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ECVAM efforts in nano- and biotechnology

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http://ecvam.jrc.it

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Biologicals

- **Meeting with EDQM and WHO March 2005:**
  Identify methods to replace intracerebral challenge in Kendrick test (potency testing of whole-cell pertussis vaccines)

  → **Start of 2 ECVAM studies to optimise serological tests**

- **Start of pyrogenicity peer review process end of June 2005**

- **ECVAM/DG SANCO workshop on 3Rs Approaches in Marine Biotoxin Testing – draft report in consultation with experts**

- **Scientific advice to CRL (Vigo) on prevalidation of functional assay for PSP testing**

- **Botulinum toxin: ECVAM in contact with EDQM regarding meeting with manufacturers, workshop with ICCVAM planned**

- **Comments on Ph.Eur. Monographs published in Pharmeuropa**

Biologicals as door-openers for a new safety toxicology for pharmaceuticals?

- **Today 50% of new drugs are biologicals (e.g. recombinant proteins, antibodies)**
- **Classical safety toxicology is useless**
- **Biologicals have failed because of side-effects in studies and clinical use**
- **A predictive toxicology e.g. based on human cells is urgently required**

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ECVAM Research activities

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Coaching and steering of DG RTD projects

- **IP ReProTect** — **started 7'04**
- **IP A-Cute-Tox** — **started 1'05**
- **IP Sens-it-iv** — **start 10'05**
- **STREP Predictomics** — **started 9'04**
- **STREP ToxDrops** — **started 1'05**
- **STREP Vitrocellomics** — **start 11'05**
- **Marie-Curie PulmoNet** — **started 4'05**

Impact: Total funding > 40 million Euro, > 120 partner institutions

Own research

- **Link to other units (ECB, PCE, BMS, GMO)**
- **Ecotoxicology test strategy (with ECB) validated 9'05 (potential of saving 60% of fish for acute ecotox.)**
- **Cancer assay now under validation**
- **8 Ph.D. (Adler, Boveri, Dodevich, Gartlon, Hoffmann Kinsner, Pellizzer, Malerba)**
- **About 50 original publications per year including Nature and PNAS, reports/reviews/citations in Nature, Science, Scientific American**

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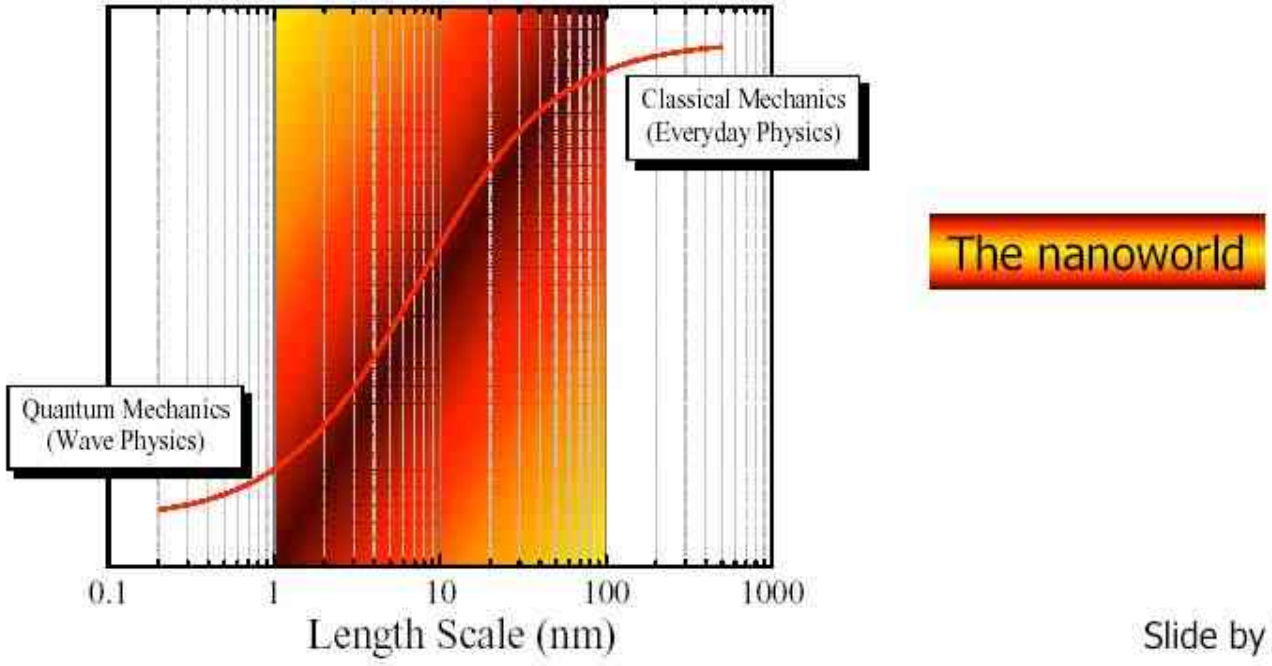
Why is Nano Different?

At the micron and larger scale, classical physics determines properties.

- **At the Angstrom (0.1 nm) scale, quantum mechanics determines properties.**
- **At the nanometer scale, fundamental properties depend on exactly how big the particle is.**



Slide by K. Ausman

From K.M.Kulinowski, Nanotechnology "The Big Science of the Very Small", CBEN

Type of exposure to metallic nanoparticles

Environmental

- Emission from automotive catalytic converters (Pt^0 on Al_2O_3, CeO_2 on Co)
- Cosmetic (sunscreen, TiO_2 ultrafine), ceramic, textile, semiconductor industries (CeO_2, ZnO, SiO_2, Ag on TiO_2, ZrO_2, Ag, Au, Pt)....

Occupational

- Large scale preparation of nanoparticles (tons of carbon nanotubes, Co_3O_4/Fe_2O_3)....

Biomedical

- Tumor diagnosis and therapy (ultrafine TiO_2 as killing compound of cancer cells by UV light), quantum dots (CdSe), supermagnetic Fe for diagnosis, Ag in catheters (antimicrobic action)

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Towards a European Strategy for Nanotechnology

On the 7th June 2005, the European Commission adopted the Action Plan "Nanosciences and nanotechnologies: An action plan for Europe 2005-2009" (COM(2005) 243) …

How to ensure that nanotechnology is safe?

