Testování nanomateriálů z hlediska současného vědeckého poznání a praxe

Pavel Rossner, PhD.
pavel.rossner@iem.cas.cz

http://iem.cas.cz/en/research/departments/department-of-nanotoxicology-and-molecular-epidemiology/

Institute of Experimental Medicine AS CR, v.v.i.
EU Centre of Excellence

Nanotoxikologie

- studies the interaction of nanomaterials with biological systems, and the consequences of these interactions

- takes into account specific physicochemical properties of NP

Zdroje nanočástic



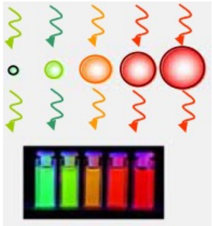
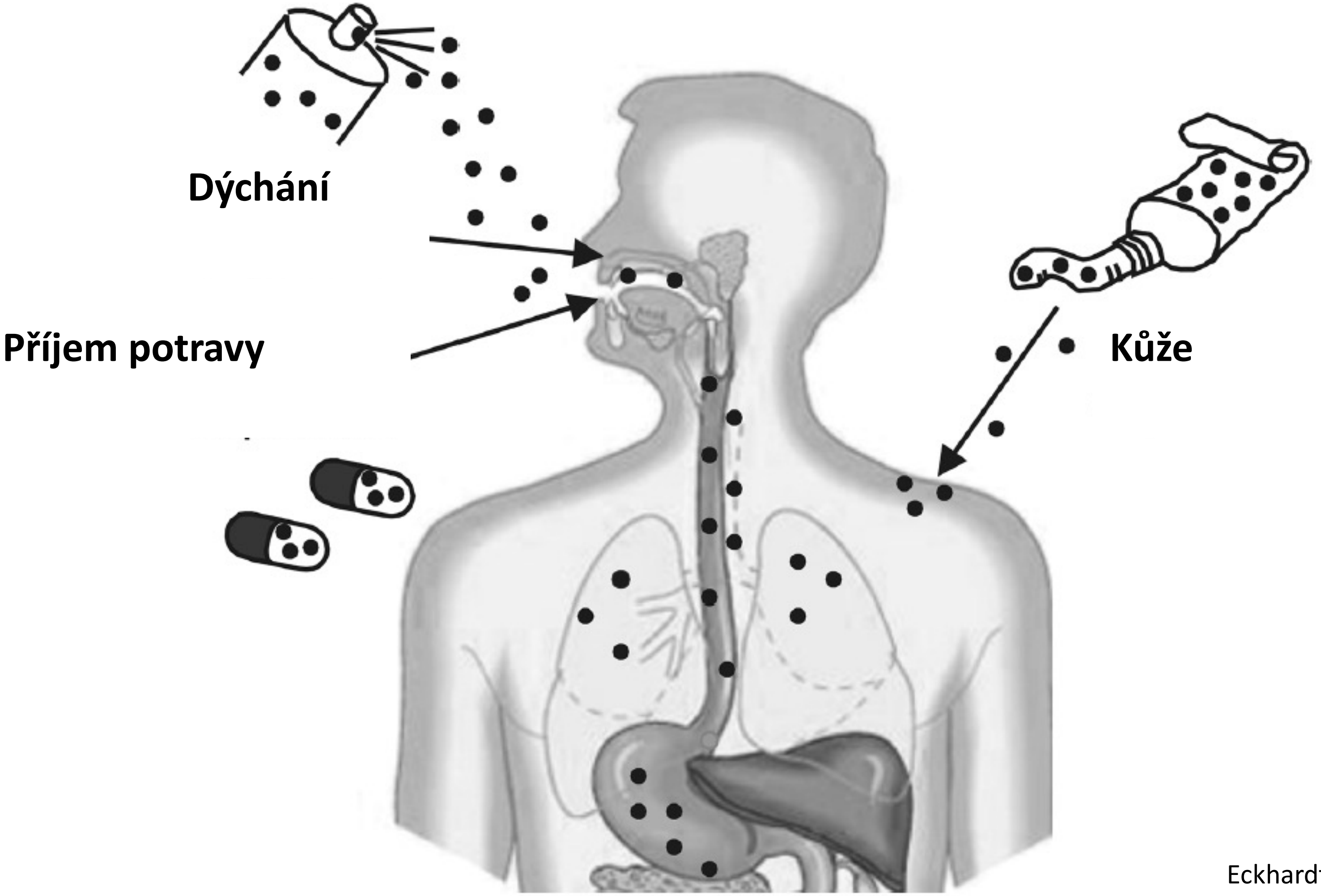



Table 1. UFPs/NPs (< 100 nm), natural and anthropogenic sources.

Natural	Anthropogenic	
	Unintentional	Intentional (NPs)
Gas-to-particle conversions	Internal combustion engines	Controlled size and shape, designed for functionality
Forest fires	Power plants	
Volcanoes (hot lava)	Incinerators	Metals, semiconductors, metal oxides, carbon, polymers
Viruses	Jet engines	
Biogenic magnetite: magnetotactic bacteria protoctists, mollusks, arthropods, fish, birds human brain, meteorite (?)	Metal fumes (smelting, welding, etc.)	Nanospheres, -wires, -needles, -tubes, -shells, -rings, -platelets
	Polymer fumes	
	Other fumes	Untreated, coated (nanotechnology applied to many products: cosmetics, medical, fabrics, electronics, optics, displays, etc.)
Ferritin (12.5 nm)	Heated surfaces	
Microparticles (< 100 nm; activated cells)	Frying, broiling, grilling	
	Electric motors	

Oberdorster et al., Environ. Health Perspect., 2005, 113, 823

Vstup nanočástic do lidského organismu

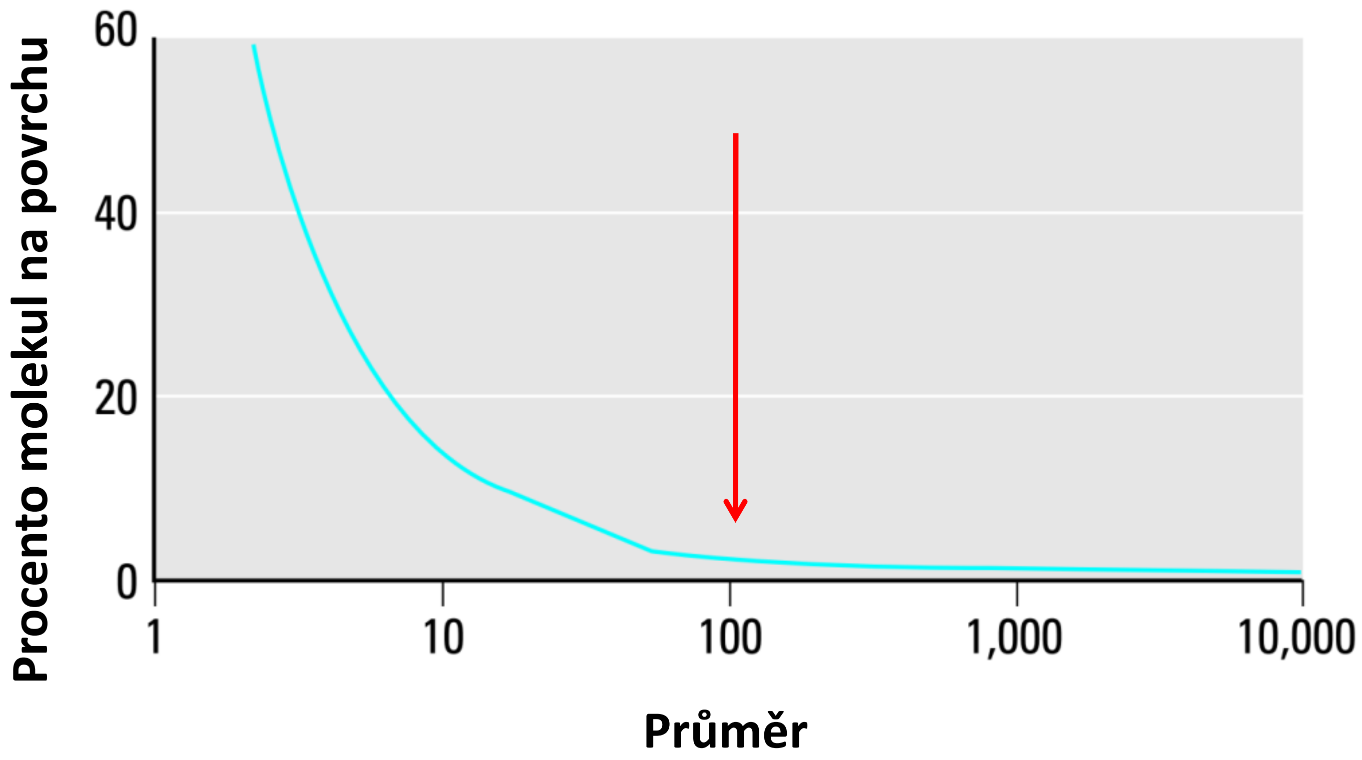


Eckhardt et al., Chem Rev. 2013

Proč jsou nanočástice potenciálně nebezpečné?

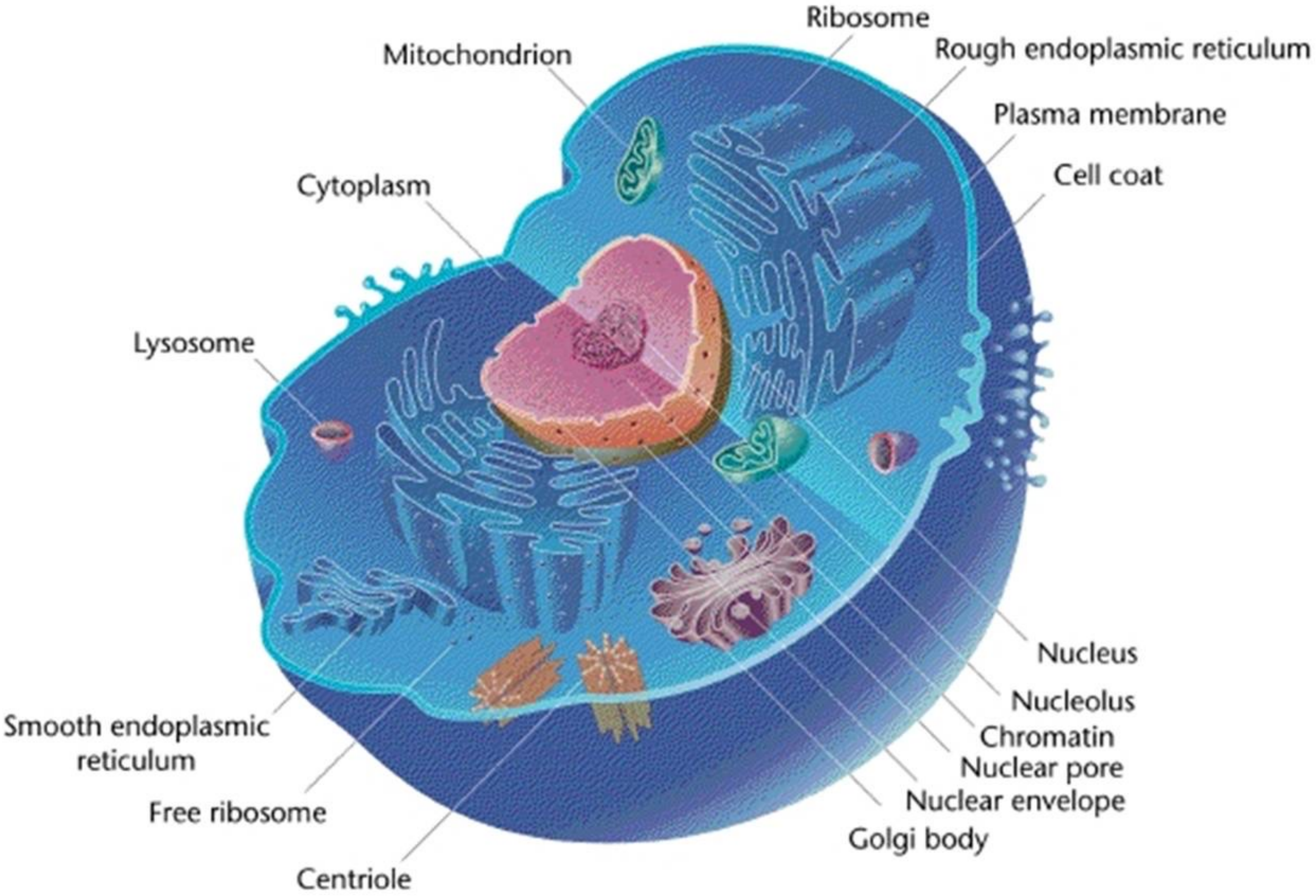
Roli hraje velikost

Exponenciální nárůst velikosti povrchu při velikosti pod 100 nm, současně narůstá reaktivita



Oberdorster et al., Environ. Health Perspect., 2005, 113, 823

Struktura lidské buňky



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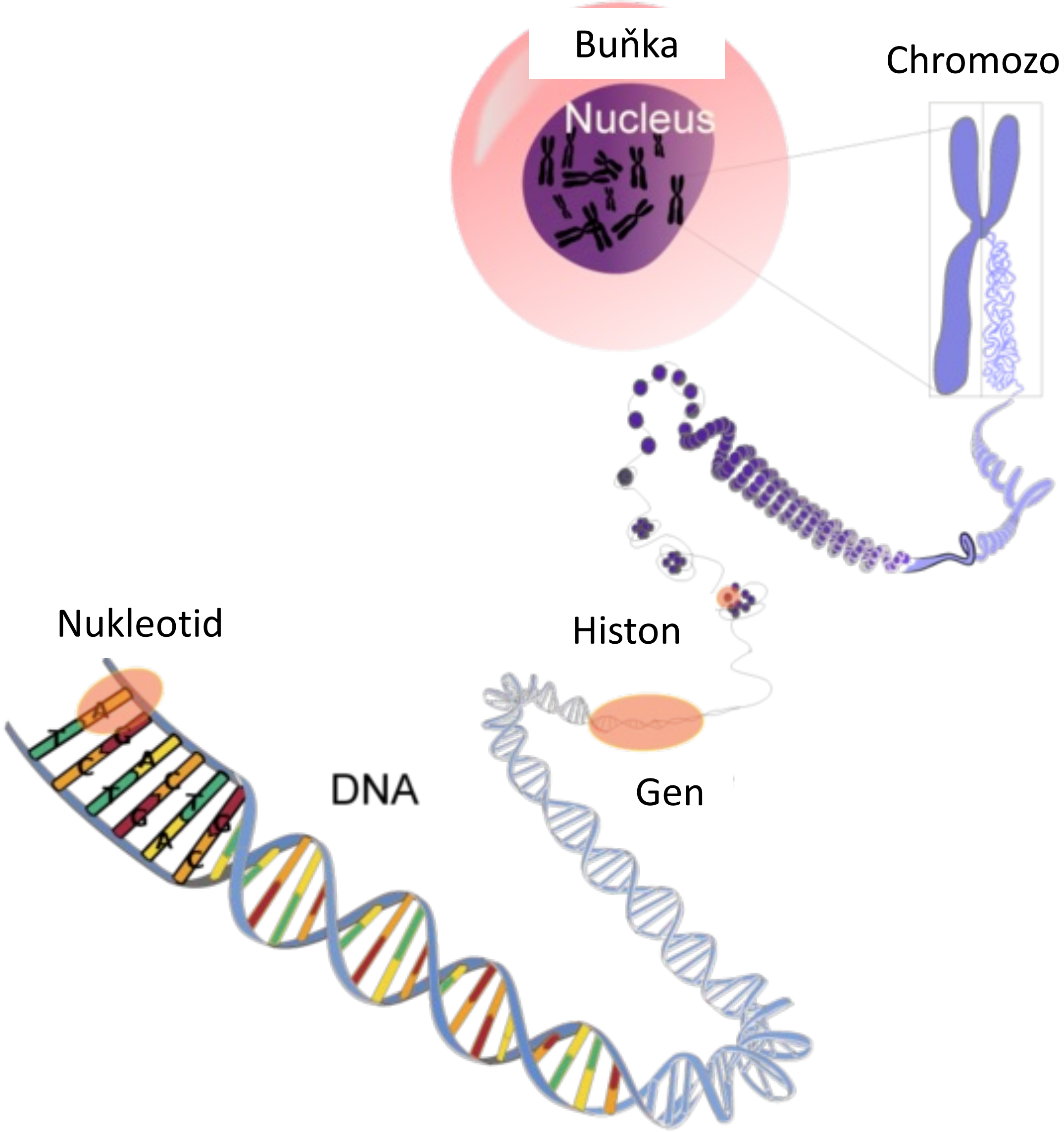
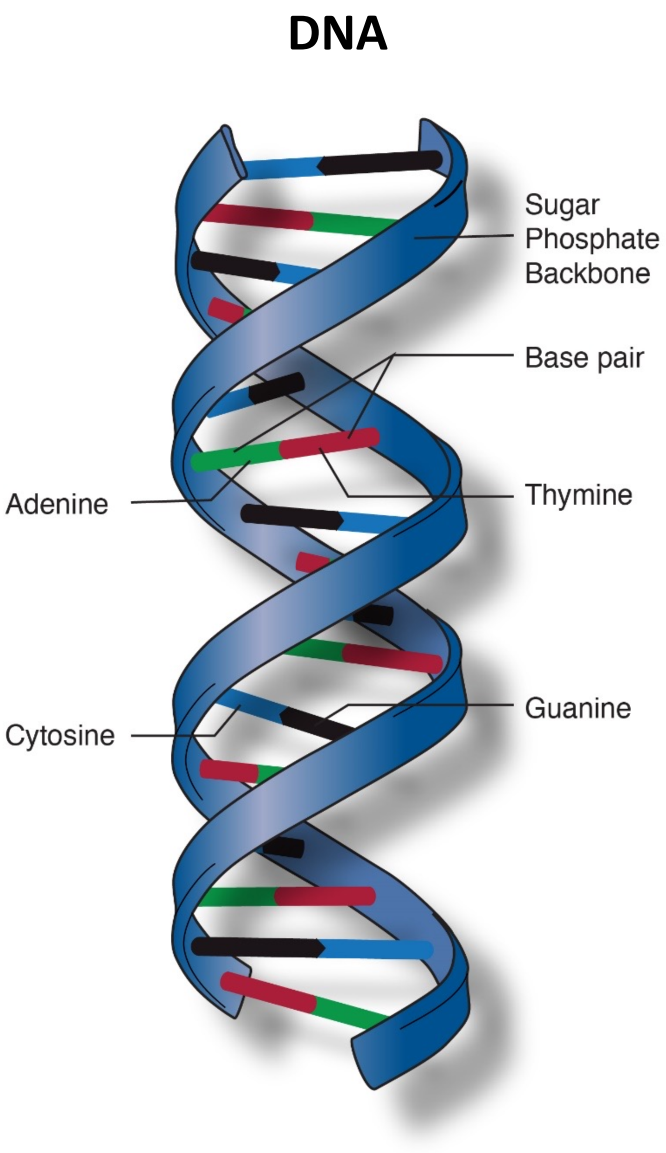
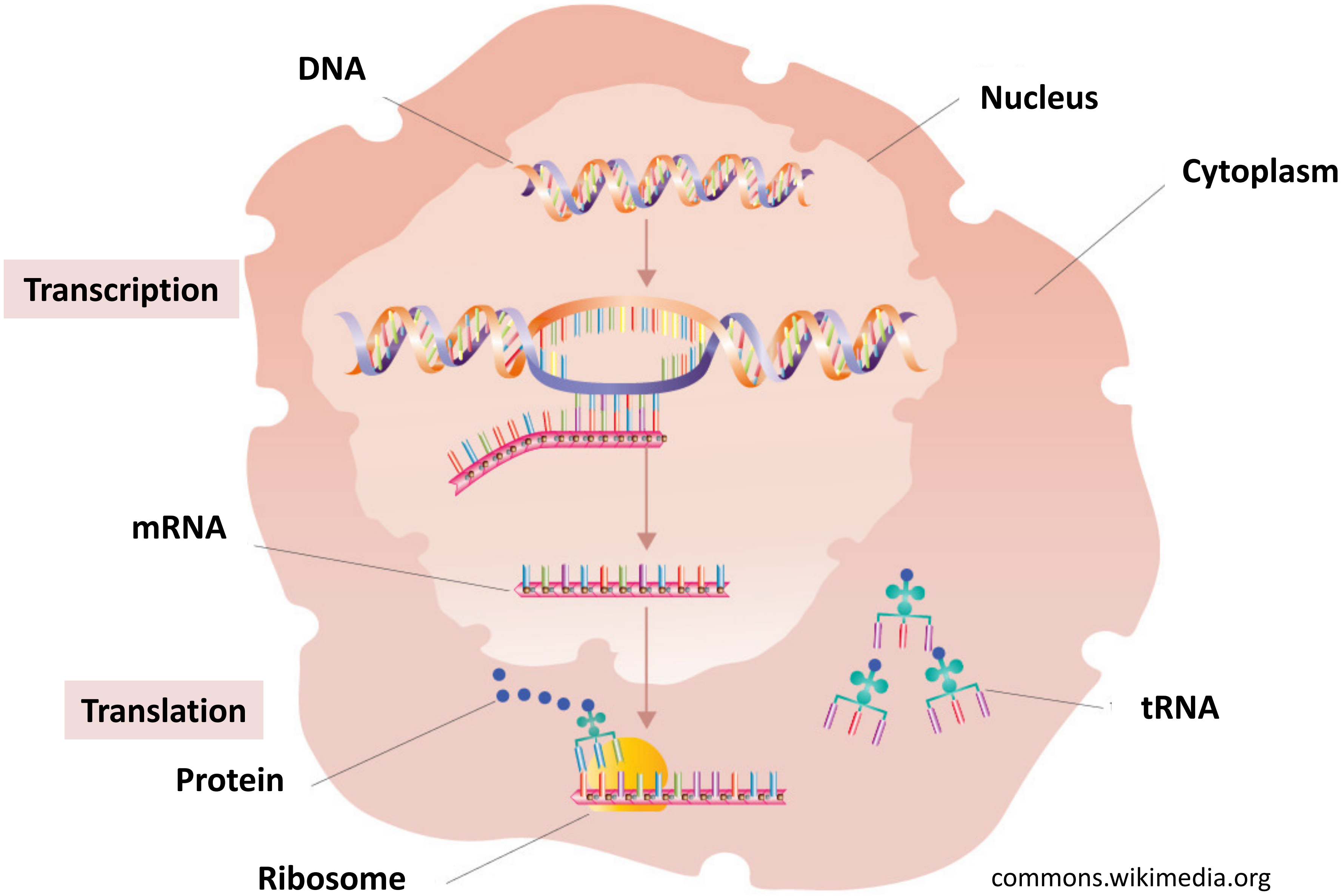
Buněčné jádro a DNA





