

Ecotoxicity testing and environmental risk assessment of iron nanomaterials for sub-surface remediation – Recommendations from the FP7 project NanoRem

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- 1 Ecotoxicity testing and environmental risk assessment
- 2 of iron nanomaterials for sub-surface remediation -

3 Recommendations from the FP7 project NanoRem

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16 Abstract

Nanoremediation with iron (Fe) nanomaterials opens new doors for treating contaminated soil and groundwater, but is also accompanied by new potential risks as large quantities of engineered nanomaterials are introduced into the environment. In this study, we have assessed the ecotoxicity of four engineered Fe nanomaterials, specifically, Nano-Goethite, Trap-Ox Fezeolites, Carbo-Iron[®] and FerMEG12, developed within the European FP7

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23 project NanoRem for sub-surface remediation towards a test battery 24 consisting of eight ecotoxicity tests on bacteria (V. fisheri, E. coli), algae (P. subcapitata, Chlamydomonas sp.), crustaceans (D. magna), worms (E. fetida, 25 26 L. variegatus) and plants (R. sativus, L. multiflorum). The tested materials are commercially available and include Fe oxide and nanoscale zero valent iron 27 (nZVI), but also hybrid products with Fe loaded into a matrix. All but one 28 29 material, a ball milled nZVI (FerMEG12), showed no toxicity in the test battery when tested in concentrations up to 100 mg/L, which is the cutoff for 30 31 hazard labeling in chemicals regulation in Europe. However it should be 32 noted that Fe nanomaterials proved challenging to test adequately due to their 33 turbidity, aggregation and sedimentation behavior in aqueous media. This 34 paper provides a number of recommendations concerning future testing of Fe nanomaterials and discusses environmental risk assessment considerations 35 related to these. 36

37 Keywords: Nanoremediation, Iron nanomaterials, Ecotoxicology, nZVI,
38 Environmental Risk Assessment, NanoRem

39

40 1 Introduction

Innovation in nanotechnology introduces new treatment options for
environmental remediation of organic compounds (notably chlorinated
solvents) and heavy metals in soil and groundwater (Karn et al., 2009;

44 Mueller et al., 2012). Especially iron (Fe) based nanomaterials have shown a 45 potential for remediation due to a larger specific surface area and 46 corresponding increased reactivity compared to micro-scale and larger Fe 47 materials traditionally used for remediation of contaminated sites (Wang and 48 Zhang, 1997). Nanoscale zerovalent iron (nZVI) has received most of the 49 attention as it is highly reactive compared to the bulk ZVI used in permeable 50 reactive barriers (Henderson and Demond, 2007). Remediation with nZVI has 51 been claimed to represent a faster, cheaper and a potentially more effective 52 treatment option than current ex situ and in situ methods (Yan et al., 2013). 53 However, nanomaterials engineered to remediate polluted soil and groundwater may constitute a risk to the environment as they are injected into 54 the subsurface in large quantities (Grieger et al., 2010). As such this could 55 represent a worst case scenario when considering possible negative 56 environmental effects of manufactured nanomaterials. Nanoremediation in 57 58 general seems associated with high uncertainty both in relation to its potential 59 environmental risks, but also towards its field scale efficacy (Grieger et al., 60 2015). Uncertainty with regards to the potential environmental impacts of Fe 61 nanomaterials hampers their use and has partly been the reason for the 62 limited implementation of Fe nanomaterials in remediation (Bardos et al., 63 2014), although no major environmental impacts have been reported in the 64 first decade of field deployments with Fe nanomaterials (Mueller et al., 65 2012).

66 Several publications in recent years have evaluated the ecotoxicity of Fe 67 nanomaterials, particular nZVI materials, with NANOFER STAR, NANOFER 25 and 25s being the most common commercially available 68 69 particles. In these publications the focus has been on aquatic and terrestrial ecotoxicity (Keller et al., 2012; Marsalek et al., 2012; Saccà et al., 2014; El-70 71 Temsah et al., 2016) and in general effect concentrations as low as 0.5 mg/L 72 to above 2.5 g/L have been reported, demonstrating considerable variation in 73 ecological response to Fe nanomaterials.

A range of Fe nanomaterials has been developed in the European FP7 project 74 75 NanoRem (Taking Nanotechnological Remediation Processes from Lab Scale 76 to End User Applications for the Restoration of a Clean Environment, for 77 more information see nanorem.eu) in order to extend the spectrum of treatable soil and groundwater contaminants from halogenated organics to 78 79 non-halogenated substances and non-reducible metals. Contrary to the three 80 materials mentioned above, the potential ecotoxicity of the materials 81 developed in NanoRem have not previously been tested. These materials are currently available on the international marked (see Table 1). Common for all 82 83 materials is that if they are to be used in field-scale remediation, their 84 production volume will easily reach 1 metric ton per year (Mueller et al., 2012). In this case, they will have to be registered under the European 85 86 chemical legislation REACH, which will be accompanied with data 87 requirements on ecotoxicity. The data generated will feed into the general

hazard identification of the nanomaterials and form the basis for a generic risk assessment (i.e. a hazard classification according to the classification, labeling and packaging (CLP) regulation). It is important to emphasize that this risk assessment does not directly relate to the safety of injecting said material into an aquifer or a contaminated soil. Such a task is done in a sitespecific risk assessment, which is outside the scope of this study.

