# Nanotoxicity

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KIRSI YLINIEMI, PÄIVI LAAKSONEN

Minilecture

This session is more about examples and raising questions.

# After This Session You Can

Explain the basics of nanotoxicity

Think possible factors for conflicting research data

Understand the roots of the knowledge gap between the nanotoxicology and nanosafety

# General remarks about nanotoxicity

- Nanomaterials interact with the living systems **differently than bulk materials**
- **High reactivity** due to the high surface area
- Popular belief that the nano-revolution is set to have a far **larger global econo-techno-political impact than the industrial revolution** of the nineteenth century or the information technology revolution of the twentieth century
- Emerging of nanotechnology increases the **emission of nanomaterials in the nature**, although they exist there already (volcano eruptions etc.)

# Toxicity of nanoparticles

- **Toxicity** = most materials are toxic when their quantity in the human system exceeds a critical acceptable limit.
- The distribution of nanoparticles in the body seems to depend on their **size, form and substance properties**
- **Biodegradable nanoparticles** are not a problem as they can be metabolized and excreted
- It has to be assumed that **accumulation** will take place predominantly in the organs of detoxification ➔ possible risk

# Nano–bio cellular interaction effects

Barriers such as **cell membranes** do not constitute obstacles for nanoparticles

**In nerve cells**, particles were observed to move along the axis cylinders

Inter- and intra-cellular accumulation

**Cytotoxicity**

- Lowered viability of the cells, they stop growing and material

**Genotoxicity, mutagenicity**

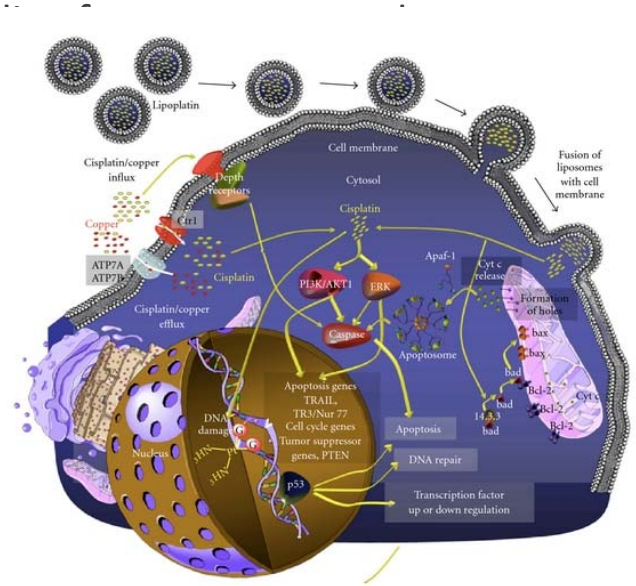
- Deleterious action on a cell's genetic material

**Necrosis**

- Cells dying due to lost membrane integrity

**Apoptosis**

- Activation of programmed cell death



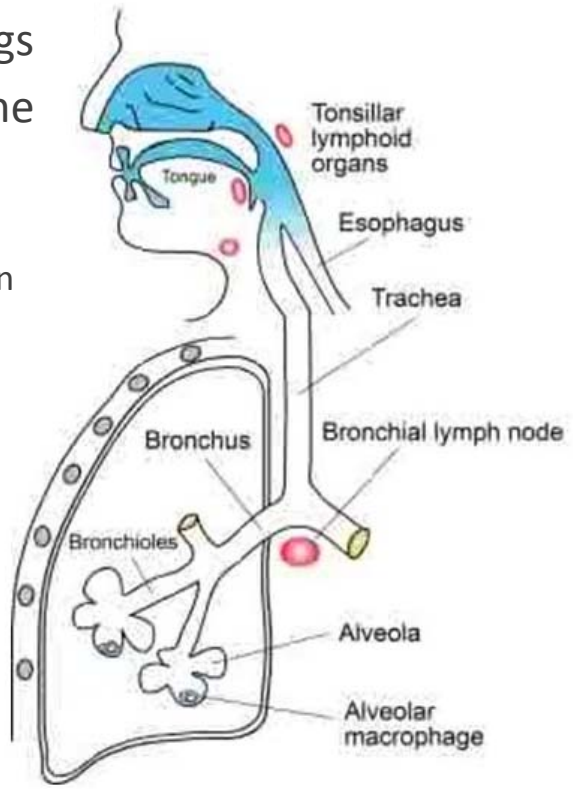

https://www.researchgate.net/publication/51636287_Lipoplatin_Formulation_Review_Article/figures?lo=1