Principy genové exprese



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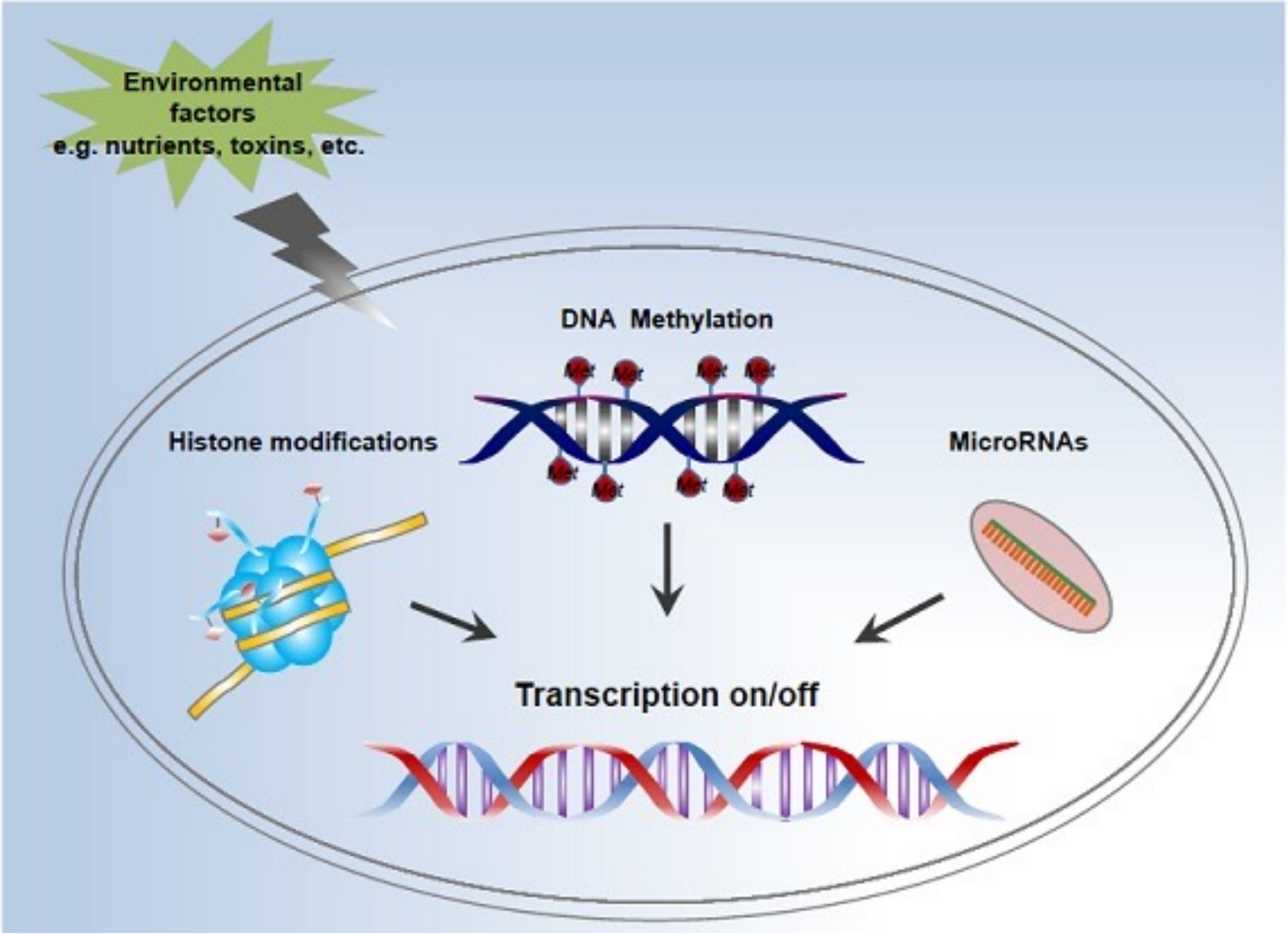
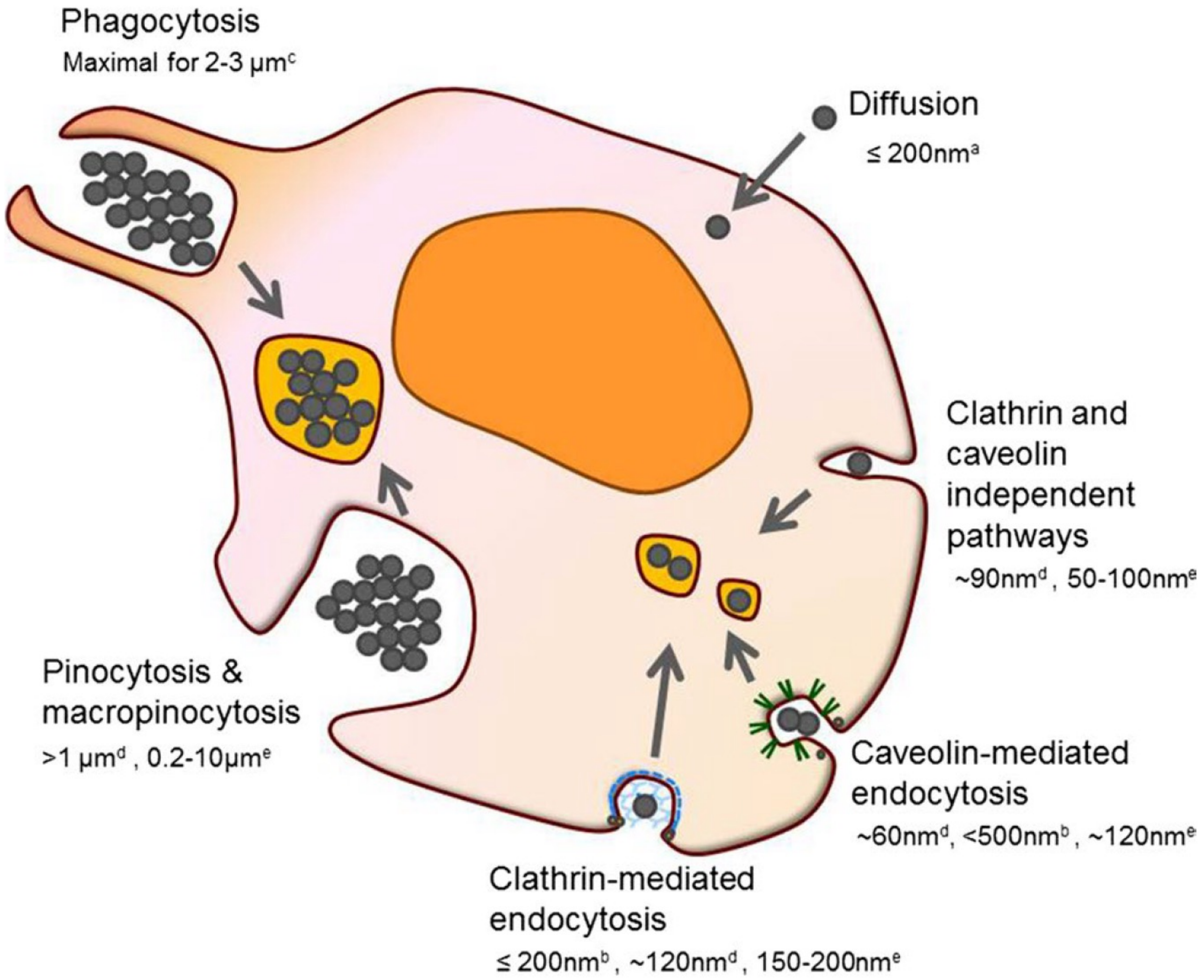
Epigenetická regulace genové exprese



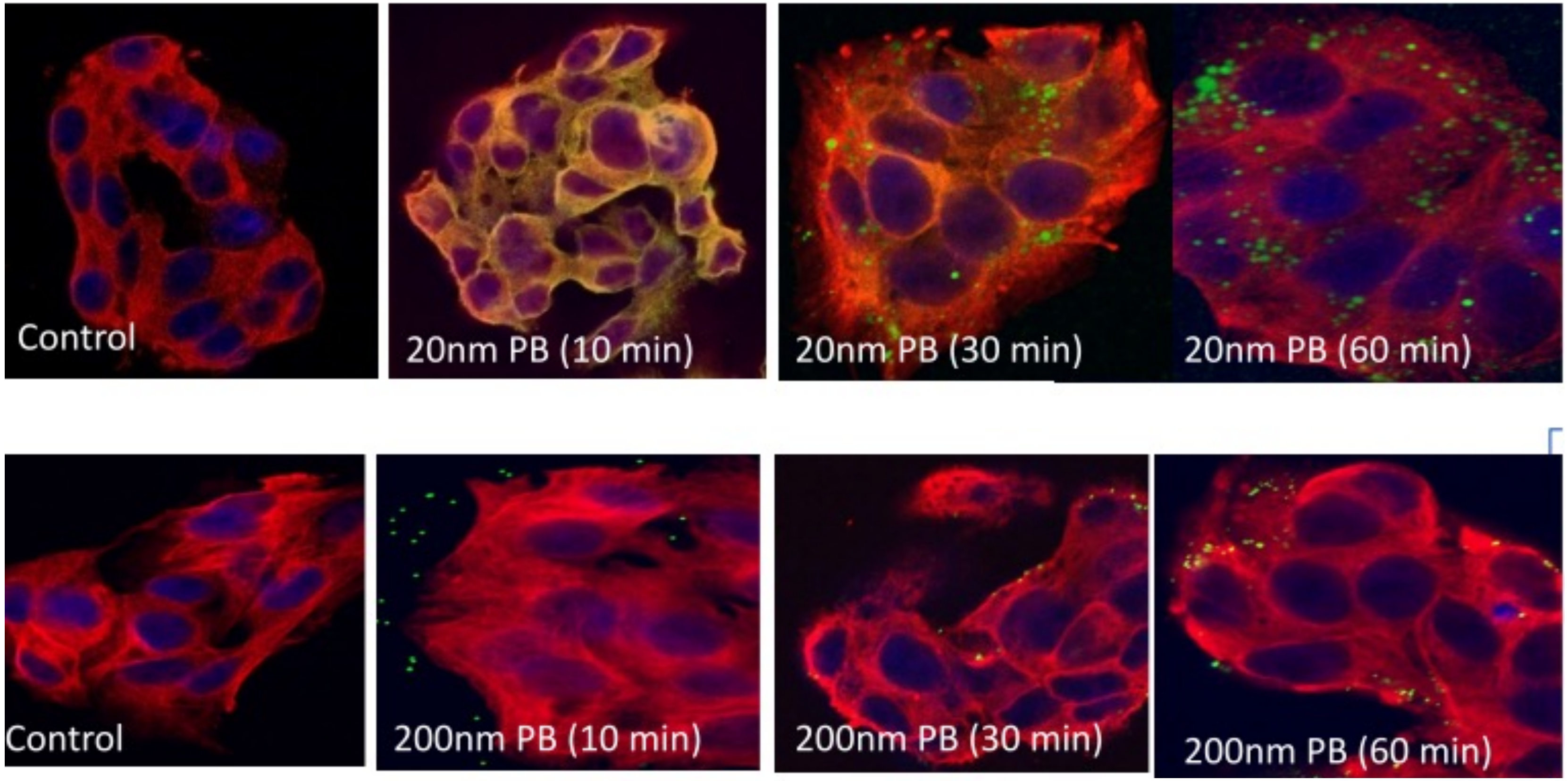
Libertas Academica

Vstup nanočástic do buněk



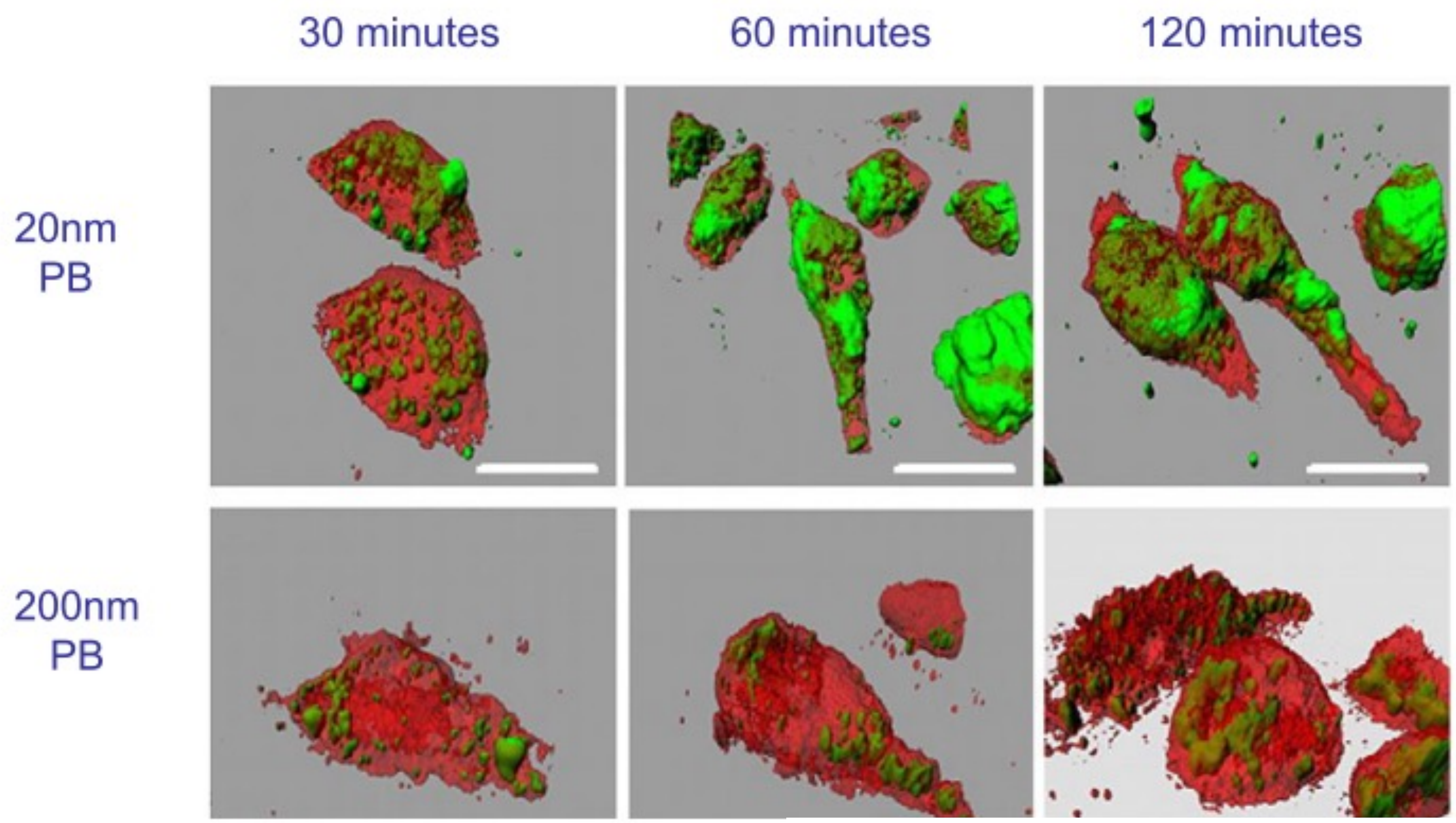
Bruinink et al., Arch. Toxicol., 2015, 89, 659

Nanočástice v hepatocytech



Johnston et al. 2010 TAAP 242(1); 66-78.

