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INTEGRANO

MULTIDIMENSIONAL INTEGRATED QUANTITATIVE APPROACH TO ASSESS SAFETY AND SUSTAINABILITY OF NANOMATERIALS IN REAL CASE LIFE CYCLE SCENARIOS USING NANOSPECIFIC IMPACT CATEGORIES

WP3

Name of the presentation

12M Annual General Meeting

Turin - Italy

29-30 January 2025

This project has received funding from the European Union's Horizon Europe research and innovation programme under GA No 101138414

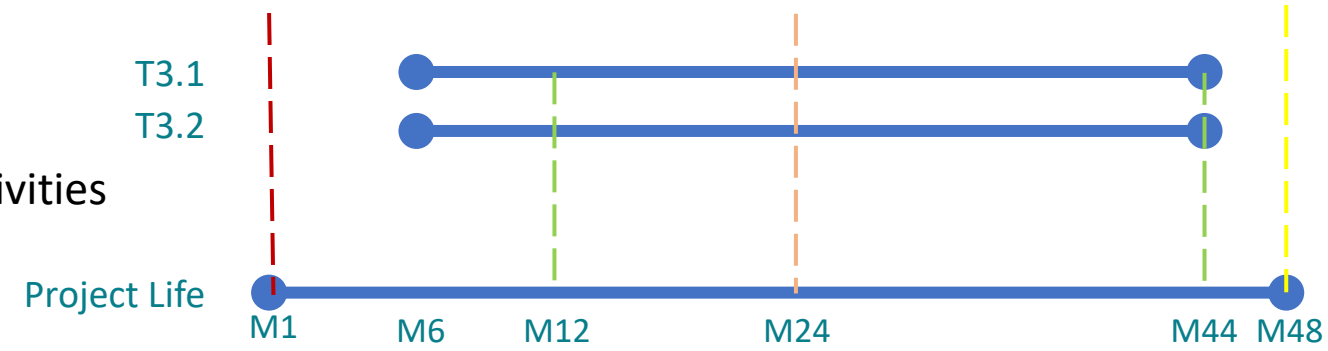
WP3

Nano Tox and nano eco-tox data generation

PLANNING OF ACTIVITIES:

Where we are…

- Beginning of the 6th month of tasks activities



Objectives

- To assess eco-toxicology of investigated composite NMs groups (T3.1)
- To provide (eco-)toxicological based on dose-response experimental outcomes (T3.1)
- To identify and correlate dimension, morphology and other p-chem features modulating impact on AOPs (T3.2)
- To assess exposure scenario and carry out studies on NMs persistence and bioaccumulation even in low concentration doses at prolonged simulated exposure (T3.1)

Task 3.1 and 3.2

Ecotoxicity: Fate and effects in biological and environmental relevant matrices
Collecting toxicity data and filling gaps for an early identification of hazard potential. CFs for toxicological assessment by in-vitro advanced models

Collection of published papers/available data from previous projects reporting p-chem and eco-toxicity

- Dataset collection on p-chem and eco-toxicological info on Ag, TiO2, SiO2, CuO, ZnO

Literature search on more endpoints to select KPI in NM ecotoxicity

PRJ-UNIMIB-CNR (11/2024)
defined during the meeting of November 18th , 2024

- Sharing of the dataset for identification of the gaps in Ecotox-Tox data

Data shown to all partners involved in NM synthesis

During the meeting (11/2024)

Task 3.1

Ecotoxicity: Fate and effects in biological and environmental relevant matrices

Ecosafety of innovative nano materials/products

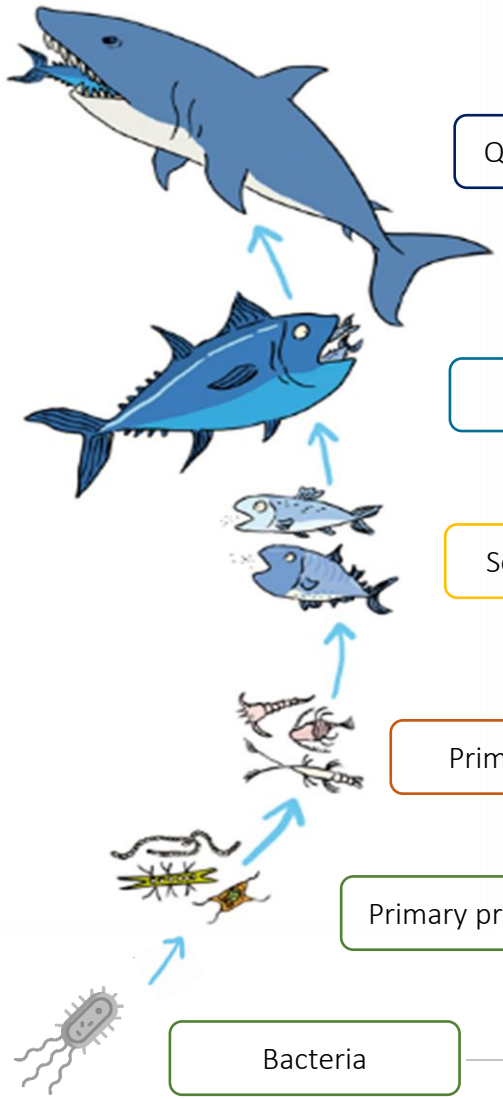
- Standard (i.e. UNI EN ISO, ISO, ASTM) and innovative methods

Nanomaterials delivering and testing

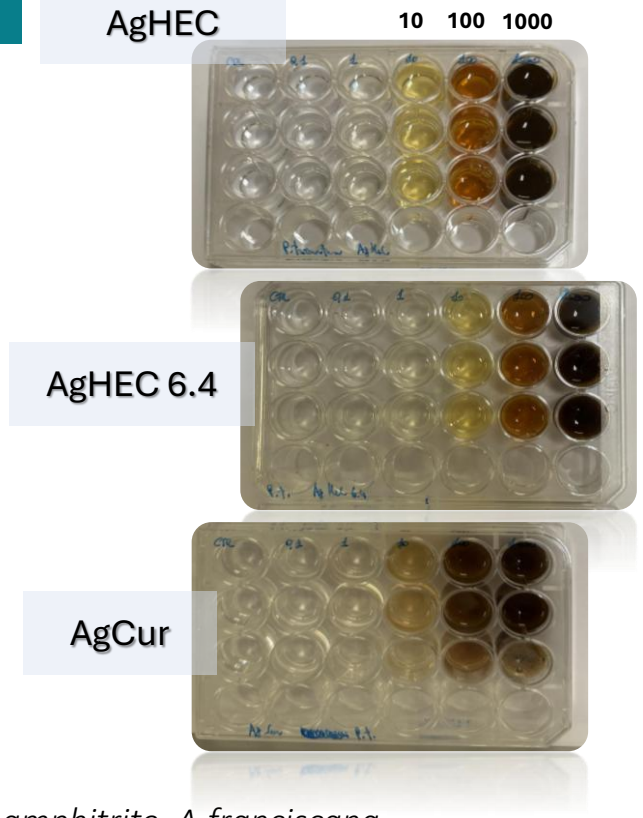
Case study			
CS - 1.1 Ag-Naked AgCur AgHEC (CS 4.2) AgHEC_6.4 (CS 4.2)	.....	Received from CNR-ISSMC (26/09/2024)	..... **Ongoing tests on** Marine bacteria (*A. fischeri*) Marine phytoplankton (*D. tertiolecta; P. tricornutum*) Marine invertebrates (*A. amphitrite; A.franciscana; P. lividus; Aurelia sp.*)
CS - 1.2 (only 2 mg) ZnO CuO	.....	Received from BIU (18/11/2024, additional batches during the meeting)	..... Ecotox tests planned in 2025 (with a high NP amount)
CS - 3 Bi-SiO2-F/D (open mould) Bi-SiO2-F/GB (open mould) Bi-SiO2-F/D (closed mould) Bi-SiO2-F/GB (closed mould)	.....	Planned shipment from CNR-IPCB and CNR-SCITEC (12/2024) *defined during the meeting of December 5th , 2024*	..... Ecotox tests planned in 2025

CNR-IAS: Ecotox

Marine food chain



- Quaternary consumers
- Tertiary consumers
- Secondary consumers
- Primary consumers — *A. amphitrite, A.franciscana, P. lividus, B. plicatilis, Aurelia sp.*
- Primary producers — *D. tertiolecta, P. tricornutum*
- Bacteria — *A. fischeri*



Tested concentrations

Range finding test:
1000 - 100 - 10 - 1 - 0.1 mg/L

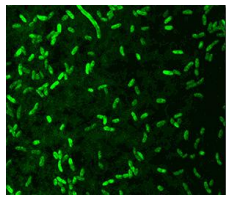
Effective concentrations:
10 - 5 - 1 - 0.5 - 0.1 mg/L

Only for *P. lividus*
0.1 - 0.05 - 0.01 - 0.005 mg/L

CS 1.1 - nAg ecotox assessment

Bacteria

A. fischeri



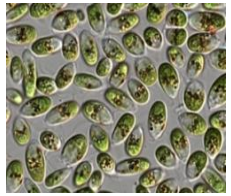
30 m

15 °C

Bioluminescence inhibition
(ISO 11348-3, 2019)

Phytoplankton

D. tertiolecta

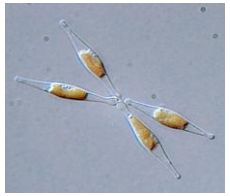


72 h

20 °C

Light condition

P. tricornutum



72 h

20 °C

Light condition

Growth inhibition
(ISO 10253, 2016)

Zooplankton

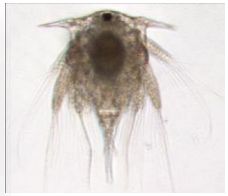
A. franciscana

24 - 48 h

20 °C

Dark condition

A. amphitrite



24 - 48 h

20 °C

Dark condition

Mortality – Immobility - Behaviour
(APAT IRSA CNR 8070, 2003;
UNICHIM, 2011; Faimali et al., 2006, 2014)

NEW!

B. plicatilis

24 - 48 h

25 °C

Dark condition

Aurelia sp.

24-48 h

20 °C

Dark condition

Immobility - Behaviour
(ISO 19820, 2016;
Famali et al., 2006, 2014)

NEW!

