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Nanosafety & Risk Assessment

- NANOTEC's best practices for promotion of human health and safety of nanotechnology -

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National Nanotechnology Center (NANOTEC), NSTDA

THAILAND

Technical Workshop for the Asia-Pacific Region on Nanotechnology and Manufactured Nanomaterials: Safety Issues

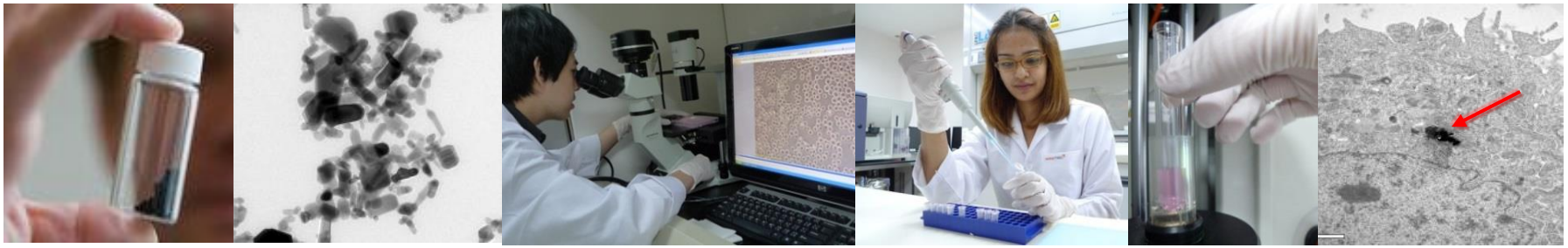
Sirindhorn Science Home, NSTDA, Thailand Science Park, Pathum Thani, Thailand

September 10, 2015

Nano Safety and Risk Assessment Laboratory (SRA)

SRA conducts the researches to address possible adverse effects of nanomaterials and novel substances on human health and the environment. The methodology for safety investigation are thoroughly implemented case-by-case, using the available guidelines and standards, as well as alternative scientific procedures for the best practice.

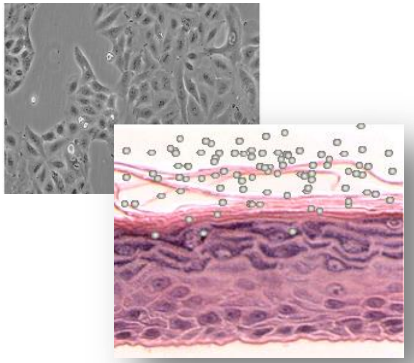
- Our group is currently focusing on development of reliable *in vitro* models for determining toxicological effects and pharmacological properties of materials upon their routes of exposure.
- SRA also introduces models of zebrafish and microorganisms into our research studies and testing services.



SRA

***In vitro* models**

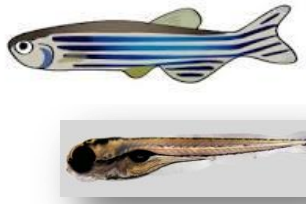
Cells/Tissues



Bacteria

***In vivo* model**

Zebrafish



Test end points: toxicity, interactions and compatibility, genetics, cellular processes, molecular mechanisms, pharmacology, and development of embryo

Test substances: nanomaterials, chemicals, cosmetics, food and dietary supplements

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Researches on Nanosafety

- Our Experiences and Inter-Lab Comparison -

Biological Effects of Nanomaterials

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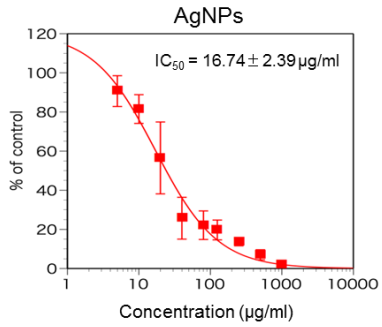
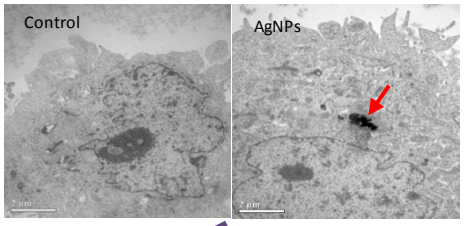
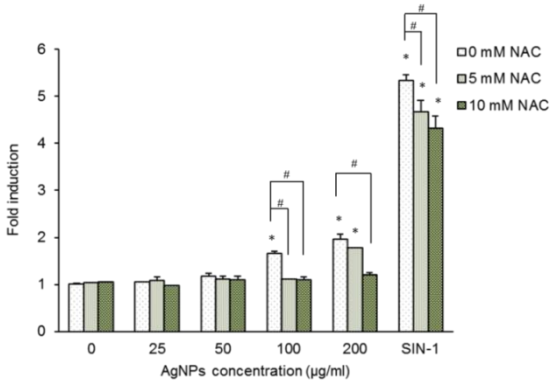
Cell viability

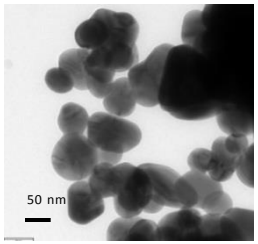


Cellular uptake

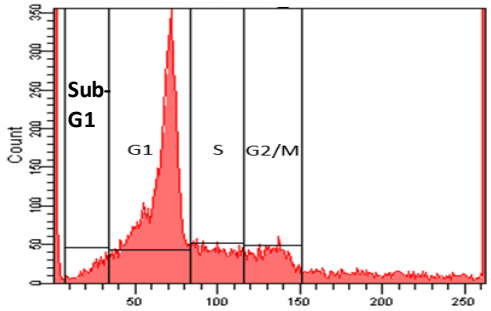


ROS generation

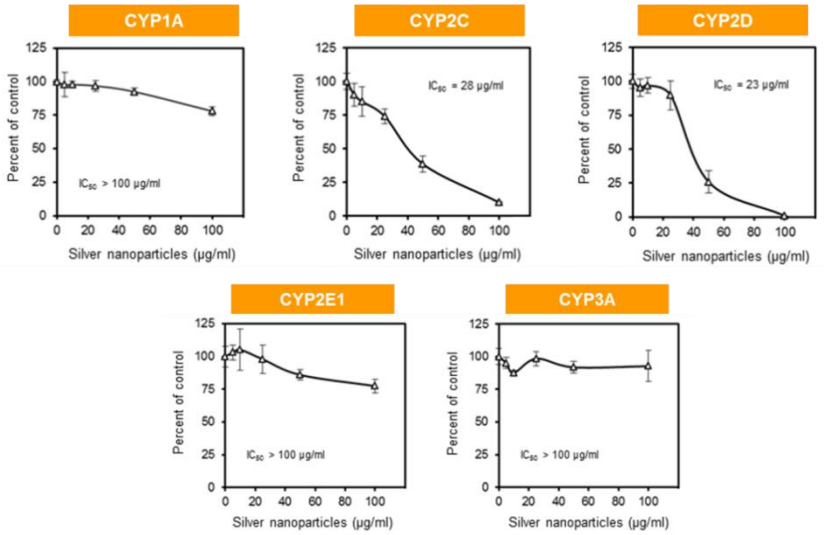




Cell cycle

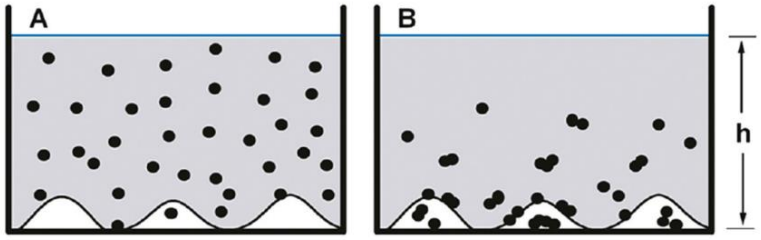


Activity of CYP enzymes



- Genotoxicity
- Immunotoxicity
- Expression of mRNA and protein

Designed experiment for study on biological effects



The conceivable interaction of insoluble particles with submersed cells grown at the bottom of a well, filled with an appropriate medium of height h. (A) Previously employed picture, (B) more appropriate concept discussed in this study. The number of particles in (A) and (B) is the same.