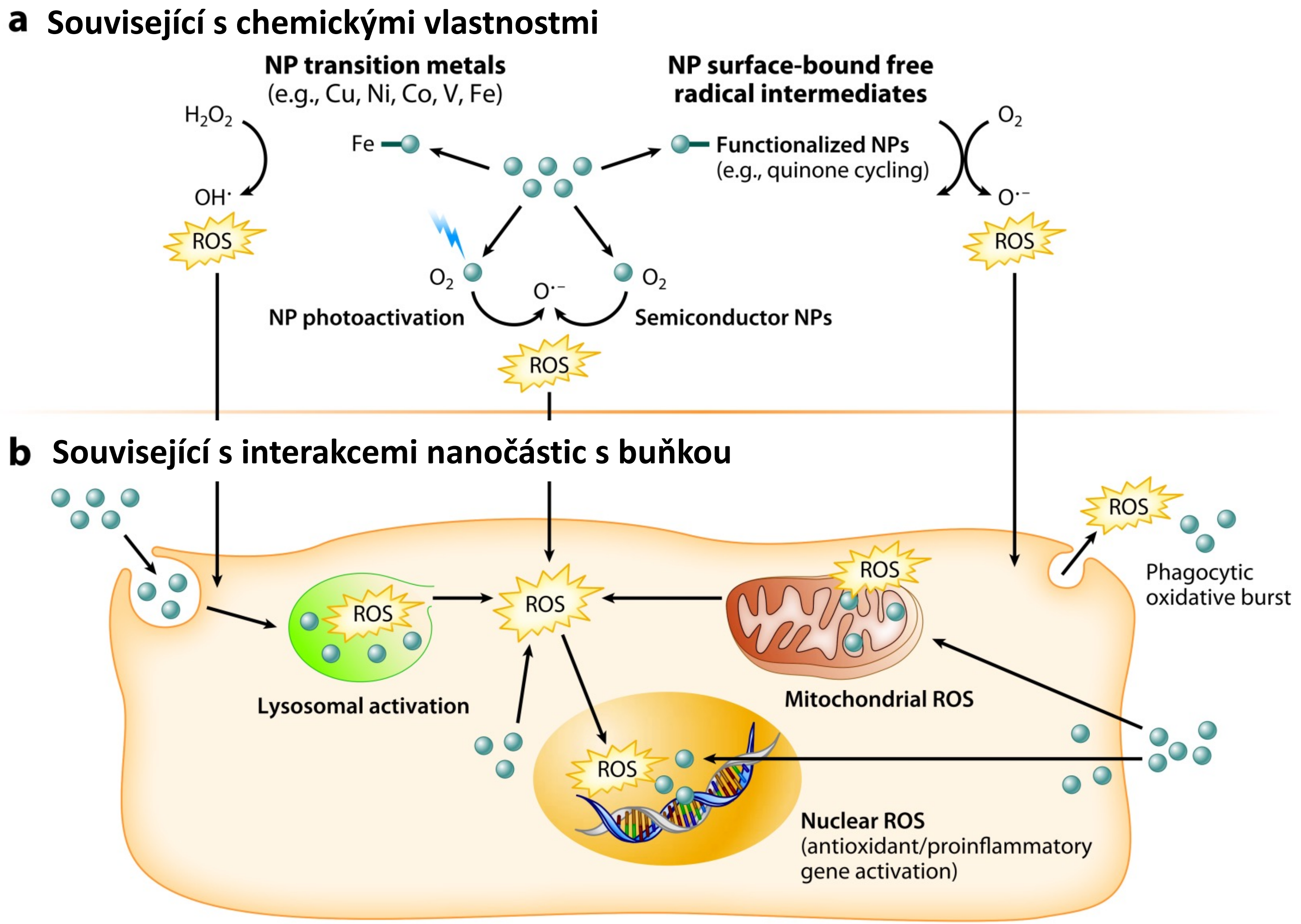
Nanočástice a makrofágy

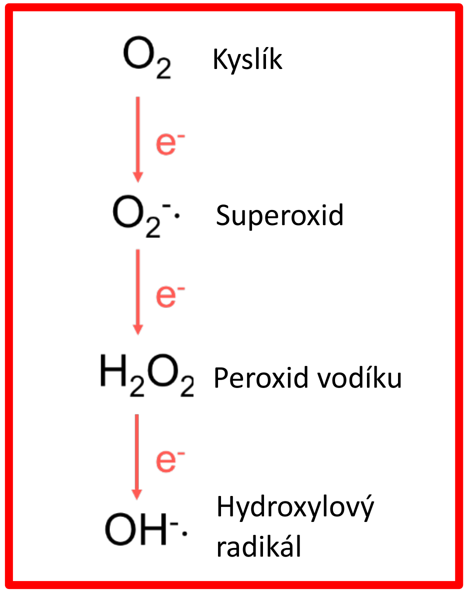


Clift *et al.* 2008 TAAP 232; 418-427.

Co se s nanočásticemi děje v buňkách?