P.lividus

72 h

20 °C

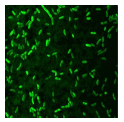
Dark condition

Larval development
(Sartori et al., 2017)

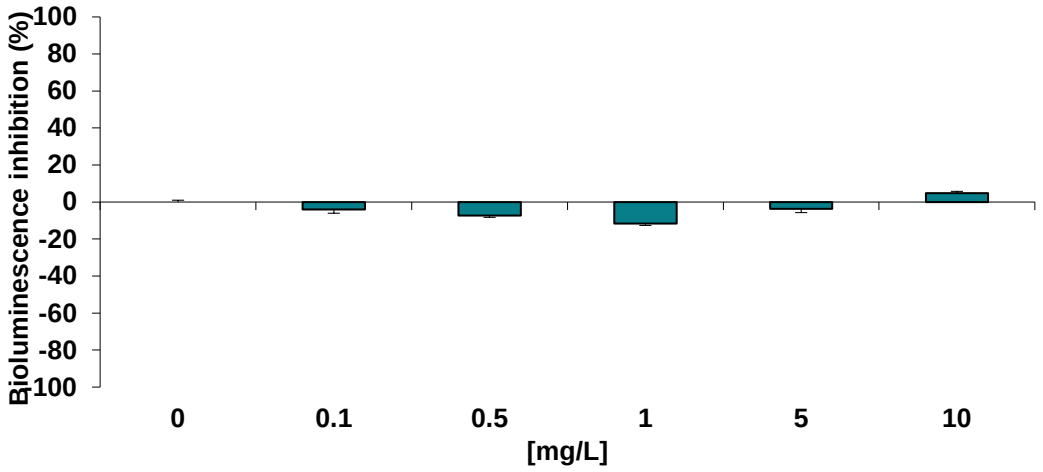
nAg: Ecotox data

Bacteria

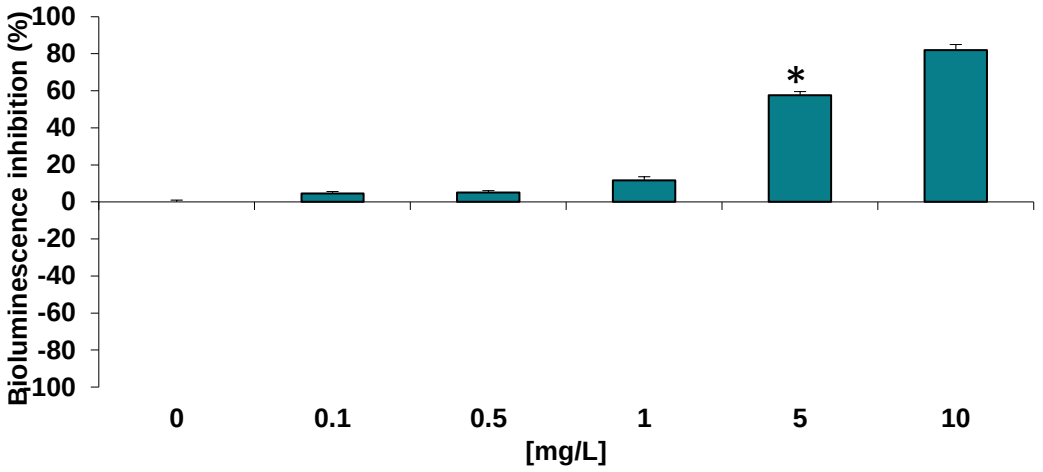
A. fischeri



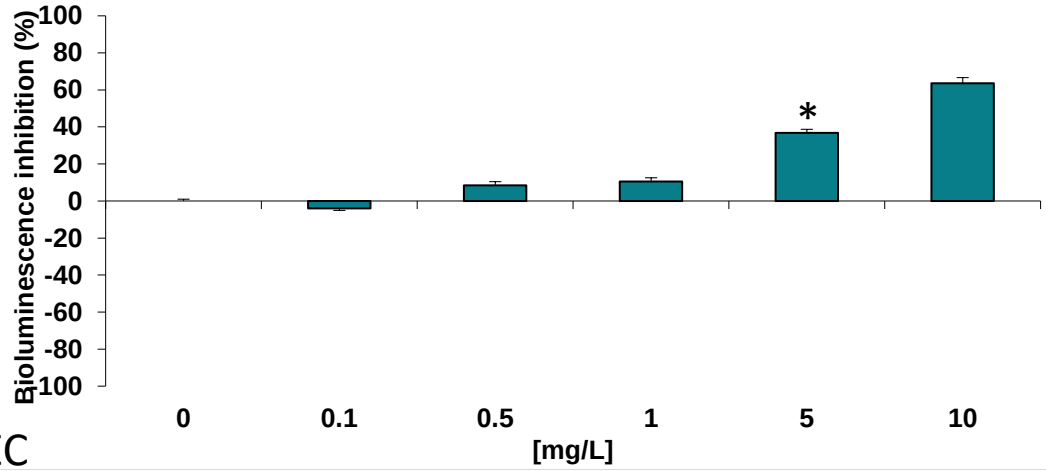
AgNaked



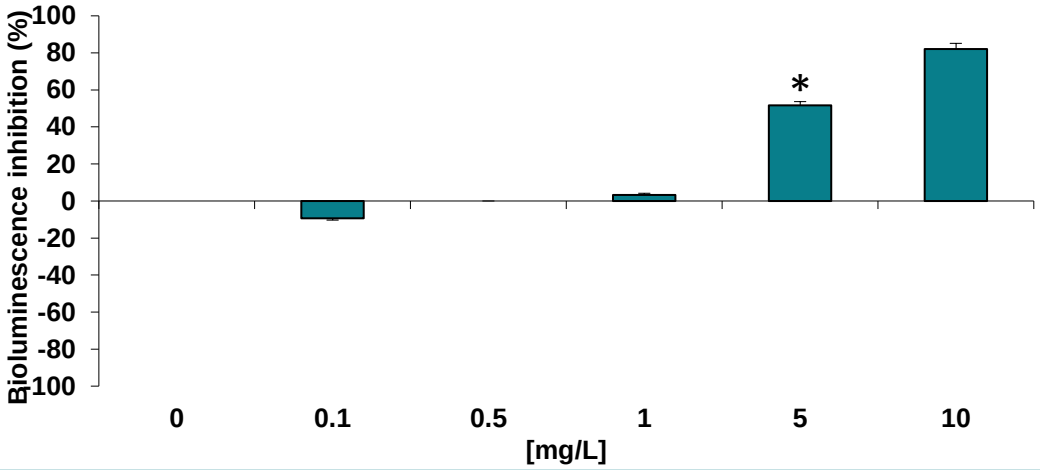
AgCur



AgHEC



AgHEC6.4



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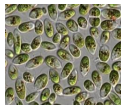
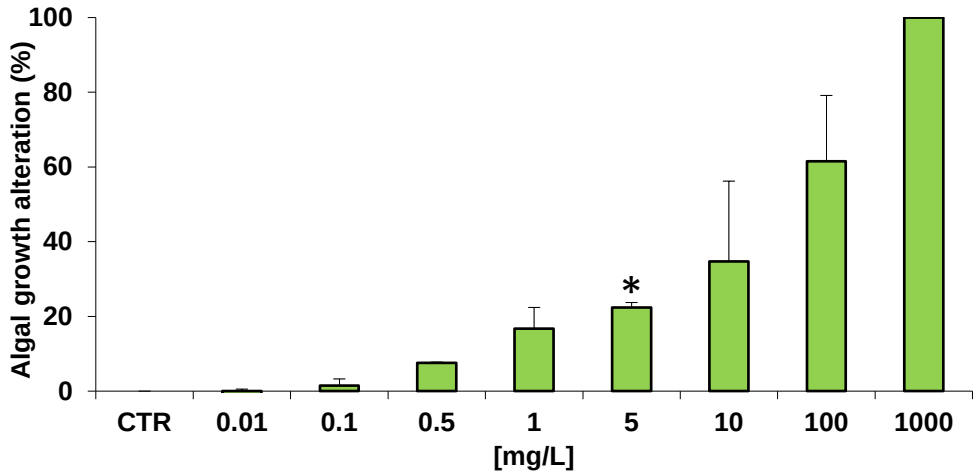
Phytoplankton

D. tertiolecta



nAg: Ecotox data

AgNaked



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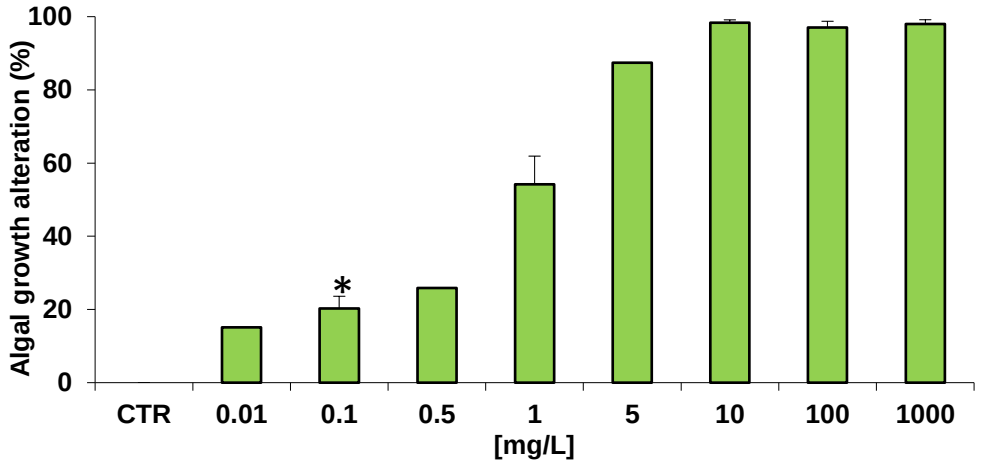
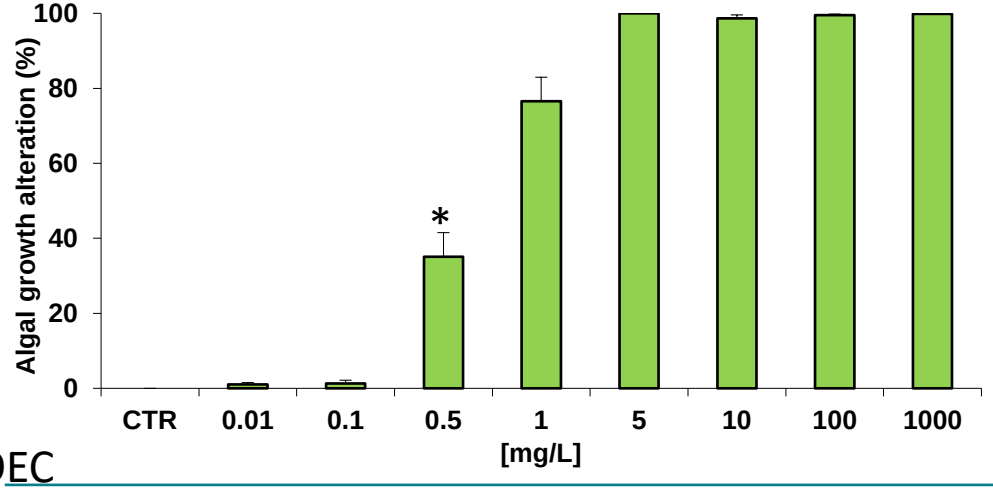