- **Applications of nanotechnology must ensure a high level of public health, safety, consumer and environmental protection.**
- **Any risks must be addressed upfront and as an integral part of the R&D process.**
- **Specific toxicological studies for e.g. nanoparticles are underway at EU and national level.**

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In Vitro nanotoxicology research on manufactured nanoparticles

Since 2003

- Starting of the *In vitro* NAno TOXicology (INATOX) project (development of multidisciplinary methodology to understand the "nanosafety" and interaction of NP with cells, subcellular structures and biomolecules (ECVAM/BMS Units))
- 6 Contributions, support to DGs (RTD, Sanco)
- 8 International Conferences/Workshops, 5 Invited Seminars at Universities
- Partnership of Integrated (NANOIMPACT, INTELTEX) and STREP (Cellnanotox, DIPNA) projects
- Workshop on Health Effects on Nanoparticles (first part of 2006)

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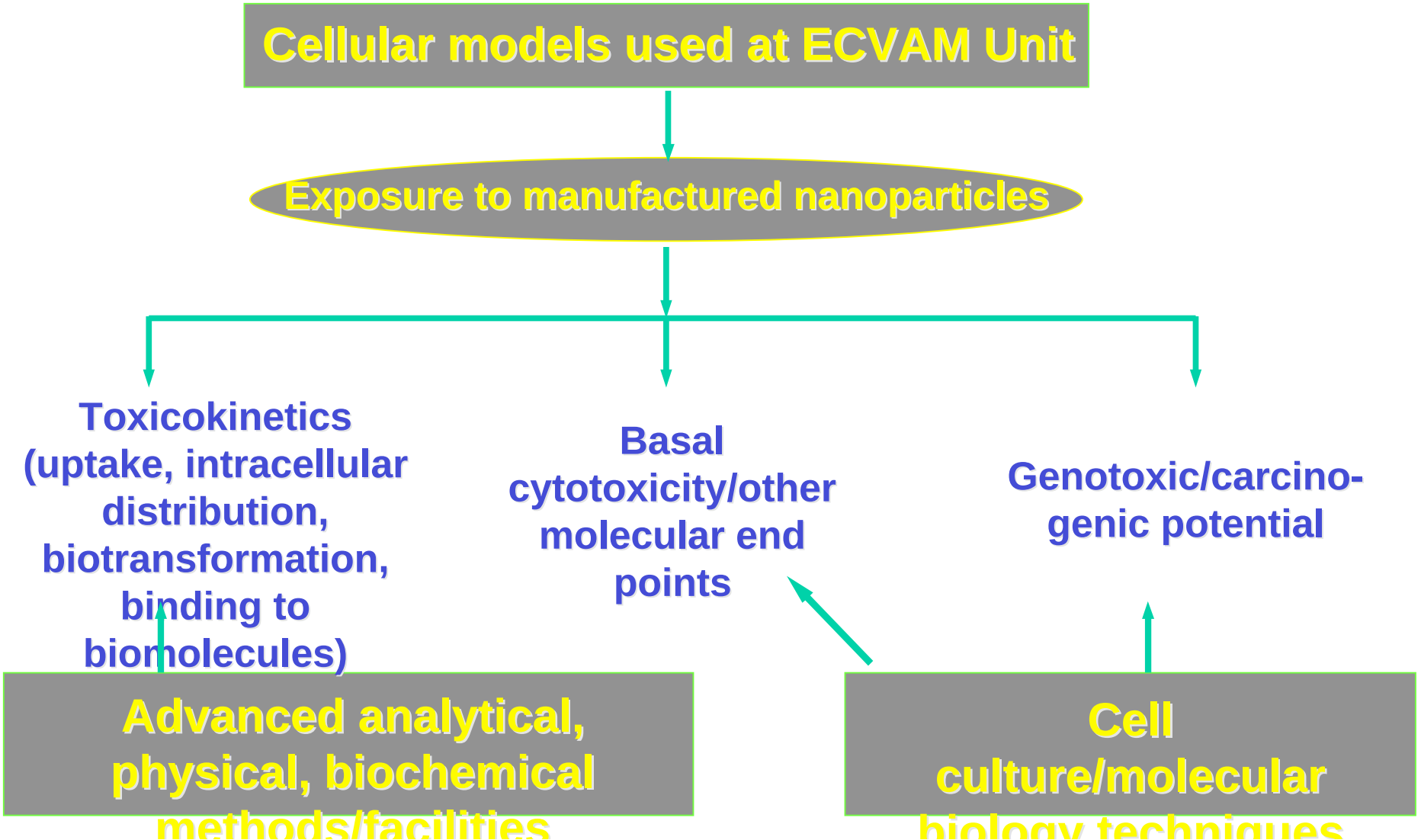
In vitro nanoparticles mechanistically-based toxicology research at the JRC



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In vitro NAnoTOXicology research (INATOX) project

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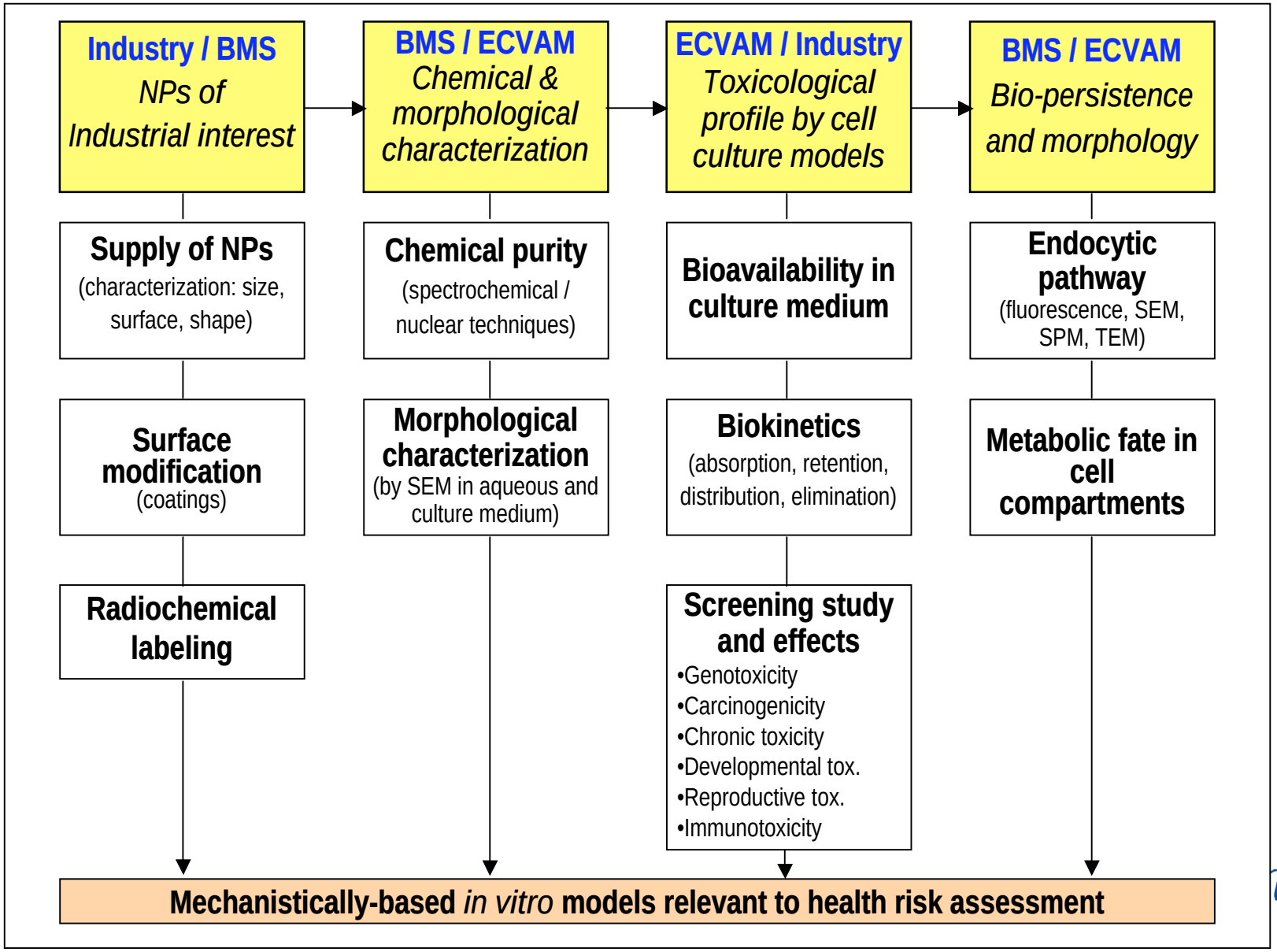