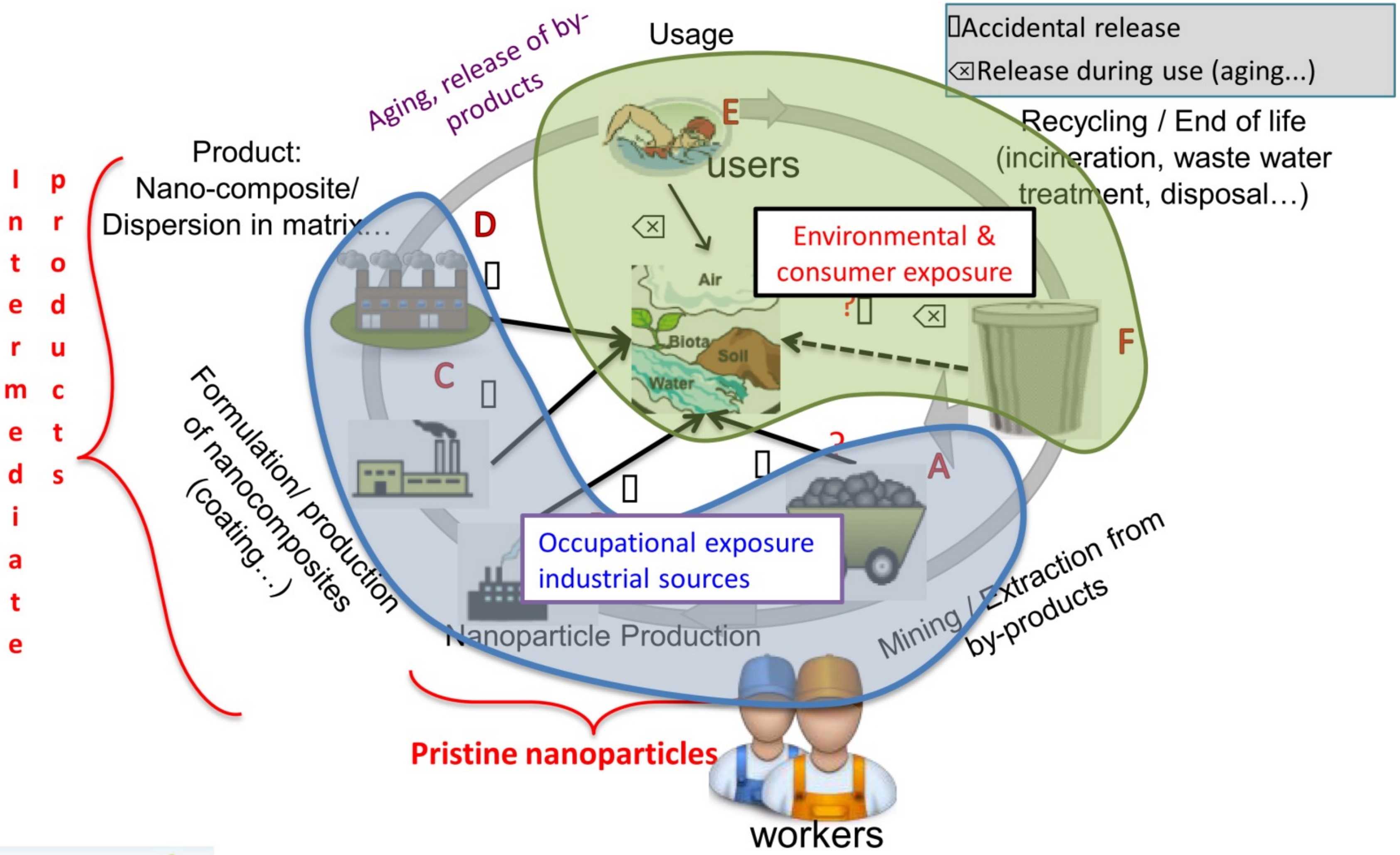
Oxidační vlastnosti nanočástic



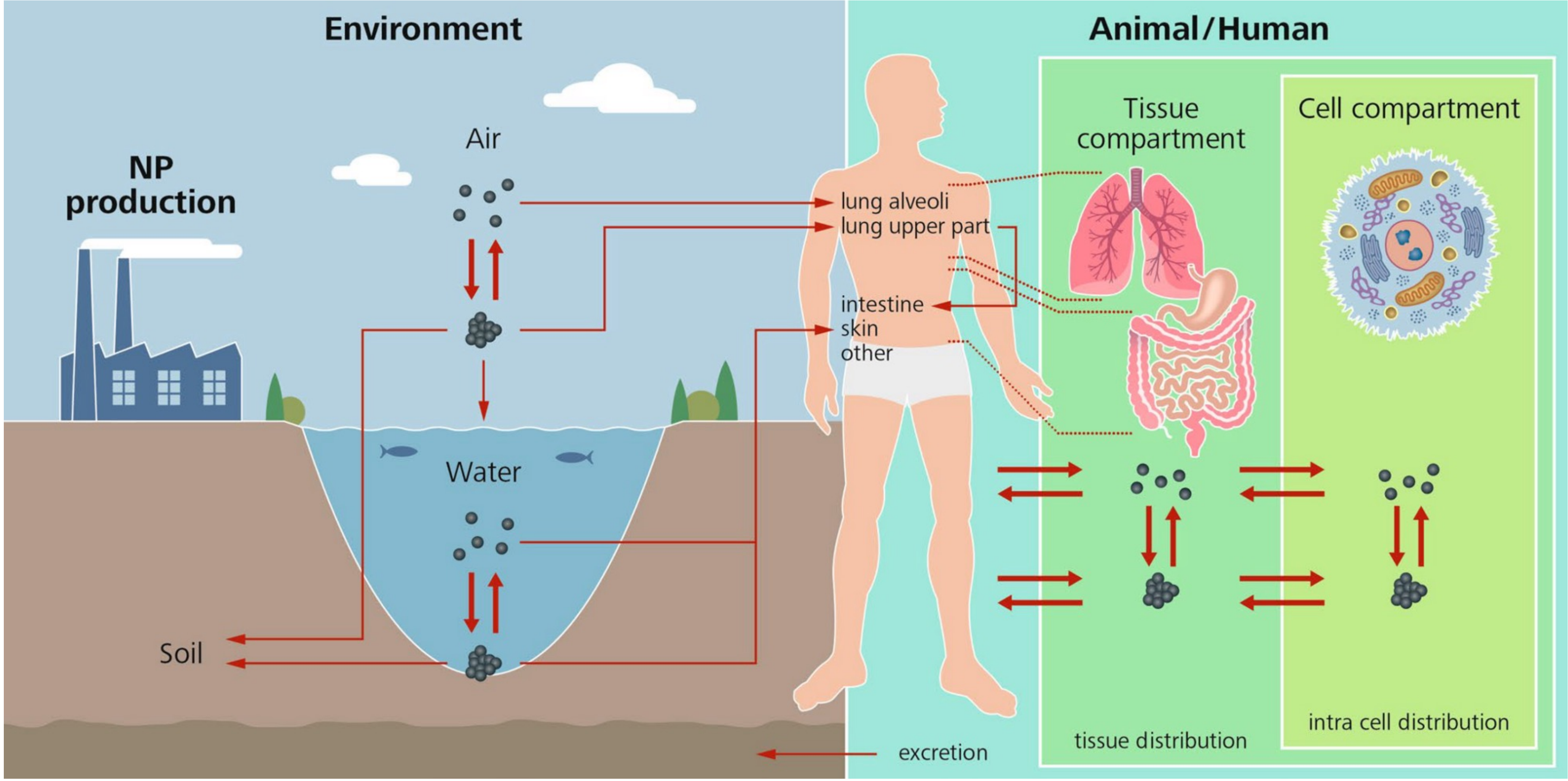


Madl et al., Annu. Rev. Physiol., 2014, 76, 447

Životní cyklus nanomateriálů

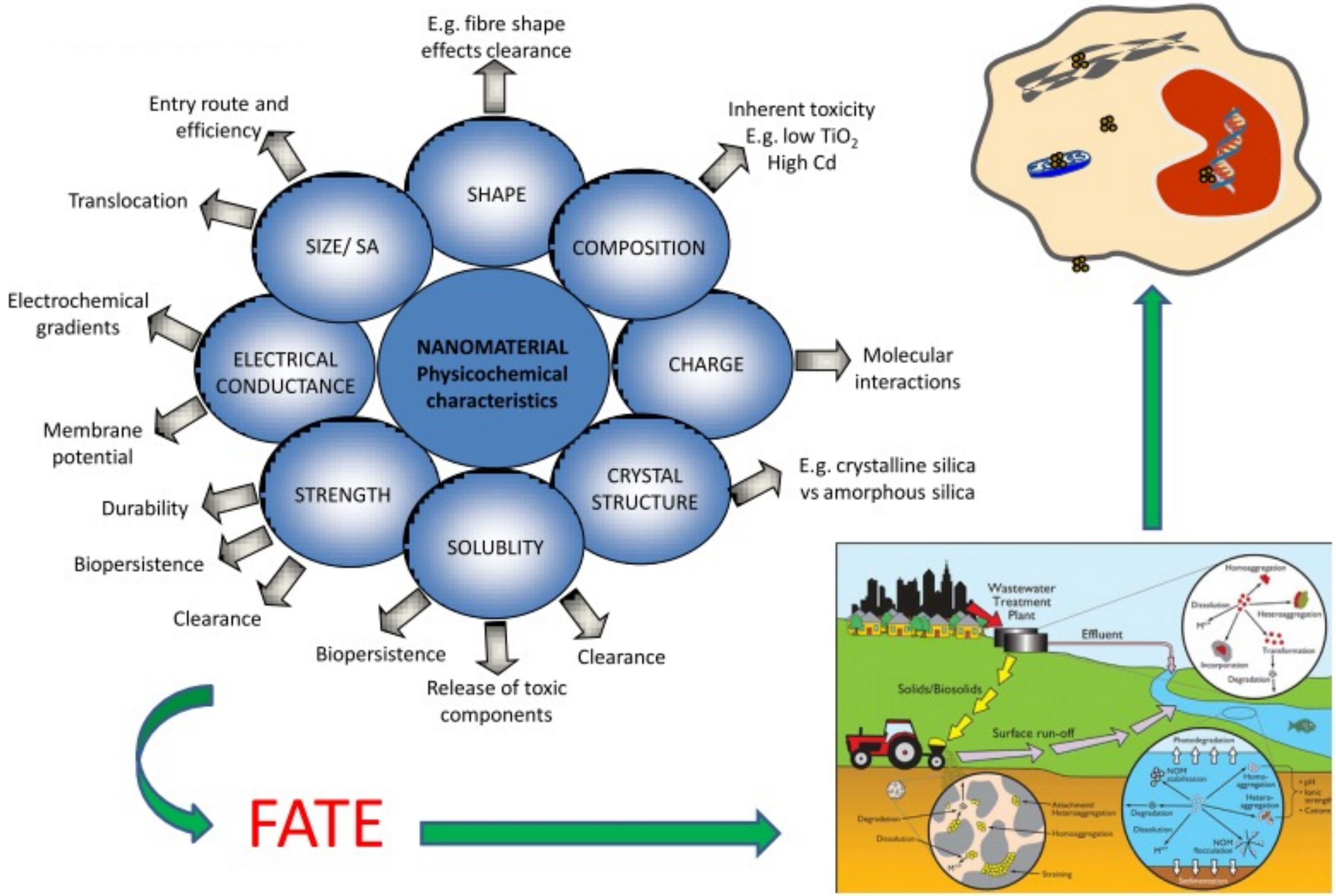


Nanomateriály v životním prostředí – vliv na člověka

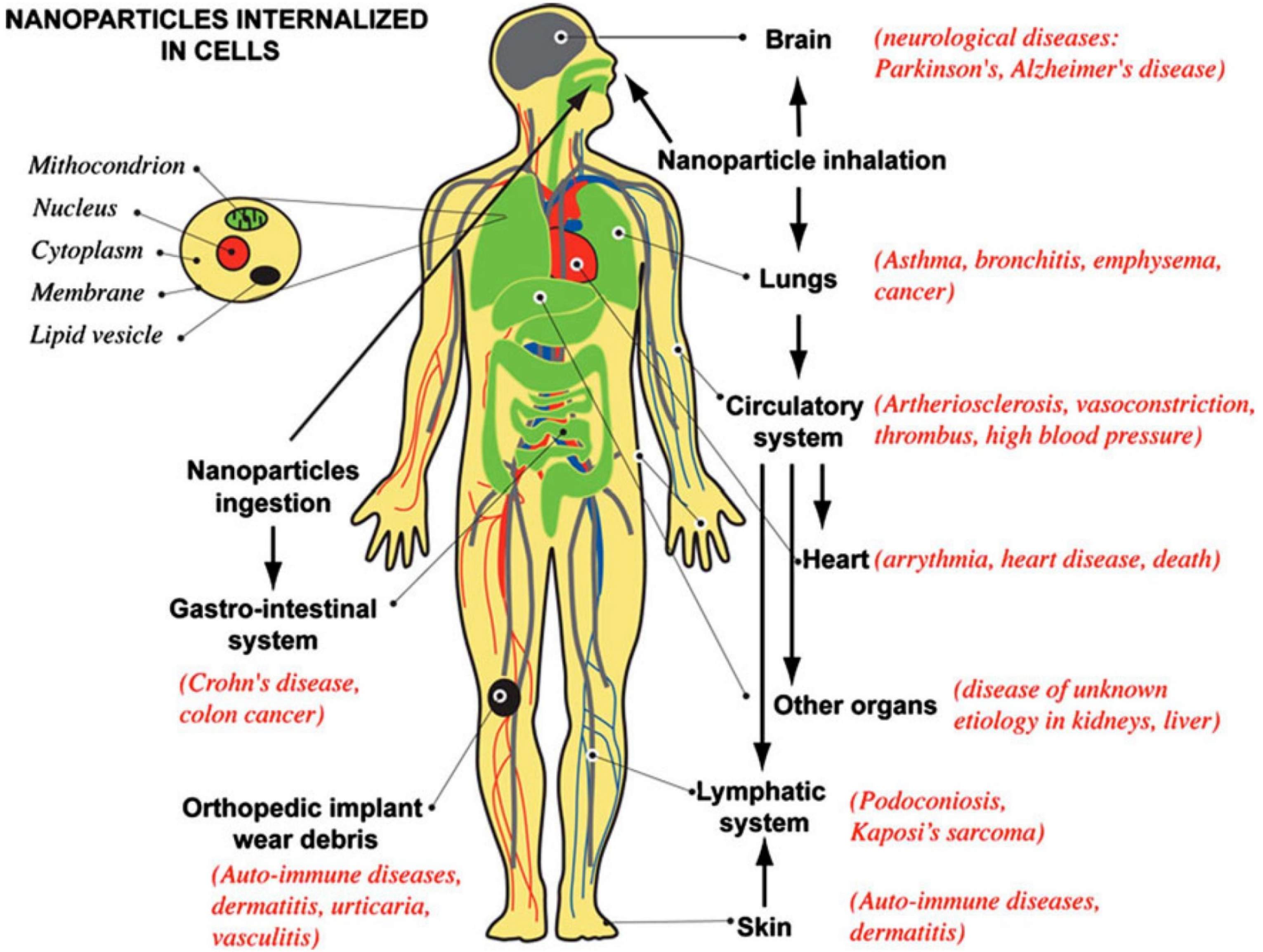


Bruinink et al., Arch. Toxicol., 2015, 89, 659

Fyzikálně-chemické vlastnosti nanočástic a biologické účinky

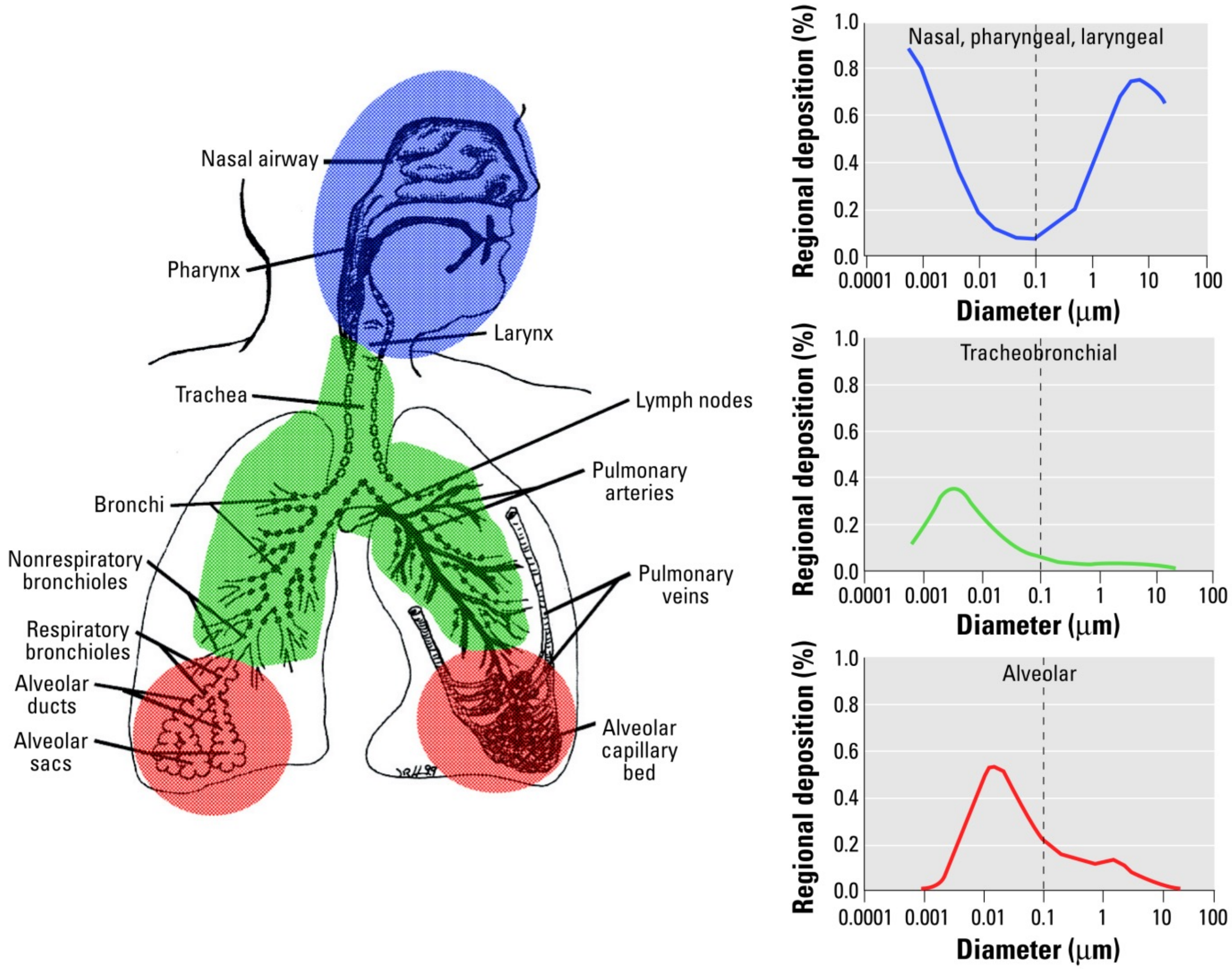


Potenciální zdravotní dopady působení nanočástic



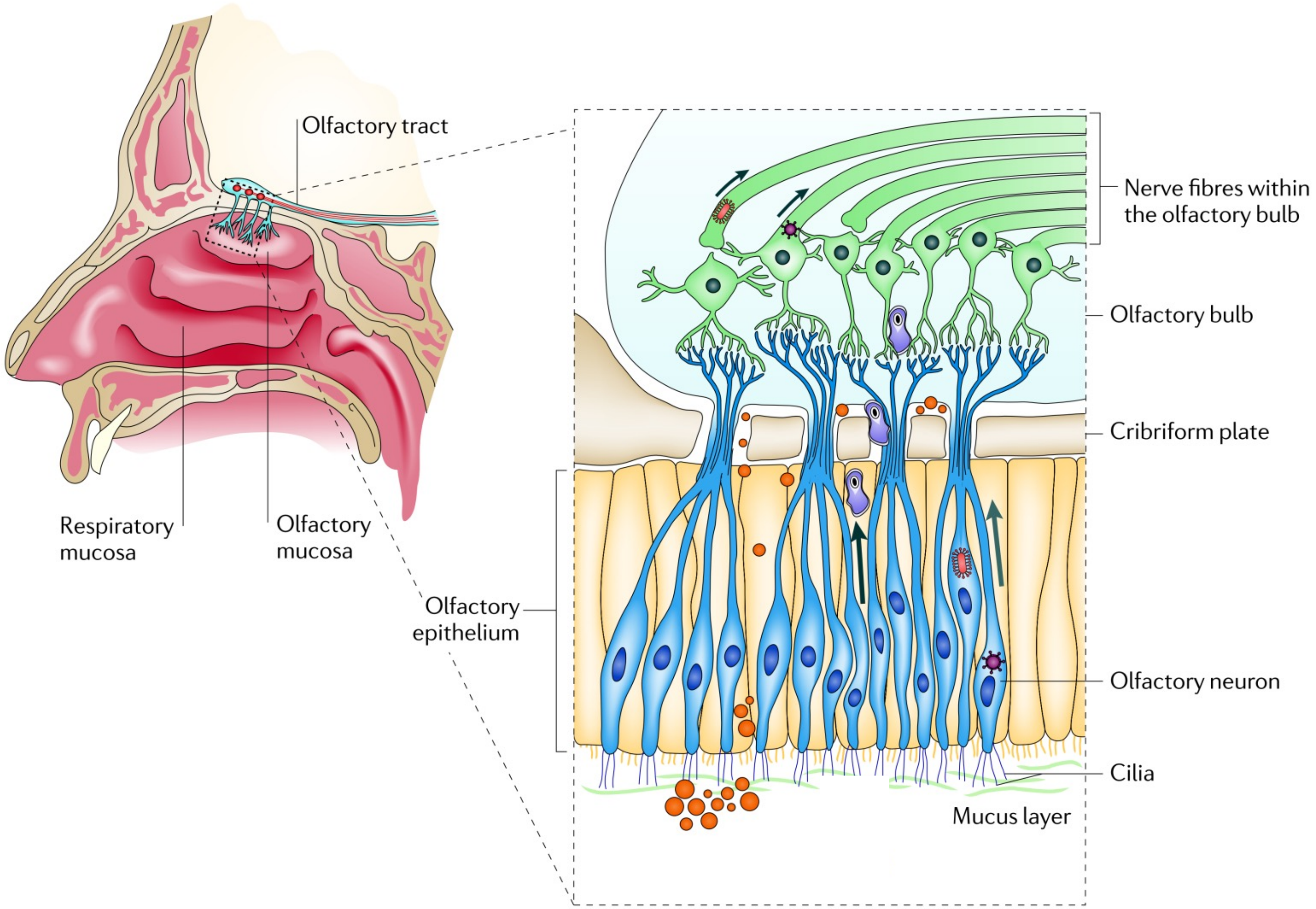
Buzea et al., Biointerphases, 2007, 2, 4

Depozice částic v dýchacím systému



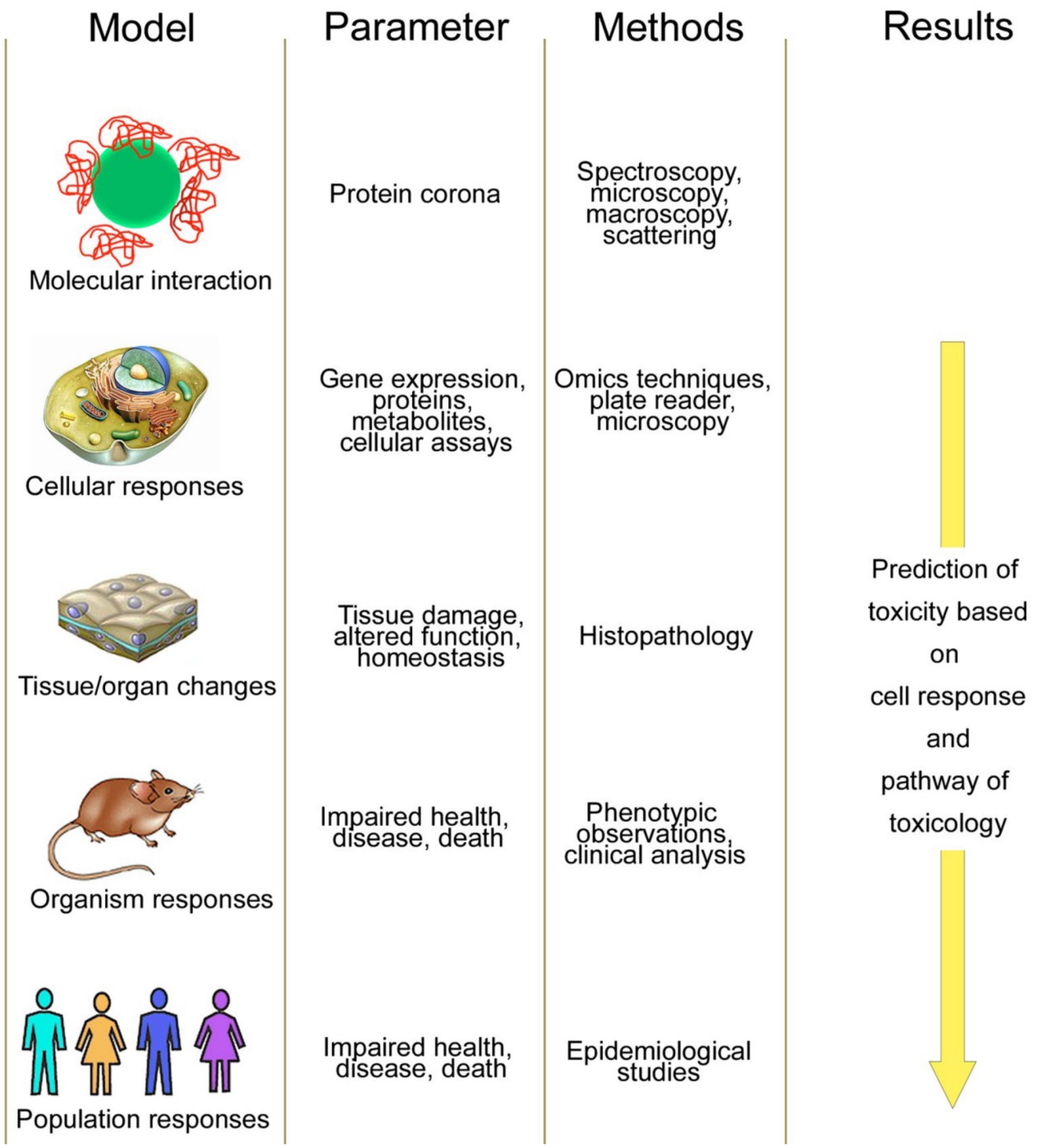
Oberdorster et al., Environ. Health Perspect., 2005, 113, 823

Vstup nanočástic do mozku



Kristensson, Nat Rev Neurosci, 2011, 12, 345; adapted

Metodické přístupy v (nano)toxikologii



Fröhlich *J Nanobiotechnol (2017) 15:84*

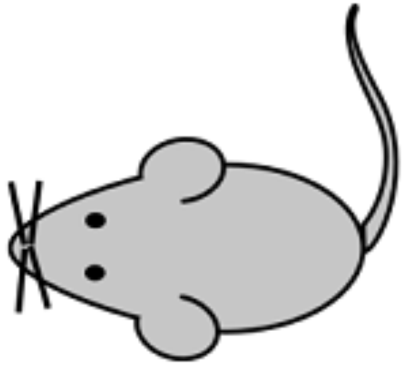
Testování biologických účinků nanočástic

V úvahu je potřeba vzít:

- Volba vhodného modelu
- Testovaná dávka
- Způsob aplikace
- Doba působení
- Měřené parametry

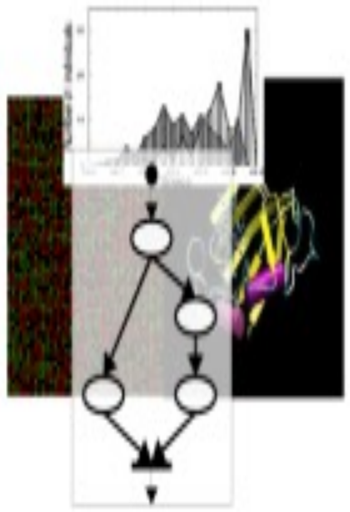
IN VITRO

Buněčné kultury



IN VIVO

Modelové organismy/lidské populace



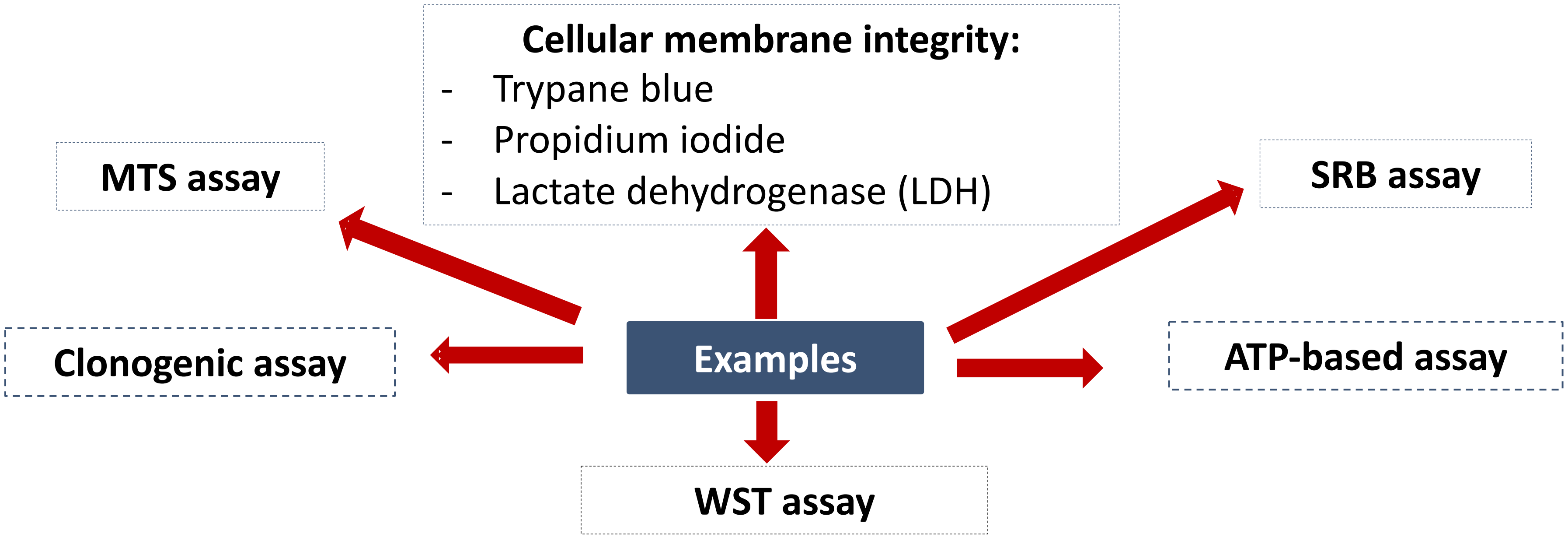
IN SILICO

Počítačové modelování

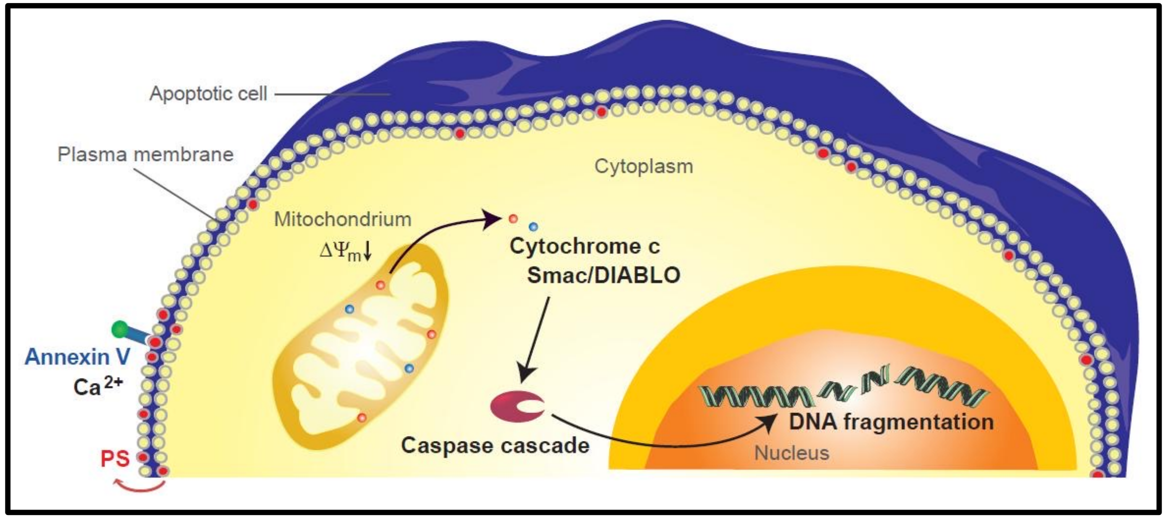
In vitro testing: Cytotoxicity

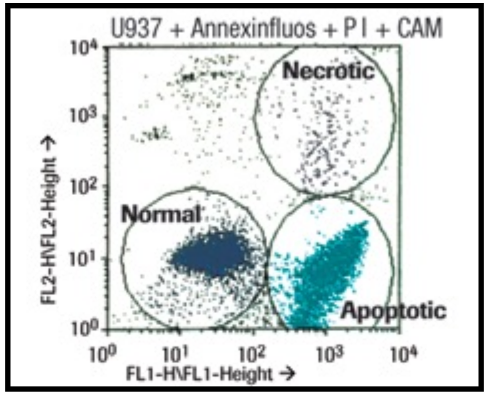
Detection of cells with reduced viability

