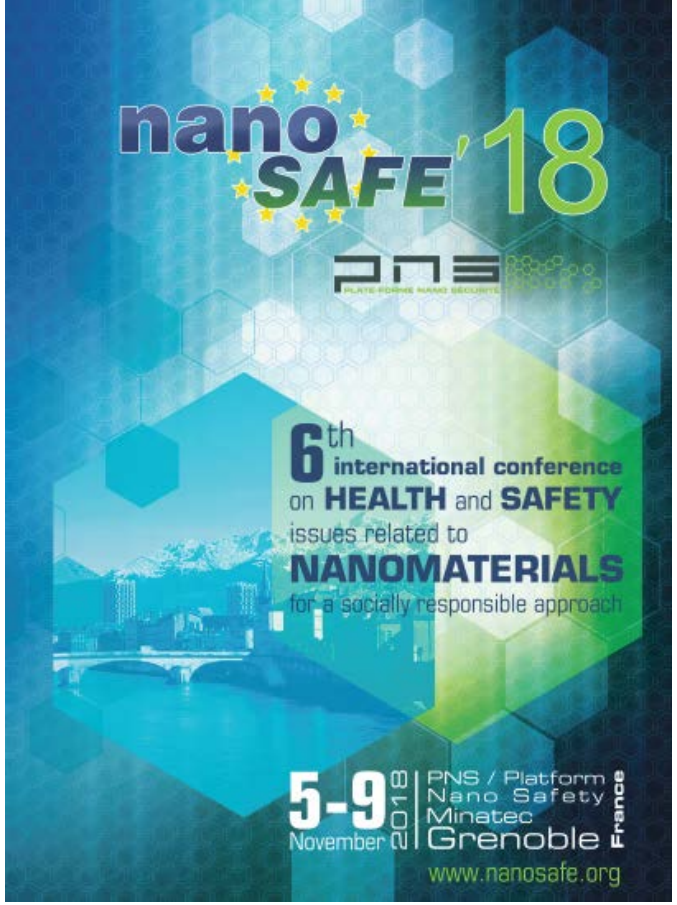
Nanoparticles, Nanotoxicology and Nanosafety in 2018: - Where are we? -



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nano SAFE 18

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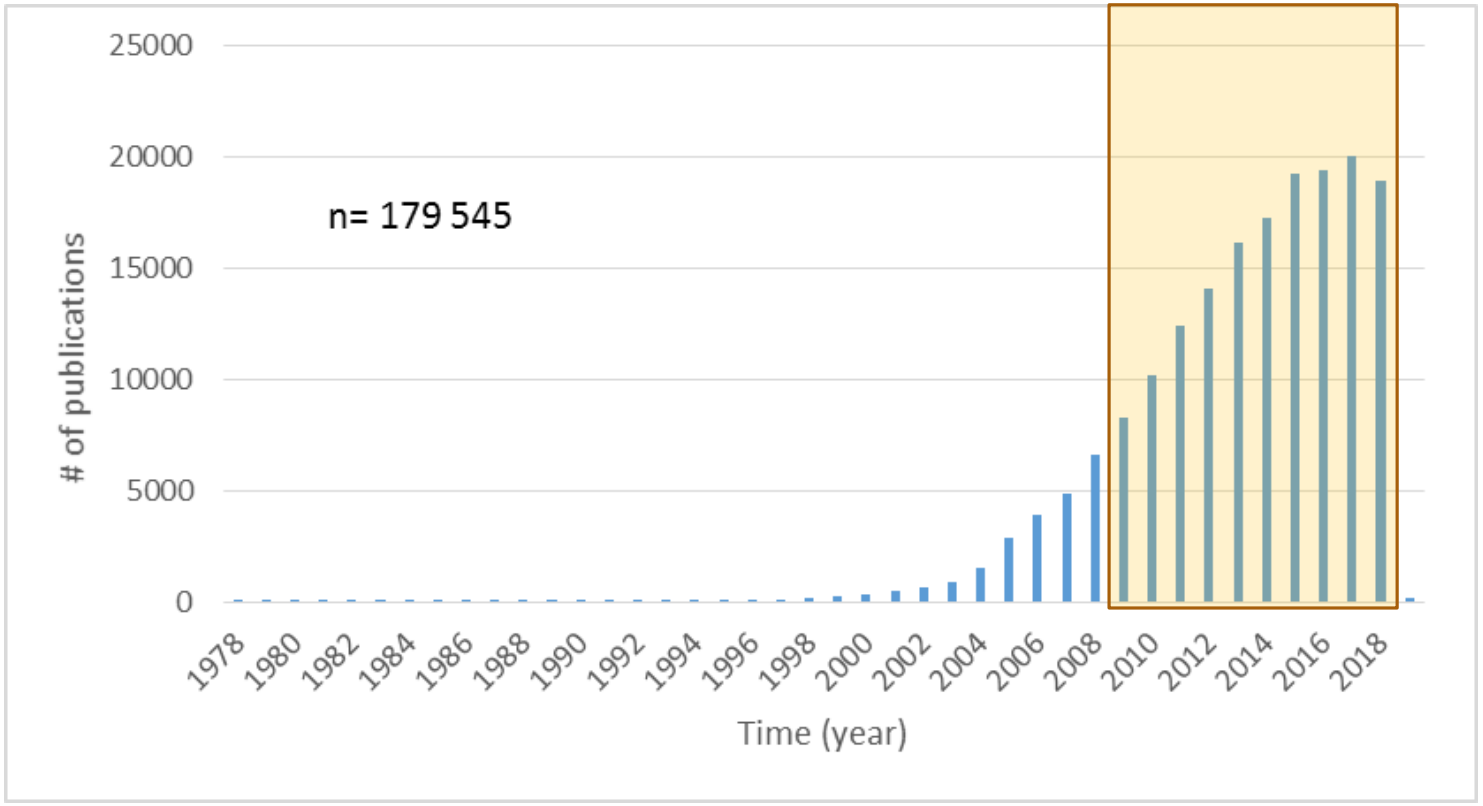
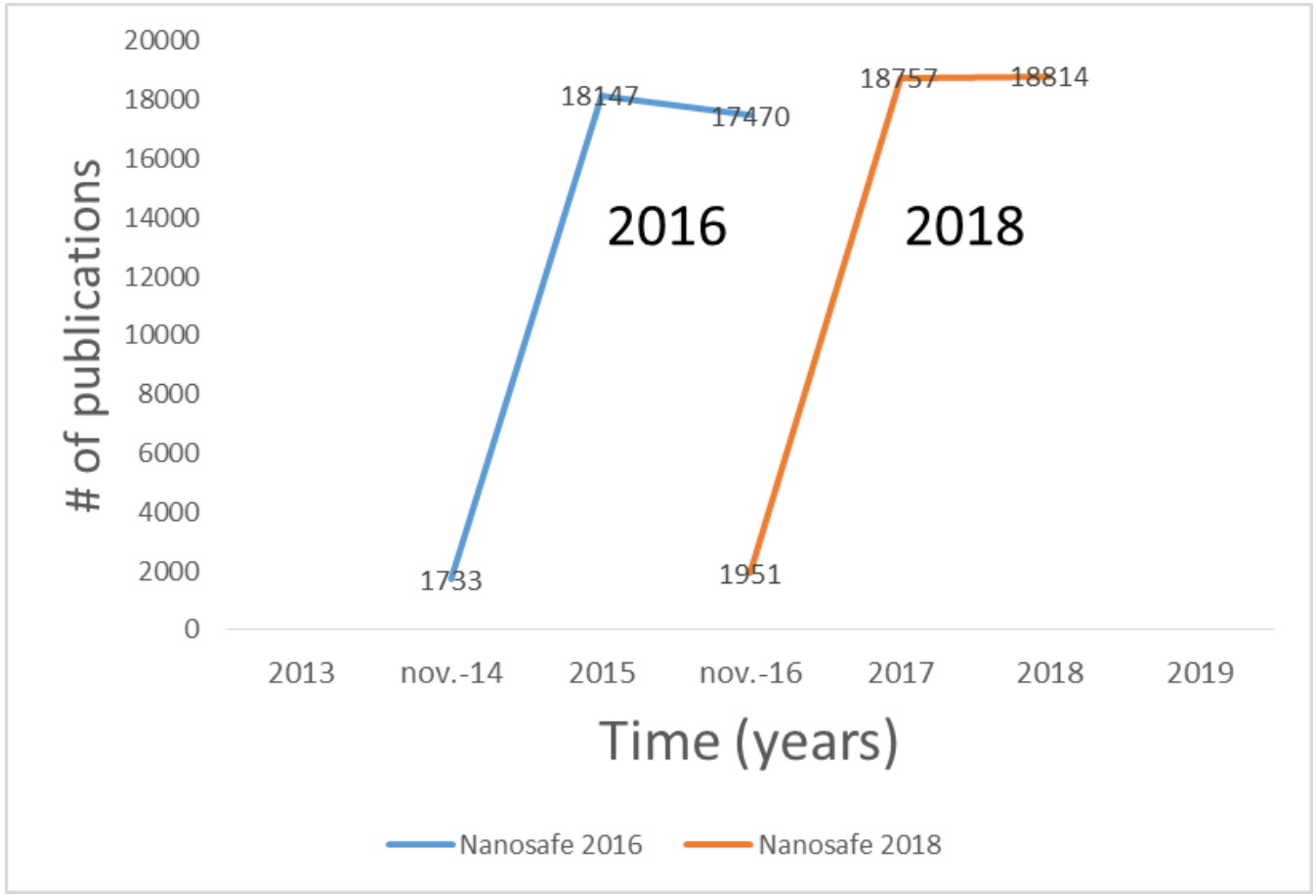
Outline