# Possible diseases that can affect different parts of the body owing to nanoparticles

| | |
|---|---|
| **Brain** | Alzheimers & Parkinson's disease |
| **Lungs** | Cancer, asthma, bronchitis |
| **Gastrointestinal system** | Colon cancer |
| **Circulation** | Blood pressure, thrombus, vasoconstriction |
| **Skin** | Dermatitis, auto-immune disease |
| **Lymphatic system** | Sarcoma |

# Exposure via respiratory tract

- **Inhalation**
  - Nanoparticles can reach the alveolar region of the lungs
  - Due to small size, they are removed insufficiently by the alveolar macrophages
    - ➔May cause inflammation
    - ➔Elongated retention in the lungs, even in blood circulation and brain
- **Through the mouth by ingestion**
  - Intestine ➔ lymphatic system

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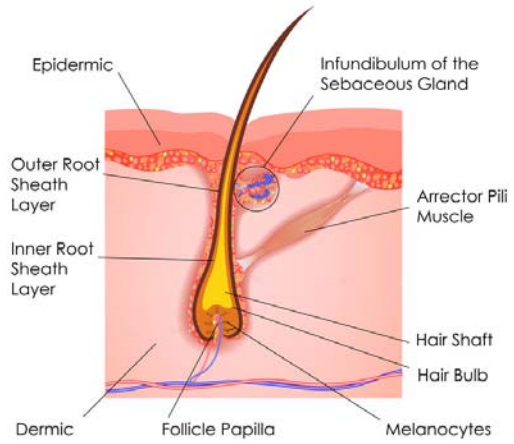
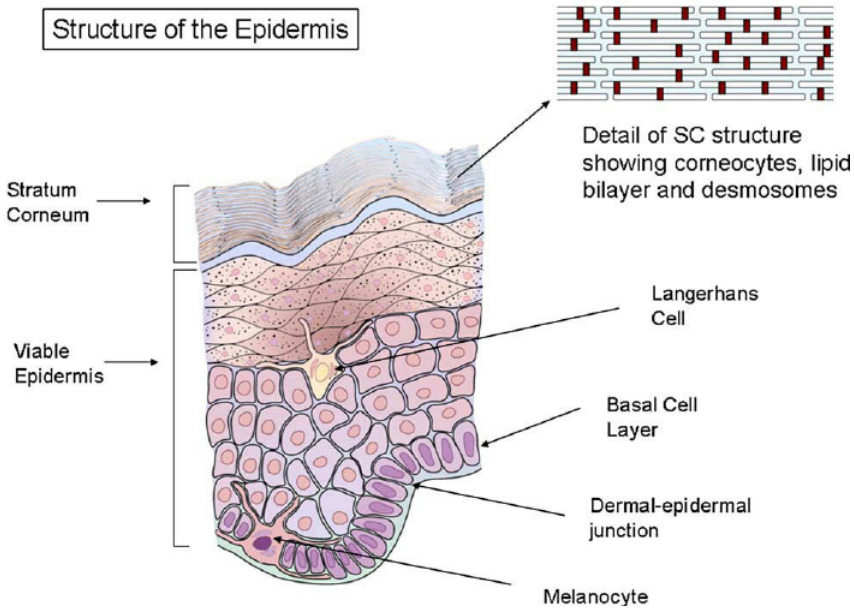
# Exposure via skin

- **Absorption** through upper layer of the skin or through the hair roots
- Intact vs. damaged skin?
- **Intact skin is a barrier that is difficult to pass**




http://www.skinpilot.de/wp-content/themes/skinpilot/images/hair2.jpg

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# Exposure via blood stream

- Bloodstream may transport nanoparticles **to a number of organs** (heart, liver, spleen, kidneys and bone marrow
- Some studies indicate that **nanoparticles can cross biological barriers**
  - the blood–brain barrier
  - through the placenta into the fetus is possible
- The same mechanisms may be utilized for therapeutic purposes

# Important questions in nanotoxicology study

- **Which particle characteristics are crucial in initiating and causing adverse effects?**
- **Do the nanomaterials penetrate the cell membranes?**
- **Do they translocate to other tissues and organs?**

# Examples of methods to study toxicity

## Cell toxicity

- The number of alive cells (cell viability) is determined
- Cell viability assays base on detection of certain molecules that tell (under defined conditions) if the cells are alive
- MTT assay: colorimetric assay indicating the level of certain cellular oxidoreductase enzymes

