Exposure to Nanoparticles and Hormesis

Ivo Iavicoli, *Catholic University of Sacred Heart, Roma, Italy*
Marc Nascarella, *Gradient, Cambridge, MA*
Edward Calabrese, *University of Massachusetts, Amherst, MA*

The 9th Annual International Conference:
Dose-Response: Implications for Toxicology, Medicine, and Risk Assessment
April 27 - 28, 2010
University of Massachusetts Amherst

Nanotechnology is an emerging multidisciplinary science that involves applications based upon the synthesis of molecules in the nanoscale (10^{-9} m) size range.

This technology has the ability to manipulate matter on a near-atomic scale to produce new structures, materials and devices with unique *physical* and *chemical properties*.

Research in nanoscale technologies is growing rapidly worldwide.

The National Science Foundation (NSF 2001) estimated that, by 2015, nanotechnology will have a $1 trillion impact on the global economy and will employ 2 million workers, 1 million of which may be in the United States.

The industrial applications of nanomaterials are very wide including those that may lead to:

- ✓ more efficient water purification;
- ✓ stronger and lighter building materials;
- ✓ increased computing power and speed;
- ✓ improved generation and conservation of energy;
- ✓ new tools for the diagnosis and treatment of diseases.

Silica (SiO_2) NPs

They are used:

- ✓ as biomarkers for leukemia cell identification, cancer therapy, drug delivery;
- ✓ for the chemical mechanical polishing;
- ✓ as additives to drugs, cosmetics, printer toners, varnishes and food.

Metals Nanoparticles

- ✓ Cerium oxide NPs have wide ranges of applications for solar and fuel cells, gas sensors, abrasives for chemical mechanical planarizations, oxygen pumps, metallurgic, glass and ceramic applications.

- ✓ Titanium dioxide NPs are used for photocatalysts, paints, sterilization, bio-medical ceramic and implanted biomaterials, cosmetics and pharmaceuticals.

- ✓ Silver NPs are used in bedding, washers, water purification, tooth paste, shampoo and rinse, infant nipples and nursing bottles, fabrics, deodorants, filters, kitchen utensils, toys and humidifiers.

Carbon Nanotubes (CNTs)

Carbon nanotubes (CNTs) are currently of interest for a variety of applications in:

- ✓ electronics;
- ✓ reinforced rods;
- ✓ micro-fabricating conjugated polymer activators;
- ✓ biosensors and enhanced electron/scanning microscopy imaging techniques.

With the increased applications of nanoparticles the concerns about their potential human toxicity and their environmental impact have also been increased.

Indeed, as a result of their small size and unique physicochemical properties, the toxicological profiles of NPs may differ considerably from those of larger particles composed of the same materials.

Consequently, nanotoxicology is emerged to elucidate the relationship of the physical and chemical properties, such as size, shape, surface chemistry, composition, aggregation and surface area, of nanomaterials with induction of toxic biological responses.

The aim of our study has been to explore the possible presence of hormesis in the studies that have investigated the adverse health effects of nanoparticles.

In the literature there are some *in vitro* and *in vivo* toxicological studies that observed a hormetic dose-response determined by the exposure to nanoparticles, in particular carbon nanotubes, quantum dots, metal nanoparticles.

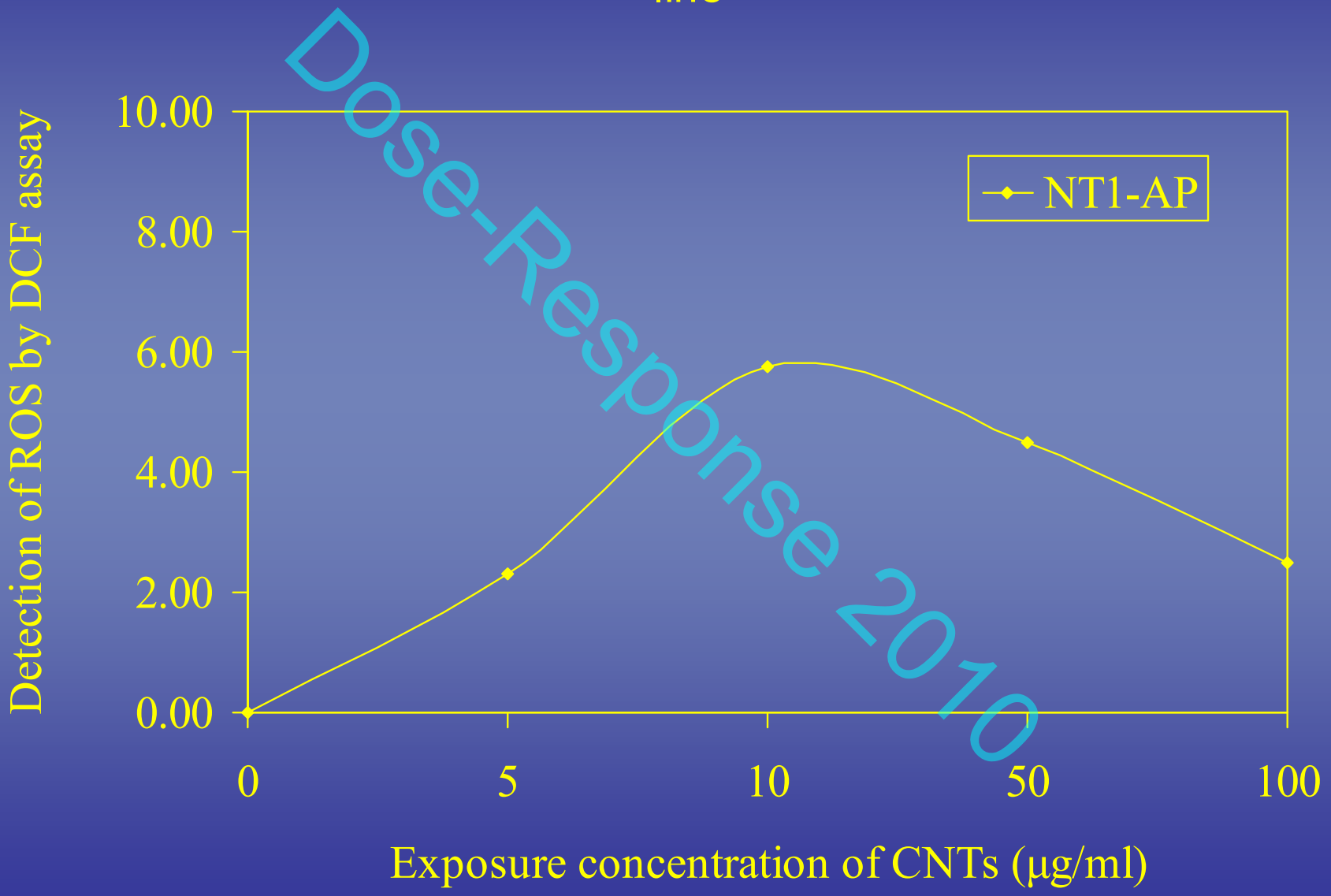
Human lung epithelial cells show biphasic oxidative burst after single-walled carbon nanotube contact

Pulskamp *et al.* (2007a) have exposed the human alveolar epithelial cells A549 at various concentrations (5-100 µg/ml) of different CNTs preparations to elicit oxidative stress response.

Cultured cells were assayed for short-term incubation of 10 minutes as well as for an extended period of 24 hours.

NT1-AP (the most impure form of CNTs) showed a stimulation of ROS production at 5 and 10 µg/ml while the ROS levels decreased at 50 and 100 µg/ml.

