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Published in:
Chemosphere

Link to article, DOI:
[10.1016/j.chemosphere.2017.05.060](https://doi.org/10.1016/j.chemosphere.2017.05.060)

Publication date:
2017

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):

Hjorth, R., Coutris, C., Nguyen, N., Sevcu, A., Gallego-Urrea, J. A., Baun, A., & Jøner, E. (2017). Ecotoxicity testing and environmental risk assessment of iron nanomaterials for sub-surface remediation – Recommendations from the FP7 project NanoRem. *Chemosphere*, 182, 525-631. <https://doi.org/10.1016/j.chemosphere.2017.05.060>

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Accepted Manuscript

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PII: S0045-6535(17)30764-6
DOI: [10.1016/j.chemosphere.2017.05.060](https://doi.org/10.1016/j.chemosphere.2017.05.060)
Reference: CHEM 19275

To appear in: *ECSN*

Received Date: 17 February 2017

Revised Date: 1 May 2017

Accepted Date: 10 May 2017

Please cite this article as: Hjorth, R., Coutris, C., Nguyen, N.H.A., Sevcu, A., Gallego-Urrea, Julià.Alberto., Baun, A., Joner, E.J., Ecotoxicity testing and environmental risk assessment of iron nanomaterials for sub-surface remediation – Recommendations from the FP7 project NanoRem, *Chemosphere* (2017), doi: [10.1016/j.chemosphere.2017.05.060](https://doi.org/10.1016/j.chemosphere.2017.05.060).

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1 Ecotoxicity testing and environmental risk assessment
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3 Recommendations from the FP7 project NanoRem

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15

16 Abstract

17 Nanoremediation with iron (Fe) nanomaterials opens new doors for treating
18 contaminated soil and groundwater, but is also accompanied by new potential
19 risks as large quantities of engineered nanomaterials are introduced into the
20 environment. In this study, we have assessed the ecotoxicity of four
21 engineered Fe nanomaterials, specifically, Nano-Goethite, Trap-Ox Fe-
22 zeolites, 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.*
25 *subcapitata*, *Chlamydomonas* sp.), crustaceans (*D. magna*), worms (*E. fetida*,
26 *L. variegatus*) and plants (*R. sativus*, *L. multiflorum*). The tested materials are
27 commercially available and include Fe oxide and nanoscale zero valent iron
28 (nZVI), but also hybrid products with Fe loaded into a matrix. All but one
29 material, a ball milled nZVI (FerMEG12), showed no toxicity in the test
30 battery when tested in concentrations up to 100 mg/L, which is the cutoff for
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
35 nanomaterials and discusses environmental risk assessment considerations
36 related to these.

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

39

40 1 Introduction

41 Innovation in nanotechnology introduces new treatment options for
42 environmental remediation of organic compounds (notably chlorinated
43 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
54 groundwater may constitute a risk to the environment as they are injected into
55 the subsurface in large quantities (Grieger et al., 2010). As such this could
56 represent a worst case scenario when considering possible negative
57 environmental effects of manufactured nanomaterials. Nanoremediation in
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,
68 NANOFER 25 and 25s being the most common commercially available
69 particles. In these publications the focus has been on aquatic and terrestrial
70 ecotoxicity (Keller et al., 2012; Marsalek et al., 2012; Saccà et al., 2014; El-
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.

74 A range of Fe nanomaterials has been developed in the European FP7 project
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
78 treatable soil and groundwater contaminants from halogenated organics to
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
82 currently available on the international market (see Table 1). Common for all
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.,
85 2012). In this case, they will have to be registered under the European
86 chemical legislation REACH, which will be accompanied with data
87 requirements on ecotoxicity. The data generated will feed into the general

88 hazard identification of the nanomaterials and form the basis for a generic
89 risk assessment (i.e. a hazard classification according to the classification,
90 labeling and packaging (CLP) regulation). It is important to emphasize that
91 this risk assessment does not directly relate to the safety of injecting said
92 material into an aquifer or a contaminated soil. Such a task is done in a site-
93 specific 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
96 nanomaterials may spread, in worst case scenarios, to both terrestrial and
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
100 respect to modes of exposure and modes of action, and to enhance the
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

110 Samples of Fe nanomaterials for ecotoxicity testing were obtained directly
111 from the manufacturers within the NanoRem project. A full list and
112 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*[®] 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

128 in 50 mL deionized water was sonicated for 15 min and the CMC solution
129 and zeolite suspension were mixed and sonicated for additional 15 min.