Metallic nanoparticles at ECVAM

(University of Modena and Colorobbia Group suppliers)

Nanoparticle	Mean size (nm)
Ag^0	20
Au^0	12
Co^0	50-100
$CoFe_2O_4$	15
$CoAl_2O_3$	15
Cr antimoniate	20
Ni^0	62
Pt^0	5
SiO_2	20-160
TiO_2	3; 20-30; 20-160
TiO_2(Ag coated)	3
$TiO_2Sb_2O_3Cr_2O_3$	15
ZrO_2	2; 5-30

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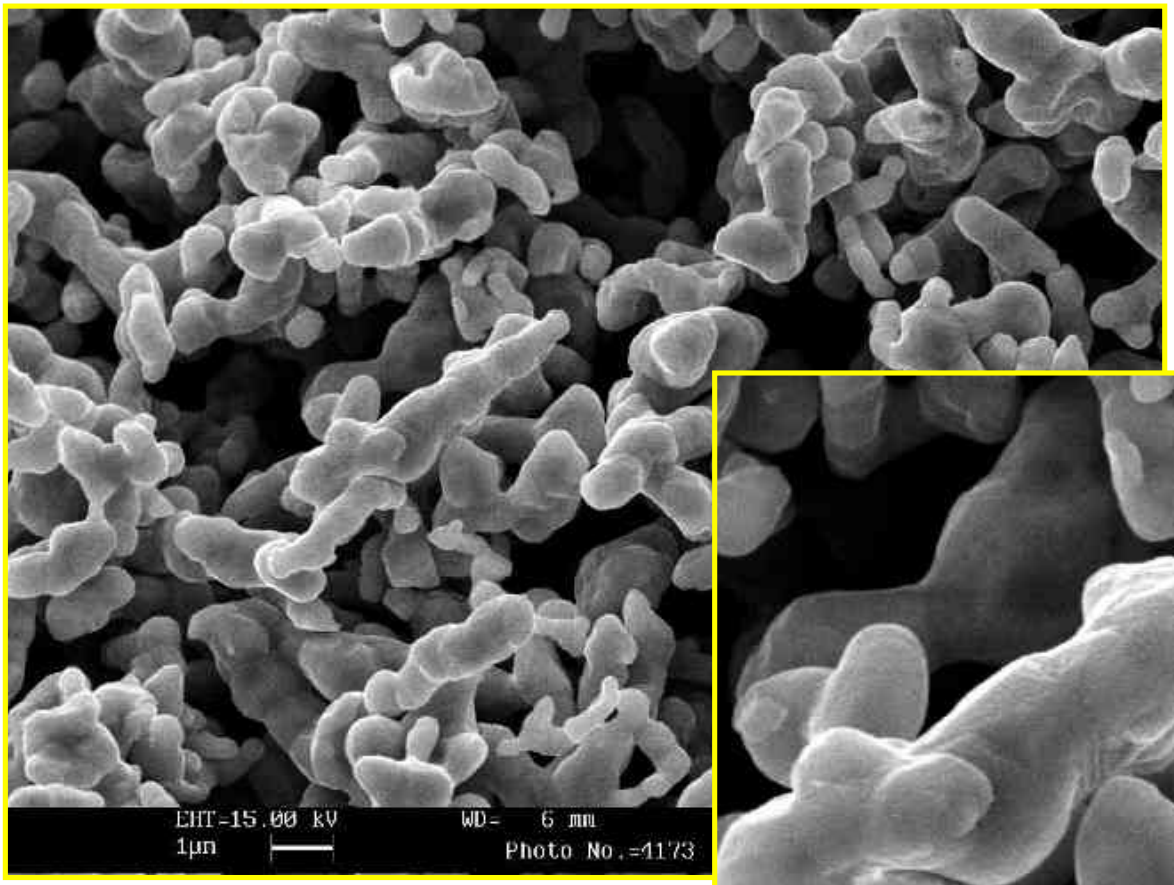
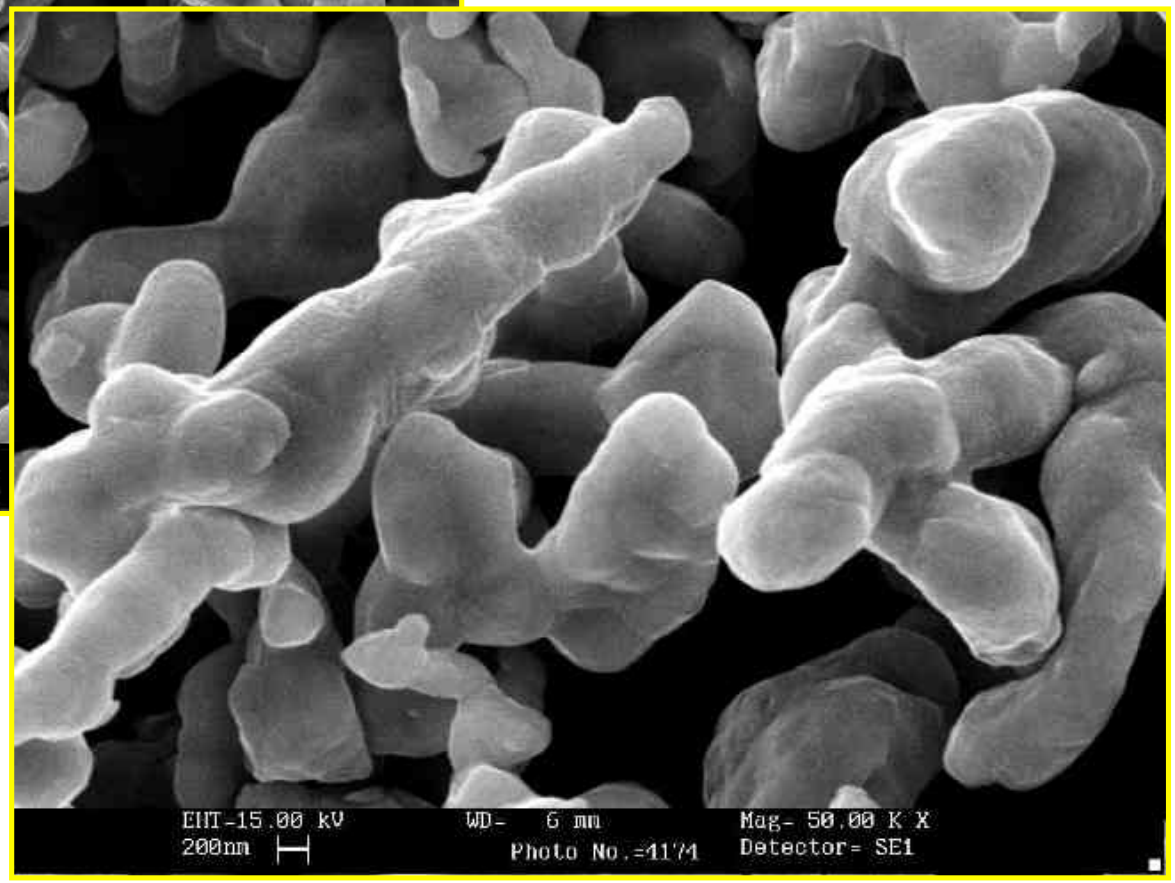
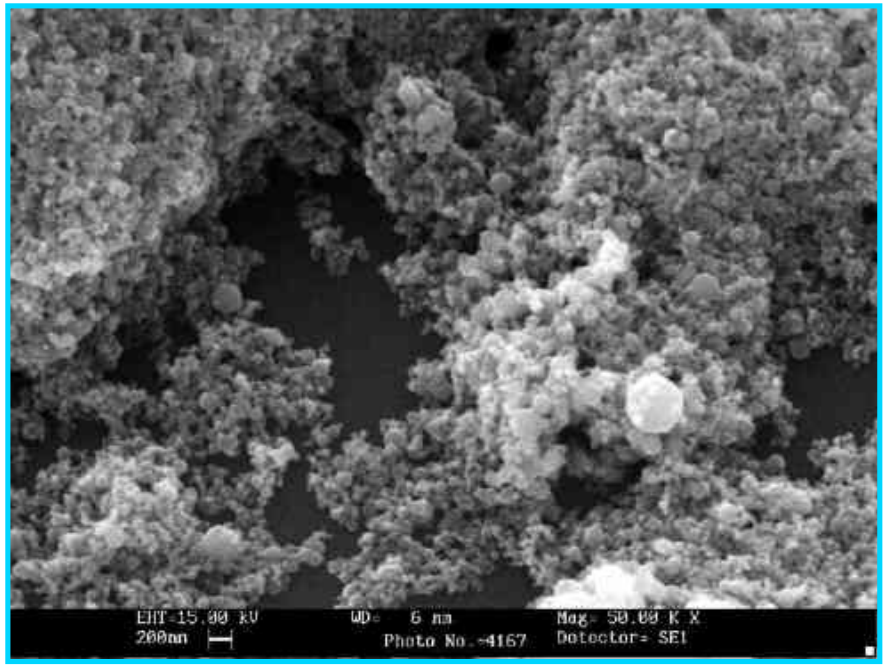
Co-nano in H_2O (SEM microphotograph)

