling process. From the precursor feeding to the	

nanopowder collection, all processes are fulfilled in the vacuum state. Silver nanoparticles ranging from 20 to 30 nm and manufactured as silver powders were introduced to the reactor using a torch and reacted with acetylene and oxygen gases. The manufactured silver nanoparticles were collected in a collector (Fig. 1). The time course of events at workplace C is described in Table 5.10. The actual silver metal concentrations ranged from 0.00002 to 0.00102 mg/m3, making them less than the silver dust 0.1 mg/m3 and silver soluble compound 0.01 mg/m3 occupational exposure limits (Table 5.11). Transmission electron micrograph of silver nanoparticles sampled in front of source material feeding entrance (Fig. 5.2 F, SKC 3 filter) showed agglomerated/aggregated silver nanoparticles (Fig. 5.3). Workplace D manufactured silver nanoparticles by mixing sodium citrate with silver nitrate or an attrition milling operation. The daily production amount was 1 kg/day. The workplace was equipped with a fume hood and well-ventilated. The samples were supplied to the attrition miller from 10:30 into the afternoon, and a large amount of sodium citrate was weighed, added to the tank, and stirred to make it soluble (Table 5.12). A sodium citrate solution and silver nitrate solution were then pumped into the reactor and mixed together to form the silver nanoparticles (Fig. 5.4 D). The silver metal concentrations ranged from 0.00003 to 0.00043 mg/m^3 (0.00118 mg/m3, 9.6 min), making them less than the current exposure limit of 0.1 mg/m3 (Table 5.11). The number concentrations of ultrafine particles in the range of 15–710.5 nm were very low, showing 393.9- 3,525.8 particles/cm^3 (Fig. 5.4 A). However, with an increase in the number of ultrafine particles, the number of fine particles larger than 0.3 μm also increased simultaneously (Fig. 5.4 A). When the sodium citrate was added and mixed, the number of particles <50 nm increased (10:54), and the number of particles larger than 100 nm increased during the cleaning (Fig. 5.4 B and 5.4 C).	