130 *Magnetite* Magnetite, received as powder, was suspended in deionized water
131 and mixed for 10 minutes with a high-shear mixer. Subsequent dilutions
132 series in exposure media were prepared and used right away.

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

136

137 2.2 Characterization of stock suspensions

138 Dynamic Light Scattering (DLS) measurements of aqueous suspensions
139 (deionized water) from 10 mg/L to 10 g/L test material were performed on a
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).

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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 particle size (nm)	Specific surface area (m ² /g)
FerMEG12	Zero-valent Fe mechanically ground through ball milling	Field tested and commercially available	Reduction	Suspension	15-30% Fe 70-85 % monoethylene glycol	-	12-18
Carbo-Iron [®]	Composite of activated carbon and zero-valent Fe	Field tested and commercially available	Adsorption + Reduction	Powder	30.3 % Fe _{tot} 20.5 % Fe ⁰ 13.1 % Fe ₃ O ₄ 55±1% C _{tot}	13440 ±20	594
Magnetite	Fe oxides (Fe ₃ O ₄)	Precursor for NANO FER 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

155

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

166

167 2.3 Ecotoxicological test battery

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

175

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

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

178 ^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
 186 aqueous stability than FerMEG12 and Carbo-Iron[®], however sedimentation of
 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

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

196

197 3.2 Ecotoxicity

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

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

210

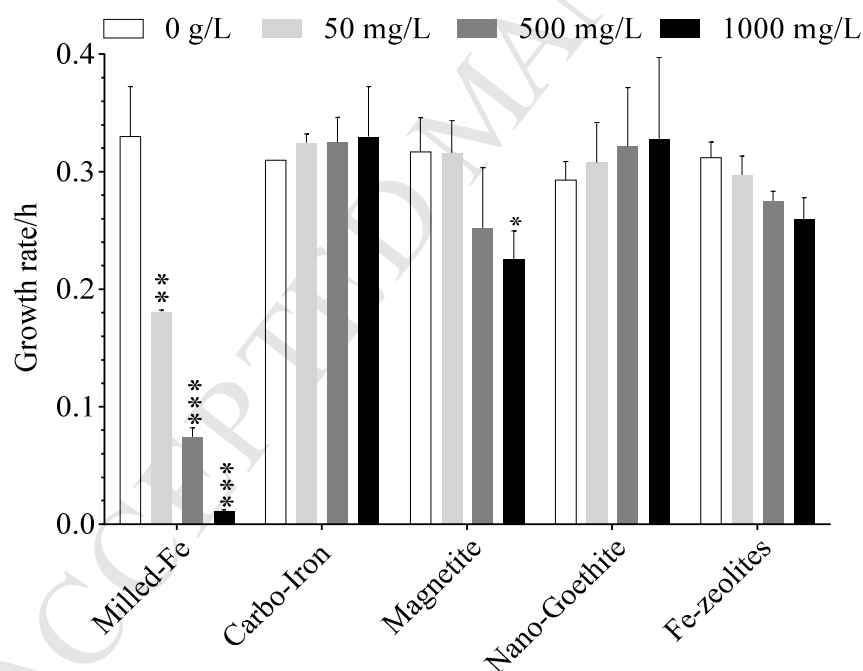
211 **Table 3** Characterization of the four tested nanomaterials dispersed in deionized
 212 water at 100 mg/L. Samples were characterized 2 and 144 h after dispersion and
 213 analyzed by DLS for hydrodynamic diameter and zeta-potential. NTA analysis was
 214 performed in samples 2 h after dispersion.