Wittmaak K (2011) *ACS nano* 5:3766–3778.

Appropriate experimental design:

96- well (0.32 cm^2) → 6- well (9.5 cm^2) → Petri dish (55 cm^2)

Volume adjustment for insoluble materials

- Concentration (w/v)
- Particles per area

Toxicological effects of AgNPs in hematological system

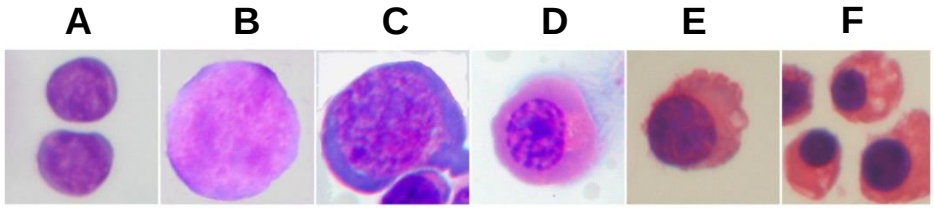
Objectives:

-To determine cytotoxic effects of AgNPs on hematological system using human erythroid cells comparing with cell lines

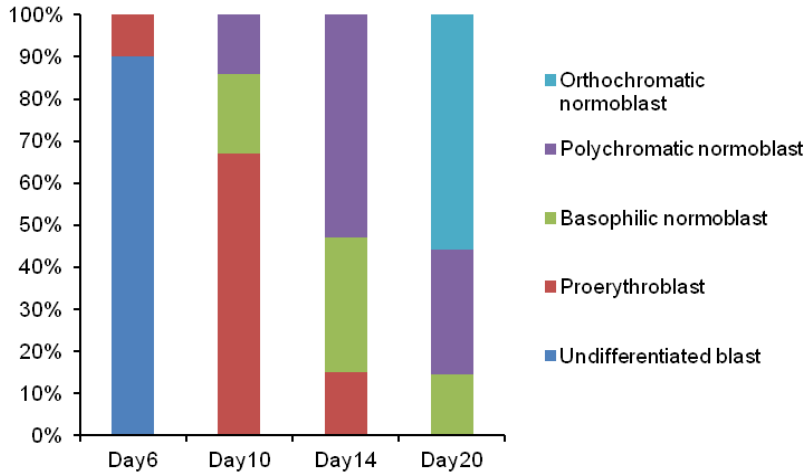
Team :NANOTEC (SRA), Mahidol University (Faculty of Medicine)

In vitro models:

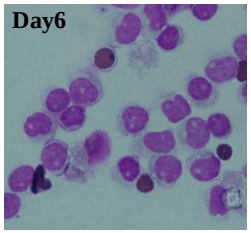
- K562 and HL60 cell lines
- Human primary erythroid cells (Healthy controls)

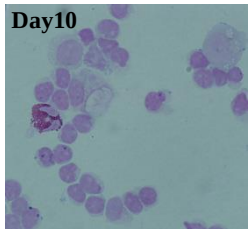


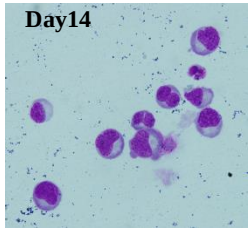
A : undifferentiated blast
B : proerythroblast
C : basophilic normoblast
D : early polychromatic normoblast
E : late polychromatic normoblast
F : orthochromatic normoblast

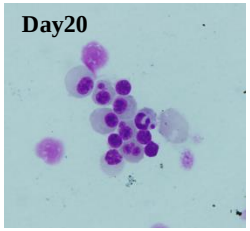


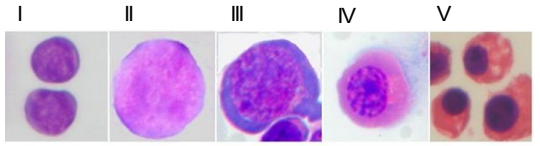
Erythroid culture by Fibach's method



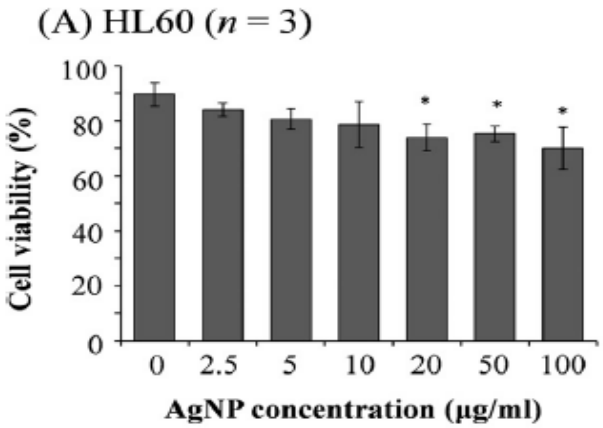


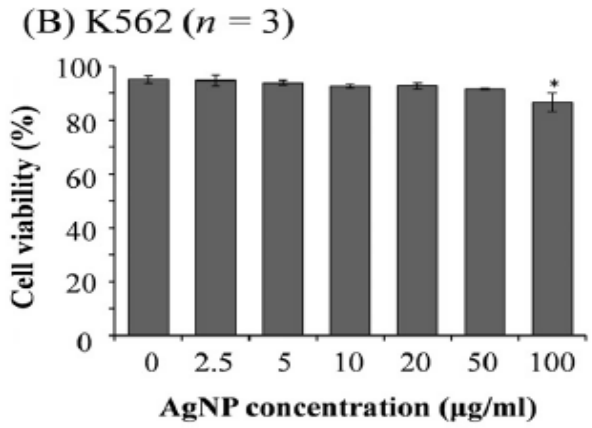


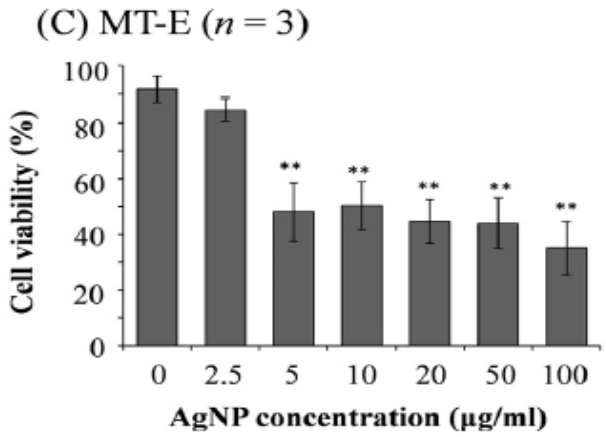


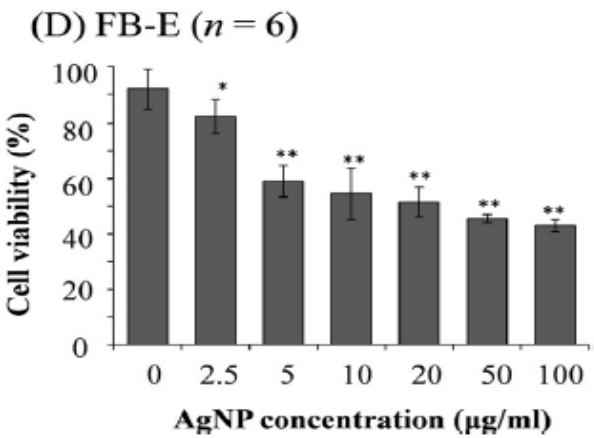


I; undifferentiated blast, II; proerythroblast III; basophilic normoblast, IV; polychromatic normoblast, V; orthochromatic normoblast