- Nanoparticles
- Nanotoxicology
- Nanosafety
- Were are we in 2018
- What do we need
- Conclusion
- Take home

Nanoparticle(s)



Nanoparticle(s)



n= 76 872 publications
Between Nov 1st, 2014 to Nov 1 2018

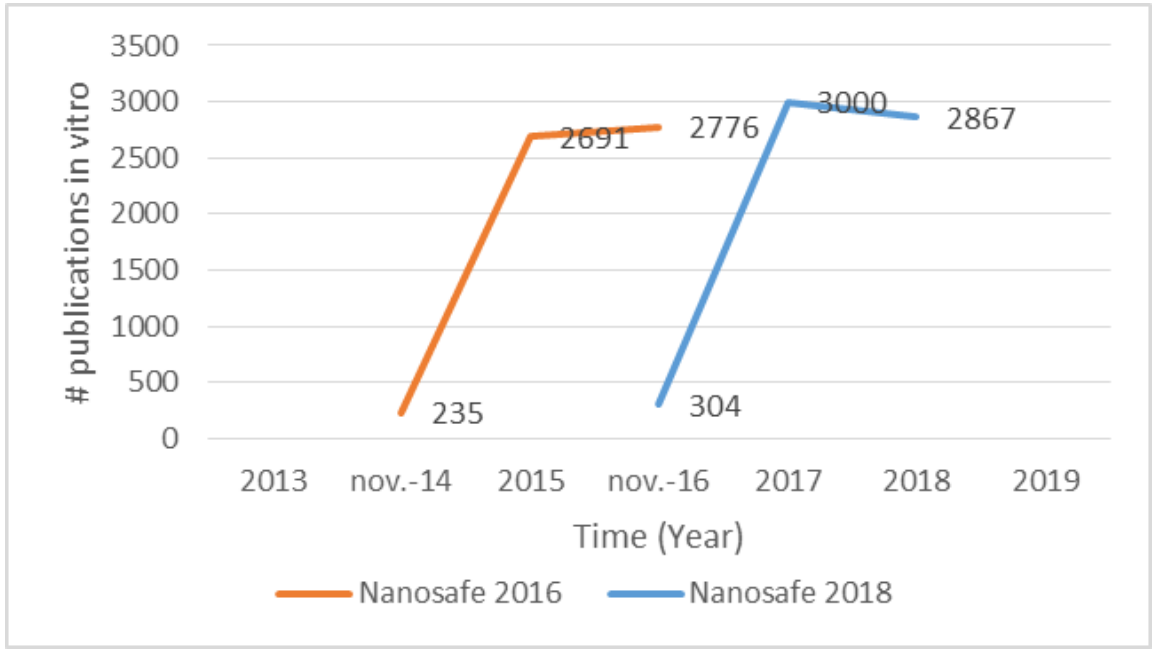
Nanoparticles most commun:
Ag, TiO_2, Al/Al_2O_3, ZnO, SiO_2, Au, ceramics, iron/iron oxide, carbon nanomaterials, nanoclays, and quantum dots

Pubmed (Nov 2018)

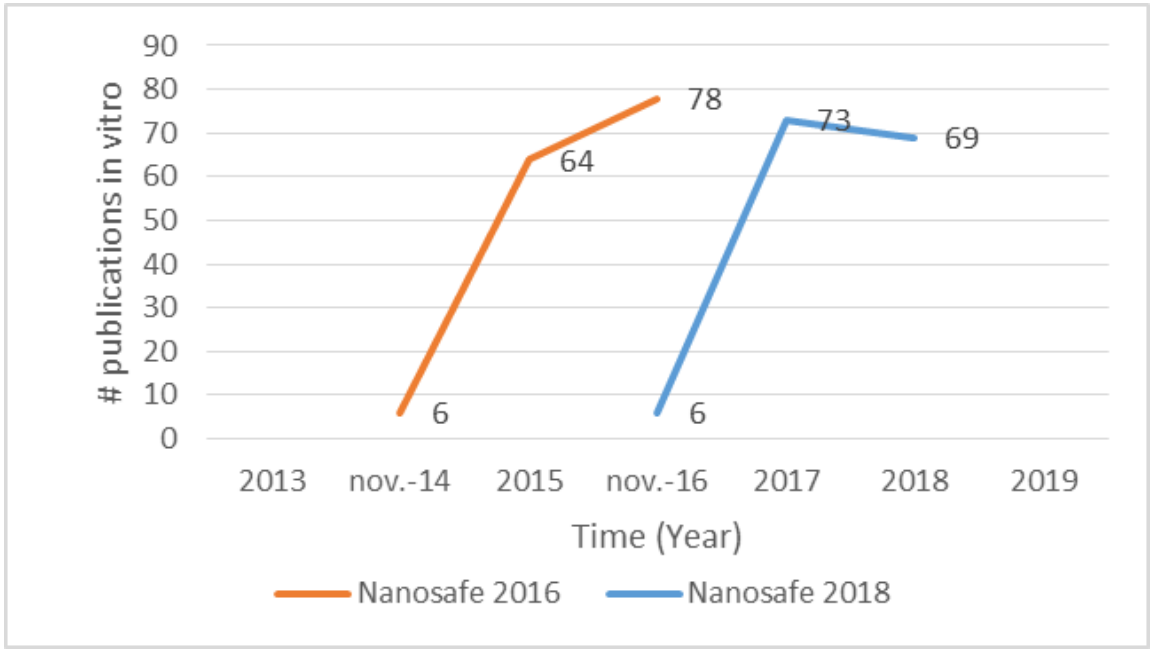
Nanotoxicology

- *in vitro*
- *in vivo*
- *in silico*

In vitro (studies)



