SUPPLEMENTARY INFORMATION for

Multilaboratory evaluation of 15 bioassays for (eco)toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID

Nanotoxicology

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SUPPLEMENTARY FIGURES

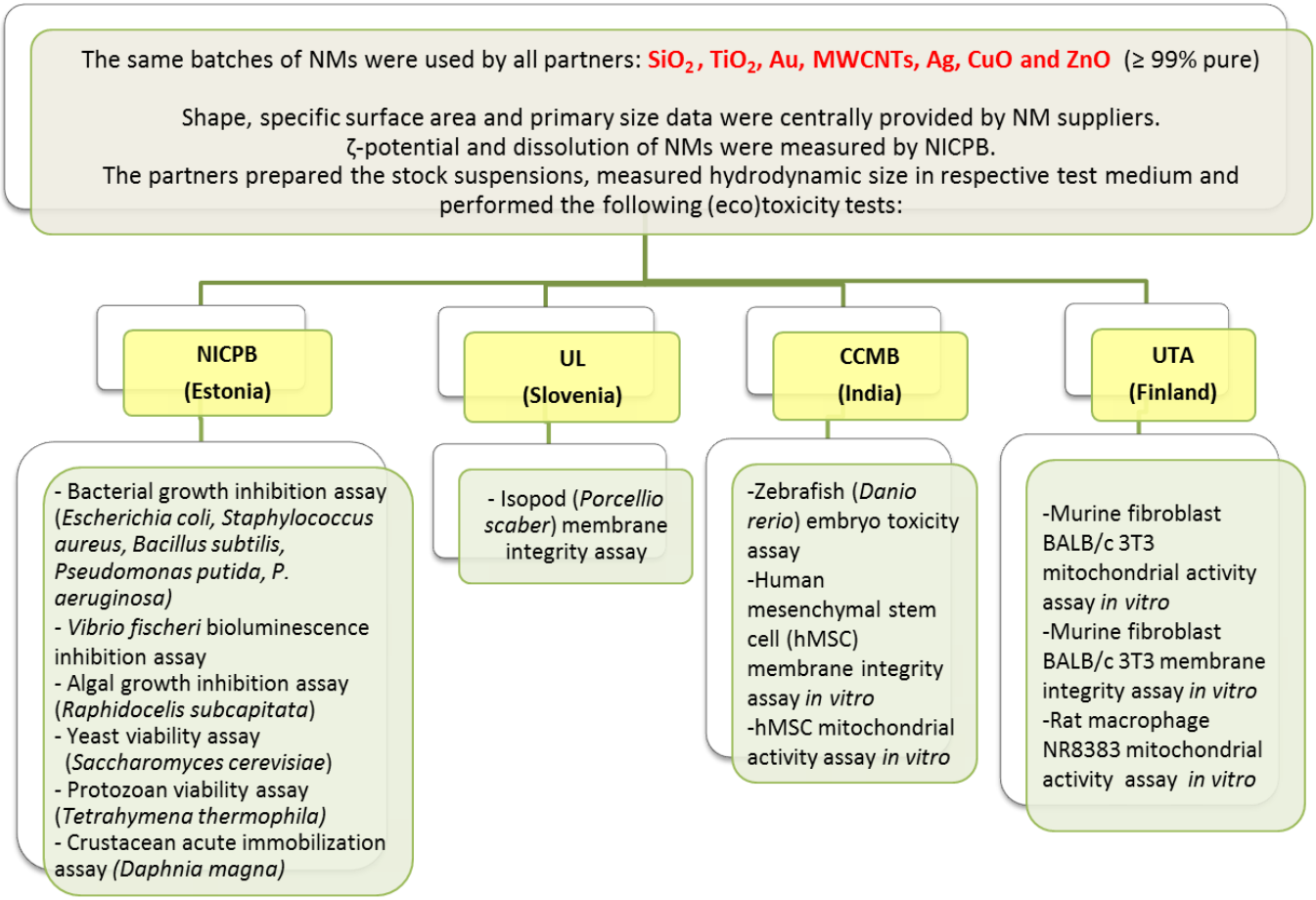

Fig. S1 Experimental set-up of this study. Yellow boxes represent partner institutions involved: National Institute of Chemical Physics and Biophysics (NICPB, Estonia), University of Ljubljana (UL, Slovenia); Centre for Cellular & Molecular Biology (CCMB, India) and University of Tampere (UTA, Finland). Green boxes present bioassays performed by partner institutions.