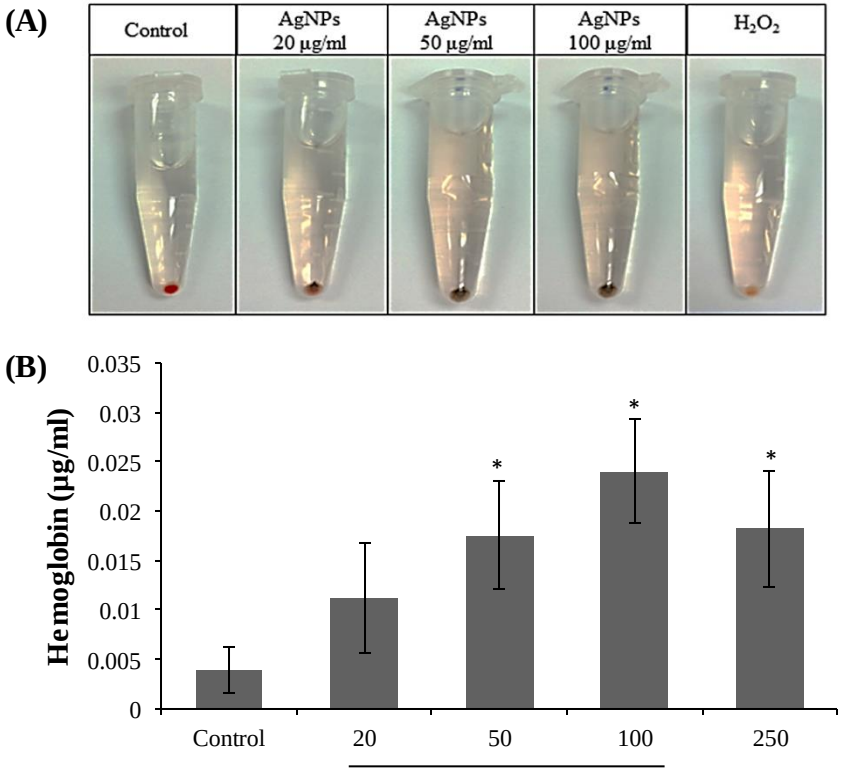




AgNPs caused significant cytotoxic effects in the primary erythroid cells, where as the immortalized HL60 and K562 cells showed much lower cytotoxic effects.

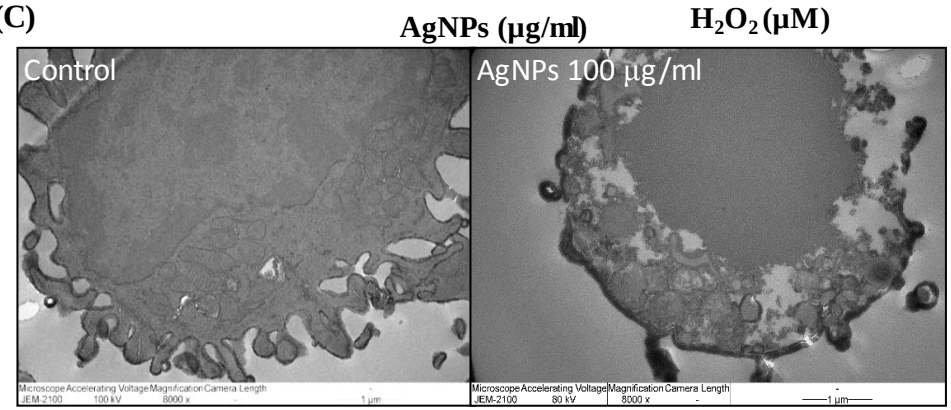
Hemolysis assay

AgNPs caused a dose-dependent hemolysis of erythroid cells.



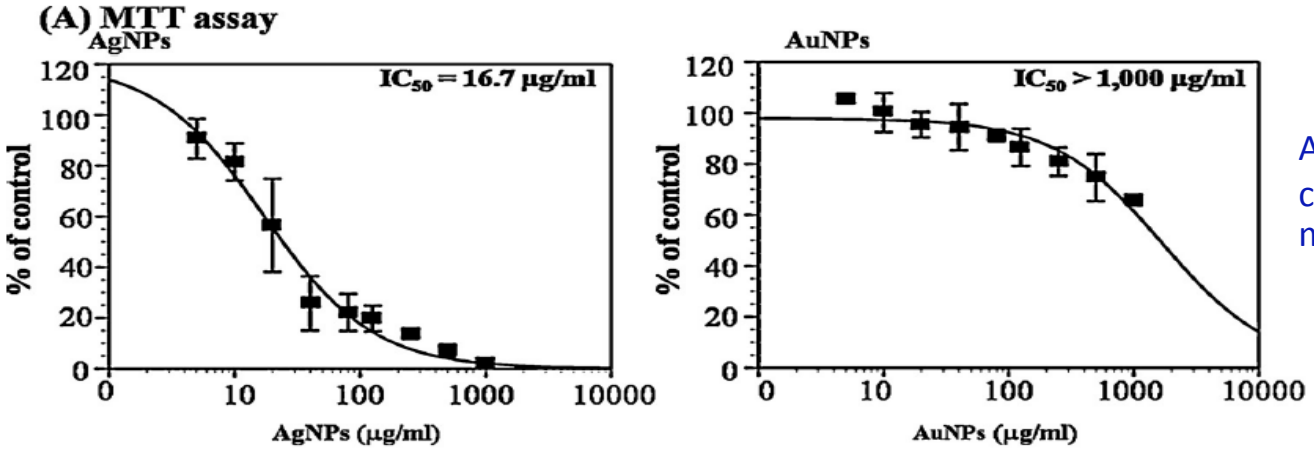
TEM analysis

AgNPs damaged the erythroid cell membrane.



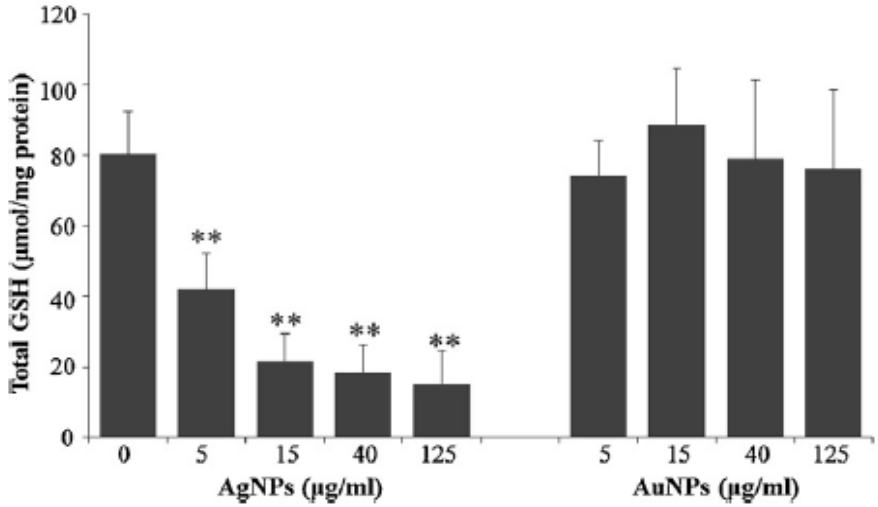
Rujanapun N, Aueviriyavit S, Viprakasit V, Maniratanachote R *et al.* *Toxicol In Vitro*. (2015) 29:1982-1992.