Effect of CNTs (NT1-AP) on ROS production in A549 cell line

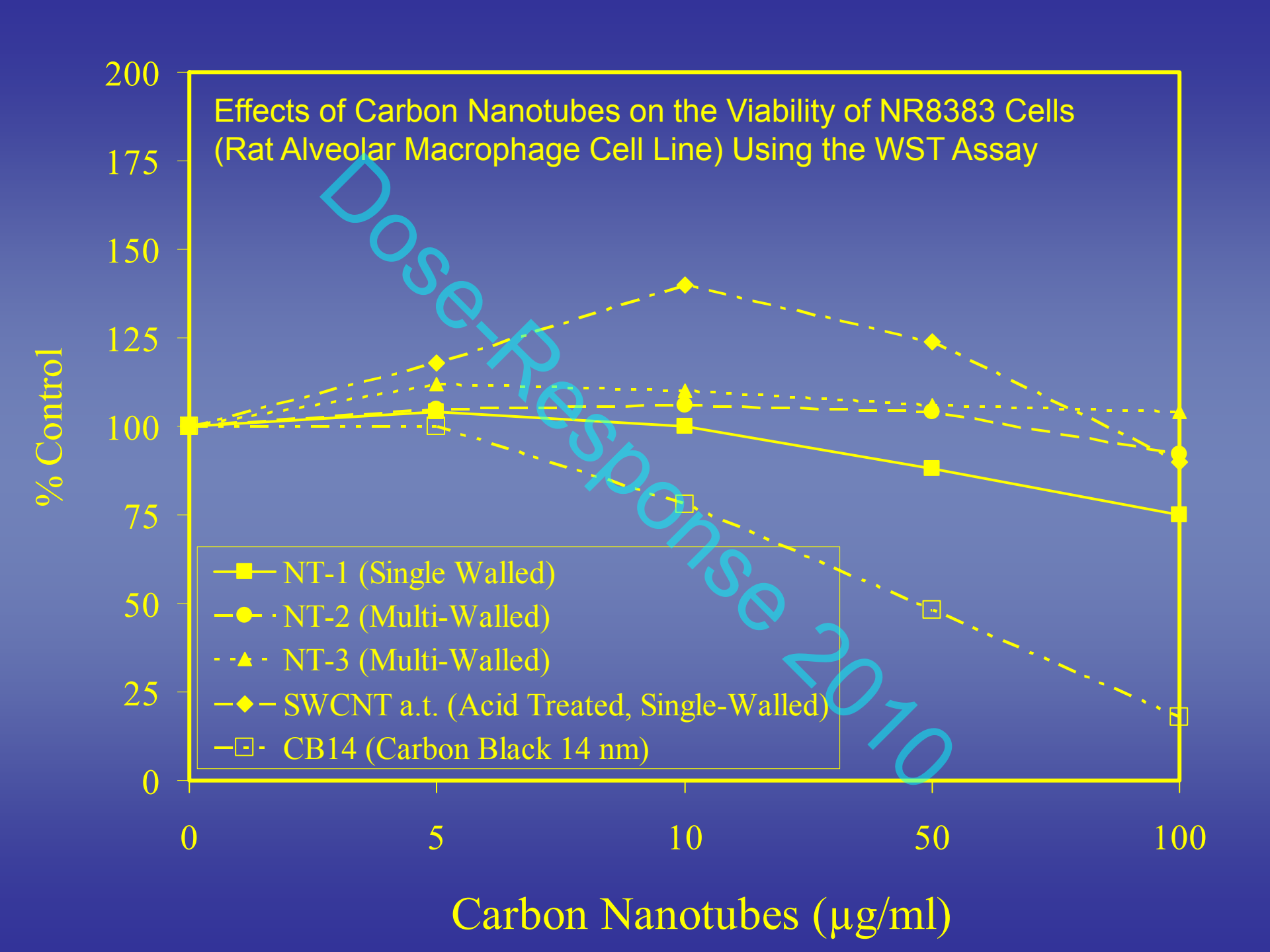


Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants

The same Authors studied, in the rat alveolar macrophage cell line NR8383, the cytotoxic effects caused by the exposure to different types of commercially available CNTs.

Cell viability of the cells, treated with 5, 10, 50 and 100 µg/ml of nanoparticles was evaluated using the WST-1 assay.

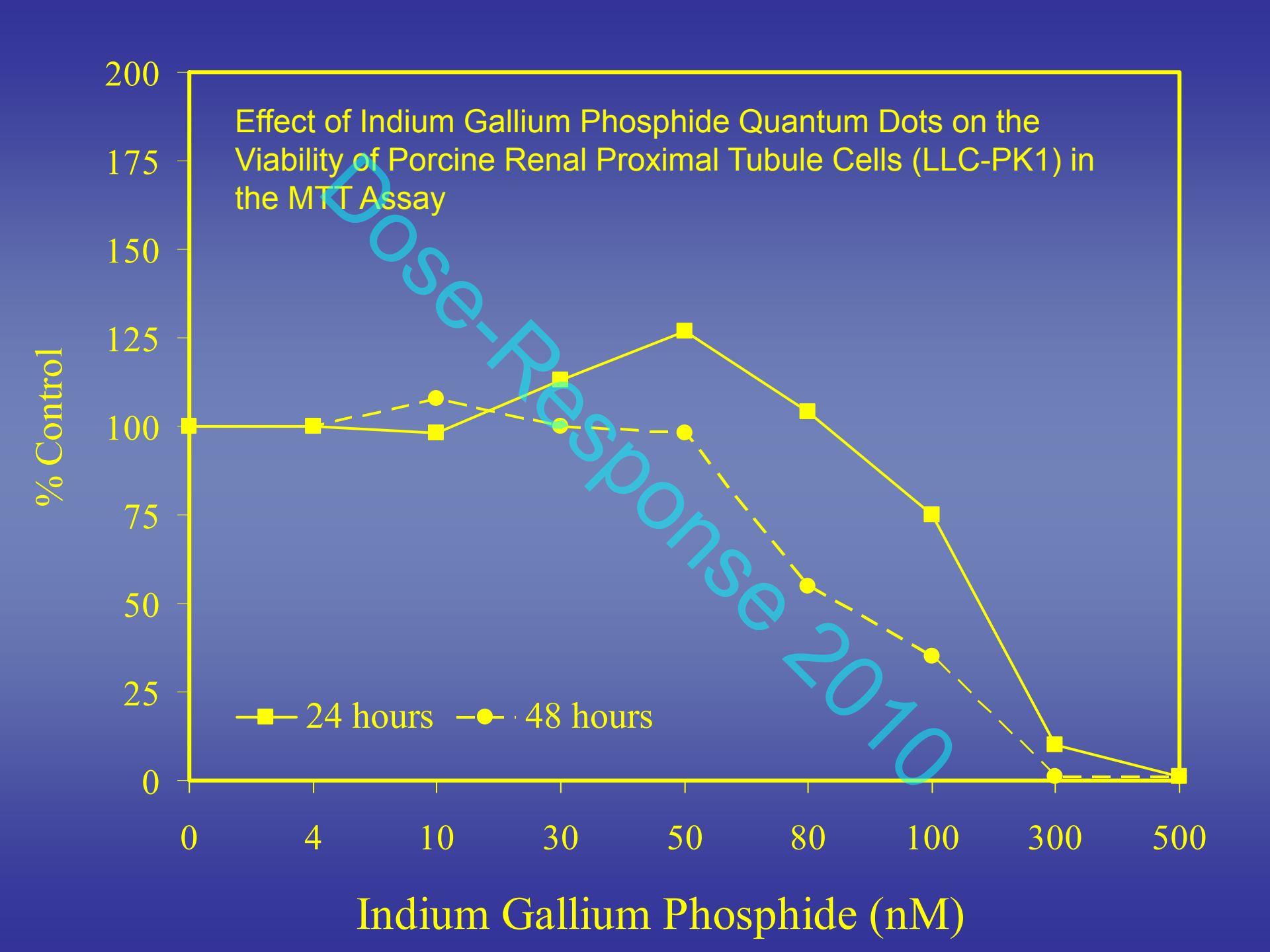
At lower concentrations it was observed an increase of viability, which rather suggested an increase of proliferation upon particle stimulation. Then, these findings showed a low dose stimulation and a high dose inhibition.


Effects of Carbon Nanotubes on the Viability of NR8383 Cells
(Rat Alveolar Macrophage Cell Line) Using the WST Assay
Dose-Response 2010
% Control
200
175
150
125
100
75
50
25
0
0
5
10
50
100
Carbon Nanotubes (µg/ml)
NT-1 (Single Walled)
NT-2 (Multi-Walled)
NT-3 (Multi-Walled)
SWCNT a.t. (Acid Treated, Single-Walled)
CB14 (Carbon Black 14 nm)

Induction of autophagy in porcine kidney cells by quantum dots: a common cellular response to nanomaterials?

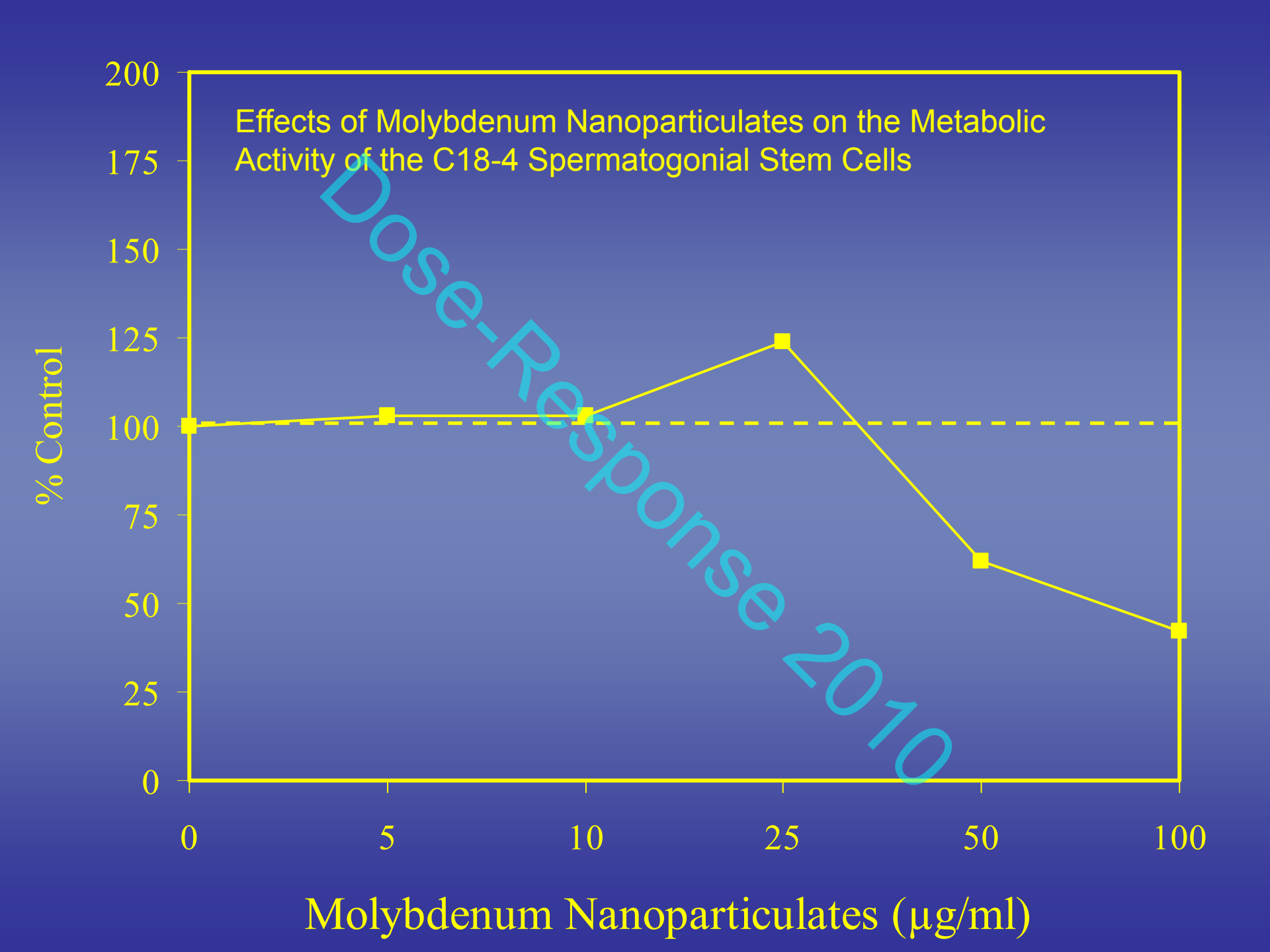
Stern *et al.* (2008) studied the toxicity of QDs, with a core material of indium gallium phosphide (InGaP-QDs), on porcine renal proximal tubule cell line (LLC-PK1) treated for 24 and 48 h with concentrations of InGaP-QDs in the range of 4 – 1000 nM.