## Animal models

- Locating the accumulated materials in certain tissues
- Monitoring of chosen indicator
- Very complex, but may not represent well the human body

## Artificial tissue (spheroids)

- Cell aggregates that model tissue

# Case study: Toxicity of carbon nanotubes

# Why are carbon nanotubes (CNTs) toxic?

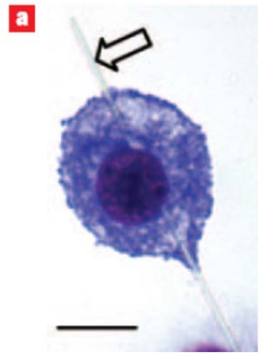
- Chemically similar to graphite ➔ non-toxic?
- Needle-like shape ➔ asbestos-like behavior?
  - Asbestos causes lung cancer and scarring of the lungs
- Slow degradation due to high biopersistence ➔ accumulation in the soft tissues
- Prolonged latency period between exposure and development of the disease

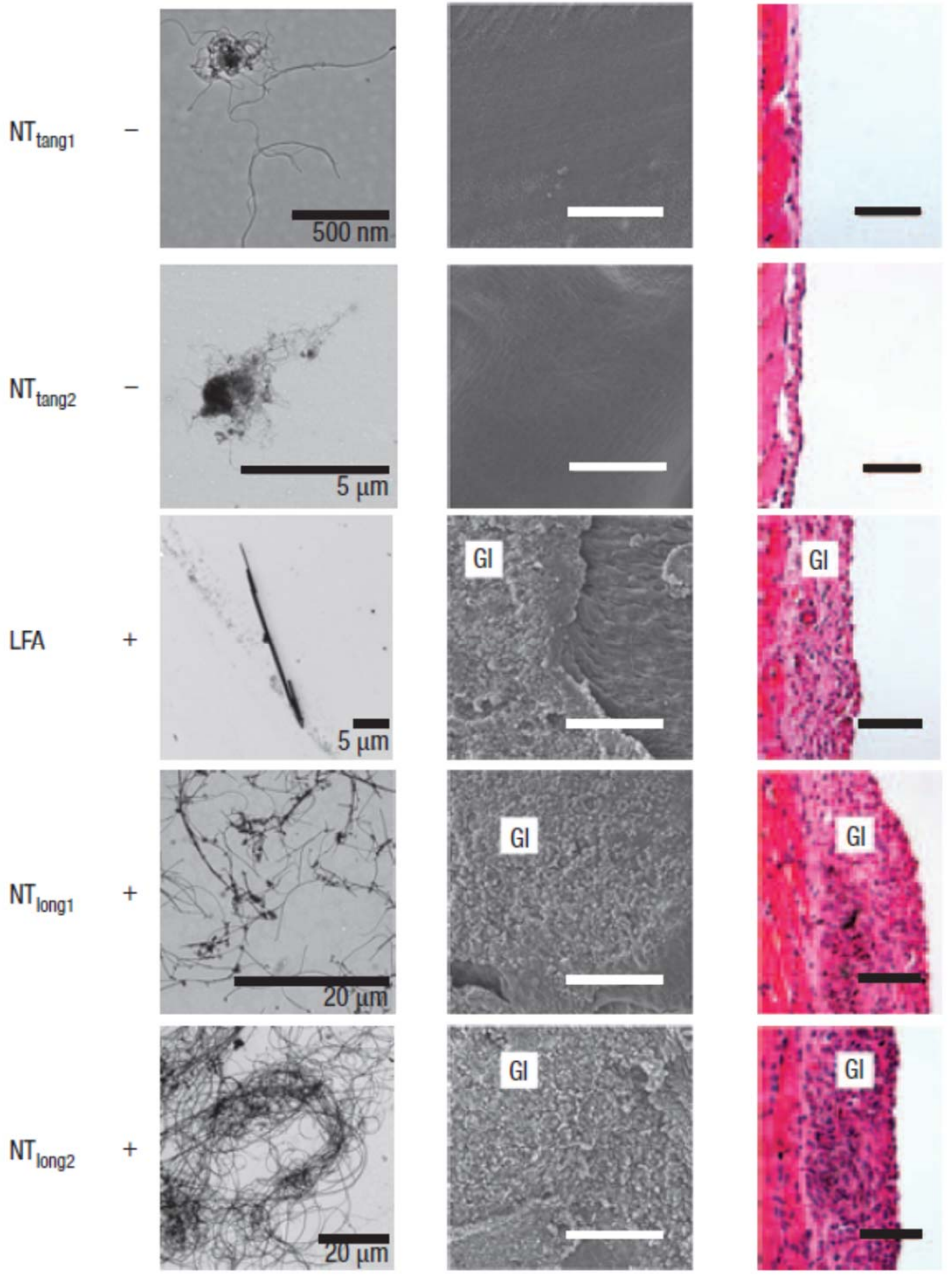
# Tube length affects the toxicity of CNTs

