



RAS AG

materials & technologies

Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products

Evaluation of active substances

DRAFT RISK ASSESSMENT REPORT (submitted by the applicant)



Silver, as a nanomaterial

Product type <PT 2, 4, 9>

Product-type 2: Disinfectants and algaecides not intended for direct applications to humans or animals
Product-type 4: Food and feed area
Product-type 9: Fibre, leather, rubber and polymerised materials preservatives

16 Active substance information

Text filter

- 0 Related information
- 1 Applicant
- 2 Identity of the active substance
- 3 Physical and chemical properties
- 4 Physical hazards and respective characteristics
- 5 Methods of detection and identification
- 6 Effectiveness against target organisms
- 7 Intended uses and exposure
- 8 Toxicological profile for humans and animals
 - 8.1 Toxicological profile for humans and animals
 - 8.1.1 Irritation
 - 8.1.1.1 (C) 8.1.1.1 (E) Eye irritation
 - 8.1.1.2 Dermatitis
 - 8.1.1.3 (C) 8.1.1.3 (E) Respiratory sensitisation
 - 8.1.2 Genetic toxicity in vivo / in vitro
 - 8.1.2.1 (C) 8.1.2.1 (E) In vivo genotoxicity study in mammalian cells
 - 8.2 Acute Toxicity
 - 8.3 Toxicokinetics and metabolism studies in mammals
 - 8.4 Repeated dose toxicity
 - 8.5 Reproductive toxicity
 - 8.6 Carcinogenicity
 - 8.7 Relevant health data, observations and treatments
 - 8.8 Additional studies
 - 8.9 Studies related to the exposure of humans to the active substance
 - 8.10 Toxic effects on livestock and pets
 - 8.11 Food and feeding stuffs studies including for food-producing animals and their products
 - 8.12 Tests to assess toxic effects of metabolites from treated plants
- 9 Ecotoxicological studies
 - 9.1 Toxicity to aquatic organisms
 - 9.2 Terrestrial toxicity, initial tests
 - 9.3 Terrestrial/terrestrial, long term
 - 9.4 Effects on birds

NIA Symposium 2018
Nanotechnology
Industries Association

Session 2 - Nanofoms

Case Study EU Regulation: Active substance dossier preparation for a nanostructured biocide

16.02.18
SR: User Admin: Plugins: Help

Main tasks

- Substance
- Material / Product
- Template
- Category
- Document

Inventories

- Legal entity
- Legal entity site
- Behavioural assessment
- Trial network
- Contacts
- Characterisation
- Literature references

Administration

- My account
- Report
- Sub-report

Plugins

- Validation assistant
- Report generator
- Help
- Dissemination preview
- Fee calculator

Gregor Schneider
RAS AG, Germany

company

RAS AG founded in 2016 as fusion of

- rent a scientist GmbH
scientific **services** since 1995
- ras materials GmbH
production and sales of **nanomaterials** since 2010

rent a scientist®
ideen bewegen



ras
materials

RAS AG offers development services, technologies and materials for the creation of technological based **product innovations**.

RAS AG

business units

rent a scientist®

- Our R+D **services** brings innovation to companies. We shape markets with creativity and knowhow.

RAS AG

agpure®

- **Antimicrobial** additive with outstanding properties and maximum safety for man and nature.

RAS AG

ECOS®

- Silver nanowire technology.
Transparent, conductive surfaces for a variety of applications.

RAS AG

new materials

- Together with our partners we are always working on absolute **new materials** and technologies.