94 Test organisms and endpoints in the ecotoxicology test battery were chosen 95 to include representatives for both terrestrial and aquatic environments as Fe nanomaterials may spread, in worst case scenarios, to both terrestrial and 96 97 aquatic habitats (Grieger et al., 2010). The ecotoxicity tests were also 98 selected to include standardized tests to ensure general regulatory acceptance 99 of test results as well as non-standardized tests to broaden the test basis with respect to modes of exposure and modes of action, and to enhance the 100 101 likelihood of seeing biological responses within the range of particles and 102 concentrations tested. The aim of the paper is to provide ecotoxicity data for 103 four newly developed Fe nanomaterials and the paper also highlights current 104 challenges in doing adequate hazard identification and environmental risk 105 assessment of Fe nanomaterials. Finally, recommendations for future 106 ecotoxicity testing of Fe nanomaterials are provided.

107

108 2 Materials and Methods

109 2.1 Nanomaterials

Samples of Fe nanomaterials for ecotoxicity testing were obtained directly
from the manufacturers within the NanoRem project. A full list and
characterization of the nanomaterials is seen in Table 1.

113 2.1.1 Dispersion of nanomaterials for toxicity testing

114 Dispersions of powder Fe nanomaterials were made according to the 115 description provided by the manufactures. Due to testing constraints (e.g. 116 infeasibility to degas exposure media) for the aquatic standard tests (on *V*. 117 *fischeri*, *P. subcapitata* and *D. magna*), all nanomaterial powders were 118 dispersed as described for magnetite.

119 $Carbo-Iron^{\ensuremath{\otimes}}$ For 100 mL of a stock suspension at 10 g/L, 20 mL of a 10 g/L 120 carboxymethyl cellulose (CMC) solution was added to 80 mL of test medium 121 and degassed with N₂ for an hour. Then, 1 g of test material was added to the 122 solution under N₂ flow, and mixed for 10 min with a high-shear mixer. 123 Dilution series were prepared under regular aerobic conditions and used right 124 away.

125 *Trap-Ox Fe-zeolites* A stock suspension with a zeolite concentration of 25 126 g/L was prepared by dissolving 2.5 g CMC in 50 mL deionized water by 127 heating the mixture to 70° C with stirring for an hour. Then, 2.5 g Fe-zeolite

in 50 mL deionized water was sonicated for 15 min and the CMC solution

and zeolite suspension were mixed and sonicated for additional 15 min. *Magnetite* Magnetite, received as powder, was suspended in deionized water
and mixed for 10 minutes with a high-shear mixer. Subsequent dilutions
series in exposure media were prepared and used right away.

Suspensions Nano-Goethite was provided as a stable suspension and was
diluted directly from the sample into the exposure media. However, the
FerMEG12 were additionally sonicated for 15 minutes due to sedimentation.

136

128

137 2.2 Characterization of stock suspensions

Dynamic Light Scattering (DLS) measurements of aqueous suspensions 138 (deionized water) from 10 mg/L to 10 g/L test material were performed on a 139 140 Malvern Zetasizer ZS (Malvern instruments Ltd, Worcestershire, UK) 141 equipped with a laser source at a wavelength of 633 nm. Zeta-averaged 142 hydrodynamic diameters and size distributions were determined using the 143 "multiple narrow modes (high resolution)" algorithm supplied by Malvern. 144 Measurements were done in triplicates of 5 runs with autocorrelation 145 functions of 10 seconds. The same instrument was used for the measurements 146 of electrophoretic mobility and the Smoluchowski approximation was used 147 for determining zeta-potentials. Three measurements with 5 runs per 148 measurement were obtained.

149 Nanoparticle Tracking Analysis (NTA) measurements of the hydrodynamic
150 diameter of individual particles suspended in deionized water at a
151 concentration of 10 mg/L to 10 g/L were done on a Nanosight LM10
152 (NanoSight Ltd, Amesbury, UK).

Chillip Marine

153 Table 1 Characterization of the pristine Fe nanomaterials. Magnetite is not used for remediation in NanoRem but was chosen as a 154 control in this study. The listed information is obtained from the manufacturer.

Name	Description	Development Status	Mode of remediation	Form	Chemical composition	Average primary	Specific surface area (m ² /g)
					R Y	particle size (nm)	
FerMEG12	Zero-valent Fe mechanically ground through hall milling	Field tested and commercially available	Reduction	Suspension	15-30% Fe 70-85 % monoethylene	-	12-18
Carbo-Iron [®]	Composite of activated carbon and zero-valent Fe	Field tested and commercially available	Adsorption + Reduction	Powder	$\begin{array}{c} 30.3 \ \% \ \ Fe_{tot} \\ 20.5 \ \% \ \ Fe^{0} \\ 13.1 \ \% \ \ Fe_{3}O_{4} \\ 55\pm1\% \ \ C_{tot} \end{array}$	13440 ±20	594
Magnetite	Fe oxides (Fe ₃ O ₄)	Precursor for NANOFER STAR	-	Powder	Fe ₃ O ₄	-	-
Nano- Goethite	Fe oxides stabilized with humic acids	Field tested and commercially available	Adsorption + Oxidation	Suspension	'pure' FeOOH with organic coating	220±20	140
Trap-Ox Fe- zeolites	Nanoporous aluminosilicate loaded with Fe(III)	Premarket	Adsorption + Oxidation	Powder	4 % Al 92 % Si 3 % Fe	1000	600

156 The light source was a solid-state, single-mode laser diode (radiation output 157 max power $<50\mu$ W, 635nm continuous wave, max power <35mW). The 158 standard camera Marlin F-033B (Allied Vision Technologies GmbH, 159 Stadtroda, Germany) was used. All data were analyzed using the instrument software (NanoSight[™] version 2.2). The analysis with NTA was done on 7 160 161 videos with 1 min length each. The solution oxidation-reduction potential and 162 pH were measured in all exposure suspensions at the beginning and the end of the tests. Total Fe concentration in stock suspensions was measured by 163 ICP-OES (Perkin Elmer, Optima 5300 DV) following microwave assisted 164 165 digestion under acidic conditions (3.7 % HCl).

