Evaluation of Toxicity and Bioaccumulation of Nanopariticles Using Aquatic Organisms

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Objectives

- Test toxicity of nanoparticles in several aquatic model organisms
- Evaluate bioconcentration, bioaccumulation, and biomagnification of nanoparticles in aquatic food chains
- Establish relationships between physicochemical properties of NPs and their bioaccumulation and toxicity.

Tested Nanomaterials and Their Bulk Counterparts

Particles	particle size	Purity (%)
C_{60}	< 200 nm	99.5
SWCNTs	D < 2 nm L = 5-15 μm	CNTs>90 SWCNTs>60
MWCNTs	D = 10-20 nm L = 5-15 μm	> 98.0
Carbon Black	20,000 nm	> 95.0
nZnO	20 nm	> 99.6
ZnO/Bulk	1,000 nm	> 99.0
$nTiO_2$	≤ 20 nm	> 99.5
TiO_2/Bulk	10,000 nm	> 99.0
nAl_2O_3	80 nm	> 99.9
Al_2O_3/Bulk	90,000 nm	> 99.0

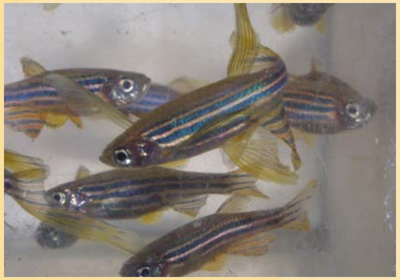
Note: "D" is diameter; "L" is length.

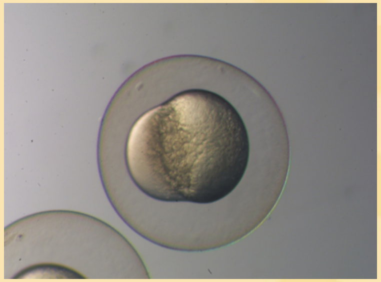
Model Organisms

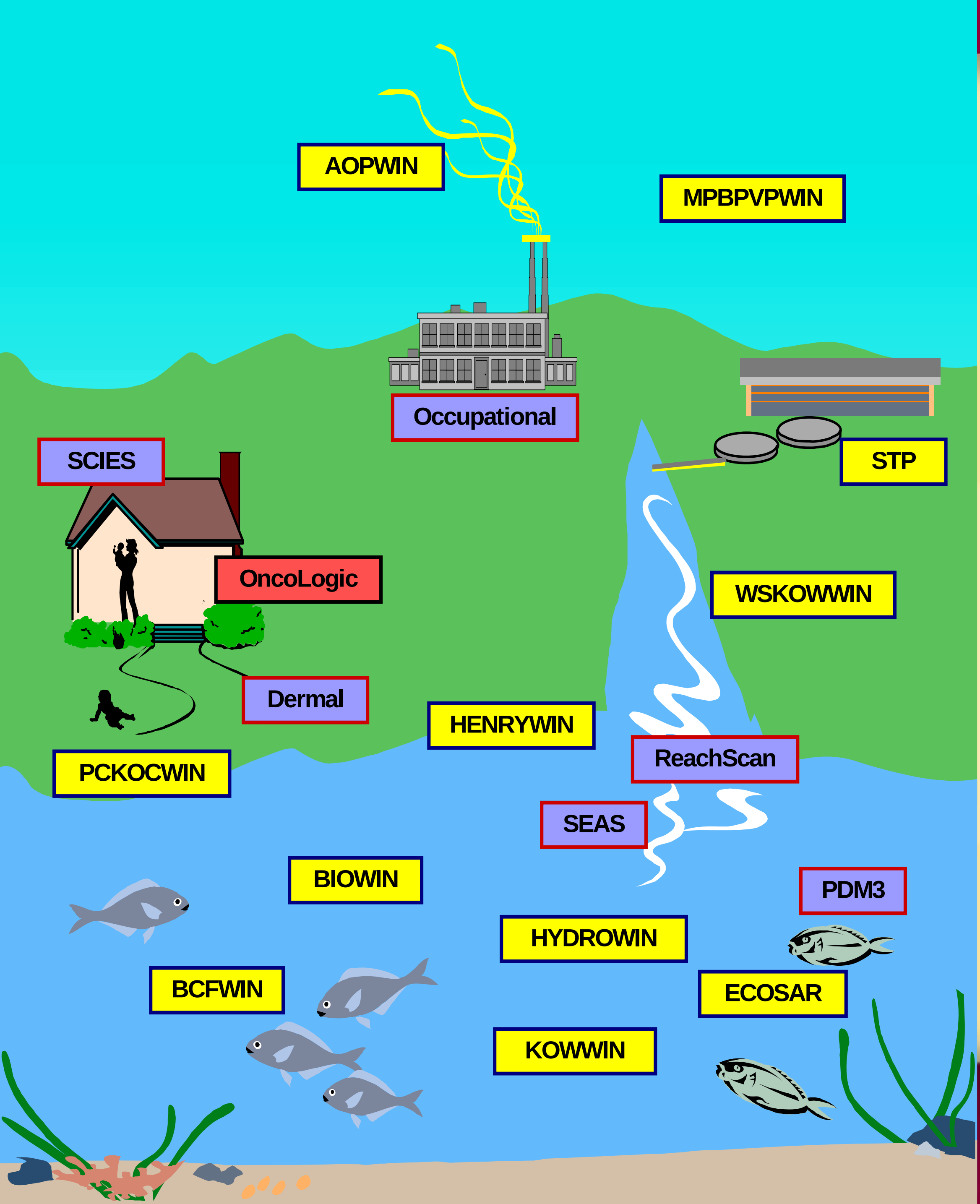
- Algae (Green Algae Chlamydomonas reinhardtii)

- Daphnia magna

- Zebra fish, Carp, and Embryo







QSAR Models

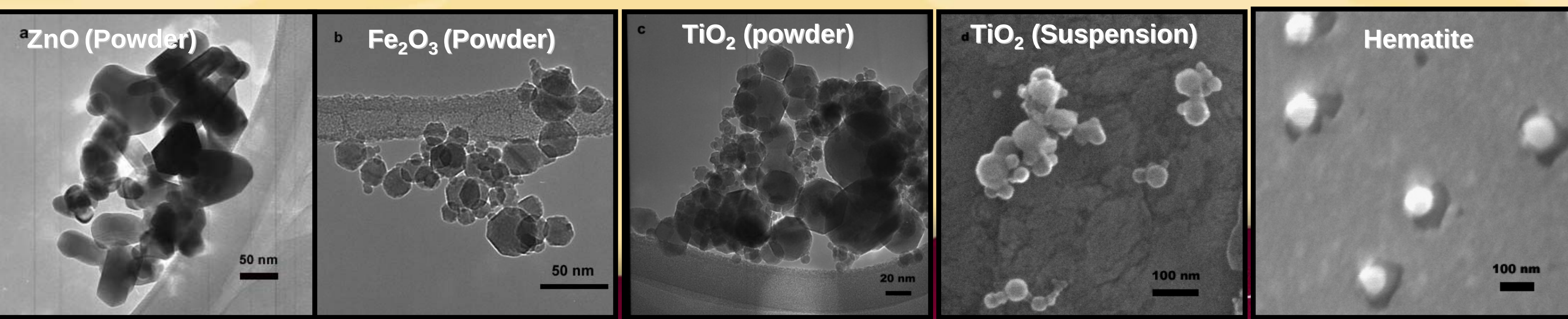
Pollution Prevention Assessment Framework Office of Pollution Prevention and Toxics - EPA

A collection of software developed to evaluate risk-related properties based on chemical structure.

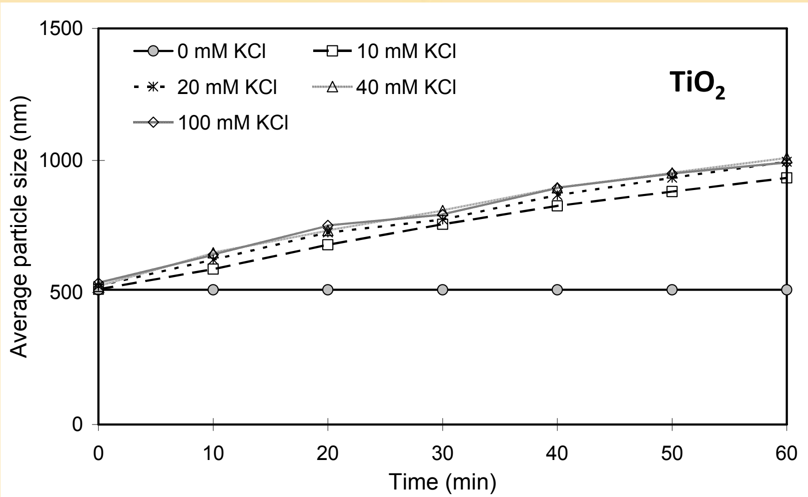
Characterization of Metal Oxides Nanoparticles

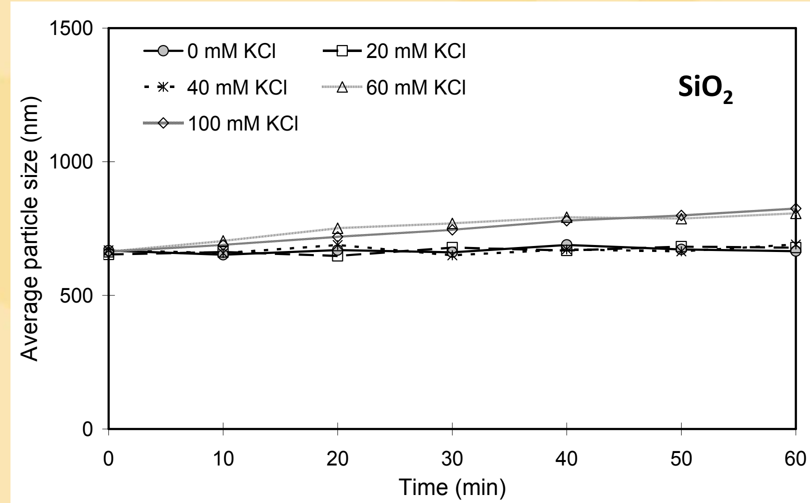
Nanoparticles	TiO_2 (powder)	TiO_2 (suspension)	Fe_2O_3	ZnO	NiO	SiO_2	Hematite
Primary particle size	5 ~ 50 nm [1]	10 ~ 60 nm [1]	5 ~ 25 nm [1]	50 ~ 70 nm [1]	10 ~ 20 nm [2]	10 nm [2]	80~ 90 nm [1]
DLS measured average sizes	530 ±30 nm	200 ±10 nm	200 ±10 nm	320 ±20 nm	750 ±30 nm	740 ±40 nm	85 ± 3 nm
DLS measured particle size distribution	Bimodal 150 & 750 nm	Bimodal 100 & 330 nm	Bimodal 60 & 200 nm	Bimodal 140 & 425 nm	Bimodal 330 & 1400 nm	Bimodal 300 & 2000 nm	Mono-modal 85 nm
MFI measured particles > 3 μm [3]	9300 ± 300 /ml	4800 ± 200 /ml	3500 ± 400 /ml	4400 ± 400 /ml	45000 ± 3000 / ml	20000 ± 200 /ml	n/a

[1] From SEM/TEM Images; [2] From Vendor Report

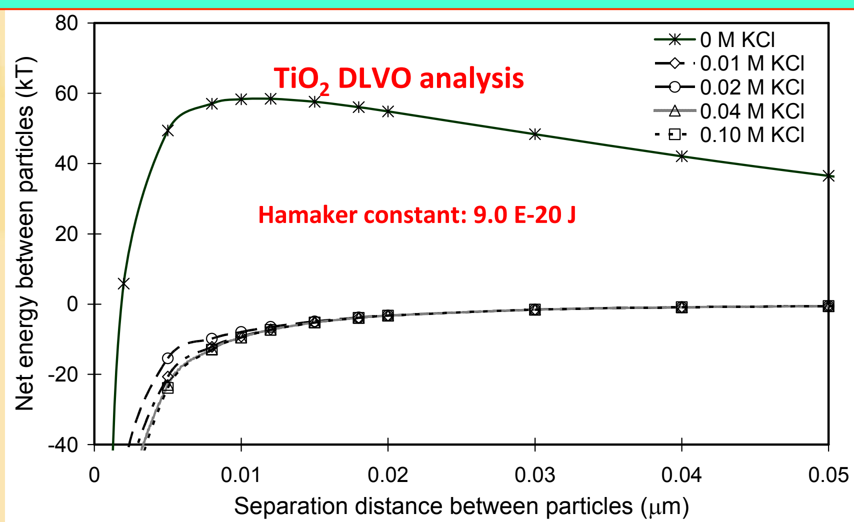


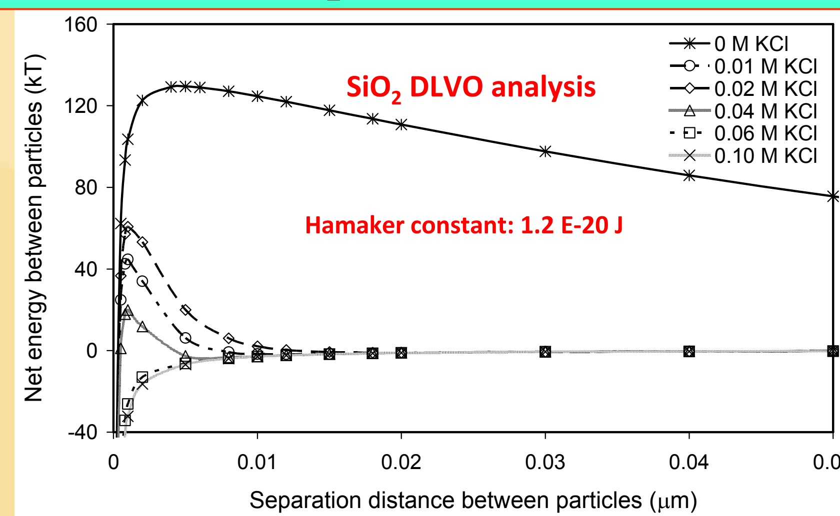
Stability of Metal Oxide NPs in Water





- The increase in ionic strength induces NP aggregation; however, SiO_2 is more stable than other NPs