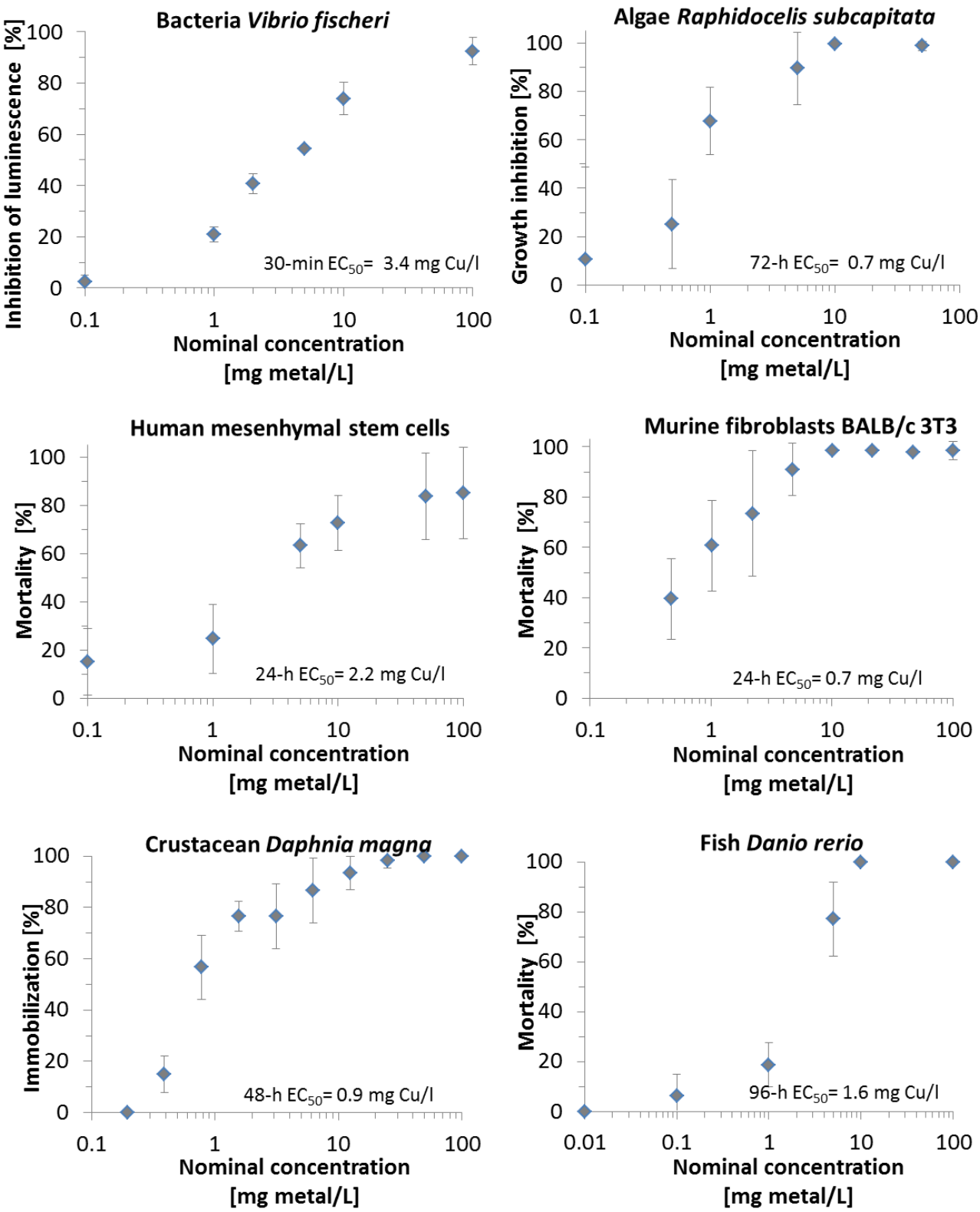

Fig. S2 Dose-response curves of test organisms to CuO NMs (average from different independent experiments ± standard deviations): 30-min *Vibrio fischeri* luminescence inhibition assay, 72-h algal *Raphidocelis subcapitata* growth inhibition assay, 24-h human mesenchymal stem cell membrane integrity assay *in vitro* (propidium iodide staining), 48-h murine fibroblast BALB/c 3T3 mitochondrial activity assay *in vitro* (WST-1), 48-h crustacean *Daphnia magna* acute immobilization assay and 96-h zebrafish *Danio rerio* embryo toxicity assay.