166

167 2.3 Ecotoxicological test battery

A test battery of eight tests (see Table 2) was used to assess and rank the nanomaterials listed in Table 1. Dilutions series were made from stock suspensions and tested in concentrations up to 1 g/L. For some tests, higher concentrations were assessed, including the root elongation test with radish *Raphanus sativus*, ryegrass *Lolium multiflorum* (up to 10 g/L) and the earthworm mortality test with *Eisenia fetida* (up to 25 g/L). Full tests protocols are enclosed in the supplementary information.

Organism	Species	Duration	Endpoint	Reference
Bacteria	Vibrio fischeri	15 min	Decrease in	ISO11348-3
			bioluminescence	100110100
Bacteria	Escherichia coli	6h/24 h	Growth/Cell viability	-
Algae	Pseudokirchneri	48 h	Growth rate	OECD 201
	ella subcapitata		inhibition	OECD 201
Algae	Chlamydomonas	48 h	Photosynthesis	
	sp.		efficiency	
Crustacean	Daphnia magna	48 h	Immobilization	OECD 202
Earthworm	Eisenia fetida	48 h	Mortality	OECD 207
Oligochaete	Lumbriculus	96 h	Mortality	OECD 225 ^a
	variegatus			
Plant	Raphanus	6 d	Root elongation	OECD 208
	sativus, Lolium			
	multiflorum			

176	Table 2 Organisms and testing endpoints of the eight ecotoxicity tests in the test
177	battery.

- ^a modified to short term water-phase exposure
- 179

180 3 Results

181 3.1 Characterization

182 Table 3 provides an overview of the characterization of the Fe nanomaterials 183 in deionized water. Differences in size distribution were observed, with NTA 184 generally finding a lower average size than DLS. Based on zeta potential 185 measurements, Nano-Goethite and Trap-Ox Fe-zeolites showed higher aqueous stability than FerMEG12 and Carbo-Iron[®], however sedimentation of 186 187 the Trap-Ox Fe-zeolites was also observed. In the more complex aquatic test 188 media, DLS measurements proved difficult due to particle sedimentation of 189 all tested materials. This violates the principle behind DLS for size

distribution measurements as the particles are affected by gravitational movement and not just Brownian movement and no reliable estimation of size distribution could be made. Characterization with DLS also revealed that all particle suspensions had a very broad size distribution with polydispersity indexes around 1, which also undermines the use of DLS measurements to characterize the suspensions.

196

197 **3.2 Ecotoxicity**

Almost all of the tests conducted showed no toxicity of the tested Fe nanomaterials at concentrations up to 100 mg/L, which is the cutoff value for hazard labeling in the EU. Only FerMEG12 gave rise to toxicity at concentrations below 100 mg/L. Effects were seen in the 6 h growth inhibition test with *E. coli* (Figure 1), the 6 d root elongation test with *R. sativus* (Figure 2) and 96 h mortality test with *L. variegatus* (Figure 3).

The growth rate (h⁻¹) of Gram-negative *E. coli* was not significantly affected in the presence of Carbo-Iron[®], Nano-Goethite and Trap-Ox Fe-zeolites at any of the tested concentrations (Figure 1). A significant effect on *E. coli* growth rate was observed for FerMEG12, from concentrations as low as 50 mg/L (P < 0.001), and for magnetite at the highest concentration tested (1000 mg/L, P < 0.05).

- 211 Table 3 Characterization of the four tested nanomaterials dispersed in deionized
- 212 water at 100 mg/L. Samples were characterized 2 and 144 h after dispersion and
- 213 analyzed by DLS for hydrodynamic diameter and zeta-potential. NTA analysis was
- 214 performed in samples 2 h after dispersion.

Nanomaterial	DLS Hydrodynamic		Zeta-potential (mV)		NTA Average size	
	diameter				(mode average;	
	(z-average; nm)				nm)	
	2 h	144 h	2 h	144 h	Y	
FerMEG12	480	720	12	1.5	210	
Carbo-Iron [®]	1300	500	-15	-17	120	
Nano-Goethite	230	270	-41	-44	-	
Trap-Ox	780*	780*	-65	-60	250	
Fe-zeolites						

- 215 -: No data
- 216 *: sedimentation after suspension in deionized water occurred
- 217



218

Figure 1 Growth rate of *E. coli* after 6h exposure to FerMEG12, Carbo-Iron[®], magnetite, Nano-Goethite and Trap-Ox Fe-zeolites at 0, 50, 500 and 1000 mg/L. Asterisks indicate treatments that differ significantly from controls (ANOVA and Dunnett's test, n=3). Significance levels were set at P<0.05 (*), P < 0.001 (**) and P < 0.0001 (***).