RAS AG

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REVIEW ARTICLE

Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria

M.K. Rai, S.D. Deshmukh, A.P. Ingle and A.K. Gade

Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India

Keywords

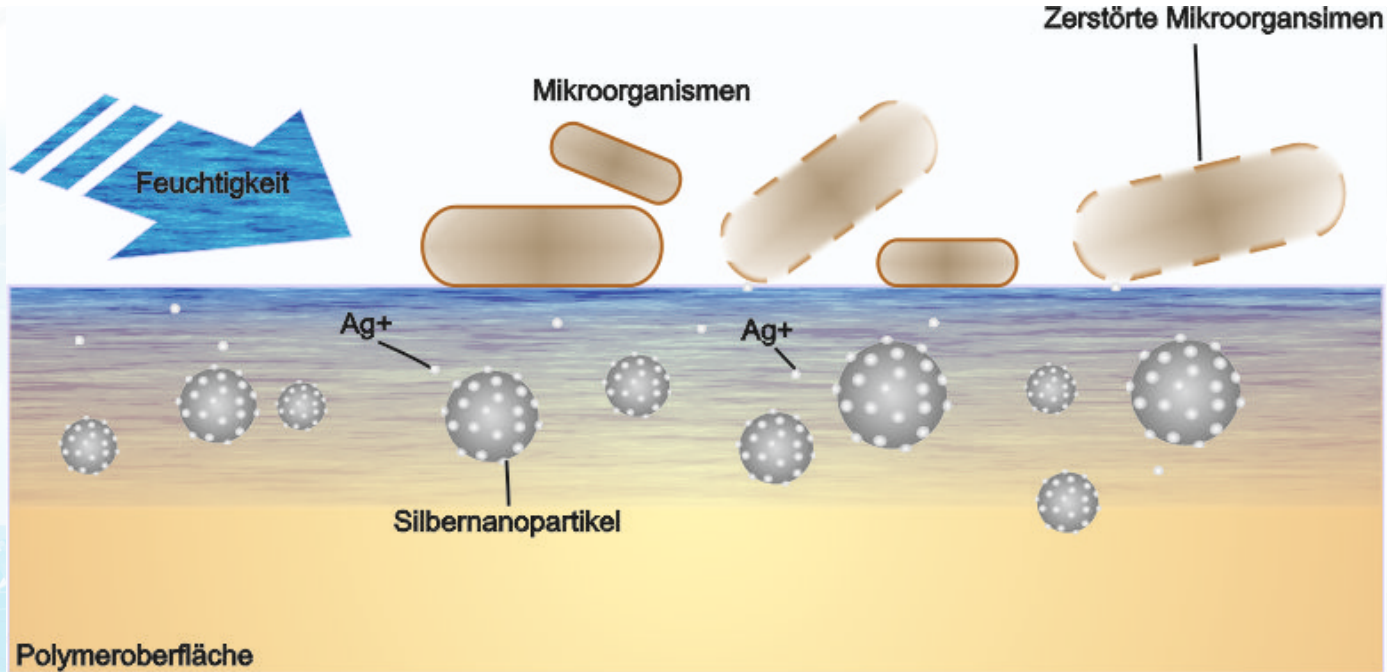
antimicrobial, methicillin-resistant

Summary

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Nanosilver – mode of action

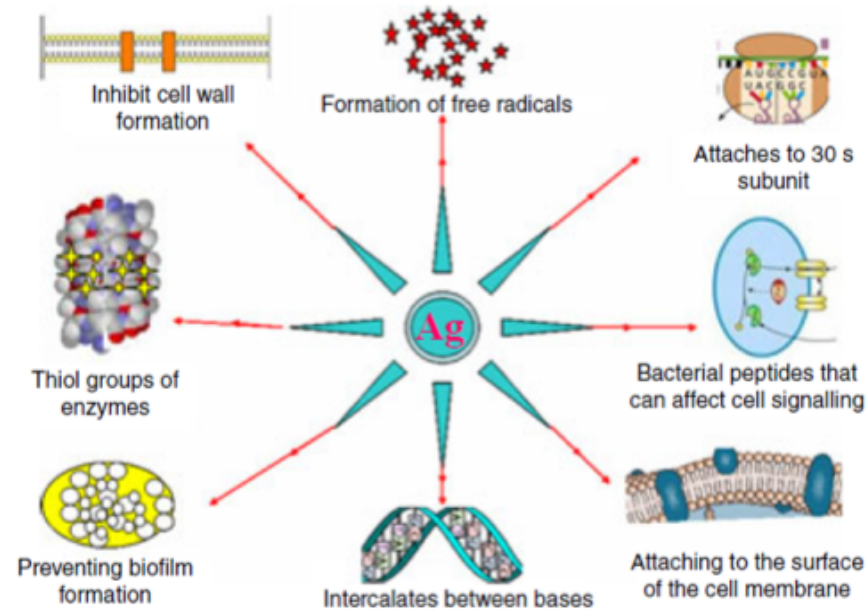
Release of Ag^+



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Nanosilver – mode of action

Antimicrobial activity



Rai, M. K., Deshmukh, S. D., Ingle, a. P., & Gade, a. K. (2012). Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. *Journal of Applied Microbiology*, 112(5), 841–852. <http://doi.org/10.1111/j.1365-2672.2012.05253.x>

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Dimensions

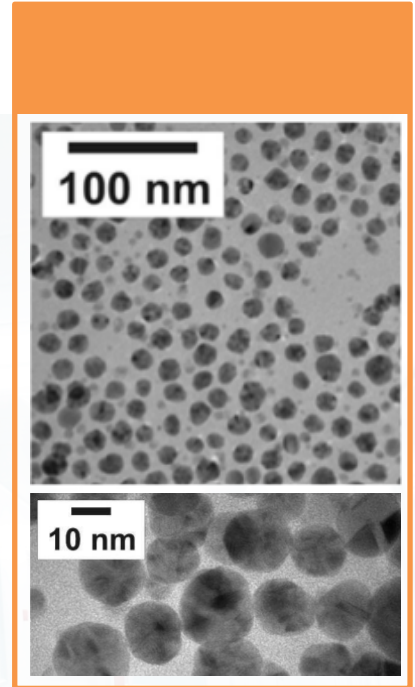
- Mean diameter 15 nm
- 99 % of particles < 20 nm

Formulations

- Stable in aqueous and organic dispersions
- Incorporation in polymers and resins

Permanence

- No release of nanoparticles (Ag^0) from solid matrices
- Continuous release of smallest amounts of silver ions (Ag^+)



*NM-series of representative manufactured nanomaterials. NM-300 Silver.
Characterisation, stability, homogeneity
Klein, C.L.; et al.*



Some of our products

agpure® W10

- 10 wt.-% nanosilver, stabilized, solvent: **water**