In the 24 h experiment, the results of MTT assay showed an important loss of cell viability at concentrations exceeding 100 nM, while in the range of 30 – 80 nM there was a significant increase of cell viability.


Effect of Indium Gallium Phosphide Quantum Dots on the Viability of Porcine Renal Proximal Tubule Cells (LLC-PK1) in the MTT Assay
Dose-Response 2010
% Control
200
175
150
125
100
75
50
25
0
24 hours
48 hours
0
4
10
30
50
80
100
300
500
Indium Gallium Phosphide (nM)

In vitro cytotoxicity of nanoparticles in mammalian germline stem cells

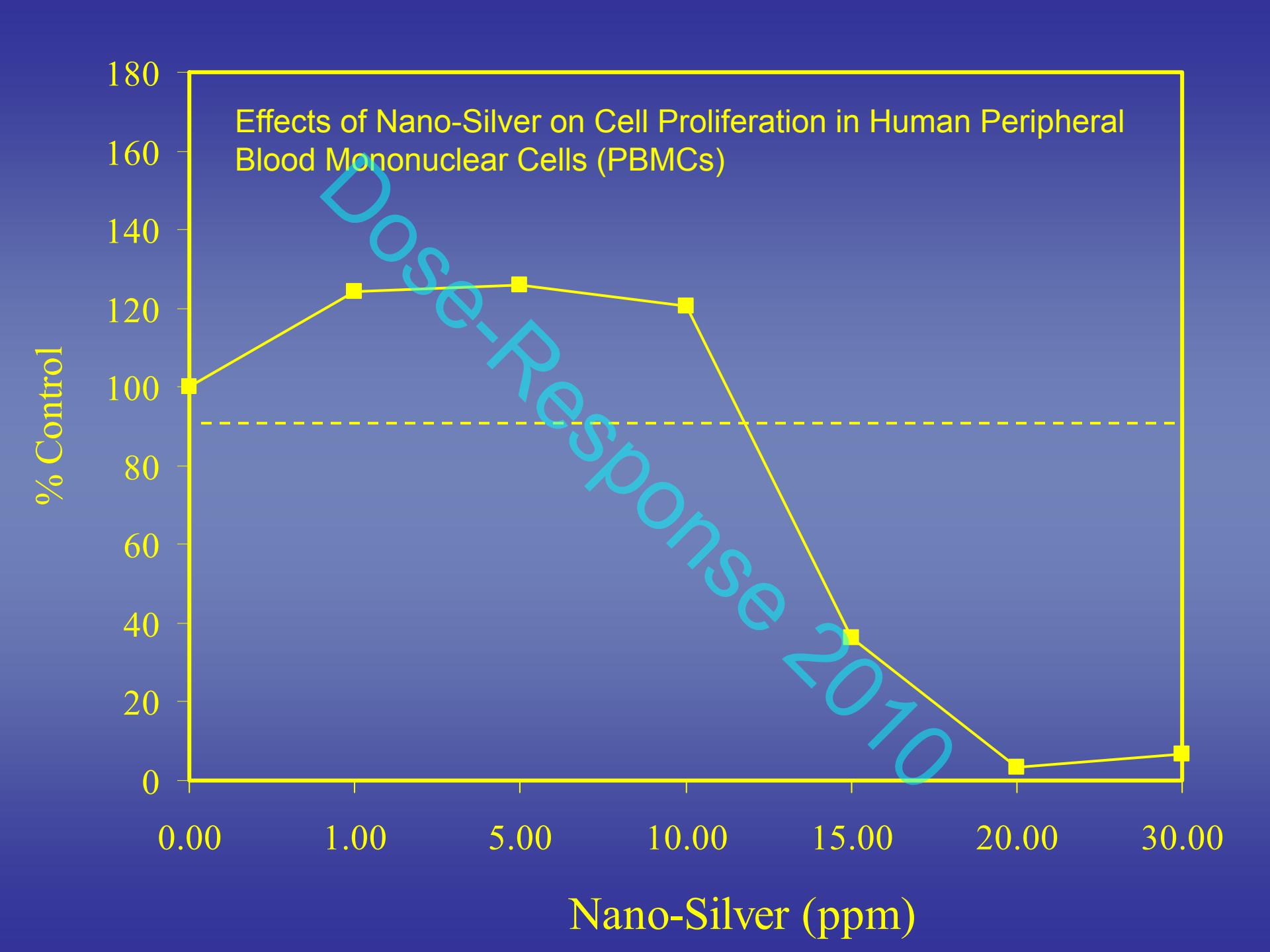
Braydich-Stolle *et al.* (2005) showed that in the C18-4 cell line, Mo-NPs exert toxic effects on cellular metabolic activity at concentrations of 50 µg/ml and above whereas, at low doses (5 – 25 µg/ml) they stimulate the mitochondrial function.


Effects of Molybdenum Nanoparticulates on the Metabolic Activity of the C18-4 Spermatogonial Stem Cells
Dose-Response 2010
% Control
200
175
150
125
100
75
50
25
0
0
5
10
25
50
100
Molybdenum Nanoparticulates (µg/ml)

The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells

The effects on the proliferation of peripheral blood mononuclear cells (PBMCs) exposed for 72 h to 1, 3, 5, 10, 20 and 30 ppm of Ag-NPs (Shin *et al.* 2007) were studied and the results showed a dose – response relationship characterized by a low dose stimulation and a high dose inhibition.

In fact, cell proliferation was found to be significantly decreased at Ag-NPs concentrations exceeding 15 ppm whereas, at lower doses it was observed an important stimulatory effect.


Effects of Nano-Silver on Cell Proliferation in Human Peripheral Blood Mononuclear Cells (PBMCs)
Dose-Response 2010
% Control
180
160
140
120
100
80
60
40
20
0
0.00
1.00
5.00
10.00
15.00
20.00
30.00
Nano-Silver (ppm)

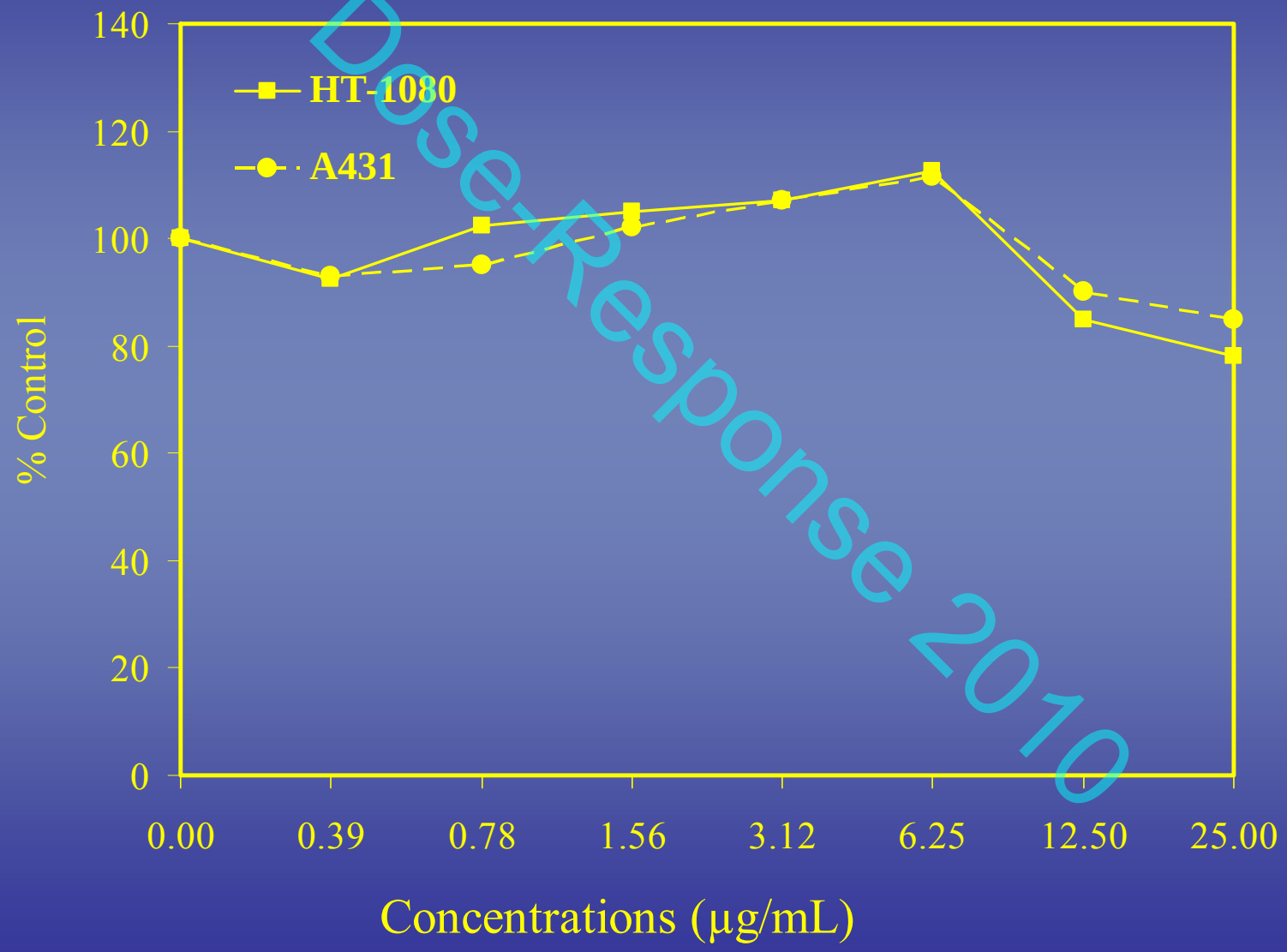
Cellular responses induced by silver nanoparticles: In vitro studies

Arora *et al.* (2008) studied the cellular responses of the cell lines A431 (human skin carcinoma) and HT-1080 (human fibrosarcoma) exposed to different concentrations of Ag-NPs.