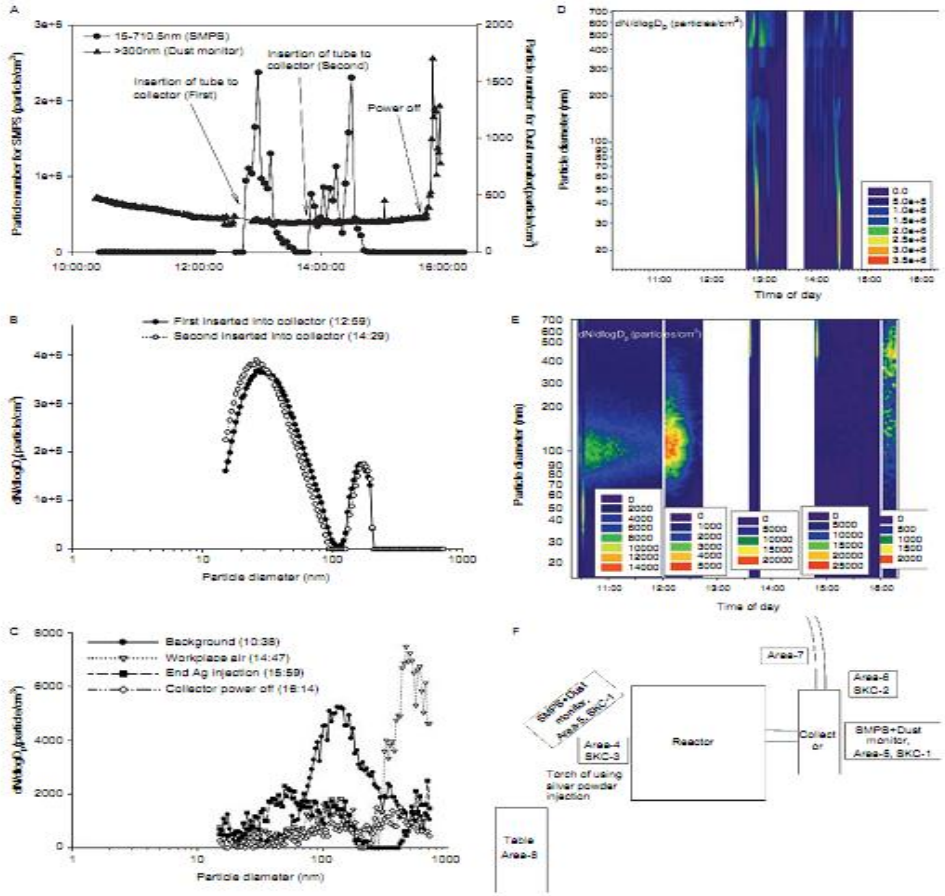


Fig.5.2. Real-time particle measurement in workplace C. (A) Particle number concentration measured by scanning mobility particle sizer (SMPS) and dust monitor; (B) particle size distributions in the collector measured by SMPS; (C) Particle size distribution in the workplace air measured by SMPS; (D) change in the particle size distributions by SMPS in the collector; (E) change in the particle size distributions by SMPS in the collector in the indoor; and (F) process and sampling locations

Table 5.10. Time course of events at workplace C

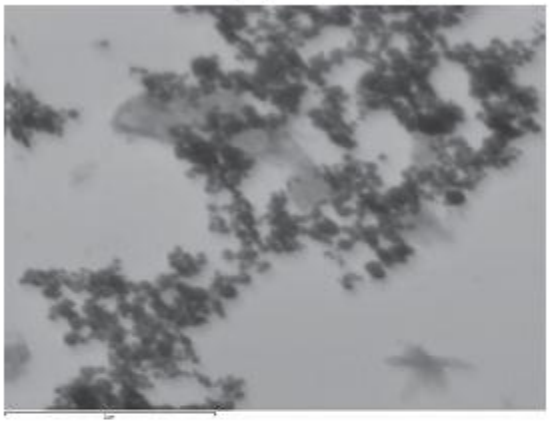
Time	Operation
10:27	Start of measurement
12:47–13:12	Powder forms of Ag were injected into the reactor using a torch (the scanning mobility particle sizer [SMPS] measurement was conducted by connecting a tube to the collector from 12:47; however, the dust monitor measurement could not be performed due to the pressure inside the collector)
13:45	Measuring the air concentration after removing the tube from the collector
13:49–14:33	Silver powder injection and tube connection to the collector
12:42	Measuring the air concentration after removing the tube from the collector
15:10–15:47	Silver powder injection and tube connection to the collector and workplace air sampling
16:02	Power off

Figure 2.

Figure 3. Table 5.11. Ag mass concentration of personal and area samples at workplaces C and D

Workplace	Process	Sampling site	Sampling time(min)	Sampling volume(L)	Metal concentraion (mg/m^3)
Workplace C	Area sampling	Area 5	229	452.3	0.00018
		SKC 1	226	1587.0	0.00028
		Area 6	222	432.2	0.00010
		SKC 2	221	1549.0	0.00024
		Area 4	237	459.8	0.00008
		SKC 3	234	1638.0	0.00002
		Area 8	237	457.6	0.00004
		Area 7	220	420.0	0.00034
	Personal sampling	Pers 9	159	315.8	0.00102
		Pers 3	160	315.2	0.00012
Workplace D	Laboratory	Area 5	222	426.9	0.00009
		SKC 1	220	1543.0	0.00004
		Area 7	223	433.3	0.00009
		SKC 2	217	1521.0	0.00003
		Area 4	219	426.2	0.00014
		SKC 3	9.6	67.7	0.00118
		Area 3	211	419.5	0.00029
		Pers 8	162	315.4	0.00038
	Other laboratory	Area 6	211	414.4	0.00010
		Pers 9	165	325.7	0.00043