agpure® W50

- 45 wt.-% nanosilver, **stabilized**, pasteous

agpure® MB6500

- 0,65 wt.-% nanosilver masterbatch,

→ No agpure® nanopowders



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Applications and regulatory framework

MDR

BPR PT 4 Food

BPR PT 2 Desinfection - Ceramics

GÜRAL | VİT



Kale



BPR PT 9 Textiles

BPR PT 2 Desinf.



Reinste Nano Ventures

HEBA Perfektion im Ohr

Leica MICROSYSTEMS MEDI-SIL Orthopädische Produkte



LAMILUX



SANPURE ANTIMICROBIAL COATING

evolon

BIONI

cleanbake

Rhenocoll Beschichtungen und Klebstoffe

FREUDENBERG INNOVATING TOGETHER

FRUTAROM SAVORY SOLUTIONS

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BPR (EU Biocidal product regulation) in practice

The legal inequality in the EU is disturbing

- In the Art. 95 list there are a manifold of silver ion releasing substances that have to be assessed separately whereas there is one general substance (new but Art. 93.) where very different substances are thrown together:

The **Ag⁺** releasing substances just differ in the inert carrier system, but they have been separated:

Reaction mass of titanium dioxide and silver chloride

Silver sodium hydrogen zirconium phosphate

Silver adsorbed on silicon dioxide (as a nanomaterial in the form of a stable aggregate with primary particles in the nanoscale)

Silver nitrate

Silver phosphate glass

Silver copper zeolite

Silver zinc zeolite

Silver

Silver zeolite

VS.