Zhang Y., Y. Chen, et. al, Water research 2008(42):2204-2212

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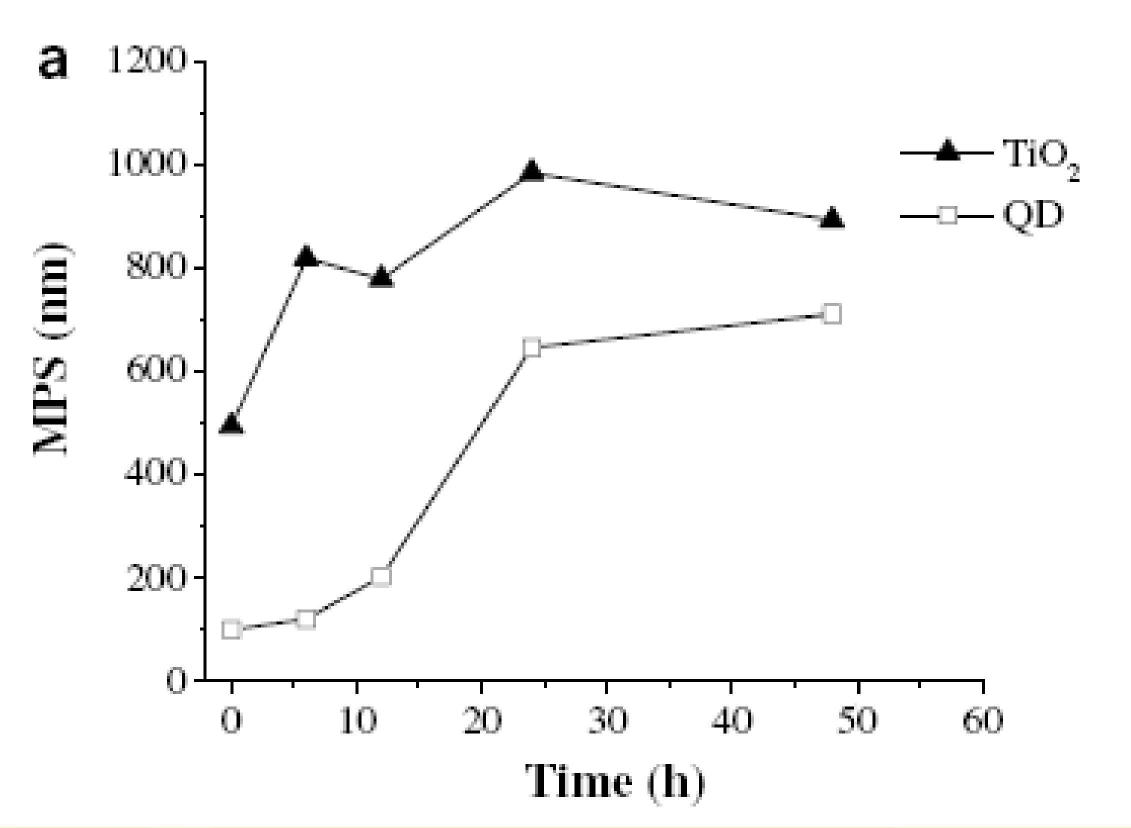
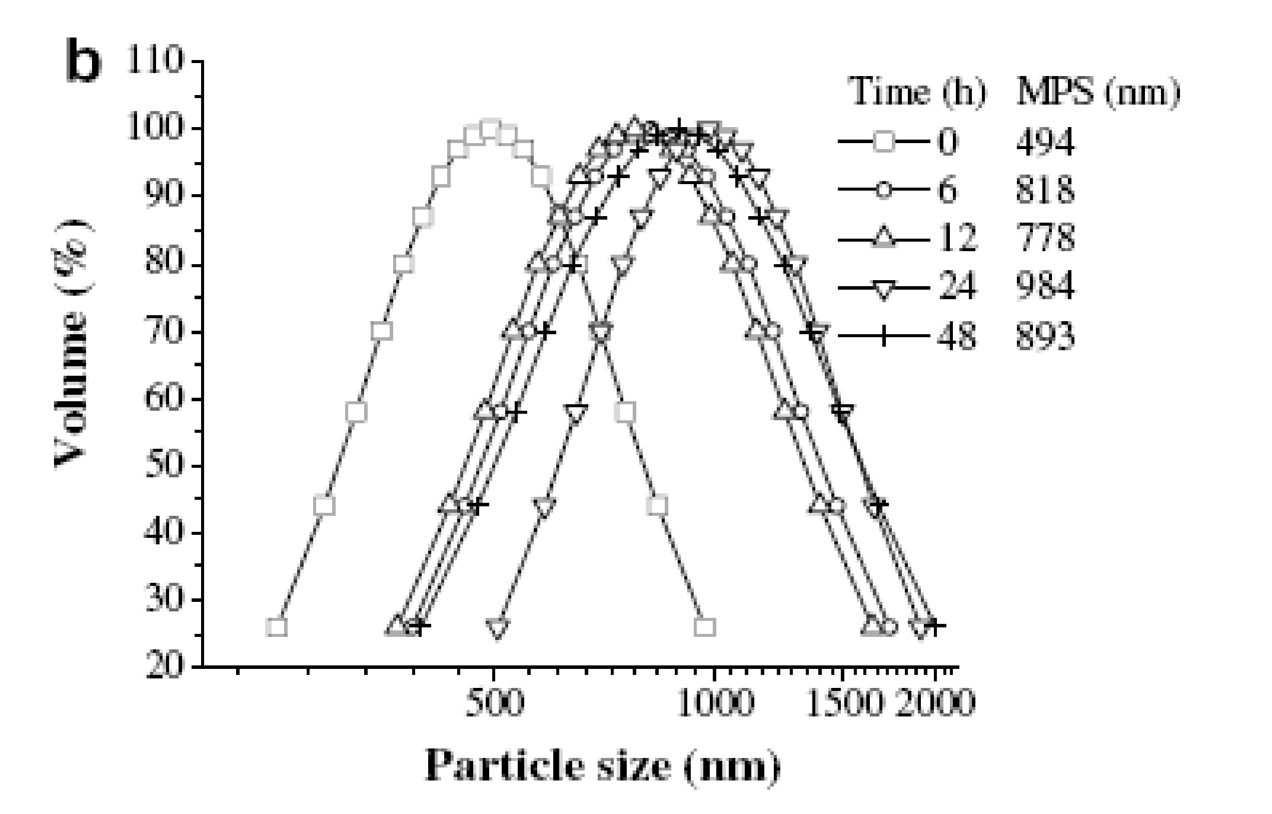
Characterization of particle size and particle number of NPs



Changes in median diameter of the particles of TiO_2 and QD NMs in buffer solution from 0 to 48 h

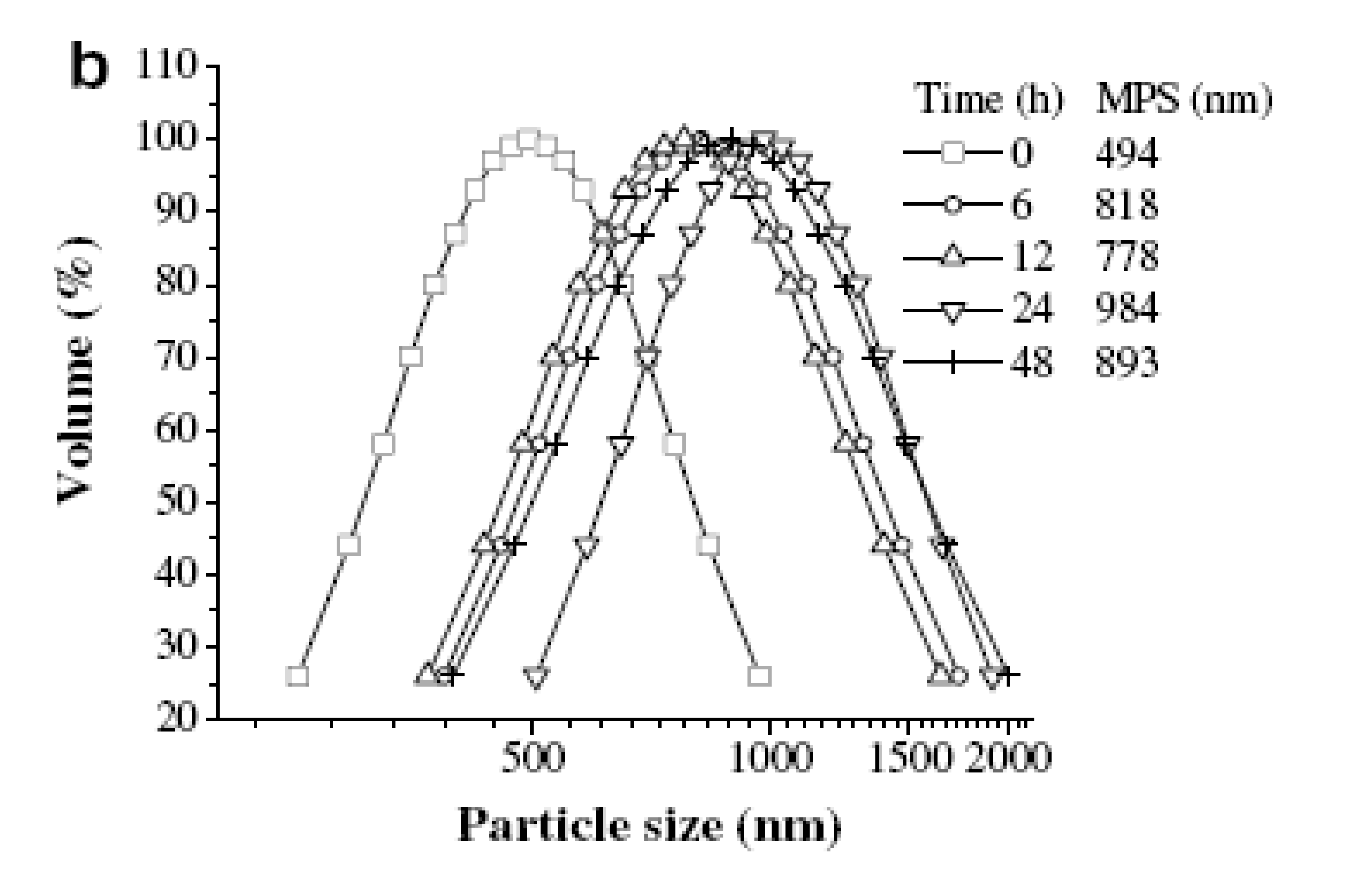
Characterization of particle size and particle number of NPs



Volume size distributions of TiO2 at different times

J. Wang, X. Zhang, Y. Chen, et. al, Chemosphere, 2008, 73: 1121–1128.

Characterization of particle size and particle number of NPs



Volume size distributions of QDs at different times

J. Wang, X. Zhang, Y. Chen, et. al, Chemosphere, 2008, 73: 1121–1128.

96 h EC50 of Nanoparticles on the Growth of Green Algae

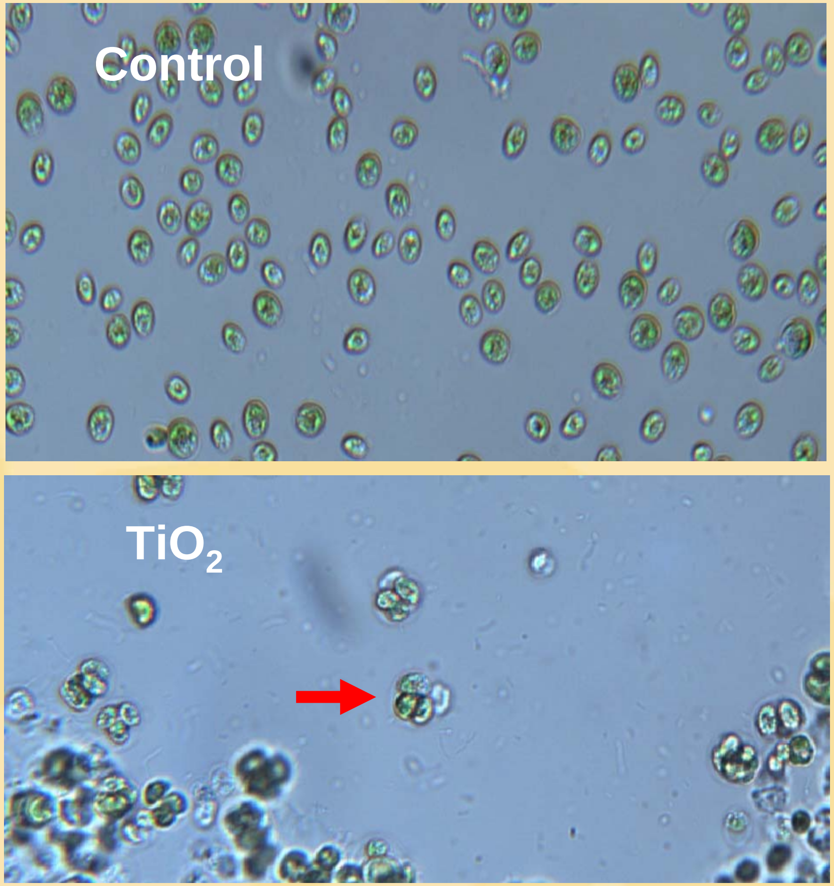
NPs	Regression Equation	Correlation Coefficient	EC_{50}（mg/L）
nZnO Suspension	y = 38.862x + 49.194	$R^2 = 0.9542$	1.049±0.565
C_{60} Suspension	y = 26.42x + 20.456	$R^2 = 0.8988$	13.122±4.182
$nTiO_2$ Suspension	y = 39.902x + 2.7719	$R^2 = 0.9275$	15.262±6.968
MWCNTs Suspension	y = 38.468x + 4.3117	$R^2 = 0.9964$	15.488±7.108
SWCNTs Suspension	y = 27.978x + 12.097	$R^2 = 0.8434$	22.633±9.605
nAl_2O_3 Suspension	y = 14.204x - 10.044	$R^2 = 0.5471$	>1000

