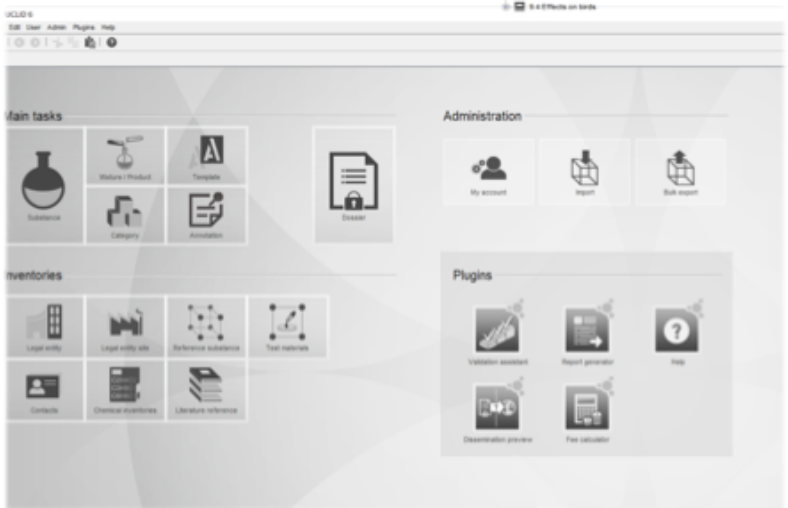

RAS AG
materials & technologies



NIA Symposium 2018

Nanotechnology Industries Association

Session 2 - Nanoforms

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

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

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

business units

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

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

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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.

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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

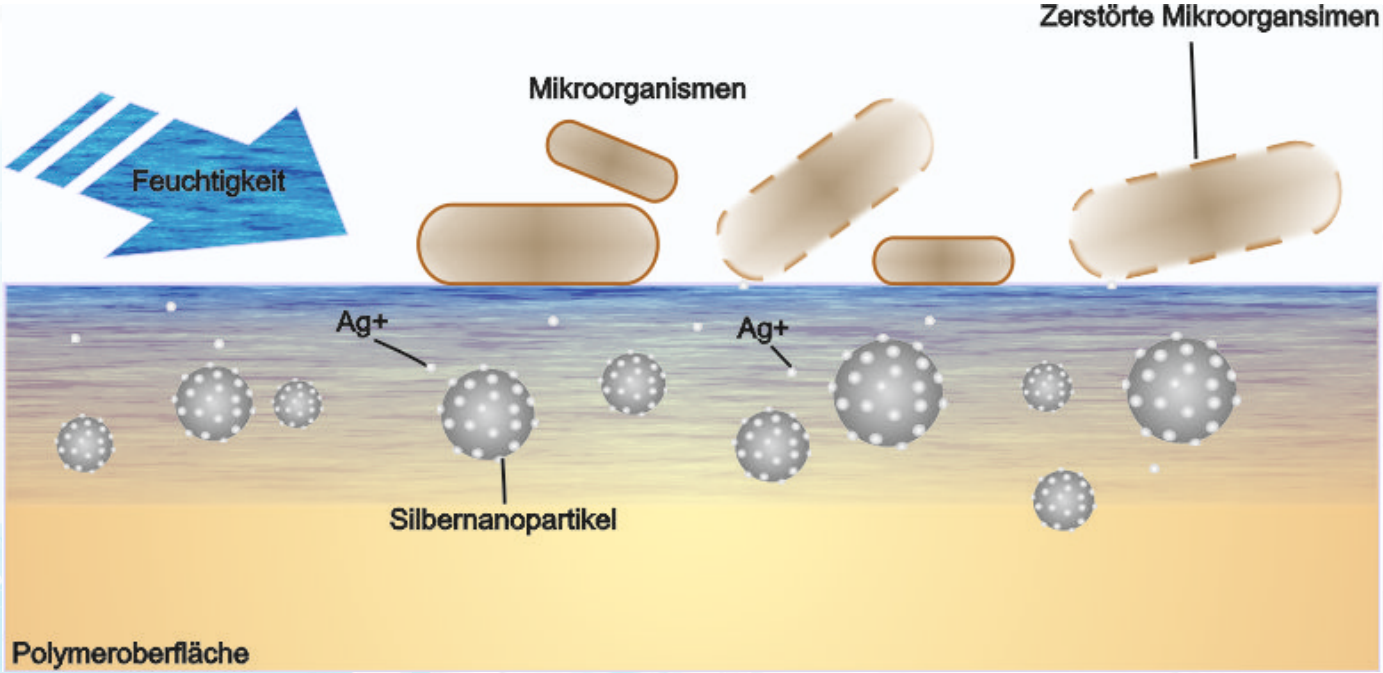
Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India