Toxicological effects of AgNPs in Caco-2 cells



AgNPs but not AgNPs reduced cell viability in intestinal cell model (Caco-2 cells)

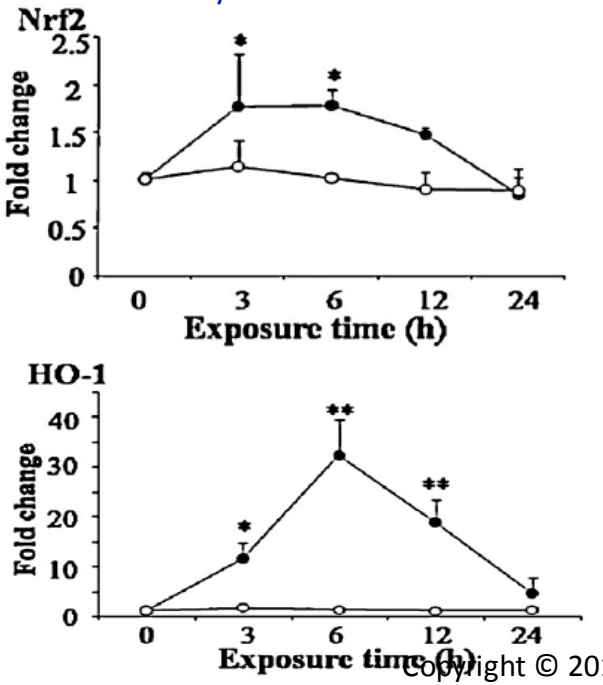
Effects of AgNP exposure on oxidative stress in Caco-2 cells



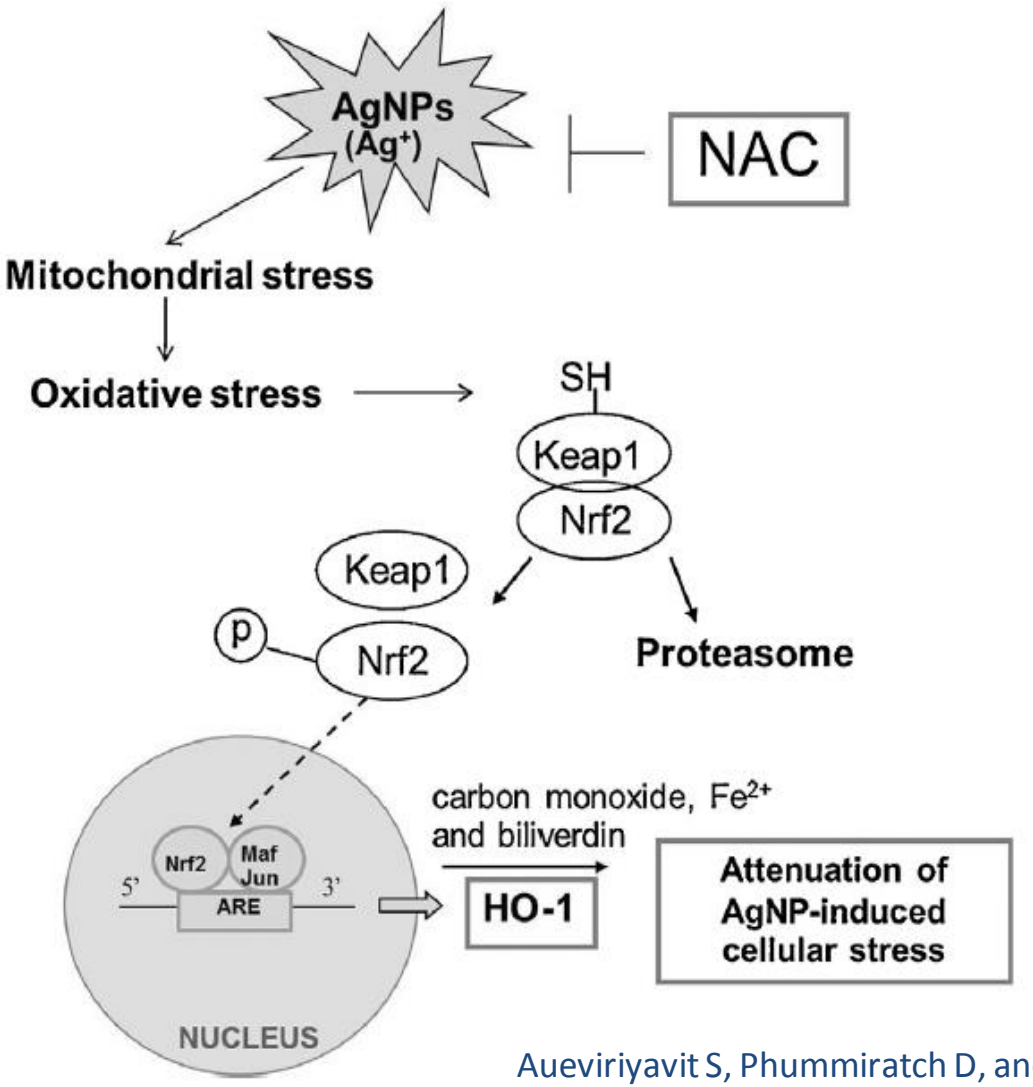
*p < 0.05; **p < 0.005 comparing the untreated and NP-exposed cells

15 µg/ml AgNPs (filled circles) and 125 µ g/ml AuNPs(open circles)

AgNPs induced biological response via Nrf2/HO-1 activation



Proposed mechanism underlying the cellular responses to AgNPs by Nrf2/HO-1 signaling pathway



Aueviriyavit S, Phummiratch D, and Maniratanachote R. *Toxicol Lett*. (2014) 224:73-83.

Occupational Health Risk

Increase production of nanomaterials in industrial sectors

Increase potential exposure and occupational health risk!

Published recommendations for assessment criteria

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OSHA Permissible Exposure Limits (PELs)- General Industry

Inert or nuisance dust (an average during normal working hours)

- Respirable dust (PM 5) 5 mg/m^3
- Total dust 15 mg/m^3

PELs are 8-hour time weighted averages (TWAs)

National Institute for Occupational Safety and Health (NIOSH)

Recommended limit value

- Titanium dioxide (< 100 nm) **0.3 mg/m^3**, 10 h/day and 40 h/week
- Carbon nanotubes and –fibers **0.007 mg/m^3** (measured as elementary carbon)

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Safety Investigation of Nanoproducts

- Our Experiences and Best Practices -

Cosmetics and Personal Care Nanoproducts

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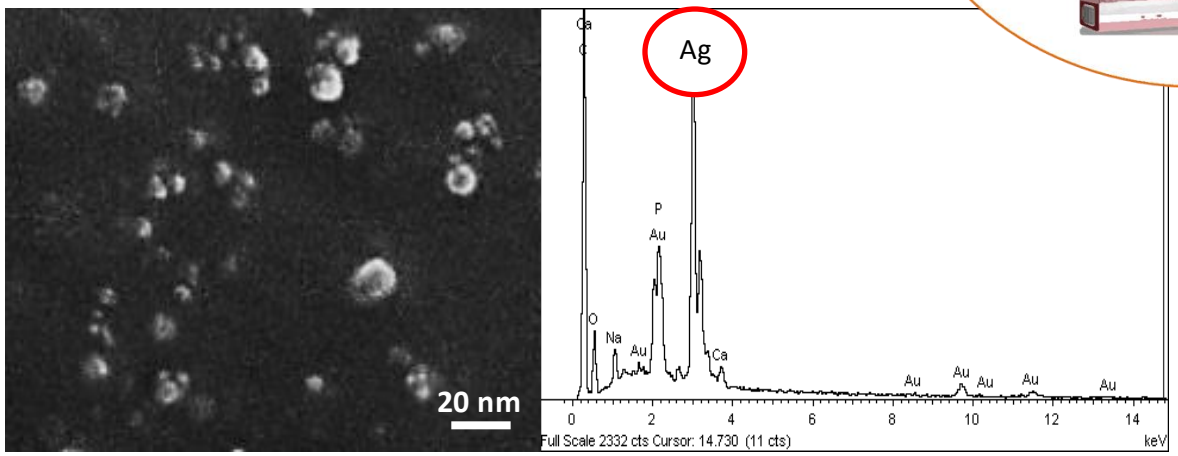
Analysis of silver in nanoproducts: 20 items

These products claimed to be “nano-silver”

Sample digestion

SEM-EDX



GFAAS

- By using SEM, silver nanomaterials can be detected in only few products
- Total Silver concentration was determined by GFAAS after wet acid digestion