In A431 cells, Ag-NPs induce caspase-3 production at concentrations in the range of 1.56 – 6.25 μg/ml but, at concentrations ≤ 0.78 and ≥ 12.5 μg/ml, data showed a lack of caspase-3 activity.

Similar results were obtained in HT-1080 cells where Ag-NPs induce caspase-3 production at concentrations in the range of 0.78 – 6.25 μg/ml whereas, at concentrations ≤ 0.39 and ≥ 12.5 μg/ml caspase-3 activity was not detected.

Effect of Silver Nanoparticles on Caspase-3 Activity

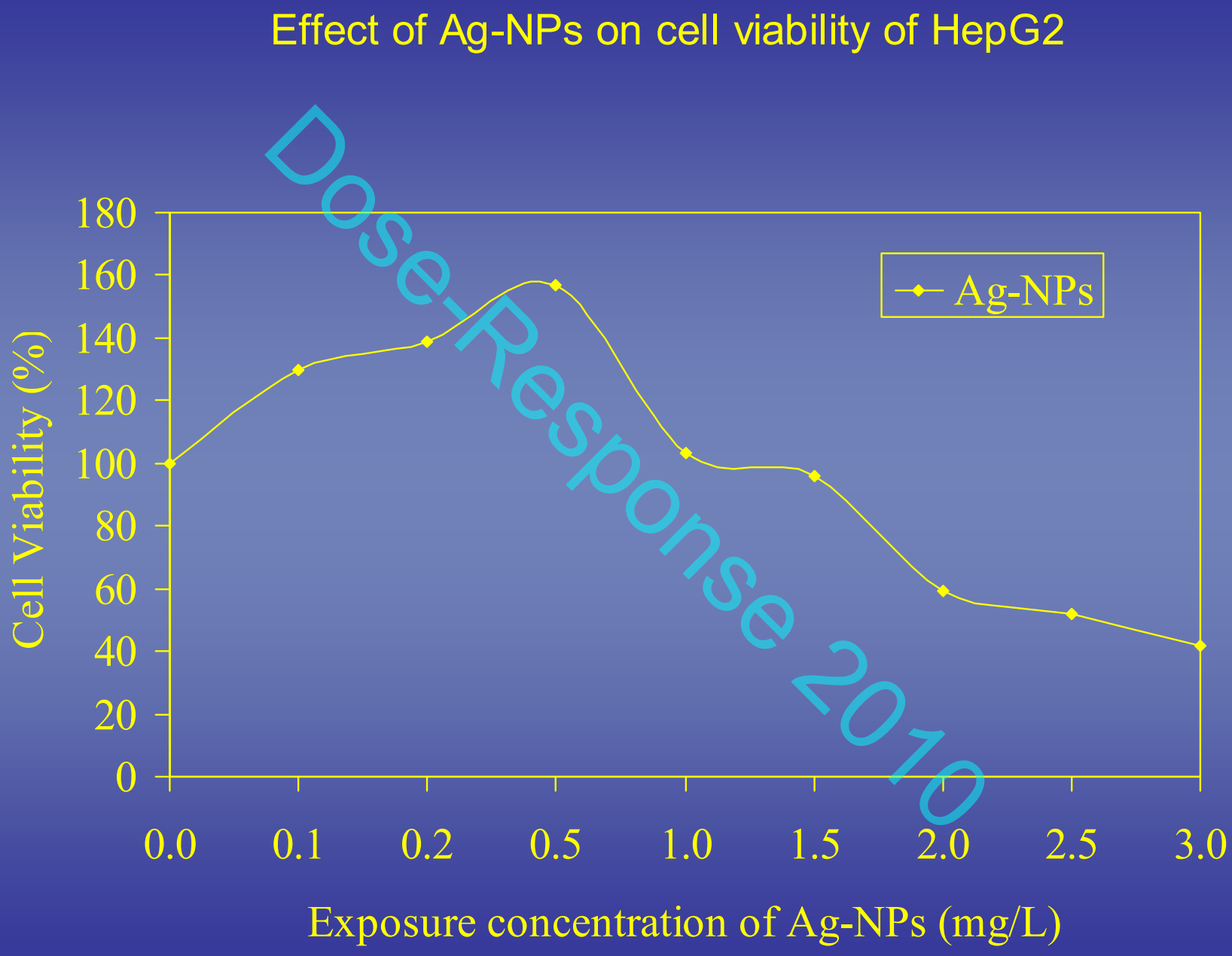


In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells

Similar results were obtained investigating the toxic effects of low exposure levels of Ag-NPs in human hepatoma derived cell line HepG2 (Kawata *et al.* 2009).

To evaluate the cytotoxicity of Ag-NPs, cells were exposed, for 24 hours, to 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l of the nanoparticles.

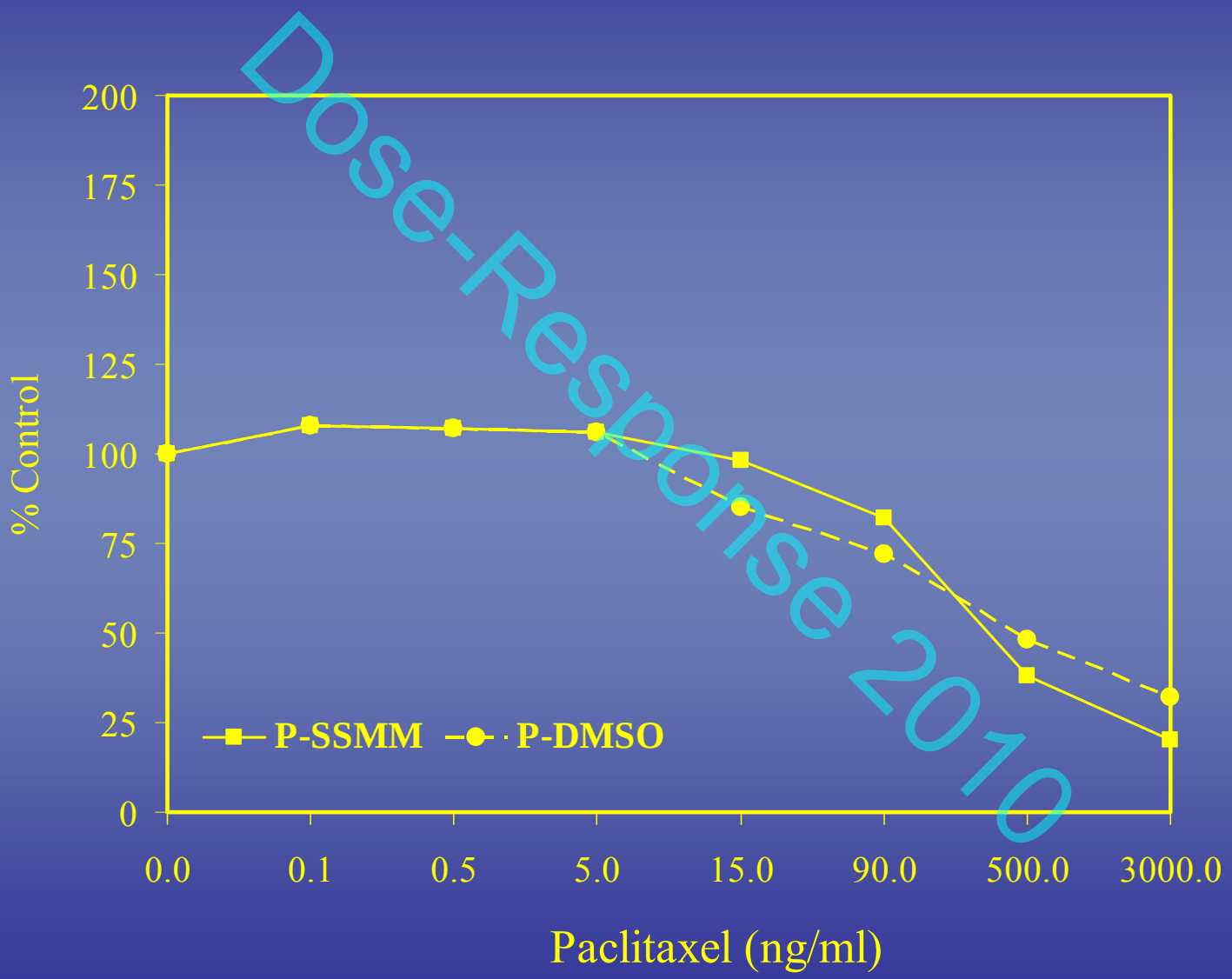
At high exposure levels, the tested material exhibited an important cytotoxicity. In fact, the cell viability of the HepG2 cells drastically decreased for Ag-NPs concentrations > 1 mg/l. Surprisingly, non cytotoxic doses (0.1 – 0.5 mg/l) of the nanoparticles significantly increased the viability of HepG2 cells.