Free radicals generated in situ
from ambient air or water

Contains:

Photocatalytic Titaniumdioxid
Photodynamic Colorants (Methylene blue)
ZnMoO₄ (pH-shift, „acid surface“)

...

+ Nanosilver as a „new existing“ substance

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Timeline

As at March 2018

2012

- BPR (EU) No. 528/2012 came into force: additional nanospecific risk assessment needed
→ Nanosilver became a „new existing“ substance

2015

- Substance dossier for nanosilver has been submitted acc. to Art. 95
- €€ ECHA fee: Submission for inclusion in the list of relevant persons; Article 95

2017

- Inclusion of nanosilver into (EU) 2017/698 (pre-stage to Art. 95)
- €€€ ECHA fee: Approval of an active substance „silver as a nanomaterial“
- Additional studies and assesement has been added to the dossier

2020

- €€€€€ KEMI fee: Evaluation of the dossier
- Evaluation finished by Swedish KEMI (RMS) and assessment report submitted to EC

2021+

- Approval of nanosilver as an existing active substance for use in PT 2, 4 and 9

Regulation and Safety Assessment

International standard reference material

- Data for nanospecific risk assessment required
 - Old data (colloidal silver 1900's etc.) are not accepted
 - Where can we find data?
- **agpure® W10**
 - The official nanosilver reference- and testing material (**NM 300 K**) for the “OECD WPMN - sponsorship program”
 - Certified Reference Material **BAM-N001** at the Federal Institute for Materials Research and Testing (German BAM)



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Regulation and Safety Assessment

OECD WPMN sponsorship program – Different Nanoforms/Grades of Nanosilver have been used

1	General information
2	Classification and Labelling
3	Manufacture, use and exposure
4	Physical and chemical properties
5	Environmental fate and pathways
6	Ecotoxicological Information
6.1	Aquatic toxicity
6.2	Sediment toxicity
6.3	Terrestrial toxicity
6.4	Biological effects monitoring
6.5	Biotransformation and kinetics
6.6	Additional ecotoxicological information
7	Toxicological information
7.1	Toxicokinetics, metabolism and distribution
7.2	Acute Toxicity
7.3	Irritation / corrosion
7.4	Sensitisation
7.5	Repeated dose toxicity
7.6	Genetic toxicity
7.7	Carcinogenicity
7.8	Toxicity to reproduction
7.9	Specific investigations
7.10	Exposure related observations in humans
7.11	Toxic effects on livestock and pets
7.12	Additional toxicological information
7.13	In vitro toxicological information
8	Analytical methods
9	Residues in food and feedingstuffs
9.1	Preliminary: Metabolism in livestock and cr
9.2	Preliminary: Residues in livestock and cr
4	Physical and chemical properties
4.0	Stability and homogeneity
4.1	Appearance
4.2	Melting point
4.3	Boiling point
4.4	Density
4.5	Particle size, size distribution
4.6	Vapour pressure
4.7	n-octanol-water partition coefficient
4.8	Water solubility, hydrophilicity, dispersibility
4.9	Solubility/dispersibility in organic solvents, of
4.10	Surface tension
4.11	Flash point
4.12	Auto flammability
4.13	Flammability
4.14	Explosiveness
4.15	Oxidising properties
4.16	Oxidation reduction potential
4.17	Stability in organic solvents and identity of re
4.18	Storage stability and reactivity towards cont
4.19	Stability; thermal, sunlight, metals
4.20	pH
4.21	Dissociation constant
4.22	Viscosity
4.23	Additional physico-chemical information
4.24	Agglomeration/aggregation
4.25	Crystalline phase
4.26	Crystallite and grain size
4.27	Aspect ratio, shape
4.28	Specific surface area
4.29	Zeta potential
4.30	Surface chemistry
4.31	Dustiness
4.32	Porosity
4.33	Pour density
4.34	Photocatalytic activity
4.35	Radical formation potential
4.36	Catalytic activity