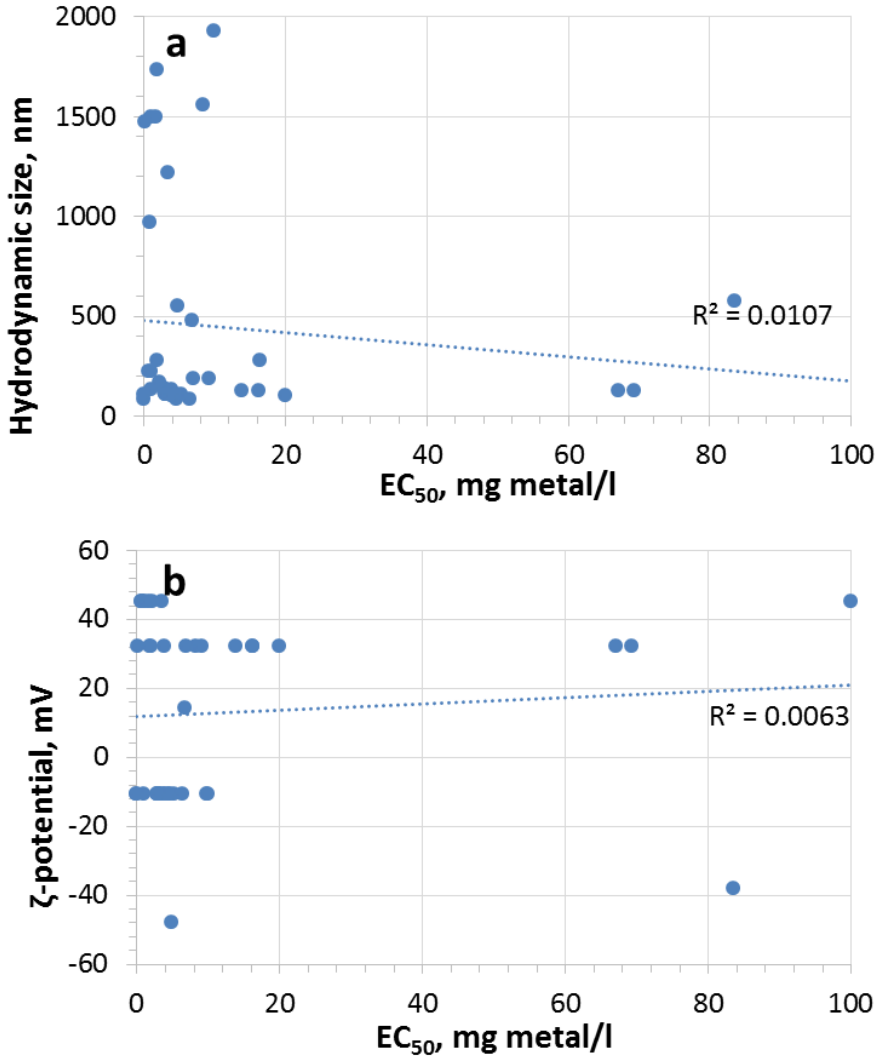

Fig. S3 Toxicity (EC_{50}) of NMs to different organisms *versus* hydrodynamic size in the respective test medium (a) and ζ-potential in DI water (b). EC_{50} >100 mg metal/l were not included. Data are plotted from Table 2, Table 4 and Table S2.

SUPPLEMENTARY TABLES

Table S1 95% confidence limits of EC_{50} values of dissolution-prone NMs (Table 4) and the number of independent experiments performed to obtain these EC_{50} values.