Nanoparticles and in vitro (n= 11 873)



Nanotoxicology and in vitro (n=303)

Pubmed (Nov 2018)

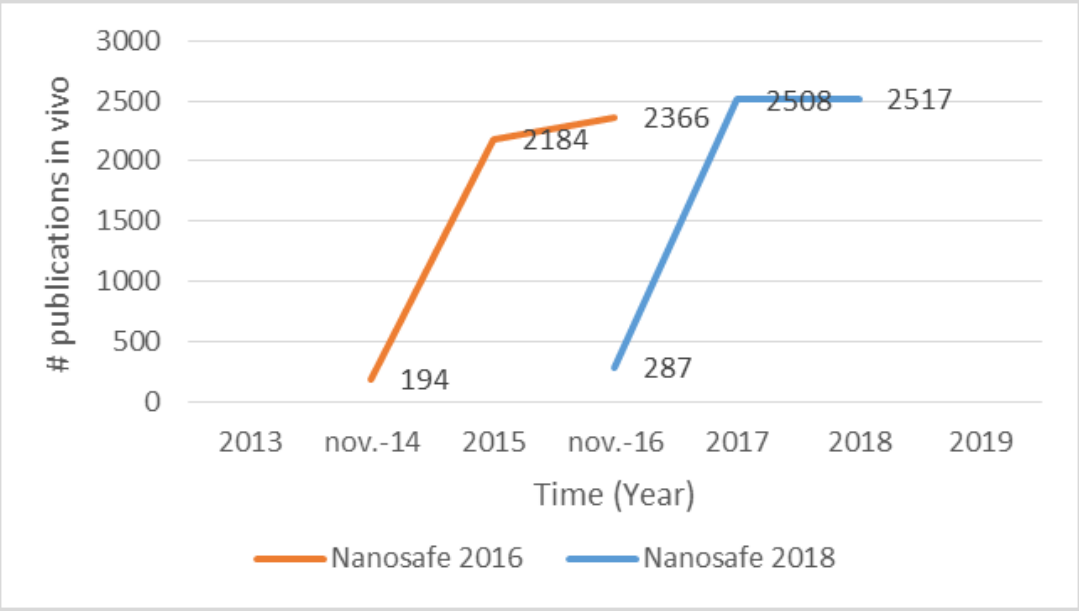
In vitro (What do we knows)

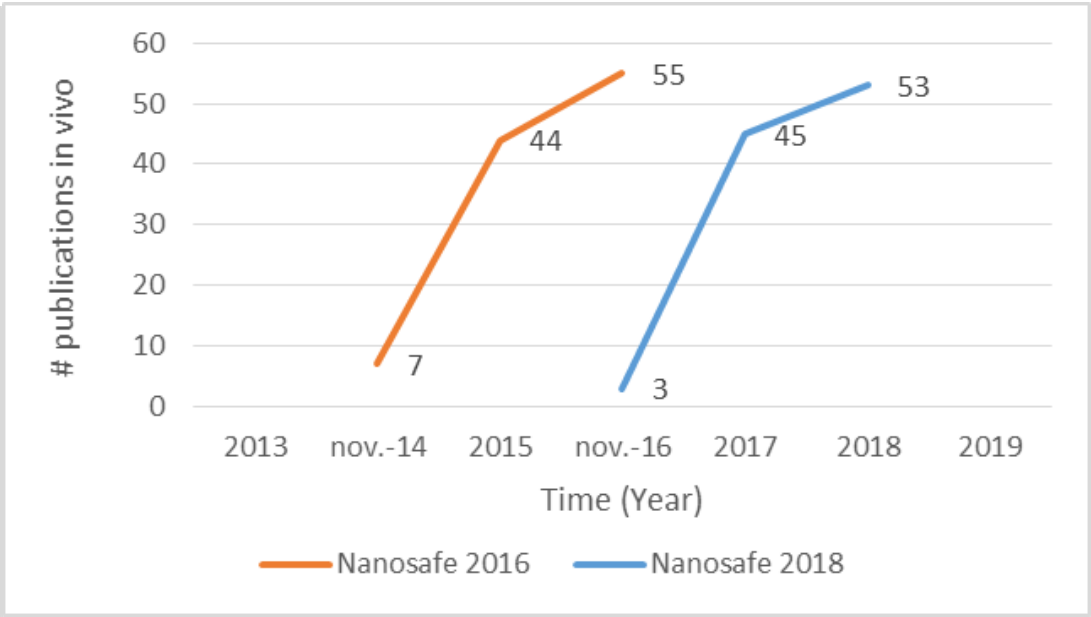
- *In vitro* tests are easy, affordable, and rapid (compare to *in vivo*)
- apoptosis assay, viability assay, oxidative stress, inflammatory assay, DNA damage
- A major challenge in nanotoxicology today is the huge discrepancy in reported toxicity studies.
- For the same type of NMs (SCNT or MWCNT) different toxicities.
- Number and complexity of the new generation of NMs (all functionalization's)
- However, traditional *in vitro* testing does not work fast enough to covert all nanoparticles send to the market.

In vitro (some ideas for the future)

- Immunotoxicity, genotoxicity, MoA, metabolic pathways
- There is a huge demand for testing NPs for safety, using in vitro approaches
- May be high-throughput screening (HTS) assay is the way to measure (such as Tox21, Toxcast)
- Maybe a solution is to study the epigenetic toxicity!
- In vitro test methods need further modifications taking into considerations challenges related to NM physicochemical properties and their interaction with surrounding, target environments, cellular uptake and novel endpoints.

In vivo (studies)





Nanoparticles and in vivo (n= 10 056)

Nanotoxicology and in vivo (n=207)

Pubmed (Nov 2018)

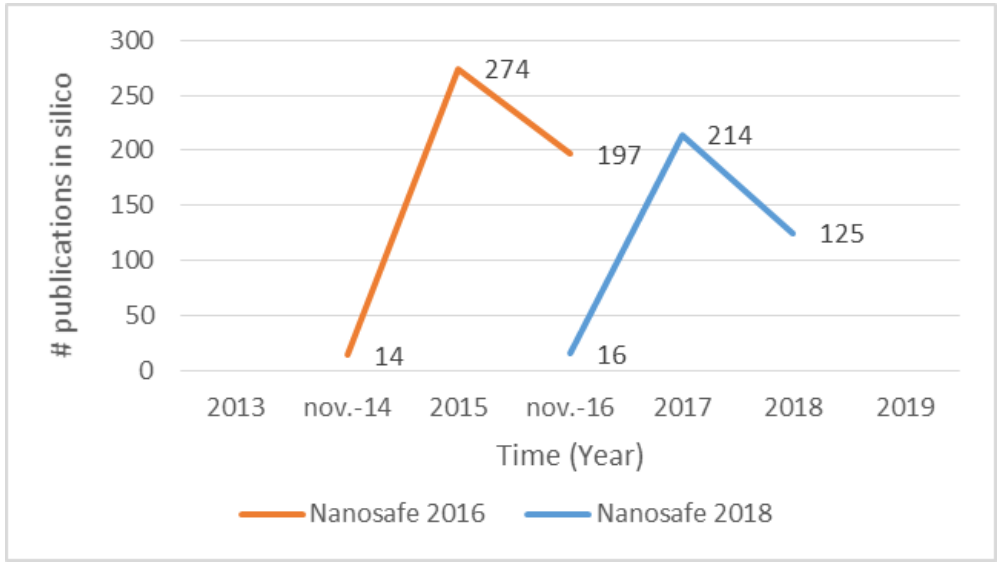
In vivo (What do we knows)