Flags	Name Type	Name	Country	Remarks
	other: 7440- 22-4 (silver)	Silver Powder	Korea, Republic Of	Reference substance: silver / silver(1+) /7440-22-4, EC number: 231-131-3, EC name: silver, CAS number: 7440-22-4, IUPAC name: silver(1+)
	other: 7440-	Citrate-stabilized AgNPs		

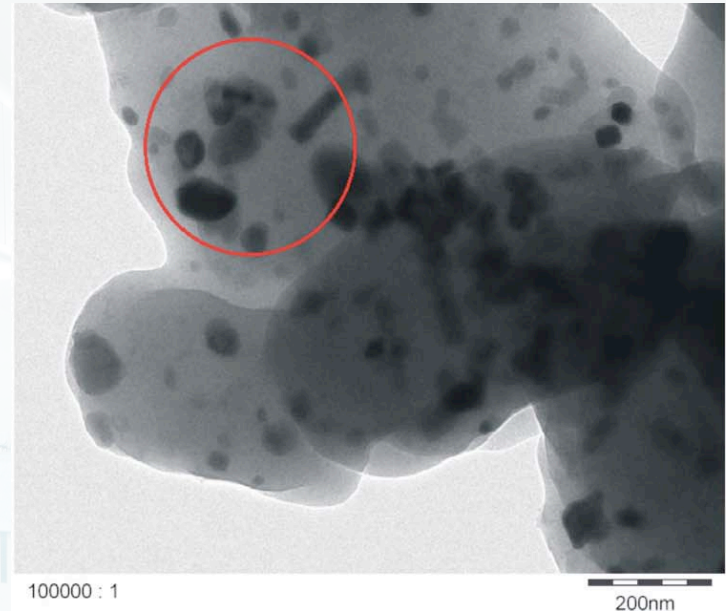
		NanoComposix uncapped nano-scale silver, 10, 20, 30, 50 nm sizes		
		NM-300K silver < 20 nm		Klein C, Comero S, Stahlmecka B (2011): NM-Series of representative manufactured nanomaterials NM-300 silver characterization, stability, homogeneity. JRC Scientific and Technical Reports. DOI:10.2788/23079
		SARPU 200KW		
		Silver nanoparticle (Korea)	Korea, Republic Of	

ENVI/MAN/ONC(2015)16P/ART1
 Organisation for Economic Co-operation and Development, Working Party on Manufactured Nanomaterials (WPMN) DOSSIER ON SILVER/MANOPARTICLES - PART 1 - Series on the Safety of Manufactured Nanomaterials No. 53 (2015)

Regulation and Safety Assessment

agpure® exposure assessment

- **Nanoparticle inside ≠ nanoparticle release**
- Safe use of silver nanoparticles:
 - Exposure of silver nano particles during production unlikely
 - *REACH-NanoHazEx: Rip-oN 3*
 - No abrasion of nano particles is detectable from polymer materials
 - *M. Vorbau, L. Hillemann, P. Fiala, M. Stintz, A. Rommert, D. Eichstädt: Kleine Teilchen in der Luft? Farbe und Lack 116 (2010) 12, 25-29.*
- Similar results on abrasion in all the other projects related to nanorisks



M. Vorbau, L. Hillemann, P. Fiala, M. Stintz, A. Rommert, D. Eichstädt: Kleine Teilchen in der Luft? Farbe und Lack 116 (2010) 12, 25-29.