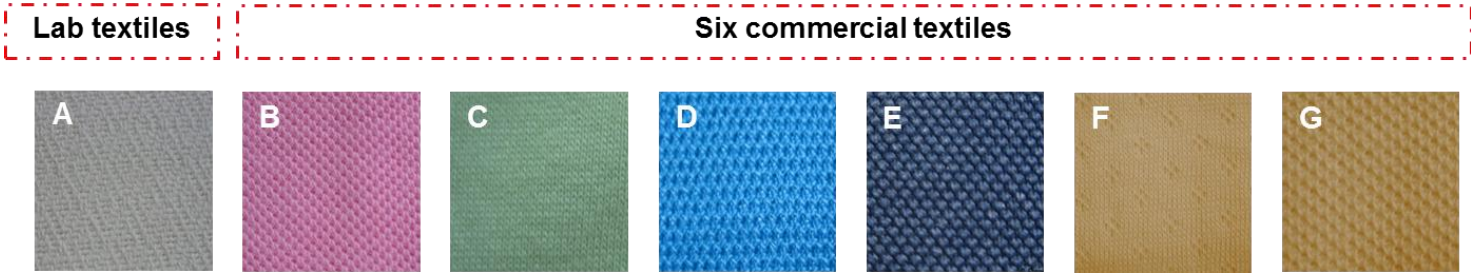
Wasukan N, Maniratanachote R et al., submitted

Textiles

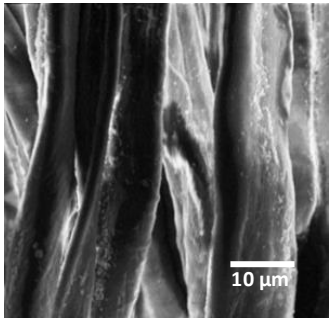
Silver release from textile nanoproducts into artificial sweat

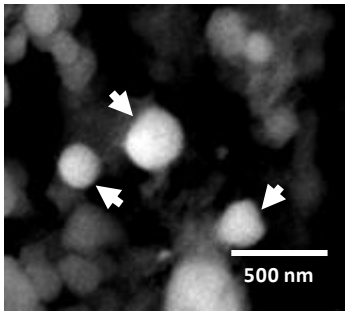
Tested samples

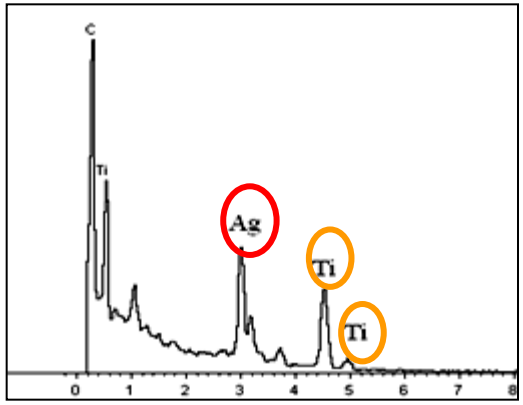
- Laboratory textiles were prepared by pad-dry-cure method (A0, A1, A2, A3, A4)
- Six commercial claimed nanosilver shirts were purchased (B, C, D, E, F and G)



Physical-chemical analysis



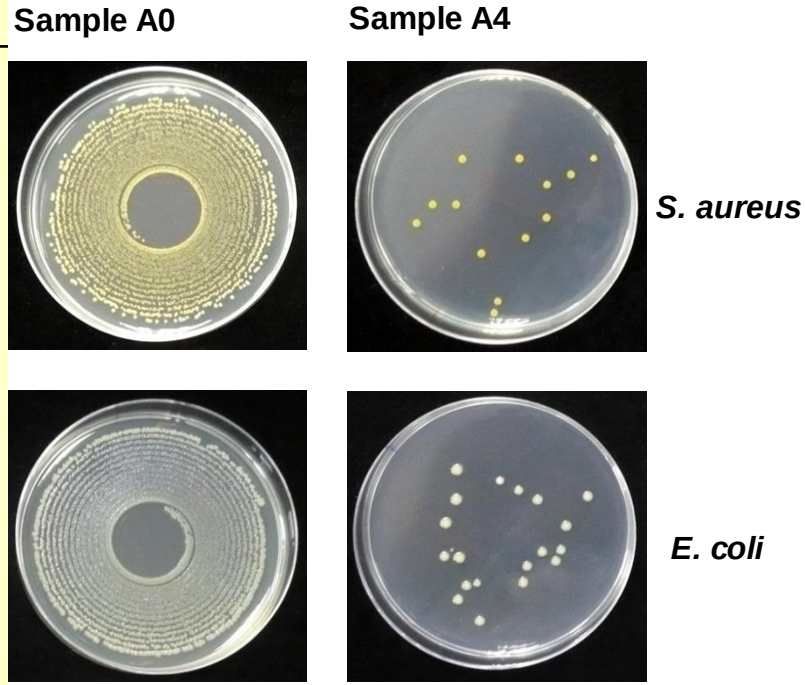




Kulthong K, Maniratanachote R *et al*., (2010) *Particle and Fibre Toxicology,* 7:8

Measurement of antibacterial properties

Sample	Percent reduction of bacteria	
	S. aureus	*E. coli*
A0	-	-
A1	98.04	-
A2	99.02	-
A3	97.30	-
A4	99.83	99.93
B	98.23	-
C	98.56	-
D	-	-
E	-	28.73
F	99.85	99.80
G	99.99	81.44



Kulthong K, Maniratanachote R *et al*., (2010) *Particle and Fibre Toxicology*, 7:8

Release of silver into artificial sweat

Sample	Initial silver content (mg/kg)	Silver released into artificial sweat (mg/kg)			
		AATCC pH4.3	ISO pH 5.5	ISO pH 8.0	EN pH6.5
A0	n.d.	n.d.	n.d.	n.d.	n.d.
A1	36.12 ± 22.42	21.01 ± 4.13	15.53 ± 3.62	34.27 ± 2.88	35.83 ± 19.68
A2	56.57 ± 34.28	33.39 ± 15.80	28.81 ± 10.34	66.54 ± 46.29	77.96 ± 23.80
A3	95.12 ± 33.12	70.15 ± 37.29	72.69 ± 11.99	82.22 ± 26.99	152.20 ± 36.54
A4	425.21 ± 93.73	217.61 ± 81.32	177.13 ± 57.13	268.31 ± 131.15	322.21 ± 87.00
B	n.d.	n.d.	n.d.	n.d.	n.d.
C	n.d.	n.d.	n.d.	n.d.	n.d.
D	n.d.	n.d.	n.d.	n.d.	n.d.
E	15.16 ± 9.90	0.08 ± 0.05	0.01 ± 0.01	0.05 ± 0.30	0.36 ± 0.10
F	1.22 ± 0.87	n.d.	n.d.	n.d.	0.05 ± 0.00
G	0.99 ± 1.53	n.d.	n.d.	n.d.	n.d.

* The amount of silver released was dependent on: Initial amount of silver, Quality of the fabrics, Artificial sweat formulations and pH.
* This information might be useful for estimating potential human exposure to silver form textile nanoproducts.