- Inhalation and ingestion are the major routes of exposure.
- Many studies outline the toxic effects of NPs (oxidative stress, inflammatory assay, DNA damage).
- However, only few addressed their potential adverse effect on target organs.
- NPs accumulated in various organs.
- Liver, spleen, kidneys and lung are probably the main target organs of NPs.
- The accumulation is not irreversible and have access to vasculature and can target others organs.
- Inhalation cause inflammation.
- The oral route limit the systemic distribution depending of NM size and intrinsic properties.
- Severity of NPs toxicity depend of exposure dose, route , NPs chemistry, size, shape, agglomeration state

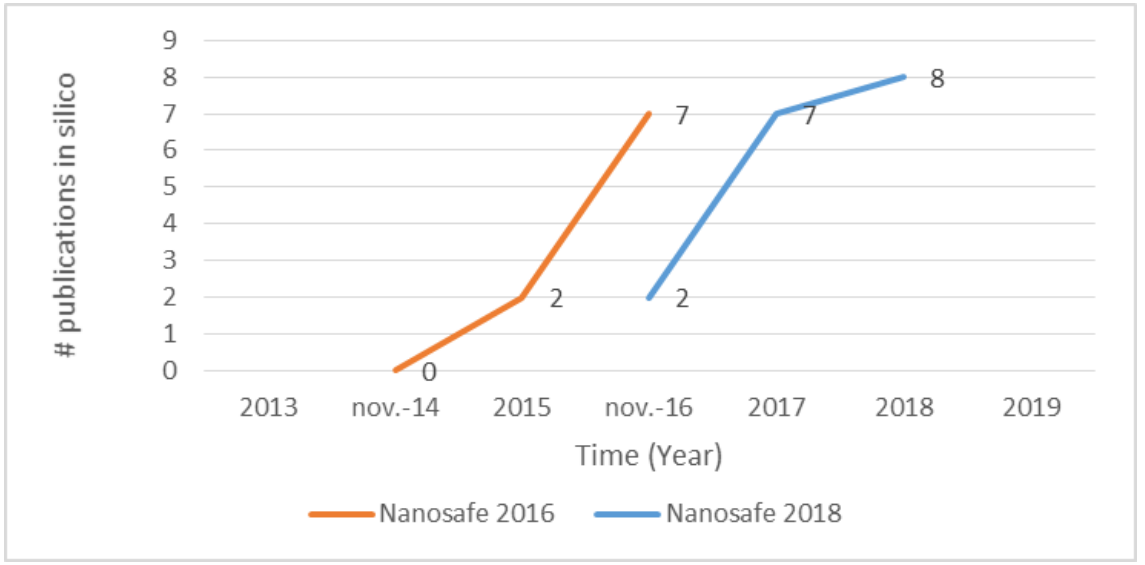
In vivo (some ideas for the future)

- Toxicity 4 days post exposure is interesting, but the kinetics of occurrence of the toxic effect is more.
- What is the most important the NPs alone or in the matrix of the final product. (Risk = exposure X hazard)
- Long term exposure to low doses
- NM properties after administration, and physicochemical characterization.
- Pristine and Aged for Nanoparticles

In silico (computer studies)



Nanoparticles and in silico (n= 840)



Nanotoxicology and in silico (n=26)

Pubmed (Nov 2018)

In silico

- Read-across
  - Read-across is a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s).
- QSAR (Quantitative Structure Activity Relationship)
  - Mathematical relationships linking chemical structure and pharmacological activity in a quantitative manner for a series of compounds (usually linked to molecular or in vitro experimental observation).
- Biological Modeling
- Protein Docking Algorithm (EADock)
  - Computational simulation of a candidate ligand binding to a receptor transporter.
- PBPK (Physiologically Based PharmacoKinetic Modeling)

In Silico (Definitions)

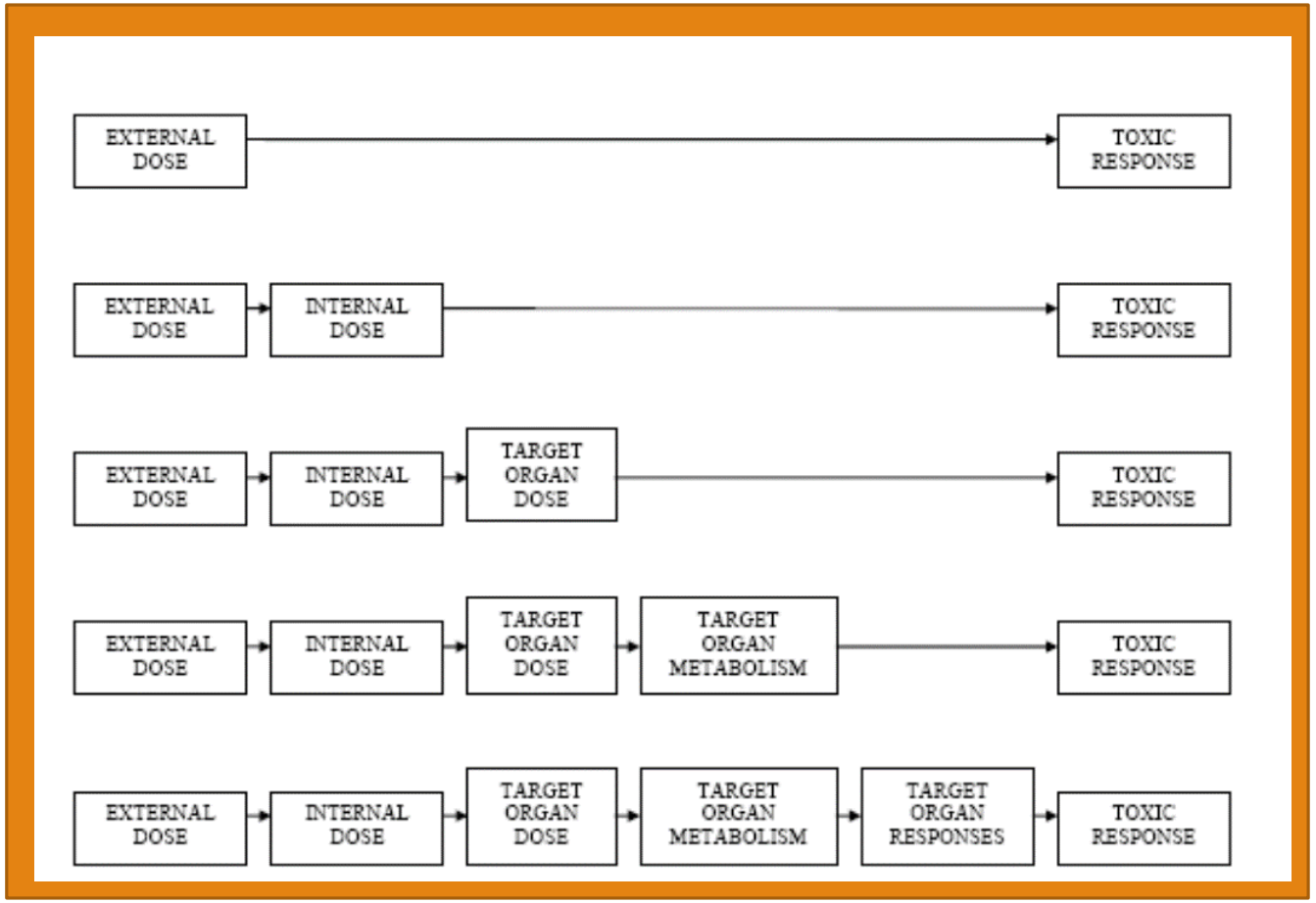
In silico model in toxicology :

- A model is a mathematical representation of biological observation.