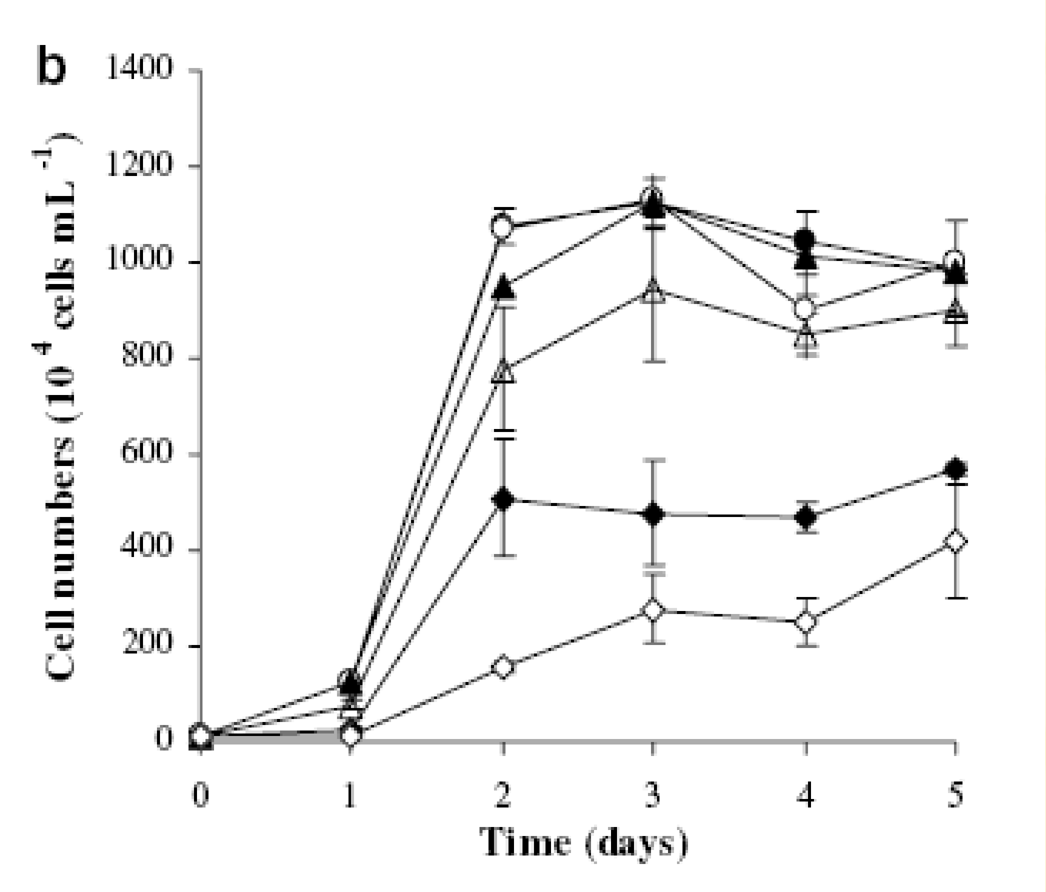
High toxicity

Low toxicity

X. Zhu, L. Zhu, Y. Chen, Journal of nanoparticle ***research***, 2009, Vol.11: 67-75

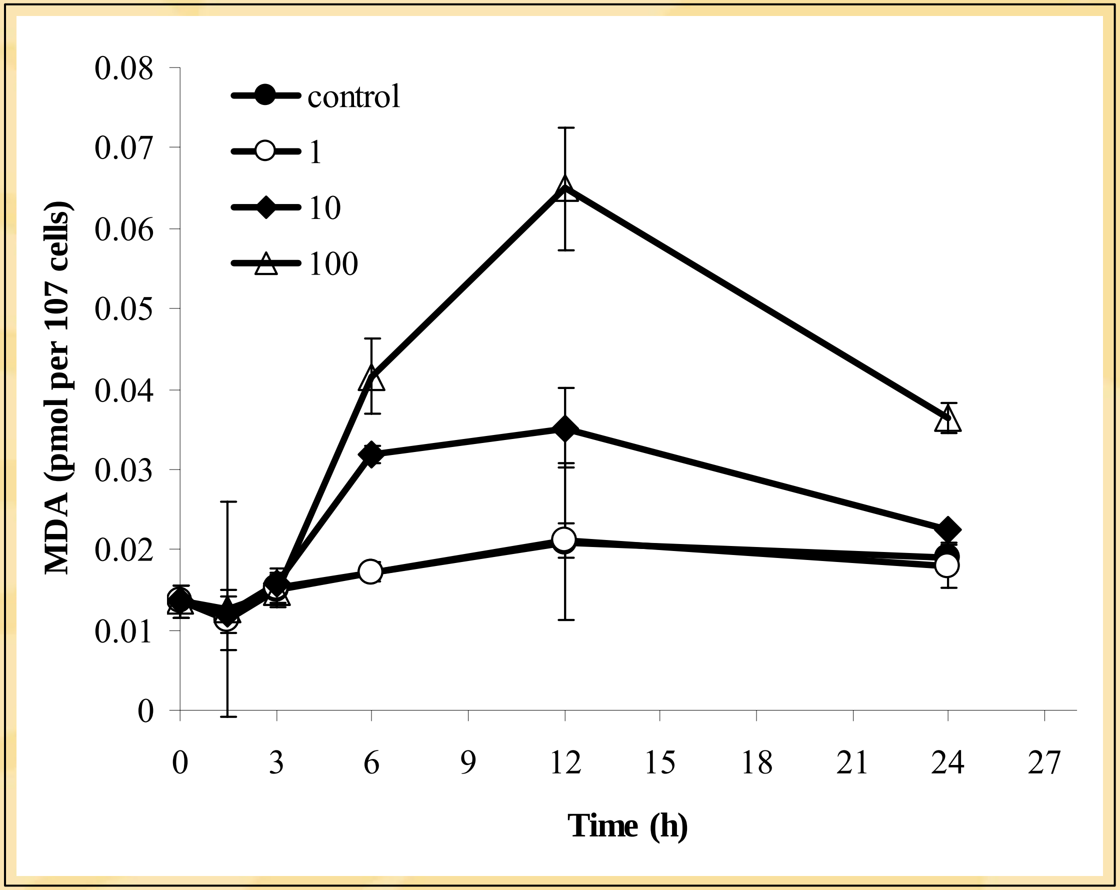
Effect of TiO2 NMs on growth





J. Wang, X. Zhang, Y. Chen, et. al, Chemosphere, 2008, 73: 1121–1128.

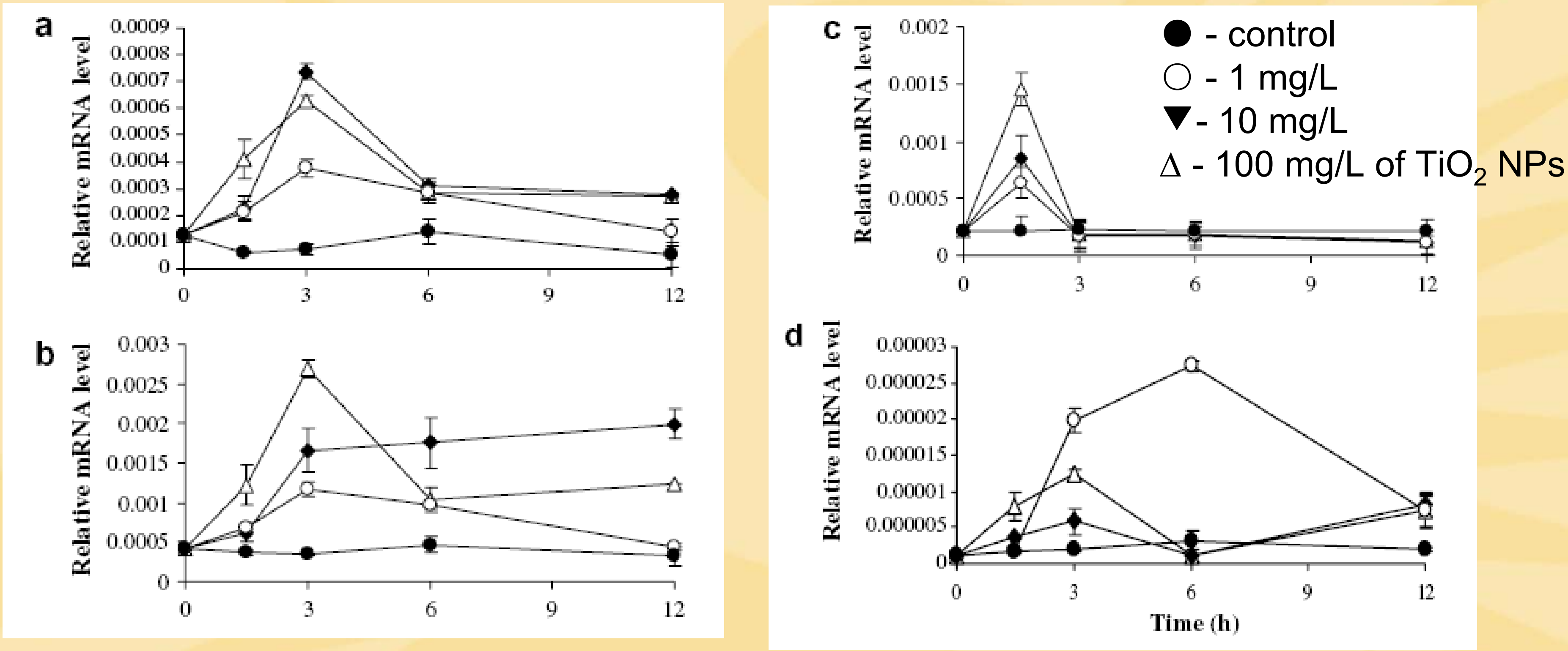
Change of lipid peroxidation paterns and gene expressions



Lipid proxidation (MDA)

J. Wang, X. Zhang, Y. Chen, et. al, Chemosphere, 2008, 73: 1121–1128.

Gene expression profiles of antioxidant enzymes



Transcriptional expression profiles of the genes in *Chlamydomonas* Exposed to 0, 1, 10 or 100 mg/L of TiO2 NMs for 12 h. (a), *sod1*, (b), *gpx*, (c), *cat*, (d), *ptox2*. Relative mRNA levels were normalized with 18S rRNA as the internal standard. d = 0 mg/L(control); s = 1 mg/L, r = 10 mg/L, D = 100 mg/L of TiO2 NMs.

J. Wang, X. Zhang, Y. Chen, et. al, Chemosphere, 2008, 73: 1121–1128.

Summery

- It suggested that both growth kinetics and molecular biomarkers can be applied for assessment of environmental nanotoxicity.

- The toxicity of the QDs to *C. reinhardtii* was ca. 10-fold greater than that of TiO2 NMs, indicative of different, yet unknown toxicological mechanisms.

48 h EC50 and LC50 of NPs to Daphnia magna

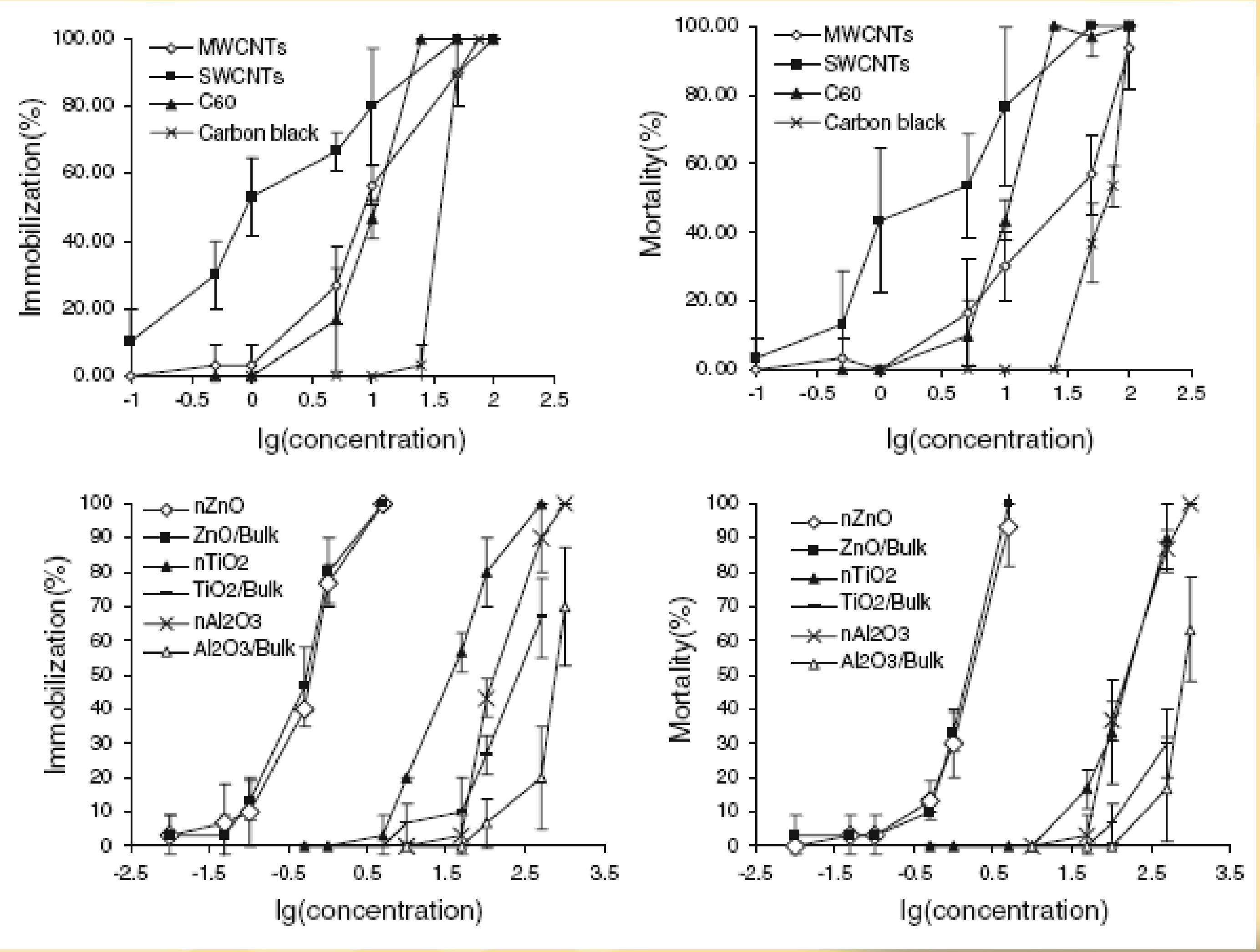
High toxicity

Material (particle size)	EC_{50} (mg/L)	95% CI	LC_{50} (mg/L)	95% CI
ZnO/Bulk (1,000 nm)	0.48	0.30-0.67	1.25	0.99-1.85
nZnO (20 nm)	0.62	0.41-0.81	1.51	1.12-2.11
SWCNTs (<2nm)	1.31	0.82-1.99	2.43	1.64-3.55
C_{60} (<200nm)	9.34	7.76-11.26	10.52	8.66-12.76
MWCNTs (10-20nm)	8.72	6.28-12.13	22.75	15.68-34.39
$nTiO_2$ (< 20nm)	35.31	25.63-48.99	143.39	106.47-202.82
nAl_2O_3 (80 nm)	114.36	111.23-191.10	162.39	124.33-214.80
TiO_2/Bulk (10,000 nm)	275.28	170.66-570.05	>500	n.d.
Al_2O_3/Bulk (90,000 nm)	>500	n.d.	>500	n.d.

Low toxicity

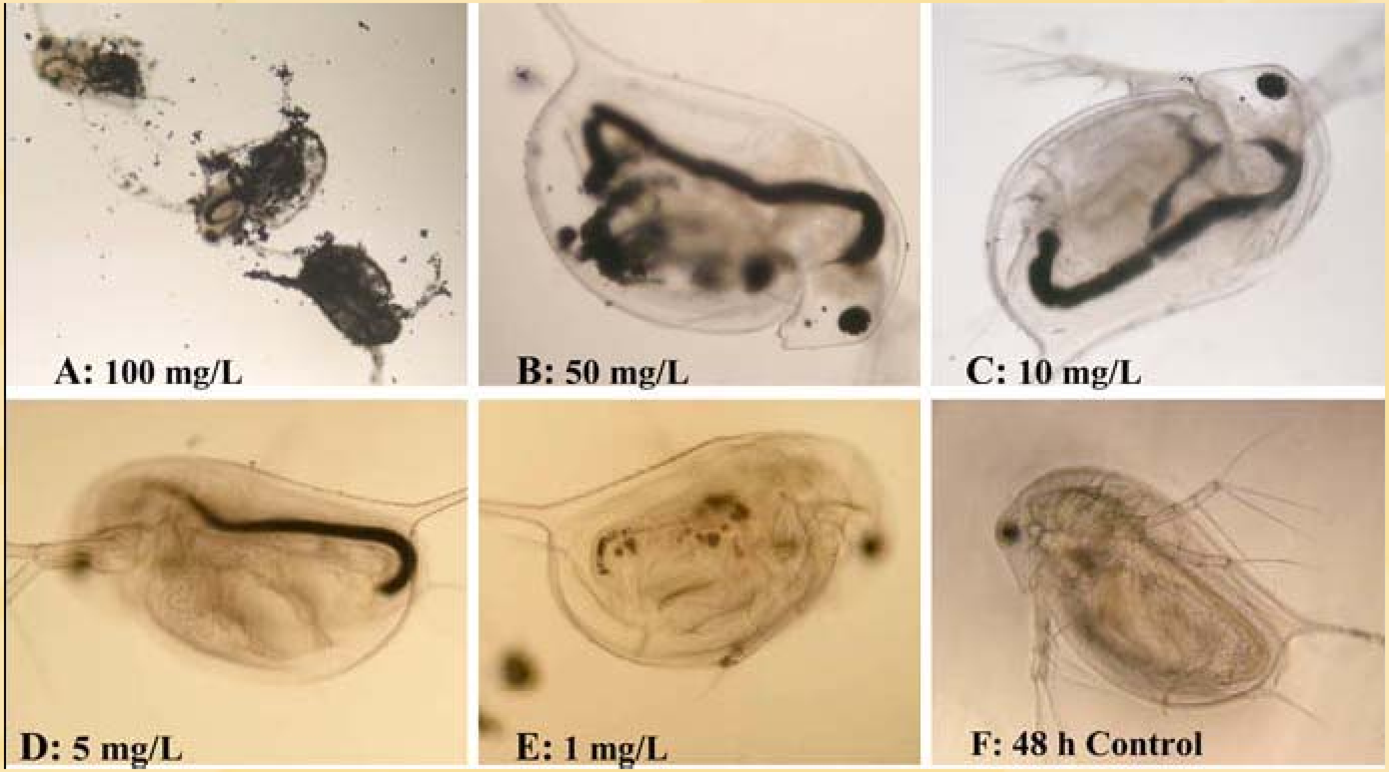
X. Zhu, L. Zhu, Y. Chen, Journal of nanoparticle ***research***, 2009, Vol.11: 67-75