AgHec



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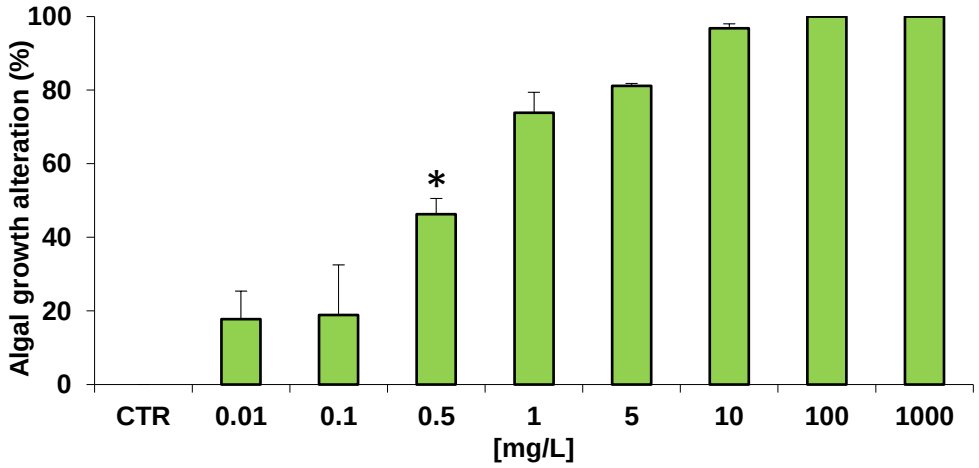

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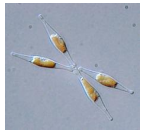
nAg: Ecotox data

Phytoplankton

P. tricornutum



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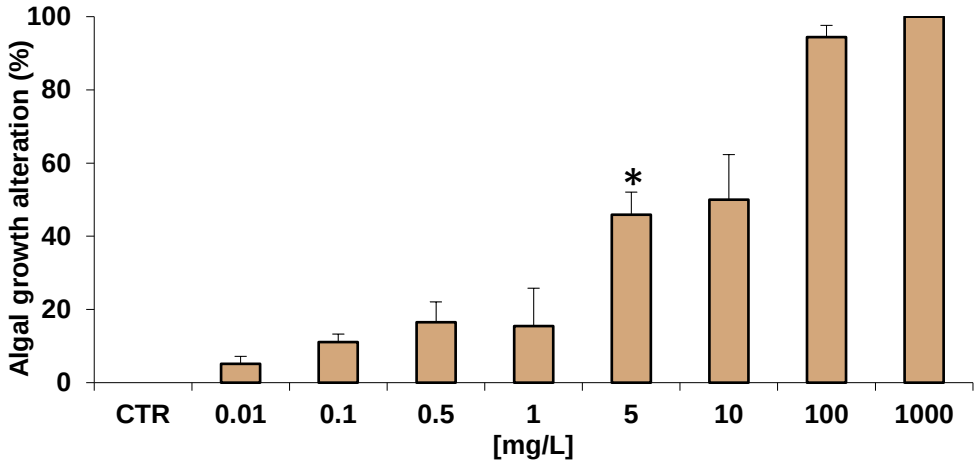

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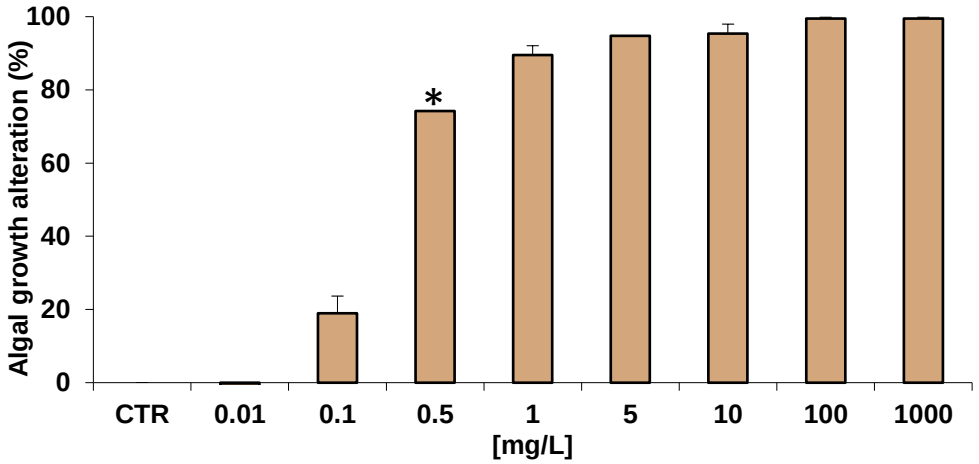
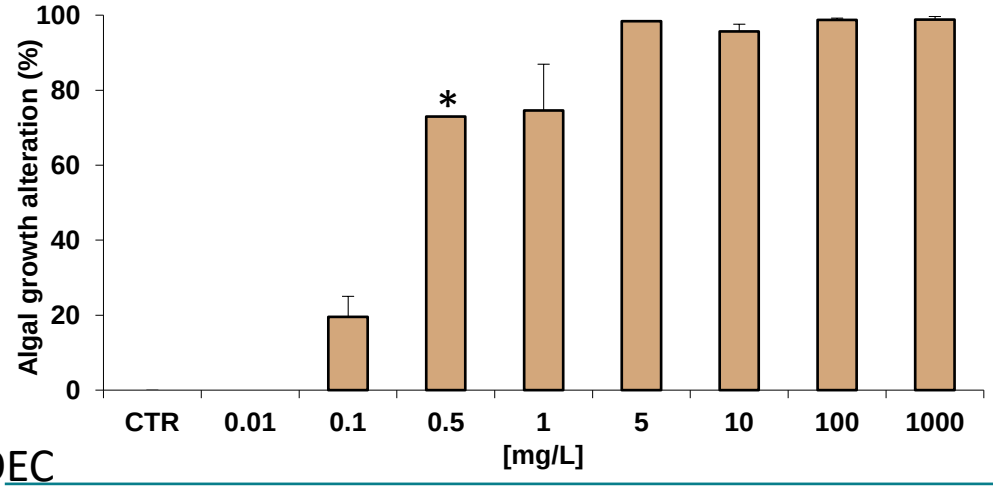


AgHec



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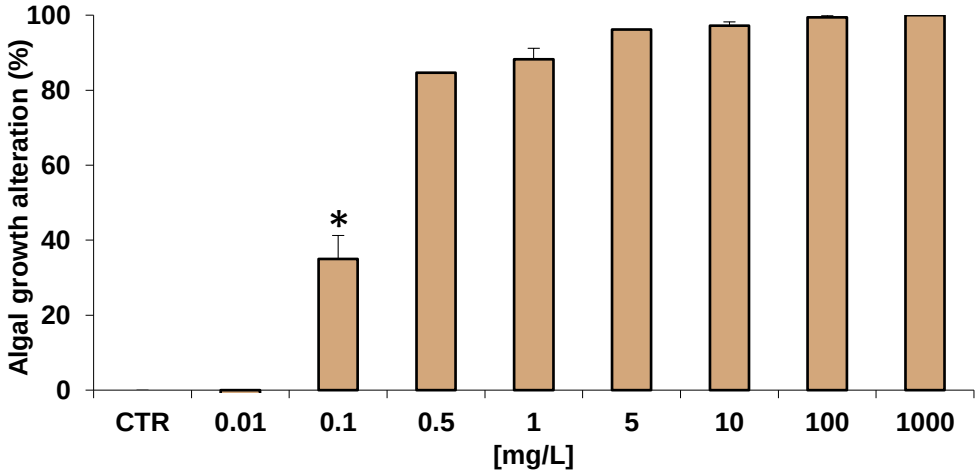