A. Transmission electron micrograph of silver nanoparticles.



B. Energy dispersive x-ray profile (silver nanoparticles on copper grid).

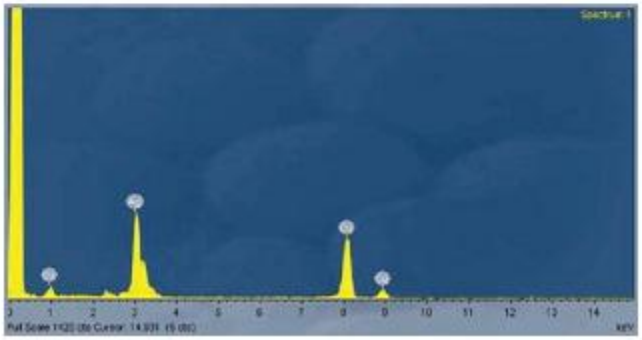


Fig. 5.3. Transmission electron micrograph of silver nanoparticles sampled in front of source material feeding entrance (Figure 1F, SKC 3 filter). (A) Transmission electron micrograph of silver nanoparticles. (B) Energy dispersive X-ray profile (silver nanoparticles on copper grid)

Table 5.12. Time course of events at workplace D

Time	Operation
10:20	Start of measurement
10:40	Samples were added to the attrition miller
10:45	Milling started at 10:45
10:53	Sodium citrate was added to tank-1 with stirring
10:58	Weighing of silver nitrate and stirring
11:04	Addition of other chemicals to tank-1
11:07	Sonication for 1 min
11:47	Stirring was ended in tank-1
11:48	Silver nitrate was added to tank-2
12:00	Tubes were inserted into tank-1 and tank-2, and connected to a pump. The mixed solutions from tank-1 and tank-2 were mixed and collected in another tank.
12:41	After ending the milling, the products were sampled and confirmed.

13:12	Pumping was ended and the silver solution operation was ended
13:21	Tanks were covered
After 13:21	Other operations were performed with and without workers and cleaned