Effect of Ag-NPs on cell viability of HepG2
Dose-Response 2010
Ag-NPs
Cell Viability (%)
180
160
140
120
100
80
60
40
20
0
0.0
0.1
0.2
0.5
1.0
1.5
2.0
2.5
3.0
Exposure concentration of Ag-NPs (mg/L)

Nanomicellar paclitaxel increases cytotoxicity of multidrug resistant breast cancer cells

A study performed to assess the efficacy of paclitaxel-loaded in biocompatible and biodegradable sterically stabilized mixed phospholipid nanomicelles (P-SSMM) and of paclitaxel dissolved in dimethyl sulfoxide (P-DMSO) in circumventing the P-glycoprotein-mediated paclitaxel resistance in the human breast cancer cell line BC19/3 revealed that the exposure of BC19/3 to P-SSMM and to P-DMSO at concentrations in the range of 0.128 – 2000 ng/ml showed a slight increase of cell survival at the lower doses and a significant inhibition of viability at concentrations exceeding 15 ng/ml.

Effect of Paclitaxel in Mixed Phospholipid Nanomicelles on Multidrug Resistant Human Breast Cancer Cells (BC19/3 Cells)

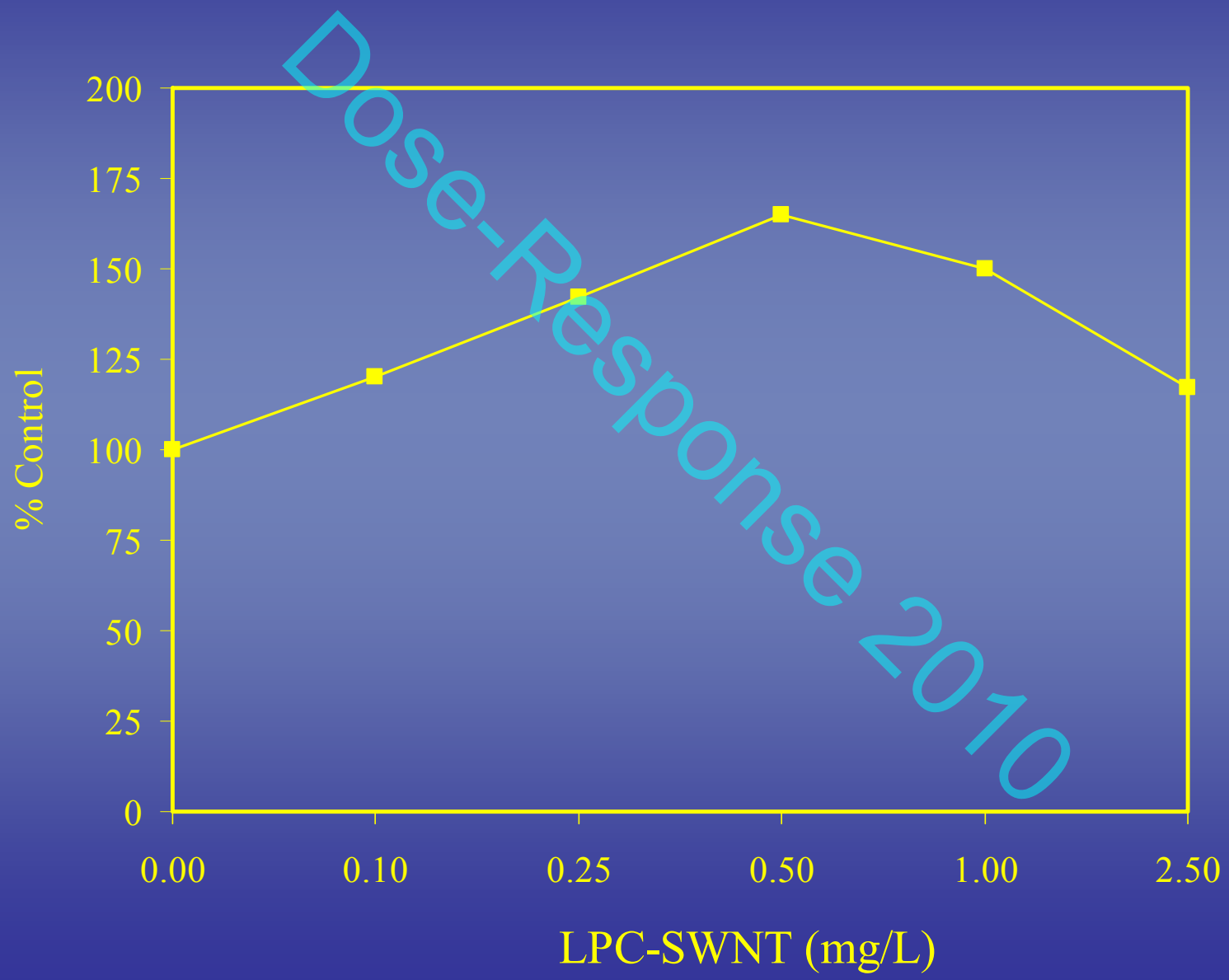


In vivo biomodification of lipid-coated carbon nanotubes by Daphnia magna

The assessment of survival of *Daphnia magna,* an aquatic invertebrate, exposed to 0.1, 0.25, 0.5, 1 and 2.5 mg/L of a water – soluble, lysophophatidylcholine coated single – walled carbon nanotube (LPC-SWCNTs) showed that the peak survival was reached at an LPC-SWCNTs concentration of 0.5 mg/L.

At higher test concentrations, survival decreased in a dose – dependent manner as opposed to survival at concentrations below 0.5 mg/L in which survival increased in a dose – dependent manner (*Roberts al.* 2007).

Effect of Lysophosphatidylcholine Coated Single-Walled Nanotubes (LPC-SWNTs) on Survival of *Daphnia Magna*