PBPK modeling (pharmacokinetic / pharmacodynamic):

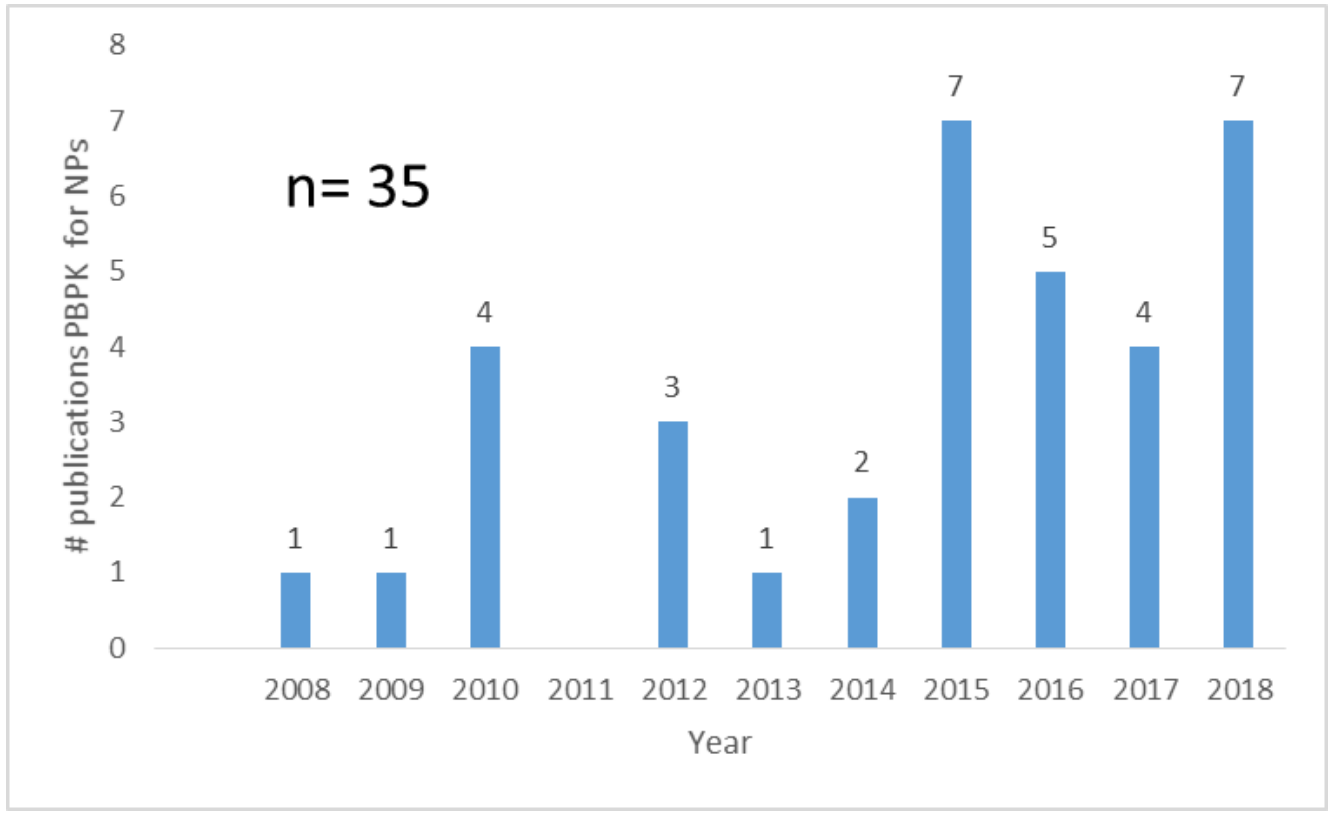
- PBPK modeling refers to the development of mathematical descriptions of the ADME of chemicals based on quantitative interrelations among the critical determinants of these processes.

In Silico (PBPK)
Levels of description we want to reach

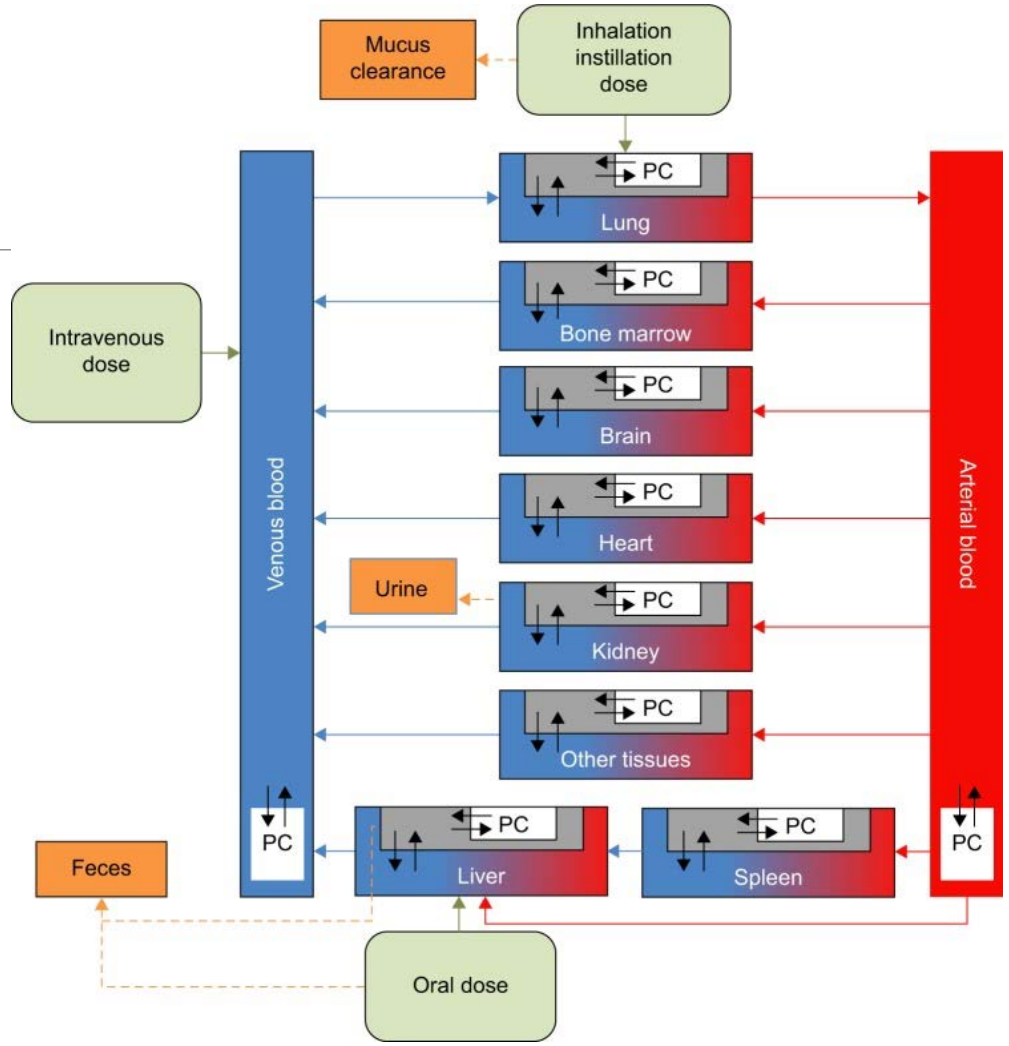


WHO 2008

In Silico Nanoparticles (PBPK)