Kulthong K, Maniratanachote R *et al*., (2010) *Particle and Fibre Toxicology*, 7:8

Structure of Nanosafety and Ethics Strategic Plan (2012-2016)

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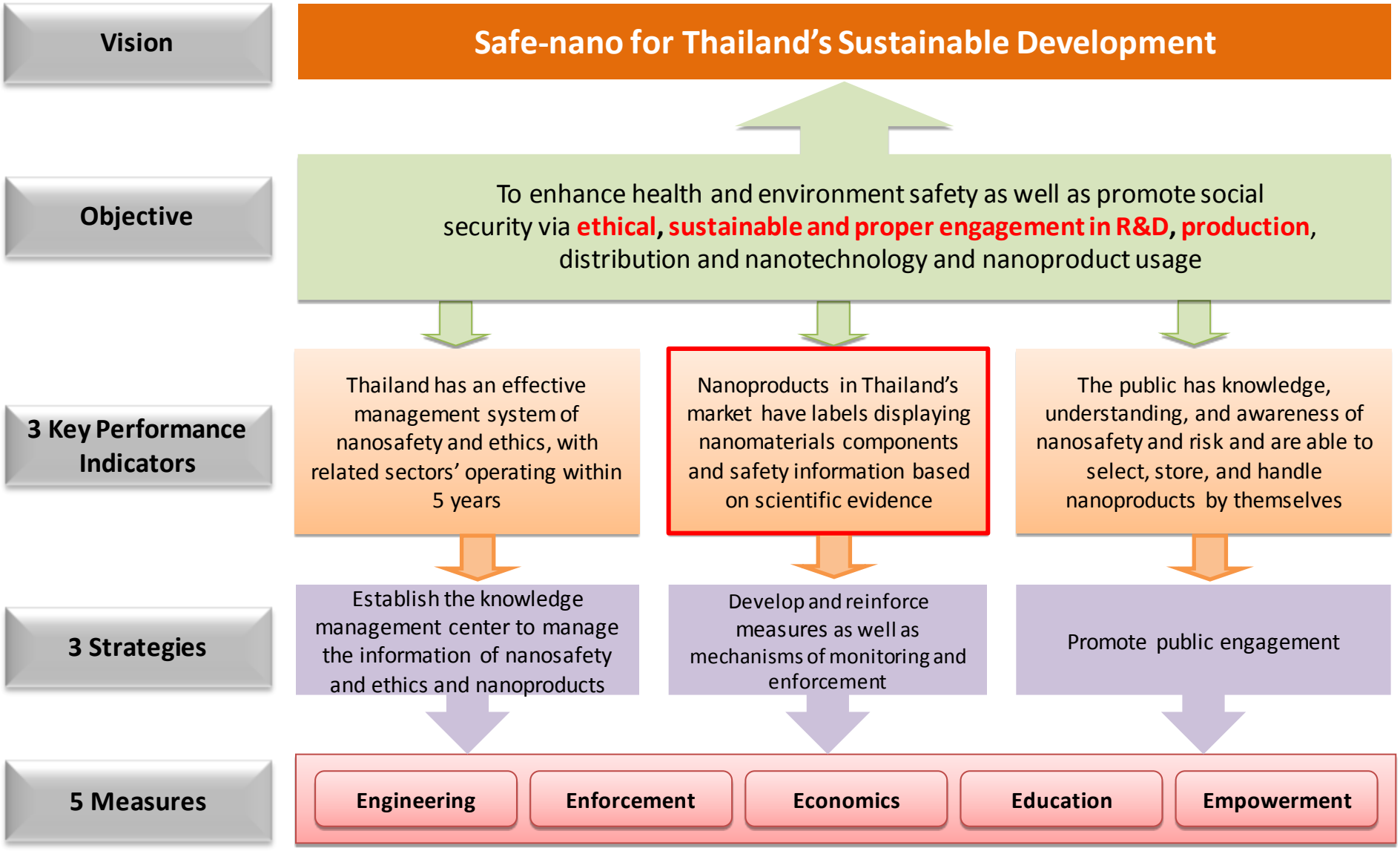

NanoMARKS Flagship

NanoMARKS Flagship

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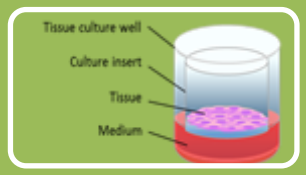
Nano Characterization Laboratory (NCL)



Nano Safety and Risk Assessment Laboratory (SRA)

Goals: NanoMARKS' targeting Thai nano-merchandises with safety, international standard, and quality acceptance from Thai consumers and trading partner countries

NanoMARKS

MEASUREMENT

ACCURACY

RELIABILITY

KNOWLEDGE

STANDARD & SAFETY

Nanoproducts in our focus

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- Health and Cosmetic Manufacturers
  (Health and Cosmetic products)
- Food and Agricultural Manufacturers
  (Agricultural products, Processed foods, Food packages)
- Post-petrochemical Manufacturers
  (Textiles, Plastic beads, Synthetic rubbers, Glue, and Coating chemicals)

NanoMARKS Flagship

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OECD
BETTER POLICIES FOR BETTER LIVES

European Commission

ISO

National Advanced NANO Characterization Center (NANC)

Nanometrology
Nanosafety

2
Standard

Income

3
Testing
Services

NANC

NCL
SRA

Expertise

Works

1
Research

Nano MARKS
Flagship

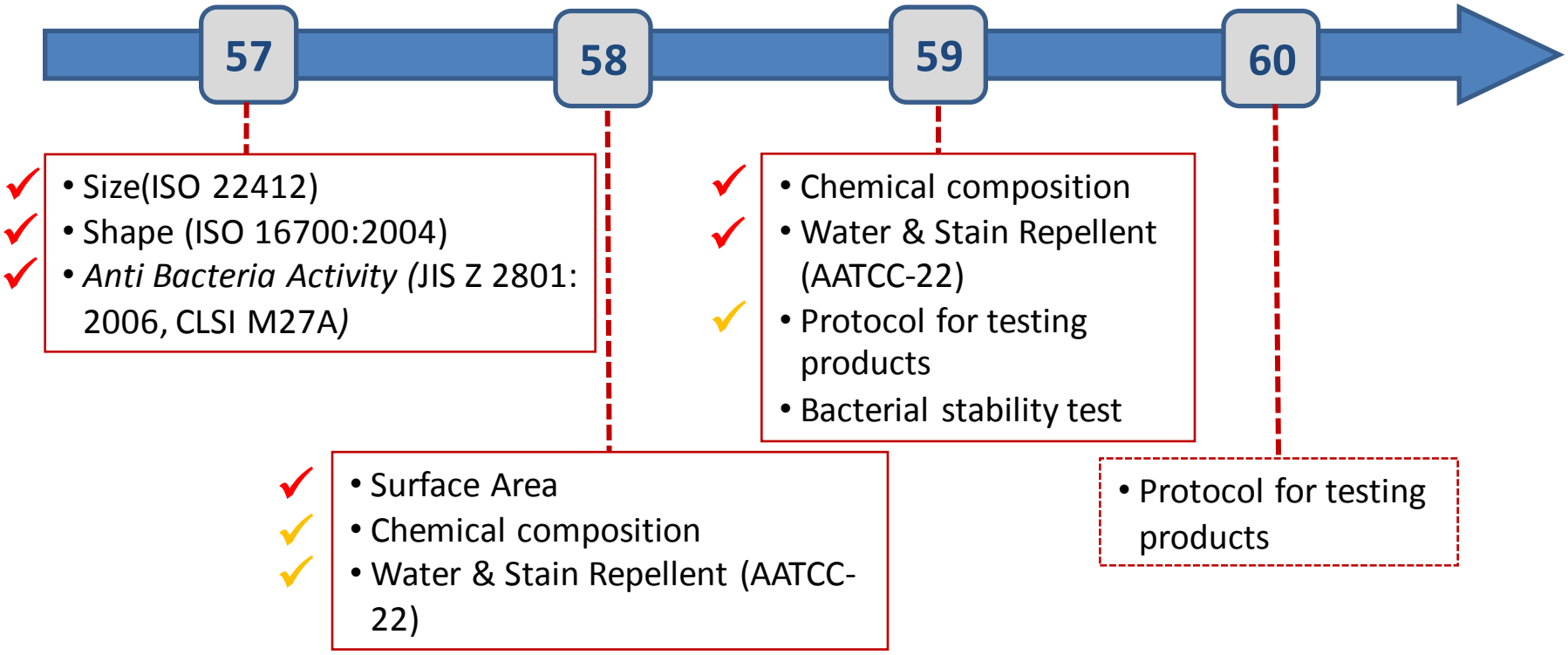
Collaboration & Training

Targeted Nanoproducts
- Cosmetics
- Food
- Petrochemicals

Product labeling

Sample tested for :
NANOTECHNOLOGY
Anti-bacteria
Water repellance
Brand : .... Model : .....
สมาคมนาโนเทคโนโลยีแห่งประเทศไทย
Nanotechnology Association of Thailand

