Toxicity of Metallic Organic Frameworks (MOFs) on cell lines and Daphnia magna

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BACKGROUND, MOTIVATION AND OBJECTIVE

Metal-organic frameworks (MOFs) are emerging classes of hybrid organic-inorganic nanostructured porous materials with potential applications in many industrial fields including gas storage, gas separation, catalysis, and chemical sensing among others. Their use in gas storage and gas separation is gaining interest due to their unique nanostructure design, tunability and properties. Within the GENESIS project, three MOFs (ZIF-8, MOF-74-Ni and ZIF-67) were developed to be incorporated in a 2nd generation of enhanced CO_2 recovery membrane systems used in post-combustion applications. As part of the nanosafety assessment, *in vitro* toxicity studies and acute toxicity studies in freshwater crustaceans (*Daphnia magna*) were performed.

MATERIALS	ZIF-8	MOF-74-Ni	ZIF-67
Molecular formula	$C_8H_{10}N_4Zn$	$C_8H_2O_6Ni$	$C_8H_{10}N_4Co$
Size by TEM / SEM (nm)	32 ± 4	20 ± 3	72 ± 25

METHODS

IN VITRO CELL TOXICITY ASSAYS

Stock dispersion: 2.56 mg/mL
Dispersion: Tip sonication 13 mm Ø, 20% amplitude, 11 min

A. Cytotoxicity and inflammatory markers in differentiated THP-1 cells

THP-1 differentiation into macrophages by 48 h exposure to 50 ng/mL of PMA

24 h - MOF exposure concentrations in cell culture media:
[1 - 3 - 10 - 30 - 100 - 300 - 1000 mg/L]

Cytotoxicity by Alamar Blue® assay + TNF-α and IL-1β quantification by ELISA

B. Cytotoxicity and genotoxicity in CHO-K1 cells

Cytotoxity : 24 h - MOF exposure concentrations : [1 - 3 - 10 - 30 - 100 - 300 - 1000 mg/L] → Alamar Blue® assay

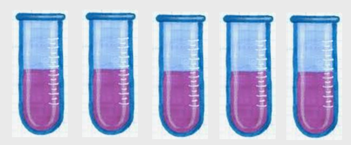
Genotoxicity: : 48 h - MOF exposure concentrations [0,1 - 3 - 10 - 30 - 50 mg/L] → Micronucleus assay

ECOTOXICITY TESTS

Stock dispersion: 1 mg/mL
Dispersion: Bath sonication, 8 min + invert 3-5 times + 8 min sonication

Daphnia Acute inmobilization test, OECD 202

[1 - 10 - 25 - 50 - 100 mg/L]



Dilutions in artificial freshwater

Inmobilization after 48h exposure

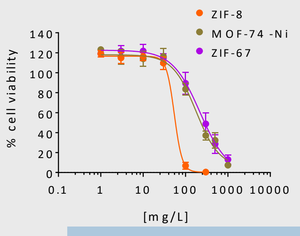
SELECTED EXPOSURE CONCENTRATIONS IN RELEVANT MEDIA WERE ULTRAFILTERED TOTAL AND % METAL IONIC FRACTION (Zn^{2+};Ni^{2+};Co^{2+}) WAS MEASURED BY ICP-MS

ION DISSOLUTION ANALYSIS BY ICP-MS ONGOING

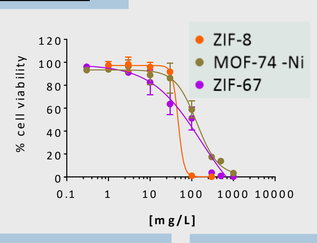
RESULTS

IN VITRO CELL TOXICITY ASSAYS

Cytotoxic effects

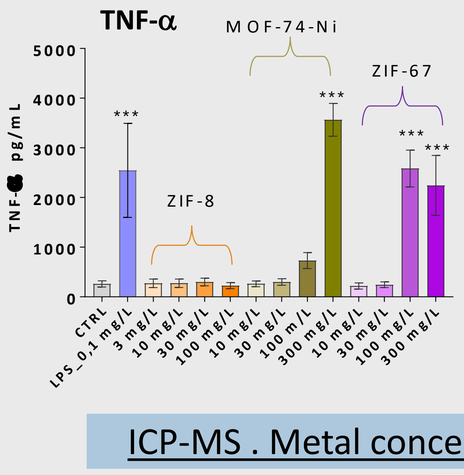


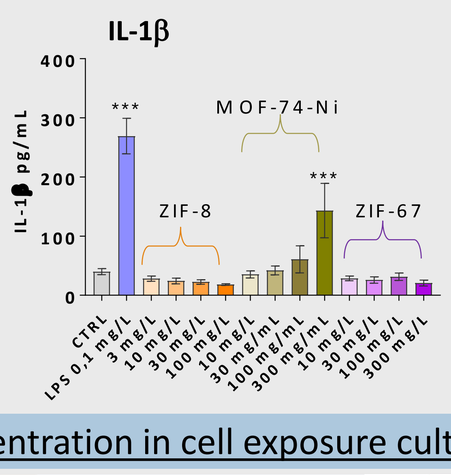
THP-1	EC_{50} mg/L	95% CI
ZIF-8	82	[79 - 101]
MOF-74-Ni	256	[181 - 361]
ZIF-67	305	[211 - 443]