Pub Med 2018

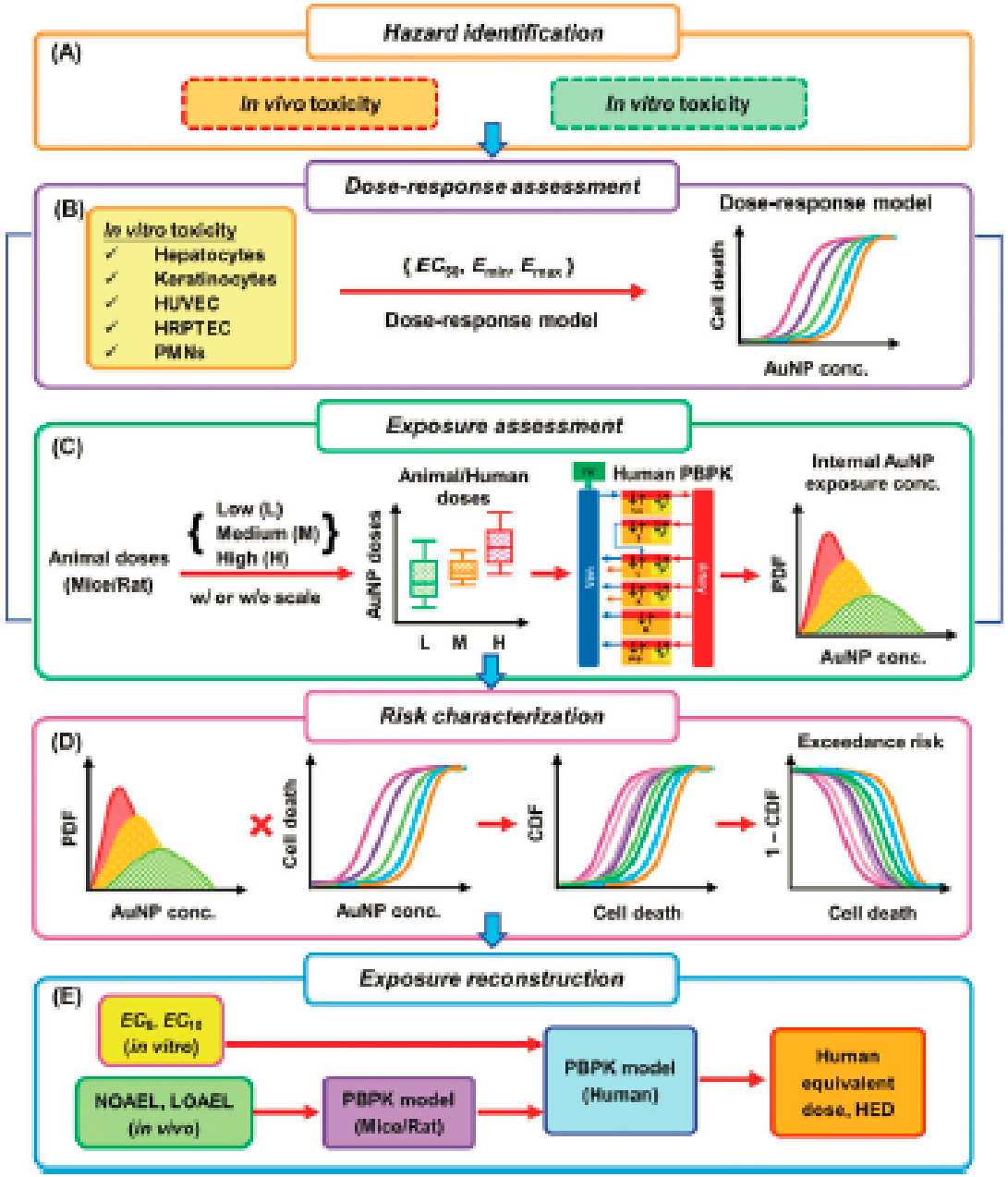


Carlander *et al.*, 2018

In Silico (PBPK)

In vitro to in vivo extrapolation (IVIVE) refers to the qualitative or quantitative transposition of experimental results or observations made *in vitro* to predicts phenomena in vivo, biological organisms.

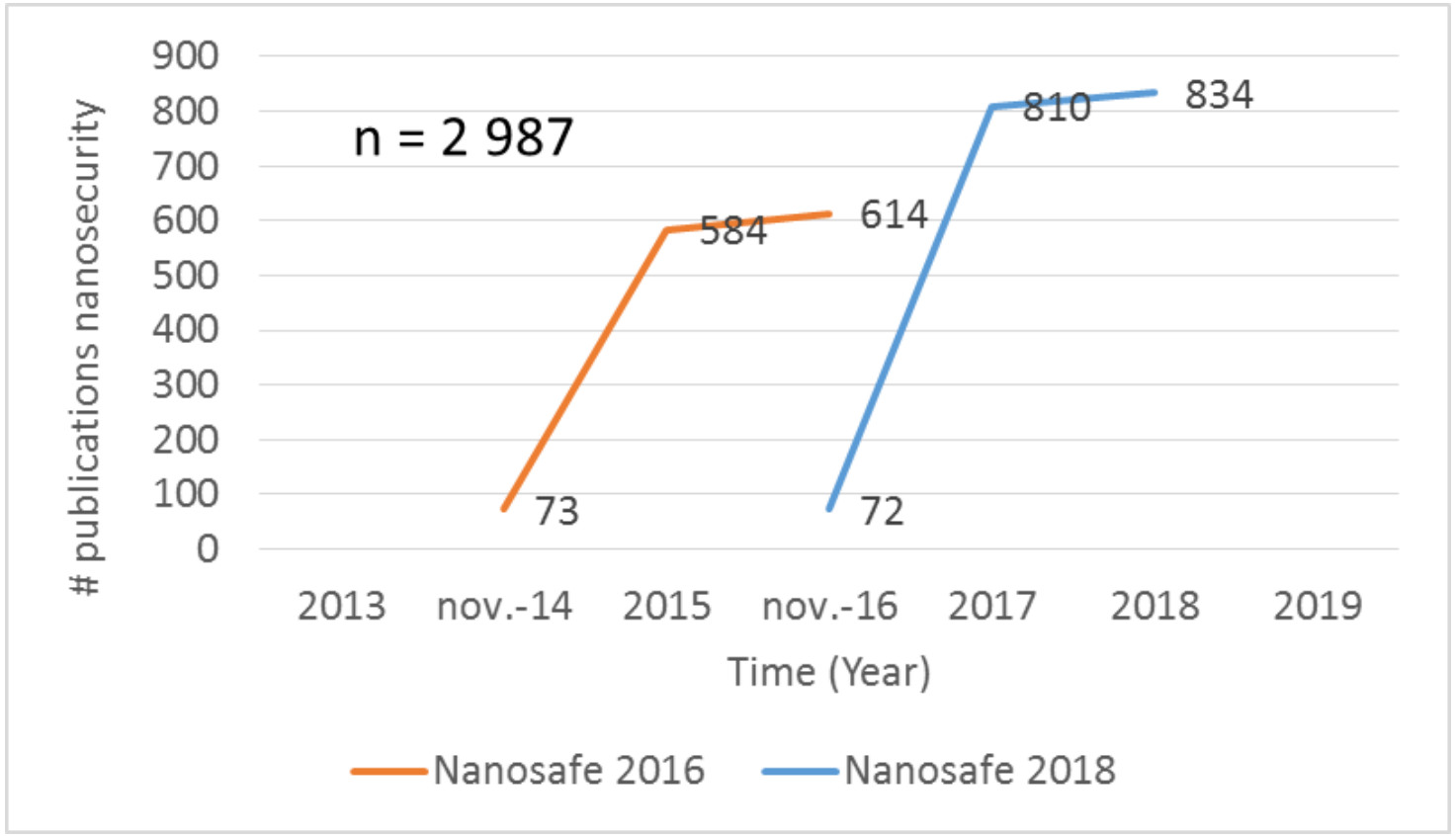
This model here is more IVIVE extrapolation model