Keywords
antimicrobial, methicillin-resistant

Summary

Nanosilver – mode of action

Release of Ag^+



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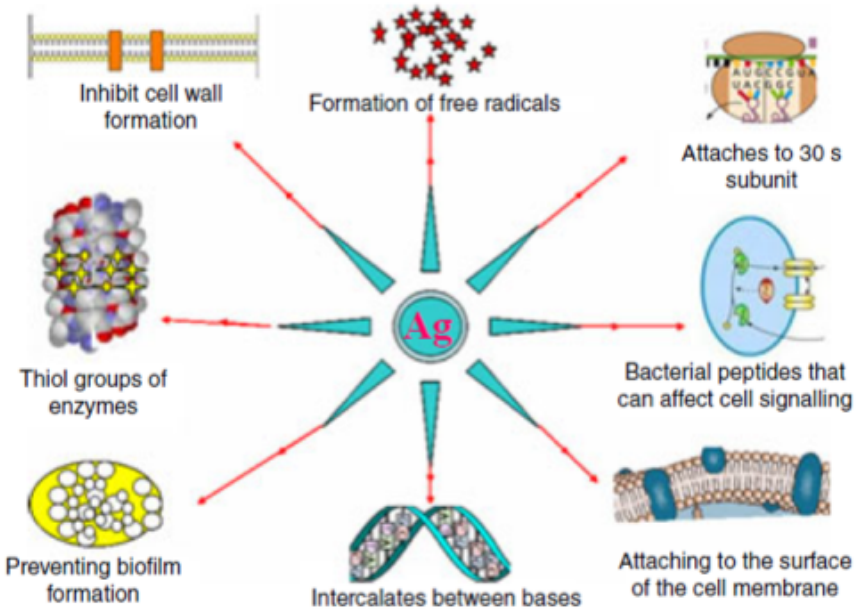
Nanosilver – mode of action

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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Technology

Dimensions

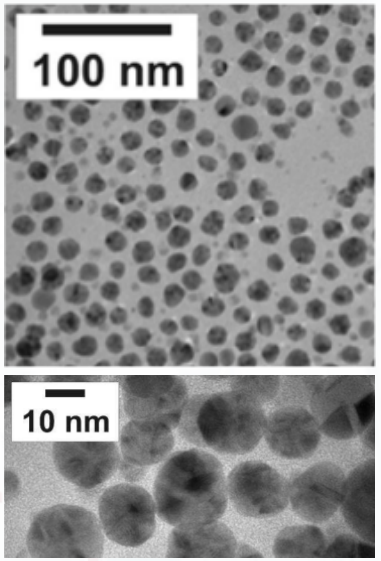
- Mean diameter 15 nm
- 99 % of particles < 20 nm

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.

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Some of our products

ag**pure**® W10

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

ag**pure**® W50

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

ag**pure**® MB6500

- 0,65 wt.-% nanosilver masterbatch,

→ No ag**pure**® nanopowders

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Applications and regulatory framework

BPR PT 2 Desinfection - Ceramics

GÜRAL | VİT

SeraPool Porselen Sanayi ve Ticaret A.Ş. SERA POOL

Kale

BPR PT 9 Textiles

evolon®

cleanbake

BPR PT 2 Desinf.

CLOU®

BIONI

Rhenocoll
Beschichtungen und Klebstoffe

MDR

aap

RAUMEDIC
Lifeline to Health

Reinste
Nano Ventures

HEBA
Perfektion im Ohr

Leica
MICROSYSTEMS

MEDI-SIL
Orthopädische Produkte

FREUDENBERG
INNOVATING TOGETHER

BPR PT 4 Food

LAMILUX

SANPURE®
ANTIMICROBIAL COATING

FRUTAROM
SAVORY SOLUTIONS

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 seperately 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 seperated:

- Silver nitrate
- Silver phosphate glass
- Silver copper zeolite
- Silver zinc zeolite
- Silver
- Silver zeolite
- 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)

+ Nanosilver as a „new existing“ substance

VS.