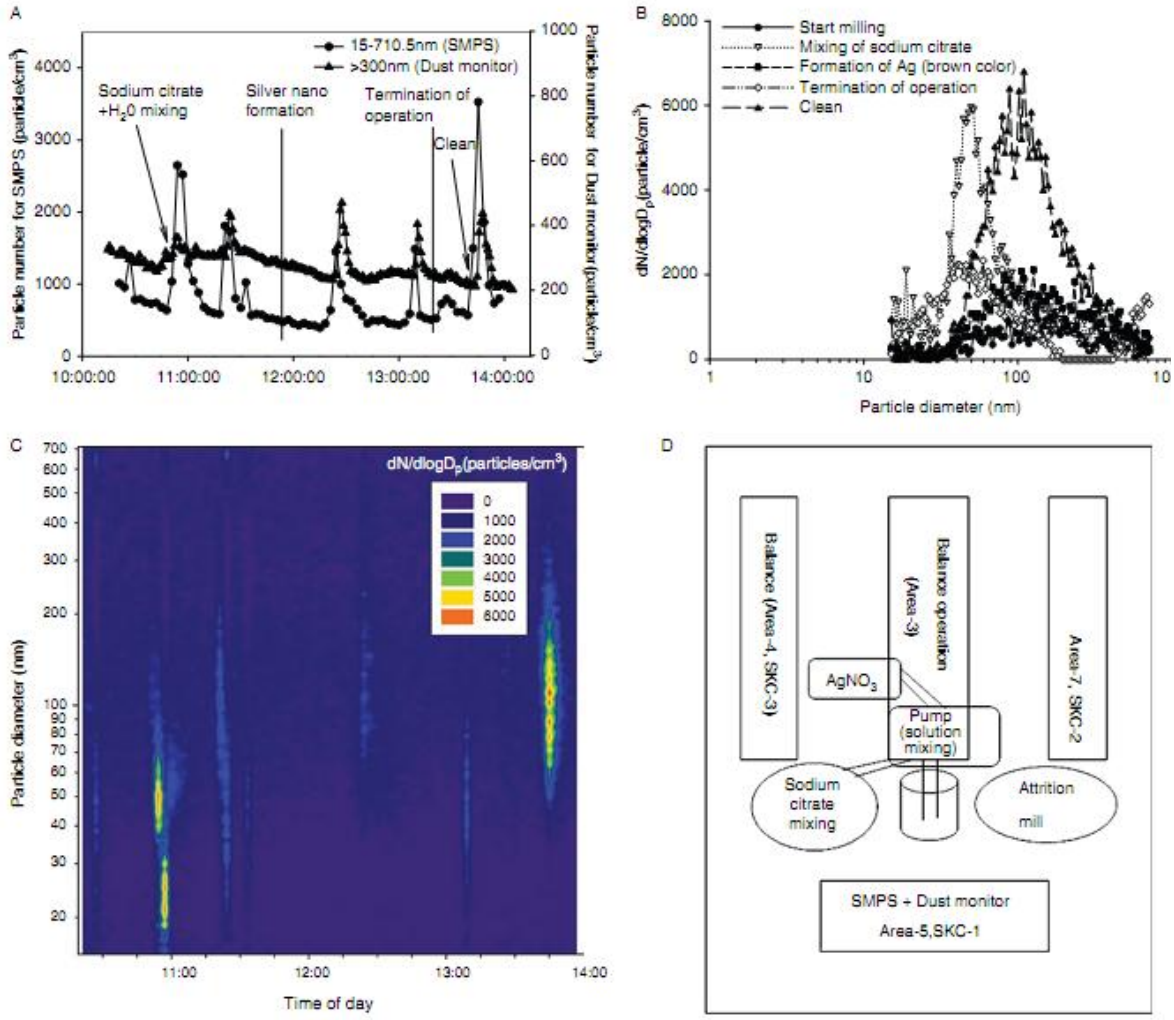


Fig.5.4. Real-time particle measurement in workplace D. (A) Particle number concentration measured by scanning mobility particle sizer (SMPS) and dust monitor; (B) particle size distributions measured by SMPS; (C) change in the particle size distributions by SMPS; and (D) process and sampling locations.

References

Ji Hyun Lee, Miran Kwon, Jun Ho Ji, Chang Soo Kang, Kang Ho Ahn, Jeong Hee Han, and Il Je Yu 2011. Exposure assessment of workplaces manufacturing nanosized TiO2 and silver. *Inhalatioin Toxicology*, 2011;23(4):226-236

Test Substances

Identity (purity): Silver, CAS No. 7440-22-4 (silver)

Methods

Sampling site: The workplace manufacturing silver nanoparticles was previously described by Lee *et al.* (2011). Essentially, a large-scale pilot reactor was used to manufacture silver nanoparticles <100 nm in size at a rate of 5 kg/day. Various types of precursor (wire type, powder type, liquid type) were fed through an inductively coupled plasma (ICP) torch, where the precursors were vaporised in the argon plasma. This vaporised precursor gas was then moved through a second zone of plasma produced from a passive ICP RF-antenna. In this zone, the atoms were condensed to nanoscale particles using a specific temperature gradient and cooling process. From the precursor feeding to the nanopowder collection, all the processes were conducted in a vacuum state. The connection from the reactor to the collector was a complete closed system, and thus very few silver nanoparticles were released from the collector into the workplace air. Silver nanoparticles ranging from 20 to 30 nm and manufactured as silver powders were introduced to the reactor using a torch and reacted with acetylene and oxygen gases. The manufactured silver nanoparticles were then amassed in a collector. A total of 5 workers were normally involved in the silver nanomaterial manufacturing (Lee et al. 2011)

Personal sampling: The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 mm nominal pore-size, and 2 in. cowl) obtained from Pall Corp. (P/N 64678; Ann Arbor, MI). The filter samples for personal sampling were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5–2.0 L/min and SKC (Leland Legacy pump) operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short. The sampling holders were also changed during the sampling period to avoid overload. The sampling with personal samplers was performed during the normal work period from 09:30 to 16:00 and typically lasted 159–350 min. The personal samplers were attached to the workers involved in the nanomaterial manufacturing. The total suspended particulate concentration was determined gravimetrically based on the NIOSH manual of analytical methods (NMAM) 0500 (1994).

Silver analysis: Silver concentrations on the filter were analysed using an ICP (Perkin Elmer optima 5300DV) based on the NMAM 7300-ICP method (NIOSH, 2003) after wet digestion using the ICP-OES Plasma Spectrometer method. The filters were digested in a microwave (MARS Xpress, CEM) for 15 min at 150 C in the presence of nitric acid. After digestion, the samples were allowed to cool and analysed by ICP.