Y-H Chang et al., 2018

Nanosafety

Nanosafety (studies)



Pubmed (Nov 2018)

Nanosafety (what we observe)

- No regulation are effective concerning NPs
- There is no toxicological reference value
- Several report and approach have been developed base on Control Banding (CB) or precautionary approaches. These methods assess the relation between hazard and exposure providing a qualitative level of risk and try to reduce it.
- No real assessment for NPs to characterize the reactivity of the NPs

Nanosafety (studies)

The NanoSafer CB tool (Jensen et al.; Kristensen et al., 2010)	The Danish Nanosafer was developed primarily for assisting SMEs and laboratories—that do not have any or have limited experience—with producing or working with nanomaterials and/or with insufficient resources to perform a full precautionary risk assessment.
Danish Nanosafer	http://nanosafer.i-bar.dk/
The IVAM Guidance (Cornelissen et al., 2011)	Guidance was developed to ensure that all work involving engineered nanomaterials is conducted safely.
Dutch Social Partners	http://www.industox.nl/Guidance_on_safe_handling_nanomats&products.pdf
Stoffenmanager Nano (Van Duuren-Stuurman et al., 2012)	A Web-based tool was developed as a practical approach for employers and employees to help prioritize risks in exposure situations. During these situations, quantitative risk assessments are currently impossible during synthesis for assessing nanomaterials in powders, in sprays, and embedded in products.
TNO and ArboUnie, Holland	https://nano.stoffenmanager.nl/)

Nanosafety (studies)

The CB Nanotool (Paik et al. [2008]) **Lawrence Livermore National Laboratory**	The Nanotool was developed to support first-line occupational health professionals and researchers to evaluate the potential risks related to production and down-stream use of nanomaterials in a research work environment. http://controlbanding.net/Services.html
The Swiss Precautionary Matrix (Höck et al., 2008) **Switzerland**	The precautionary matrix was developed for synthetic nanomaterials to guide SMEs to apply a precautionary approach to identify possible sources of risk from the production, use, and disposal of synthetic nanomaterials, considering employees, consumers, and the environment. http://www.bag.admin.ch/nanotechnologie/12171/12174/14653/index.html?lang=en
ANSES CB Nanotool **France**	ANSES developed an operational CB approach for small- to large-sized enterprises during synthesis and for the downstream use of nanomaterials in workplace settings, but also in laboratories. http://www.anses.fr/

Assessment Tool of Nanomaterials Based on Physico-Chemical and Biological Characterisations

Physico Chemical test

- Size shape
- charge surface
- chemical composition
- crystallinity
- aggregation state

Biologic test

- apoptosis assay
- viability assay
- oxidative stress
- inflammatory assay
- DNA damage

	PCtest 1	PCtest 1	PCtest 1	PCtest 1	PCtest 1	PCtest 1
Biotest 1	HL3	HL2	HL2	HL3	HL2	HL2
Biotest 2	HL4	HL2	HL3	HL4	HL2	HL3
BiotEST 3	HL1	HL1	HL3	HL1	HL1	HL3
Biotest 4	HL2	HL1	HL4	HL2	HL1	HL4
Biotest 5	HL4	HL1	HL4	HL4	HL1	HL4
Biotest 6	HL3	HL2	HL2	HL3	HL2	HL2

Color Key of Toxicity category rating:

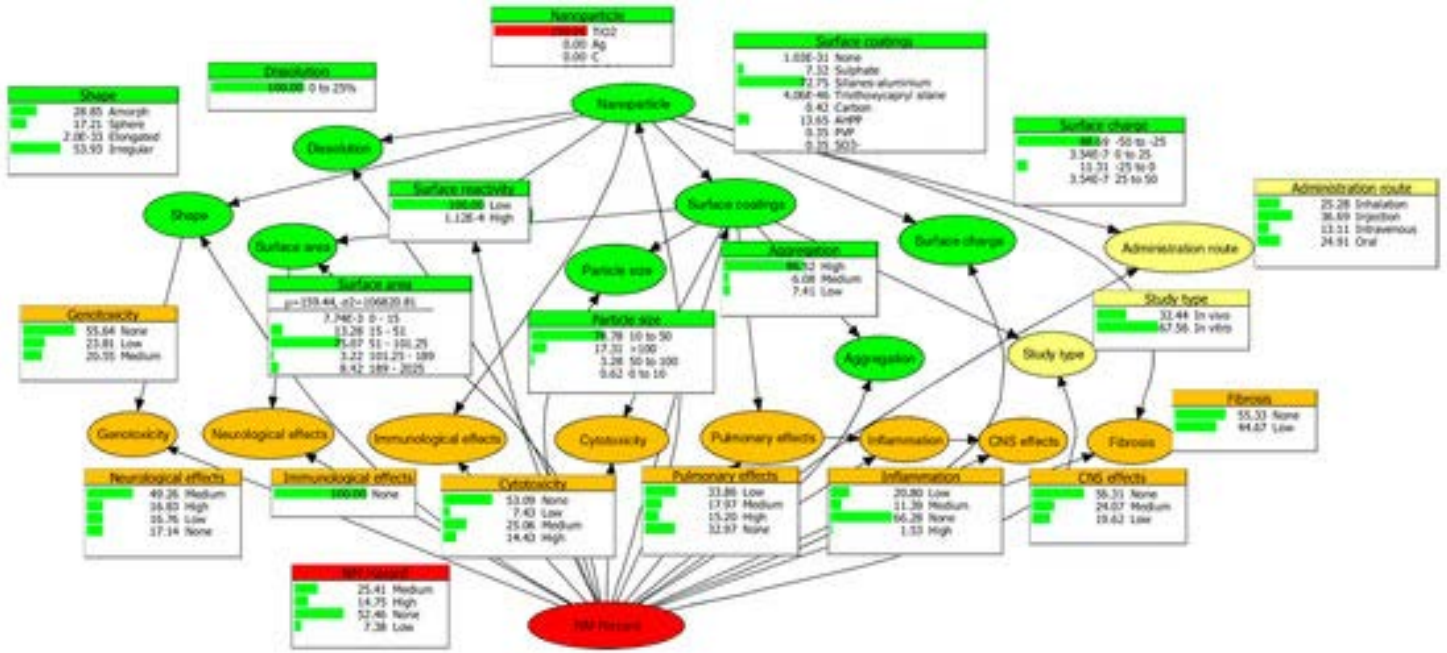
H4: Hazard level red =Toxicity category I is Highly toxic;