Immobilization and mortality of D. magna



X. Zhu, L. Zhu, Y. Chen, ***Journal of nanoparticle research***, 2009, Vol.11: 67-75

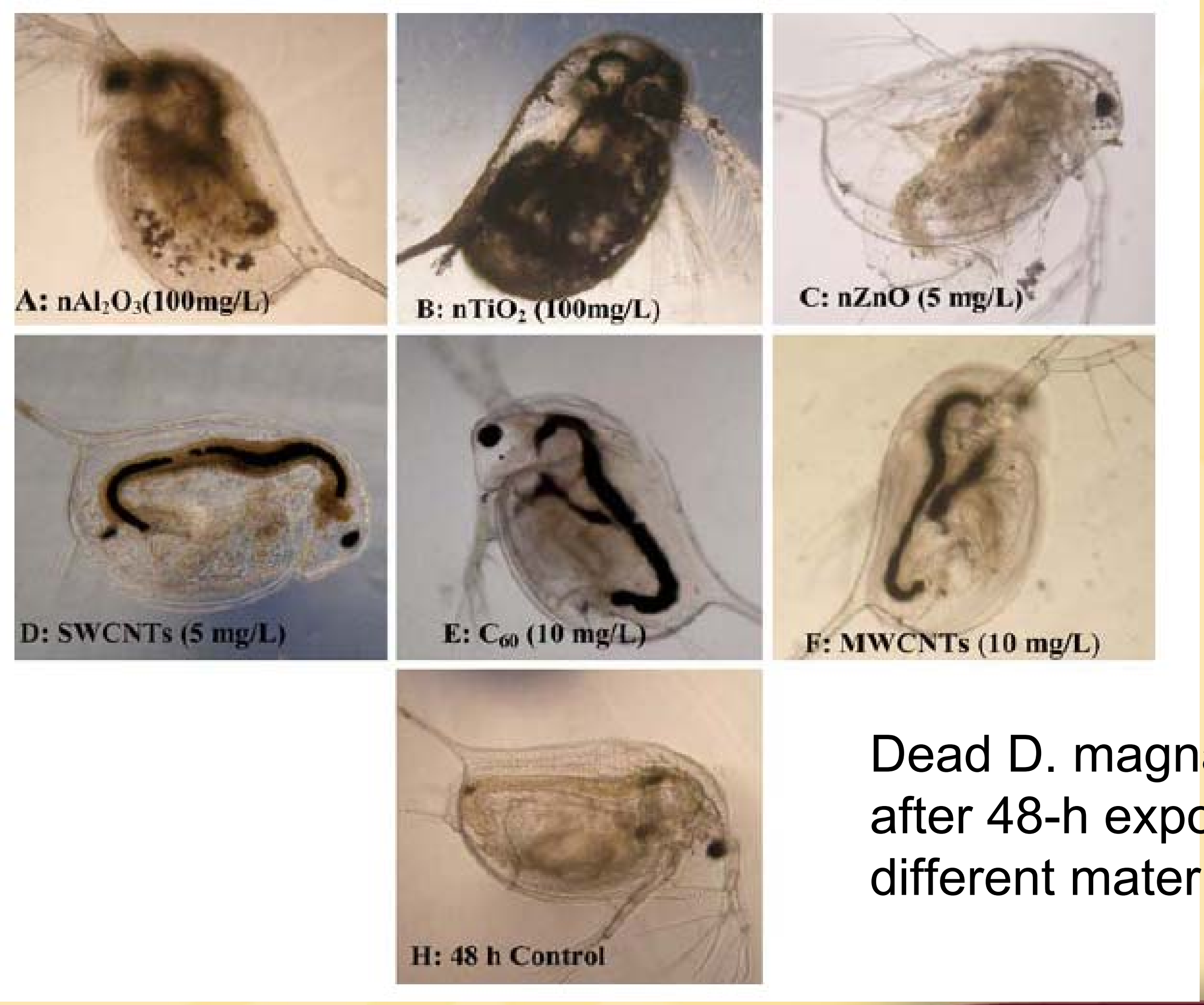
Uptake and Adsorption of Daphania



The uptake and adsorption of MWCNTs by D. magna after 48 h exposure

X. Zhu, L. Zhu, Y. Chen, Journal of nanoparticle research, 2009, Vol.11: 67-75

Uptake and Adsorption of Daphania



Dead D. magna
after 48-h exposure to
different materials

X. Zhu, L. Zhu, Y. Chen, Journal of nanoparticle research, 2009, Vol.11: 67-75

Summery

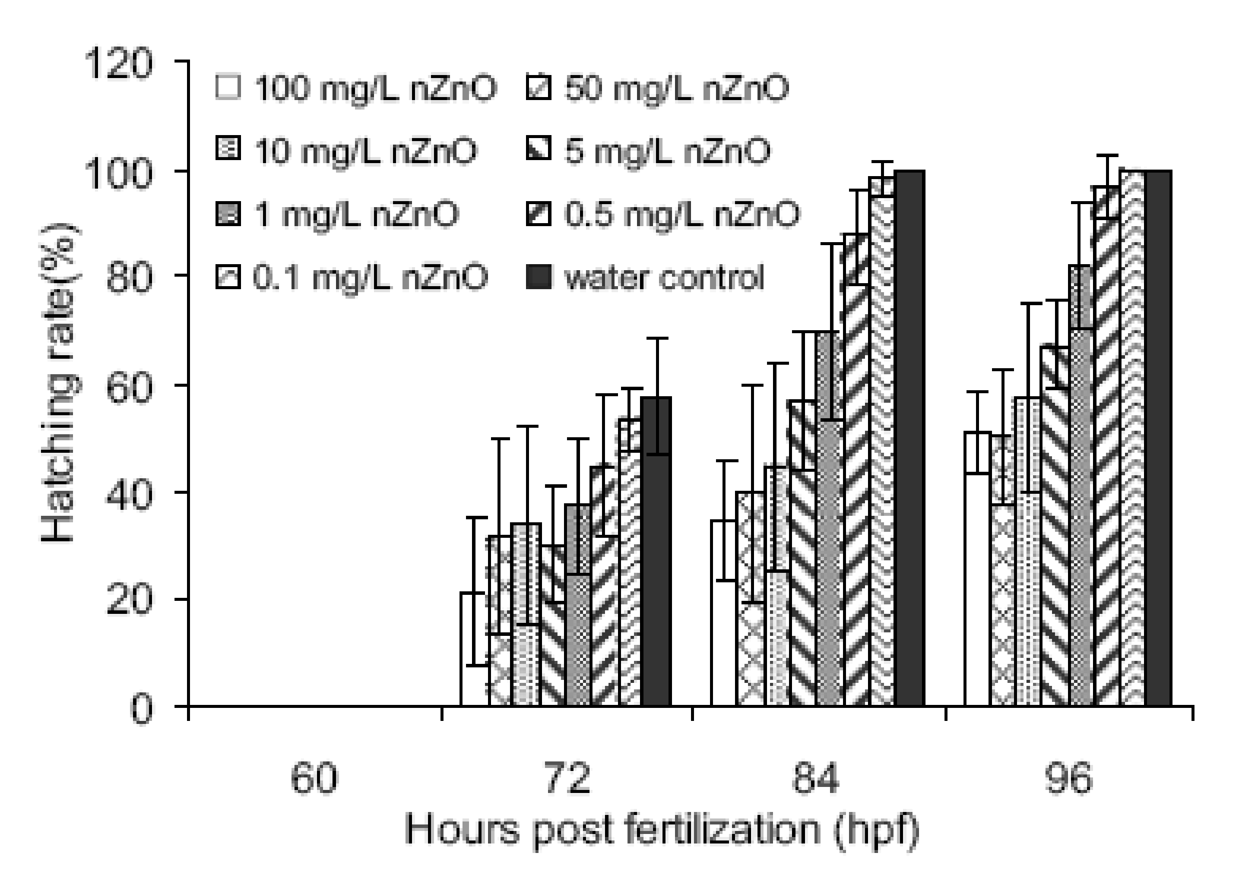
(1) The NPs may have acute, dose-dependent ecotoxicological effects on freshwater zooplankton.

(2) NPs with different compositions exhibited different toxicities to D. magna. The nZnO suspension was observed to be the most toxic of those tested, while nAl_2O_3 had the least toxicity.

(3) New regulations should be established that are based not only on chemical components but also on size, as chemical properties change at the nanoscale.

(4) D. magna is able to ingest NPs from test solutions. Other grazing and filter-feeding aquatic organisms are likely to ingest such NPs as well. The potential for subsequent transfer of NPs to other trophic levels should receive additional attention.

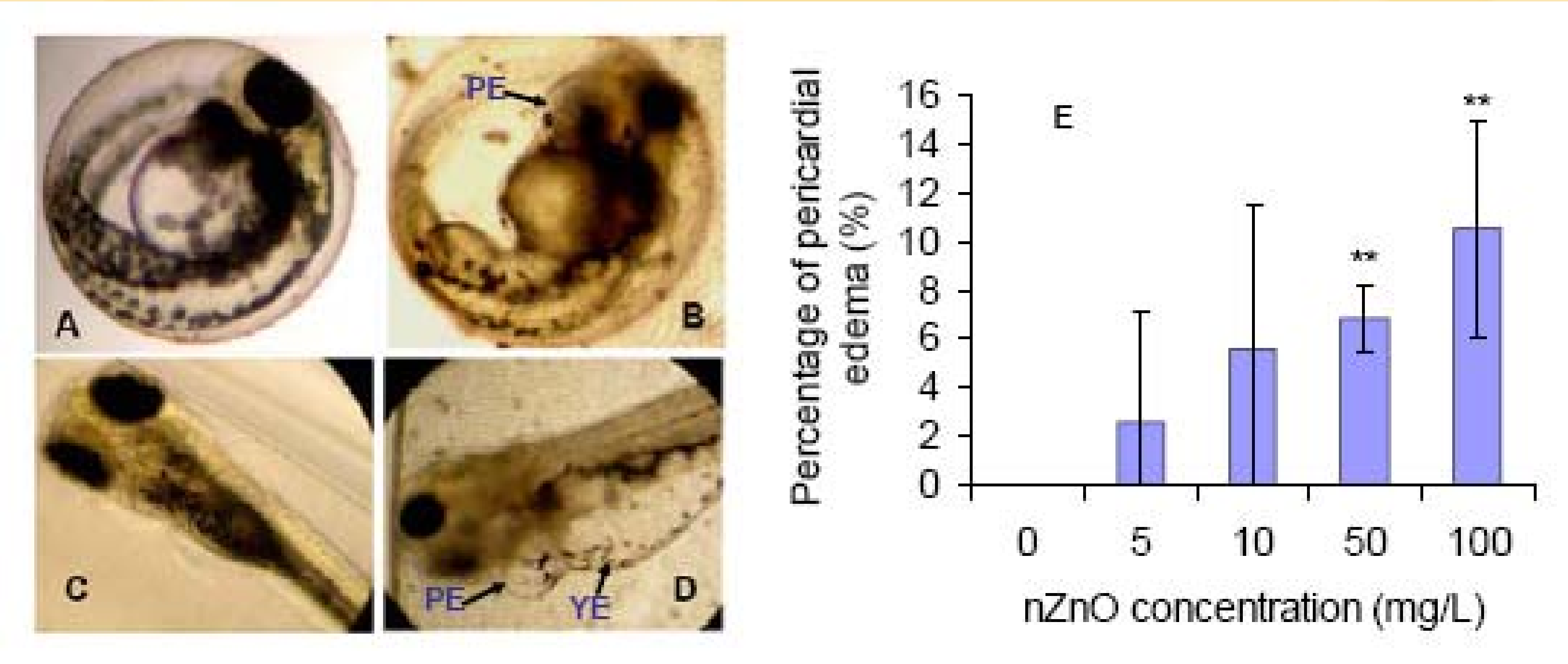
Toxicity of ZnO Nanoparticle Aggregates on the Embryo Development of Zebrafish (Danio rerio)



Hatching rate (%) of zebrafish embryos exposed to different treatments over 96 h.

X. Zhu, Y. Chang, Y. Chen, Nanotechnology, in press, 2009

Toxicity of ZnO Nanoparticle Aggregates on the Embryo Development of Zebrafish (Danio rerio)



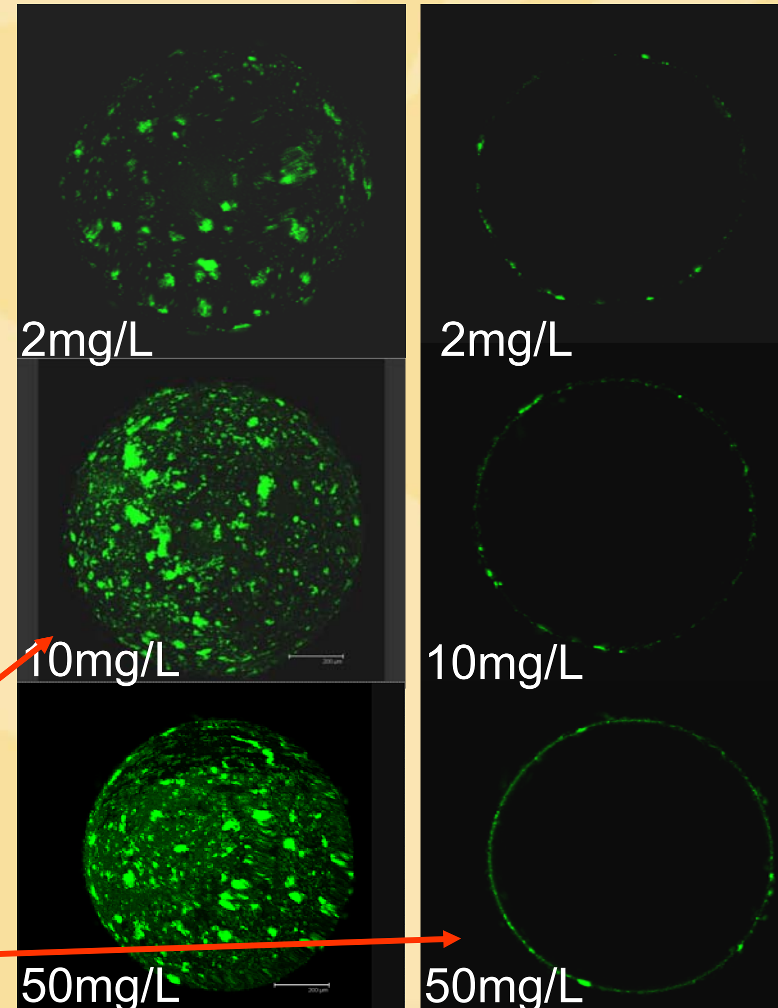
Examples of pericardial and yolk sac edemas (indicated by arrows) in zebrafish embryos and larvae exposed to nZnO aggregates

X. Zhu, Y. Chang, Y. Chen, Nanotechnology, in press, 2009

Chorion penetration property of nanoparticle

Embryos exposed to FITC-$nSiO_2$ for 20 h (24hpf)