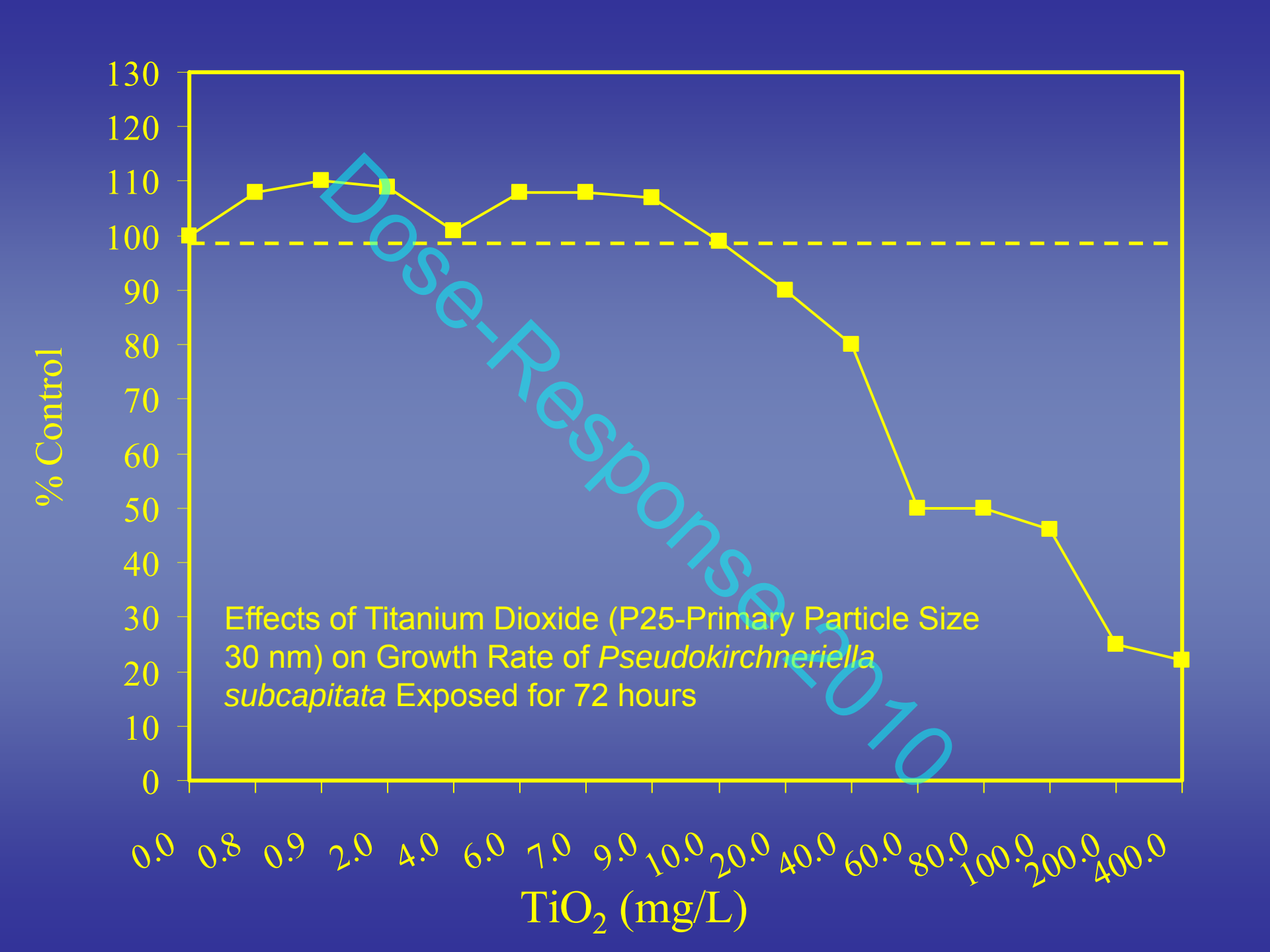


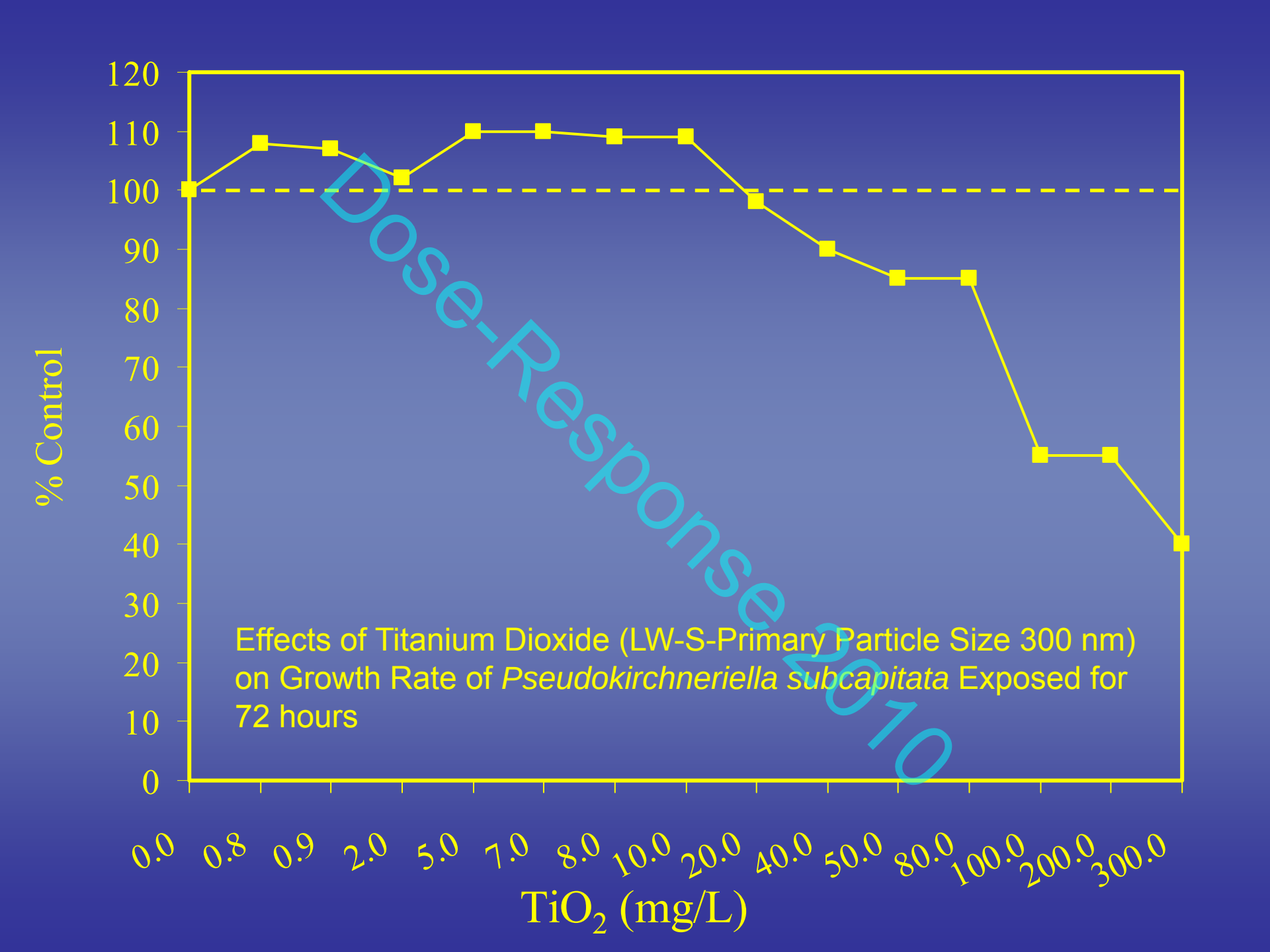
Algal testing of titanium dioxide nanoparticles-testing considerations, inhibitory effects and modification of cadmium bioavailability

TiO_2 nanoparticles of different sizes (10, 30 and 300 nm) were also used to assess the ecotoxicity to the freshwater green alga *Pseudokirchneriella subcapitata* (Hartmann *et al.* 2010).

In particular, the Authors assessed the growth rate inhibition of algae exposing them for 72 h to 16 concentrations (0.6 – 250 mg/L) of several TiO_2 nanoparticles.

Results showed a tendency of the smallest (10 nm) nanoparticles to induce higher inhibition at lower concentrations. However, for the larger nanoparticles (30 and 300 nm), a biphasic relationship with a statistically significant stimulation of the algal growth rate at lower concentrations and an inhibition at higher doses.


130
120
110
100
90
80
70
60
50
40
30
20
10
0
% Control
Dose-Response 2010
Effects of Titanium Dioxide (P25-Primary Particle Size 30 nm) on Growth Rate of *Pseudokirchneriella subcapitata* Exposed for 72 hours
0.0
0.8
0.9
2.0
4.0
6.0
7.0
9.0
10.0
20.0
40.0
60.0
80.0
100.0
200.0
400.0
TiO_2 (mg/L)

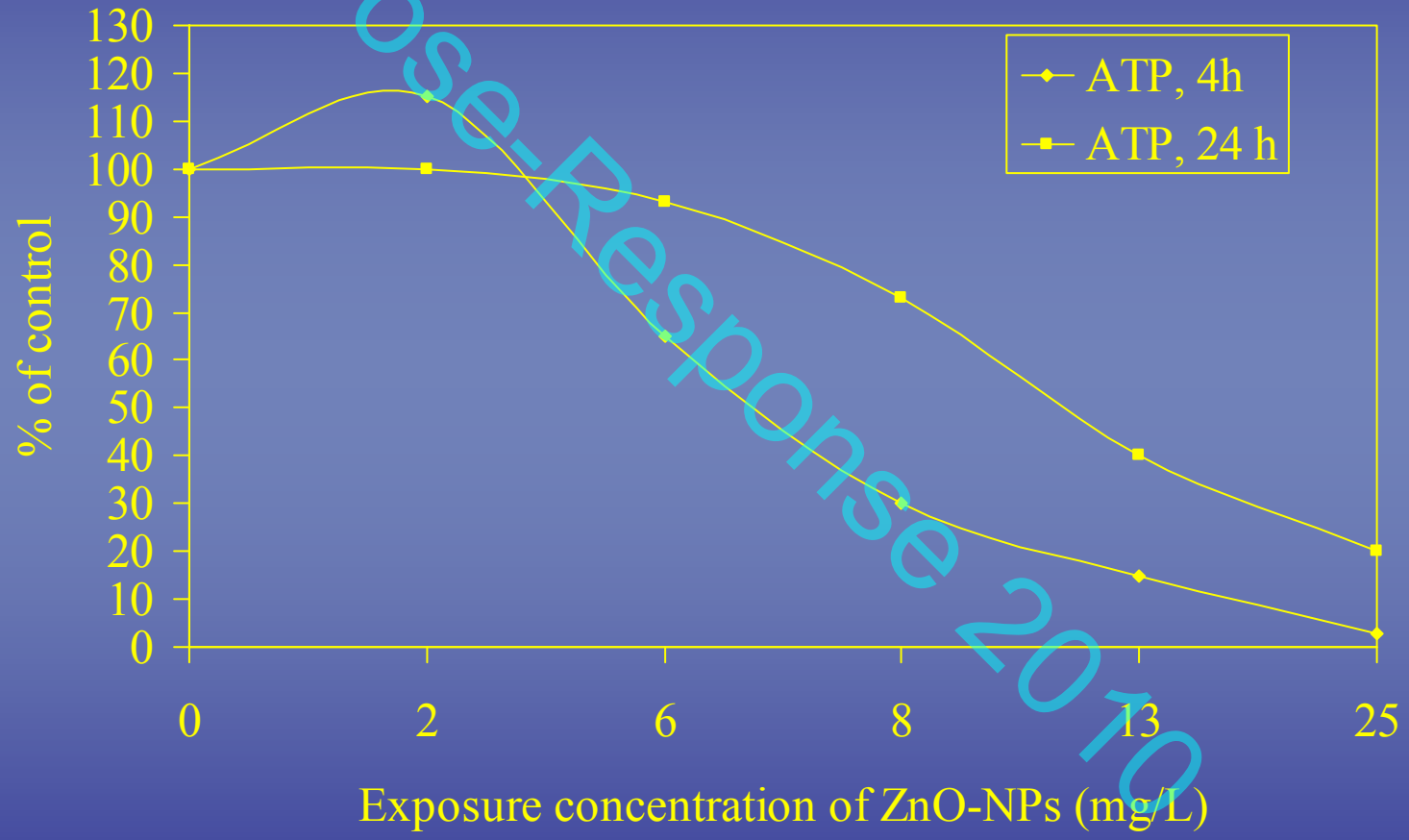

120
110
100
90
80
70
60
50
40
30
20
10
0
% Control
Dose-Response 2010
Effects of Titanium Dioxide (LW-S-Primary Particle Size 300 nm) on Growth Rate of *Pseudokirchneriella subcapitata* Exposed for 72 hours
0.0
0.8
0.9
2.0
5.0
7.0
8.0
10.0
20.0
40.0
50.0
80.0
100.0
200.0
300.0
TiO_2 (mg/L)