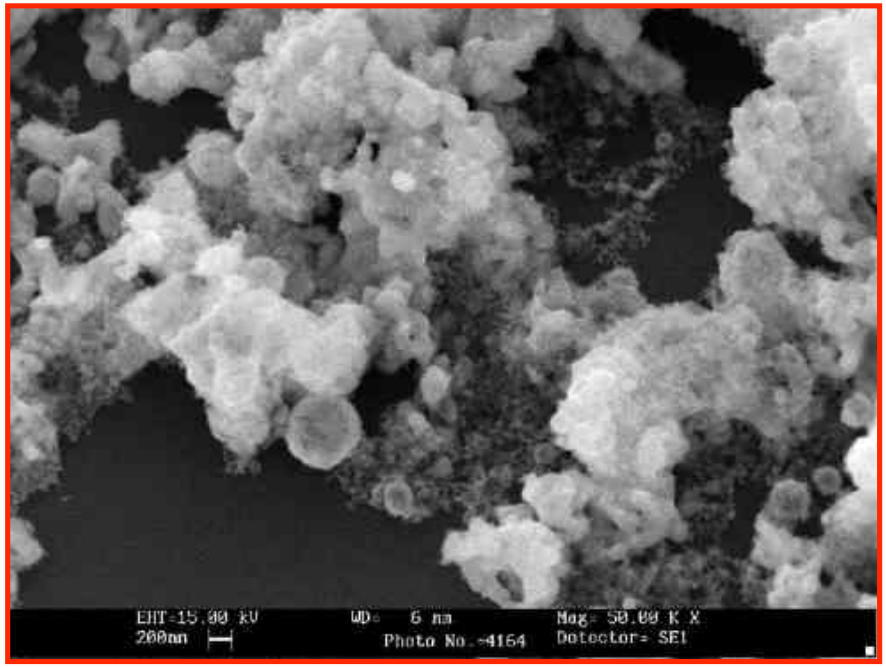




TiO_2-nano in H_2O (SEM microphotograph)



ZrO_2-nano in H_2O (SEM microphotograph)



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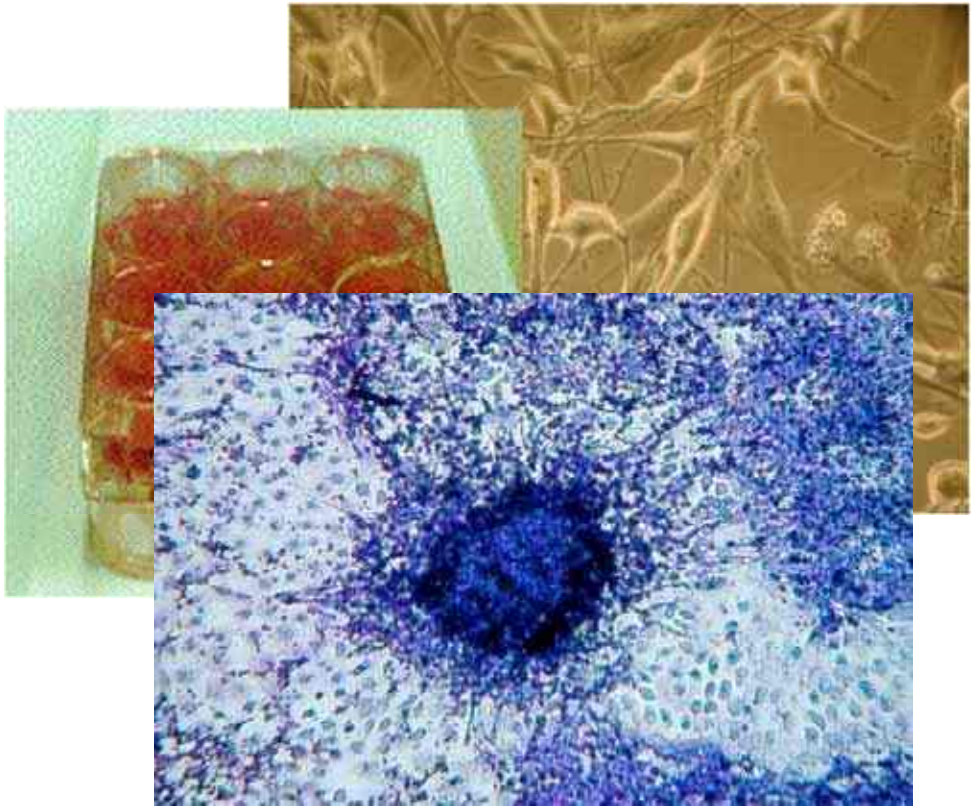
1st study involving EU, USA & Japan