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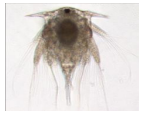
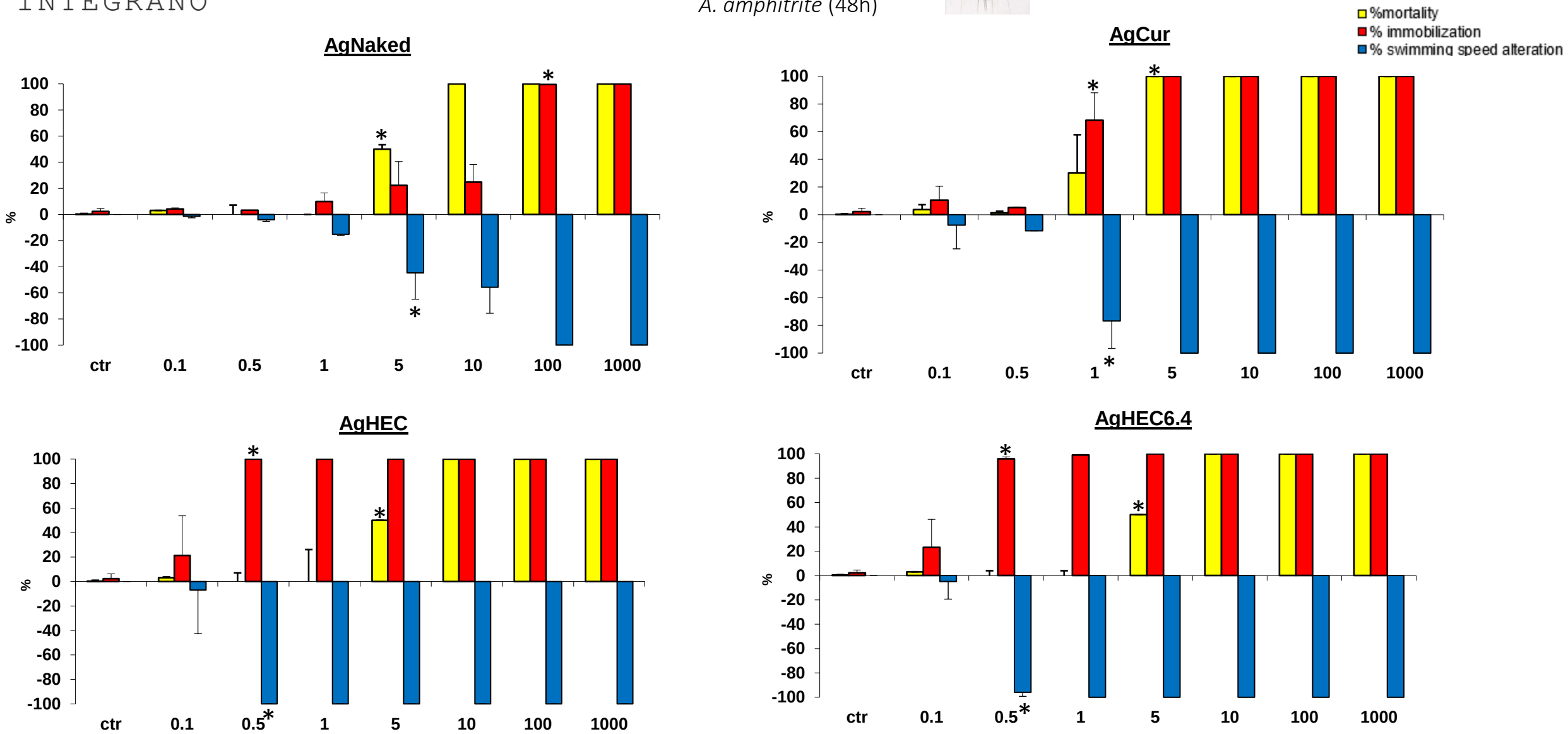
nAg: Ecotox data

Zooplankton

A. amphitrite (48h)





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nAg: Ecotox data

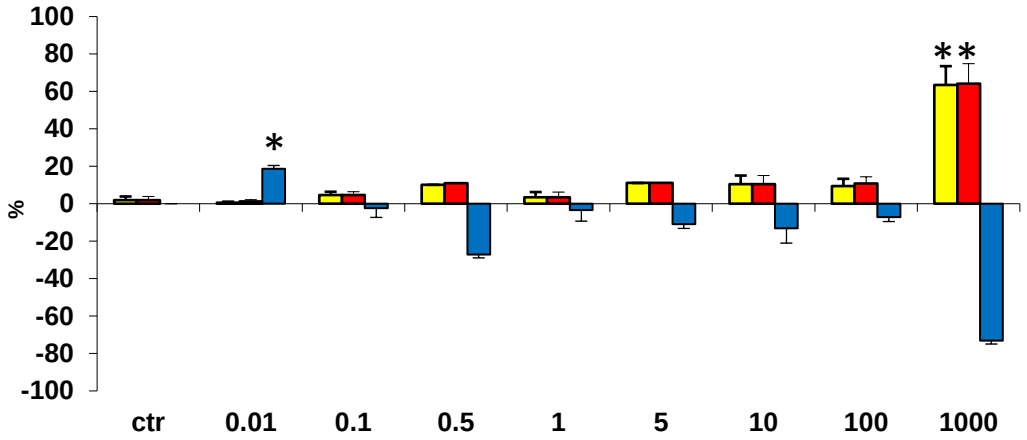
Zooplankton

A. franciscana (48h)

%mortality
% immobilization
% swimming speed alteration

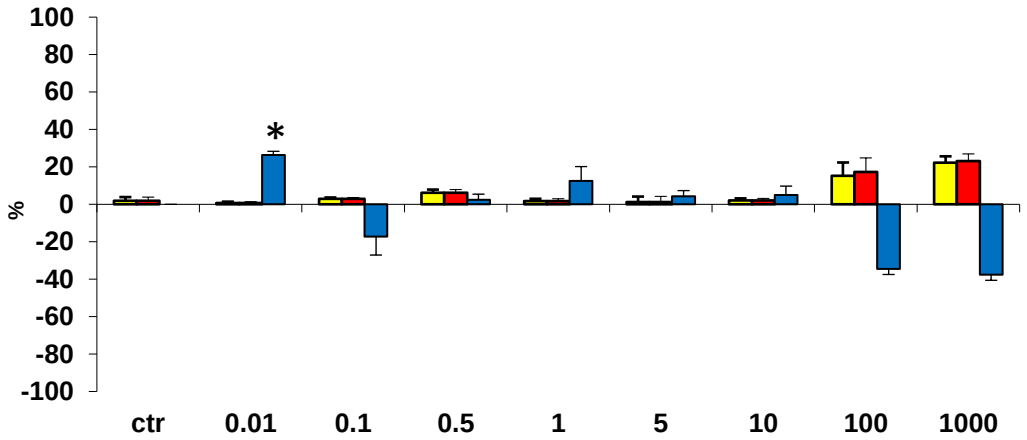
***A. franciscana* - 48h**

AgNaked



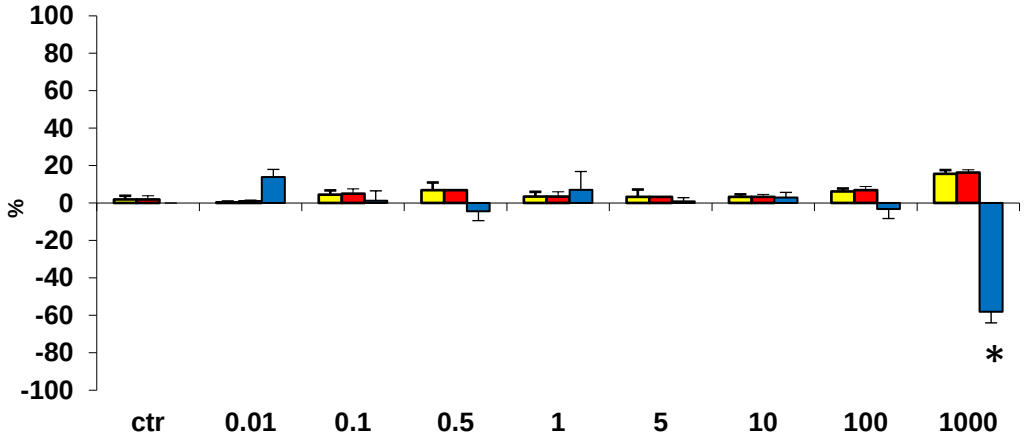
***A. franciscana* - 48h**

AgCur



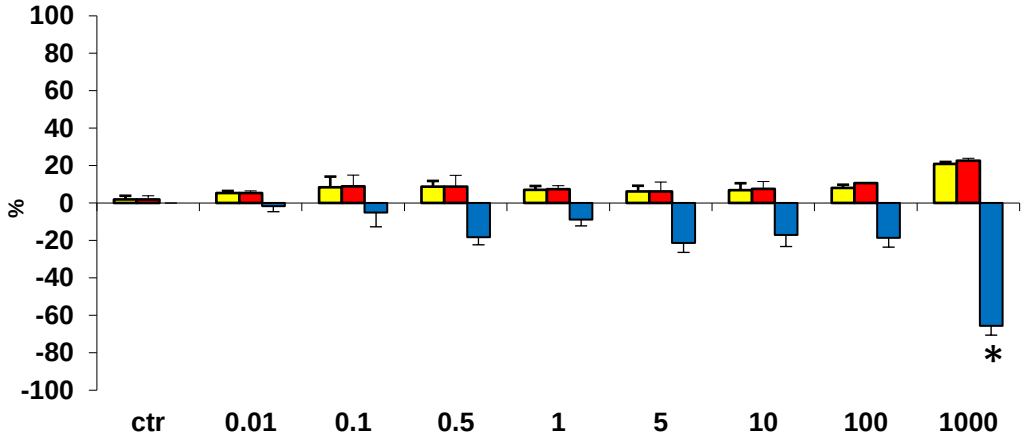
***A. franciscana* - 48h**

AgHEC



***A. franciscana* - 48h**

AgHEC 6.4



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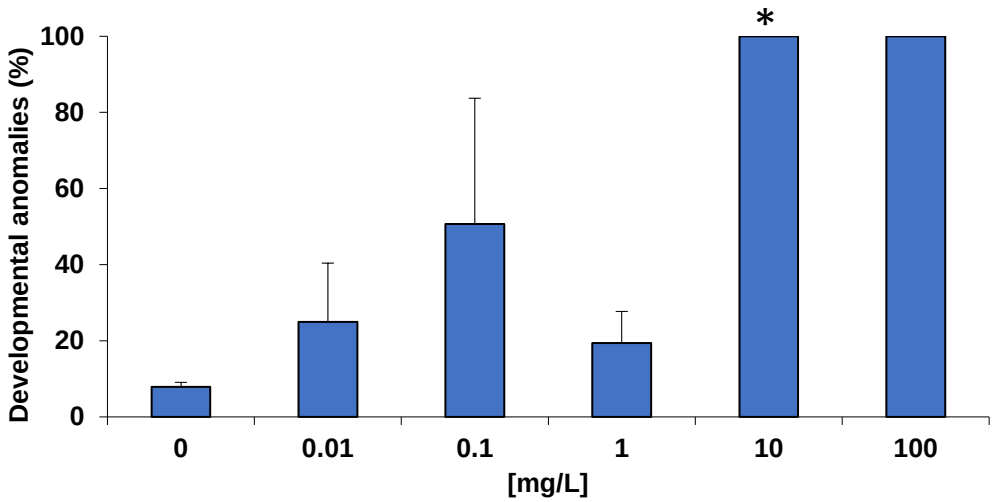
Zooplankton