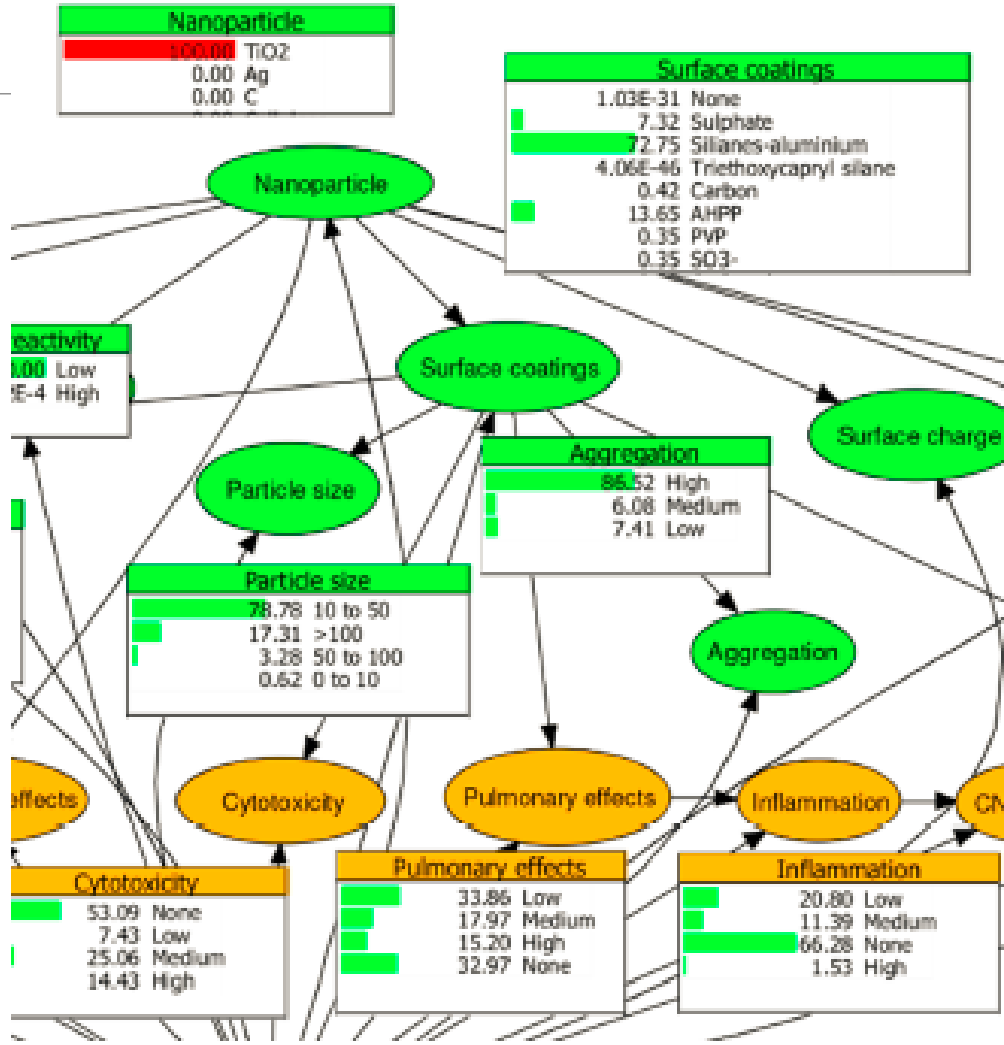
H3: Hazard level orange= Toxicity category II is Moderately toxic;

H2: Hazard level yellow= Toxicity category III is Slightly toxic;

H1: Hazard level green =Toxicity category IV is Practically non-toxic

Graphical structure and parametrization of the Bayesian networks (BN) with TiO2 NPs.





Bayesian networks (BN) are a probabilistic, machine learning approach This comparative study investigates the efficacy of quantitative WoE and Bayesian methodologies in ranking the potential hazard

Sheehan et al., (2018)

Where are we in 2018

- Number of NPs increases every year.
- The characterization of NPs is performed using different technic, but we need reference tests.
- The nanotoxicology test chemicals and bring new information in the literature
- No regulation yet around the world
- No real consensus about the metric need to be used (We dose animals in mg/kg and we observed toxicity in square area.
- Efforts are made to recently to regroup NPs by characteristic.

What do we need?

- Need to think about the in vitro test. There is no consensus yet on the optimal set of technique and procedures required.
- Debate about ‘’characterization’’ (individually / final product).
- Developed in silico tools like PBPK generic model. But also implement the QSAR approach, read-across approach or any other computer biology.
- Tend to do more in vitro tests because they are fast, cost-effective and can be performed as high-throughput screening (HTS) assays on relevant cells from humans and other mammals.
- In vitro assays adapted for testing of NPs for taking physicochemical characterization and cellular uptake into account.
- Explore using epigenetic signatures of exposure as potential biomarkers of NPs exposure.

Conclusions

- We need to sit and think what should be the best way to do both in vitro and in vivo. Then, elaborate a plan to do testing useful for risk assessment. (increase in vitro/reduce in vivo)
- Prediction of the toxicity of compounds can be optimized using in vitro toxicity screening methods by means of a battery of tests for different kinds of toxicity.
- The challenges in evaluating the large number of NMs and their complex variations using resource intensive and time-consuming in vivo assays, are a motivating factor for developing faster, more economical and reliable assays.
- An important aspect of impact assessment of NPs is the grouping and ranking of NMs and NM-containing products according to their inherent toxicity.
- Robust and standardized methods are of great importance in nanotoxicology.
- Standards characterization and in vitro test for more efficient risk assessment.

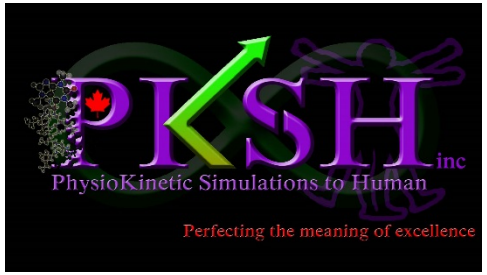
Take Home

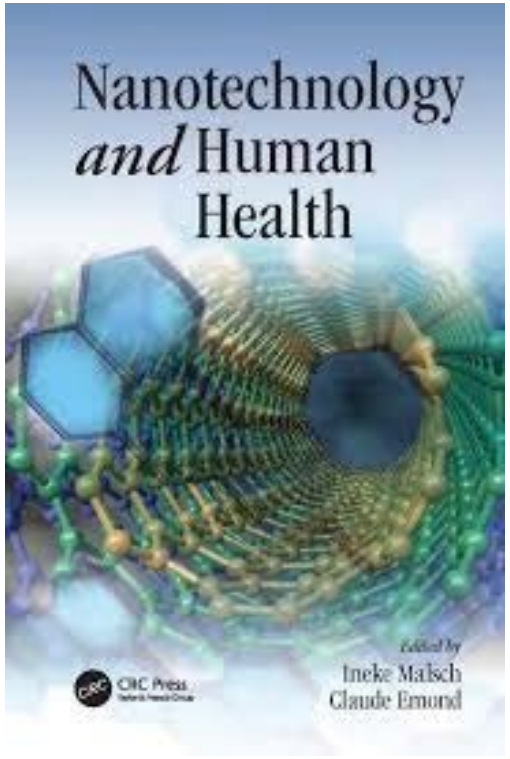
- 180 000 papers have been published about nanoparticles.
- The amount of study related to nanotoxicology is maybe underestimated (3%).
- We need to improve in vitro technique.
- HTS maybe an important step to increase our efficiency
- In silico PBPK is an important alternative especially because of the IVIVE
- Alternative interesting tools like QSAR or read across are also important to considerate support by strategy analysis like WoE and MoA, AOP.

Acknowledgment

Thank you !

TITANT
The International Team in NanosafeTy





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