Determination of tissue silver: Blood and urine were digested with concentrated nitric acid by using the microwave digestion system (MARS 230/60,CEM). The concentration of silver in digested fluid was analysed with a flameless method using an atomic absorption spectrophotometer equipped with a Zeeman graphite furnace (Perkin Elmer 5100ZL, Zeeman Furnace Module, USA) based on the NIOSH 7300 method. The concentration of silver in the tissue was expressed as mg/dl wet weight.

Collection and analysis of biological samples: A trained occupational nurse collected urine and drew blood samples from 2 workers after their shift. The analysis of the blood samples included the blood biochemistry and a haematological examination. The data were reported to an occupational physician for an evaluation of their health status. The blood and urine were digested with concentrated nitric acid using the microwave digestion system(MARS 230/60, CEM). The

concentration of silver in the digested fluid was then analysed by a flwas the method using an atomic absorption spectrophotometer equipped with a Zeeman graphite furnace (Perkin Elmer 5100ZL, Zeeman Furnace Module, USA) based on the NIOSH 7300 method (2003).

Results

Only 2 workers participated in the voluntary biomonitoring program. The workers were 37 and 42 years of age, male, and had worked for 7 years in the nanosilver manufacturing industry. Despite being exposed to 0.15777 and 0.10869 mg/m3 of total suspended particulate, the silver concentrations were 0.35 and 1.35 mg/m3 (Table 5.13), which are lower than the current occupational exposure limit for both silver dust and soluble silver compounds. The levels of silver in their blood and urine were also low. When the blood chemistry and haematology data were evaluated by an occupational physician, the blood data were determined to be within a normal range

Table 5.13. Demographic data and silver concentrations related to personal exposure, and blood and urine samples

Worker	Gender/age	Occupational history (years)	TSP (mg/m3)	Air Ag (mg/m3)	Blood (mg/dl)	Urine (mg/dl)
1	Male/37	7	0.15755	0.00035	0.034	0.043
2	Male/42	7	0.10869	0.00135	0.030	ND

Conclusions

Remarks: The health surveillance data in this report indicated that the nanomaterial manufacturing workers were exposed to much less than the concentrations of silver dust (100 mg/m3) or soluble silver (10 mg/m3) threshold limit values for the workplace air or silver nanoparticle 90-day inhalation toxicity NOAEL of 100 mg/m3 (Sung *et al*. 2009). Furthermore, their blood and urine concentrations of silver were within the normal reported range, and their health status estimated by their blood biochemistry and haematology showed no significant findings. Thus, engineering control such as a closed system from the generation of silver nanoparticles to collection can reduce exposure to silver nanoparticles and contribute to the health status of workers who manufacture silver nanomaterials.

References

Ji Hyun Lee, Jehyeok Mun, Jung Duck Park and Il je Yu. 2011. A health surveillance case study on workers who manufactures silver nanomaterials. *Nanotoxicology*, 2011; Early Online, 1-3

Applicant's summary and conclusion

Conclusions

The silver metal concentrations ranged from 0.00002 to 0.00118 mg/m3, which were also lower than the silver dust 0.1 mg/m3 and silver soluble compound 0.01 mg/m3 occupational exposure limits set by the ACGIH.