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

215 -: No data

216 *: sedimentation after suspension in deionized water occurred

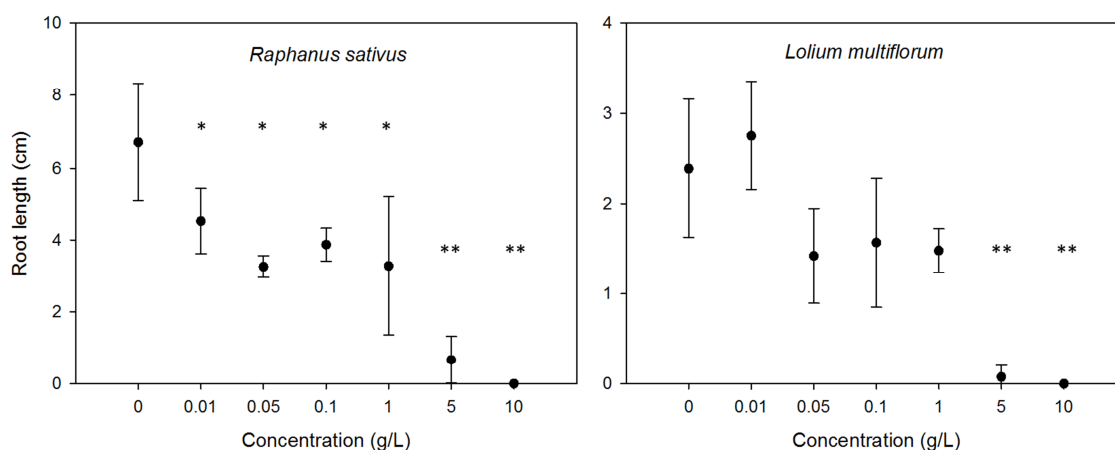
217



218

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

224

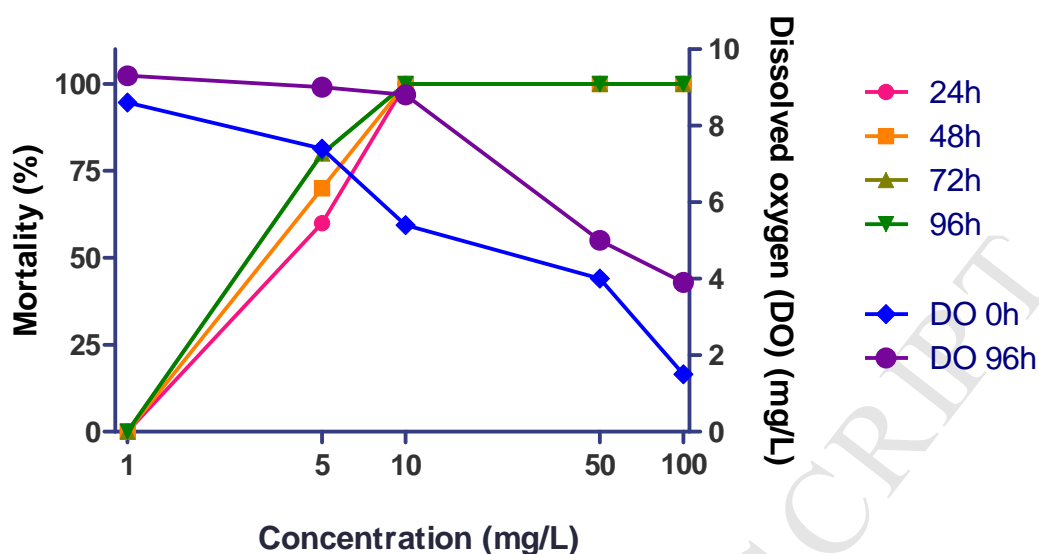


225

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

230

231 The root elongation of *R. sativus* was reduced by 33 % by FerMEG12
 232 particles at a nominal Fe concentration as low as 10 mg/L. Root elongation
 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.*
 235 *multiflorum* was significantly reduced at nominal Fe concentrations >1 g/L,
 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).



242
 243 **Figure 3** Mortality of *L. variegatus* after 24-96 h exposure to FerMEG12 (left axis)
 244 and dissolved oxygen (DO) concentration in test beakers after 0-96 h (right axis).

245
 246 For the *L. variegatus* test, no toxicity was observed at 1 mg/L, however more
 247 than 50 % mortality was observed at 5 mg/L and 100 % at 10 mg/L. Mortality
 248 was observed already after 24 h, together with a rapid decrease in the
 249 dissolved oxygen concentration in test beakers containing FerMEG12 (see
 250 Figure 3).