Figure 2 Root length of radish (*Raphanus sativus*) and ryegrass (*Lolium multiflorum*) exposed to various nominal Fe concentrations from FerMEG12
particles for 6 d. Asterisks indicate treatments that differ significantly from controls
(Holm-Sidak, n=3, P<0.05 (*), P<0.001 (**)).

225

The root elongation of R. sativus was reduced by 33 % by FerMEG12 231 particles at a nominal Fe concentration as low as 10 mg/L. Root elongation 232 233 was increasingly reduced in a concentration-dependent manner and 234 completely inhibited at 10 g/L (Figure 2, left). The root elongation of L. multiflorum was significantly reduced at nominal Fe concentrations >1 g/L, 235 236 and completely inhibited at 10 g/L (Figure 2, right). The pH of the exposure 237 suspensions at the beginning of the experiment was 6.0 ± 0.5 over the whole 238 concentration range. In contrast, the oxidation reduction potential, measured 239 in exposure suspensions at the beginning of the experiment, was dramatically 240 different among concentrations and ranged from +250 mV (control), +50 mV 241 (0.01-0.1 g/L), to -590 mV (5 and 10 g/L).



Figure 3 Mortality of *L.variegatus* after 24-96 h exposure to FerMEG12 (left axis)
and dissolved oxygen (DO) concentration in test beakers after 0-96 h (right axis).

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For the *L. variegatus* test, no toxicity was observed at 1 mg/L, however more than 50 % mortality was observed at 5 mg/L and 100 % at 10 mg/L. Mortality was observed already after 24 h, together with a rapid decrease in the dissolved oxygen concentration in test beakers containing FerMEG12 (see Figure 3).

251

252 3.3 Technical challenges of ecotoxicity testing of

253 Fe nanomaterials

The higher concentrations of Fe nanomaterials did in several cases influence the measurement principles or assumptions behind the tests. Especially the turbidity of the suspensions caused issues with limited light transmission

257 through the suspensions. In the V. fischeri test, the quantification of the bioluminescence could be influenced by quenching of the emitted light before 258 259 it reaches the detector. This can easily be measured in a double vial setup that 260 ensures no actual bacterial exposure to the suspension, with the inner vial 261 containing the bacteria. A way to account for this is by spiking the suspension with V. fischeri emitting a known amount of bioluminescence. In 262 263 this way, the added bacteria can act as an internal standard and the effect of quenching can be estimated and corrected for. Using this method, it was clear 264 265 that the tested materials did quench light emission, giving rise to potential 266 erroneous conclusions if unaccounted for.

For the algal growth inhibition, issues with the quantification of algal biomass also started to appear at higher concentrations (>100 mg/L). At high Fe concentrations the fluorescence spectrum was altered significantly and obfuscated the presence and the size of the chlorophyll peak. During the testing, the turbidity can also prevent the algae to obtain sufficient light for exponential growth, a shading effect that can be difficult to account for (Hjorth et al., 2015).

In the tests with *D. magna* and *L. variegatus*, oxidation, aggregation, precipitation and ultimately sedimentation of Fe resulted in a change of exposure route (which was intended to be through the water phase alone) directly affecting the mobility of *D. magna*. Due to the described stratification of Fe particles, *L. variegatus* was exposed to an increased

concentration, as both the particles and the oligochaeta stay at the bottom ofthe beaker.

281

282 4 Discussion

283 4.1 Ecotoxicity of Fe nanomaterials

284 Based on the performed ecotoxicity tests in the present study, only the 285 FerMEG12 particles would be classified as toxic to aquatic organisms in 286 accordance to the CLP regulation. As none of the other tested materials showed toxicity below 100 mg/L, none of them would receive any 287 environmental hazard classification. The highest toxicity of the FerMEG12 288 289 particles was observed towards the oligochaeta L. variegatus. However particle sedimentation during the test consequently exposed L. variegatus to 290 291 higher Fe concentrations than what was initially dispersed, which could 292 explain why toxicity was observed for L. variegatus and not e.g. for D. magna which spends more time in the water column. Ageing the particles for 293 294 1 h in media alleviated the toxicity, which is in agreement with an earlier 295 study on milled particles reporting low toxicity after oxidation (Köber et al., 2014). Similarly, a recent study on zebrafish found no adverse effects of aged 296 Carbo-Iron[®] (Weil et al., 2015). 297

298 The FerMEG12 particles were also tested in the standard algal test with P. subcapitata with acute effects evidenced by a decrease in fluorescence right 299 300 after the onset of the test (data not shown). However, during the incubation 301 period the algal population recovered and exhibited growth rates similar (or higher) than the non-exposed controls. As the only material out of the four 302 tested, FerMEG12 was dispersed in a solvent (ethylene glycol) and although 303 304 ethylene glycol in itself showed no toxicity when tested, it seems to have preserved the reactivity of FerMEG12's elemental iron. Other studies confirm 305 306 the toxicity of freshly prepared, non-oxidized nZVI. For instance, Keller et 307 al. (2012) studied the response of microalgae and D. magna exposed to 308 NANOFER STAR and 25S. D. magna proved the most sensitive with LOEC 309 values of 0.5 mg/L for the NANOFER STAR and NANOFER 25s, compared 310 to a LOEC of 1 mg/L for Fe^{2+} . Whereas the growth of the marine microalga *I*. 311 galbana was inhibited after exposure to NANOFER 25s starting at 3 mg/L 312 (Keller et al., 2012), no effect was observed for NANOFER STAR at 313 concentrations up to 100 mg/L and effects from dissolved Fe did not occur at 314 concentrations lower than 50 mg/L. For the freshwater microalgae, P. subcapitata. Fe^{2+} exposure proved the most toxic with a LOEC value of 5 315 mg/L, which was lower than for any of the particles or Fe^{3+} (Keller et al., 316 317 2012).