Metabolic activity of the cells, protein content, proliferation, membrane integrity



In vitro testing: Apoptosis



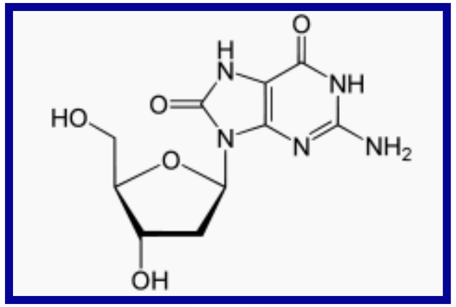


In early apoptosis stages phosphatidylserine (PS) translocates to outer membrane

Annexin V is a protein with high affinity to PS. Annexin V conjugated with fluorescent compounds is used to detect apoptotic cells

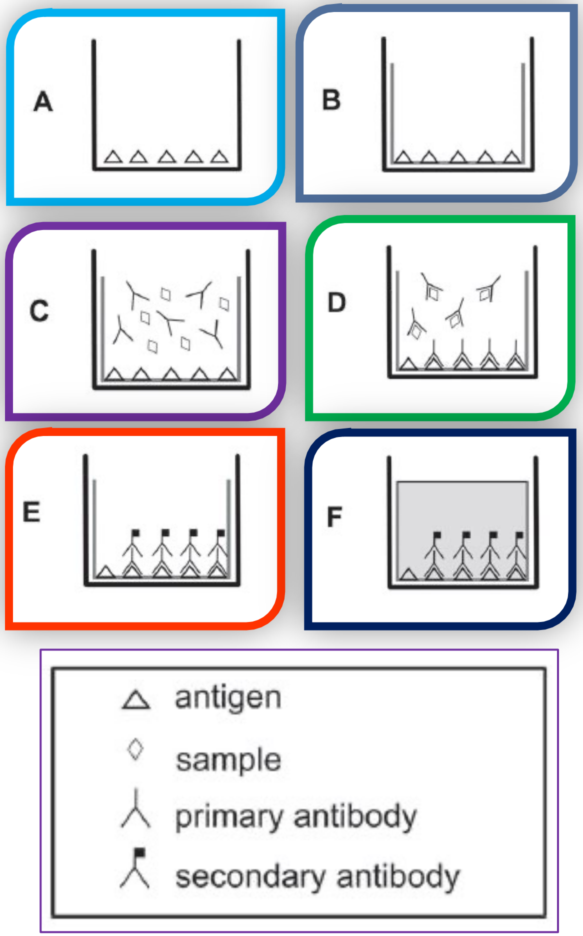
In vitro testing: Oxidative DNA damage

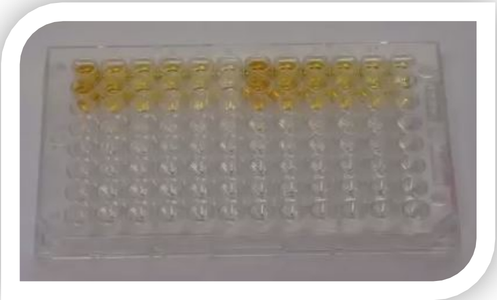
8-oxodeoxyguanosine – the most common oxidized DNA nucleoside; its presence may induce mutations



Detection: ELISA; chromatography

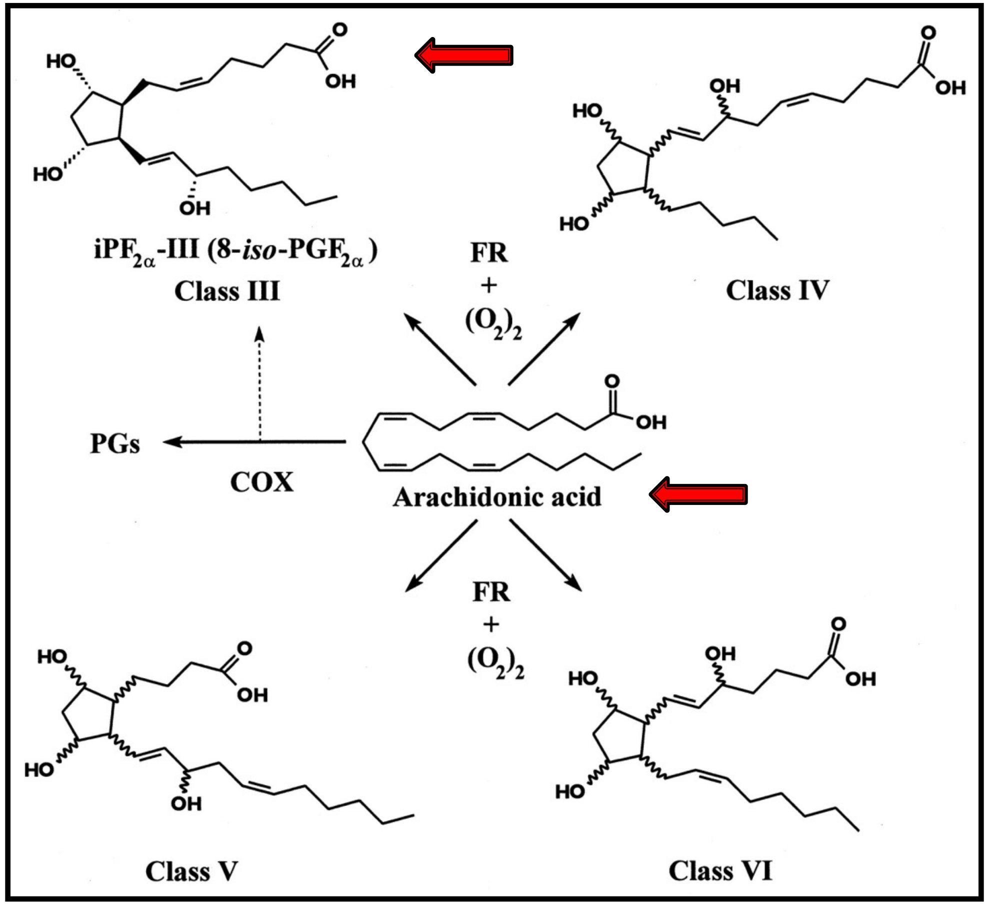
2. A-coating with 8-oxoG-BSA
3. B-blocking with FCS
4. C-incubation with samples and primary anti-8-oxodG antibody
5. D-competition
6. E- incubation with secondary antibody conjugated with enzymes
7. F-incubation with chromogenic substrate and color development



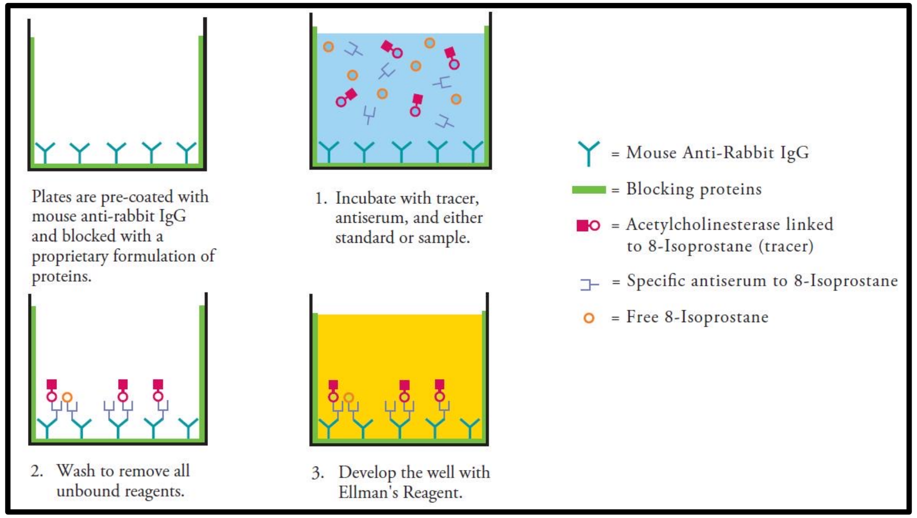


In vitro testing: Lipid peroxidation

15-F_{2t}-isoprostane – formed by ROS attack on arachidonic acid in cellular membranes



Detection:
ELISA
Chromatography



In vitro testing: Protein oxidation

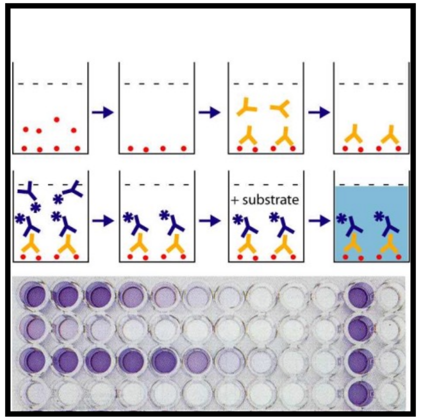
Carbonyl groups in proteins

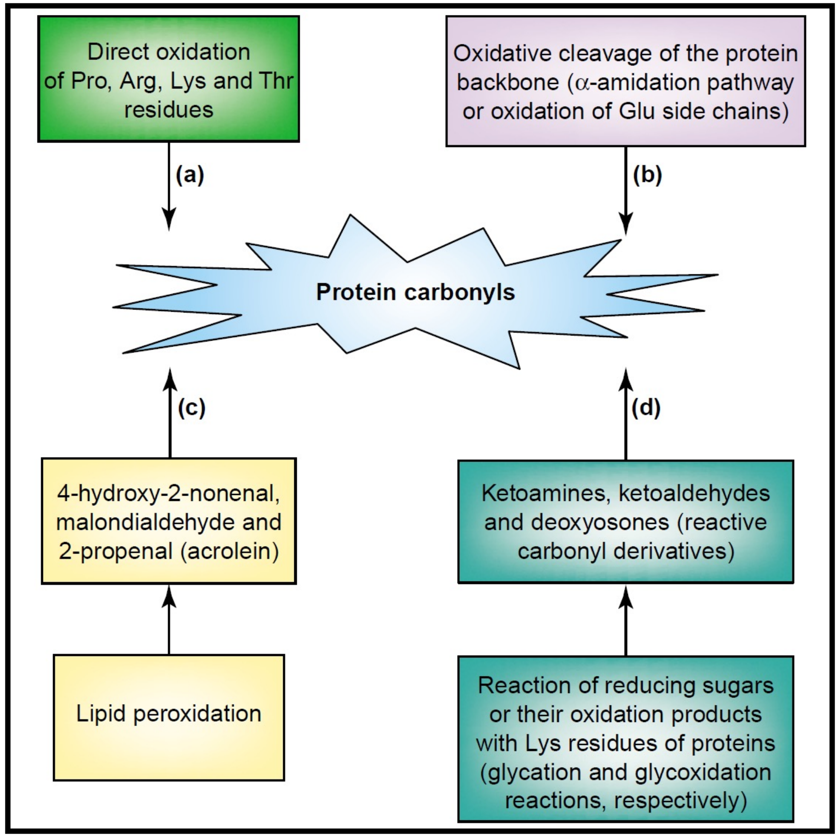
Formed by the ROS attack on side chains of amino acids or protein backbone

Negatively affect protein functions

Associated with a number of diseases

Detection:
ELISA





Dalle-Donne et al., Trends Mol Med 9 (2003) 169-176

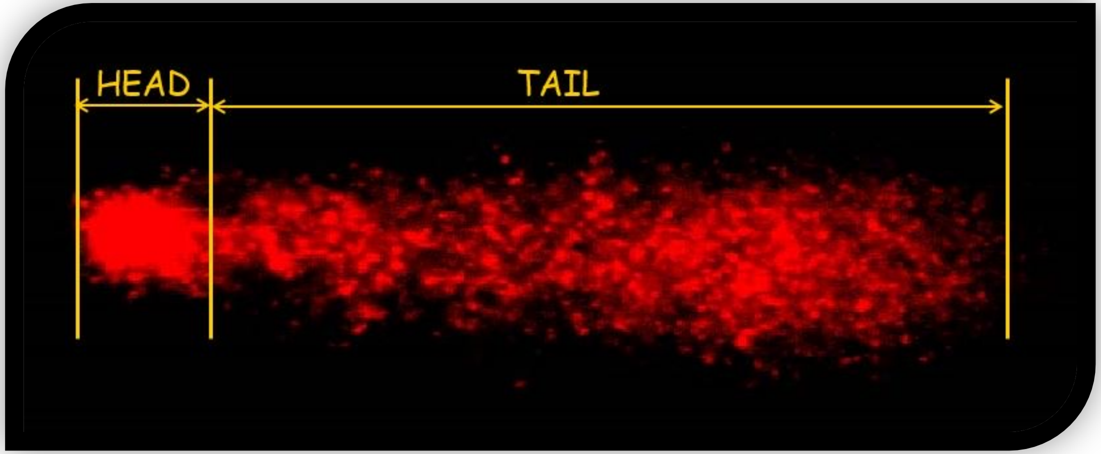
In vitro testing: Comet assay

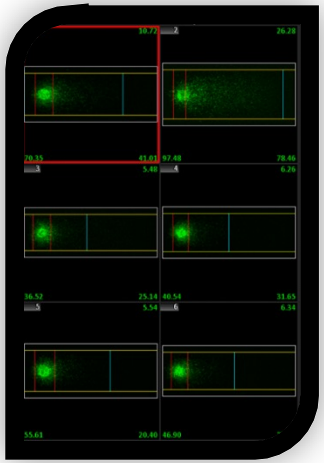
Commonly used method for genotoxicity testing

DNA damage is expressed as a percentage of DNA in the comet tail relative to the total DNA content

Comet assay variants: neutral, alkaline

Oxidative DNA damage detection: DNA breaks are formed at the sites of activity of endonuclease (ENDO III) and glykosylase (FPG)





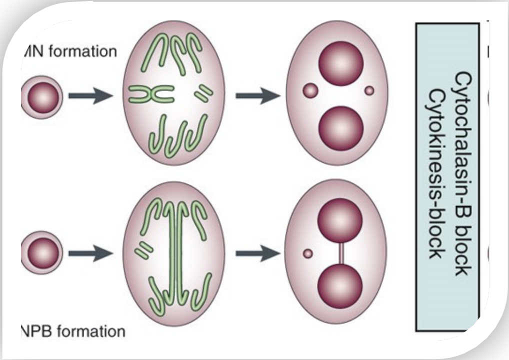
In vitro testing: Micronuclei analysis

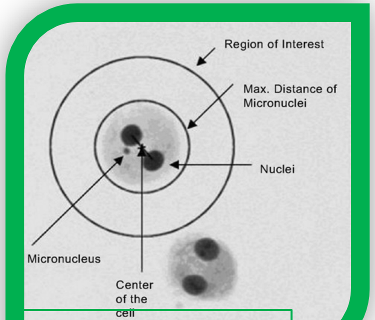
Detection of chromosome breaks or losses

Methods of analysis:

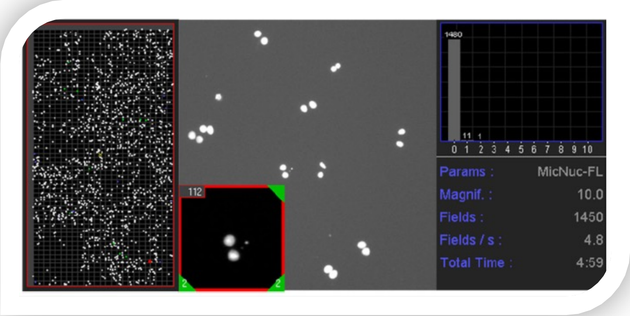
manual – cheap, time-consuming

automated – analysis of a large number of cells; higher quality of data, faster





Varga et al., 2004



OMICS approach in toxicology: toxicogenomics

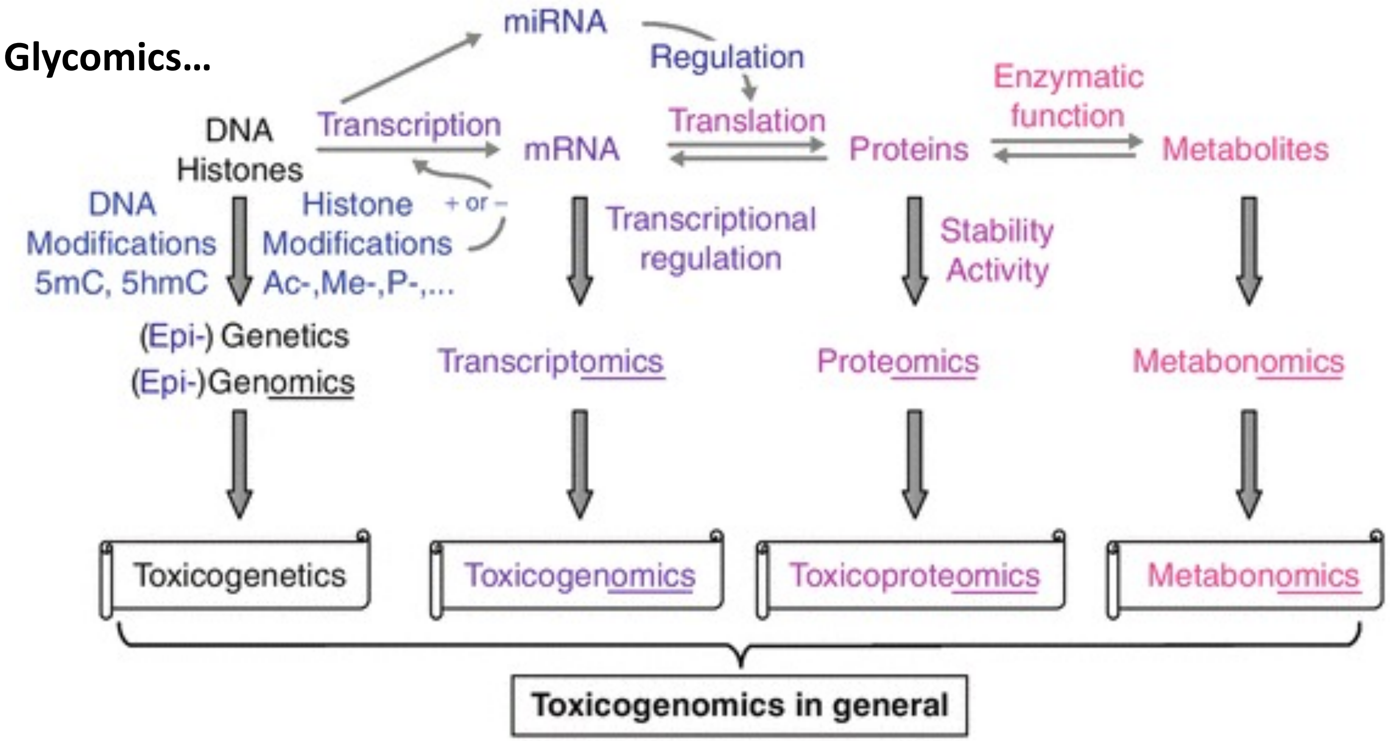
Genomics – DNA sequencing, genetic profiling, genetic mapping, structural and functional analysis of genome

Transcriptomics – RNA sequencing, expression profiling, transcritpional regulation

Proteomics – protein identification, quantification, post-translational modification

Metabolomics – study metabolite profiles, metabolic intermediates, hormones and other signaling molecules