In vitro Cell Transformation Assay



Prevalidation initiated in 11/2004

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The beam line for the production of carrier-free radiotracers

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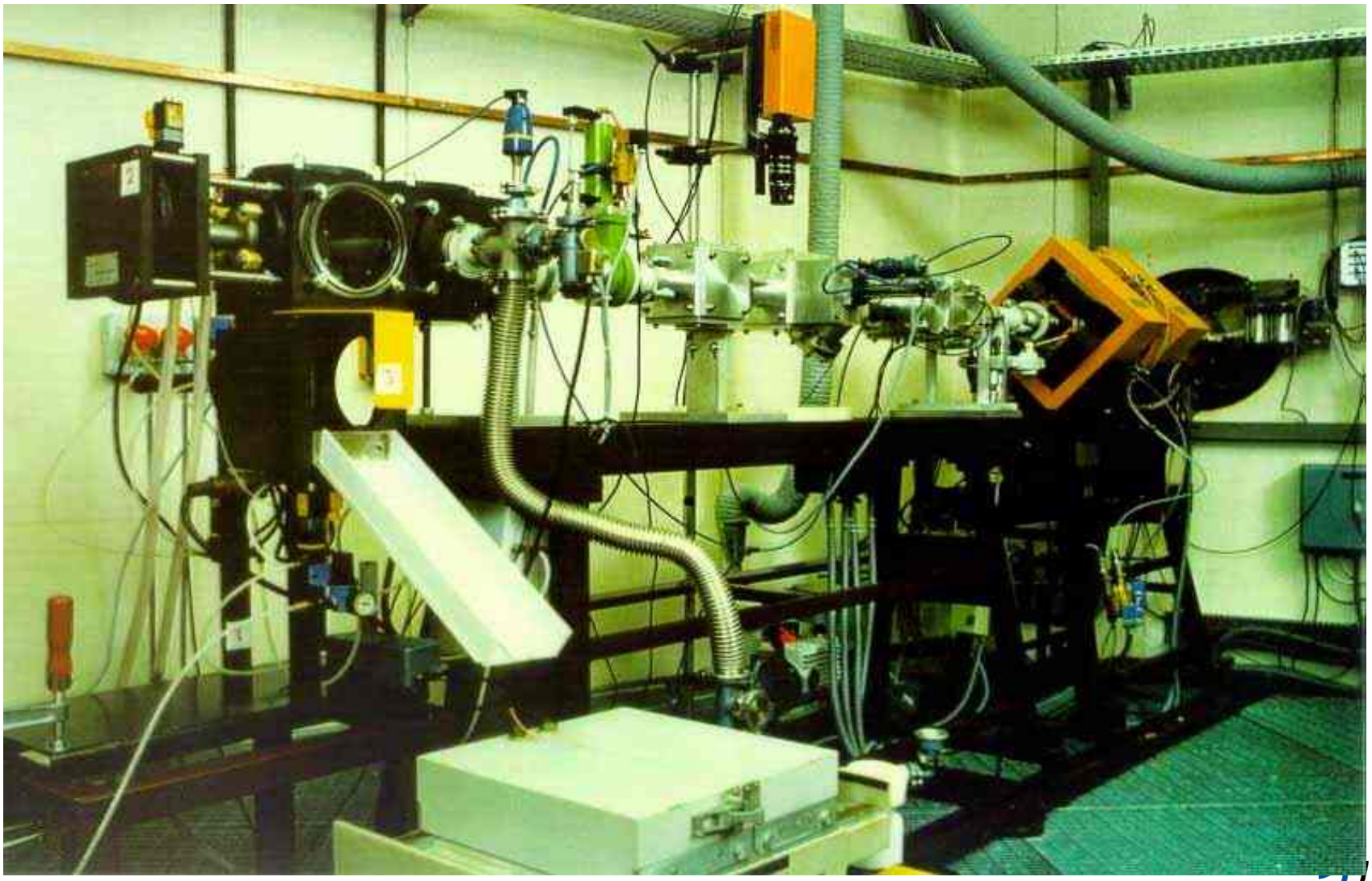

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"Radiolabelling" di Co-nano

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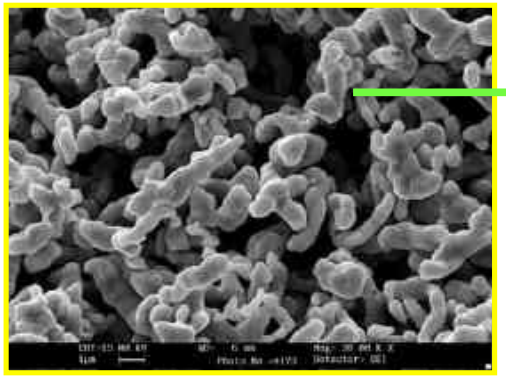


Co_{nano}

Neutron activation

HFR reactor

$^{59}Co + n \rightarrow {}^{60}Co^{*} + \gamma$

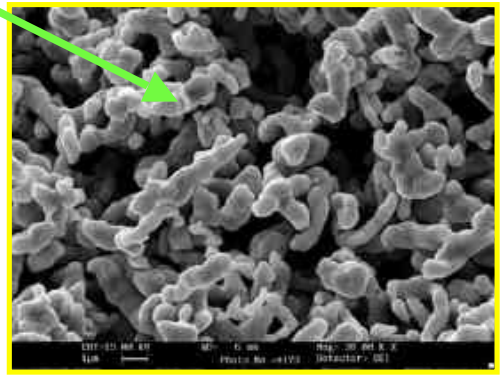
^{60}Co

β^-

$\gamma 1$ 1332 KeV

$\gamma 2$ 1173 KeV

^{60}Ni



$^{60}Co_{nano}$

Uptake of cobalt in BALB/3T3 cells exposed simultaneously to $^{57}Co_{sol}$ and $^{60}Co_{nano}$ for 4h

Concentration (µM) $^{60}Co_{nano}$ and $^{57}Co^{2+}$	fgCo·cell^{-1}[(a)]	
	$^{60}Co_{nano}$	$^{57}Co^{2+}$
1	120	0.1
5	720	1.0
10	1900	2.2
30	7300	13.3
50	10000	26.8
70	15000	31.1
100	17385	27.4

(a): mean of 3 experiments;
RSD<11%.