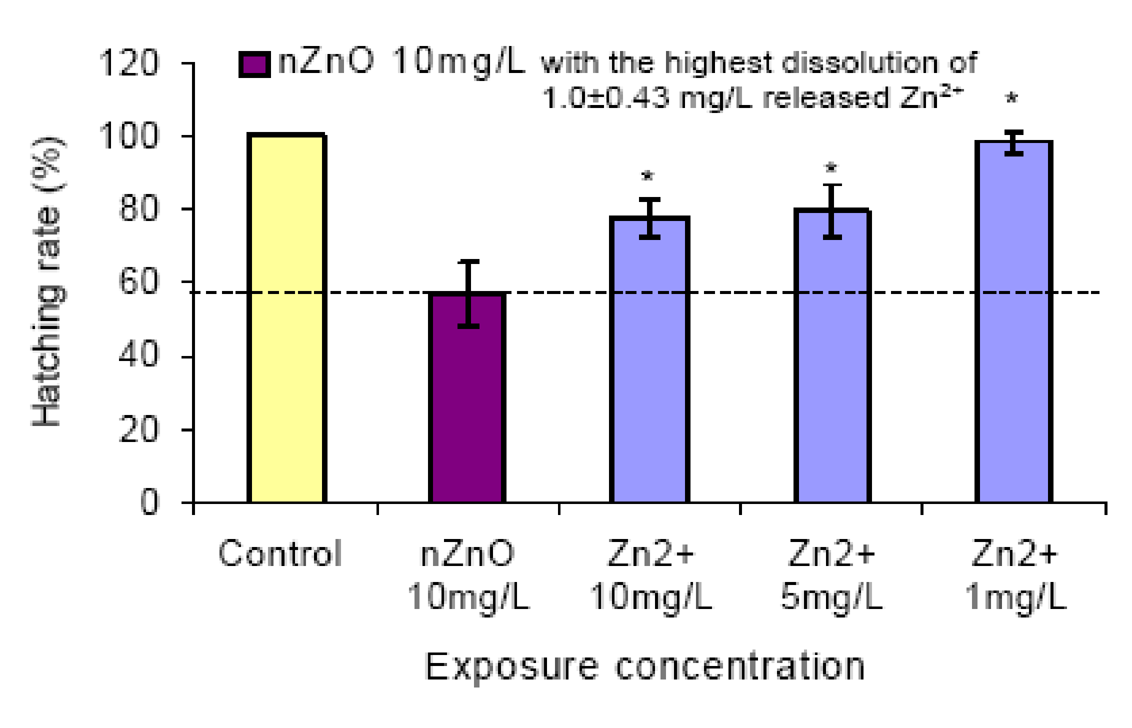
$nSiO_2$ adsorbed on the surface of embryos but can not penetrate the chorion of zebrfish embryos



Embryo’s Three-dimensional Image

Embryo’s intermediate layer Image

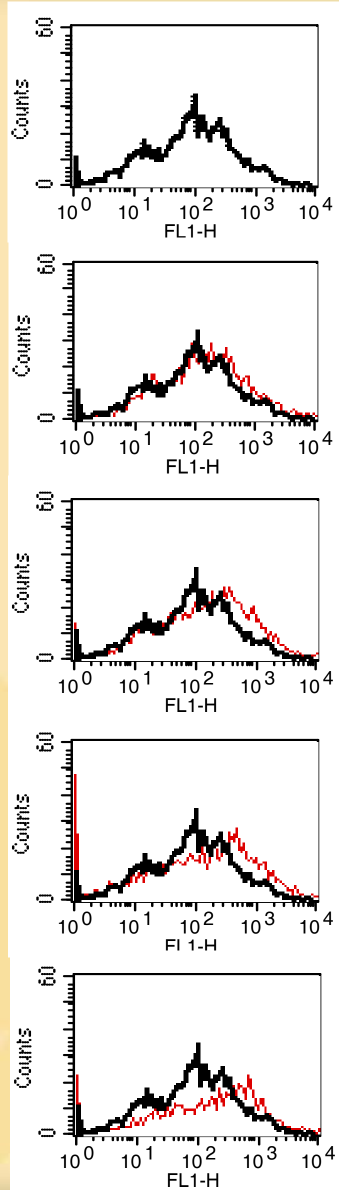
Toxicity of ZnO Nanoparticle Aggregates on the Embryo Development of Zebrafish (Danio rerio)



Comparative toxicity between Zn2+ and nZnO aggregates using hatching rate as endpoint at 96 hpf.

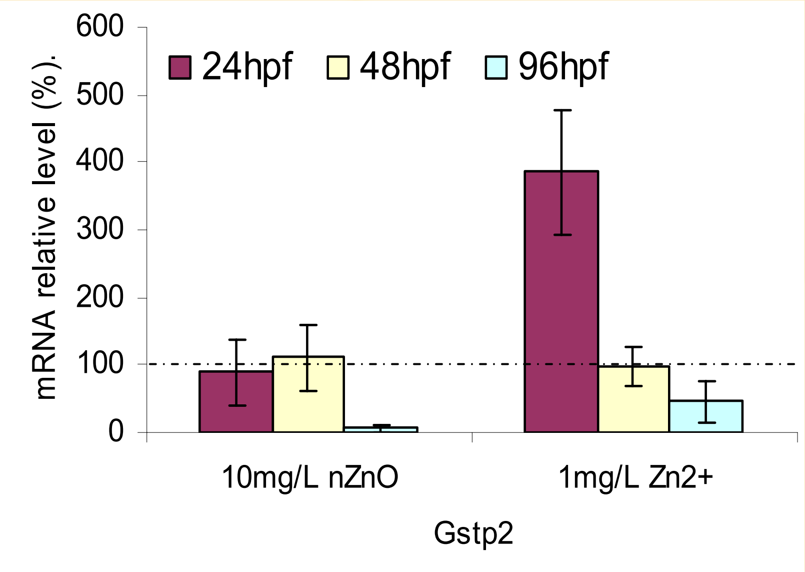
X. Zhu, Y. Chang, Y. Chen, Nanotechnology, in press, 2009

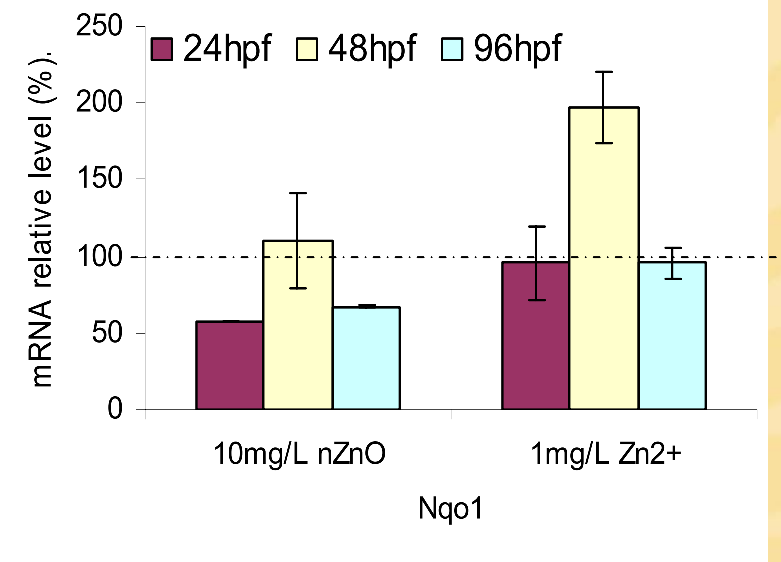
Intracellular ROS in zebrafish embryo cells treated with Zn2+ or nZnO



→	Control	PC=90
→	1mg/L Zn^{2+}	PC=77
→	1 mg/L nZnO	PC=257
→	10 mg/L nZnO	PC=429
→	100 mg/L nZnO	PC=609

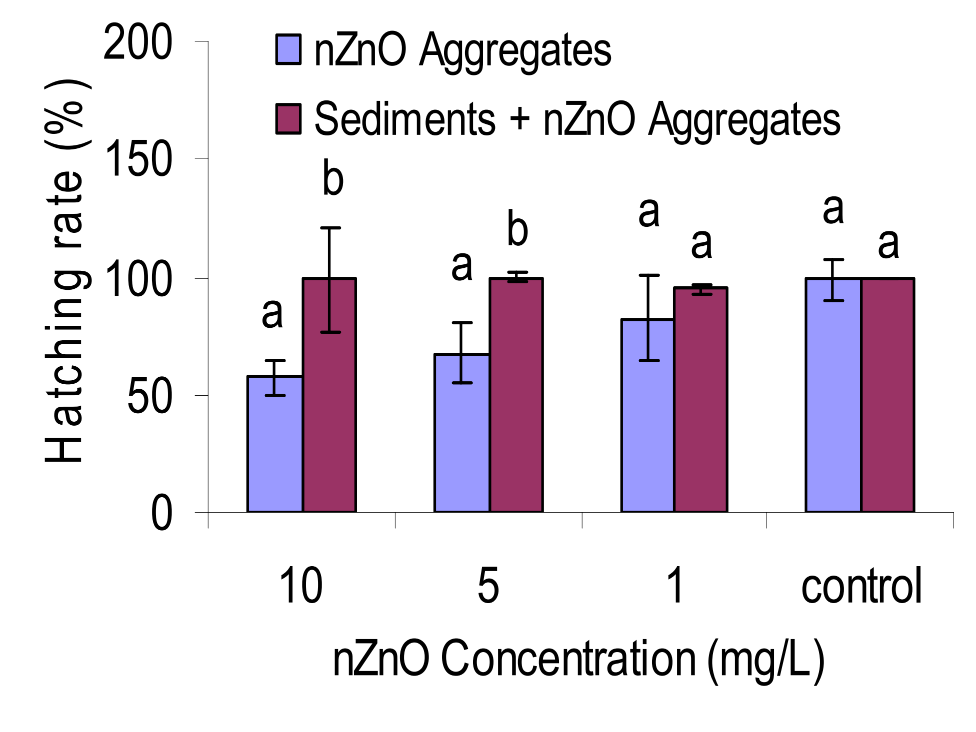
X. Zhu, Y. Chang, Y. Chen, Nanotechnology, in press, 2009

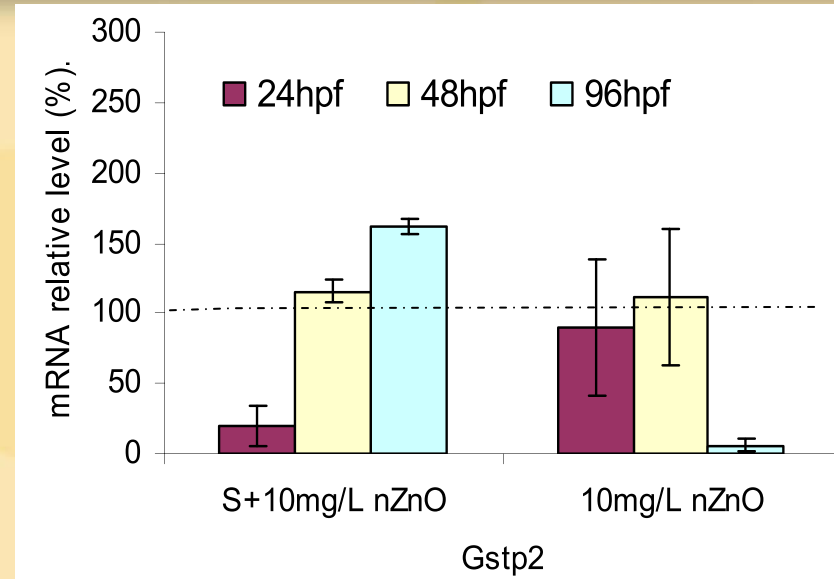


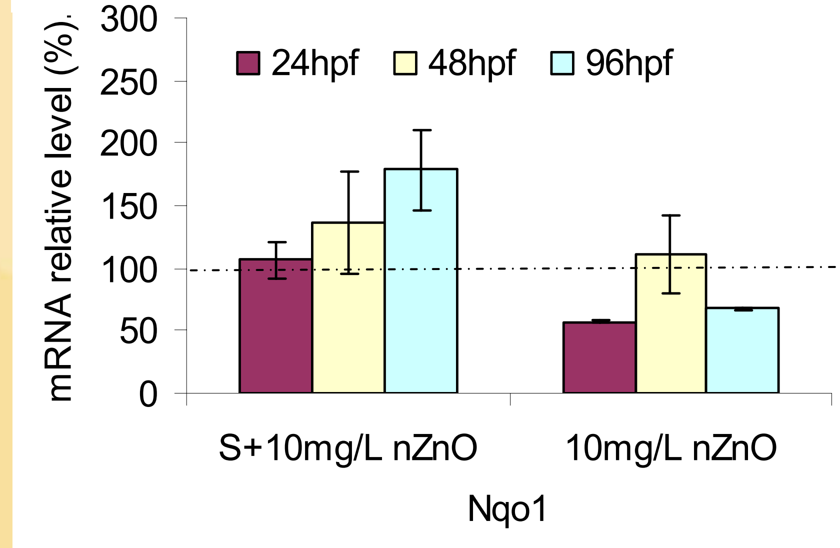


Results of quantitative RT-PCR for the oxidant-associated genes: gstp2, Nqo1: lower expression in nZO treated embryos than those treated with Zn++.

X. Zhu, Y. Chang, Y. Chen, Nanotechnology, in press, 2009







Comparison toxicity of nZnO aggregates with and/or without sediments on zebrafish embryos at 96 hpf.

X. Zhu, Y. Chang, Y. Chen, Nanotechnology, in press, 2009

Summary

•nZnO exerts dose-dependent toxicity to zebrafish embryos and larvae, causing malformation in the cardiovascular system, and blocking hatching, leading to mortality in some embryos.

•the observed toxicity as a result of exposure to nZnO aggregates might be mediated through elevated ROS.

•Sediments in aquatic environments, however, could mitigate the toxicity of nZnO aggregates.