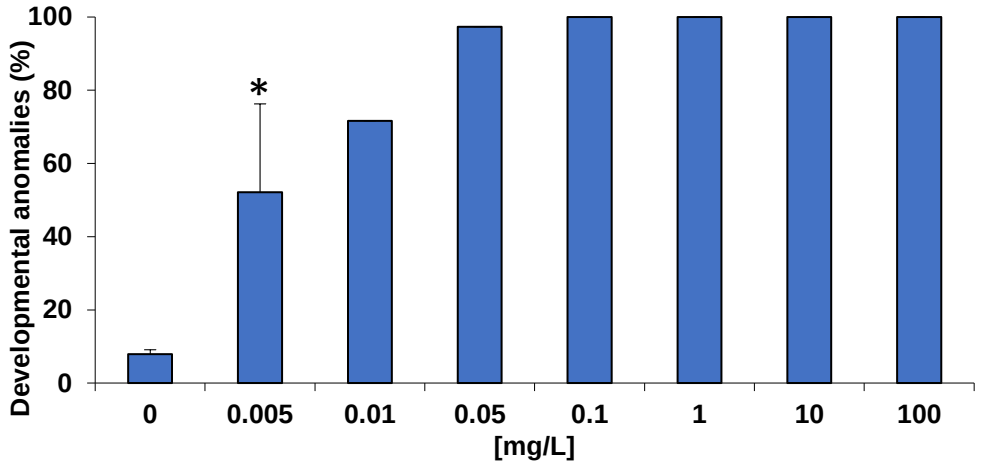
P. lividus

nAg: Ecotox data

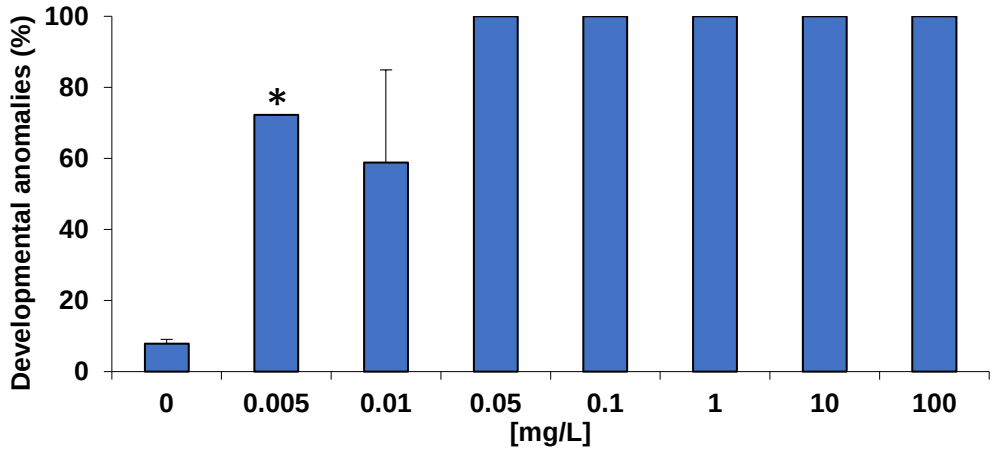
***P.lividus* - 72h** **AgNaked**



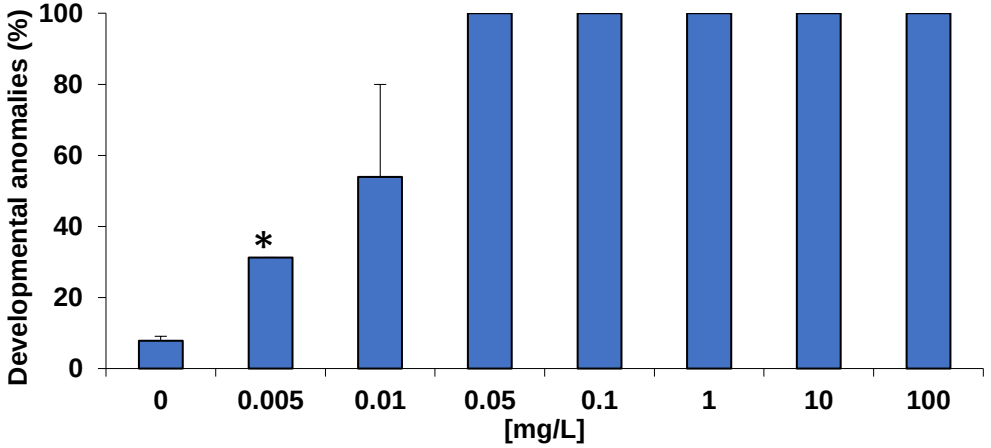
***P.lividus* - 72h** **AgCur**



***P.lividus* - 72h** **AgHEC**



***P.lividus* - 72h** **AgHEC6.4**



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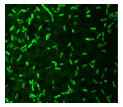
CS 1.1 - nAg ecotox assessment in seawater

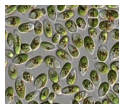
Based on ≥ 3 tests

A. fischeri



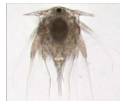
P. tricornutum

D. tertiolecta



A. franciscana

A. amphitrite



P.lividus

				EC50 (mg/L)			
		Exposure time	End-point	Ag-Naked	AgCur	AgHEC	AgHEC6.4
Bacteria	*A. fischeri*	30m	Bioluminescence inhibition	>10	3.68 (3.03-4.47)	6.98 (3.59-13.56)	4.35 (3.57-5.29)
Phytoplankton	*D. tertiolecta*	72h	Growth inhibition	77.20 (14.30-418.30)	0.42 (0.22-0.81)	0.08 (0.004-1.48)	0.16 (0.07-0.40)
	P. tricornutum	72h	Growth inhibition	18.79 (9.58-36.86)	0.25 (0.21-0.30)	0.046 (0.007-0.27)	0.17 (0.13-0.20)
Zooplankton	*A. amphitrite*	24h	Mortality	302.0 (283-322)	5.00 (4.69-5.33)	3.34 (3.15-3.54)	1.020 (-)
			Immobility	31.12 (29.81-32.50)	0.48 (0.42-0.55)	0.30 (0.30-0.31)	0.51 (0.49-0.52)
			Behaviour	14.32 (8.13-25.21)	0.29 (0.28-0.29)	0.54 (0.49-0.60)	0.17 (0.16-0.17)
		48h	Mortality	5.0 (4.97-5.03)	0.35 (0.34-0.36)	0.33 (0.31-0.35)	0.35 (0.32-0.38)
			Immobility	19.76 (0.29- 1000)	0.164 (0.163-0.166)	~ 0.09	0.06 (0.05-0.06)
			Behaviour	16.03 (9.55-26.91)	0.77 (0.70-0.85)	0.11	0.22 (0.21-0.22)
	A. franciscana	24h	Mortality	>1000	>1000	>1000	>1000
			Immobility	>1000	>1000	>1000	>1000
			Behaviour	>1000	>1000	599.5 (432.7-830.6)	338.39 (256.88-445.76)
		48h	Mortality	~ 900	>1000	>1000	>1000
			Immobility	~ 900	>1000	>1000	>1000
			Behaviour	448.24 (362.93- 593.62)	>1000	715.39 (502.24- 1000)	485.30 (289.9-812.5)
	P. lividus	72h	Larval development	0.098 (0.002-4.977)	0.005 (0.001-0.022)	0.004 (0.0006-0.0268)	0.009 (0.005-0.15)

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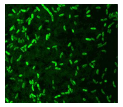
CS 1.1 - nAg ecotox assessment in seawater

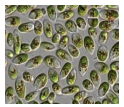
Based on ≥ 3 tests

A. fischeri



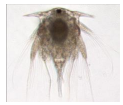
P. tricornutum

D. tertiolecta



A. franciscana

A. amphitrite



P.lividus

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		48h	Mortality	5.0 (4.97-5.03)	0.35 (0.34-0.36)	0.33 (0.31-0.35)	0.35 (0.32-0.38)
			Immobility	19.76 (0.29- 1000)	0.164 (0.163-0.166)	~ 0.09	0.06 (0.05-0.06)
			Behaviour	16.03 (9.55-26.91)	0.77 (0.70-0.85)	0.11	0.22 (0.21-0.22)
	A. franciscana	24h	Mortality	>1000	>1000	>1000	>1000
			Immobility	>1000	>1000	>1000	>1000
			Behaviour	>1000	>1000	599.5 (432.7-830.6)	338.39 (256.88-445.76)
		48h	Mortality	~ 900	>1000	>1000	>1000
			Immobility	~ 900	>1000	>1000	>1000
			Behaviour	448.24 (362.93- 593.62)	>1000	715.39 (502.24- 1000)	485.30 (289.9-812.5)
	P. lividus	72h	Larval development	0.098 (0.002-4.977)	0.005 (0.001-0.022)	0.004 (0.0006-0.0268)	0.009 (0.005-0.15)

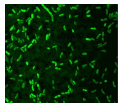
Species used in the Italian Legislative Decree on wastewater treatment (DL 152/2006):
No toxicity up to 1000 mg/L by using standardized methods

Funded by the European Union

CS 1.1 - nAg ecotox assessment in seawater

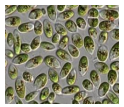
Based on ≥ 3 tests

A. fischeri



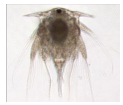
P. tricornutum

D. tertiolecta



A. franciscana

A. amphitrite



P.lividus

		Exposure time	End-point	EC50 (mg/L) Ag-Naked	AgCur	AgHEC	AgHEC6.4
Bacteria	*A. fischeri*	30m	Bioluminescence inhibition	>10	3.68 (3.03-4.47)	6.98 (3.59-13.56)	4.35 (3.57-5.29)
Phytoplankton	*D. tertiolecta*	72h	Growth inhibition	77.20 (14.30-418.30)	0.42 (0.22-0.81)	0.08 (0.004-1.48)	0.16 (0.07-0.40)
	P. tricornutum	72h	Growth inhibition	18.79 (9.58-36.86)	0.25 (0.21-0.30)	0.046 (0.007-0.27)	0.17 (0.13-0.20)
Zooplankton	*A. amphitrite*	24h	Mortality	302.0 (283-322)	5.00 (4.69-5.33)	3.34 (3.15-3.54)	1.020 (-)
			Immobility	31.12 (29.81-32.50)	0.48 (0.42-0.55)	0.30 (0.30-0.31)	0.51 (0.49-0.52)
			Behaviour	14.32 (8.13-25.21)	0.29 (0.28-0.29)	0.54 (0.49-0.60)	0.17 (0.16-0.17)
		48h	Mortality	5.0 (4.97-5.03)	0.35 (0.34-0.36)	0.33 (0.31-0.35)	0.35 (0.32-0.38)
			Immobility	19.76 (0.29- 1000)	0.164 (0.163-0.166)	~ 0.09	0.06 (0.05-0.06)
			Behaviour	16.03 (9.55-26.91)	0.77 (0.70-0.85)	0.11	0.22 (0.21-0.22)
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			Immobility	>1000	>1000	>1000	>1000
			Behaviour	>1000	>1000	599.5 (432.7-830.6)	338.39 (256.88-445.76)
		48h	Mortality	~ 900	>1000	>1000	>1000
			Immobility	~ 900	>1000	>1000	>1000
			Behaviour	448.24 (362.93- 593.62)	>1000	715.39 (502.24- 1000)	485.30 (289.9-812.5)
	P. lividus	72h	Larval development	0.098 (0.002-4.977)	0.005 (0.001-0.022)	0.004 (0.0006-0.0268)	0.009 (0.005-0.15)