Conclusion: obvious different accumulation of Co from Co-nano and Co-sol

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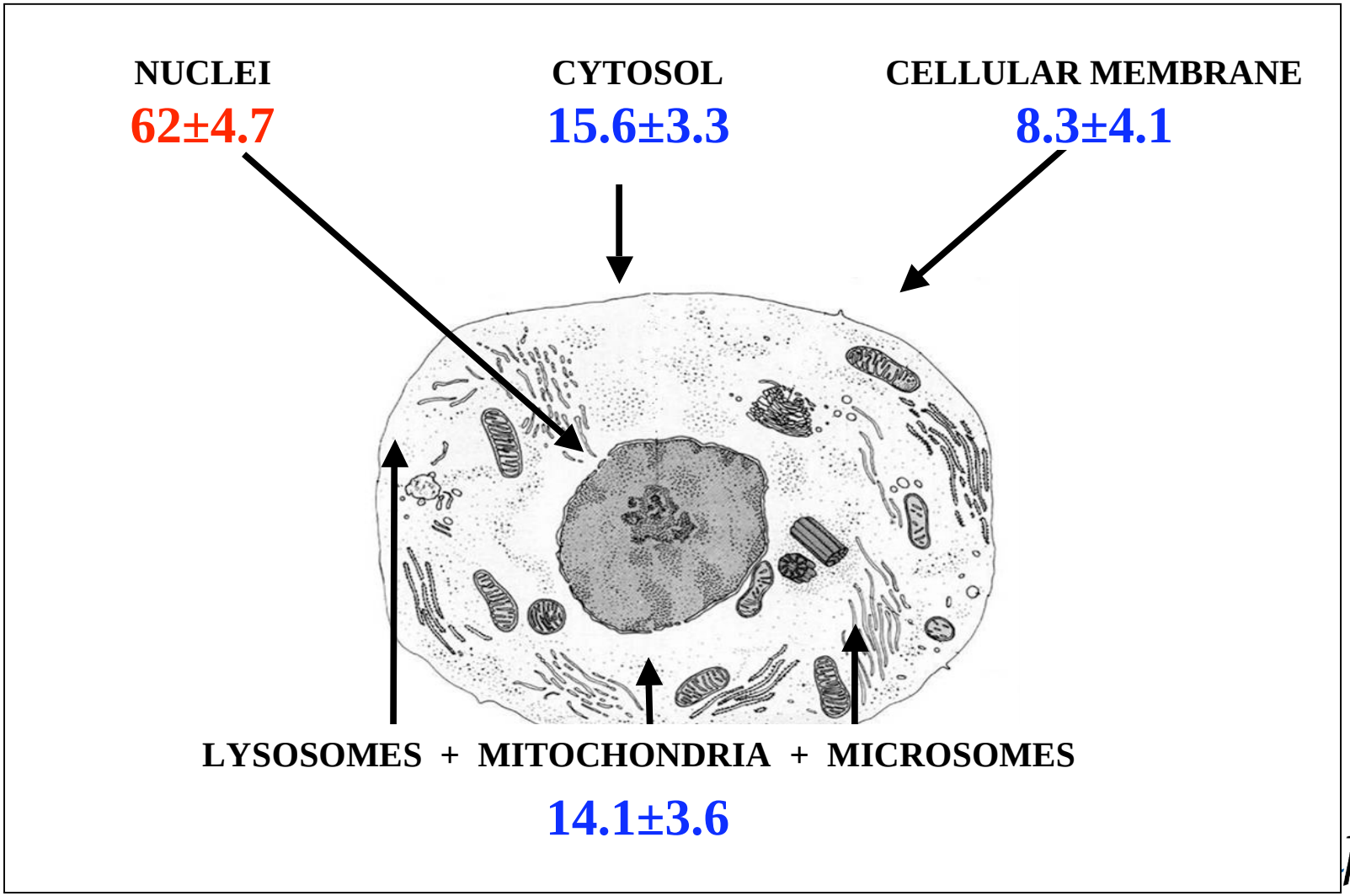
Intracellular distribution of Co in Balb/3T3 cells exposed for 4h to 100µM of ^{60}Co-nano (% of total homogenate)



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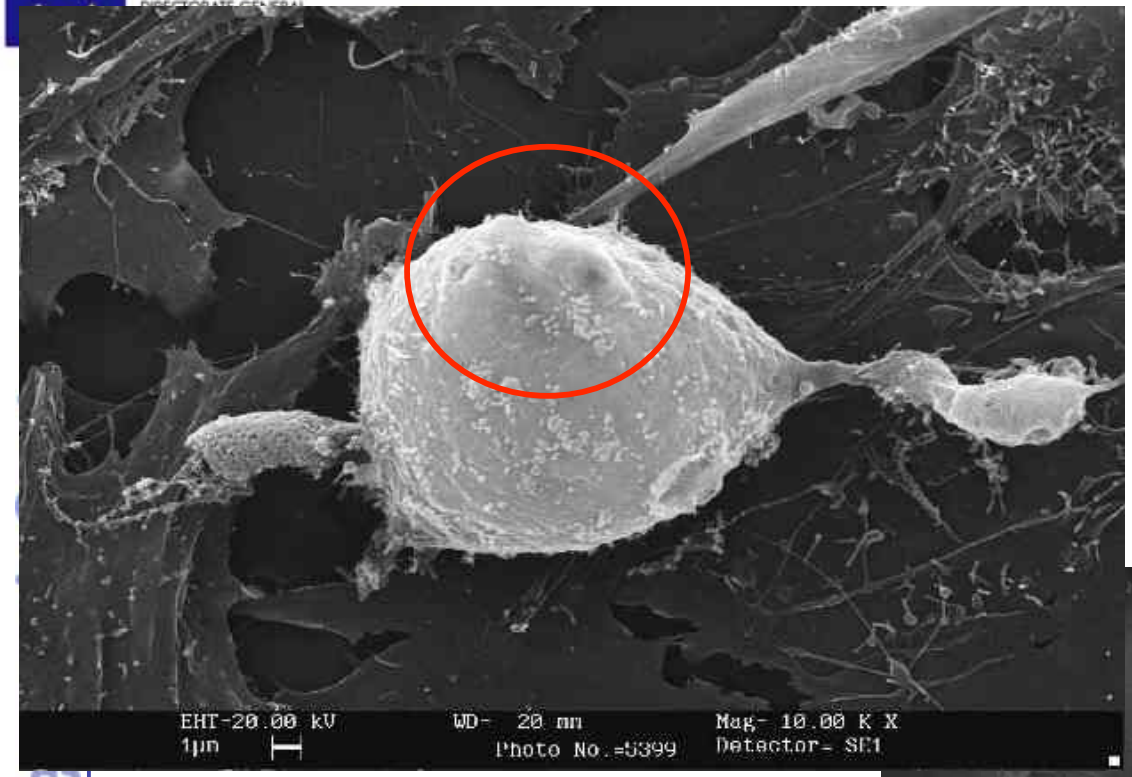
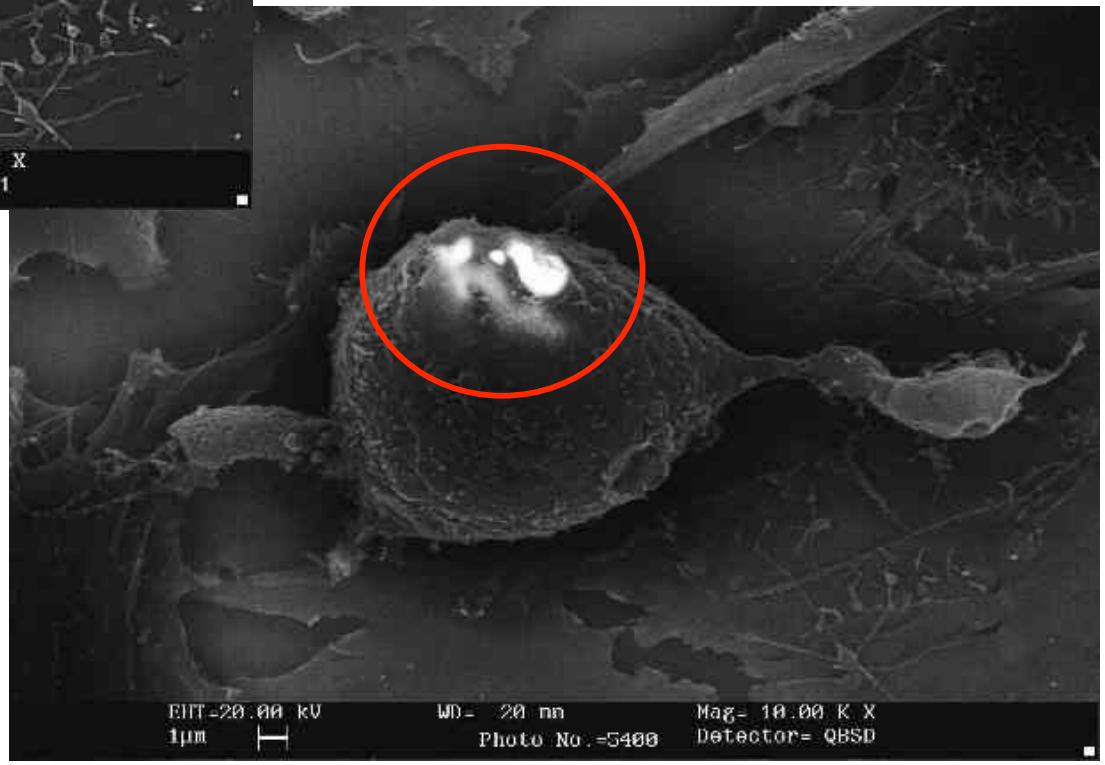
SEM pictures of Balb/3T3 cells exposed to 100µM Co-nano prepared by Critical Point Dryer technique