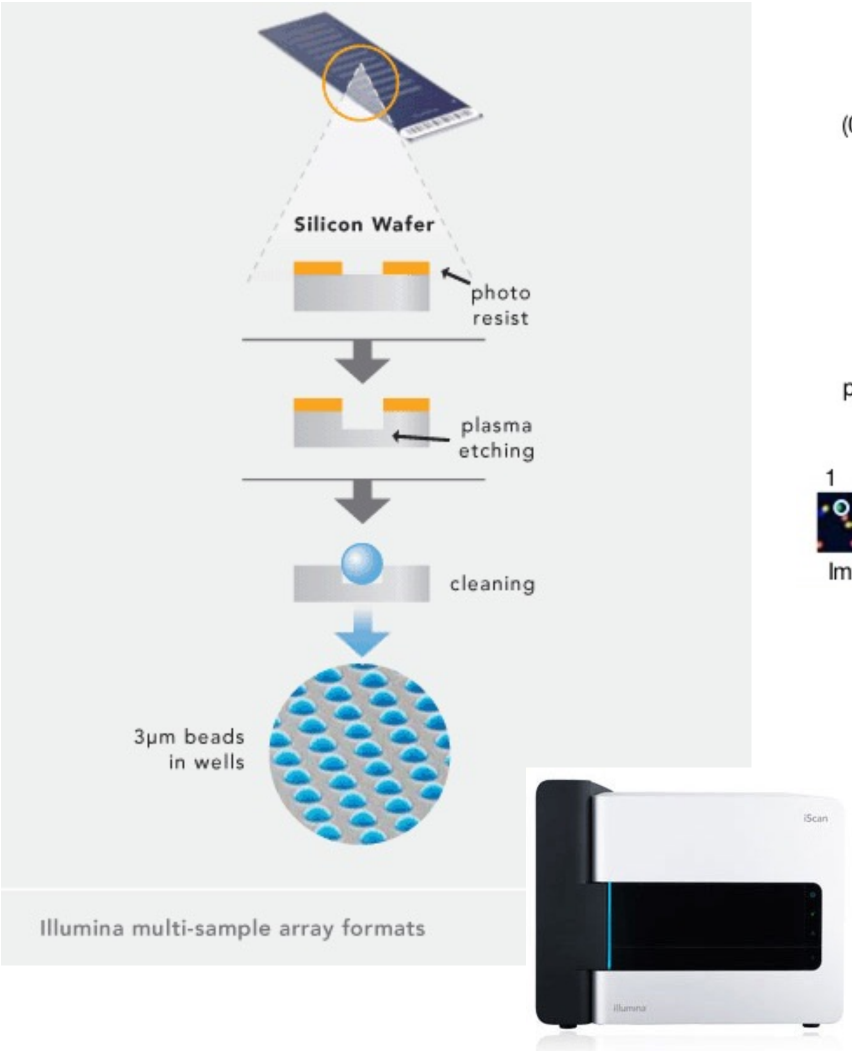
Epigenomics, Lipidomics, Glycomics…



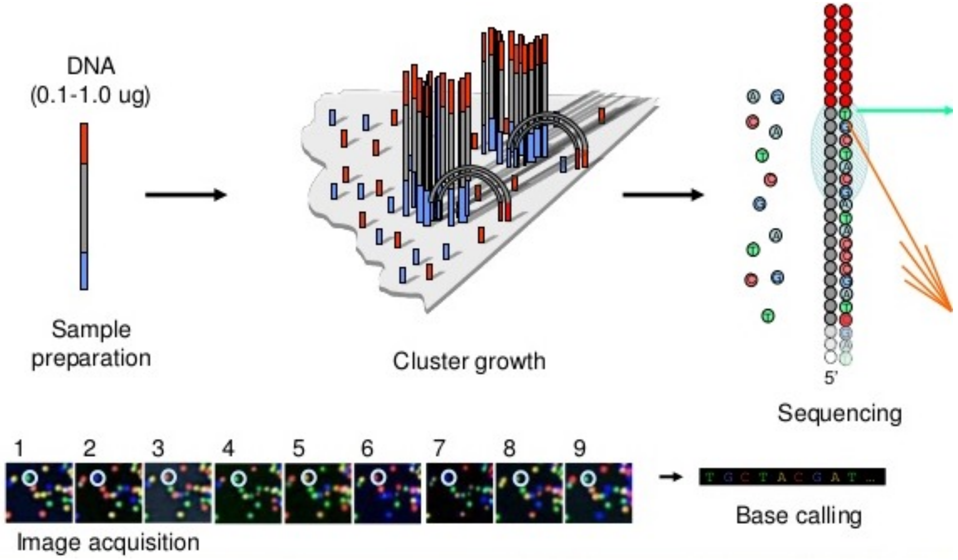
Ellinger-Ziegelbauer *et al.*, 2014 (*Regulatory Toxicology*)

Advanced genomics methods

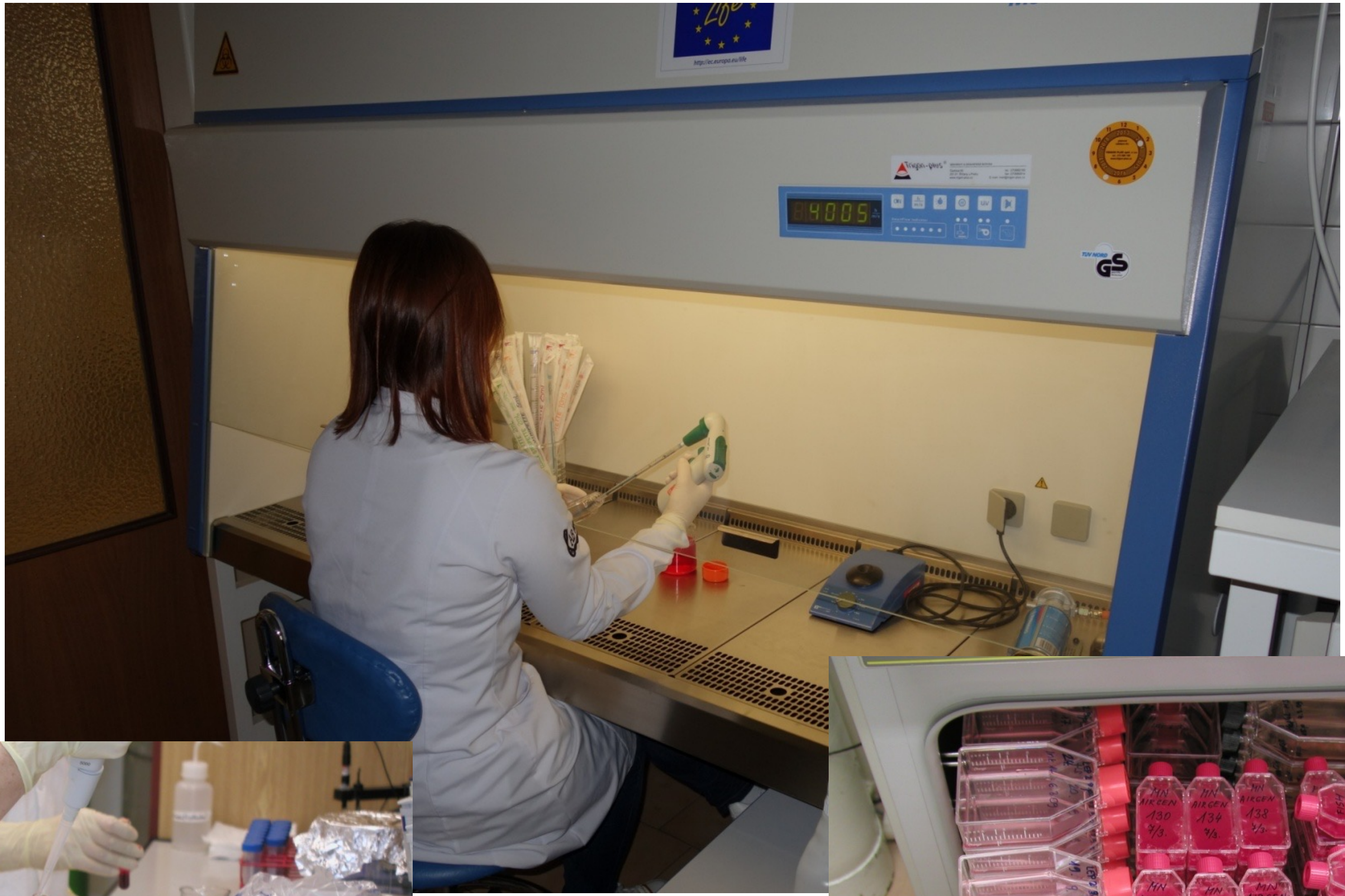
Microarrays

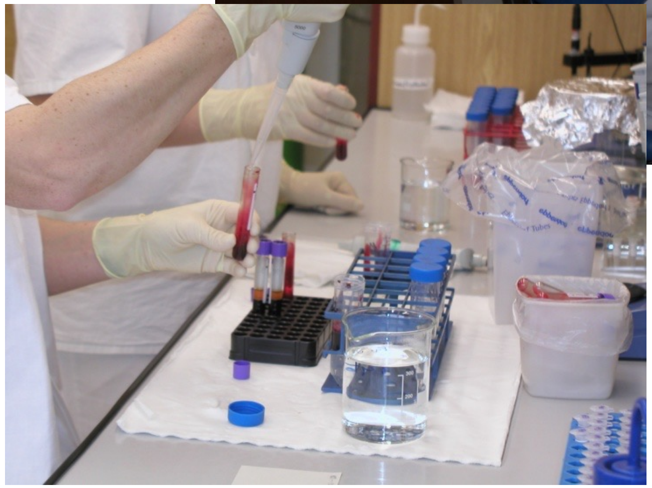


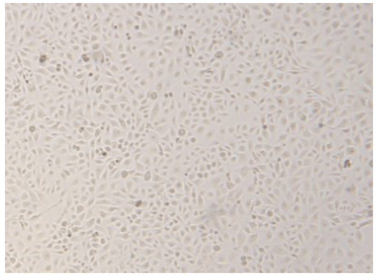
Next generation sequencing



In vitro tests – incubation of cells

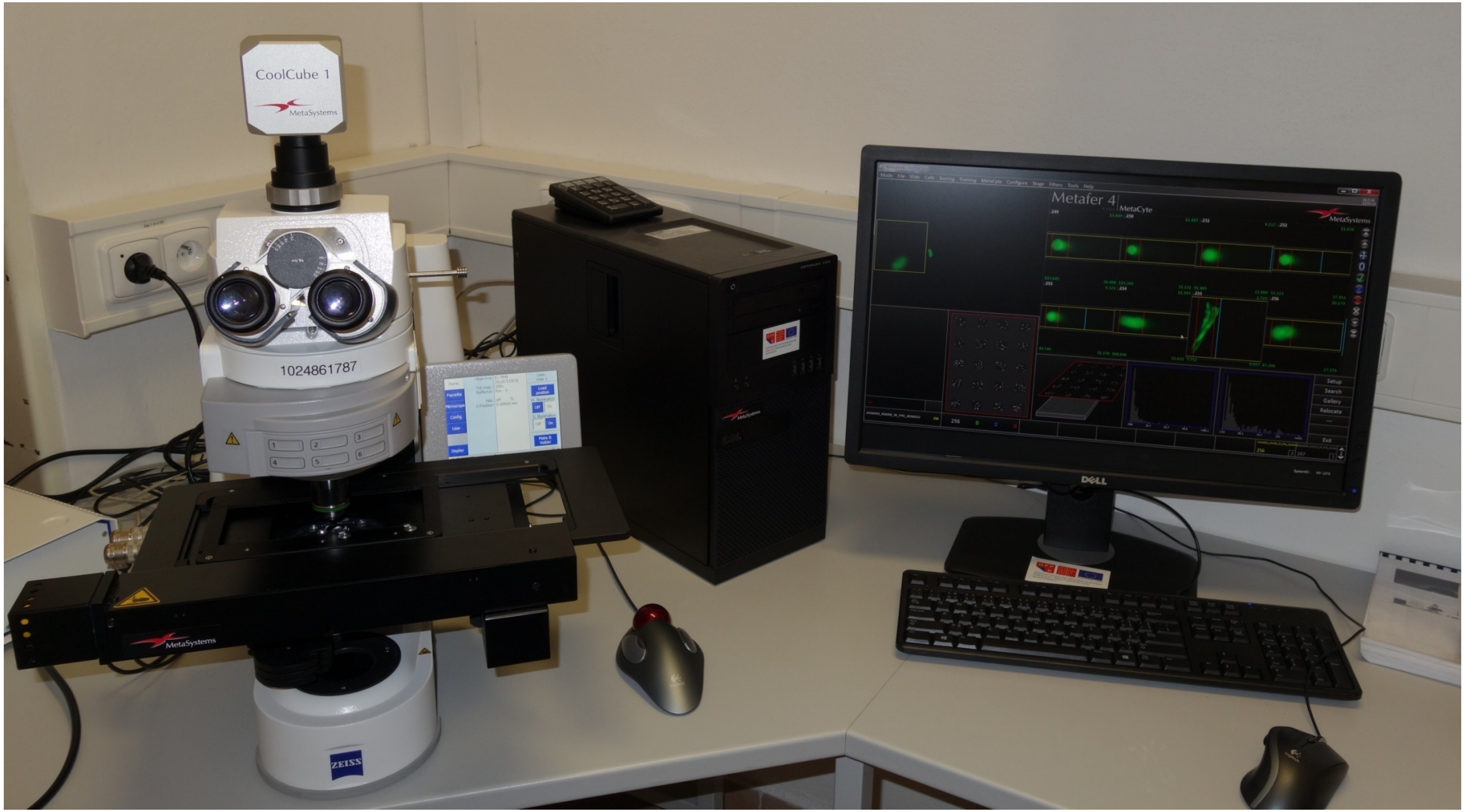




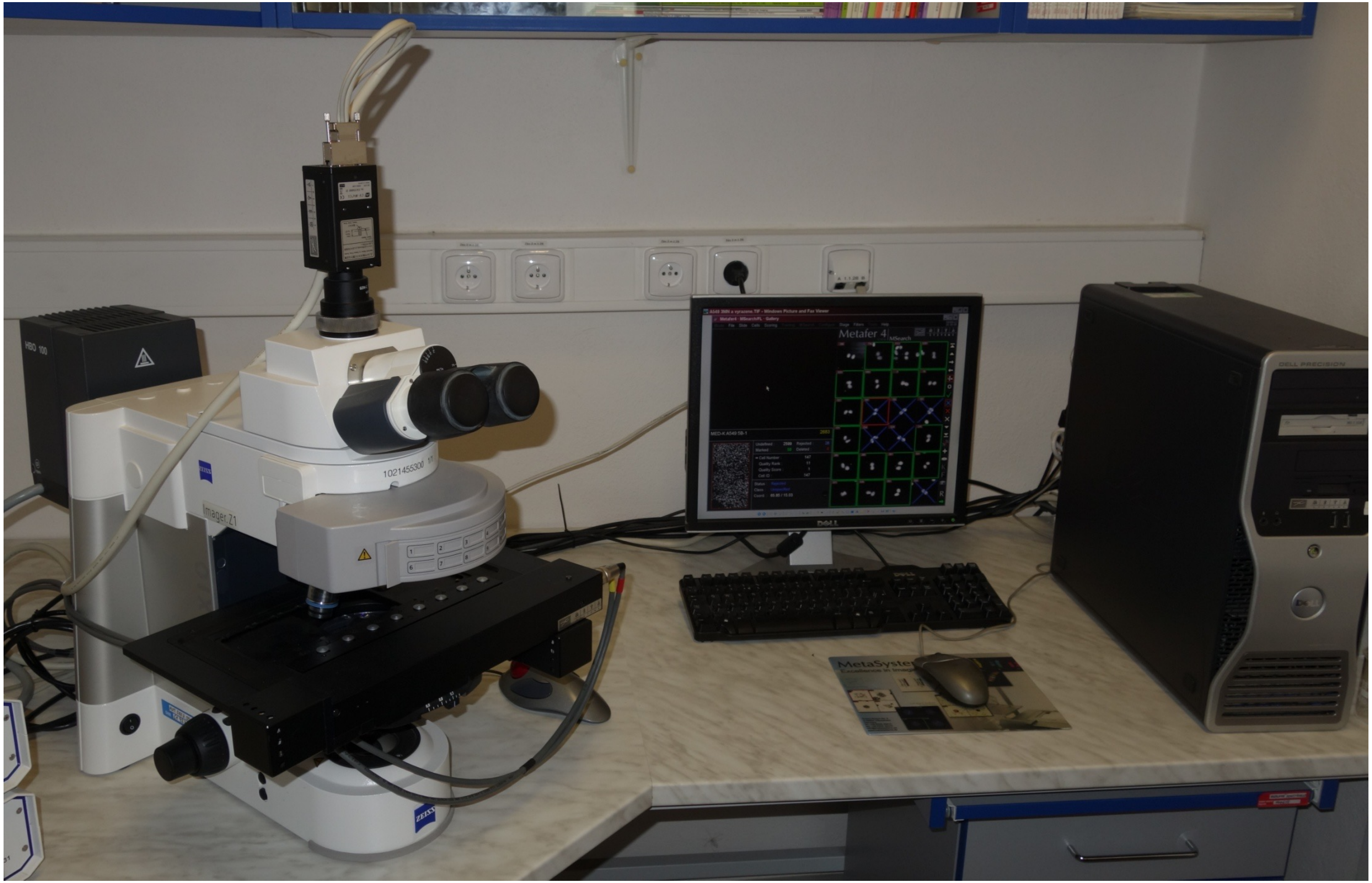


Spectrophotometer (cytotoxicity…)

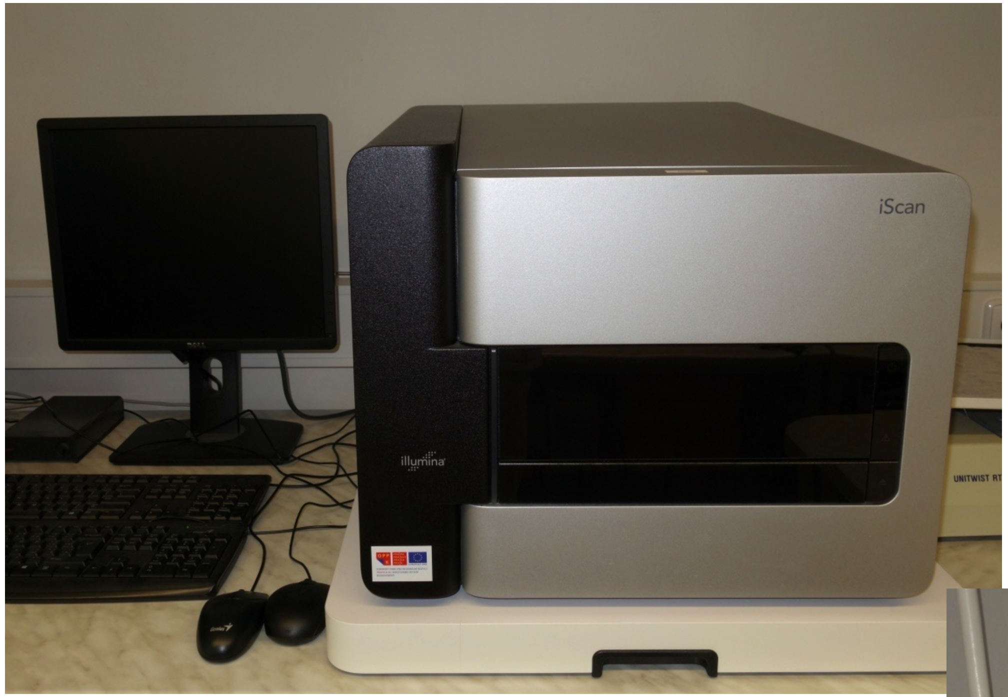
Comet assay


CoolCube 1
MetaSystems
1024861787
MetaSystems
ZEISS
Metafer 4
MetaCyte
MetaSystems
DELL

Micronuclei analysis



Genomics techniques



Microarray scanner

MiSeq system

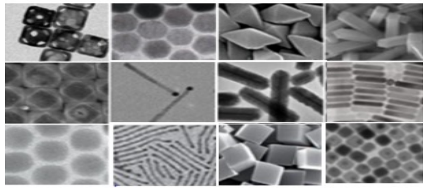
Overcoming the limitations: computational modeling?