How to prepare a BPR-„Nano-Dossier“

Find the data and just do it

3PR Active substance information

Text filter

- 0 Related information
- 1 Applicant
- 2 Identity of the active substance
- 3 Physical and chemical properties
 - 3 Physical and chemical properties of the active substance
 - 3.1 Appearance
 - Appearance
 - Nanosilver-NM300K- Appearance.002**
 - 3.2 Melting point / freezing point
 - 3.3 Acidity, alkalinity
 - 3.4 Boiling point
 - 3.5 Relative density
 - 3.6 Absorption spectral data (UV/VIS, IR, NMR) and a mass spectrum, molar extinction at relevant wavelengths
 - 3.7 Vapour pressure and Henry's law constant
 - 3.8 Surface tension
 - 3.9 Water solubility
 - 3.10 Partition coefficient (n-octanol/water) and its pH dependency
 - 3.11 Thermal stability, identity of breakdown products
 - 3.12 Reactivity towards container material
 - 3.13 Dissociation constant
 - 3.14 Granulometry
 - 3.15 Viscosity
 - 3.16 Solubility in organic solvents, including effect of temperature on solubility
 - 3.17 Stability in organic solvents used in biocidal products and identity of relevant breakdown products
 - 3.18 Additional physico-chemical properties of nanomaterials
 - 4 Physical hazards and respective characteristics
 - 5 Methods of detection and identification

Data source

Title	Author	Reference type	Year	Bibliographic source	Test
Safety Data Sheet	RAS AG	other Company Data			
DOSSIER ON SILVER N...	OECD Environment, He...	other OECD WPMN DO...	2015	ENV/JIMMONO(2015)1...	
Metallic Nanomaterials	Dr. Chala S.S.R. Kumar	review article or handb...	2009	ISBN: 978-3-527-32151-3	

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test guideline

Qualifier	Guideline
no guideline followed	

Principles of method if other than guideline

GLP compliance

Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products

Evaluation of active substances

DRAFT RISK ASSESSMENT REPORT
(submitted by the applicant)



Silver, as a nanomaterial

Product type <PT 2, 4, 9>

Product-type 2: Disinfectants and algaecides not intended for direct application to humans or animals
Product-type 4: Food and feed area
Product-type 9: Fibre, leather, rubber and polymerised materials preservatives

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How to prepare a BPR-„Nano-Dossier“

Selection: Different grades of nanosilver for particular endpoints

- Σ 8 Toxicological profile for humans and animals
 - + 8.1 Irritation
 - 8.2 (Cf. 8.1.2) Eye irritation
 - + 8.3 Sensitisation
 - 8.4 (Cf. 8.3.2) Respiratory sensitisation
 - + 8.5 Genetic toxicity in vivo / in vitro
 - 8.6 (Cf. 8.5.5) In vivo genotoxicity study in mammalian cells
 - + 8.7 Acute Toxicity
 - + 8.8 Toxicokinetics and metabolism studies in mammals
 - + 8.9 Repeated dose toxicity
 - + 8.10 Reproductive toxicity
 - + 8.11 Carcinogenicity

- Σ 9 Ecotoxicological studies
 - + 9.1 Toxicity to aquatic organisms
 - + 9.2 Terrestrial toxicity, initial tests
 - + 9.3 Terrestrial tests, long term
 - + 9.4 Effects on birds
 - + 9.5 Effects on arthropods
 - + 9.6 Bioconcentration terrestrial
 - 9.7 (Cf.9.6) Bioaccumulation: terrestrial
 - 9.8 Effects on other non-target, non-aquatic organisms
 - + 9.9 Effects on mammals
 - + 9.10 Identification of endocrine activity

Take home message

Nanosilver as a biocidal substance in the EU

- Scientific studies show a very strong antimicrobial activity of silver nanoparticles against multidrugresistant germs.
- agpure[®] NM 300K nanosilver: Responsible development for sustainable and safe use of nanosilver is reality
- Silver and nanosilver are well regulated within the EU
- Substance dossier has been submitted and is currently under evaluation
- OECD WPMN data have been created by using different nanosilver grades – regulating authorities have to consider
- Nanospecific risk assessment of nanosilver:
Silver ion related risk >>> nanoparticle specific risk