Free radicals generated in situ from ambient air or water

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

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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 assessement 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

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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

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	

ENV/JM/MONO(2015)16/PART1
Organisation for Economic Co-operation and Development. Working Party on Manufactured Nanomaterials (WPMN) DOSSIER ON SILVER NANOPARTICLES - PART 1 - Series on the Safety of Manufactured Nanomaterials No. 53. (2015)

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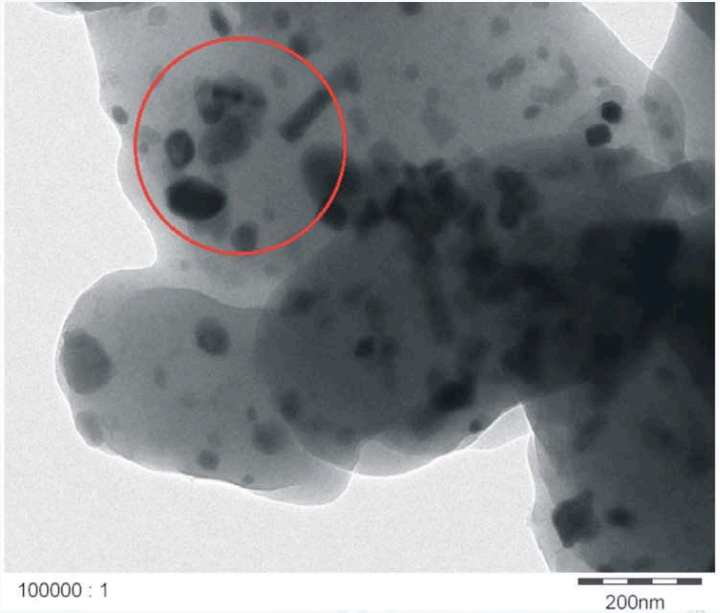
Regulation and Safety Assessment

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.

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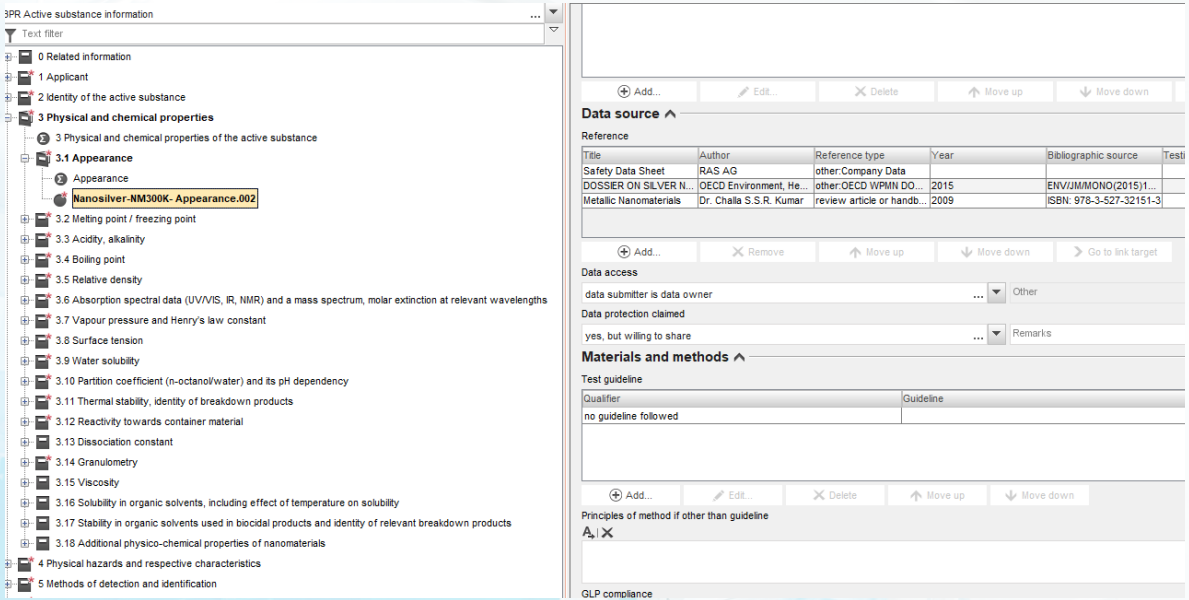
How to prepare a BPR-„Nano-Dossier“