WHY?

increased use of NM

ethical issues

variety of NM (physicochemical properties)



a great number of NM interactions with biological systems

TOO MANY VARIABLES TO BE TESTED IN THE LABORATORY

Laboratory testing may be replaced by mathematical modeling

Ultimate goal of computational modeling: safe-by-design NM

Safe by design

production of NM with properties that minimize negative biological and environmental impact of NM application and use

Limitations of computational modeling

in vitro **tests may not cover all possible negative biological impacts**

selection of the model system

Occupational exposure to NPs - examples

Occupational exposure to (nano)TiO_2

Workers exposed to (nano)TiO_2 pigment during production (36 subjects)

40% of the shift spent in the close vicinity of units emitting NP
60% of the shift in the separate control room

Researchers from the same factory

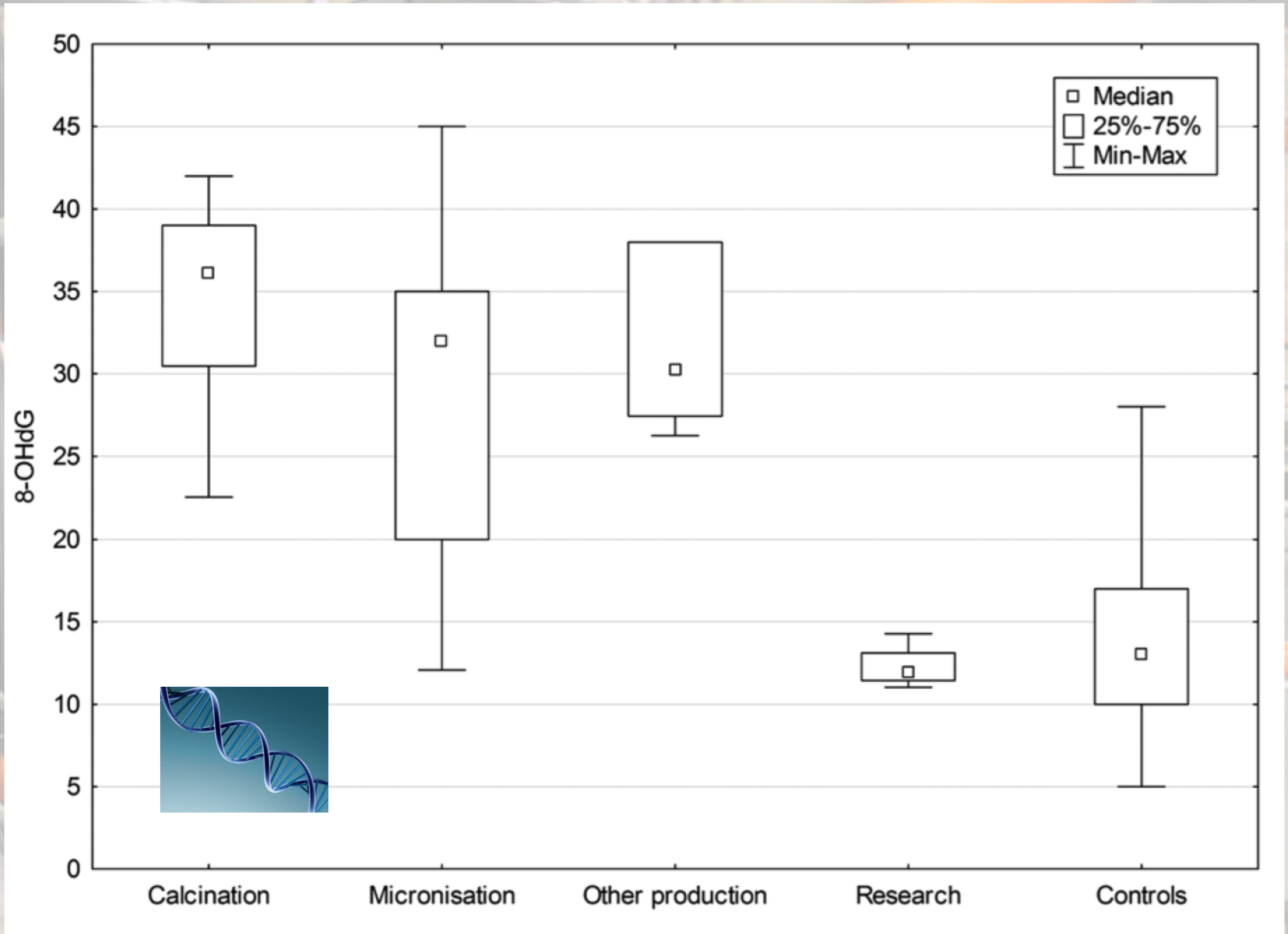
Controls not employed in the plant (healthcare workers, 45 subjects)

NP measurement:

scanning mobility particle sizer
aerodynamic particles sizer

Pelclova et al., Occup Environ Med 2016, 73, 110; Nanotoxicology, 2017, 11, 1

Occupational exposure to (nano)TiO_2: DNA oxidation



Pelclova et al., Occup Environ Med 2016, 73, 110

Occupational exposure to (nano)TiO_2: conclusions

Exposure to (nano)TiO_2 during the production results in elevated **oxidative damage to DNA, proteins and lipids**

Dose-dependent effect of occupational (nano)TiO_2 exposure was observed

The exposure has possible **chronic or subacute effect**

Pelclova et al., Occup Environ Med 2016, 73, 110; Nanotoxicology, 2017, 11, 1

Occupational exposure to iron oxide pigments

Workers exposed to iron oxide aerosol (14 subjects)

exposure for 10 ± 4 years

Controls not employed in the plant, no occupational exposure to dust (14 subjects)

NP measurement:

scanning mobility particle sizer
aerodynamic particles sizer

Journal of Breath Research

CrossMark

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PAPER

Oxidative stress markers are elevated in exhaled breath condensate of workers exposed to nanoparticles during iron oxide pigment production

Daniela Pelclova[1], Vladimir Zdimal[2], Petr Kacer[3], Zdenka Fenclova[1], Stepanka Vlckova[1], Kamila Syslova[3], Tomas Navratil[4], Jaroslav Schwarz[2], Nadezda Zikova[2], Hana Barosova[5], Francesco Turci[6], Martin Komarc[7,8], Tomas Pelcl[9], Jaroslav Belacek[7], Jana Kukutschova[5] and Sergey Zakharov[1]

1 Charles University in Prague and General University Hospital in Prague, First Faculty of Medicine, Department of Occupational Medicine, Na Bojišti 1, 128 00 Prague 2, Czech Republic
2 Institute of Chemical Process Fundamentals of the AS CR, v.v.i., Rozvojová 1/135, 165 02 Prague 6, Czech Republic
3 Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic
4 J. Heyrovský Institute of Physical Chemistry of the AS CR, v.v.i., Dolejškova 3, 182 23 Prague 8, Czech Republic
5 Nanotechnology Centre, VSB—Technical University of Ostrava, 17. Listopadu 15, 708 33 Ostrava, Czech Republic
6 Interdepartmental Centre 'G. Scansetti' for Studies on Asbestos and Other Toxic Particulates and NIS Interdepartmental Centre for Nanostructured Interfaces and Surfaces, University of Torino, Via P. Giuria 7, I-10125 Torino, Italy
7 Charles University in Prague and General University Hospital in Prague, First Faculty of Medicine, Institute of Biophysics and Informatics, Salmovská 1, 120 00 Prague 2, Czech Republic
8 Charles University in Prague and General University Hospital in Prague, First Faculty of Medicine, Faculty of Physical Education and Sport, José Martího 31, 162 52 Prague 6, Czech Republic
9 Charles University in Prague and General University Hospital in Prague, First Faculty of Medicine, 3rd Medical Department, U Nemocnice 1, 128 08 Praha 2, Czech Republic

E-mail: daniela.pelclova@lf1.cuni.cz

Keywords: nanoparticles, Fe_2O_3, Fe_3O_4, exhaled breath condensate, urine, oxidative stress, occupational exposure

Pelclova et al., J Breath Res 2016, 10, 1

Occupational exposure to iron oxide pigments

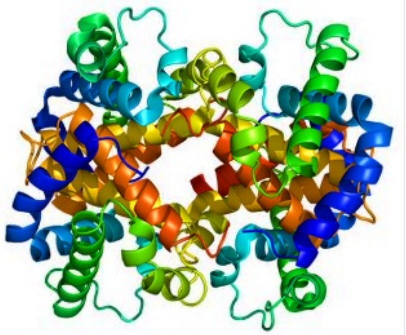
Biological analyzes in exhaled breath condensate and urine:

oxidative DNA damage:

- 8-hydroxy-2-deoxyguanosine
- 8-hydroxyguanosine
- 5-hydroxymethyl uracil

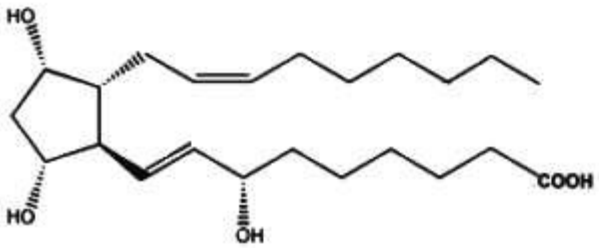
protein oxidation:

- o-tyrosine
- 3-chlorotyrosine
- 3-nitrotyrosine



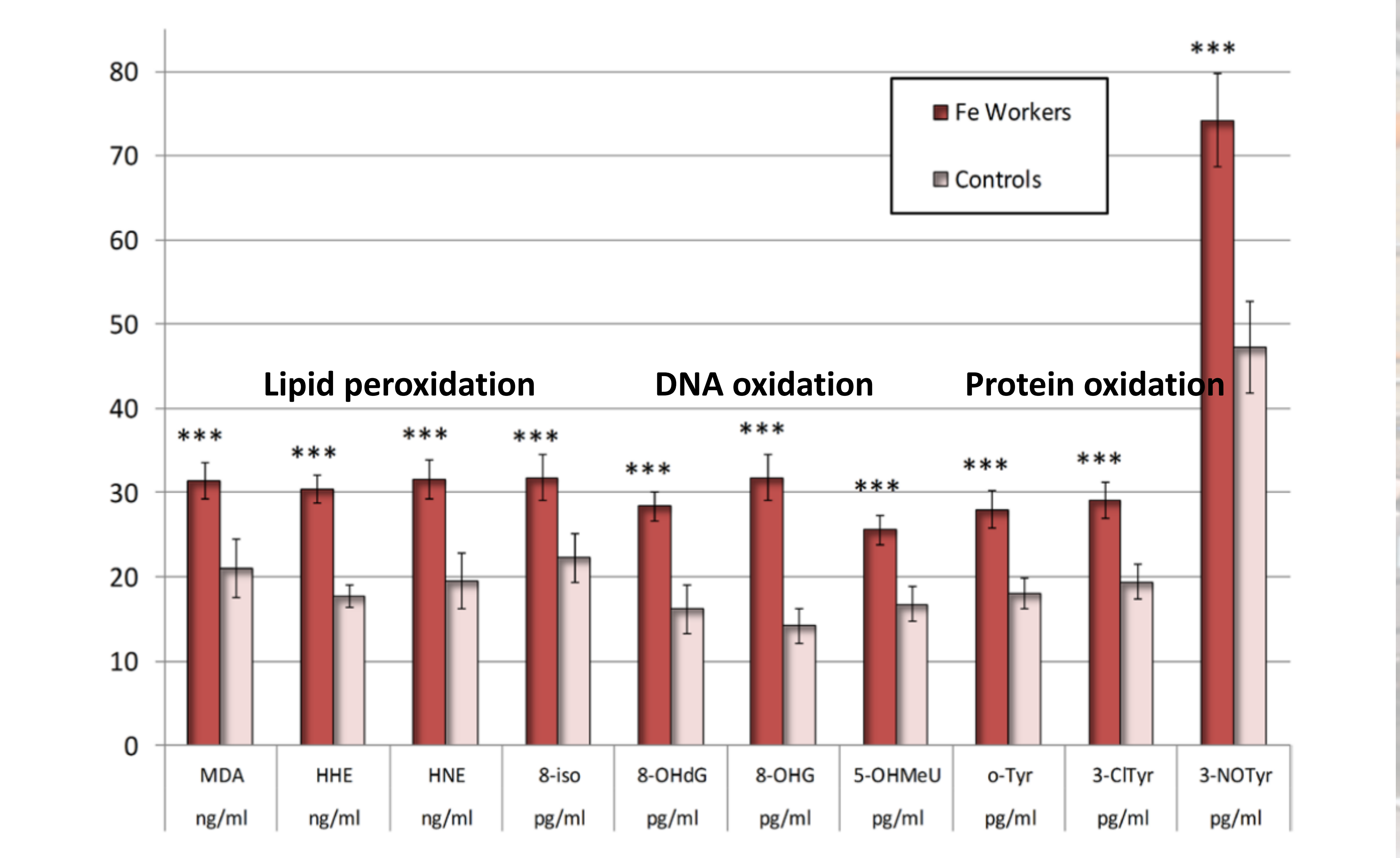
lipid peroxidation:

- malondialdehyde
- 15-F_{2t}-isoprostane
- 4-hydroxynonenale…



Pelclova et al., J Breath Res 2016, 10, 1

Occupational exposure to iron oxide pigments



Pelclova et al., J Breath Res 2016, 10, 1

Occupational exposure to iron oxide pigments

Exposure to iron oxide pigments during the production induced **oxidative damage to DNA, lipids and proteins** in exhaled breath condensate

No effects for markers in urine were observed

The exposure **does not result in systemic induction of oxidative stress**

Pelclova et al., J Breath Res 2016, 10, 1

Occupational exposure to nanocomposites

Workers exposed to nanocomposites during material processing
(20 subjects)

exposure for 17.8 ± 10 years

Controls from the same location, not exposed to nanocomposites
(21 subjects)

NP measurement:
scanning mobility particle sizer
aerodynamic particles sizer

Mutagenesis, 2020, 35, 331–340
doi:10.1093/mutage/geaa016
Original Manuscript
Advance Access publication 23 July 2020

OXFORD

Original Manuscript

The genotoxic effects in the leukocytes of workers handling nanocomposite materials

Bozena Novotna[1], Daniela Pelclova[2], Andrea Rossnerova[3], Vladimir Zdimal[4], Jakub Ondracek[4], Lucie Lischkova[2], Stepanka Vlckova[2], Zdenka Fenclova[2], Pavlina Klusackova[2], Tana Zavodna[3], Jan Topinka[3], Martin Komarc[2], Stepanka Dvorackova[5] and Pavel Rossner Jr[1,*]

Mutagenesis, 2019, 34, 253–263
doi:10.1093/mutage/gez016
Original Manuscript
Advance Access publication 24 June 2019

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Original Manuscript

The repeated cytogenetic analysis of subjects occupationally exposed to nanoparticles: a pilot study

Andrea Rossnerova[1*], Daniela Pelclova[2], Vladimir Zdimal[3], Pavel Rossner Jr.[1], Fatima Elzeinova[1], Kristyna Vrbova[1], Jan Topinka[1], Jaroslav Schwarz[3], Jakub Ondracek[3], Martin Kostejn[3], Martin Komarc[4], Stepanka Vlckova[2], Zdenka Fenclova[2] and Stepanka Dvorackova[5,6,7]

International Journal of *Molecular Sciences*

MDPI

Article

DNA Methylation Profiles in a Group of Workers Occupationally Exposed to Nanoparticles

Andrea Rossnerova [1,†,*], Katerina Honkova [1,†], Daniela Pelclova [2], Vladimir Zdimal [3], Jaroslav A. Hubacek [4], Irena Chvojkova [1], Kristyna Vrbova [5], Pavel Rossner Jr. [5], Jan Topinka [1], Stepanka Vlckova [2], Zdenka Fenclova [2], Lucie Lischkova [2], Pavlina Klusackova [2], Jaroslav Schwarz [3], Jakub Ondracek [3], Lucie Ondrackova [3], Martin Kostejn [3], Jiri Klema [6] and Stepanka Dvorackova [7]

Rossnerova et al., Mutagenesis 2019, 34, 253; Rossnerova et al., Int. J. Mol. Sci. 2020, 21, 2420; Novotna et al., Mutagenesis 2020, 36, 331

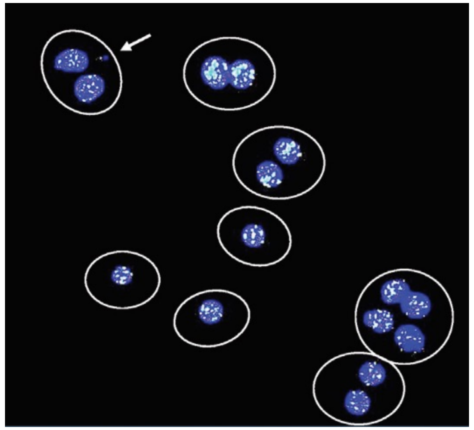
Occupational exposure to nanocomposites

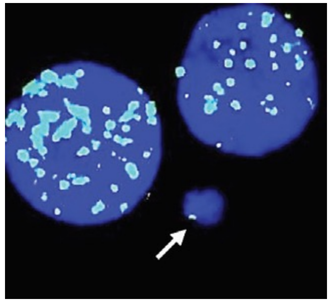
Biological analyzes in blood samples:

1. Micronuclei detection (losses and breaks)

centromere positive

centromere negative





Rossnerova et al., Mutagenesis 2019, 34, 253331

Occupational exposure to nanocomposites

Biological analyzes in blood samples:

2. DNA breaks and oxidative damage

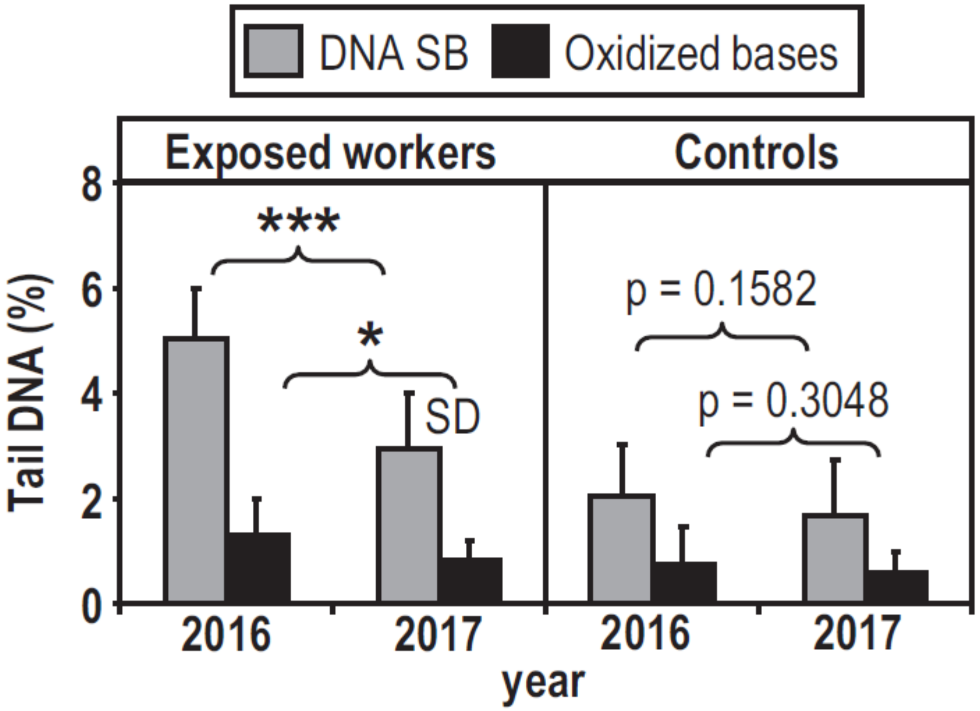


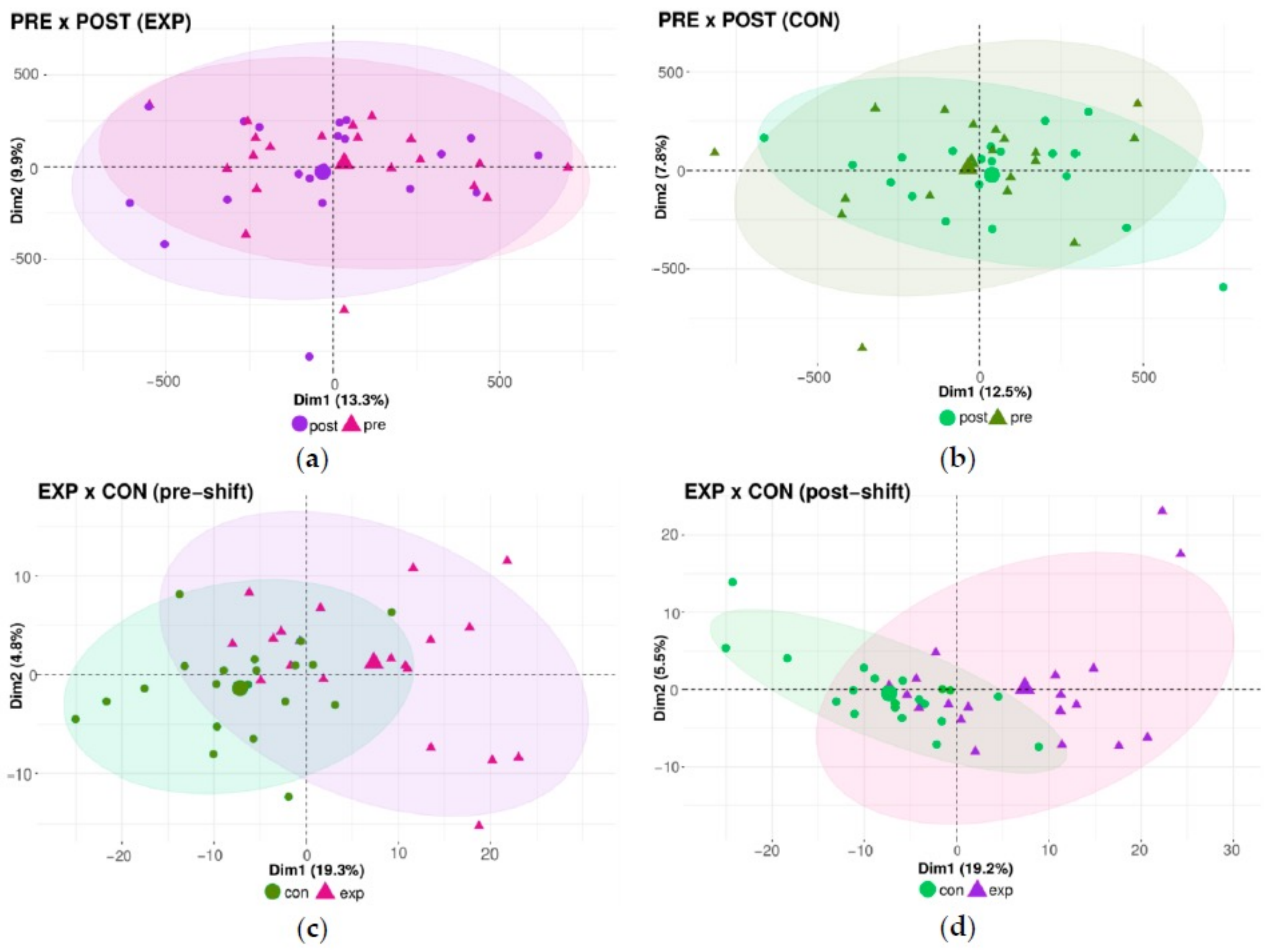
Figure 1. DNA damage in the leukocytes of controls and workers long-term exposed to nanoparticles: comparison of pre-shift values obtained from subjects examined repeatedly in the years 2016 and 2017. Number of exposed workers—14; number of controls—11; $*P \leq 0.05$; $***P \leq 0.001$.

Novotna et al., Mutagenesis 2020, 36, 331

Occupational exposure to nanocomposites

Biological analyzes in blood samples:

3. DNA methylation profiles



(a) (b) (c) (d)

Rossnerova et al., Int. J. Mol. Sci. 2020, 21, 2420

Occupational exposure to nanocomposites

Results:

Increased MN frequency after short-term, but not long-term exposure

DNA breaks and oxidative damage were increased after NP inhalation, particularly after acute exposure

DNA methylation profiles significantly differed between the exposed and control subjects when long-term exposure was considered

Rossnerova et al., Mutagenesis 2019, 34, 253; Rossnerova et al., Int. J. Mol. Sci. 2020, 21, 2420;
Novotna et al., Mutagenesis 2020, 36, 331

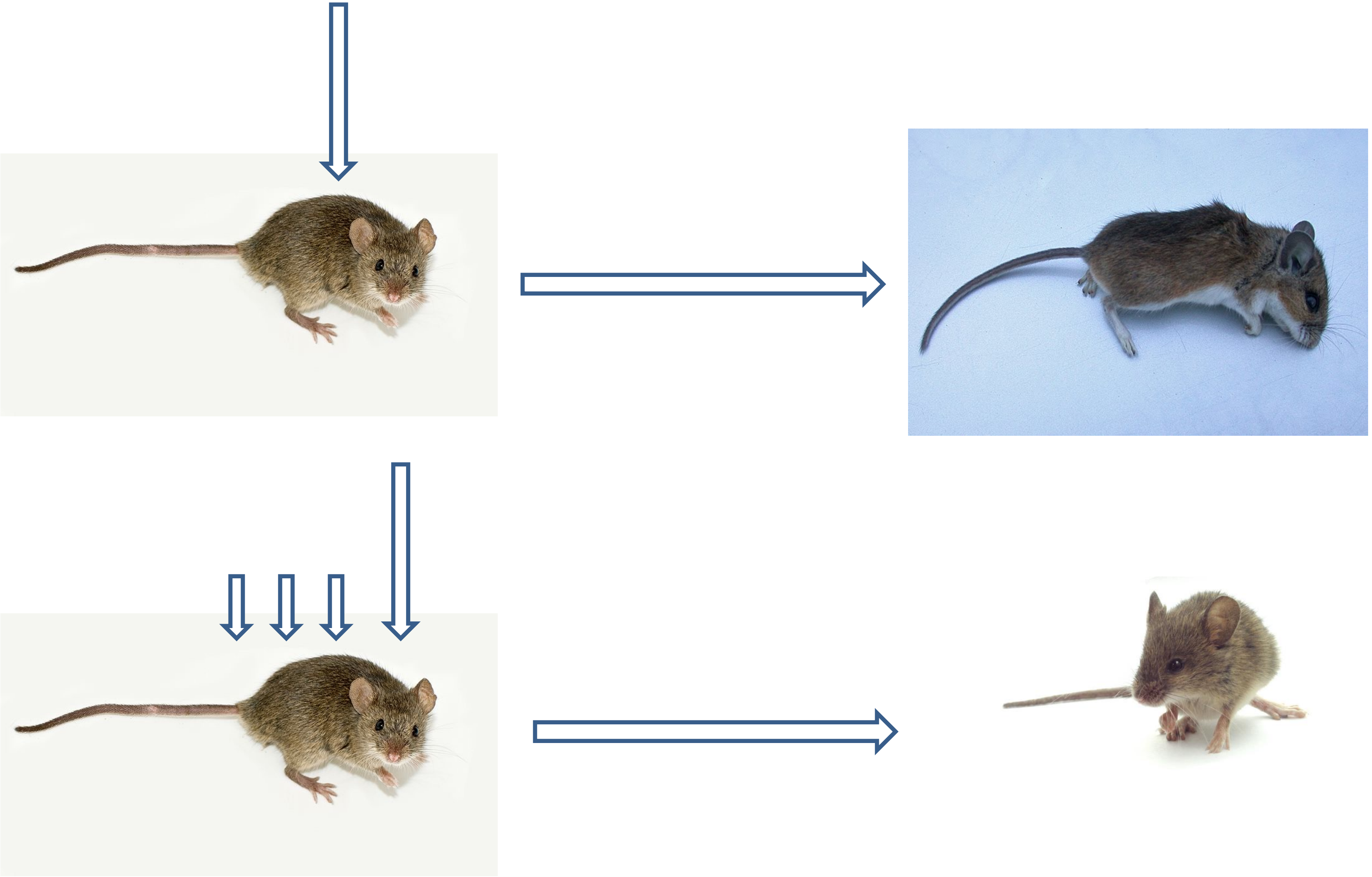
Occupational exposure to nanocomposites

The exposure to nanocomposites causes DNA damage and affects DNA methylation profiles

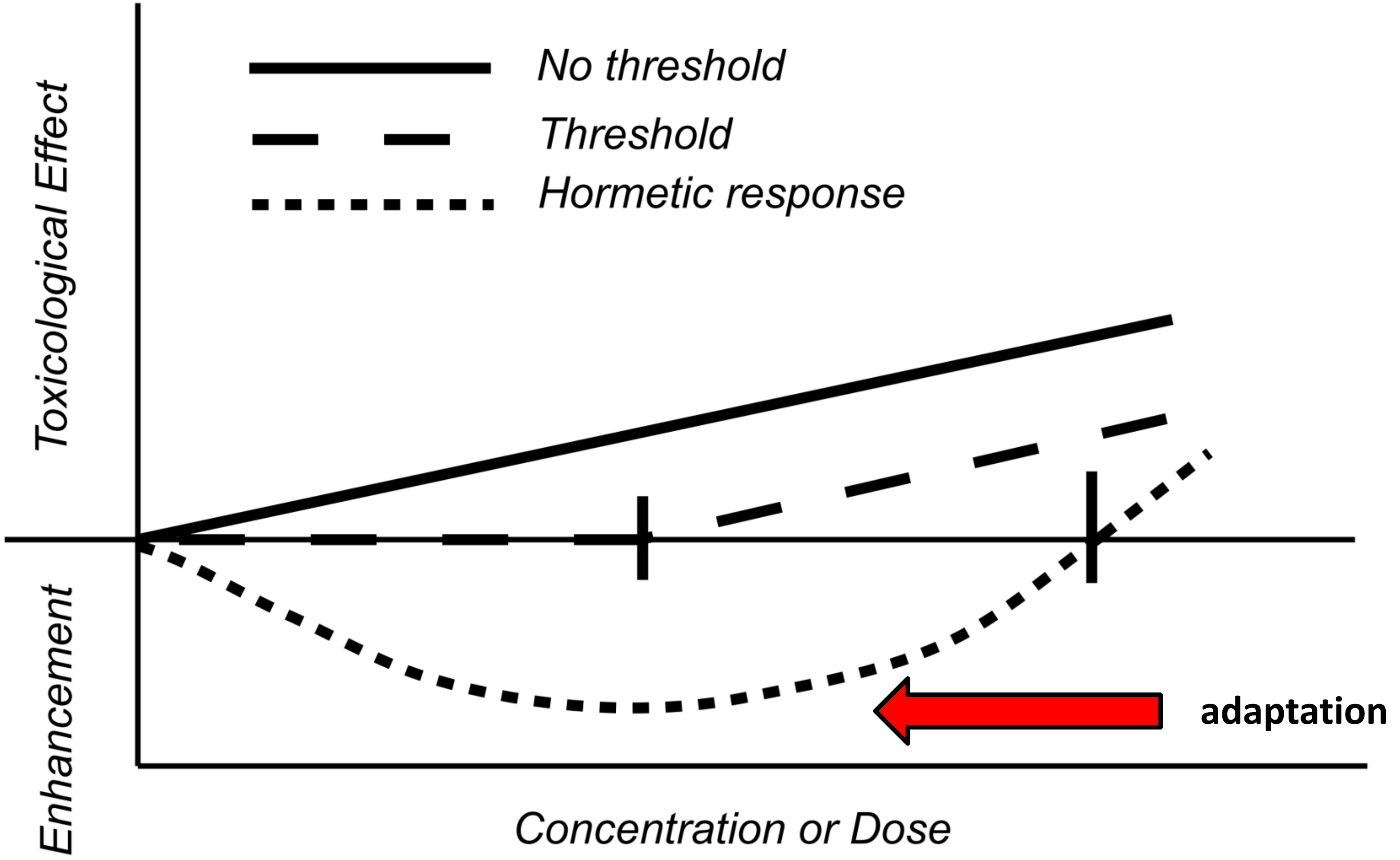
For some parameters, the effect was observed after acute (short-term), but not chronic (long-term) exposure suggesting adaptation to NP exposure

Rossnerova et al., Mutagenesis 2019, 34, 253; Rossnerova et al., Int. J. Mol. Sci. 2020, 21, 2420; Novotna et al., Mutagenesis 2020, 36, 331

Adaptive response: a clue to (human) adaptation?



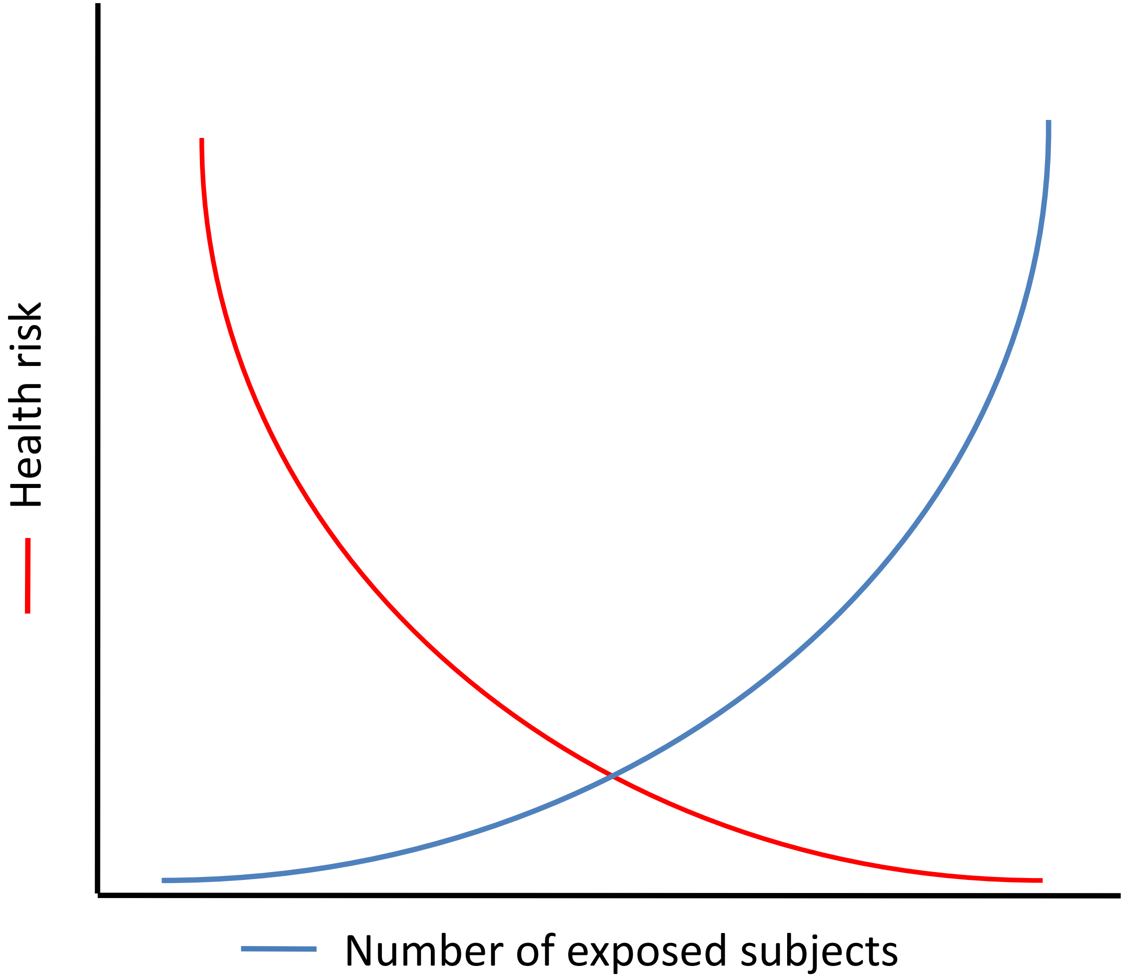
Effect of xenobiotics in the organism: Dose-response curves



Kritika konceptu LNT (linear non-threshold dose-response model): **Calabrese, https://doi.org/10.1016/j.envres.2021.111025**

Take-home message

Be careful but use common sense



NanoEnviCZ

Research infrastructure

Supported by the Ministry of Education, Youth and Sports (2016-2022) (LM2015073)

Partners:

J. Heyrovský Institute of Physical Chemistry AS CR
Palacký University Olomouc
Technical University in Liberec
Jan Evangelista Purkyně University in Ústí nad Labem
Institute of Inorganic Chemistry AS CR
Institute of Experimental Medicine AS CR

http://www.nanoenvicz.cz/en

NANO ENVICZ • CZ

NanoEnviCZ

The principal aim is to provide infrastructure for external and internal partners (research institutions, industry) to achieve the following goals:

1. Development of new NM and technologies applicable in:
    a. degradation of environmental pollutants
    b. transformation of solar energy to hydrogen
    c. production of biosensors
    d. catalysts for production of next generation fuels and chemical products

2. **Assessment of (eco)toxicity of NM, construction of predictive models of the effect on NM on the environment and application of the models to design eco-friendly materials**

NanoEnviCZ

NanoEnviCZ at the Institute of Experimental Medicine:

Cytotoxicity analyses

Oxidative damage assays

DNA damage (comet aassay, micronuclei analysis)

Gene expression analyses and next-generation sequencing

Contact persons:

Jan Topinka, DSc., jan.topinka@iem.cas.cz
Pavel Rossner, Jr., PhD., pavel.rossner@iem.cas.cz