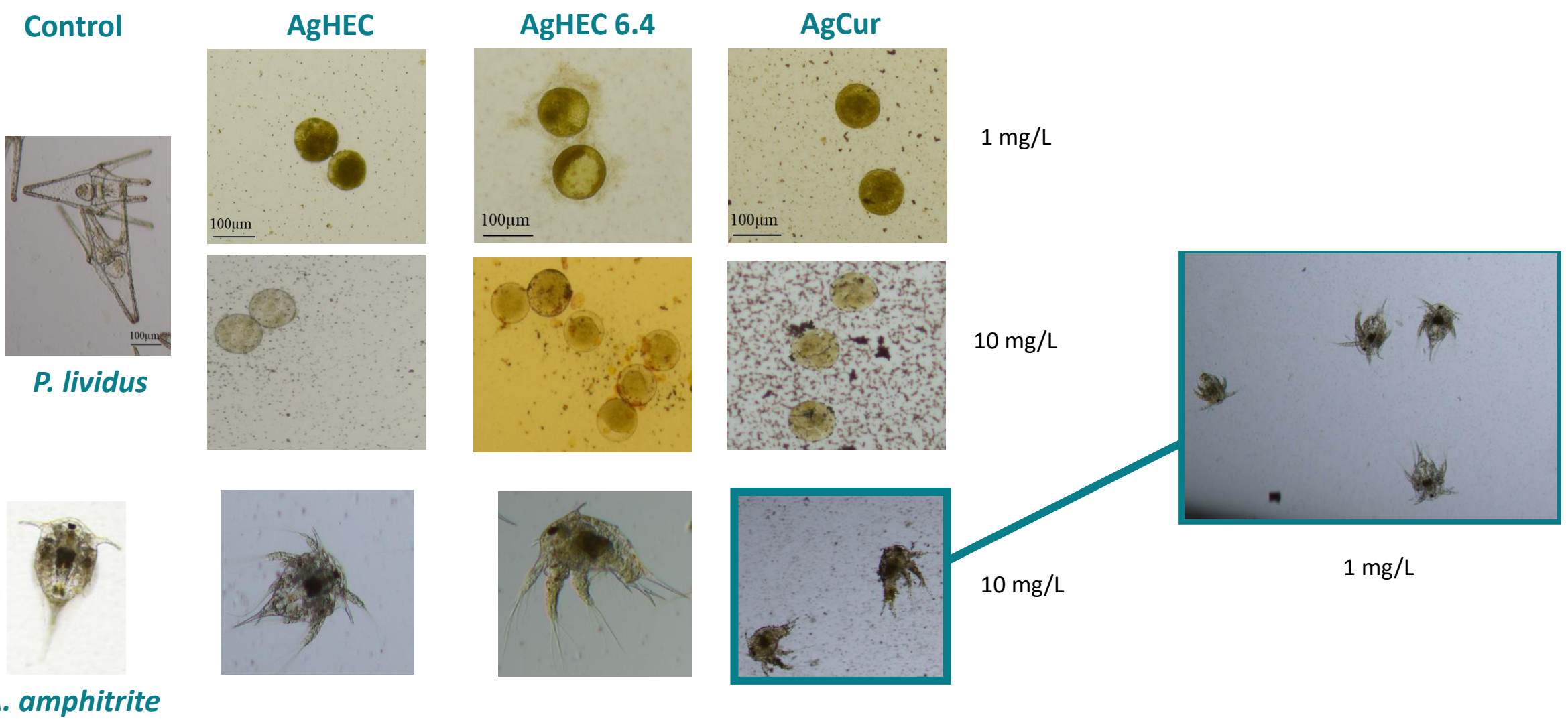
Overall,
nAg- more toxic than Ag-Naked for bacteria, phyto- and zooplankton

Data agree with previous results on the same NPs on *Danio rerio*

Funded by the European Union

nAg: Ecotox data

Acute effects at 1 mg/L in two species out of 6



Funded by the European Union

Task 3.2

Collecting toxicity data and filling gaps for an early identification of hazard potential. CFs for toxicological assessment by in-vitro advanced models

Data gaps filling:

- Primary objective is to fill in the gaps of missing data, starting with NMs already available, namely ZnO and CuO (genotox and data from FET test).
- Ongoing test to assess the properties in relevant media of NMs and evaluation of toxicity reference outcomes ("old" and new)

New data collection

- Confirmation of delivering time is essential. Please send us the NMs as soon as they are ready and preliminary characterization is available – please consider few grams per material.

Data gaps filling:

- Data from previous projects collected and organized to identify missing data on p-chem and eco-tox data.

Nano Material	Type	Other forms available *	NF Provider	Project 1	Project 2	Commercial property	Commercial property 2	NPs / NEPs	Form	Life cycle stage	Test category	Core Conc (g/L)	Doping conc (M)	Z-average nm (water)	Dev.st Z-aver nm (water)	Z-average t0 nm (MQ)	Dev.st Z.averT0 nm	Z-average t0 nm (Medium)	Dev.st Z.averT0 nm	PdI (water)	Err_PdI (water)	PdI T0 (MQ)	Err_PdI T0 (MQ)	PdI T0 (Medium)	Err_PdI T0 (Medium)	Medium type	Z-average tech	Z-pot (water)
AgHECs2.8			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	5,117	0,14	170,8	0,6	na	na	na	na	0,27	0,006	na	na	na	na	DMEM 1%	DLS	12,2
AgHECs5.5			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	na	na	na	na	122,16	5,89	80,37	2,4	na	na	0,14	0,02	0,2	0,02	DMEM 1%	DLS	
AgHECs6.4			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	5,19	0,32	329	12	na	na	na	na	0,404	0,062	na	na	na	na	DMEM 1%	DLS	21,1
AgHECp			CNR-ISSM	ASINA		antibacter	antiviral	NP	powder	synthesis	in vitro assay		na	na	na	304,89	34,89	150,29	19,7	na	na	0,37	0,07	0,35	0,06	DMEM 1%	DLS	
AgCUR			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	5,8	0,009	114	1	91,27	5,79	na	na	0,389	0,009	0,31	0,01	na	na	DMEM 1%	DLS	-35
AgCUR_A			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	6,02	0,004	2921	475	na	na	na	na	1	0	na	na	na	na	DMEM 1%	DLS	-38,8
AgCUR_B			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	5,78	0,018	93	0,8	na	na	na	na	0,39	0,008	na	na	na	na	DMEM 1%	DLS	-47
Ag			Market	ASINA		reference		NP	powder	synthesis	in vitro assay	na	na	na	na	270,6	53,18	328,71	76,9	na	na	0,45	0,004	0,37	0,13	DMEM 1%	DLS	na
AgPVP			Market	ASINA		reference		NP	powder	synthesis	in vitro assay	na	na	na	na	695,91	617,49	545,96	386,17	na	na	0,7	0,26	0,43	0,06	DMEM 1%	DLS	na
TiO2			JRC	ASINA		reference		NP	powder	synthesis	in vitro assay	na	na	na	na	18,26	3,97	na	na	na	na	0,13	0,04	na	na	na	DLS	na
TiO2-N			CNR-ISSM	ASINA		antibacterial		NP	textiles/polymer		in vitro assay	na	na	na	na	-17,45	3,36	na	na	na	na	0,96	0,05	na	na	na	DLS	na
wCuO		water bas	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis	in vitro assay	na	na	220	14	na	na	1191	259	0,27	na	na	na	1,44	na	Optimem	DLS	27,9
eCuO		ethanol ba	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis/	in vitro assay	na	na	174	6	na	na	932	126	0,17	na	na	na	0,39	na	Optimem	DLS	32,3
wZnO		water bas	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis/	in vitro assay	na	na	2015	222	na	na	1335	209	0,76	na	na	na	0,75	na	Optimem	DLS	11,4
eZnO		ethanol ba	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis/	in vitro assay	na	na	1239	577	na	na	1109	259	0,87	na	na	na	0,84	na	Optimem	DLS	13,6

Nano Material	Type	Other forms available *	NF Provider	Project 1	Project 2	Commercial property	Commercial property 2	NPs / NEPs	Form	Life cycle stage	Test category et	Viability (EC50/LC50 ug/ml)	Viability LOAEC (ug/ml)	note	Viability test	IL8 (EC50)	IL8 LOAEC	Inflammation test	EC50 Ox Resp (ug/mL)	LOAEC Ox Resp (ug/mL)	Ox Resp test	yH2AX EC50	yH2AX LOAEC
AgHECs2.8			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	91,53	10		Alamar Bl	na	na	ELISA	na	na	DCFH ROS	na	ns
AgHECs5.5			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	na	20		Alamar Bl	52,32	100	ELISA	na	100	DCFH ROS	6,98	20
AgHECs6.4			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	7,29	4		Alamar Bl	na	na	ELISA	na	na	DCFH ROS	4,93	6
AgHECp			CNR-ISSM	ASINA		antibacter	antiviral	NP	powder	synthesis	in vitro assay	57,05	100		Alamar Bl	21,69	50	ELISA	na	100	DCFH ROS	6,48	20
AgCUR			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	na	50		Alamar Bl	na	10	ELISA	na	100	DCFH ROS	121,77	50
AgCUR_A			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	5,65	10		Alamar Bl	na	na	ELISA	na	na	DCFH ROS	na	na
AgCUR_B			CNR-ISSM	ASINA		antibacter	antiviral	NP	colloidal	synthesis	in vitro assay	na	100		Alamar Bl	na	na	ELISA	na	na	DCFH ROS	na	na
Ag			Market	ASINA		reference		NP	powder	synthesis	in vitro assay	na	100	Max conc	Alamar Bl	29,73	100	ELISA	42,74	50	DCFH ROS	19,02	50
AgPVP			Market	ASINA		reference		NP	powder	synthesis	in vitro assay	na	100	Max conc	Alamar Bl	na	100	ELISA	49,33	50	DCFH ROS	20,7	20
TiO2			JRC	ASINA		reference		NP	powder	synthesis	in vitro assay	na	100	Max conc	Alamar Bl	na	100		na	na	DCFH ROS	70,16	100
TiO2-N			CNR-ISSM	ASINA		antibacterial		NP	textiles/polymer		in vitro assay	na	100	Max conc	Alamar Bl	na	100		na	na	DCFH ROS	128,15	50
wCuO		water bas	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis	in vitro assay	29,9	20		MTT	0,68	20	IL-8	na	na	DCFH ROS	na	na
eCuO		ethanol ba	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis/	in vitro assay	33,85	20		MTT	2,2	20	IL-8	na	na	DCFH ROS	na	na
wZnO		water bas	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis/	in vitro assay	16,22	20		MTT	3,94	20	IL-8	na	na	DCFH ROS	na	na
eZnO		ethanol ba	BIU	PROTECT	AMROCE	anitbacterial		NP	powder	synthesis/	in vitro assay	28,92	25		MTT	16,32	25	IL-8	na	na	DCFH ROS	na	na