318 NANOFER 25s has also been found to affect the growth of the nematode C. 319 *elegans* at 0.5 mg/L, whereas at 5 mg/L a decrease in survival and

reproduction occurred. However, when tested in soil, no toxicity to *C*. *elegans* was observed for NANOFER 25s at concentrations up to 17 mg/g.
On the contrary, their growth and reproduction increased (Saccà et al., 2014).
Similarly, the survival of the earthworm, *E. fetida*, was not affected by nZVI
even at 3 g/kg, although DNA damage and lipid oxidation was observed
(Yirsaw et al., 2016).

326 Chen et al. (2011) investigated continuous exposure of carboxymethyl cellulose stabilized nZVI (CMC-nZVI) towards medaka fish larvae and 327 concluded that the toxicity was caused by hypoxia, Fe^{2+} toxicity, and ROS-328 mediated oxidative damage. In their experiment Fe^{2+} proved the most acutely 329 330 toxic with 100 % mortality at 75 mg/L. In a similar study, Chen et al. (2012) reported Fe²⁺ being the most toxic form of Fe tested, followed by CMC-331 332 nZVI, nZVI and lastly the aged nZVI. Yet in Chen et al. (2013) CMC-nZVI had a higher acute toxicity than both Fe^{2+} and aged nZVI. 333

Marsalek et al. (2012) have reported low aquatic toxicity for NANOFER 25 with $EC_{50} > 1$ g/L for *D. magna* and >2.5 g/L for fish (*P. reticulate*) and similar values for willows (*S. alba*), duckweed (*L. minor*), and microalgae (*D. subspicatus*). Effects on the cyanobacteria *M. aeruginosa* were observed at the lowest concentrations in the test battery and with an EC_{50} -value of 50 mg/L.

Although there are signs of increased ecotoxicity of nZVI compared to Fe^{2+} , which seems rational due to the additional oxidative capacity of Fe^{0} , the

342 opposite is also sometimes the case. However none of the studies in the 343 scientific literature on Fe nanomaterials have reported a higher toxicity than 344 what has been shown for dissolved Fe (Johnson et al., 2007). It seems likely 345 that varying, and generally lower, bioavailability of nZVI in media has the 346 potential to offset any increase in toxicity compared to dissolved Fe.

347

348 4.2 Environmental risk assessment of Fe in surface

349 water

350 Whereas hazard and risk assessment of Fe nanomaterials is a novel task, the toxic effects of Fe and Fe salts on aquatic life is well described. Fe^{2+} is 351 generally considered bioavailable and can induce toxicity in aquatic 352 organisms, whereas when oxidized to Fe^{3+} it hydrolyses and precipitates out 353 of solution as hydroxides at normal pH, which then can give rise to indirect, 354 physical effects (Vuori, 1995). Ecotoxicity testing of Fe has traditionally 355 made a distinction between total Fe and dissolved Fe, ideally signifying Fe^{2+} , 356 however in practice meaning anything that passes through a 0.45 µm filter, 357 358 which includes colloidal Fe(III) stabilized mostly by organic material (Vuori, 359 1995) and Fe(III) complexed by organic ligands. In this way, many tests have 360 already, indirectly, assessed the toxicity of suspended Fe in the nano range. 361 Even for Fe, hazard and risk assessment as well as setting appropriate

362 environmental quality standards (EQS) has been difficult for decades due to363 this redox cycling, inorganic speciation, complexation and precipitation.

As an example, the company American Electric Power (AEP) argued in 1983 that the USEPA water quality standard of 1 mg/L for Fe was too low, stating that field data showed that Fe concentrations "need to be far over 1 mg/L to adversely affect" aquatic life, and that toxicity testing is not fit to assess Fe toxicity (Loeffelman et al., 1985). AEP also called for a use of Fe^{2+} , and not total Fe, as the foundation for setting the water quality standard, based on the fact that Fe^{2+} is the bioavailable fraction of Fe.

More recently, Linton et al. (2007) acknowledged that the USEPA "metal 371 policy" generally is to derive "aquatic life criteria" based on the dissolved 372 metal and therefore Fe should be regulated based on Fe^{2+} toxicity. Yet, it 373 374 makes sense to use field data on total Fe, as lab-based toxicity testing of Fe is poor at assessing colloidal and indirect effects such as the impact on 375 respiration and food consumption. With reference to a review by Vouri 376 (1995) who states "the effect of Fe on aquatic animals and their habitats are 377 378 mainly indirect", Linton et al. (2007) argue that field studies therefore would do a better job of assessing the overall environmental impact of Fe. 379 Accordingly, the assessment still has a foundation in field observations partly 380 381 due to this issue of indirect effects (Linton et al., 2007). Based on additional 382 field data, Linton et al. (2007) however proposed a new and differentiated

383 EQS of 0.21 mg/L for sensitive groups and 1.74 mg/L for "slight to moderate384 changes" in the aquatic community.

385 In Europe, the Environment Agency in England proposed a new EQS for Fe 386 in 2007 under the Water Framework Directive (Johnson et al., 2007). A long 387 term PNEC of 16 μ g/L was derived, based on a NOEC value of 0.16 mg/L 388 obtained in a 21-d study on D. magna. Similarly, a 96 h study on brook trout 389 (S. fontinalis) with a LC_{50} value of 0.41 mg/L gave rise to a short term PNEC 390 of 41 μ g/L. They rationalized the use of the considerably lower PNEC values 391 with the emergence of new data as well as the fact that the old EQS was 392 based on field data and not on standardized toxicity tests.