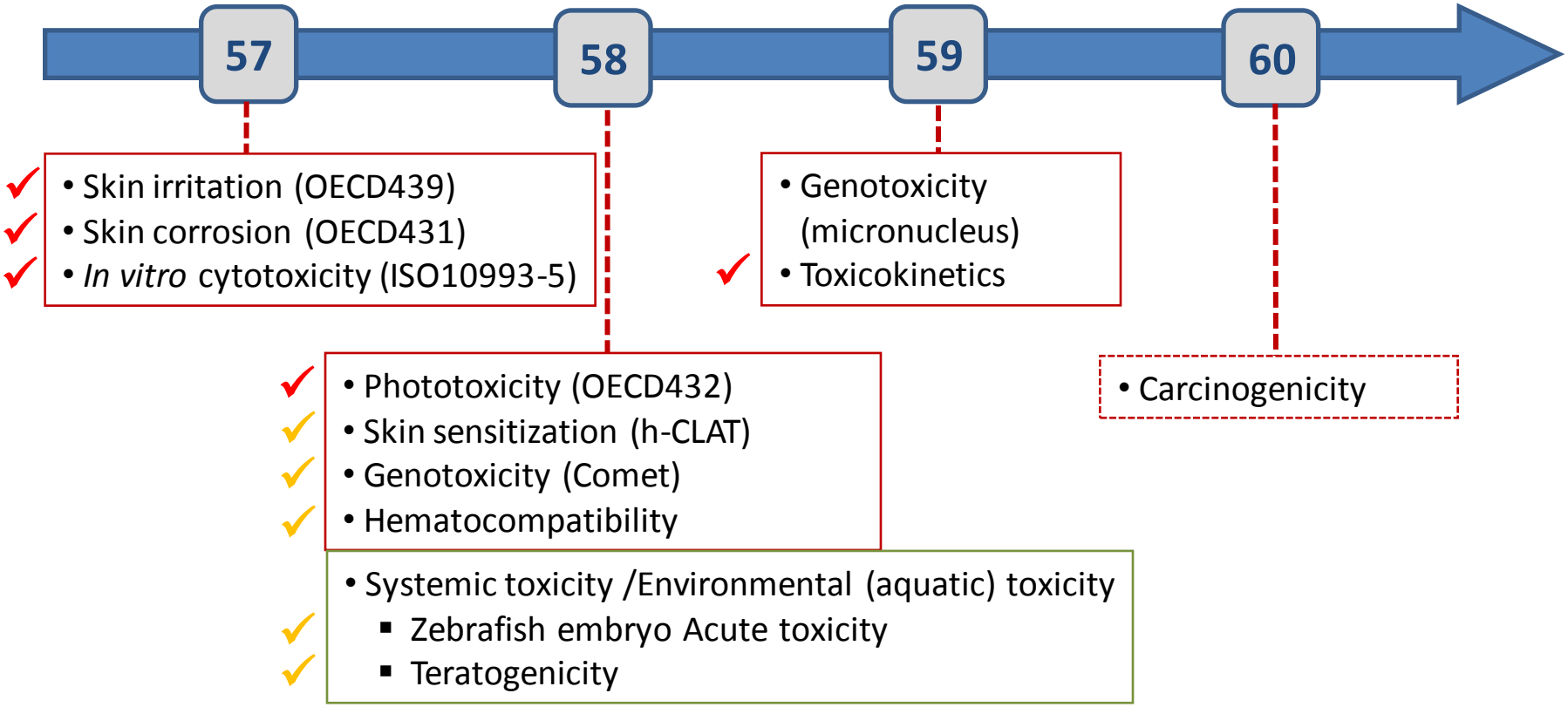
TRM: Physico-Chemical techniques



Size characterization: ISO 22412:2008, ISO 16700:2004, ISO/TS 24597:2011

Functions: ISO 22916: 2011, JIS Z 2801: 2006, AATCC 100, ASTM E 2149, ISO 27447:2009, ASTMG21 : 1992 reapprove 2002, JIS Z 2911 :1992, CLSI M27A

TRM: Safety testing techniques



Biological effects and Biocompatibility test matrix (ISO 10993-1)

Current status

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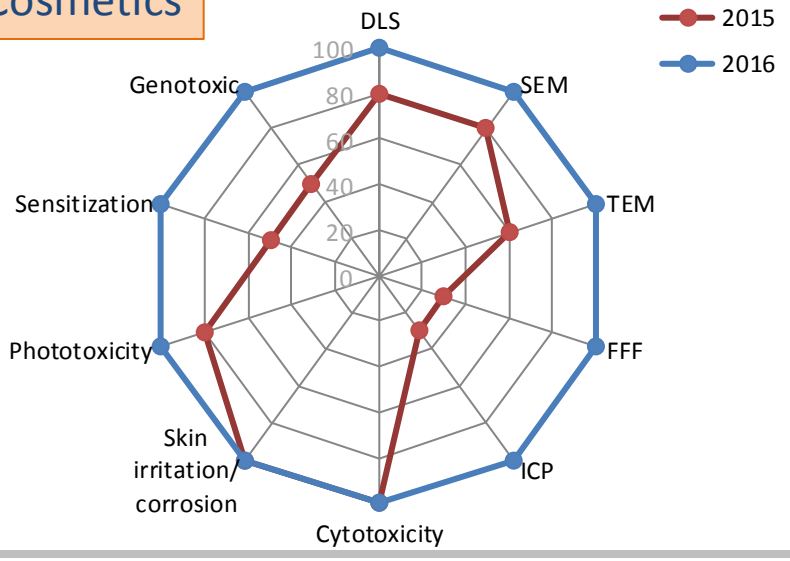
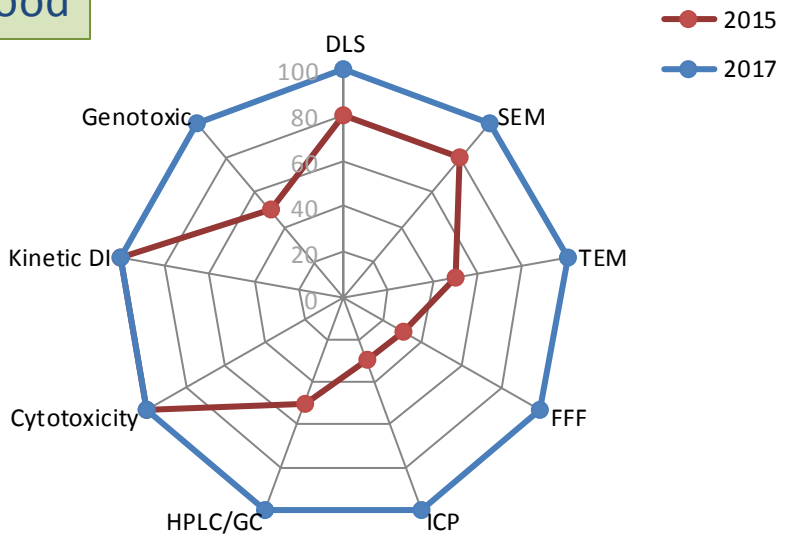
Quality test