- CNTs were injected into abdominal cavity of mice
  - Samples containing long fibers
  - Samples containing short and entangled fibers
- Response was assessed by protein levels and cell populations in the peritoneal cavity
  - Scar-like structures were studied
  - Foreign body giant cells (fused macrophages)
- Long fibers caused a foreign body response typical for indigestible and non-biodegradable material that the macrophages cannot destroy

Poland C.A. *et al. Nature Nanotechnology*, **2008**, *3*, 423.

- The long fibers showed increased signals and inflammation
- The other components included in the samples did not cause any response
- Short fibers may have some other toxic influences due to their small size
- Macrophages try to engulf the foreign body particles and fail with long ones




Poland C.A. *et al. Nature Nanotechnology*, **2008**, *3*, 423.

# Other toxicity studies on carbon nanotubes

| Organ | Test | What happens? | Biological response | Research Group(s) |
|---|---|---|---|---|
| Lungs | Rat | Bundle together | Stimulation of immune system | Dr CW Lam at Wyle Labs (NASA Johnson Space Center), Dr Robert Hunter, University of Texas (Houston) |
| Trachea | Rat | NTs blocking the bronchial passageways | Suffocation | Dr David Warheit (DuPont's Haskell Labs) |
| Trachea | Guinea pigs | Nothing | No health risk | University of Warsaw |

Textbook of Nanoscience and Nanotechnology, B. S. Murty, P. Shankar, B. Raj, B B Rath, J. Murday, Universities Press (India) Private Limited 2013, Ch. 7 pp. 214-223.