In response to this, Crane et al. (2007) stated that 16 µg/L was "substantially 393 394 below concentrations associated with impaired invertebrate assemblages in 395 the field". Based on their analysis of data from 253 sites in England and 396 Wales, Crane et al. (2007) proposed an EQS between 43-250 µg/L based on dissolved Fe. In 2012, the UK Technical Advisory Group proposed an EQS 397 398 of 0.73 mg/L total Fe, based on field data, in a very thorough review (Peters 399 et al., 2012). The suggested EQS from the Environment Agency of 16 µg/L was described as well below background levels and therefore "not adopted 400 401 for regulatory use".

402 In accordance with Linton et al. (2007), Peters et al. (2012) state that the 403 effects of Fe are difficult to isolate and, in contrast to most metals, we cannot 404 just focus on the dissolved fraction as there is also a physical effect from the 405 total Fe, which perhaps could even be dominating. This line of reasoning also
406 advocates for the use of total Fe as the dose metric for ecotoxicity studies on
407 Fe nanomaterials.

408

409 4.3 Applicability of standardized ecotoxicity tests

410 for hazard identification of Fe nanomaterials

411 As shown above, the current consensus seems to move away from using 412 standardized ecotoxicity tests on Fe for risk assessment purposes; rather the use of field data and mesocosm studies are encouraged. Peters et al. (2012) 413 414 point towards the issue of Fe solubility as well as the issue with assessing 415 physical effects as the key points against using standardized ecotoxicity 416 testing of Fe. Wess (2015) also questions the adequacy of ecotoxicity tests on 417 Fe to inform risk assessment as they fail to uphold various criteria for 418 assessing causation, e.g. issues with establishing dose response relationships 419 and incoherence with field data. If these ecotoxicity tests are not suitable for 420 Fe salts due to precipitation and exposure control issues, then clearly they are 421 not suitable to assess Fe nanomaterials, which by definition are not dissolved 422 entities.

However, having the test limitations and challenges in mind, such as the ones
mentioned in section 3.3, as well as the general testing considerations in
nanoecotoxicology (Petersen et al., 2014; Skjolding et al., 2016) and indirect

426 physical effects (Sørensen et al., 2015), standardized ecotoxicity tests can 427 still provide valuable information, as a screening and ranking tool for hazard 428 identification. Trying to overcome these limitations will sometimes result in 429 deviations from the standard tests, which at times are necessary to obtain 430 meaningful data. Additionally, the information generated from standardized 431 ecotoxicity tests is still required by regulatory agencies to achieve market 432 access.

433 On the other hand, as shown for Fe salts, standardized ecotoxicity tests are 434 challenged in terms of their usefulness and accuracy for site-specific and case 435 oriented risk assessments, and it is recommended to rely more on field data 436 when assessing the environmental impact of Fe (EC, 2011). The relevance of standard organisms for site-specific risk assessment is questionable for 437 438 remediation cases, as the extrapolation value from these organisms to ecosystems may be low. In addition, the transformation of the pristine 439 440 material to the oxidized form expected in the environment and the corresponding change in toxicity is also better assessed in the field. As such, 441 it makes sense to rely less on laboratory ecotoxicity testing and instead 442 443 incorporate mesocosm and field data into site-specific risk assessment of Fe 444 nanomaterials.

445 5 Conclusion and recommendations

Ecotoxicity testing of four nanomaterials engineered for sub-surface 446 remediation revealed low toxicity for all Fe oxides materials as well as 447 Carbo-Iron[®], and the results do not lead to any hazard classification 448 449 according to current EU regulation. FerMEG12 was the only material 450 exhibiting toxicity towards bacteria and plants at 50 mg/L and oligochaetes at 451 5 mg/L. Standard ecotoxicity testing of nanoparticles has in general proven technically difficult and it may be questioned whether proper hazard 452 453 identification of engineered nanoparticles needed for environmental risk 454 assessment is currently feasible. Aggregation, agglomeration, sedimentation, 455 shading, and other physical effects are known to confound the measuring principles behind the tests and these interferences were also observed for the 456 tested particles. This was pronounced for tests on algae, bacteria, and 457 458 crustaceans and requires inclusion of additional controls to ensure a correct data interpretation. While Fe^0 nanomaterials have the potential to be toxic at 459 low concentrations, a potential environmental impact downstream of the 460 461 injection of Fe nanomaterials seems more likely to originate from the large 462 amount of Fe injected in *in-situ* remediation, than from novel particle related effects, especially since Fe is abundant in nature as particulate matter. In 463 464 conclusion we recommend:

465	٠	Testing concentrations up to 100 mg/L is relevant for hazard identification
466		and classification purposes. Testing Fe nanoparticles at higher concentrations
467		not only decreases the environmental relevance, but also increases the
468		influence of physical effects such as turbidity and concentration-dependent
469		agglomeration. Therefore, the main focus in testing should be on
470		concentrations <100 mg/L, and care should be taken when conducting
471		ecotoxicological testing of Fe nanomaterials at higher concentrations.
472		
473	•	For Fe nanomaterials, agglomeration and sedimentation challenge the validity
474		of the standard test setups for which a constant exposure during incubation is
475		required. These issues are currently under scrutiny in the OECD WPMN and
476		in several EU projects (Lynch, 2016). We recommend the use of these tests
477		for hazard identification and ranking, where these tests still provide valuable
478		information.
479		
/80	•	We recommend studying the effects of Fe nanomaterials in more

nanomaterials more studying ге 1n 480 the enecus environmentally realistic conditions to support site-specific hazard 481 assessment. E.g. through testing of relevant organisms, media and more 482 complex testing systems closer related to the field, as the scope of 483 standardized ecotoxicity testing is limited and not designed to assess the 484 485 indirect effects of Fe exposure.

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498 6 References

499 Bardos, P., Bone, B., Daly, P., Elliott, D., Jones, S., Lowry, G. & Merly, C. (2014).

500 A Risk / Benefit Appraisal for the Application of Nano-Scale Zero Valent Iron

- 501 (*nZVI*) for the Remediation of Contaminated Sites. WP9 NanoRem. Available
 502 at
- 503 <u>http://www.nanorem.eu/Stream.aspx?p=/App_Data/docs/user7Gallery/NANOR</u>
- 504 EM%20NZVI%20risk%20benefit%20issues%20paper%20FINAL_2.0.pdf
- 505 Chen, P.-J., Su, C.-H., Tseng, C.-Y., Tan, S.-W. & Cheng, C.-H. (2011). Toxicity
 506 assessments of nanoscale zerovalent iron and its oxidation products in medaka
 507 (*Oryzias latipes*) fish. *Marine pollution bulletin*. 63 (5–12). p.pp. 339–46.

508	Chen, PJ., Tan, SW. & Wu, WL. (2012). Stabilization or oxidation of
509	nanoscale zerovalent iron at environmentally relevant exposure changes
510	bioavailability and toxicity in medaka fish. Environmental Science &
511	<i>Technology</i> . 46 (15). p.pp. 8431–9.

- 512 Chen, P.-J., Wu, W.-L. & Wu, K.C.-W. (2013). The zerovalent iron nanoparticle
 513 causes higher developmental toxicity than its oxidation products in early life
 514 stages of medaka fish. *Water research*. 47 (12). p.pp. 3899–909.
- 515 Crane, M., Kwok, K.W.H., Wells, C., Whitehouse, P. & Lui, G.C.S. (2007). Use of
 516 field data to support European Water Framework Directive quality standards
 517 for dissolved metals. *Environmental Science & Technology*. 41 (14). p.pp.
 518 5014–5021.
- EC, (2011). Common Implementation Strategy for the Water Framework Directive
 (2000/60/EC). Guidance Document No. 27. Technical Guidance For Dericing
 Environmental Quality Standards. European Commission, Brussels, Belgium.
- El-Temsah, Y.S., Sevcu, A., Bobcikova, K., Cernik, M. & Joner, E.J. (2016). DDT
 degradation efficiency and ecotoxicological effects of two types of nano-sized
 zero-valent iron (nZVI) in water and soil. *Chemosphere*. 144. p.pp. 2221–2228.
- Grieger, K.D., Fjordbøge, A., Hartmann, N.B., Eriksson, E., Bjerg, P.L. & Baun, A.
 (2010). Environmental benefits and risks of zero-valent iron nanoparticles
 (nZVI) for in situ remediation: risk mitigation or trade-off? *Journal of contaminant hydrology*. 118 (3–4). p.pp. 165–83.
- 529 Grieger, K.D., Hjorth, R., Rice, J., Kumar, N. & Bang, J. (2015). Nano-

- *remediation : tiny particles cleaning up big environmental problems*. Blog
 entry for the International Union for Conservation of Nature (IUCN),
 Available at http://cmsdata.iucn.org/downloads/nanoremediation.pdf.
- Henderson, A.D. & Demond, A.H. (2007). Long-Term Performance of Zero-Valent
 Iron Permeable Reactive Barriers: A Critical Review. *Environmental Engineering Science*. 24 (4). p.pp. 401–423.
- Hjorth, R., Sørensen, S.N., Olsson, M.E., Baun, A. & Hartmann, N.B. (2015). A
 certain shade of green: Can algal pigments reveal shading effects of
 nanoparticles? *Integrated Environmental Assessment and Management*. 15(1),
 p.pp 200-202.
- Johnson, I., Sorokin, N., Atkinson, C., Rule, K., Hope, S.-J. & Agency, E. (2007). *Preconsultation report: Proposed EQS for Water Framework Directive Annex VIII substances: iron (total dissolved)*. Environment Agency, Bristol, UK.
- Karn, B., Kuiken, T. & Otto, M. (2009). Nanotechnology and in situ remediation: a
 review of the benefits and potential risks. *Environmental health perspectives*.
 117 (12). p.pp. 1813–31.
- Keller, A. a, Garner, K., Miller, R.J. & Lenihan, H.S. (2012). Toxicity of nano-zero
 valent iron to freshwater and marine organisms. *PloS one*. 7 (8). p.pp 1-10.
- 548 Köber, R., Hollert, H., Hornbruch, G., Jekel, M., Kamptner, A., Klaas, N., Maes,
- 549 H., Mangold, K.M., Martac, E., Matheis, A., Paar, H., Schäffer, A., Schell, H.,
- 550 Schiwy, A., Schmidt, K.R., Strutz, T.J., Thümmler, S., Tiehm, A. & Braun, J.
- 551 (2014). Nanoscale zero-valent iron flakes for groundwater treatment.