	Tests model	95% confidence limit			Number of independent experiments		
		Ag	CuO	ZnO	Ag	CuO	ZnO
Prokaryotes, single cell	BACTERIA:						
	Escherichia coli	1.36-4.76	not applicable*	55.4-78.9	4	2	2
	Staphylococcus aureus	1.83-8.55	not applicable*	12.2-20.2	4	2	2
	Bacillus subtilis	2.71-6.33	not applicable*	8.8-18.8	4	2	2
	Pseudomonas putida	2.31-5.33	not applicable*	26.9-81.6	4	2	2
	Pseudomonas aeruginosa	2.0-4.34	not applicable*	not applicable*	4	2	2
	Vibrio fischeri	2.48-3.36	3.29-3.82	7.88-12.55	4	2	1
Eukaryotes, single cell	YEAST: *Saccharomyces cerevisiae*	not applicable**	not applicable**	not applicable**	3	2	3
	ALGAE: *Raphidocelis subcapitata*	0.00597 - 0.01003	0.41-1.07	0.06 -0.27	2	2	2
	PROTOZOA: *Tetrahymena thermophila*	3.11-5.11	not applicable*	3.53-8.12	5	1	4
Mammalian cell cultures in vitro	human mesenchymal stem cells (PI)	1.94-10.74	0.72-6.78	0.91-4.29	4	4	5
	human mesenchymal stem cells (MTT)	2.80-8.56	not applicable*	11.4-21.22	3	3	3
	murine fibroblasts BALB/c 3T3 (WST-1)	2.7-3.3***	0.55-0.87	6.55-11.6	2	2	2
	murine fibroblasts BALB/c 3T3 (NRU)	2.7-2.9***	0.93-1.11	5.95-7.73	2	2	2
	rat macrophages NR8383 (WST-1)	9.4-10.2	not determined	not determined	2	not determined	not determined
Multicellular organisms	CRUSTACEAN: *Daphnia magna*	0.00206 - 0.00266	0.82-1.22	1.56-1.91	3	5	5
	CRUSTACEAN: *Porcellio scaber* (isolated digestive gland) (AO/EB)	not applicable**	not applicable**	not applicable**	2	2	2
	FISH: zebrafish (*Danio rerio*) embryo	2.19-4.02	0.84-2.24	not determined	3	3	not determined

*EC_{50} >100 mg/l; **Toxicity endpoint is minimal bactericidal concentration (yeast *S. cerevisiae*) or LOEC (crustacean *P. scaber*); ***Published in Zou et al. 2014. AO/EB, acridine orange/ethidium bromide; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NRU, neutral red uptake; PI, propidium iodide; WST-1, 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium.

Table S2 Hydrodynamic size of the studied nanomaterials in different test media.

Test medium	Respective assay	Hydrodynamic size, nm					
		SiO_2	**TiO_2**	**Au**	**Ag**	**CuO**	**ZnO**
LB medium[1]	Bacterial growth inhibition assay	921±138	759±48	24±2	107±1.3	1039±141	125±2
2% NaCl	*Vibrio fischeri* bio-luminescence inhibition assay	1101±76	865±354	552±34	137±0.9	1221±250	1561±123
Deionised (DI) water	Yeast spot assay; Protozoan viability assay	854±38	367±60	23±4	132±0.5	152±2	102±1
Algal growth medium	Algae growth inhibition test	575±65	478±44	not determined	83±2.9	970±253	1476±576
Artificial freshwater[2]	*Daphnia magna* immobilization assay and zebrafish embryo toxicity assay	not determined	767±13	528±41	111±0.6	1497±77	1733±185
DMEM containing 10% foetal bovine serum (FBS)	Assays with human mesenchymal stem cells	6858±1120	6513±2783	489±120	87±1	171±4	277±44
DMEM containing 5.0% new born calf serum	Assays with murine fibroblasts BALB/c 3T3	493±266	365±169	48±31	121±53	227±89	187±100
Kaighn's Modification of Ham's F-12 with 7.5% FBS	Assays with rat alveolar macrophages NR8383	620±285	not determined	52±43	1929±372	not determined	not determined

[1] Composition: 10 g tryptone and 5 g yeast extract *per* liter, pH=7 (NaCl was not added to the medium to reduce the agglomeration of NMs).
[2] 294 mg/l $CaCl_2*2H_2O$, 123.3 mg/l $MgSO_4*7H_2O$, 65 mg/l $NaHCO_3$, 5.75 mg/L KCl dissolved DI water.