Toxicity to Fish– Carp Oxidative Stress and Growth Inhibition

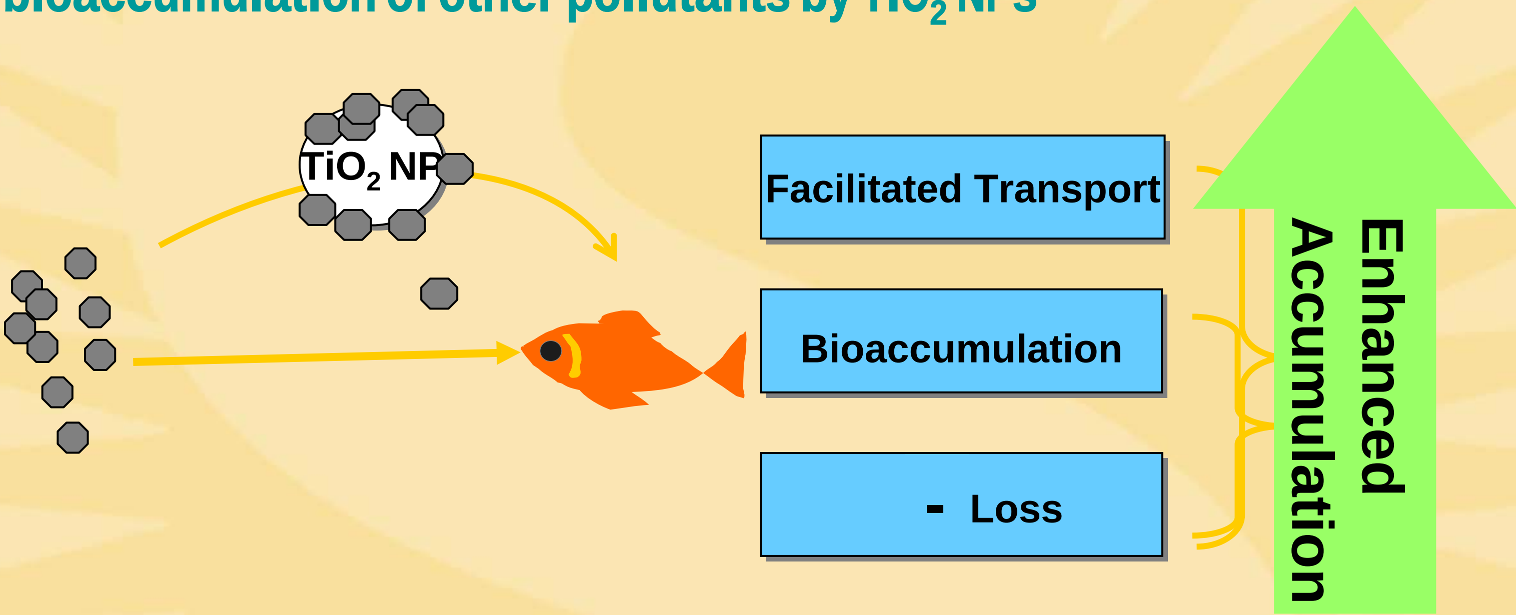
Tissue	Biomarker	Exposure groups (mg/L)			
		control	0.04	0.20	1.0
Brain	SOD (U/mgprot)	49.4±5.28 (a)	46.8±6.69 (a)	58.7±10.4 (a)	49.8±14.7 (a)
	CAT (U/gprot)	4.25±1.10 (a)	4.24±0.54 (a)	6.47±1.54 (a)	4.78±1.57 (a)
	GSH (mg/gprot)	442.5±15.1 (a)	393.6±9.2 (b)	380.1±5.5 (b)	346.6±0.0 (c)
	LPO (nmoL/mgprot)	4.19±0.11 (a)	3.21±0.11 (b)	2.42±0.06 (c)	2.49±0.03 (c)
Liver	SOD (U/mgprot)	188.1±9.3 (a)	215.9±4.8 (b)	207.2±4.2 (b)	208.8±4.9 (b)
	CAT (U/gprot)	708.5±51.5 (a)	859.7±38.5 (b)	828.8±18.8 (bc)	755.9±35.1 (ac)
	GSH (mg/gprot)	213.4±5.8 (a)	223.6±5.3 (a)	186.0±1.8 (b)	192.8±2.0 (b)
	LPO (nmoL/mgprot)	3.54±0.02 (a)	2.88±0.02 (b)	2.83±0.07 (b)	3.81±0.03 (c)
Gill	SOD (U/mgprot)	17.0±4.9 (a)	22.6±1.2 (a)	13.7±3.7 (a)	16.9±4.8 (a)
	CAT (U/gprot)	4.75±0.86 (a)	6.86±0.39 (ab)	9.70±1.60 (c)	8.90±0.77 (bc)
	GSH (mg/gprot)	548.4±8.0 (a)	453.4±10.4 (b)	504.5±8.1 (c)	415.2±4.0 (d)
	LPO (nmoL/mgprot)	6.50±0.05 (a)	2.46±0.02 (b)	2.28±0.05 (c)	2.49±0.05 (b)

Note: Values for the control and C_{60} aggregates exposed groups are based on 32 d exposure and expressed as mean ± S.D. ($n = 3$). Different letters in the same row indicate significant differences between treatments within each tissue (ANOVA with Tukey's test, $p < 0.05$).

Zhu X., L. Zhu, Y. Lang, Y. Chen
Environ. Toxicol. & Chem. 2008(27) 9 Apr 24:1

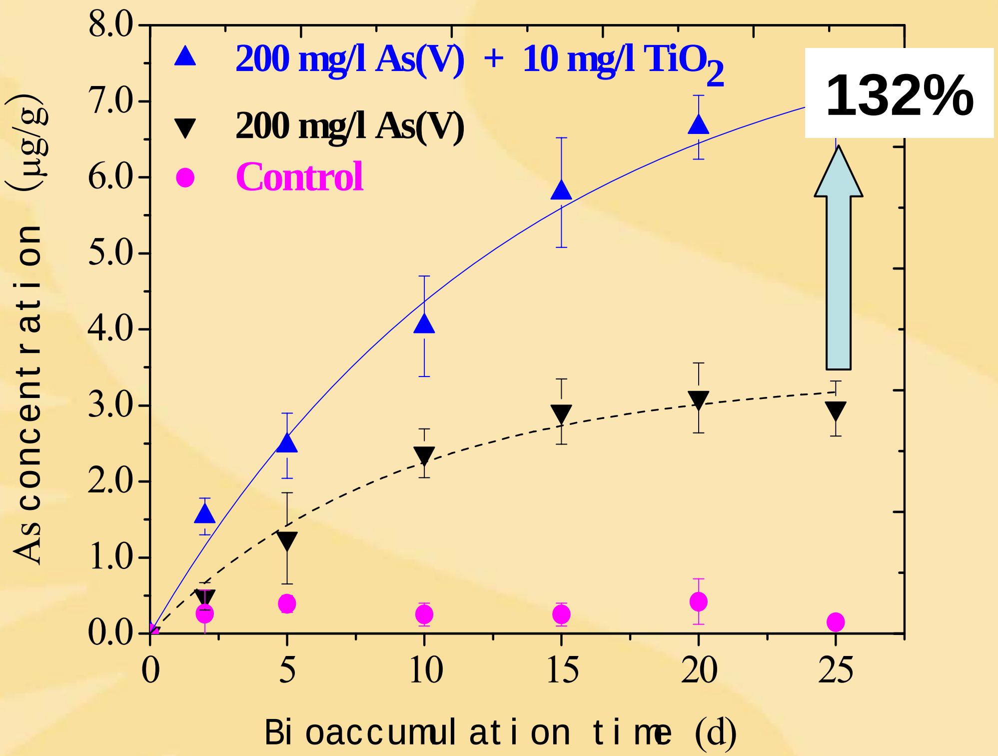
Bioconcentration of NPs in carp (Cyprinus carpio)

Facilitated bioaccumulation of other pollutants by TiO_2 NPs



As(III), As(V) and Cd can be adsorbed onto NPs, as TiO_2 NPs accumulate in body of carp, the adsorbed pollutants are transported to carp by TiO_2 NPs. As a result, the accumulation of and Cd were enhanced in the presence of TiO_2 NPs.

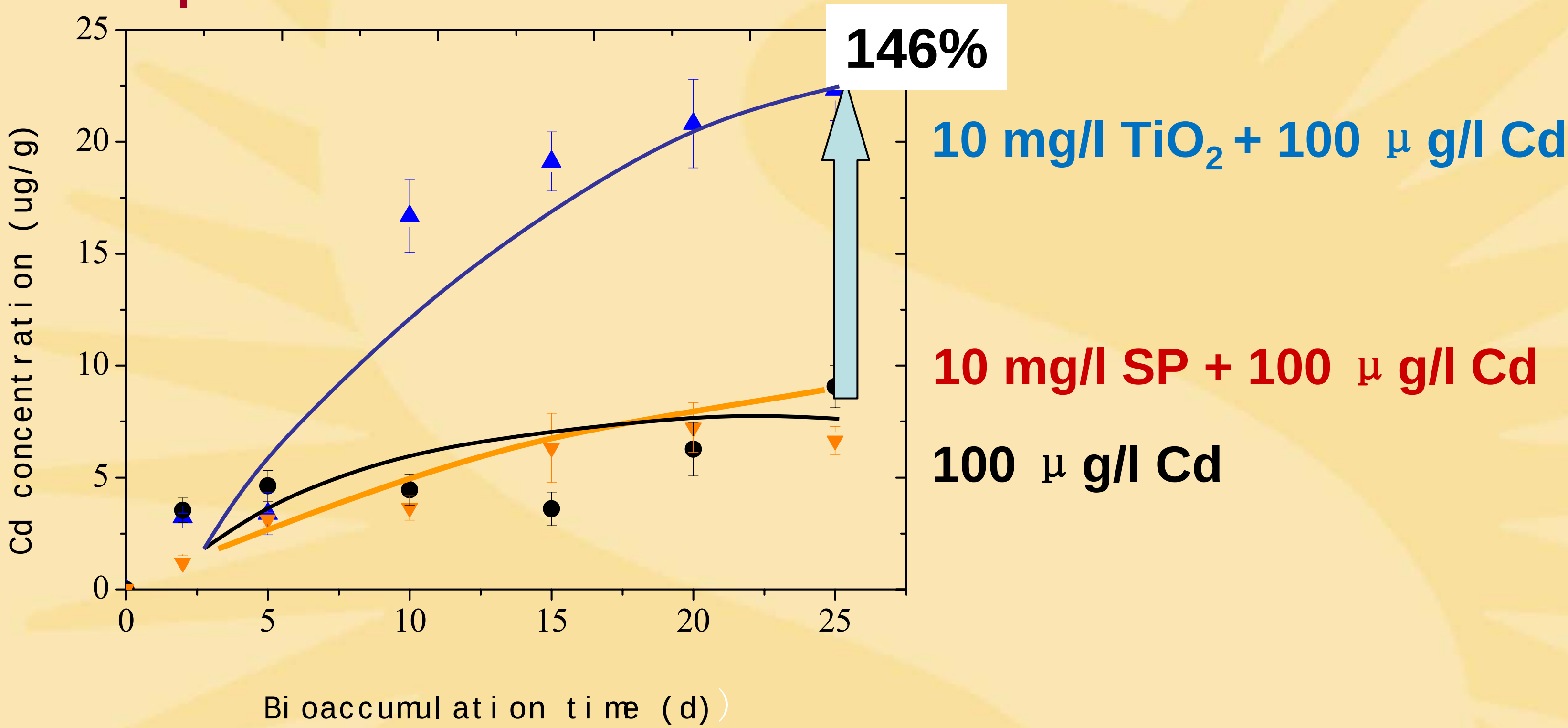
Biological Fate and Transport – Enhanced Bioaccumulation



In the presence of TiO_2 NPs, As concentration in carp increased 132%.

Zhang, X., H. Sun, Z. Zhang, Q. Niu, Y. Chen, J. Crittenden
Water, Air & Soil Pollution. 2007(178):245–254

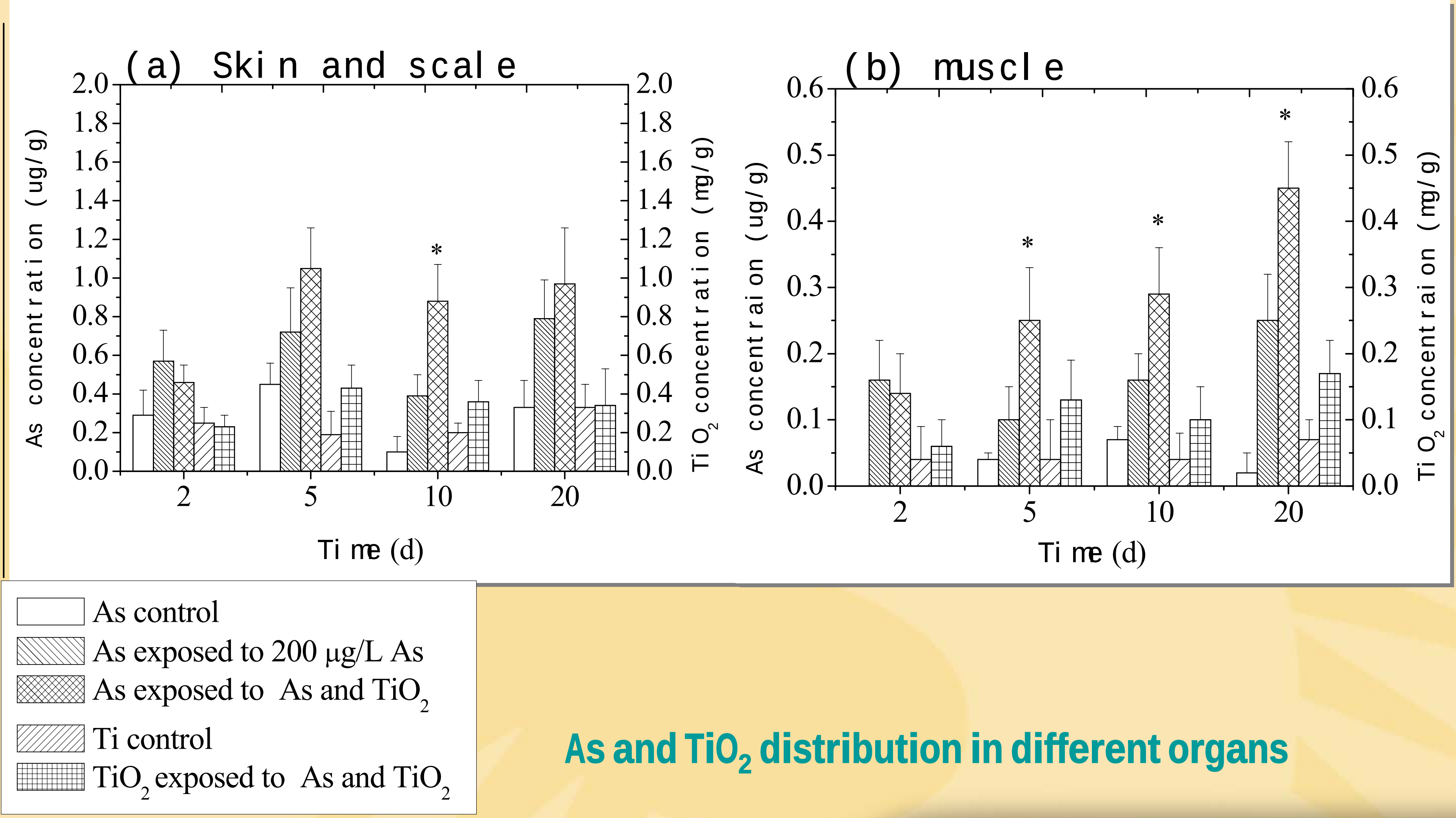
Enhanced bioaccumulation of Cd in carp by TiO2 NPs and Suspended Particles



The presence of SP did not have significant influence on the accumulation of Cd in carp during the 25 d of exposure. However, the presence of TiO_2 NPs, after 25 d of exposure Cd concentration in carp increased by 146%.

Chemosphere *2007, 67:160–166.*

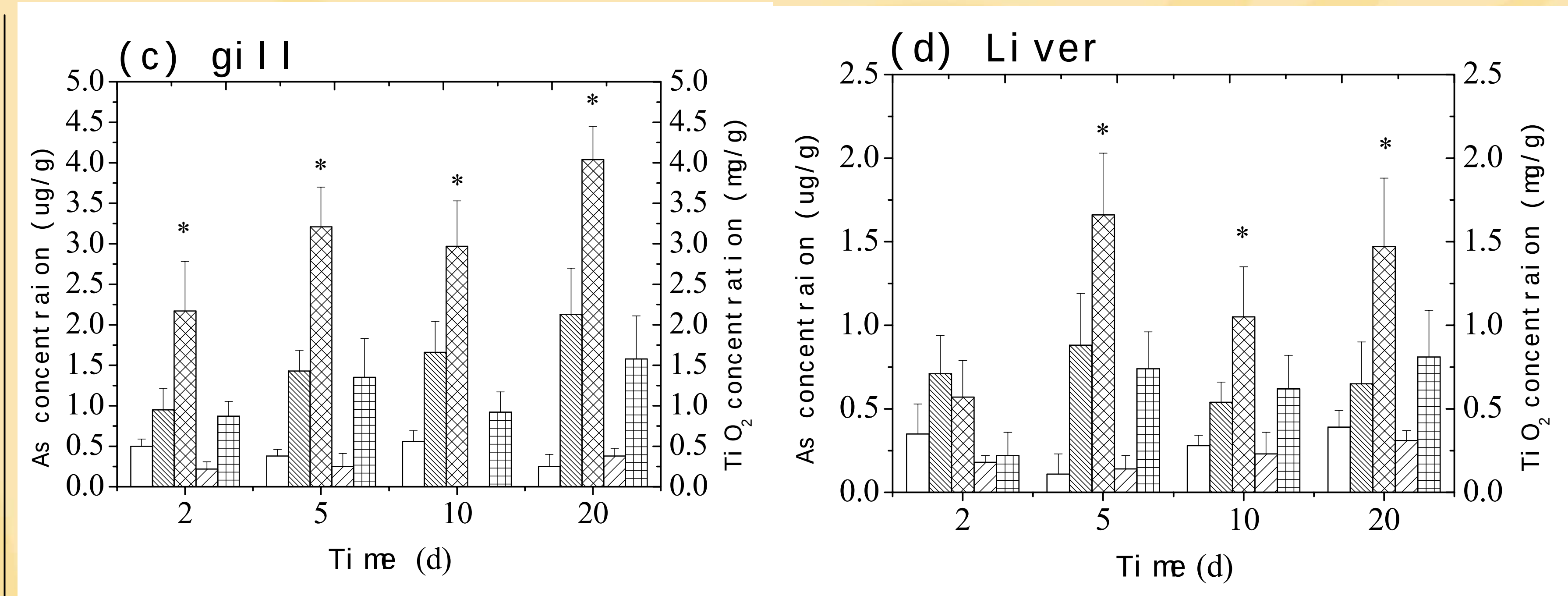
Bioconcentration of NPs in carp (Cyprinus carpio)



As and TiO_2 distribution in different organs

X. Zhang, H. Sun, Y. Chen, et. al, Water, Air & Soil Pollution. 2007, 178:245–254.

Bioconcentration of NPs in carp (Cyprinus carpio)



As control
As exposed to 200 μg/L As
As exposed to As and TiO_2
Ti control
TiO_2 exposed to As and TiO_2

As and TiO_2 distribution in different organs

Accumulation of As and TiO_2 in gills is significant.