251

252 3.3 Technical challenges of ecotoxicity testing of 253 Fe nanomaterials

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

257 through the suspensions. In the *V. fischeri* test, the quantification of the
258 bioluminescence could be influenced by quenching of the emitted light before
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
262 suspension with *V. fischeri* emitting a known amount of bioluminescence. In
263 this way, the added bacteria can act as an internal standard and the effect of
264 quenching can be estimated and corrected for. Using this method, it was clear
265 that the tested materials did quench light emission, giving rise to potential
266 erroneous conclusions if unaccounted for.

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

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

279 concentration, as both the particles and the oligochaeta stay at the bottom of
280 the 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
287 showed toxicity below 100 mg/L, none of them would receive any
288 environmental hazard classification. The highest toxicity of the FerMEG12
289 particles was observed towards the oligochaeta *L. variegatus*. However
290 particle sedimentation during the test consequently exposed *L. variegatus* to
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.*
293 *magna* which spends more time in the water column. Ageing the particles for
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.,
296 2014). Similarly, a recent study on zebrafish found no adverse effects of aged
297 Carbo-Iron[®] (Weil et al., 2015).

298 The FerMEG12 particles were also tested in the standard algal test with *P.*
299 *subcapitata* with acute effects evidenced by a decrease in fluorescence right
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
302 higher) than the non-exposed controls. As the only material out of the four
303 tested, FerMEG12 was dispersed in a solvent (ethylene glycol) and although
304 ethylene glycol in itself showed no toxicity when tested, it seems to have
305 preserved the reactivity of FerMEG12's elemental iron. Other studies confirm
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 NANO FER STAR and 25S. *D. magna* proved the most sensitive with LOEC
309 values of 0.5 mg/L for the NANO FER STAR and NANO FER 25s, compared
310 to a LOEC of 1 mg/L for Fe²⁺. Whereas the growth of the marine microalga *I.*
311 *galbana* was inhibited after exposure to NANO FER 25s starting at 3 mg/L
312 (Keller et al., 2012), no effect was observed for NANO FER 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.*
315 *subcapitata*, Fe²⁺ exposure proved the most toxic with a LOEC value of 5
316 mg/L, which was lower than for any of the particles or Fe³⁺ (Keller et al.,
317 2012).

318 NANO FER 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

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

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

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

340 Although there are signs of increased ecotoxicity of nZVI compared to Fe^{2+} ,
341 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
351 toxic effects of Fe and Fe salts on aquatic life is well described. Fe^{2+} is
352 generally considered bioavailable and can induce toxicity in aquatic
353 organisms, whereas when oxidized to Fe^{3+} it hydrolyses and precipitates out
354 of solution as hydroxides at normal pH, which then can give rise to indirect,
355 physical effects (Vuori, 1995). Ecotoxicity testing of Fe has traditionally
356 made a distinction between total Fe and dissolved Fe, ideally signifying Fe^{2+} ,
357 however in practice meaning anything that passes through a 0.45 μm filter,
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 to
363 this redox cycling, inorganic speciation, complexation and precipitation.

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

371 More recently, Linton et al. (2007) acknowledged that the USEPA “metal
372 policy” generally is to derive “aquatic life criteria” based on the dissolved
373 metal and therefore Fe should be regulated based on Fe^{2+} toxicity. Yet, it
374 makes sense to use field data on total Fe, as lab-based toxicity testing of Fe is
375 poor at assessing colloidal and indirect effects such as the impact on
376 respiration and food consumption. With reference to a review by Vouri
377 (1995) who states “the effect of Fe on aquatic animals and their habitats are
378 mainly indirect”, Linton et al. (2007) argue that field studies therefore would
379 do a better job of assessing the overall environmental impact of Fe.
380 Accordingly, the assessment still has a foundation in field observations partly
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 moderate
384 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 µg/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 LC₅₀ value of 0.41 mg/L gave rise to a short term PNEC
390 of 41 µg/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.