Thank you

Gregor Schneider

RAS AG

An der Irlter Hoehe 3a

D-93055 Regensburg

GERMANY

Fon: +49 (0)941/60 717-305

Mail: GS@ras-ag.com

www.ras-ag.com



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Regulation and Safety Assessment

national and international R&D projects

- NANEX
- Global-NanoMaPPP
- Several other EU-Projects
 - ENPRA
 - INLIVETOX
 - MARINA
 - LICARA
- TECHNOtox
 - Textiles functionalised with nanotechnology to eliminate toxicological risks

- UMSICHT
 - Ecological fate of nanosilver in polymer textiles
 - <http://www.umsicht.uni-bremen.de/>
- NANOSILBERPARTIKEL
 - Nanosilver particles - mechanisms of action and study of possible interaction with tissue, cells and molecules. <http://nanosilver-project.info/>



Regulation and Safety Assessment

Biocompatibility

- In-Vitro-Cytotoxicity:

- ISO 10993-5

- Mutagenicity :

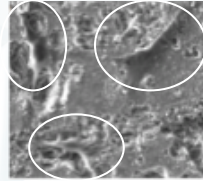
- OECD TG 471

- Allergic potential:

- Local Lymph Node Assay (LLNA)
- Epicutaneous test

- Skin irritation:

- OECD TG 402
- OECD TG 404
- OECD TG 406



- Eye irritation:

- HET-CAM-Test
- OECD TG 406



- Inhalation:

- OECD TG 413

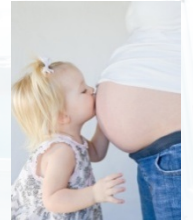


- Oral:

- OECD TG 408

- Reproductive and Developmental toxicity:

- OECD TG 413
- OECD TG 422



Regulation and Safety Assessment

Ecology

- Environmental toxicity studies:
 - Pelagic species:
OECD TG 201, 202, 203, 210, 211, 221
 - Microorganism:
OECD TG 217, 201, ISO 15685, DIN 38412 L 48, DIN ISO 17155



Soil species:
OECD TG 232, 222, 219, 207,
ISO/DIS 17512-1

Additional Eco-Studies

- Activated sludge:
OECD TG 303, 209
- Wastewater Treatment Plant:
Nitrification works optimal, even in a worst case scenario (>1 ppm nAg)
- **Studies show, that nanosilver particles have always been present on any surface made of bulk silver**

Vermeintliche Silber Resistenz

- Wissenschaftliche Daten führen zu folgender Schlussfolgerung
 - Molekularbiologische Methoden lassen ein Silberresistenz-Gen vermuten
 - Genotype ≠ Phenotype
 - Studie der Forschungsinstitute Hohenstein (mit *S. aureus*):
 - “Gewöhnung an steigende Silberkonzentrationen über 2000 Generationen, um eine Resistenz hervorzurufen.
 - Nach einer Generation auf Silber-freiem Medium verhält er sich wie jeder andere Stamm und wächst nicht auf Silber
 - Es fand also keine Resistenzbildung statt.
 - Es wurde bis jetzt keine Resistenz von Bakterien auf subinhibitorische Silberkonzentrationen bewiesen.
 - Bakterien sind solchen Konzentrationen seit Milliarden Jahren in verschiedensten Habitaten ausgesetzt ohne dass sich eine Resistenz gebildet hätte (Silber ist ubiquitär vorhanden. [Daunderer et al 2006])
 - Es gibt keine direkte Evidenz, dass Silberresistenz-Gene eine Kreuzresistenz gegenüber Antibiotika verursachen kann.
 - Eine Resistenz speziell gegen Nansilber kann es nicht geben.
- Es gibt keinen Beweis, dass der Gebrauch von Silber zu Resistenzen führt.