*X. Zhang, H. Sun, Y. Chen, et. al, Water, Air & **Soil Pollution***. 2007, 178:245–254.

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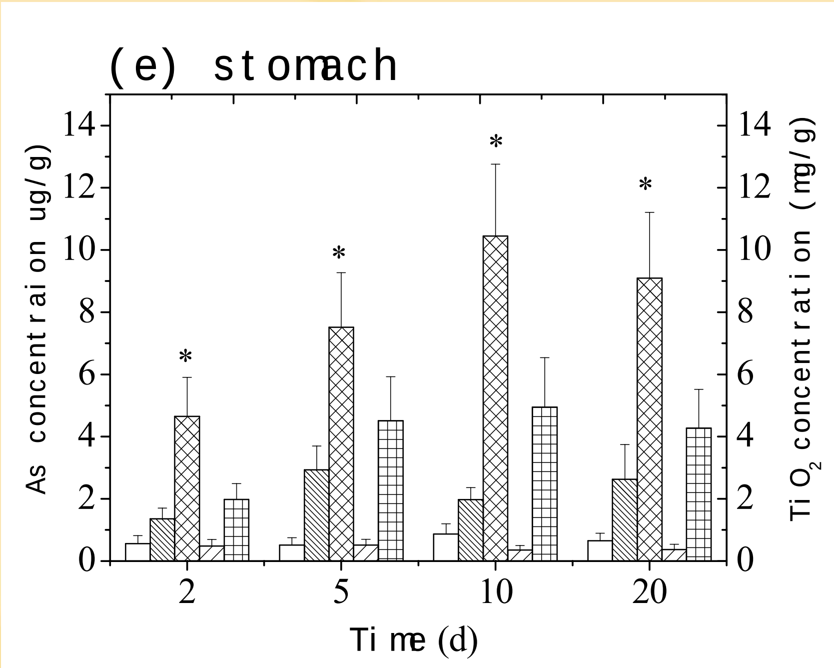
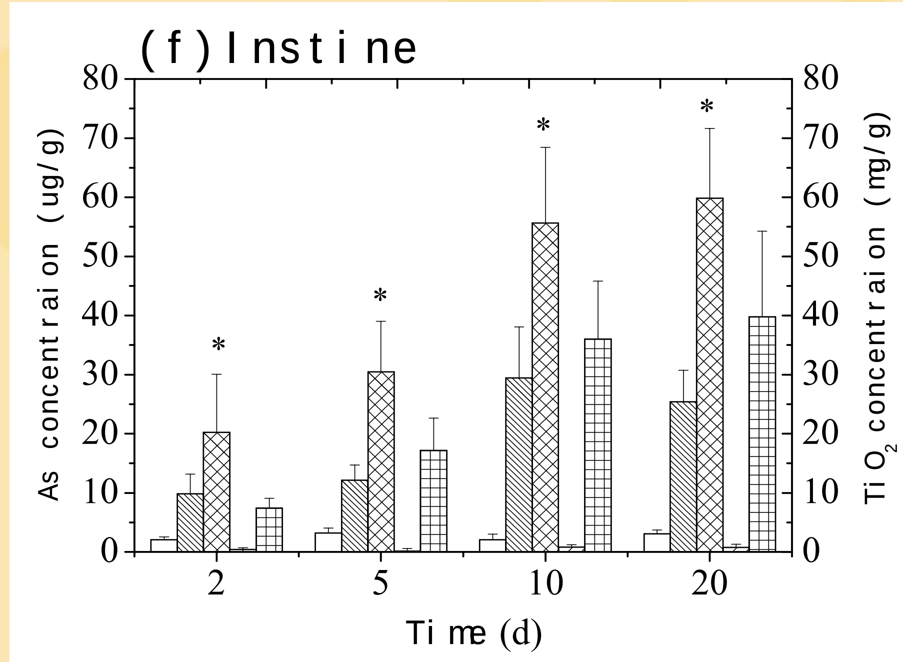
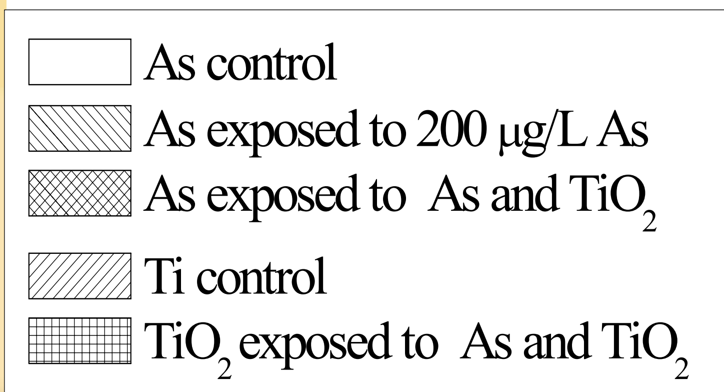
Bioconcentration of NPs in carp (Cyprinus carpio)





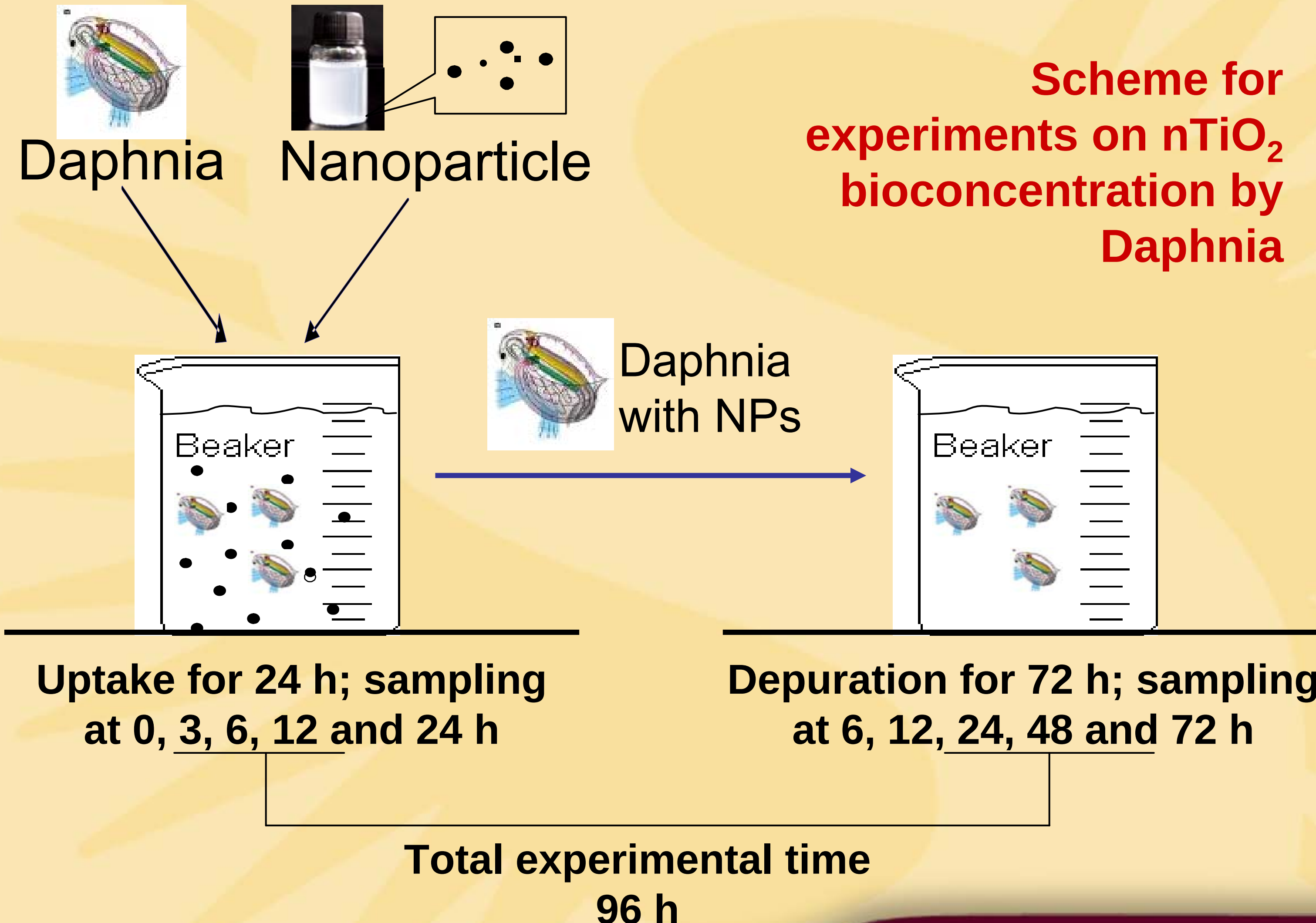


As and TiO_2 distribution in different organs

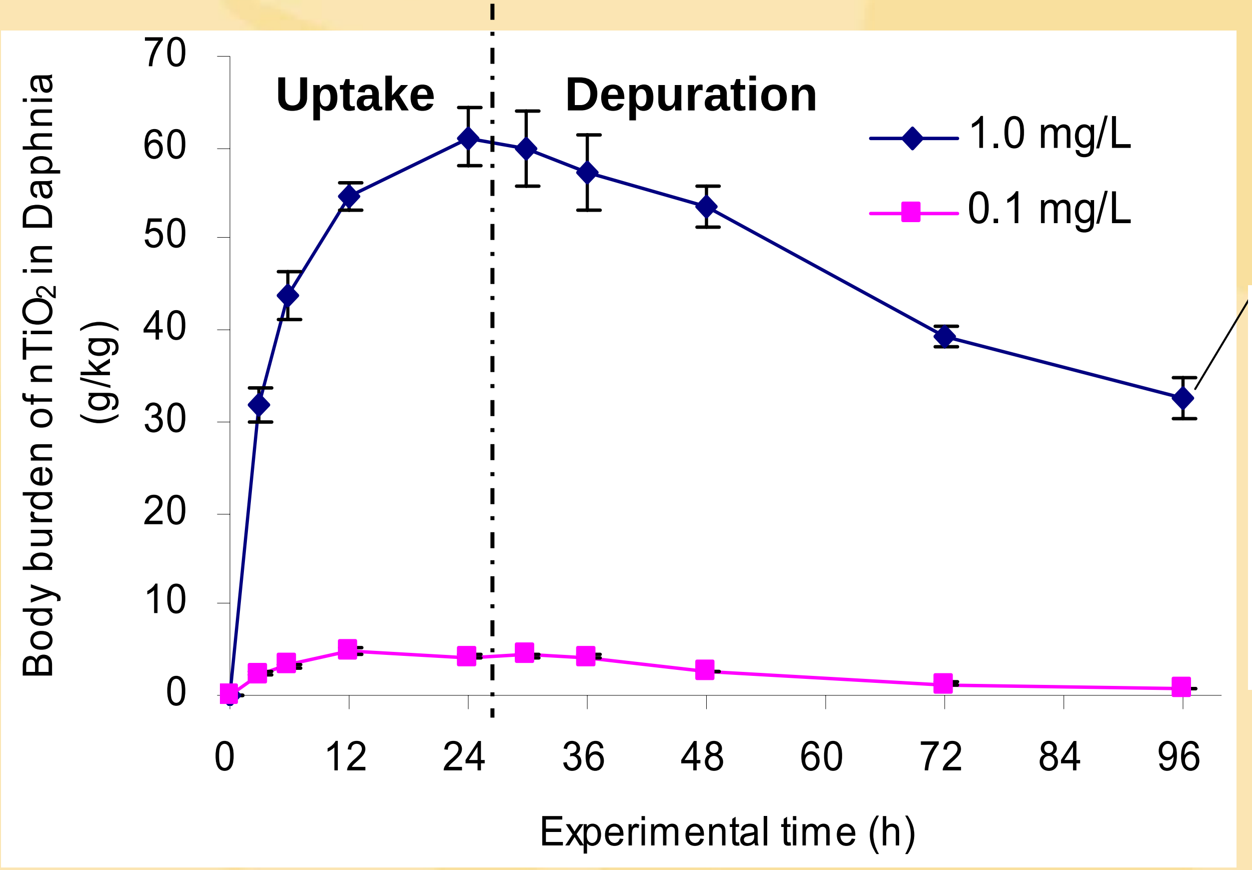
Accumulation of As and TiO_2 in stomach, intestine and gills are significant.