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SEM of Balb/3T3 cells, 100µM Co_{nano}

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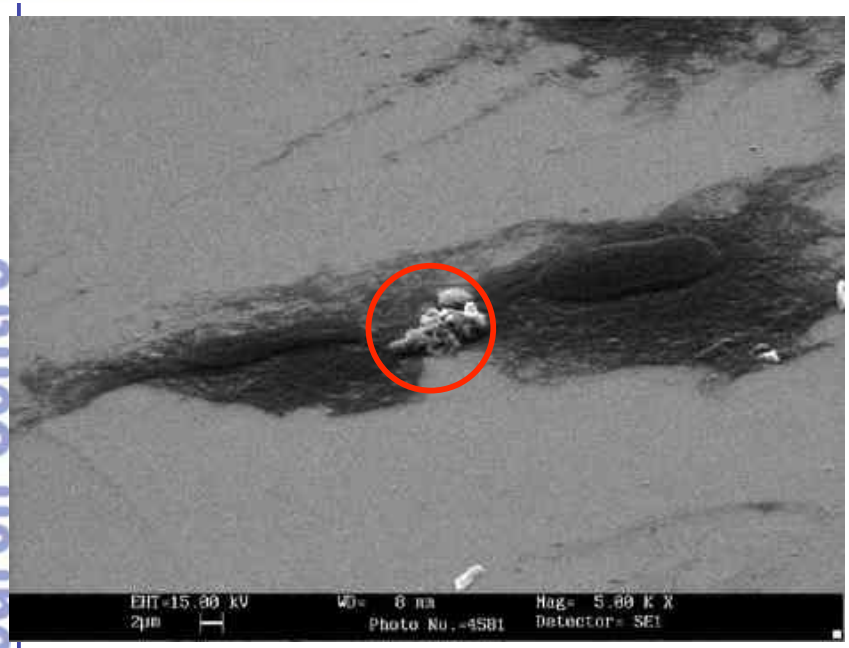
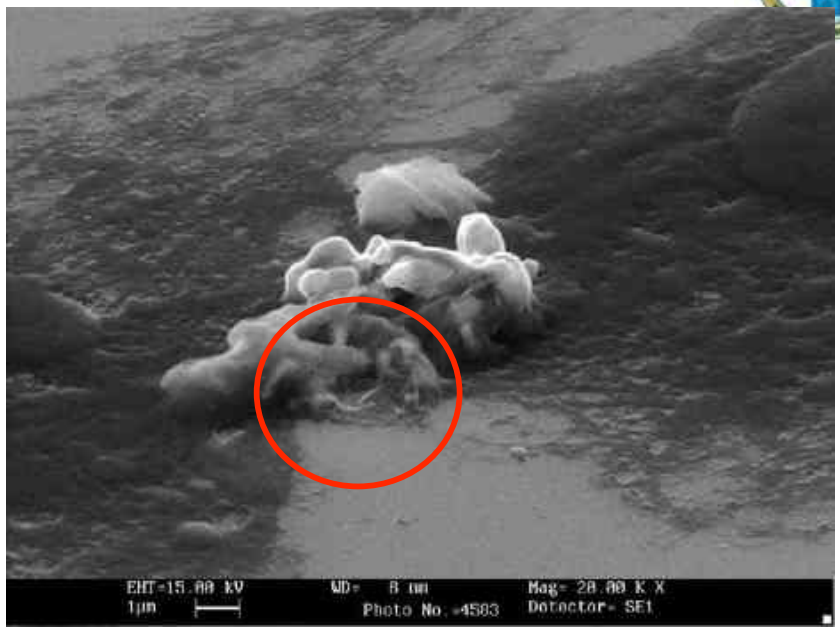
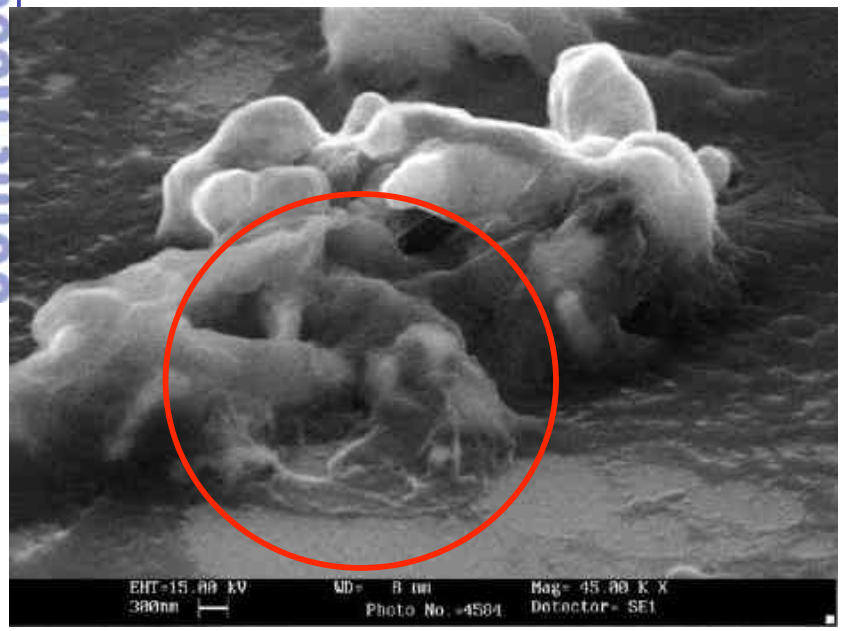
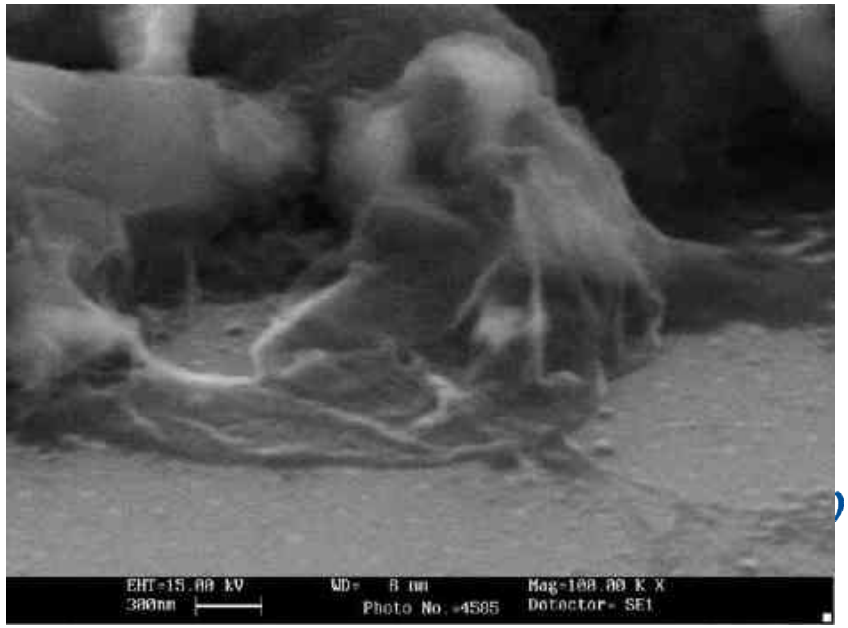
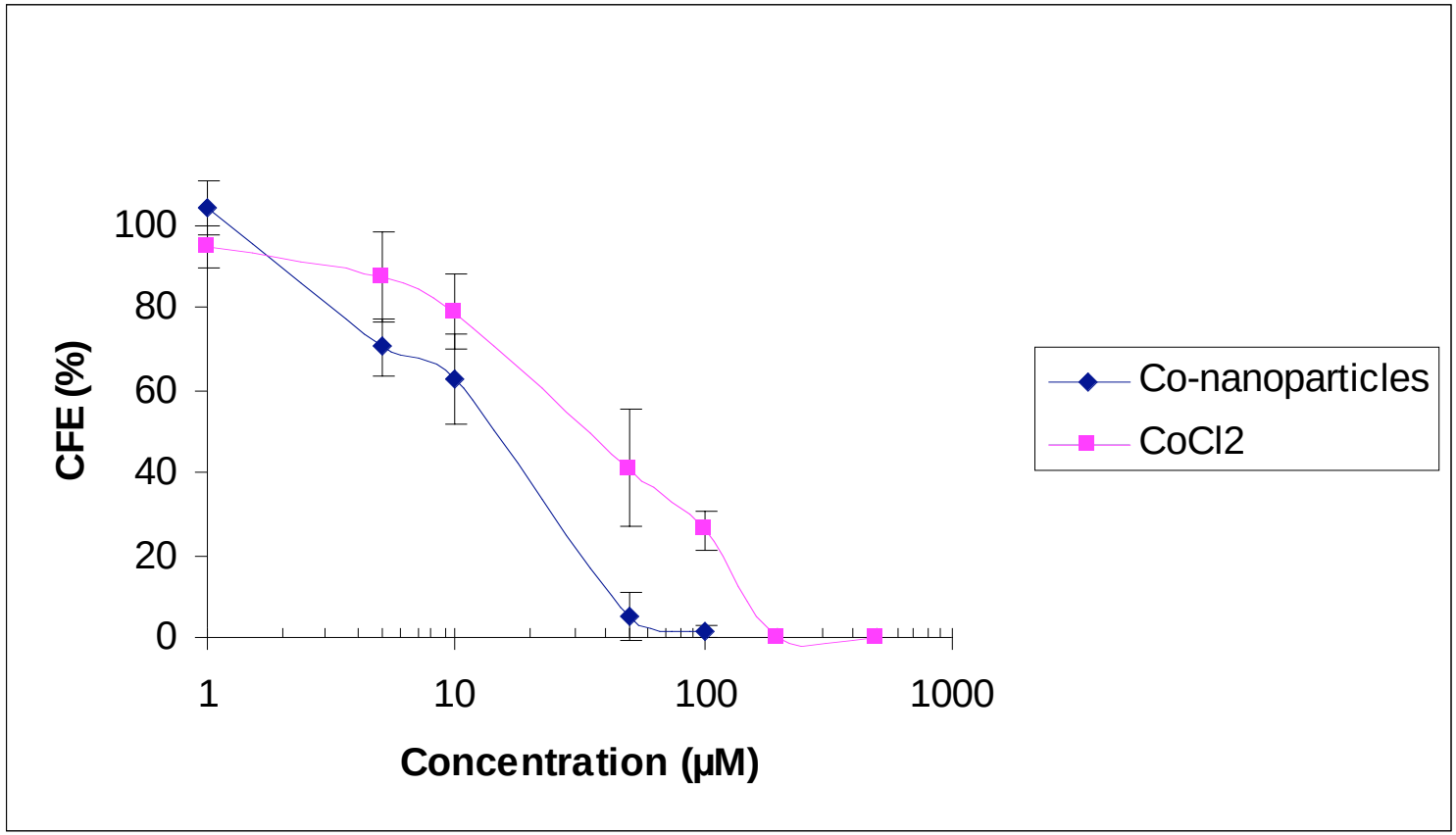








Cytotoxicity induced by Co-sol and Co-nano in Balb/3T3 cell line at 4h exposure



IC_{50}

Co_{sol} 41µM

Co_{nano} 9µM

Conclusion: quantitative difference of the induced cytotoxic effect

In Vitro nanotoxicology research on manufactured nanoparticles

The future …

- **Full integration of alternative (non animal) methods into "An Integrated and Responsible Approach" concerning a R&D European Strategy for Nanotechnology.**
- **Creation of a Key Area at ECVAM**

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