Data gaps filling:

- Primary objective is to fill in the gaps of missing data, starting with NMs already available, namely ZnO and CuO (genotox and data from FET test) and **AgNPs (p-chem properties)**

NP	Temperature	Concentration	Medium	Time	z-average	PdI
					average	average
AgHEC	25°C	10 ug/mL	mQ	0	192,90	0,36
AgCUR					56,32	0,53
AgHEC6.4					428,40	0,54
AgHEC		100 ug/mL			181,65	0,28
AgCUR					547,53	0,45
AgHEC6.4					324,07	0,37
AgHEC		10 ug/mL	mQ	24	112,90	0,35
AgCUR					51,99	0,52
AgHEC6.4					128,10	0,61
AgHEC		100 ug/mL			209,12	0,31
AgCUR					139,15	0,67
AgHEC6.4					185,33	0,33
AgHEC		10 ug/mL	H2O marine	0	124,67	0,32
AgCUR					518,90	0,32
AgHEC6.4					190,93	0,43
AgHEC		100 ug/mL			149,03	0,30
AgCUR					1019,87	0,39
AgHEC6.4					237,90	0,43
AgHEC		10 ug/mL	H2O marine	24	119,40	0,33
AgCUR					2696,00	1,00
AgHEC6.4					146,13	0,39
AgHEC		100 ug/mL			141,13	0,30
AgCUR					2325,58	0,77
AgHEC6.4					228,30	0,41

Different agglomeration/aggregation of AgCUR in mQ and marine water. AgHEC more comparable in the two media.

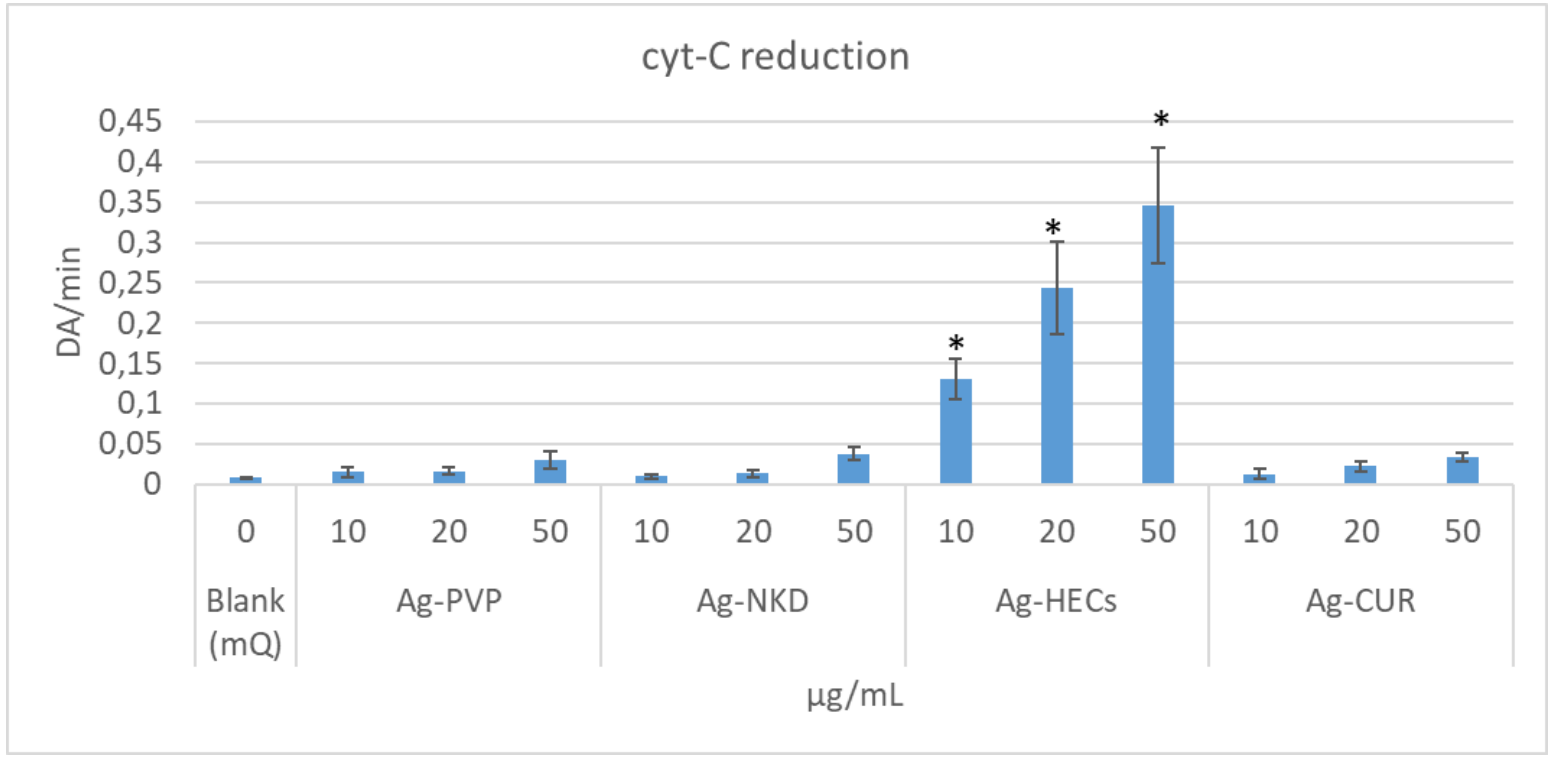
Data gaps filling:

- Primary objective is to fill in the gaps of missing data, starting with NMs already available, namely ZnO and CuO (genotox and data from FET test) and **AgNPs (p-chem properties)**

					z-average		PdI	
					average	SD	average	SD
AgHEC	**37°C**	**10 ug/mL**	**AS pH4.7**	**0**	4,03	0,18	0,31	0,01
AgCUR					126,10	22,36	0,13	0,02
AgHEC6.4								
AgHEC		**100 ug/mL**			137,67	6,96	0,27	0,01
AgCUR					356,37	48,81	0,70	0,52
AgHEC6.4								
AgHEC		**10 ug/mL**		**18**	3,69	0,35	0,31	0,01
AgCUR					599,83	137,82	0,37	0,04
AgHEC6.4								
AgHEC		**100 ug/mL**			96,23	6,52	0,52	0,05
AgCUR					820,27	44,93	0,40	0,10
AgHEC6.4								

Data gaps filling:

- Primary objective is to fill in the gaps of missing data, starting with NMs already available, namely ZnO and CuO (genotox and data from FET test) and **AgNPs (p-chem properties)**

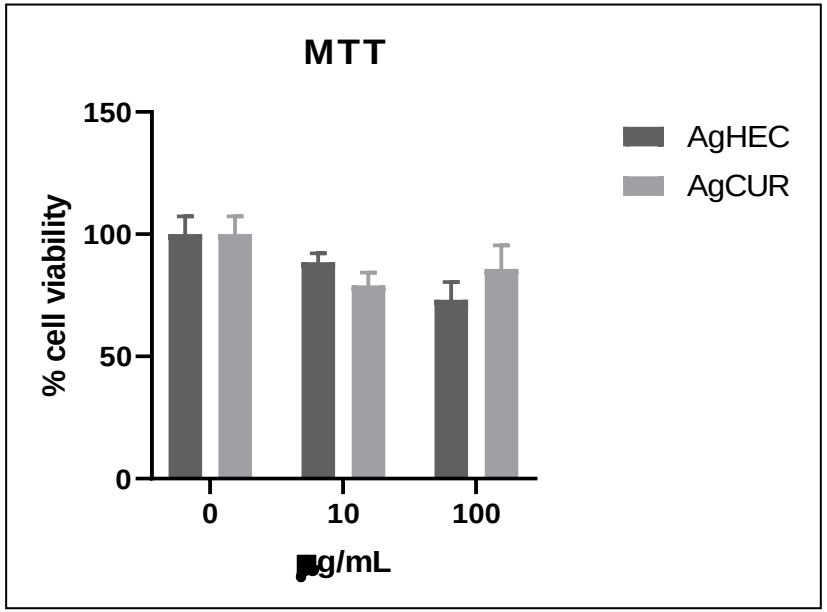


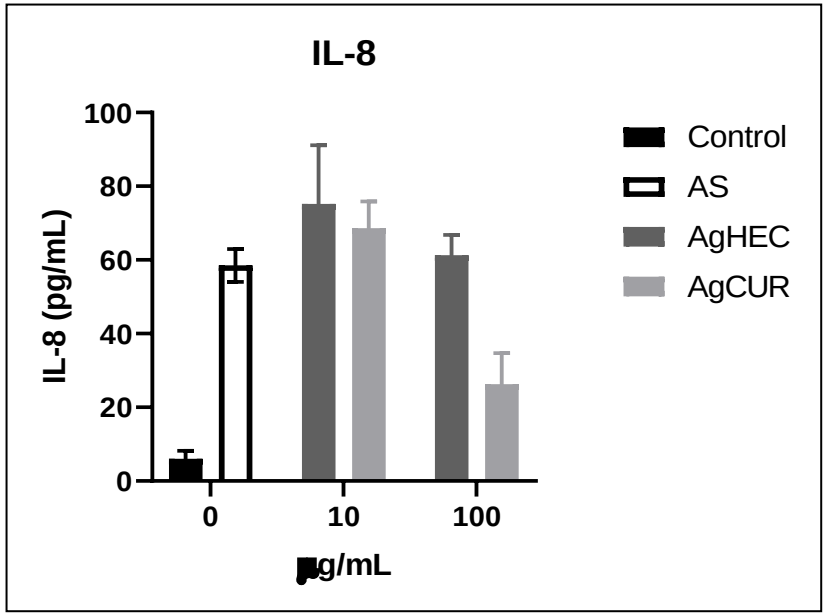
AgHEC particles trigger the formation of reactive oxygen species (ROS) in the cell-free system (therefore without the need of cell metabolism). ROS may in turn damage cellular macromolecules and cause adverse effects. All the other AgNPs cause only minor and not significant modifications.