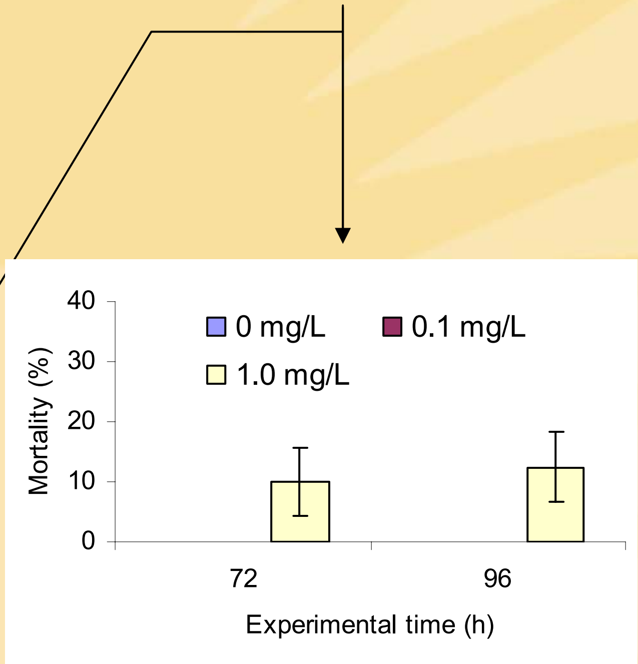
Bioconcentration of nanoparticles in aquatic organisms

Scheme for experiments on $nTiO_2$ bioconcentration by Daphnia



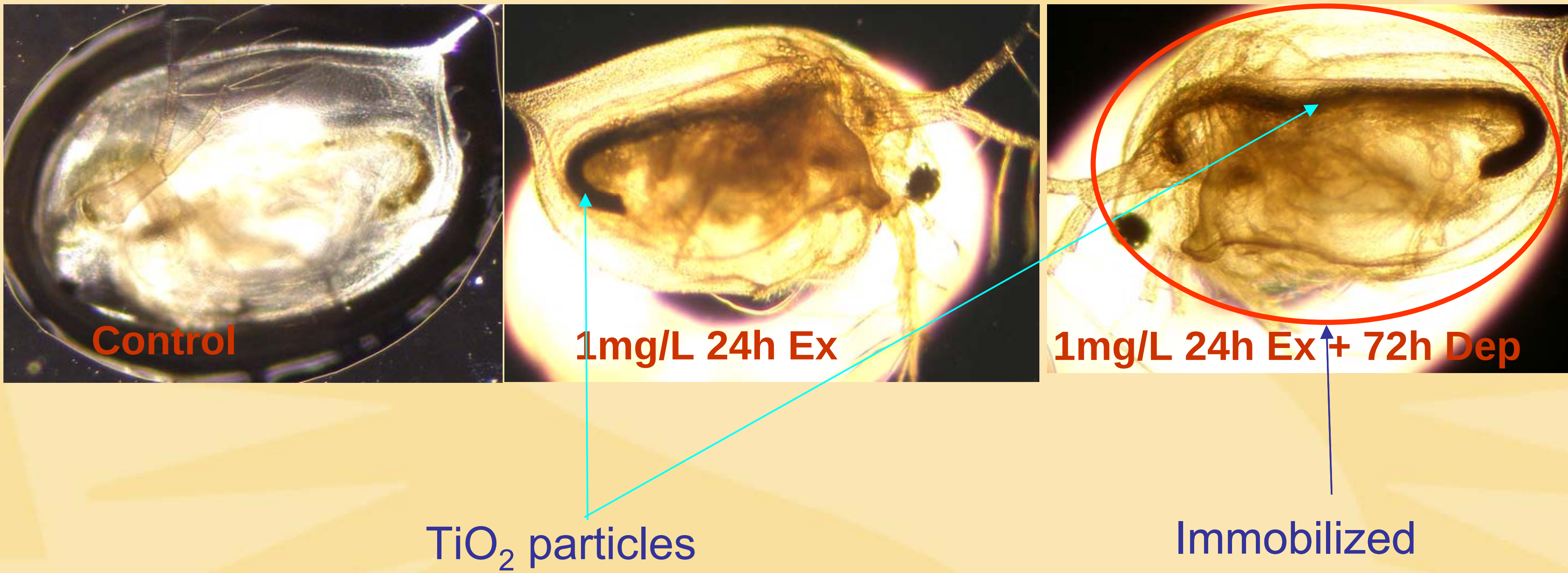
Uptake and depuration of nTiO2 in Daphnia magna





Mortality of Daphnia was found at 72 h

Uptake of nTiO2 by daphnia



Ex means Exposure; Dep means Depuration

$$\frac{1}{C} = \left(\frac{K_M}{C_{sat}}\right)\frac{1}{t} + \left(\frac{1}{C_{sat}}\right)$$

Uptake and depuration rate constants and bioconcentration factors (BCFs) for $nTiO_2$ in *Daphnia magna*

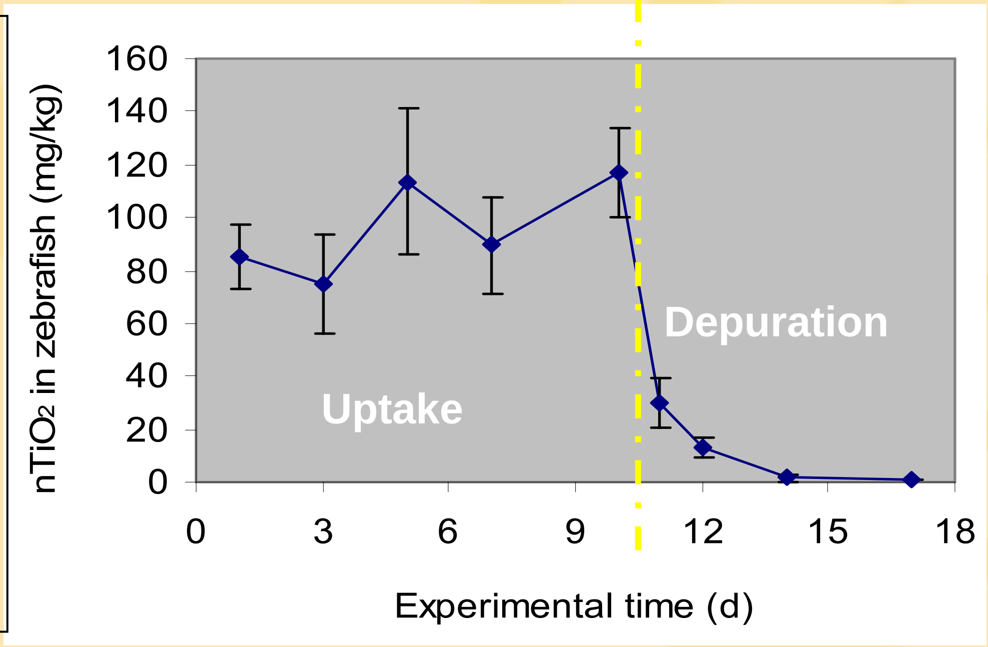
Dose (mg/L)	Exact dose[a] (mg/L)	Whole body concentration(dw) (g/kg)	BCFs (l/kg)	$K_M = t_{u0.5}$ (h)	$t_{u0.9}$ (h)	$t_{d0.5}$ (h)	$t_{d0.9}$ (h)
0.10	0.08	4.52[b]	56562.50	3.87	34.84	26.76	88.90
1.0	0.517	61.09	118062.84	3.72	33.51	74.52	247.59

a Exact concentration of $nTiO_2$ in culture medium after exposure for 24 hours.
b Average of the concentrations at 12 and 24 hours.

Daphnia (8-10 days old): exposed to 0.1 mg/L $nTiO_2$ for 24 hours

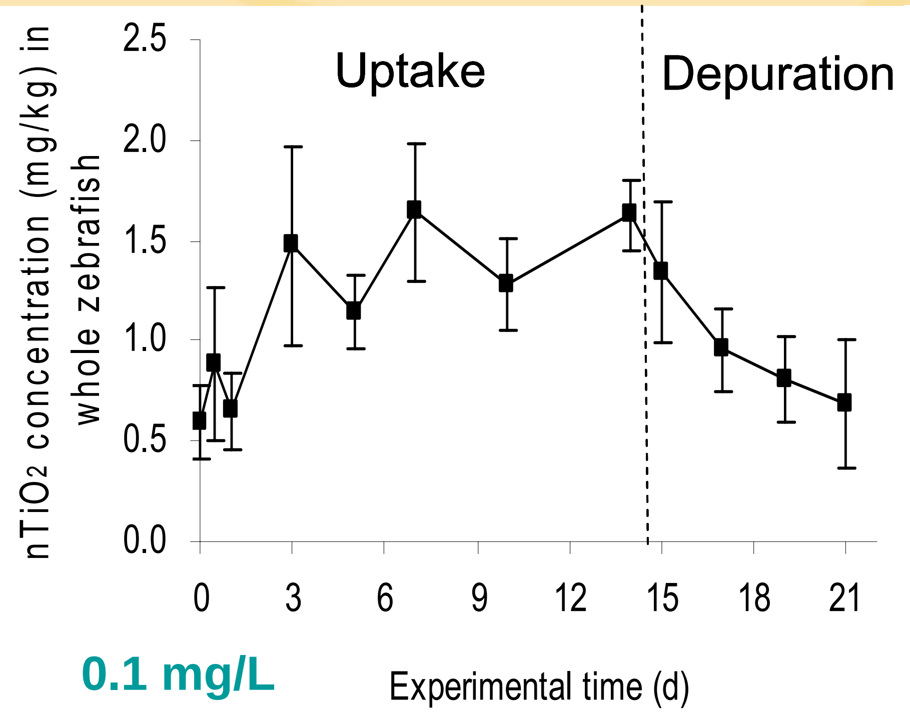
Fed two times each day (about 8% wet weight daily ration)

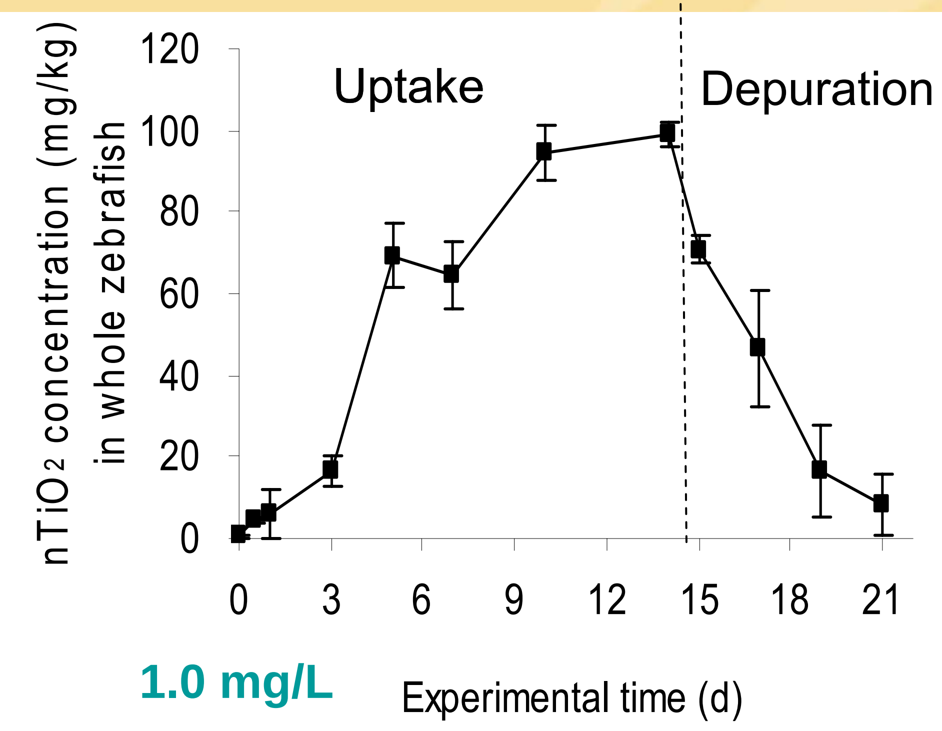
Zebrafish (Danio rerio) (5-8 months old)



Biomagnification factor (BMF) = 0.0259. Thus, it can be speculated that there is **no biomagnification of $nTiO_2$ from Daphnia to zebrafish**.

Uptake and depuration of nTiO2 in Zebrafish : waterborne

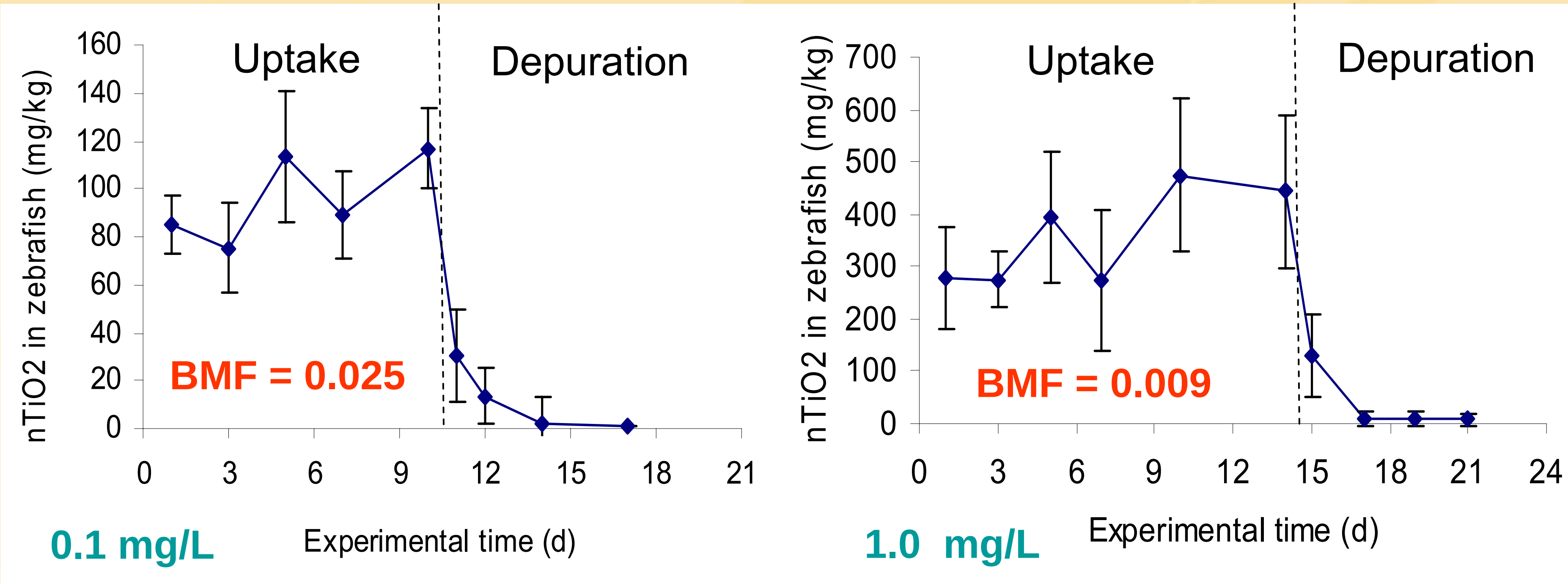




BCF = 25.38

BCF = 181.38

Uptake and depuration of nTiO2 in Zebrafish : dietary-borne



no biomagnification of $nTiO_2$ from Daphnia to zebrafish.

Uptake, depuration rate constants, bioconcentration factors (BCFs), and biomagnifications factors (BMFs) in Danio rerio

Initial dose (mg/L)	Exact dose	Whole body concentration [c]	BCFs or BMFs	$K_M = t_{u0.5}$ (d)	$t_{d0.5}$ (d)
0.1	0.06 mg/L[a]	1.52 ± 0.26 mg/kg	25.38	1.42	5.69
1.0	0.55 mg/L[a]	96.76 ± 3.02 mg/kg	181.38	16.75	1.92
--	4.23±0.41 g/kg[b0.1]	106.57 ± 14.89 mg/kg	0.025[d]	--	--
--	56.91±3.20 g/kg[b1.0]	522.02 ± 12.94 mg/kg	0.009[d]	--	--

[a] Exact dose in culture medium: 24 h time weight exposure concentrations.
[b] Exact dose in Daphnia: b0.1- after exposure to 0.1 mg/L $nTiO_2$ for 24 h; b1.0- after exposure to 1.0 mg/L $nTiO_2$ for 24 h.
[c] Concentrations at steady state conditions.
[d] BMFs at steady state conditions.

$nTiO_2$ accumulation profile comparison between Daphnia and Zebrafish

Test organism	$nTiO_2$ 0.1 mg/L				$nTiO_2$ 1.0 mg/L			
	$t_{u0.5}$		$t_{d0.5}$	BCFs	$t_{u0.5}$		$t_{d0.5}$	BCFs
Daphnia	3.87 h	$<$	26.76 h	56562.50	3.72 h	$<$	74.52 h	118062.84
zebrafish	1.42 d	$<$	5. 69 d	25.38	16.75 d	$>$	1.92 d	181.38

Future works

- Determine the other NPs (such as C_{60} NPs) bioconcentration and bioaccumulation in aquatic organisms.
- Determine the distribution (or fate) of NPs in different parts of exposure system, including water, organism body and the excretion, based on the mass balance profile or used a stable isotopic tracer approach.
- Determine the influence of NPs physicochemical properties (such as particle size, shape) and the environmental factor (such as food, salinity and illumination) on the bioaccumulation behavior of NPs.
- Determine the bioaccumulation behavior of NPs under different exposure conditions, such as static, semi-static and flow-through system.

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