Find the data and just do it



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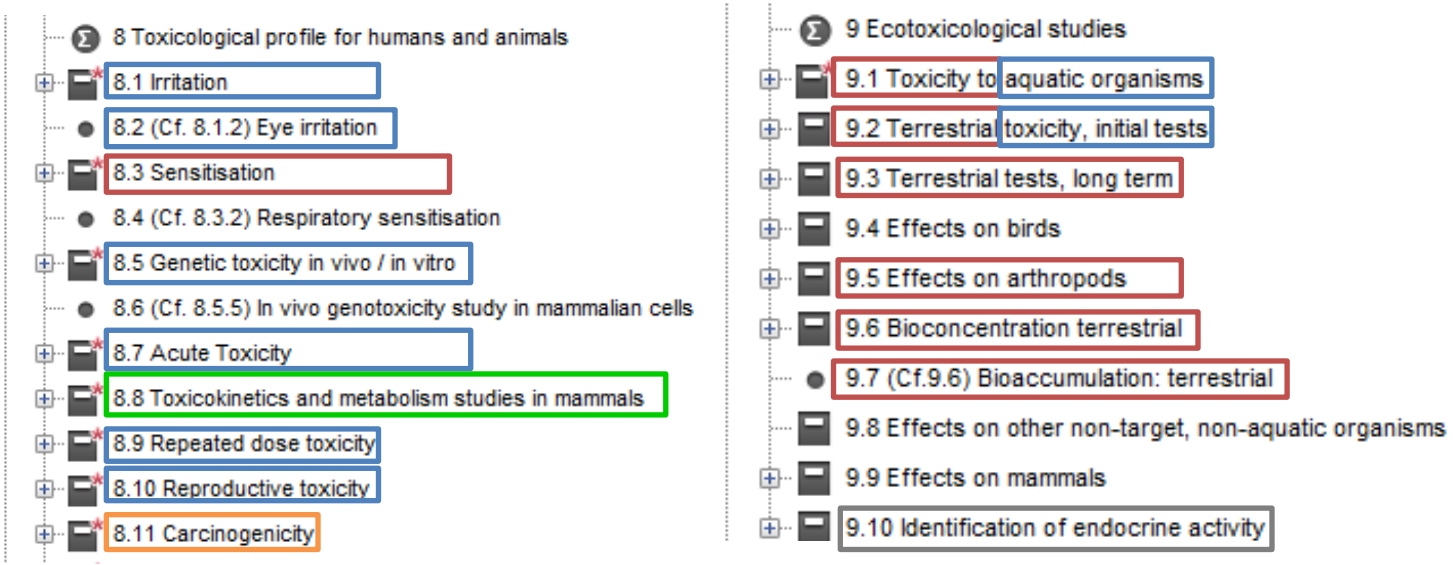
How to prepare a BPR-„Nano-Dossier“

Selection: Different grades of nanosilver for particular endpoints



Take home message

Nanosilver as a biocidal substance in the EU

- Scientfic 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

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Thank you

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Regulation and Safety Assessment

national and international R&D projects

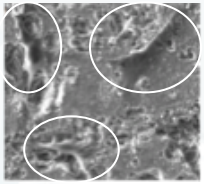
- NANEX
- Global-NanoMaPPP
- Several other EU-Projects
  - ENPRA
  - INLIVETOX
  - MARINA
  - LICARA
- TECHNOTOX
  - Textiles functionalised with nanotechnology to eliminate toxicological risks

- 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/

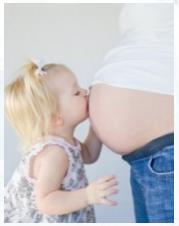
Regulation and Safety Assessment

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



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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**

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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.