# Nanotoxicology and Nanosafety

# Nanotoxicology ≠ Nanosafety

## NANOTOXICOLOGY

Physiology

Pathology

Biomolecular mechanisms

**Results from the lab**

## NANOSAFETY

Risk assessment of materials

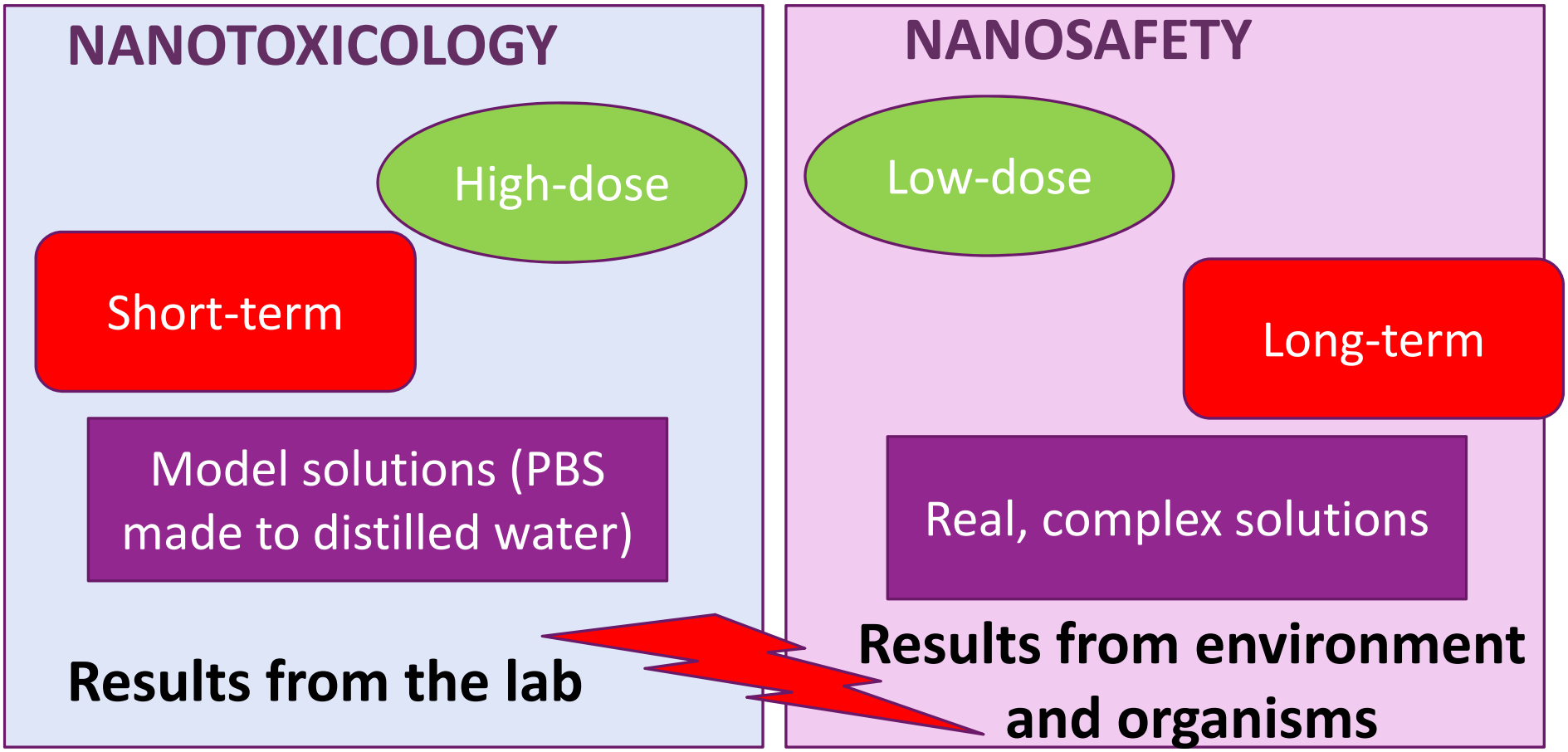
In environment

In organisms

**Results from environment and organisms**

X. Hu, D. Li, Y. Gao, L. Mu, Q. Zhou, Knowledge gaps between nanotoxicological research and nanomaterial safety, *Environment International* **94** (2016) 8–23

# Knowledge Gap

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# Methodological Deficiencies 1

**Quantification *in vivo***

1. How to determine very low concentrations in real environment?
2. How to detect carbon nanomaterials (and their transport) in organisms?
3. Safety issues when making research (animal and environmental safety)

**Modelling**

1. Simple models “useless”
2. More realistic models too expensive
3. Fully realistic models currently impossible

# Methodological Deficiencies 2

**Several processes**

1. In real environments, impossible to “change” only one parameter

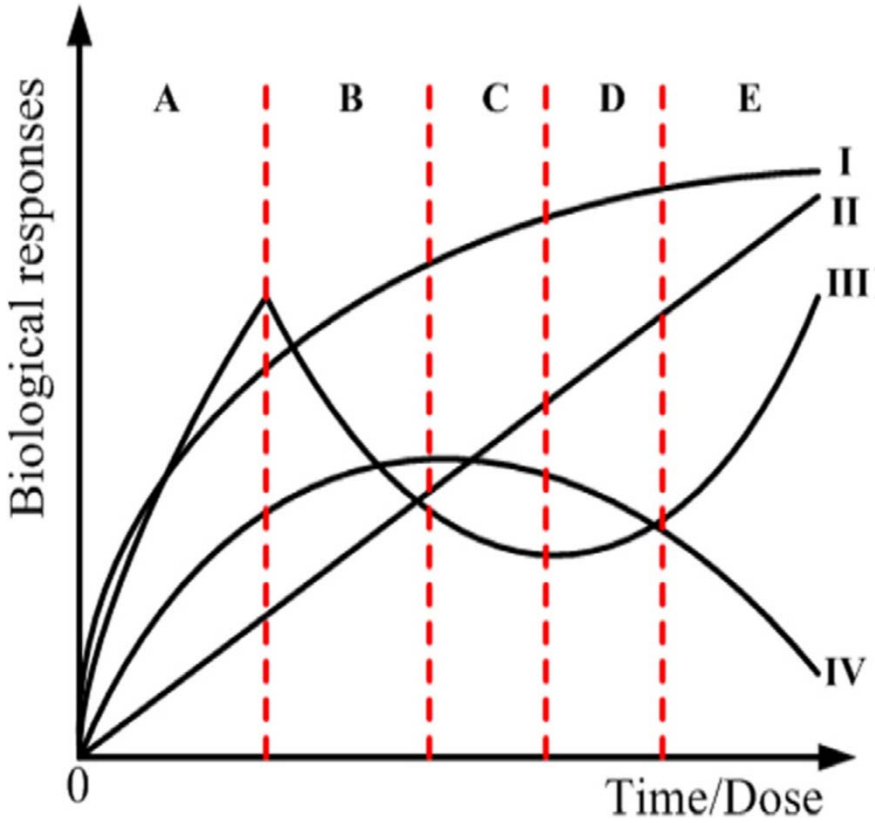
- Response is always a “sum” of many factors