Safety test

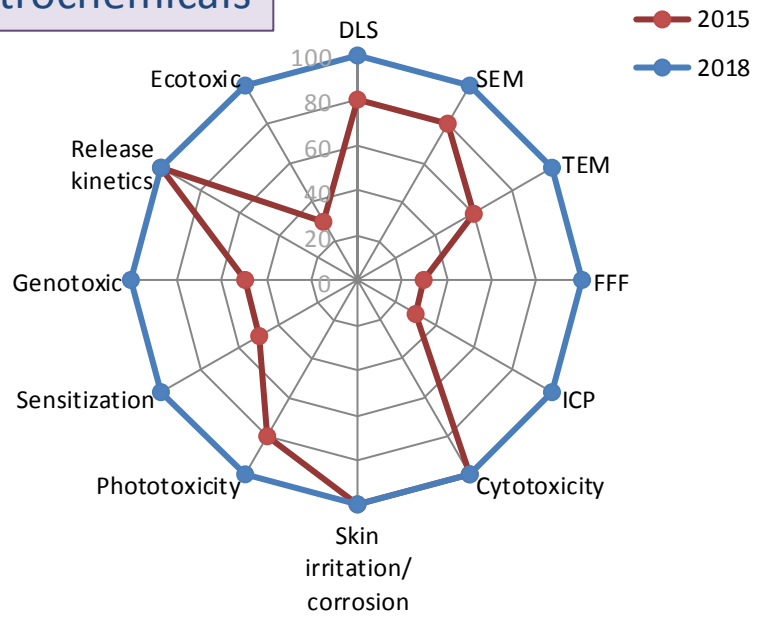
Cosmetics



Food

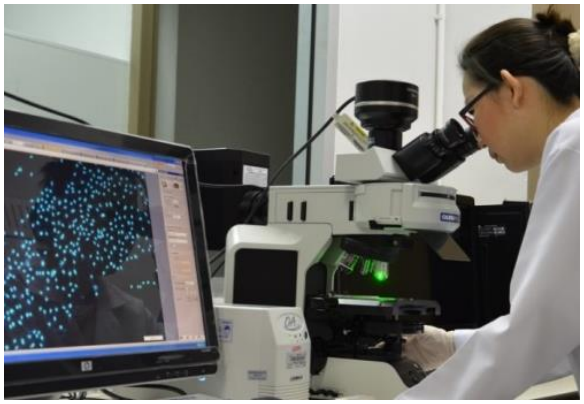


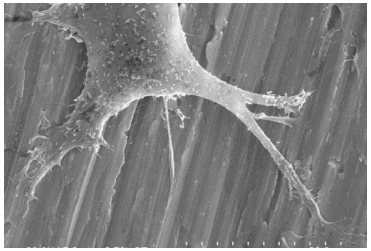
Petrochemicals



Our supporting roles

- The labs support and promote manufacturer's capability to compete in national and international markets.
- Scientific-based characterization and toxicological testing can aid the manufacturers in the selection of raw materials in order to manufacture safe and high-quality products.
- The testing information of the products also help for consumer decisions in product selection.





Our supporting roles

NANOTEC
a member of NSTDA

SCG
SIAM CEMENT GROUP

IRPC

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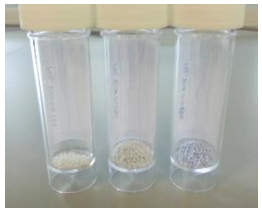
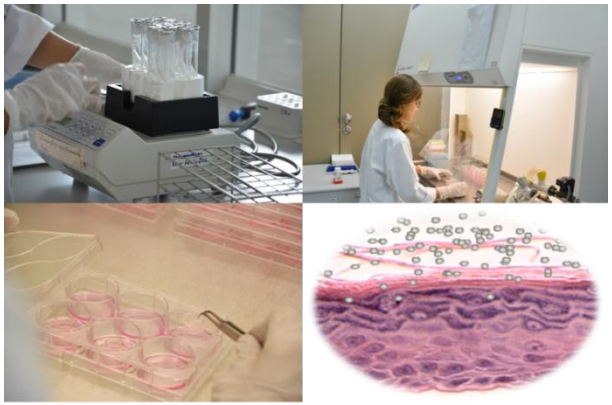
ECO COAT CO.,LTD.



Finished products
and raw materials

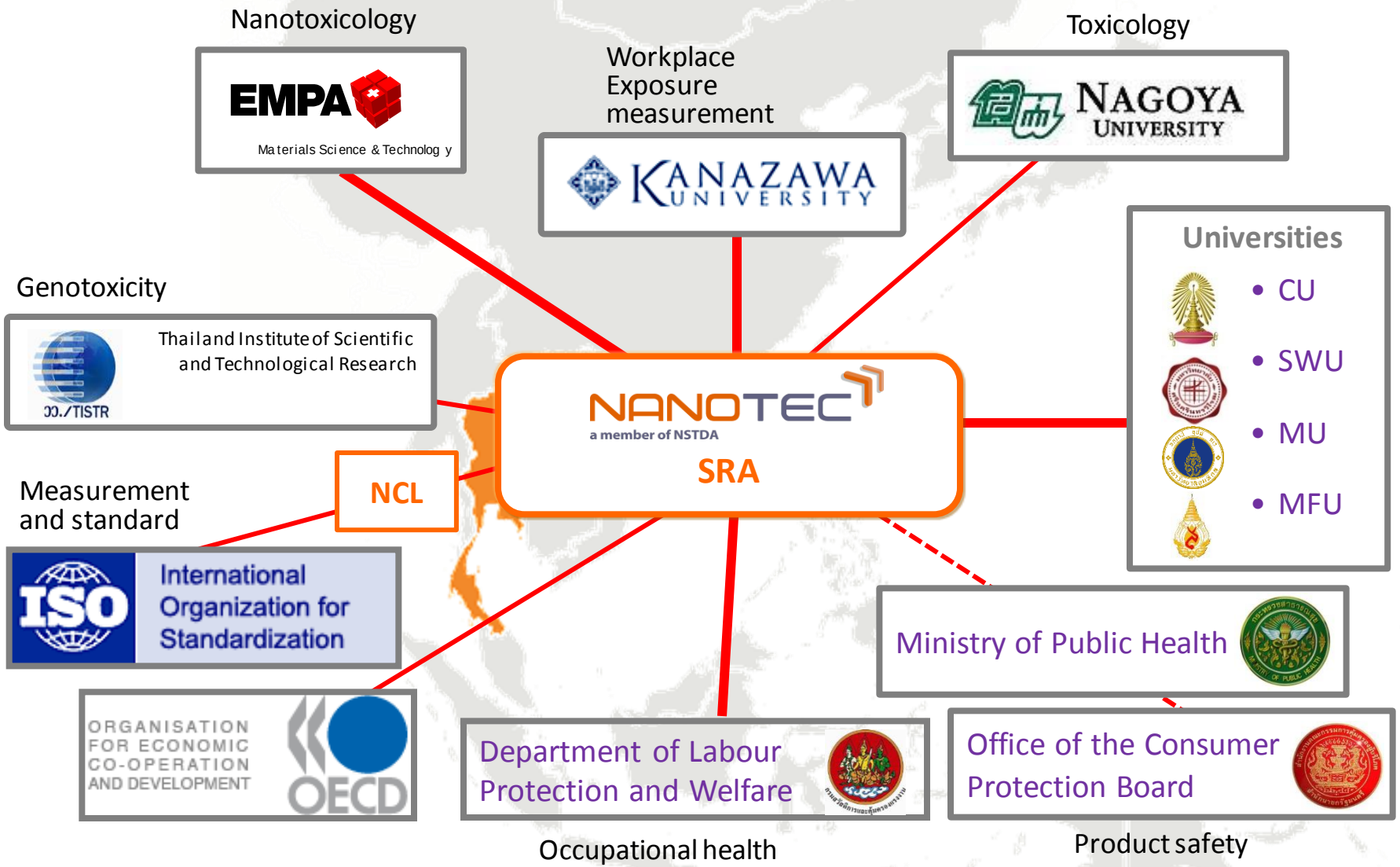
- Skin corrosion/irritation (OECD TG431 and TG439)
- Cytotoxicity (ISO 10993-5)
- Release and sustainability (ASTM F619-03)
- Toxicity and human health effects



Impact value on promoting commercialized Nanotechnology > 1,500 million THB
(From a company in 2013 and 2014)

Our Collaborations and Networks

NANOTEC
a member of NSTDA



Thank you!