Executive summary

A study was conducted at workplaces where the workers handle nanomaterials, personal sampling, area monitoring, and real-time monitoring using a scanning mobility particle sizer (SMPS) and dust monitor were performed to estimate the potential exposure of workers.The air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (37 mm diameter, 0.8 mm nominal pore-size, and 2 in. cowl) obtained from Pall Corp. (P/N 64678; Ann Arbor, MI). The filter samples for personal sampling were collected in the breathing zone using MSA (Escort Elf pump)-operated sampling pumps at a flow rate of 1.5–2.0 L/min and SKC (Leland Legacy pump) operated sampling pumps at a flow rate of 6.9–7.3 L/min when the work duration was short. The sampling with personal samplers was performed during the normal work period from 09:30 to 16:00 and typically lasted 159–350 min.An scanning mobility particle sizer (SMPS) combining a differential mobility analyzer (DMA, 4220, HCT Co., Ltd., Korea) and condensation particle counter (CPC, 4312, HCT Co., Ltd., 0–108 particles/cm3 detection range) was used to monitor the particle size distribution with the electrical mobility diameter ranging from 15 to 710.5 nm. A dust monitor (Model 1.108, Grimm) was used to observe the particle size distribution with the diameter ranging from 0.3 to 20 μm. After wet digestion using the inductive coupled plasma (ICP)-OES Plasma Spectrometer method, silver concentrations on the filter were analyzed using an ICP (Perkin Elmer optima 5300DV) based on the NIOSH Manual 7300-ICP method (NIOSH, 2003a). Ag nanoparticles were morphologically identified using a scanning transmission electron microscope.
The silver metal concentrations ranged from 0.00002 to 0.00118 mg/m^3, which were also lower than the silver dust 0.1 mg/$m^3$and silver soluble compound 0.01 mg/ m^3occupational exposure limits set by the ACGIH. Similarly, the particle concentrations at the silver nanoparticle manufacturing workplaces increased when the sodium citrates were weighed or reacted with the silver nitrates, and during the cleaning of the workplace. The number of silver nanoparticles in the samples obtained from the workplace manufacturing silver nanoparticles using induced coupled plasma ranged from 57,789 to 2,373,309 particles/cm^3inside the reactor with an average size of 20-30 nm and 535-25,022 particles/$cm^3$with a wide range of particle sizes due to agglomeration or aggregation after the release of nanoparticles into the workplace air. In contrast, the silver nanoparticles manufactured by the wet method ranged from 393 to 3526 particle/$cm^3$with an average size of 50 nm. Thus, when taken together silver nanoparticle concentrations were relatively lower than existing occupational exposure limits.

Cross-reference to other study

No cross-reference

7.11 Toxic effects on livestock and pets

7.12 Additional toxicological information

Endpoint study record: In vitro toxicological information.001

Administrative Data

Materials and methods

Test materials

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

yes

Test material identity

Identifier	Identity
CAS number	7440-22-4
EC number	231-131-3
IUPAC name	silver (1+)

8. ANALYTICAL METHODS

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE

12. LITERATURE SEARCH

13. ASSESSMENT REPORTS

14. INFORMATION REQUIREMENTS

ANNEXES

ANNEXE 1: SOP_Preparing Ag-NP-Suspension_TUDr.pdf / 337.17 KB (application/pdf)

UMSICHT

TECHNISCHE UNIVERSITÄT DRESDEN MVT

Preparing aqueous silver nanoparticle suspensions of NM-300k

Aim of the SOP

The aim of this Standard Operating Procedure (SOP) is to provide stable and reproducible silver nanoparticle suspensions for use in tests on ecotoxicology and environmental fate within UMSICHT-Project and to ensure comparable conditions in different laboratories. The procedure is focused on the concentrated silver nanoparticle suspension NM-300k, which was provided by the Joint Research Centre (JRC) in the OECD Sponsorship Programme of Working Party on Manufactured Nanomaterials (WPMN).

It is important that the required steps of an SOP are accomplishable by each partner. For this reason, a simple but nevertheless robust method of the preparation and control of the suspension was developed.

Preparation of Dispersions

To prepare stable and reproducible solutions for ecotoxicology and environmental tests, the following steps are recommended:

1. Pre-Treatment of original suspension
2. Dilution to final exposure concentration
3. Characterisation of (stock and exposure) suspension

Prior to the preparation step, the user is recommended to create an experimental design with number and volume of the test samples and reference samples. This ensures that the silver nanoparticle suspension is fully and effectively used once it has been opened.

Pre-Treatment of original suspension

The NM-300k is provided by the JRC in separate containers (vials) with a small amount of suspension (2000 mg) and a high solid concentration (10.16 wt.%). Because of the high concentration and density the silver nanoparticles can separate over the time. Furthermore, formation of dry agglomerates on the vial wall was observed. Therefore, it is necessary to pre-treat the original suspension in its vial to a stock suspension.