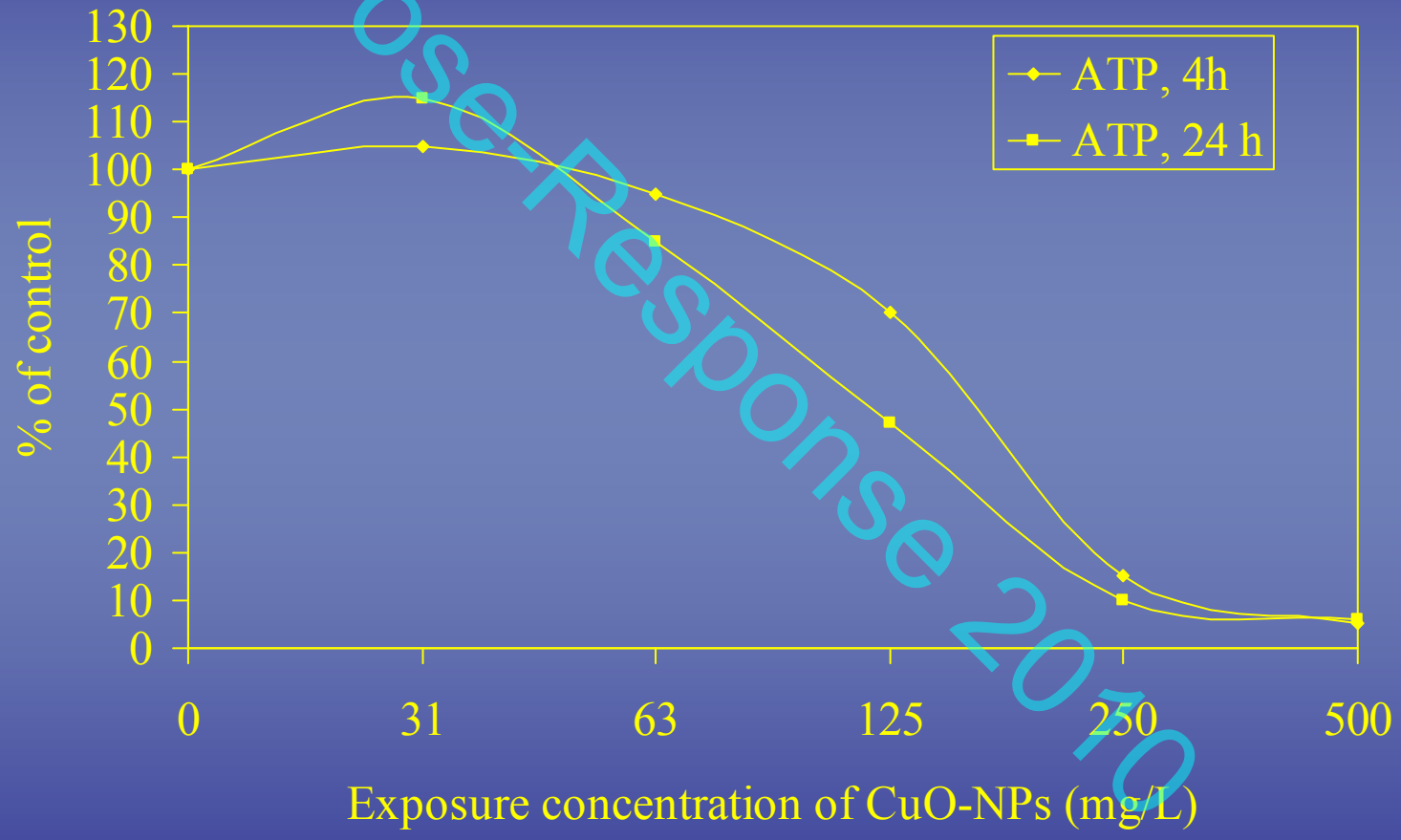
Toxicity of ZnO and CuO nanoparticles to ciliated protozoa Tetrahymena thermophila

Recently, a study by Mortimer *et al.* (2010), conducted on the ciliated protozoa *Tetrahymena thermophila* exposed to 31.25, 62.5, 125, 250 and 500 mg/L of copper nanoparticles (Cu-NPs) and to 1.85, 5.55, 8.33, 12,5 and 25 of zinc nanoparticles (Zn-NPs) showed the presence of a hormetic response.

The toxic effects of these nanoparticles to protozoa were evaluated, at two exposure times (4 and 24 h), using cellular ATP concentration that is correlated to the cell viability.

Interestingly, at the lowest and sub-toxic concentrations tested (31.25 mg/L and 1.85 mg/L for Cu-NPs and Zn-NPs, respectively) the nanoparticles had a stimulatory effect on ATP concentration of *Tetrahymena thermophila*.


Effect of ZnO-NPs on ATP concentration of Tetrahymena thermophila
Dose-Response 2010
ATP, 4h
ATP, 24 h
% of control
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
6
8
13
25
Exposure concentration of ZnO-NPs (mg/L)

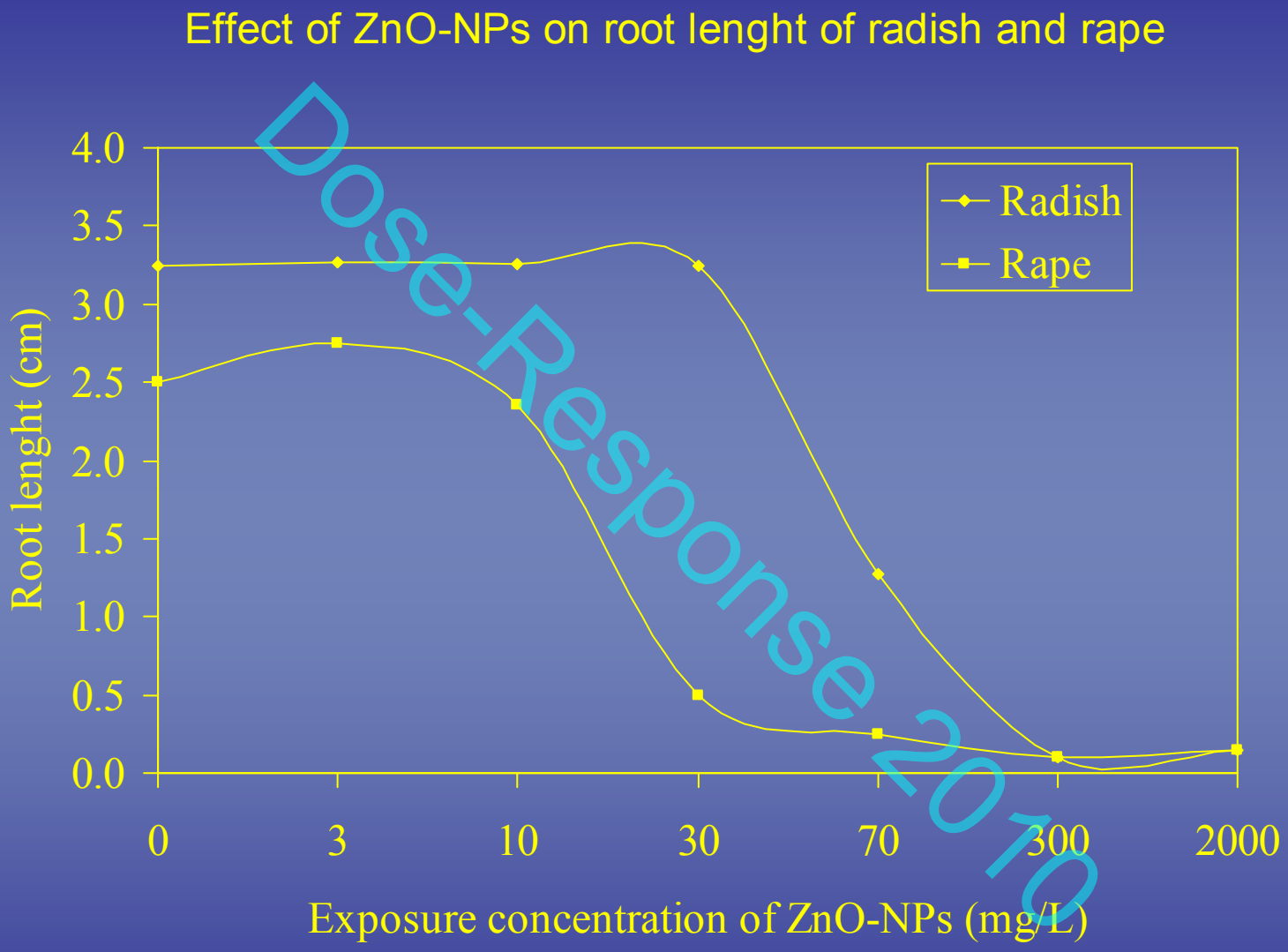

Effect of CuO-NPs on ATP concentration of Tetrahymena thermophila
Dose-Response 2010
ATP, 4h
ATP, 24 h
% of control
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
31
63
125
250
500
Exposure concentration of CuO-NPs (mg/L)