Data gaps filling:

- Primary objective is to fill in the gaps of missing data, starting with NMs already available, namely ZnO and CuO (genotox and data from FET test) and AgNPs (skin irritation test)
- Results from triplicate independent experiments on skin 3D model (Corrosion and irritation skin test (OECD 431, 439).

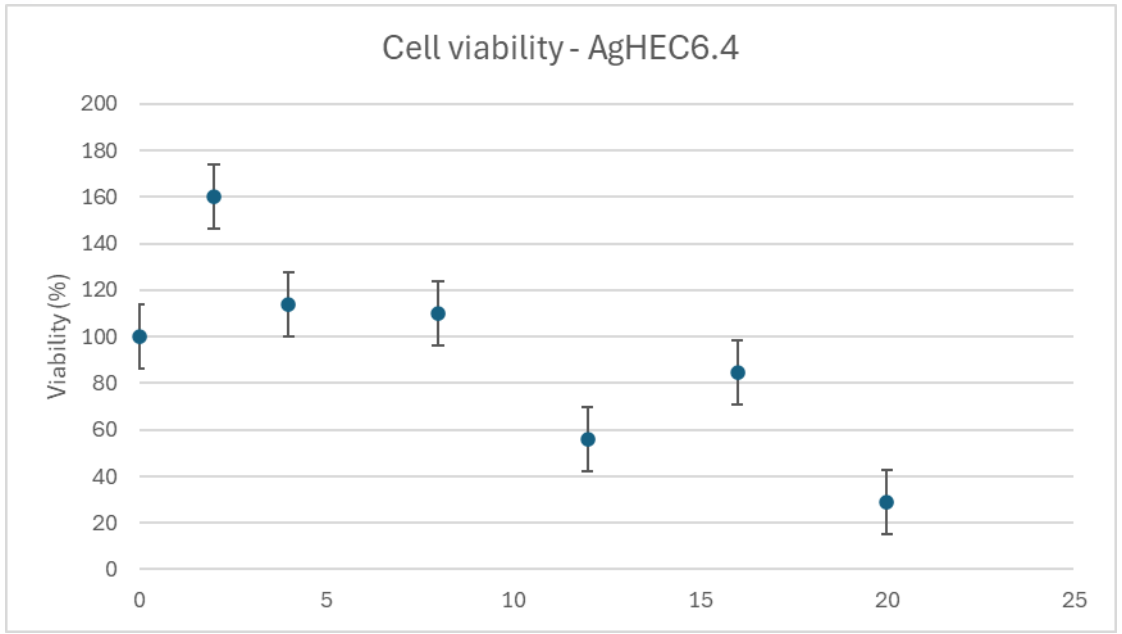
  - Minor reduction of model viability, some increase in inflammatory mediator (IL-8) release





Data gaps filling:

- Primary objective is to fill in the gaps of missing data, starting with NMs already available, namely ZnO and CuO (genotox and data from FET test) **and AgNPs (control tests on viability and inflammatory responses)**
- Results from triplicate independent experiments on A549 alveolar cells. A newly arrived Ph.D. student (dott. Stefano Cervellera is working on this)
  - Reduction of viability is dose dependent



Work planned for the NEXT 6 MONTHS

Task 3.1

Ecotoxicity: Fate and effects in biological and environmental relevant matrices

- CS1.1 AgNPs ecotox assessment in seawater: Ecotoxicological assays with marine rotifers and cnidarians and freshwater organisms
- CS 1.2 – CuO and ZnO ecotox assessment in seawater: Ecotoxicological assays with a battery of marine species belonging to different trophic levels by using standard and innovative endpoints
- CS 3 – bio-SiO2 nanoparticles: Ecotoxicological assays with a battery of marine species belonging to different trophic levels by using standard and innovative endpoints

Task 3.2

Collecting toxicity data and filling gaps for an early identification of hazard potential. CFs for toxicological assessment by in-vitro advanced models

- CS1.1 AgNPs completion of reference experiments
- CS 1.2 – CuO and ZnO collection of missing data genotox and FET endpoints
- CS2 Perovskites collection of new data
- CS 3 – bio-SiO2 and SiO2@TiO2 nanoparticles collection of new data, the bio-SiO2 NPs will be considered as a new NMs

Work planned for the NEXT 6 MONTHS

Task 3.1

Ecotoxicity: Fate and effects in biological and environmental relevan

- CS1.1 AgNPs ecotox assessment in seawater: Ecotoxicologi
- CS 1.2 – CuO and ZnO ecotox assessment in seawat arine species belonging to different trophic levels by using
- CS 3 – bioSiO2 nanoparticles: Ecotoxi es belonging to different trophic levels by using standard and in

Task 3.2

Collecti dentification of hazard potential. CFs for toxicological assessment b

- ce experiments
- C tion of missing data genotox and FET endpoints
- CS ction of new data (p-chem and eco-tox)
- CS 2 and bio-SiO2@TiO2 nanoparticles collection of new data (p-chem and eco-tox), the bio-SiO2 NPs will be co idered as a new NMs

When NMs are available please contact us to organize the shipment to UNIMIB and CNR!!! The soon the better ☺

nAg: Ecotox data

Weight of Evidence

0.1 – 1 mg/L

Concentration <EC50 values

High Concentration

Sample code	Specie	Endpoint	Conc	Contr % HQ	$HQ_{Battery}$	Level of hazard	Conc	Contr % HQ	$HQ_{Battery}$	Level of hazard
AgNaked	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	4,9	0,49	ABSENT	1 mgL	15,8	0,55	ABSENT
	Artemia franciscana	Survival		1,5				0,8		
	Balanus amphitrite	Behaviour		0,7				30,7		
	Balanus amphitrite	Survival		6,9				4,8		
	Dunaliella tertiolecta	Algal Growth		0,5				12,3		
	Paracentrotus lividus	Development		79,3				17,8		
	Phaeodactylum tricornutum	Algal Growth		6,2				17,8		
AgCur	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	2,5	0,97	ABSENT	1 mgL	2,0	4,71	MAJOR
	Artemia franciscana	Survival		0,2				0,0		
	Balanus amphitrite	Behaviour		1,4				19,6		
	Balanus amphitrite	Survival		2,3				17,2		
	Dunaliella tertiolecta	Algal Growth		8,3				12,8		
	Paracentrotus lividus	Development		79,7				25,5		
	Phaeodactylum tricornutum	Algal Growth		5,6				23,0		
AgHEC	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	2,2	1,01	SLIGHT	1 mgL	1,5	5,99	MAJOR
	Artemia franciscana	Survival		0,7				0,1		
	Balanus amphitrite	Behaviour		1,2				21,2		
	Balanus amphitrite	Survival		5,0				21,2		
	Dunaliella tertiolecta	Algal Growth		0,3				16,4		
	Paracentrotus lividus	Development		80,3				21,2		
	Phaeodactylum tricornutum	Algal Growth		10,4				18,6		
AgHEC6.4	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	3,4	1,73	MODERATE	1 mgL	0,4	5,96	MAJOR
	Artemia franciscana	Survival		1,2				0,6		
	Balanus amphitrite	Behaviour		0,6				21,2		
	Balanus amphitrite	Survival		3,5				21,0		
	Dunaliella tertiolecta	Algal Growth		17,0				16,9		
	Paracentrotus lividus	Development		55,2				21,2		
	Phaeodactylum tricornutum	Algal Growth		19,1				18,7		

nAg: Ecotox data

Weight of Evidence

0.1 – 1 mg/L

Concentration <EC50 values

High Concentration

Sample code	Specie	Endpoint	Conc	Contr % HQ	$HQ_{Battery}$	Level of hazard	Conc	Contr % HQ	$HQ_{Battery}$	Level of hazard
AgNaked	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	4,9	0,49	ABSENT	1 mgL	15,8	0,55	ABSENT
	Artemia franciscana	Survival		1,5				0,8		
	Balanus amphitrite	Behaviour		0,7				30,7		
	Balanus amphitrite	Survival		6,9				4,8		
	Dunaliella tertiolecta	Algal Growth		0,5				12,3		
	Paracentrotus lividus	Development		79,3				17,8		
	Phaeodactylum tricornutum	Algal Growth		6,2				17,8		
AgCur	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	2,5	0,97	ABSENT	1 mgL	2,0	4,71	MAJOR
	Artemia franciscana	Survival		0,2				0,0		
	Balanus amphitrite	Behaviour		1,4				19,6		
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	Dunaliella tertiolecta	Algal Growth		8,3				12,8		
	Paracentrotus lividus	Development		79,7				25,5		
	Phaeodactylum tricornutum	Algal Growth		5,6				23,0		
AgHEC	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	2,2	1,01	SLIGHT	1 mgL	1,5	5,99	MAJOR
	Artemia franciscana	Survival		0,7				0,1		
	Balanus amphitrite	Behaviour		1,2				21,2		
	Balanus amphitrite	Survival		5,0				21,2		
	Dunaliella tertiolecta	Algal Growth		0,3				16,4		
	Paracentrotus lividus	Development		80,3				21,2		
	Phaeodactylum tricornutum	Algal Growth		10,4				18,6		
AgHEC6.4	*Aliivibrio fischeri*	Bioluminescence	0.1 mgL	3,4	1,73	MODERATE	1 mgL	0,4	5,96	MAJOR
	Artemia franciscana	Survival		1,2				0,6		
	Balanus amphitrite	Behaviour		0,6				21,2		
	Balanus amphitrite	Survival		3,5				21,0		
	Dunaliella tertiolecta	Algal Growth		17,0				16,9		
	Paracentrotus lividus	Development		55,2				21,2		
	Phaeodactylum tricornutum	Algal Growth		19,1				18,7		