393 In response to this, Crane et al. (2007) stated that 16 µg/L was “substantially
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
397 dissolved Fe. In 2012, the UK Technical Advisory Group proposed an EQS
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
400 was described as well below background levels and therefore “not adopted
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
413 use of field data and mesocosm studies are encouraged. Peters et al. (2012)
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.

423 However, having the test limitations and challenges in mind, such as the ones
424 mentioned in section 3.3, as well as the general testing considerations in
425 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
437 standard organisms for site-specific risk assessment is questionable for
438 remediation cases, as the extrapolation value from these organisms to
439 ecosystems may be low. In addition, the transformation of the pristine
440 material to the oxidized form expected in the environment and the
441 corresponding change in toxicity is also better assessed in the field. As such,
442 it makes sense to rely less on laboratory ecotoxicity testing and instead
443 incorporate mesocosm and field data into site-specific risk assessment of Fe
444 nanomaterials.

445 5 Conclusion and recommendations

446 Ecotoxicity testing of four nanomaterials engineered for sub-surface
447 remediation revealed low toxicity for all Fe oxides materials as well as
448 Carbo-Iron[®], and the results do not lead to any hazard classification
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
452 technically difficult and it may be questioned whether proper hazard
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
456 principles behind the tests and these interferences were also observed for the
457 tested particles. This was pronounced for tests on algae, bacteria, and
458 crustaceans and requires inclusion of additional controls to ensure a correct
459 data interpretation. While Fe⁰ nanomaterials have the potential to be toxic at
460 low concentrations, a potential environmental impact downstream of the
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
463 effects, especially since Fe is abundant in nature as particulate matter. In
464 conclusion we recommend:

- 465 • 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
- 480 • We recommend studying the effects of Fe nanomaterials in more
481 environmentally realistic conditions to support site-specific hazard
482 assessment. E.g. through testing of relevant organisms, media and more
483 complex testing systems closer related to the field , as the scope of
484 standardized ecotoxicity testing is limited and not designed to assess the
485 indirect effects of Fe exposure.

486

487 Acknowledgements

488 This project has received funding from the European Union's Seventh
489 Framework Programme for research, technological development and
490 demonstration under grant agreement no 309517 (NanoRem). RH is also
491 funded by the project 'Integrated water technology' - an initiative between
492 the Korea Advanced Institute of Science and the Technical University of
493 Denmark. NN and AŠ acknowledge the assistance provided by the Research
494 Infrastructure NanoEnviCz, supported by the Ministry of Education, Youth
495 and Sports (CZ) under project No. LM2015073. The authors are also grateful
496 to E. Maremonti for her help during plant growth testing.

497

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 http://www.nanorem.eu/Stream.aspx?p=/App_Data/docs/user7Gallery/NANOR
504 [EM%20NZVI%20risk%20benefit%20issues%20paper%20FINAL_2.0.pdf](http://www.nanorem.eu/Stream.aspx?p=/App_Data/docs/user7Gallery/NANOR)
- 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, P.-J., Tan, S.-W. & Wu, W.-L. (2012). Stabilization or oxidation of
509 nanoscale zerovalent iron at environmentally relevant exposure changes
510 bioavailability and toxicity in medaka fish. *Environmental Science &*
511 *Technology*. 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.
- 519 EC, (2011). Common Implementation Strategy for the Water Framework Directive
520 (2000/60/EC). Guidance Document No. 27. Technical Guidance For Deriving
521 Environmental Quality Standards. European Commission, Brussels, Belgium.
- 522 El-Temsah, Y.S., Sevcu, A., Bobcikova, K., Cernik, M. & Joner, E.J. (2016). DDT
523 degradation efficiency and ecotoxicological effects of two types of nano-sized
524 zero-valent iron (nZVI) in water and soil. *Chemosphere*. 144. p.pp. 2221–2228.
- 525 Grieger, K.D., Fjordbøge, A., Hartmann, N.B., Eriksson, E., Bjerg, P.L. & Baun, A.
526 (2010). Environmental benefits and risks of zero-valent iron nanoparticles
527 (nZVI) for in situ remediation: risk mitigation or trade-off? *Journal of*
528 *contaminant hydrology*. 118 (3–4). p.pp. 165–83.
- 529 Grieger, K.D., Hjorth, R., Rice, J., Kumar, N. & Bang, J. (2015). *Nano-*