1.1. Dilution

Dilute the silver suspension with deionised water to 2 wt.% in the original vial (brown 15 ml glass bottles from JRC).

1.2. Dispersion

Close the vial and homogenise the suspensions by shaking carefully. Afterwards disperse the sample for 15 minutes in an ultrasonic bath to destroy loose agglomerates and air bubbles. Note that the sample should be fixed in the bath to prevent buoying upwards (e.g. use a small basket for the ultrasonic unit).

Store the pre-diluted stock suspension in the original vial under controlled storage conditions (in the dark).

Dilution to final exposure concentration

Solutions for final tests and applications should be prepared from the pre-treated 2 wt.% stock suspension by following steps:

2.1. Dispersion of pre-treated stock suspension

If there are several hours or even days between the preparation of the stock suspension and the preparation of the exposure solution, the stock suspension should be homogenised with a magnetic stirrer in the original container. If this is not possible, repeat step 1.2.

2.2. Dilution to final exposure concentration

Now the stock suspension can be diluted using the respective test medium. This should be done with a sterile pipette to a clean glass beaker.

Characterisation of (stock and exposure) suspension

The stock and exposure suspensions will be characterized with respect to quality and content of particulate matter before and after each test. Therefore, the following measurements should be done:

- pH-value
- conductivity
- particle size distribution
- solid content

Before the characterisation the suspension has to be homogenised reasonably for at least 15 minutes.

The characterisation of particle size may be achieved by dynamic light scattering or analytical ultra-centrifugation. Additionally, a quantification of the particulate matter should be done with atomic spectroscopy like ICP-MS/OES, AAS or uv/vis-spectroscopy.

Results should be expressed in a standardised protocol and in relation to the original solution.

ANNEXE 2: A. GLP CERTIFICATE 2006

Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz
des Landes Nordrhein-Westfalen

Postanschrift: 40190 Düsseldorf

Aktenzeichen: VI-3- 31.11.79.06

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Fraunhofer Institut
für Molekularbiologie und Angewandte Ökologie
Bereich Angewandte Ökologie
Auf dem Aberg 1
D-57392 Schmallenberg

(unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV-GLP Nr. 5.3/OECD guidance)
Kategorie 1	category 1
Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	physical-chemical testing
Kategorie 4	category 4
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	environmental toxicity studies on aquatic and terrestrial organisms
Kategorie 5	category 5
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	studies on behaviour in water, soil and air; bioaccumulation

Kategorie 6	category 6
Prüfungen zur Bestimmung von Rückständen	residue studies
Kategorie 7	category 7
Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme	studies on effects on mesocosms and natural ecosystems

Datum der Inspektion	Date of Inspection
(Tag.Monat.Jahr)	(day.month.year)
23. bis 25. August 2006	on 23 until 25 August 2006
Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.	The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.
Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.	Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Düsseldorf, den 21.03.2007
Im Auftrag

(Prof. Dr. David)

Dienstsiegel/official-seal

ANNEXE 2: B. GLP CERTIFICATE_2011

Ministerium für Arbeit, Integration und Soziales
des Landes Nordrhein-Westfalen

Fürstenwall 25, 40219 Düsseldorf — Aktenzeichen III 5 – 31.11.79.07

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Fraunhofer Institut für Molekularbiologie
und Angewandte Oekologie IME
Auf dem Aberg 1
57392 Schmallenberg

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	**Areas of Expertise** (according ChemVwV GLP Nr. 5.3/OECD guidance)
Kategorie 1	category 1
Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	physical-chemical testing
Kategorie 3	category 3
Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	mutagenicity studies
Kategorie 4	category 4
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	environmental toxicity studies on aquatic and terrestrial organisms

Kategorie 5	category 5
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	studies on behaviour in water, soil and air; bioaccumulation
Kategorie 6	category 6
Prüfungen zur Bestimmung von Rückständen	residue studies
Kategorie 7	category 7
Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme	studies on effects on mesocosms and natural ecosystems

Datum der Inspektion	Date of Inspection
14.09.2010	September, 14th 2010
Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.	The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.
Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung / diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.	Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Düsseldorf, 07.02.2011 Im Auftrag (Dr. Deden)	Dienstsiegel/official-seal