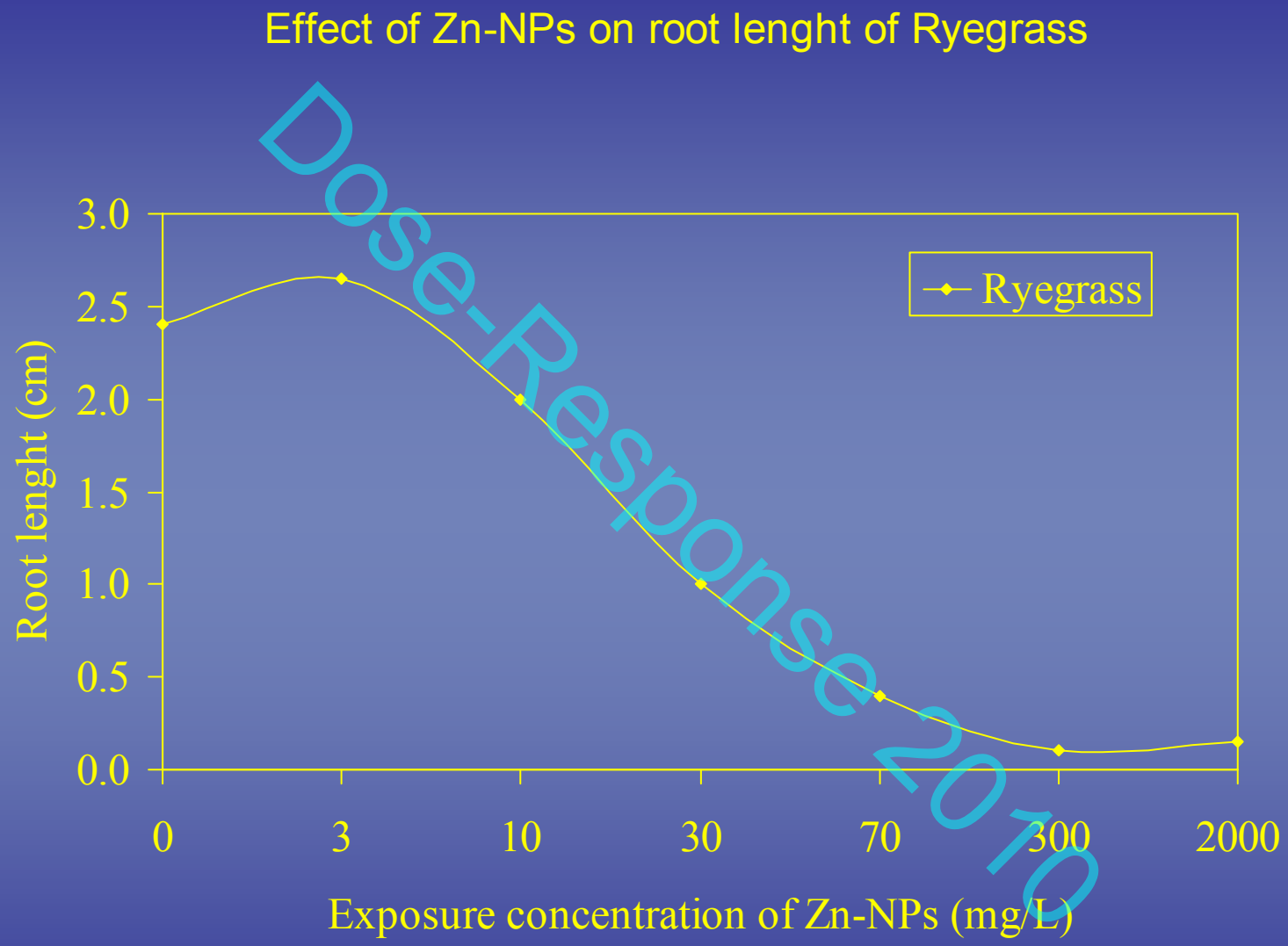
Phytotoxicity of nanoparticles: inhibition of seed germination and root growth

The assessment of the phytotoxicity of Zn-NPs and zinc oxide nanoparticles (ZnO-NPs) on seed germination and root growth of radish, rape and ryegrass showed a hormetic dose – response (Lin and Xing 2007).

No significant root growth inhibition was observed under low concentrations (less than 10 mg/L for rape and ryegrass and 20 mg/L for radish).

In particular, the exposure of radish and rape to lower doses of ZnO-NPs and of ryegrass to lower doses of Zn-NPs caused a slight increase of root length whereas, root growth of these plant species was clearly restricted with increasing concentration and was almost terminated at 200 mg/L.


Effect of ZnO-NPs on root lenght of radish and rape
Dose-Response 2010
Root lenght (cm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Radish
Rape
0
3
10
30
70
300
2000
Exposure concentration of ZnO-NPs (mg/L)


Effect of Zn-NPs on root lenght of Ryegrass
Dose-Response 2010
Root lenght (cm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Ryegrass
0
3
10
30
70
300
2000
Exposure concentration of Zn-NPs (mg/L)

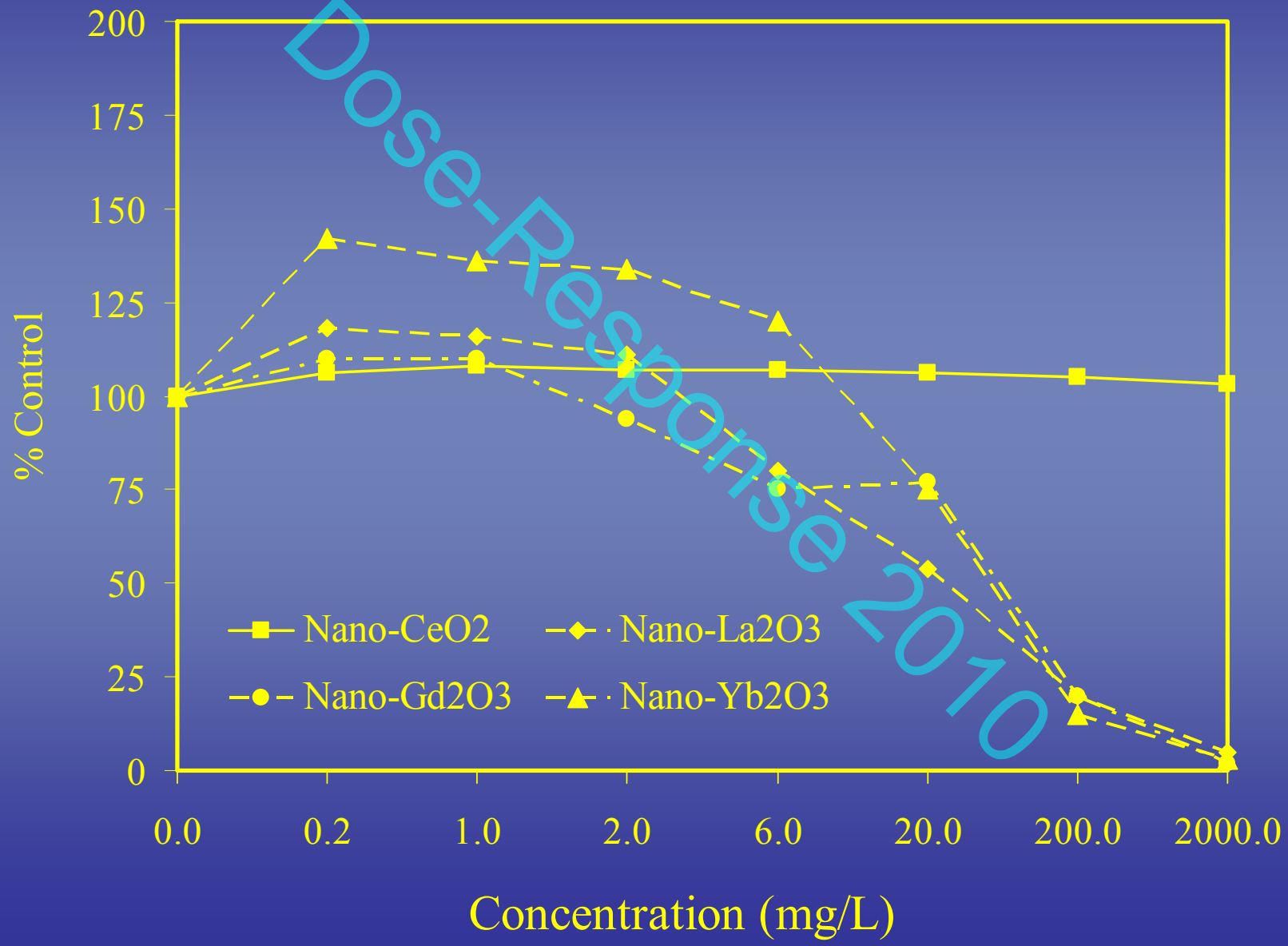
Effects of rare earth oxide nanoparticles on root elongation of plants

Ma *et al.* (2010), studied the effects of exposure to different concentrations (0.2 – 2000 mg/L) of rare earth oxide NPs on root elongation of rape.

After exposure to La_2O_3-NPs and Yb_2O_3-NPs, the root growth was promoted at less than 0.8 mg/L but, as the concentration increasing, the root growth was restricted and it was almost halted at 200 and 2000 mg/L.

Therefore, at low concentrations (< 0.8 mg/L) La_2O_3-NPs and Yb_2O_3-NPs had positive effects on root elongation but negative effects at higher concentrations.

Effects of Rare Earth Oxide Nanoparticles on the Root Growth of Rape



In the last years the number of studies that have investigated the adverse health effects of nanoparticles increased significantly.

However:

the toxicity of the nanoparticles has not been fully evaluated and, at present, there are many uncertainties as to whether the unique properties of engineered nanoparticles also pose a health risks for humans. These uncertainties arise because of gaps in knowledge about the factors that are essential for predicting health risks such as the nature of the dose-response curve at low level exposures below the toxic threshold.

Current evidence suggests that the biological impacts and the biokinetics of nanoparticles are dependent on their small size (surface area and size distribution), chemical composition (purity, crystallinity, electronic properties, etc.), surface structure (surface reactivity, surface groups, inorganic or organic coatings, etc.), solubility, shape, and aggregation (Nel *et al.* 2006).

Hence, a correct evaluation of the induction of a hormetic response by nanoparticles is not possible without a preliminary, accurate and precise characterization of these materials.

Conclusions

- ✓ The results of some studies suggest the possibility that nanoparticles are able to induce, in some experimental settings, a hormetic response for specific endpoints.
- ✓ Nevertheless, currently available data regarding this topic are extremely limited and fragmentary and for this reason at the present time it is not possible to achieve comprehensive conclusions or a broad consensus.
- ✓ More research is needed on the occurrence of the hormetic dose – response elicited by the exposure to the nanoparticles.
- ✓ To be able to detect the real presence of hormesis, future studies should carry out a depth and accurate characterization of nanoparticles, with a broad range of exposure doses and a series of temporal evaluations.

Thank you!