- 552 Environmental Earth Sciences. 72 (9). p.pp. 3339–3352.
- 553 Linton, T., Pacheco, M., McIntyre, D., Clement, W. & Goorich-Mahoney, J. (2007).
- 554 Development of bioassessment-based benchmarks for iron. *Environmental*555 *Toxicology*. 26 (6). p.pp. 1291–1298.
- 556 Loeffelman, P.H., Van Hassel, J.H., Arnold, T.E. & Hendricks, J.C. (1985). A new
- approach for regulating iron in water quality standards. Aquatic Toxicology
 and Hazard assessment: Eight Symposium. ASTM STP 891. p.pp. 137–152.
- 559 Lynch, I. (2016). Compendium of Projects in the European NanoSafety Cluster.
- 560 2016 edition. Available at
- 561 http://www.nanosafetycluster.eu/www.nanosafetycluster.eu/home/european-
- 562 nanosafety-cluster-compendium.html
- Marsalek, B., Jancula, D., Marsalkova, E., Mashlan, M., Safarova, K., Tucek, J. &
 Zboril, R. (2012). Multimodal action and selective toxicity of zerovalent iron
 nanoparticles against cyanobacteria. *Environmental Science and Technology*.
 46 (4). p.pp. 2316–2323.
- Mueller, N.C., Braun, J., Bruns, J., Černík, M., Rissing, P., Rickerby, D. &
 Nowack, B. (2012). Application of nanoscale zero valent iron (NZVI) for
 groundwater remediation in Europe. *Environmental science and pollution research international*. 19. p.pp. 550–558.
- 571 Peters, A., Merrington, G., Simpson, P. & Crane, M. (2012). Proposed Quality
 572 Standards for Iron in Freshwaters Based on Field Evidence. Water Framework
 573 Directive United Kingdom Technical Advisory Group (WFD-UKTAG).

- 574 Edinburgh, Scotland.
- 575 Petersen, E.J., Henry, T.B., Zhao, J., Maccuspie, R.I., Kirschling, T.L.,
 576 Dobrovolskaia, M.A., Hackley, V., Xing, B. & White, J.C. (2014).
 577 Identification and avoidance of potential artifacts and misinterpretations in
 578 nanomaterial ecotoxicity measurements. *Environmental science & technology*.
 579 48. p.pp. 4226–4246.
- Saccà, M.L., Fajardo, C., Costa, G., Lobo, C., Nande, M. & Martin, M. (2014).
 Integrating classical and molecular approaches to evaluate the impact of
 nanosized zero-valent iron (nZVI) on soil organisms. *Chemosphere*. 104. p.pp.
 184–189.
- 584 Skjolding, L.M., Sørensen, S.N., Hartmann, N.B., Hjorth, R., Hansen, S.F. & Baun,
 585 A. (2016). A Critical Review of Aquatic Ecotoxicity Testing of Nanoparticles 586 The Quest for Disclosing Nanoparticle Effects. *Angewandte Chemie*587 *International Edition*. 55 (49). p.pp. 15224-15239.
- Sørensen, S.N., Hjorth, R., Delgado, C.G., Hartmann, N.B. & Baun, A. (2015).
 Nanoparticle toxicity Physical and/or chemical effects? *Integrated Environmental Assessment and Management*. 11(4). p.pp. 722-724.
- 591 Vuori, K.-M. (1995). Direct and indirect effects on iron on river ecosystems.
 592 Annales Zoologici Fennici. (32). p.pp 317-329.
- Wang, C.-B. & Zhang, W. (1997). Synthesizing Nanoscale Iron Particles for Rapid
 and Complete Dechlorination of TCE and PCBs. *Environmental Science* & *Technology*. 31 (7). p.pp. 2154–2156.

- Weil, M., Meißner, T., Busch, W., Springer, A., Kühnel, D., Schulz, R. & Duis, K.
 (2015). The oxidized state of the nanocomposite Carbo-Iron® causes no
 adverse effects on growth, survival and differential gene expression in
 zebrafish. *Science of the Total Environment*. 530–531. p.pp. 198–208.
- 600 Wess, R.A. (2015). The question of causation and adequacy iron as an example of
- 601 intrinsic toxicity and other effects. *Integrated Environmental Assessment and*602 *Management.* 12 (1). p.pp. 202–204.
- Yan, W., Lien, H.-L., Koel, B.E. & Zhang, W. (2013). Iron nanoparticles for
 environmental clean-up: recent developments and future outlook. *Environmental Science: Processes & Impacts.* 15. p.pp. 63-77.
- Yirsaw, B.D., Mayilswami, S., Megharaj, M., Chen, Z. & Naidu, R. (2016). Effect
 of zero valent iron nanoparticles to Eisenia fetida in three soil types. *Environmental Science and Pollution Research*. 23 (10). p.pp. 9822–9831.

Highlights

- This study summarizes the outcome of nanoecotoxicity testing in NanoRem.
- We assessed four novel engineered nanomaterials in an ecotoxicological test battery.
- Only one of the tested materials gave rise to toxicity below 100 mg/L.
- Standardized testing is inadequate to inform site-specific risk assessment.