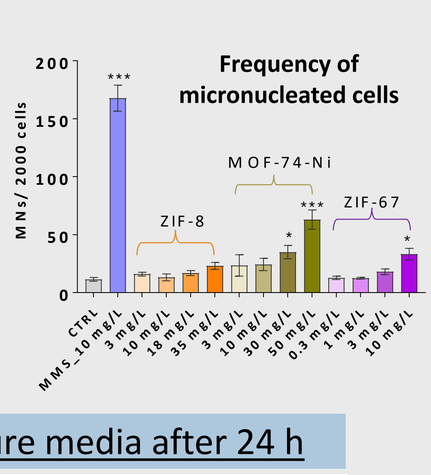
CHO-K1	EC_{50} mg/L	95% CI
ZIF-8	45	[29 -71]
MOF-74-Ni	118	[83 -167]
ZIF-67	64	[40 - 102]

Inflammogenic response in differentiated THP-1





Genotoxic effects in CHO- K1

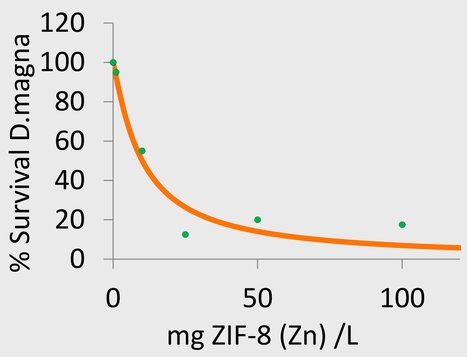


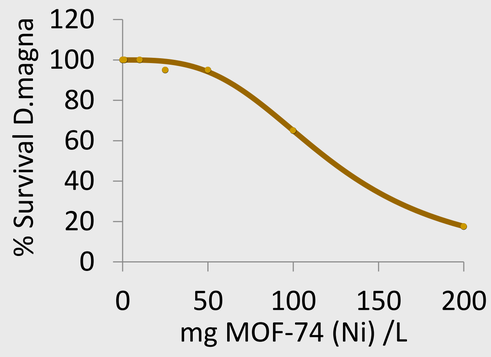
ICP-MS . Metal concentration in cell exposure culture media after 24 h

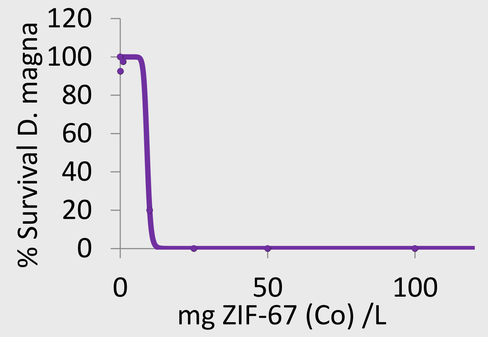
	ZIF-8				MOF-74-Ni				ZIF-67			
	Nominal		Actual	Ionic fraction (%)	Nominal		Actual	Ionic fraction (%)	Nominal		Actual	Ionic fraction (%)
	ZIF-8 [mg/L]	Zn [mg/L]	Zn [mg/L]		MOF-74 [µg/mL]	Ni [µg/mL]	Ni [µg/mL]		ZIF-67 [µg/mL]	Co [µg/mL]	Co [µg/mL]	
Total Fraction	300	86,2	45,5 (± 7,7)	---	300	69,7	70,4 (± 4,3)	---	300	80,0	75,4 (± 5,7)	---
	30	8,6	7,1 (± 0,3)	---	30	7,0	6,8 (± 0,4)	---	30	8,0	7,8 (± 0,3)	---
	3	0,9	0,8 (± 0,04)	---	3	0,7	0,75 (± 0,003)	---	3	0,8	0,8 (± 0,07)	---
Ultra Filtered	300	---	12,9 (± 4,0)	**32,5 (± 12,3)**	300	---	50,0 (± 1,8)	**72,0 (± 7,1)**	300	---	53,0 (± 7,5)	**69,7 (± 4,9)**
	30	---	2,4 (± 0,6)	**34,7 (± 9,4)**	30	---	5,6 (± 0,4)	**83,7 (±10,9)**	30	---	6,2 (± 0,7)	**78,4 (± 5,1)**
	3	---	0,3 (± 0,21)	**38,8 (± 23,0)**	3	---	0,67 (± 0,11)	**89,3 (±14,3)**	3	---	0,86 (± 0,28)	**106,0 (± 25,4)**

ECOTOXICITY TESTS

Daphnia Acute inmobilization test, OECD 202







Compound	EC_{50} (mg MOF/L)
ZIF-8	10.1 [5.94-15.3]
MOF-74-Ni	121 [113-123]
ZIF-67	9.14 [8.90 - 9.14]

Comparison of EC_{50} for the metal organic frameworks and their main metallic constituents:

Compound	EC_{50} (mg metal/L)
ZIF-8	1.27 [0.74-1.90]
MOF-74-Ni	11.2 [10.5-11.3]
ZIF-67	1.10 [1.07 - 1.10]

(Okamoto et al., 2015)

Compound	EC_{50} (mg metal/L)
Zn	0.72
Ni	0.65
Co	0.71

CONCLUSION

- ✓ For ZIF-8, the effects in cell viability occurred in general at lower concentrations than the effects in MOF-74-Ni or ZIF-67.
- ✓ No inflammogenic or genotoxic effects were observed for ZIF-8. MOF-74-Ni and ZIF-67 showed genotoxic effects and an increase of release in at least one of the two measured proinflammatory cytokines.
- ✓ MOFs dissolved to a high extend during the cellular assays, suggesting a large contribution of the dissolved ions on the observed adverse responses.
- ✓ ZIF-8 and ZIF-67 exhibited toxic effects on *Daphnia magna* at much lower concentration than MOF-74-Ni.
- ✓ Dissolution data is not yet available for the *D. magna* studies. This data is necessary to conclude on the relative contribution of particles versus dissolved fraction on the effects observed.

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