*How to determine which factor influences what?*

# Exercise 1

Discuss about the difference between nanotoxigology and nanosafety.

# Time- or Dose –profiles of biological Responses




X. Hu, D. Li, Y. Gao, L. Mu, Q. Zhou, Knowledge gaps between nanotoxicological research and nanomaterial safety, *Environment International* **94** (2016) 8–23

# Exercise 2

**Determine to which time/dose-profiles the following cases belong to?**

i. Graphene oxide induces Parkinson's like symptoms in zebra fish at low concentrations, but at certain concentrations the effect is strongly reduced due to agglomeration.

ii. Graphene nanosheets inhibit plant growth but overtime graphene nanosheet are oxidised (in biological environment) to nanoribbons which in turn increase the wheat growth.

iii. In short-term exposure, Ag nanoparticles induce DNA breakage in cell culture but at longer exposure times, more silver resistant genes are formed. In the end, there was no difference in DNA breakage between cells which were exposed to Ag nanoparticles and cells which were not (control measurement).

What type of questions/problems did you encounter in this exercise?

# Uncertainties

**1) Is the described end-point actually the real end-point?**

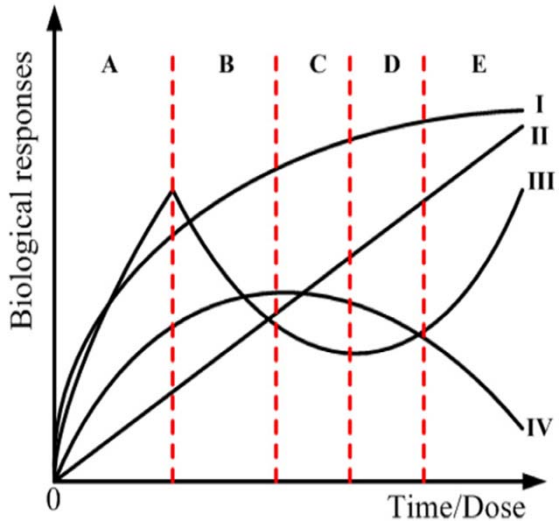
- What if the study would have been continued longer or performed at different dose?

**2) What is the time intervals the measurements were done?**

- What if we take samples too seldom?

**3) What biological response we are looking for?**

- Are we studying markers of diseases or actual symptoms?



# Exercise 3 - Toxicity of gold nanoparticles

**In Pairs**

Based the provided two papers about toxicity of AuNP:

**1) What is the main conclusion of both paper (separately)?**

**2) How did the authors end up to this result?**

# Exercise 3 - Toxicity of gold nanoparticles

**In Pairs**

Based the provided two papers about toxicity of AuNP:

**1) What is the main conclusion of both paper (separately)?**

**2) How did the authors end up to this result?**

**Two pairs combined**

Based the provided two papers about toxicity of AuNP:

**1) What are the main differencies between these studies?**

- **Methodological?**
- **Material?**
- **Interpretation of the results?**

# Exercise 3 - Toxicity of gold nanoparticles

**Combine groups again**

Based the provided two papers about toxicity of AuNP:

1) **What are the main differencies between these studies?**

- **Methodological?**
- **Material?**
- **Interpretation of the results?**

# Literature

Murty B.S., Shankar, P. , Raj,B., Rath, B.B., Murday, J., **Textbook of Nanoscience and Nanotechnology**, Springer (2013), pp. 212-223.

X. Hu, D. Li, Y. Gao, L. Mu, Q. Zhou, Knowledge gaps between nanotoxicological research and nanomaterial safety, *Environment International* **94** (2016) 8–23.

AuNP examples:

Li et al., *Adv. Mater.* **20** (2008) 138–142**.**

Connor et al., *Small* **1** (2005) 325 –327.