- 530 *remediation : tiny particles cleaning up big environmental problems*. Blog
531 entry for the International Union for Conservation of Nature (IUCN),
532 Available at <http://cmsdata.iucn.org/downloads/nanoremediation.pdf>.
- 533 Henderson, A.D. & Demond, A.H. (2007). Long-Term Performance of Zero-Valent
534 Iron Permeable Reactive Barriers: A Critical Review. *Environmental*
535 *Engineering Science*. 24 (4). p.pp. 401–423.
- 536 Hjorth, R., Sørensen, S.N., Olsson, M.E., Baun, A. & Hartmann, N.B. (2015). A
537 certain shade of green: Can algal pigments reveal shading effects of
538 nanoparticles? *Integrated Environmental Assessment and Management*. 15(1),
539 p.pp 200-202.
- 540 Johnson, I., Sorokin, N., Atkinson, C., Rule, K., Hope, S.-J. & Agency, E. (2007).
541 *Preconsultation report: Proposed EQS for Water Framework Directive Annex*
542 *VIII substances: iron (total dissolved)*. Environment Agency, Bristol, UK.
- 543 Karn, B., Kuiken, T. & Otto, M. (2009). Nanotechnology and in situ remediation: a
544 review of the benefits and potential risks. *Environmental health perspectives*.
545 117 (12). p.pp. 1813–31.
- 546 Keller, A. a, Garner, K., Miller, R.J. & Lenihan, H.S. (2012). Toxicity of nano-zero
547 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
557 approach for regulating iron in water quality standards. *Aquatic Toxicology*
558 *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-](http://www.nanosafetycluster.eu/www.nanosafetycluster.eu/home/european-nanosafety-cluster-compendium.html)
562 [nanosafety-cluster-compendium.html](http://www.nanosafetycluster.eu/www.nanosafetycluster.eu/home/european-nanosafety-cluster-compendium.html)
- 563 Marsalek, B., Jancula, D., Marsalkova, E., Mashlan, M., Safarova, K., Tucek, J. &
564 Zboril, R. (2012). Multimodal action and selective toxicity of zerovalent iron
565 nanoparticles against cyanobacteria. *Environmental Science and Technology*.
566 46 (4). p.pp. 2316–2323.
- 567 Mueller, N.C., Braun, J., Bruns, J., Černík, M., Rissing, P., Rickerby, D. &
568 Nowack, B. (2012). Application of nanoscale zero valent iron (NZVI) for
569 groundwater remediation in Europe. *Environmental science and pollution*
570 *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.
- 580 Saccà, M.L., Fajardo, C., Costa, G., Lobo, C., Nande, M. & Martin, M. (2014).
581 Integrating classical and molecular approaches to evaluate the impact of
582 nanosized zero-valent iron (nZVI) on soil organisms. *Chemosphere*. 104. p.pp.
583 184–189.
- 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.
- 588 Sørensen, S.N., Hjorth, R., Delgado, C.G., Hartmann, N.B. & Baun, A. (2015).
589 Nanoparticle toxicity – Physical and/or chemical effects? *Integrated*
590 *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.
- 593 Wang, C.-B. & Zhang, W. (1997). Synthesizing Nanoscale Iron Particles for Rapid
594 and Complete Dechlorination of TCE and PCBs. *Environmental Science &*
595 *Technology*. 31 (7). p.pp. 2154–2156.

- 596 Weil, M., Meißner, T., Busch, W., Springer, A., Kühnel, D., Schulz, R. & Duis, K.
597 (2015). The oxidized state of the nanocomposite Carbo-Iron® causes no
598 adverse effects on growth, survival and differential gene expression in
599 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.
- 603 Yan, W., Lien, H.-L., Koel, B.E. & Zhang, W. (2013). Iron nanoparticles for
604 environmental clean-up: recent developments and future outlook.
605 *Environmental Science: Processes & Impacts*. 15. p.pp. 63-77.
- 606 Yirsaw, B.D., Mayilswami, S., Megharaj, M., Chen, Z. & Naidu, R. (2016). Effect
607 of zero valent iron nanoparticles to *Eisenia fetida* in three soil types.
608 *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.